EVALUATION OF GENETIC DIVERSITY, DRUG RESISTANCE MUTATIONAL AND CYTOKINE PATTERNS ON HEPATOTOXICITY MARKERS AMONG HIV PATIENTS IN NORTHWEST REGION, CAMEROON

LEM EDITH ABONGWA, (M.Sc.)

(I84F/30736/2015)

A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (MEDICAL MICROBIOLOGY) IN THE SCHOOL OF PURE AND APPLIED SCIENCES OF KENYATTA UNIVERSITY

MAY, 2019
DECLARATION

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

Signature ……………………… Date ……………………………

LEM Edith ABONGWA
Department of Biochemistry, Microbiology, and Biotechnology

We confirm that the work reported in this thesis was carried out by the candidate under our supervision.

SUPERVISORS

Sign…………………… Date…………………………

Dr. Anthony Kebira
Department of Biochemistry, Microbiology, and Biotechnology
Kenyatta University

Prof. Okemo Paul
Department of Biochemistry, Microbiology, and Biotechnology
Kenyatta University

Prof. Fokunang Charles
Department of Pharmacotoxicology and Pharmacokinetics
University of Yaounde, Cameroon

Prof. Judith Torimiro
Chantal Biya International Center for Research on HIV/AIDS (CIRCB)
Yaounde, Cameroon
DEDICATION

This work is dedicated to my parents; Susana and Michael Abongwa, my kids; Abongwa Clinton Luzah, Sama Clifford Luzah and Chofor Anthony Luzah and to my late brother Anthony Ategha Abongwa.
I thank God Almighty for His gift of good health and protection throughout my life. All I have ever been and will ever be in life, I owe it all to you, Lord.

I do acknowledge Kenyatta University for the opportunity to undertake this study.

I am greatly indebted to my supervisors Dr. Anthony Kebira, Profs: Okemo Paul, Fokunang Charles, and Judith Torimiro who have diligently directed this work. I appreciate your invaluable support, unreserved advice, guidance, constructive suggestions that made my entire research study a success.

Special appreciation also goes to Drs: Nchinda G., Fokam J., Teto G. Aubain N., Mr. Takou D. and my lab mates especially Rene K., and Loveline N. of CIRCB Yaounde for your support and fruitful discussions on various aspects of this study.

I am equally indebted to my loving husband Dr. Alus Luzah, and our kids, for supporting and enduring my absence during this period. A big thank you to my brothers and sisters; Emmanuel, Eric, Irene, Annabella, Christian, Stella and Augustina, In a special way, I thank Mr. Akenji M. and Mr. (late)/Mrs. Kikah I., of Ntenefor Credit union for their financial and emotional support that motivated me to realize this project. To all my friends, I say thank you.

Special thanks to the health care providers and laboratory technicians of the study sites. To the study participants I say thank you and may God grant you good health. This work was partly supported by funds from Centre International de Référence “Chantal Biya” (CIRCB), Yaounde and an EDTCP Grant (TA.2010.40200.016), TWAS 12059RG /bio/af/aciG), and Grand Challenge Canada Grant (0121-01) to Dr. Godwin W. Nchinda.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>TITLE</th>
<th>i</th>
</tr>
</thead>
<tbody>
<tr>
<td>DECLARATION</td>
<td>ii</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF APPENDICES</td>
<td>x</td>
</tr>
<tr>
<td>ABBREVIATIONS AND ACRONYMS</td>
<td>xi</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>xiv</td>
</tr>
<tr>
<td>CHAPTER ONE: INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Background information</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Statement of problem</td>
<td>9</td>
</tr>
<tr>
<td>1.3 Justification of the study</td>
<td>11</td>
</tr>
<tr>
<td>1.4 Research hypothesis</td>
<td>12</td>
</tr>
<tr>
<td>1.5 Objectives</td>
<td>13</td>
</tr>
<tr>
<td>1.6 Significance of the study</td>
<td>13</td>
</tr>
<tr>
<td>CHAPTER TWO: LITERATURE REVIEW</td>
<td>15</td>
</tr>
<tr>
<td>2.1 HIV structure</td>
<td>15</td>
</tr>
<tr>
<td>2.2 The HIV genome</td>
<td>16</td>
</tr>
<tr>
<td>2.3 HIV replication</td>
<td>18</td>
</tr>
<tr>
<td>2.4 HIV genetic diversity</td>
<td>19</td>
</tr>
<tr>
<td>2.5 HIV treatment</td>
<td>21</td>
</tr>
<tr>
<td>2.6 HIV drug resistance</td>
<td>27</td>
</tr>
<tr>
<td>2.7 HIV and hepatotoxicity</td>
<td>38</td>
</tr>
</tbody>
</table>
2.8 Cytokines in response to HIV infection......................................................... 43

CHAPTER THREE: MATERIALS AND METHODS .............................................. 47

3.1 Study sites ........................................................................................................ 47
3.2 Study design ...................................................................................................... 49
3.3 Sampling technique .......................................................................................... 50
3.4 Sample population and size determination ...................................................... 50
3.5 Inclusion and exclusion criteria ......................................................................... 50
3.6 Demographic data ............................................................................................ 51
3.7 Sample collection .............................................................................................. 52
3.8 Hepatitis B and hepatitis C screening ............................................................... 52
3.9 Measurement of liver function enzymes (ALT, AST, and ALP) ................. 52
3.10 Cytokine measurement ................................................................................... 54
3.11 HIV subtyping and drug resistance determination ........................................ 54
3.12 Data analysis .................................................................................................... 59

CHAPTER FOUR: RESULTS .............................................................................. 62

4.1 Study population .............................................................................................. 62
4.2 Socio-demographic and clinical characteristics of the study population ...... 62
4.3: Variation of liver biochemical markers .......................................................... 64
4.4 Risk factors associated with severe hepatotoxicity .......................................... 67
4.5 Inflammatory markers and hepatotoxicity ....................................................... 73
4.6 HIV-1 genetic diversity .................................................................................... 76
4.7 Prevalence of transmitted drug resistance ...................................................... 80

CHAPTER FIVE: DISCUSSION, CONCLUSIONS, AND
RECOMMENDATIONS ....................................................................................... 84

5.1 Discussion ......................................................................................................... 84
5.1.1a Variation of liver biochemical markers in relation to the duration of
treatment .................................................................................................................. 84
LIST OF TABLES

Table 2.1: Resistance mutations to NRTIs .................................................................32
Table 2.2: Resistance mutation to NNRTIs .................................................................33
Table 2.3: Mutation resistance to protease inhibitors ...............................................35
Table 2.4: Resistance mutation to integrase inhibitors ..............................................36
Table 2.5: Resistance mutation to fusion inhibitors....................................................37
Table 4.1: Socio-demographic and clinical characteristics of the study population by
drug type ..................................................................................................................63
Table 4.2: Clinical and hepatic markers at baseline ....................................................64
Table 4.3: Variation of ALT, AST and ALP during the study duration.......................65
Table 4.4: Variation of ALT (U/L), AST (U/L), and ALP (U/L) with drug type and
duration .....................................................................................................................66
Table 4.5: Cox proportional univariate analysis for baseline characteristics of patients71
Table 4.6: Cox proportional multivariate analysis for baseline characteristics of patients
with or without severe hepatotoxicity ........................................................................73
Table 4.7: Descriptive characteristics of study participants (n=81) .........................78
Table 4.8: Demographic and immunologic characteristics of patients with TDR and
predicted ARV drug resistance ................................................................................82
Table 4.9: Assessing the presence of TDR and prevalence of hepatotoxicity at end of
D180 .............................................................................................................................83
LIST OF FIGURES

Figure 2.1: The structure of Human immunodeficiency virus.................................16
Figure 2.2: HIV-1 genome.........................................................................................17
Figure 2.3: The life cycle of HIV..................................................................................19
Figure 2.4: Targets for ARVs .......................................................................................22
Figure 3.1: Map of the Northwest region (Bamenda) showing the study sites..........48
Figure 3.2: Schematic representation of the study design .........................................49
Figure 3.3: Verification of amplified RT-PCR product on 1% agarose gel ...............56
Figure 3.4: Verification of amplified semi-nested PCR product on 1% agarose gel...57
Figure 4.1: Flow chart showing the study population..................................................62
Figure 4.2: Prevalence (%) of hepatotoxicity by ALT relating to treatment duration ........................................................................................................67
Figure 4.3: Prevalence (%) of hepatotoxicity by AST with regard to treatment duration ........................................................................................................68
Figure 4.4: Prevalence (%) of hepatotoxicity by ALP regarding treatment duration..68
Figure 4.5: Prevalence (%) of hepatotoxicity within the study duration .................69
Figure 4.6: Relationship between CD4⁺ T-cell counts with ALT and AST at D0 ......74
Figure 4.7: Mean cytokine variation between patients presented with and without hepatotoxicity at D30.........................................................................................75
Figure 4.8: Mean cytokine variation between patients presented with and without hepatotoxicity at D180..........................................................................................76
Figure 4.9: A phylogenetic tree of HIV-1 pol gene.......................................................79
Figure 4.10: Prevalence (%) of hepatotoxicity by HIV subtypes .................................80
LIST OF APPENDICES

Appendix I: First and second lines HIV treatments currently recommended in Cameroon.................................................................................................................128

Appendix II: List of amino acids ...............................................................................................................................................................................................129

Appendix III: Ethical clearance ..........................................................................................................................................................................................130

Appendix IV: Information sheet (in English) .........................................................................................................................................................131

Appendix V: Information sheet (in French) .........................................................................................................................................................133

Appendix VI: Informed consent form (in English) ........................................................................................................................................135

Appendix VII: Informed consent form (in French) ........................................................................................................................................136

Appendix VIII: Questionnaire ......................................................................................................................................................................................137

Appendix IX: Composition of reagents (R) used for ALT, AST, and ALP measurement .......................................................................................................................139

Appendix X: Sequences and accession numbers in GenBank............................................................................................................................140
### ABBREVIATIONS AND ACRONYMS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$:</td>
<td>US dollars</td>
</tr>
<tr>
<td>(/r:)</td>
<td>Administered with ritonavir for pharmacokinetic boosting</td>
</tr>
<tr>
<td>3TC:</td>
<td>Lamivudine</td>
</tr>
<tr>
<td>Ab:</td>
<td>Antibodies</td>
</tr>
<tr>
<td>ABC:</td>
<td>Abacavir</td>
</tr>
<tr>
<td>ADR:</td>
<td>Acquired drug resistance</td>
</tr>
<tr>
<td>AIDS:</td>
<td>Acquired Immune Deficiency Syndrome</td>
</tr>
<tr>
<td>ALP:</td>
<td>Alkaline Phosphatase</td>
</tr>
<tr>
<td>ALT:</td>
<td>Alanine Amino Transferase</td>
</tr>
<tr>
<td>ART:</td>
<td>Antiretroviral Therapy</td>
</tr>
<tr>
<td>ARV:</td>
<td>Antiretroviral</td>
</tr>
<tr>
<td>AST:</td>
<td>Aspartate Amino Transferase</td>
</tr>
<tr>
<td>ATV:</td>
<td>Atazanavir</td>
</tr>
<tr>
<td>AZT:</td>
<td>Zidovudine</td>
</tr>
<tr>
<td>cART:</td>
<td>Combination of Antiretroviral Therapy</td>
</tr>
<tr>
<td>CD4$^+$:</td>
<td>Cluster of Differentiation 4</td>
</tr>
<tr>
<td>CDC:</td>
<td>Center for Diseases Control</td>
</tr>
<tr>
<td>CRFs:</td>
<td>Circulating recombinant forms</td>
</tr>
<tr>
<td>CrS:</td>
<td>Serum Creatinine Levels</td>
</tr>
<tr>
<td>D:</td>
<td>Day</td>
</tr>
<tr>
<td>D4T:</td>
<td>Stavudine</td>
</tr>
<tr>
<td>DDI:</td>
<td>Didanosine</td>
</tr>
<tr>
<td>DNA:</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>dNTP:</td>
<td>Deoxynucleoside triphosphate</td>
</tr>
<tr>
<td>DR:</td>
<td>Drug resistance</td>
</tr>
<tr>
<td>DRM:</td>
<td>Drug resistance mutation</td>
</tr>
<tr>
<td>DRV:</td>
<td>Darunavir</td>
</tr>
<tr>
<td>Ds DNA:</td>
<td>Double stranded deoxyribonucleic Acid</td>
</tr>
<tr>
<td>EFV:</td>
<td>Efavirenz</td>
</tr>
<tr>
<td>env:</td>
<td>Viral envelope</td>
</tr>
<tr>
<td>ETR:</td>
<td>Etravirine</td>
</tr>
<tr>
<td>FBC:</td>
<td>Full Blood Count</td>
</tr>
</tbody>
</table>
FPV: Fosamprenavir
FTC: Emtricitabine
gag: Group specific antigen gene
gp: Glycoprotein
HAART: Highly active anti-retroviral therapy
HBsAg: Hepatitis B soluble Antigen
HCC: Hepatocellular cancer
HIV: Human Immune Virus
HIV-1: HIV type 1
HIV-2: HIV type 2
IDV: Indinavir
IFN-γ: Interferon gamma
IL: Interleukin
INI: Integrase Inhibitors
IQR: Interquartile range
LANL: Los Alamos National Library
LDH: Lactate dehydrogenase
LPV: Lopinavir
LTR: Long terminal repeats; repetitive sequence of bases
M: Major or Main group
MDH: Malate dehydrogenase
Min: Minute
MTCT: Mother-to-child-transmission
N: Non-M, non-O group
NAD⁺: Oxidized Nicotinamide adenine dinucleotide
NADH: Reduced Nicotinamide adenine dinucleotide
Nef: encodes for negative regulator protein
NFV: Nelfinavir
NK cells: Natural killer cells
NNRTI: Non-nucleoside Reverse Transcriptase Inhibitors
NRTI: Nucleoside reverse transcriptase inhibitors
NVP: Nevirapine
NWR: North West Region
O: Outlier group
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD</td>
<td>Optical Density</td>
</tr>
<tr>
<td>P</td>
<td>Protease</td>
</tr>
<tr>
<td>p</td>
<td>Viral protein</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction.</td>
</tr>
<tr>
<td>PI</td>
<td>Protease Inhibitor</td>
</tr>
<tr>
<td>PMTCT</td>
<td>Prevention of mother to child transmission</td>
</tr>
<tr>
<td>Pol</td>
<td>Polymerase gene</td>
</tr>
<tr>
<td>RAM</td>
<td>Resistance-associated mutations</td>
</tr>
<tr>
<td>Rev</td>
<td>Regulator of expression of viral protein</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
</tr>
<tr>
<td>Rpm</td>
<td>Revolution per minute</td>
</tr>
<tr>
<td>RPV</td>
<td>Rilpivirine</td>
</tr>
<tr>
<td>RT</td>
<td>Reverse Transcriptase</td>
</tr>
<tr>
<td>S</td>
<td>Second</td>
</tr>
<tr>
<td>SDRM</td>
<td>surveillance drug resistance mutation</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of mean</td>
</tr>
<tr>
<td>SQV/r</td>
<td>Saquinavir</td>
</tr>
<tr>
<td>SsDNA</td>
<td>Single-stranded deoxyribonucleic Acid</td>
</tr>
<tr>
<td>ssRNA</td>
<td>Single-stranded ribonucleic Acid</td>
</tr>
<tr>
<td>TAM</td>
<td>Thymidine analogue mutation</td>
</tr>
<tr>
<td>Tat</td>
<td>Transactivator protein</td>
</tr>
<tr>
<td>TDF</td>
<td>Tenoforvir</td>
</tr>
<tr>
<td>TDR</td>
<td>Transmitted drug resistance</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumour necrosis factor-α</td>
</tr>
<tr>
<td>TPV/r</td>
<td>Tipranavir</td>
</tr>
<tr>
<td>US</td>
<td>United State</td>
</tr>
<tr>
<td>Vif</td>
<td>Viral infectivity</td>
</tr>
<tr>
<td>Vpr</td>
<td>Viral protein R</td>
</tr>
<tr>
<td>Vpu</td>
<td>Viral protein U</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
ABSTRACT

Highly active anti-retroviral therapy (HAART) has been known to cause hepatotoxicity despite its benefit to improve the morbidity and mortality associated with HIV infections. However, the impact of HAART on HIV subtypes, drug resistance mutations and cytokine profiles, is not yet fully elucidated especially in the Northwest Region (NWR) of Cameroon. Therefore, this study was carried out with aim of determining the effect of HIV-1 subtypes, drug resistance mutations, cytokine profiles and risk factors on hepatotoxicity markers among HIV-1 drug naïve adults in the NWR of Cameroon. This was a longitudinal study conducted from February to November 2016 and newly diagnosed HIV-1 drug naïve patients were recruited into the study. Stratified and simple random sampling techniques were used in selecting the five study sites. Blood samples were collected prior to HAART initiation Day (D) 0 and at D30 and D180. The participants were placed on either Tenofovir (TDF) + Lamivudine (3TC) + Efavirenz (EFV) or Zidovudine (AZT) + 3TC + Nevirapine (NVP) or AZT+3TC+EFV regimens. Serum levels of alanine aminotransferases (ALT), aspartate aminotransferases (AST) and alkaline phosphatase (ALP) were analyzed. Human Th1/Th2/Th17 cytokines were measured using a cytometric bead array assay. Genotypic and transmitted drug resistance (TDR) analyses were performed by sequencing HIV virus using an in-house protocol. Data were analyzed using SPSS vs. 23 and Graph pad prism 6. HIV-1 subtypes were determined phylogenetically using MEGA vs. 7 while TDR and resistance-associated mutations (RAMs) were identified using the Stanford HIVDR interpretation program. The level of significance was set at 5%. In all, 100 individuals participated in the study with a mean (age range) of 36.5 (18-61) years. Of this, 37(37%) and 49(49%) patients presented with hepatotoxicity while 15% and 28% of patients had severe hepatotoxicity at D30 and D180 respectively. Serum levels of ALT, AST and ALP increased significantly (p<0.05) with increased treatment duration and in patients on AZT+3TC+NVP treatment. Multivariate analysis showed that age <30 years, low BMI, low monthly income and the use of AZT+3TC+NVP regimen were independent risk factors for severe hepatotoxicity. Mean interleukin (IL)-6 and IL-17A, were significantly (p<0.05) high in patients with hepatotoxicity compared to patients without hepatotoxicity at D30 and D180. HIV-1 subtype revealed that CRF02 _AG (75.3.0%) was the most predominant subtype. The prevalence of hepatotoxicity was highest at 70.3% (26/37) among individual harboring CRF02_AG virus. This study shows that 9 (11.1%) patients were infected with HIV variants that carried RAMs associated with NRTI; 8.6% (7/81), NNRTI; 4.9% (4/81) and PI; 1.2% (1/81). Seven of the nine patients with TDR developed hepatotoxicity of different grades at D180. Thus the use of NVP based regimen should not be recommended for patients less than 30 years and with low baseline BMI. IL-6 and IL-17A seem to play a significant role in the pathophysiology of hepatotoxicity. CRF02_AG was the most predominant HIV strain. Despite the sample size, these findings highlight a caution in the management of hepatotoxicity and the need for continuous surveillance for TDR.
CHAPTER ONE: INTRODUCTION

1.1 Background information

Human immunodeficiency virus (HIV) infection still remains a global health problem despite the intense medical and scientific research being done (Elmi Abar et al., 2012; UNAIDS, 2018). Since the beginning of the epidemic, about 77.3 [59.3–100.0] million people have been infected with HIV (WHO, 2018). Globally, there were about 1.8 million new cases of HIV in 2017 (UNAIDS, 2018) and about 36.7 [31.1–43.9] million persons were living with HIV in 2017. Sub-Saharan Africa carries the highest burden of HIV and AIDS universally and accounts for about 64% of all new HIV infections (USAIDS, 2018; WHO, 2018). HIV prevalence in Cameroon was 4.3% in 2016 (Fokam et al., 2015; Patino-Galindo et al., 2017; Teto et al., 2017; WHO, 2017) and has dropped to 3.4% in 2018 affecting about 680,000 inhabitants with close to 28,000 new infections (www.cameroononline.org/cameroon-hiv-aids-prevalence).

The advent of highly active antiretroviral therapy (HAART) has significantly reduced the morbidity and mortality of HIV infected patients by more than 51% since the peak in 2004 (UNAIDS, 2018). Thus has significantly improved their quality of life (Kan et al., 2017; Onywera et al., 2017). This has led to increased access to HAART from 7.7 million in 2010 and 17.1 million in 2015 to 21.7 million in 2017 (UNAIDS, 2018; WHO, 2018). HAART was introduced in Cameroon in 2002 where patients had to pay US $23–$100 for their drugs monthly. The Government of Cameroon with financial support from the Global Fund to Fight AIDS, Tuberculosis and Malaria reduced the cost from US $23–$100 in 2002, to US $10 at the beginning of 2004 and by May 2007 all eligible individuals received ARV drugs free of charge through a national distribution program (Burda et al., 2010). As such there has been a rapid
scale-up of HAART with an increasing national coverage from 0% in 2001, 2% in 2003, 32% in 2016 and 49.3% by the end of June 2018 of the eligible patients (Billong et al., 2016; Teto et al., 2017; National AIDS control committee, 2018).

Although HAART helps to reduce HIV associated morbidity and mortality, toxicity and drug resistance remains a challenge in this field of medicine as it has resulted in other complications such as liver toxicity, hematuria, decreased bone density, cardiovascular disease, gastrointestinal tract infection, hypersensitivity reaction, lactic acidosis, lipodystrophy, myopathy, Steven Johns syndrome, among others (Soriano et al., 2008; Wambani et al., 2015; De Luca et al., 2017). These complications are associated with both short and long-term toxicity effects which may be life-threatening and likely to cause significant morbidity and mortality. These effects vary from organ to organs such as the kidney and the liver (Wondemagegn et al., 2013; Osakunor et al., 2015; Abongwa et al., 2017). In the era of effective HAART, 1–54.0% of patients are shown to have liver toxicity (also known as hepatotoxicity) and have been attributed to discontinuation of HAART which has increased the morbidity and mortality rates among HIV-infected patients (Wondemagegn et al., 2013; Priyanka and Ezhilarasan, 2014; Wambani et al., 2015; Wenderlein et al., 2016). Nevertheless, the hepatic complications due to the use of antiretroviral therapy (ART) have not yet received enough attention in our African milieu in general and the Northwest region in particular.

The liver is one of the vital organs in the reticuloendothelial system and is useful in the metabolism of drugs and their detoxification. Its thus contains several enzymes which are markers of cellular damage such that their serum levels are increased in individuals with various forms of liver disease (Osakunor et al., 2015; Seki and
Example of these enzymes include alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), among others (Elameen and Abdrabo, 2013). With the widespread use of HAART and the availability of new ART, antiretroviral (ARV) drugs have gained prominent attention owing to its negative impact on these biochemical enzymes (Wambani et al., 2015; Wenderlein et al., 2016).

Hepatotoxicity has been reported across all families of HAART which include; non-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleoside/nucleotide reverse transcriptase inhibitors (Ns/NtRTIs) and protease inhibitors (PIs) though some may cause liver injury more frequently than others in individuals and different regions (Ofotokun et al., 2007; Wenderlein et al., 2016). Previous studies have shown that the contribution of each particular drug to the development of hepatotoxicity varies and is difficult to assess (Mugusi et al., 2012; Wambani et al., 2015; Wenderlein et al., 2016). Thus, with the increased use and development of new HAART, there is a need to continuously monitor its involvement in the liver cells via the estimation of serum levels of these hepatic enzymes.

Hepatotoxicity evaluation can be done by both invasive and non-invasive methods. The invasive method includes liver biopsy while non-invasive methods include molecular serum biomarkers tests and imaging techniques which have gained interest recently in identifying and describing hepatotoxicity (Liu et al., 2009; Sumida et al., 2014). However, little work has been done in evaluating the effect of these biomarkers in HIV patients on HAART in resource-limited countries. Identifying such markers may lead to defining common pathways in the pathophysiology of
hepatotoxicity (McBurney et al., 2012) which may be helpful in the management of these patients.

Although HAART has been associated with increases in serum transaminases, little is known about the risk factors associated with severe hepatotoxicity. Treatment initiation and switch are only based on clinical and immunological assessments with little or no assessment on its level of hepatotoxicity. Apart from ARV drugs, it has been shown that other factors such as baseline levels of CD4+ T cells count, AST, and ALT, hepatitis B and C, alcohol, age, gender, other drugs (for example tuberculosis drugs, paracetamol, among others), jaundice, poor nutrition among others, can influence hepatotoxicity. These risk factors vary by studies from different regions and localities (Kontorinis, and Dieterich, 2003; Wondemagegn et al., 2013). As such, additional studies are needed to gain a better appreciation for the different risk factors associated with hepatotoxicity.

Cytokine levels are known to control the nature, amount and duration of the immune response in a particular individual (Tudela et al., 2014; Akase et al., 2017) and are produced by virtually all nucleated cells in the body including the Kupffer cells of the liver (Neuman, 2003). Both in vivo and in vitro studies conducted indicate that HIV infection results in dysregulation of the cytokine profiles (Reuter, et al., 2012; De Medeiros et al., 2016; Akase et al., 2017). Most research studies carried out between cytokines and HIV have relied on evaluating the impact of different cytokines on HIV replication or disease progression (De Medeiros et al., 2016; Akase et al., 2017), comparing the cytokine expression profile of HIV-infected and uninfected donors (Spear et al., 2005; Williams et al., 2013), measuring cytokine production from in vitro infected cells (Reuter et al., 2012; Akase et al., 2017) or its approach with
respect to diagnosis, treatment and vaccine development (Reuter, et al., 2012; Williams et al., 2013). In addition, many studies have shown that a switch from type 1 cytokines to type 2 patterns is indicative of HIV disease progression and replication (Vishwanath et al., 2011; Akase et al., 2017). As such, cytokine measurements are considered evolution markers regarding ART markers (Meira et al., 2008).

A few studies have revealed that there is a possible relationship between cytokines and liver injury in patients with Hepatitis B virus (Lian et al., 2014; Deng et al., 2015). As such an imbalance in cytokine production plays a key role in the development of liver damage, necro-inflammation and subsequent fibrosis (Galun and Axelrod, 2002; Seki and Schwabe, 2015). However, the existence of any relationship between cytokine and HAART induced hepatotoxicity has not been proven and there is, therefore, an urgent need to investigate if cytokines play a vital role in HAART-induced hepatotoxicity. This study will, therefore, provide information for the first time, on the cytokine profile among HIV patients on HAART with and without hepatotoxicity. The alterations of cytokines levels following HAART induced hepatotoxicity might favor viral replication which leads to the development of resistant mutations. In addition, it might also stimulate the production of free radicals that may weaken the host immunity (Vishwanath et al., 2011). On the other hand, the release of cytokines into circulation may regulate the internal conditions of the cells and thus enhance immunity against chronic intracellular infections in almost all situations (Xing et al., 1998). Thus regulating the level of these cytokines might serve as a potential means of therapy for HIV-1 and other hepatic diseases. Results from this study may allow new cytokine therapeutic approaches based on the use of either recombinant cytokines or specific cytokine antagonists, with the aim of limiting the spread of HIV and the clinical manifestations associated with it.
Genetic diversity remains one of the major hindrances to the eradication of HIV and has been shown to have consequences in global distribution, vaccine design, therapy success and disease progression (Nyamache, 2012; Santoro and Perno, 2013; Patino-Galindo et al., 2017). The upward trend in global HIV-1 diversity has continued to increase, with newer groups, subtypes, unique and circulating recombinants forms being identified in most African countries (Courtney et al., 2016; Teto et al., 2017; Fokam et al., 2018) and Western countries (Li et al., 2013; Siemieniuk et al., 2013; Patino-Galindo et al., 2017). These subtypes, however, vary significantly in types, regions and in range (Veras et al., 2011; Patino-Galindo et al., 2017). The differences among subtypes have been reported mostly in relation to prevalence, drug studies, vaccine development and disease progression (Santoro and Perno, 2013; Sallam et al., 2017). However, it has not been proven if the genetic variation has any impact on the development of hepatotoxicity. As such there exist critical gaps that limit the understanding of the effect of the different HIV-1 subtypes on biochemical markers of hepatotoxicity. Furthermore, there is a need to regularly monitor and screen for new or rare HIV variants, as increasing variants are being reported in different areas and localities.

Cameroon still remains an ideal country to explore the genetic diversity of HIV-1 and its impact on the global pandemic. In Cameroon, despite the low infection rate among the general population, phylogenetic analysis of the gag, pol and env gene sequences have shown that the genetic diversity of the virus is very broad and all group M clades: A (A1, A2), B, C, D, F (F1, F2) G H, J, K and several circulating recombinant forms (CRFs) have been identified. These CRFs include; CRF01_AE, CRF02_AG, CRF06_cpx, CRF09_cpx, CRF11_cpx, CRF13_cpx, CRF18_cpx, CRF22_01A1, CRF36_cpx, and CRF37_cpx with CRF02_AG being the predominant type (Powell et
al., 2010; Ragupathy et al., 2011; Agyingi et al., 2014; Negedu-Momoh et al., 2014; Teto et al., 2017). Although several studies have reported a high genetic variability of non recombinant and different recombinant forms of HIV-1 from the different parts of Cameroon such as in the Centre, Northern, Southwest, Eastern, and Littoral regions (Veras et al., 2011; Aghokeng et al., 2013; Billong et al., 2013; Agyingi et al., 2014; Teto et al., 2017), adequate information is lacking in the Northwest region (NWR). Furthermore, research on genetic diversity in Cameroon with many distinct subtypes and recombinant forms is of great importance as there is a possibility of viral recombination with recombinant viruses. This might lead to the complexity of new circulating HIV strains. This diverse genetic diversity is a public health concern in the area of diagnosis, therapeutics, and development of an effective HIV vaccine. However, studies carried out in the NWR have identified some new stains despite the low sample size used (Tebit et al., 2002; Sherri et al., 2004). This study, therefore, seeks to find the different variants and to determine if HIV-1 genetic diversity has an effect on biochemical markers of hepatotoxicity. As such it is of prime importance to identify possible new strains especially in the NWR with dearth data on genetic diversity. Results from such findings will anticipate the complex forms that may be introduced with time.

In 2017, it was found that over half of all the people harboring the HIV (59%) now have access to life-saving treatment compared to 8 million in 2010 (UNAIDS, 2018; WHO, 2018). With increased treatment coverage, HIV drug resistance (HIVDR) and onward transmission of drug resistance mutations (DRMs) are expected to increase, especially in settings relying on clinical and CD4 monitoring to detect treatment failure. However, the emergence of drug-resistant HIV strains have been shown to compromise the effectiveness of HIV management and this is a major threat to the
scaling up of ART especially in developing countries (Li et al., 2015; Abdissa et al., 2014; Barennes et al., 2014; Ferreira et al., 2017; Machnowska et al., 2017).

The increased survival of individuals on HAART favors the onward transmission of HIV resistant strains. Transmission of ARV drug-resistant HIV strains has been reported in both developed and developing countries with limited project-driven access to treatment (Xiaobai et al., 2014; Onywera et al., 2017; Teto et al., 2017). This has limited ARV treatment responses to first-line ART among naive HIV infected persons and increases the risk of treatment failure. Consequently, the development and transmission of drug resistant viruses have become a major concern with the scaling up of ART (Pennings, 2013; Barennes et al., 2014; Xiaobai et al., 2014). Although transmitted drug resistance (TDR) mutations have been studied extensively in high-income countries such as France, United States and Denmark (Abdissa et al., 2014; Pennings, 2013; Xiaobai et al., 2014), there is still a paucity of data from resource-limited settings like ours. Previous studies in other countries have identified DRM in naïve patients ranging from 3.4-29% (Xiaobai et al., 2014; De Luca et al., 2017; Onywera et al., 2017) and from 3.6 to 44%, in Cameroon (Laurent et al., 2005; Burda et al., 2010; Ragupathy et al., 2011; Ceccarelli et al., 2012; Agyingi et al., 2014; Zoufaly et al., 2014; Teto et al., 2017). Currently, TDR testing is not consistent in most resource-limited settings, including Cameroon. It has been shown that TDR surveillance is required to evaluate the appearance and spread of drug-resistant HIV strains so as to update HIV treatment guidelines. However, the prevalence and pattern of HIV TDR in the Northwest region of Cameroon remain uncertain. Studies on TDR mutation patterns in drug naïve patients are more likely to reveal the right drug resistance pattern. Thus, this study will examine the recent HIV drug resistance pattern in patients naïve to treatment in the NWR.
Studies on HIV-1 DR are mostly prevalence studies or are associated with drug types. Results from these studies vary across regions and countries (Vergne \textit{et al.}, 2006, Aghokeng \textit{et al.}, 2013; Teto \textit{et al.}, 2017). However, it has not been shown if the HIV DR pattern has an effect on the development of hepatotoxicity which we seek to investigate. Such a study is essential especially with the scale-up of the ART program that has led to a tremendous increase in ART coverage.

Since new HIV-1 viral variants emerge due to recombination with well documented or existing recombinant forms or from entirely new viral variants, it is important to continuously evaluate the development of HIV-1 genetic diversity and the effectiveness of ART in different populations of the world as the potential for recombination plays an important role in the development of resistance against ARV drugs (Kantor \textit{et al.}, 2005; Agyingi \textit{et al.}, 2014). Thus, in this study, we shall also be identifying naïve individuals with drug-resistant mutations and their effect on liver toxicity.

\textbf{1.2 Statement of problem}

In Cameroon, research work on HIV is mostly on the prevalence, treatment outcome, genetic diversity, drug resistance, mother to child transmission, hepatotoxicity and prevention in regions like the Centre and Littoral regions with little attention in the Northwest region. Although several patterns of response after initiation of HAART have been observed in persons with HIV infection, adequate knowledge on its toxicity effects in organs like the liver and kidney is not established. Taking into consideration the important role the liver plays in the body in metabolism and drug detoxification, it is of prime importance to monitor the biochemical markers of hepatotoxicity and risk.
factors associated with HAART. It is, therefore, imperative that the liver is monitored; and the HAART regimens that may be toxic to it identified so that changes or modifications can be made to enhance patient care.

Despite recorded low HIV prevalence of 3.4% in Cameroon (5.1% in the NWR) compared to most Sub Saharan countries (CAMPHIA; 2018; www.cameroononline.org, 2018), all circulating HIV-1 subtypes have been detected in Cameroon hence the possibility of viral combinations (Billong et al., 2013; Agyingi et al., 2014; Teto et al., 2017). This HIV-1 diversity causes variations in immune response and transmission fitness by the different subtypes and therefore causes challenges in vaccine design. Nevertheless, the distribution of these HIV subtypes in other regions, especially in the Northwest region of Cameroon, is not fully determined. In addition, with the increasing number of patients on HAART, there is a high demand for new drugs. Based on the high prevalence (1.1-44%) of DR variants in drug-naive clients there is need to evaluate treatment response to first-line drugs and advise to a switch in treatment in case of treatment failure.

Even though some cytokines have been shown to play a vital role in the pathogenesis or up-regulator of HIV replication, approaches to evaluate the relationship between cytokines and HIV have relied only on comparing the cytokine expression profile and not of its importance in the development of hepatotoxicity. As such, there is a need to evaluate immune response through induced inflammation due to viral infection and drug toxicity. This, therefore, calls for serious consideration to determine the role of genetic diversity, HIV-1 drug resistance mutation and cytokine profiles on biochemical markers of hepatotoxicity.
1.3 Justification of the study

The role of monitoring hepatotoxicity has not been fully studied in resource-limited settings. Since the introduction of ART in 2003 in Cameroon, there has been little evidence on the trend, analysis studies to determine this effect and risk factors associated with it. This is mostly because laboratory equipment is either not available, not functioning properly, reagents are expired or out of stock or there is a shortage of skilled laboratory personnel. This makes access to laboratory diagnostic tests limited and very expensive for most HIV clients on treatment. With the adoption of the WHO new policies of “test and treat” where any individual who is tested positive for HIV is placed on HAART irrespective of the clinical and immunological status (www.who.int/mediacentre), there is an increase in the number of HIV infected patients on HAART. Several factors have been identified to cause hepatotoxicity in HIV persons on HAART (Wambani et al., 2015; Wenderlein et al., 2016). However, these findings do not fully convince researchers as being universally applicable based on diverse regions, cultures, and specific human adaptations. It is for this reason that researchers are still being encouraged to carry out such studies in other regions and setups to determine if similar findings will result.

Although cytokines have shown to play an essential role in the pathogenesis or replication of many infections (Vishwanath et al., 2011; Akase et al., 2017), its role in hepatotoxicity has not been done. In addition, despite the low infection rate among the general population, the genetic diversity of the viruses infecting individuals in Cameroon is broad (De Oliveira et al., 2017). However, studies carried out in the NWR have identified some new strains despite the low sample size used (Tebit et al., 2002; Sherri et al., 2004). The small sample size used and the diversity in strains make it important to carry out similar research with a larger population size.
Moreover, studies on genetic diversity in Nigeria, a neighbouring country to the NWR, revealed that, in addition to the subtypes which are common in Cameroon, there exist some rare complex recombinant forms like CRF15_01B and CRF19_cpx, (Chaplin et al., 2011; Negedu-Momoh et al., 2014) which are not common in Cameroon. Furthermore, studies on DR in Cameroon have shown that DR ranges from 3.6%-44% (Billong et al., 2013; Agyingi et al., 2014; Teto et al., 2017) in different parts of the country after a certain period of drug initiation. However, this information is lacking in the NWR especially among drug naïve clients. Consequently, the need to monitor the evolving diverse strains and its impact on ART regimens in Cameroon in order to ensure continuous efficacy of the limited available ART as new antiretroviral agents become available with the increasing number of clients on HAART. Therefore, the findings of this present study would provide baseline information on the magnitude of liver toxicity in relation to antiretroviral agents, genetic diversity, cytokine profile, and drug-resistant pattern. This study will also identify possible risk factors of hepatotoxicity in this area.

1.4 Research hypothesis

i. Different ARV drugs and duration of treatment do not affect liver biochemical markers

ii. The different risk factors associated with HAART does not induce hepatotoxicity

iii. Some inflammatory markers are not associated with hepatotoxicity

iv. HIV-1 subtypes do not differ among individuals of the Northwest Region and also have no effect on the development of hepatotoxicity

v. The difference in the HIV drug resistance patterns has no effect on the development of hepatotoxicity
1.5 Objectives

1.5.1 General objective

To determine the effect of HIV-1 genetic diversity, drug resistance, patterns and cytokine profiles on hepatotoxicity markers among HIV patients.

1.5.2 Specific objectives

i. To determine the variation of liver biochemical markers in relation to drug type and duration of treatment

ii. To determine risk factors associated with severe hepatotoxicity

iii. To determine inflammatory markers associated with hepatotoxicity

iv. To determine HIV-1 genetic diversity and its effect on the development of hepatotoxicity among individuals on treatment in the NWR of Cameroon

v. To determine the HIV drug resistance pattern and its effects on hepatotoxicity

1.6 Significance of the study

It is expected that findings from this study will provide a better understanding of direct and indirect factors responsible for hepatotoxicity from the Cameroonian settings. This would also impart an important message to policymakers to integrate certain cytokine profiles as a monitoring tool of assessing biochemical markers of hepatotoxicity at a rate affordable and accessible by all. The identification of reliable biochemical markers linked with the progression of hepatotoxicity will also be used by pharmaceutical companies to overcome the negative impact of HAART. Similarly, the identification of the circulating HIV genotypes in the NWR of Cameroon will contribute to the knowledge required for vaccine development. These research findings will facilitate a better understanding of the rapidly evolving ART guidelines.
Furthermore, the identification of resistant genes is critically important to the continued success of ART scale-up as it will optimize strategies to help clinicians to identify predictive treatment failure and advice on second-line drugs. Lastly, it is hoped that the results of this study would act as a pioneering piece of study in Cameroon that would help the Ministry of Health, health-related institutions, health practitioners and academicians to conduct wider research into the dynamics of HAART induced hepatotoxicity.
CHAPTER TWO: LITERATURE REVIEW

2.1 HIV structure

HIV is a retrovirus (enveloped virus) of the genus lentivirus (McGovern et al., 2002). HIV, as shown in figure 2.1, is spherical in structure with a diameter of about 120 nm and has a nucleocapsid also known as viral protein (p) 24. Present within p24 is two identical, single strands (ss) of RNA genetic material that contain enzymes such as reverse transcriptase, protease, and integrase needed for the development of the virion (Engelman and Cherepanov, 2012). It also has a matrix which is made up of the viral protein (p17) that surrounds the capsid and ensures the integrity of the virion particle. The p17 is surrounded by the viral envelope (env) and is made up of phospholipids that are taken from the human cell membrane when the newly viral particle buds from the cell. Entrenched in the env are peg-like HIV proteins that protrude through the surface of the viral particle. Each peg consists of three or four glycoproteins (gp) 41 (the stem), capped with three or four gp120 (the head). The glycoprotein molecules allow the virus to attach to and fuse with target cells in order to begin the infectious cycle (Engelman and Cherepanov, 2012).

HIV is divided into two major types; HIV type 1 (HIV-1) and HIV type 2 (HIV-2). The HIV-1 is reported to be related to viruses that originate from Chimpanzees and Gorillas existing in the western part of Africa. On the other hand, HIV-2 viruses are closely related to viruses found in West African primate sooty mangabey. HIV-1 and HIV-2 share many similarities and differences (Santiago et al., 2005; Kannangai et al., 2012). Their similarities include their essential gene arrangement, clinical findings, methods of transmission, replication pathways and both of them result in AIDS. In terms of disease progression, both viruses demonstrate similar pathological
processes (Kannangai et al., 2012; Nyamweya et al., 2013). The differences between these two infections enlighten broader issues of retroviral pathogenesis, which remain incompletely understood (Nyamweya et al., 2013; Santoro and Perno, 2013). Unlike HIV-2, HIV-1 includes four groups: M (main), O (outlier), N (non-M, non-O), and P (putative), which have different geographic distributions but similar clinical symptoms (Veras et al., 2011; Santoro and Perno, 2013; Negedu-Momoh et al., 2014).

Figure 2.1: The structure of Human immunodeficiency virus (Rajarapu, 2014)

2.2 The HIV genome

The HIV virus is characterized by broad genomic diversity as a result of multiple factors which include multiple zoonotic transmissions and high viral evolution and recombination rates (Santoro and Perno, 2013; Agyingi et al., 2014; De Oliveira et al., 2017). The HIV genome (Figure 2.2) contains nine genes that encode fifteen viral
proteins that control the ability of HIV to replicate, infect new cells and cause disease (Engelman and Cherepanov, 2012; www.hiv.lanl.gov/content/sequence/HIV/MAP/landmark.html). These include the three major genes known as the gag (Matrix [p17], Capsid [p24], Nucleocapsid [p6 and p7]), pol (Protease, Reverse transcriptase (RT), Integrase), and env (gp120, gp41) which codes the viral structural proteins. The remaining genes code for the regulatory (Tat, Rev) and accessory proteins (Vif, Vpr, Vpu/Vpx, Nef). The vpu is present only in HIV-1 while vpx is present only in HIV-2. At each end of its genome is found the Long terminal repeat (LTR) that act as “sticky ends” and enable the integrase protein to insert the HIV genome into the host DNA (www.hiv.lanl.gov/content/sequence/HIV/MAP/landmark.html).

Figure 2.2: HIV-1 genome
(www.hiv.lanl.gov/content/sequence/HIV/MAP/landmark, 2017)
2.3 HIV replication

In figure 2.3, HIV recognizes and attaches to T helper cells specifically the CD4+T cell receptors and causes a conformational change that allows the chemokine co-receptors (CCR5) and/or CXCR4 co-receptors to interact with extracellular gp120 and transmembrane gp41 viral surface proteins (Chan and Kim, 1998). This allows gp41 to insert its hydrophobic terminals into the cell membrane and enable the fusion of the two membranes (step 1). The viral nucleocapsid then enters the host cell and releases the 2 viral ssRNA and the 3 essential enzymes; reverse transcriptase, integrase, and protease (step 2). Reverse transcriptase transcribed HIV RNA into single-strand (ss) and eventually double-strand (ds) proviral DNA (Oguntibeju, 2012). Ribonuclease breaks down RNA and the polymerase complete the remaining ssDNA to a dsDNA helix (step 3). The integrase then transfers the dsDNA into the host cell genome. Activation of the cell induces transcription of proviral DNA into mRNA (step 4). The viral mRNA migrates into the cytoplasm where building blocks for a new virus are synthesized some of which is processed by the virus using the enzyme protease. Protease clip longer proteins to smaller core proteins (step 5). The two viral RNA strands and a replication enzyme then come together and more proteins assemble around them forming the capsid. This immature viral protein leaves the cells acquiring envelope of host and viral proteins (step 6) the virion matures and is ready to infect other cells (step 7).
2.4 HIV genetic diversity

Among other virus studied, HIV-1 displays a greater degree of genetic and antigenic variability due to its high mutation rate during viral replication (Santoro and Perno, 2013; Siemieniuk et al., 2013). So far, HIV-1 has 4 distinct groups (M, O, N and P) with at least 9 different subtypes (A [A1, A2, A3, A4], B, C, D, F [F1, F2], G, H, J and K), 98 circulating recombinant forms (CRFs) and numerous unique recombinant forms (URFs) within the major M Group (http://www.hiv.lanl.gov/content/sequence/HIV/CRFs/CRFs.html). This is due to the high mutation rate of the reverse transcriptase (RT) enzyme that lacks the proof-reading activity and high potential for recombination (Santoro and Perno, 2013; Recordon-Pinson et al., 2018). Phylogenetic classifications are presently based either
on nucleotide sequences derived from multiple subgenomic regions (*gag, pol* and *env*) of the same isolates or the complete genome sequence analysis (Veras *et al.*, 2011; Elmi Abar *et al.*, 2012; De Oliveira *et al.*, 2017; Patino-Galindo *et al.*, 2017). Studies have shown that the genetic variation within a subtype ranges from 15 to 20%, whereas variation between subtypes ranges from 25 to 35% (Santoro and Perno, 2013). The diversity and distribution of HIV-1 subtypes and CRFs have influenced the spectrum of disease progression, antiretroviral therapy as well as complicated the development of effective vaccines (Kantor *et al.*, 2005; Abdissa *et al.*, 2014; Recordon-Pinson *et al.*, 2018).

It is estimated that the HIV prevalence in Cameroon is about 3.4% in about 20 million inhabitants (www.cameroononline.org). Although this prevalence is low, Cameroon has the widest genetic arrays where all known groups and multiple variants strains of HIV-1 (M, N, O, and P) have been identified. These multiple variants include; CRF02_AG, the predominant strain with a prevalence of 48.6–80%. Other subtypes A1, A2, A3, A4, B, D, C, F1, F2, G, H, J, K, CRF01_AE, CRF02_AG, CRF06_cpx, CRF09_cpx, CRF11_cpx, CRF13-cpx, CRFs 14_BG, CRF16_A2D, CRF18-cpx, , CRF22_01A1, CRF22-cpx, CRF25_cpx, CRF27_cpx, CRF36_cpx, CRF37_cpx, and CRF43_02G (Tebit *et al.*, 2002; Ndembí *et al.*, 2008; Powell *et al.*, 2010; Ragupathy *et al.*, 2011; Agyingi *et al.*, 2014; Negedu-Momoh *et al.*, 2014; Courtney *et al.*, 2016; Teto *et al.*, 2017). Recently a unique new inter-group HIV-1/MO has been identified (De Oliveira *et al.*, 2017) suggesting that Cameroon is considered as one of the epicenters of the HIV epidemic.
2.5 HIV treatment

One of the greatest accomplishments in the fields of biology, pharmacology, and medicine has been the transformation of AIDS from an unavoidably fatal illness to chronic and controllable disease in most countries where HIV infected persons have complete access to HAART (Simon et al., 2006; Oguntibeju, 2012). Of the 36.7 million people living with HIV, 20.9 million had access to ART in 2017 (USAIDS, 2018). HAART has extensively reduced morbidity and mortality from HIV-1 infection. Nevertheless, it is still not curative (Aghokeng et al., 2013; Li et al., 2013).

The goal of ART is to improve the quality of life by reducing viral load and reconstitution of immune competence (Teklay et al., 2013). Although ART does not completely destroy the virus and cannot cure the infection/disease, it has greatly reduced the viral load and significantly slows down the disease progression rates, thereby increasing life expectancy and improving the quality of life of people living with HIV and AIDS (Oguntibeju, 2012; Wambani et al., 2016). In addition, HAART has shown to also contribute to the prevention of HIV-1 spread, as low viral load diminishes the risk of HIV-1 transmission (Dimitrov et al., 2016; Sallam et al., 2017).

The use of ART in medical practice depends on their ease or complexity of use and efficacy based on clinical evidence, practice guidelines, and clinician preference (Oguntibeju, 2012; Minister of Public Health [MPH], 2015).

2.5.1 Types of antiretroviral drugs

Six classes of antiretroviral agents currently exist; nucleoside/nucleotide reverse-transcriptase inhibitors (NsRTIs/NtRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase inhibitors (INIs), fusion inhibitors, and chemokine receptor antagonists (Oguntibeju, 2012). Each class of
ART targets a different step in the viral life cycle (Figure 2.4) as the virus infects CD4+ T lymphocyte or other target cells (Oguntibuje, 2012). The mechanisms of action of these ARV inhibitors occur in two phases which include the enzymatic and the entry processes. The enzymatic inhibitors that target HIV-1 replication comprise of the protease inhibitors, reverse transcriptase, and integrase while the entry inhibitors that prevent the entry process fall into three major classes based on the specific entry process and include the attachment, co-receptor and fusion inhibitors (Iyidogan and Anderson, 2014, https://www.webmd.com/hiv-aids/aids-hiv-medication).

**Figure 2.4: Targets for ARVs** (Berns and Kasbekar, 2006)

2.5.1.1 **Nucleoside/nucleotide RT inhibitors (NRTIs/NtRTIs)**

These were the first drug available for the treatment of HIV infection (Simon *et al.*, 2006). Although NsRTIs/NtRTIs have been found to be less potent against HIV than
NNRTIs and PIs, the NRTIs have a principal role in ARV treatment. They remain part of the current standard of care and have been shown to inhibit both HIV-1 and HIV-2 (Oguntibeju, 2012). NRTIs are reverse transcriptase inhibitors (RTI) known as DNA chain terminators or faulty DNA building blocks. They are structurally similar to the DNA nucleoside bases and become efficiently incorporated into the viral DNA at the 3’-end as an NRTI monophosphate by HIV-1 RT while competing with the natural deoxynucleoside triphosphates (dNTPs). Upon incorporation, they inhibit the elongation of viral DNA chain due to the lack of a 3’-hydroxyl group or an altered sugar moiety (purine and pyrimidine nucleosides) that prevents the incorporation of next incoming nucleotide (Iyidogan and Anderson, 2014). When one of these defective building blocks is added to a growing HIV DNA chain, further HIV DNA synthesis is blocked. Examples include Lamivudine (3TC), Abacavir (ABC) didanosine (DDI), emtricitabine (FCT) Tenofovir disoproxil fumarate (TDF), Zidovudine (AZT), Stavudine (D4T), Tenofovir alafenamide (TAF) (https://www.webmd.com/hiv-aids/aids-hiv-medications).

### 2.5.1.2 Non-nucleoside reverse transcriptase inhibitors (NNRTIs)

The NNRTIs are RTIs that causes an allosteric inhibition of the reverse transcriptase enzyme by binding directly to the enzyme at a site different from the nucleoside binding component. The binding of NNRTIs changes the spatial conformation of the substrate-binding site and reduces the polymerase activity. This prevents the conversion of viral RNA to DNA, thereby preventing it from being copied into the cell’s DNA (Arts and Hazuda, 2012). Thus they act as non-competitive inhibitors of the enzyme since they do not interfere with dNTP binding. Examples include; Nevirapine (NVP), Efavirenz (EFV), Etravirine (ETR), Rilpivirine (RPV),

2.5.1.3 Protease inhibitors (PI)

PIs prevent viral replication by competitively binding to the active site of the HIV protease and inhibit its action (Oguntibeju, 2012). Thus they prevent the HIV protease from cleaving the polypeptide precursors of the virus proteins and enzymes into structural proteins and mature enzymes that go on to infect new cells (Oguntibeju, 2012; Iyidogan and Anderson, 2014). Example of PIs includes Atazanavir (ATV), Darunavir (DRV), Fosamprenavir (FPV), Indinavir (IDV), Lopinavir (LPV), Nelfinavir (NFV), Saquinavir (SQV), Tipranavir (TPV) and Ritonavir (RTV) (https://www.webmd.com/hiv-aids/aids-hiv-medication).

2.5.1.4 Integrase inhibitors (INIs)

INIs chelate the divalent cation (Mg$^{2+}$ or Mn$^{2+}$) in the active site of the enzyme that is required for integrase enzymatic activity thereby preventing the integration of viral DNA into the host DNA (Wang et al., 2011). This inhibition is called strand transfer inhibition and prevents HIV from replicating (Arts and Hazuda, 2012). Examples include Raltegravir (RAL), Elvitegravir (EVG), Dolutegravir (DTG), Bictegravir (BIC) (www.webmd.com/hiv-aids/aids-hiv-medication).

2.5.1.5 Entry and co-receptor inhibitors

They are relatively new generation of antivirals drugs which interfere with the virus ability to bind to host cells. The entry inhibitors block the attachment of HIV gp120 to either the CD4$^+$ T cell receptor, while the co-receptor inhibitors block the interaction between gp120 and CCR5 or CXCR4 (Arts and Hazuda, 2012; Iyidogan and

2.5.1.6 Fusion inhibitors

Fusion inhibitors prevent membrane fusion by averting the interaction between the heptad repeat (HR) regions 1 (HR1) and 2 (HR2). This blocks the formation of the fusion pore thereby preventing the release of the viral core into the host cytoplasm (Miller and Hazuda, 2004). An example includes Enfuvirtide (ENF, T-20) that mimics an HR2 fragment (https://www.webmd.com/hiv-aids/aids-hiv-medication).

2.5.2 Combination therapy

ART which is also known as HAART is a term that refers to the use of the combination of three or more antiretroviral agents to suppress a wide variety of HIV infection effectively (Osakunor et al., 2015; Teto et al., 2017). The advent of combined therapy has significantly changed the perception of HIV/AIDS from a fatal to a chronic and potentially manageable disease, thus decreasing the morbidity and mortality rates of the people living with HIV/AIDS (Oguntibeju, 2012; Iyidogan and Anderson, 2014; Zhang et al., 2017).

Combination therapy uses three drugs from at least two classes. Most of the combination therapies include two NRTIs and one NNRTI or PI. In addition, combinations of integrase or entry inhibitors with RT inhibitors and PIs are used as an alternative treatment strategy (WHO, 2001; Iyidogan and Anderson, 2014; Aidsinfo, 2015). The main aim of employing HAART in HIV infected individuals is that if one drug is not able to suppress the virus at a particular stage, the other drugs used in this combination will be more likely to suppress the HIV (Priyanka and Ezhilarasan,
Combination antiretroviral therapy dramatically suppresses viral replication and reduces the plasma HIV-1 viral load to below the limits of detection thereby resulting in a significant reconstitution of the immune system which is measured by an increase in the circulating CD4+ T cell-lymphocytes (Arts and Hazuda, 2012). In addition, it helps to decrease the likelihood of resistance because it reduces HIV replication at multiple steps in viral replication (Iyidogan and Anderson, 2014; Aidsinfo, 2015).

Increased access to ART in Cameroon has been improved through the implementation of the WHO simplified approach and the decentralization of ART services (Lucien et al., 2010; Aghokeng et al., 2013). The standard first and second line therapy consists of 2 NRTIs plus 1 NNRTI and one ritonavir-boosted protease inhibitor [PI/r], plus two NRTIs (Billong et al., 2013, MPH, 2015) respectively. In 2010 these regimens were modified when WHO recommended the replacement of Stavudine with Tenofovir. As such the reference first-line ARVs in Cameroon (Appendix I) included; AZT, TDF, FTC, ABC plus 3TC as NRTIs and NVP or EFV as NNRTIs (MPH, 2015). First-and second-line treatments have been freely provided to eligible patients since 2007, and treatment initiation and monitoring has been guided by clinical and/or immunological data (Aghokeng et al., 2013). First-line ARV regimens are recommended for use in naive patients whereas a change to second-line occurs when there is treatment failure of the first-line as proven by clinical, immunological and virological factors or when the virus has the ability to tolerate a drug (WHO, 2001; Aidsinfo, 2015).
2.6 HIV drug resistance

This is the ability of HIV to mutate and reproduce itself in the presence of drugs (Suraj and Prasanna, 2015). Drug resistance is a major contributing factor to the failure of ART (WHO, 2001; Wensing et al., 2017). Antiretroviral drugs were developed using the subtype B isolates. However, ARV drugs have reduced the morbidity and mortality of both HIV B and non-B subtypes patients (Kantor et al., 2005; Negedu-Momoh et al., 2014; Kan et al., 2017; Onywera et al., 2017). This has led to increased access to ARTs during the past decade to reach millions of HIV-1 infected persons (Agyingi et al., 2014; Billong et al., 2016; De Luca et al., 2017; Teto et al., 2017). Despite the remarkable achievement derived from ART, drug resistance mutations (DRM) are known to develop and are major hindrances in the management of both HIV treated and therapy-naïve patients (Negedu-Momoh et al., 2014; Teto et al., 2017). These mutations develop as a result of an amino acid (AA) changes in the virus genome compared to the wild type (HXB2) taken as a reference (Wensing et al., 2017).

Mutations can either be major mutations or accessory mutations. Major mutations are the first mutations selected during therapy and causes decreased binding of the drug to its enzyme target to confer phenotypic resistance (WHO, 2001; Burda et al., 2010). While accessory mutations contribute to drug resistance by improving the fitness of viruses carrying the primary mutations or increase resistance in the presence of major mutations. They have very little direct effect on inhibitor binding or on the level of resistance in the absence of primary mutations (WHO, 2001; Wensing et al., 2017).

Mutations are described using a number to denote the mutant codons or amino acids (Appendix II) with letters before and after the codon number denoting the AA
associated with “wild-type” and mutant virus respectively. For example, M184V indicates that 184 is the AA position, M (methionine) is the wild type AA, and V (valine) is the mutant AA. When both wild-type and mutant AA are detected it is represented as M184M/V and is known as a quasi-species.

Factors contributing to drug resistance include both viral and host factors. The viral factors include extreme high degree of genetic variability and evolution rate of HIV-1 as a result of the rapid turnover in HIV replication (estimated at $10^9$ new viral particles each day) and the high error rate of the reverse transcriptase enzyme during viral replication (Arts and Hazuda, 2012; Suraj and Prasanna, 2015). This is explained by the fact that the virus population within an infected person consists of a complex mixture of heterogeneous strains, termed “quasi-species” (Santoro and Perno, 2013; Suraj and Prasanna, 2015). Patient factors on the other hand include poor adherence (the regimen, toxicity and social and personal issues), insufficient drug level (use of substandard ARV regimens, wrong dose, poor potency, host genetics, poor absorption, rapid clearance, drug interaction), gaps in service delivery (inadequate drug supply systems, patient retention and inadequate human resources) and lack of plasma viral load monitoring (Nyamache, 2012; Aghokeng et al., 2013; Hamers et al., 2013; Teto et al., 2017).

2.6.1 Types of resistance

Drug resistance can be categorized into transmitted drug resistance (TDR) or acquired drug resistance (ADR), both of which present serious threats to the health sector (Sallam et al., 2017; Zhang et al., 2017).
2.6.1.1 Primary or transmitted drug resistance (TDR)

Transmitted drug resistance is defined as resistance to one or more ARV drugs classes in patients not previously exposed to any ARV therapy as a result of direct transmission from a patient harboring DR mutants (Suraj and Prasanna, 2015; Sallam et al., 2017). As such, there is evidence for a rapid increase in TDR in the years after the rollout of ART (Gupta et al., 2012). Evidence arising from the prevalence of TDR has been reported in almost all classes of ARV that is NRTIs, NNRTIs, PI, INI, and CCR5 (Agyingi et al., 2014; Barennes et al., 2014; De Luca et al., 2017; Wensing et al., 2017; https://hivdb.stanford.edu). Previous studies from other regions in Cameroon have identified TDR ranging from 3.6-44% (Billong et al., 2013; Agyingi et al., 2014; Zoufaly et al., 2014; De Luca et al., 2017; Onywera et al., 2017) in the adult population and about 3.6-40.7% in children (Ikomey et al., 2016). Increase in TDR has been shown to causes early treatment failures in the newly infected individuals and thus, jeopardizes both preventive and treatment efforts in HIV patients (Wittkop et al., 2011; Hamers et al., 2013; Ferreira et al., 2017; Sallam et al., 2017).

2.6.1.2 Secondary or acquired drug resistance (ADR)

This is the development of resistance while on ART (Suraj and Prasanna, 2015). The prevalence of acquired resistance is on the rise and ranges from 16.4-81.5% in Cameroon (Laurent et al., 2005; Burda et al., 2010; Aghokeng et al., 2013; Agyingi et al., 2014; Zoufaly et al., 2014; Teto et al., 2017) and 9.5-89.6% in other countries (Elmi Abar et al., 2012; Negedu-Momoh et al., 2014; De Luca et al., 2017; Onywera et al., 2017). The dynamics of the rapid evolution of HIV-1 facilitated by its error-prone reverse transcriptase, the rapid rate of HIV replication, alongside patients,
physicians, and drug-related factors, has led to the emergence of HIV resistant strains in most patients (Billong et al., 2016; Sallam et al., 2017).

2.6.2 Mechanism of drug resistance

Drug resistance mutation in HIV develops differently depending on the drug class and can evolve through different pathways for specific drugs. Resistance mutations have been identified in all the major classes of ARVs. The most common resistance-associated mutations are within the NNRTI, followed by the NRTI and the PIs drug classes (Suraj and Prasanna, 2015; Teto et al., 2017). The development of DR further complicates clinical management because of the high level of cross-resistance within all three drug classes; NRTI, NNRTI, and PI (WHO, 2001; Li et al., 2015; Onywera et al., 2017).

2.6.2.1 Resistance to reverse transcriptase inhibitors (RTIs)

Studies have identified mutations to NRTIs and NNRTIs from patients on treatment within a range of 16.4-84.4%, and in drug naïve individuals within the range from 1.4-48% in most African countries (Laurent et al., 2 005; Burda et al., 2010; Ragupathy et al., 2011; Hamers et al., 2013; Agyingi et al., 2014; De Luca et al., 2017; Onywera et al., 2017).

2.6.2.1.1 Resistances to nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs)

There are two principal resistance mechanisms to NRTIs which include the discrimination and excision mechanisms.
2.6.2.1.2 Discrimination mechanism

Several mutations in reverse transcriptase can promote resistance by selectively impairing the ability of reverse transcriptase to incorporate its analogue into DNA. In this mechanism, the RT enzyme prevents the binding of the NRTI while retaining the ability to recognize the analogous natural deoxynucleoside triphosphate (dNTP) substrates (WHO, 2001). Once this false substrate is incorporated into the viral reverse transcriptase, it will prevent the synthesis of new viral DNA due to the absence of the OH group in the 3’ direction. The most implicated mutations in this mechanism are the K65R, L74V, V75I, Y115F, Q151M and M184V (WHO, 2001; Iyidogan and Anderson, 2014).

2.6.2.1.3 Excision mechanism

In this mechanism, RT utilizes ATP or inorganic pyrophosphate as a co-substrate to remove the incorporated nucleoside analog after it has been incorporated into the viral DNA allowing reverse transcription to resume (Iyidogan and Anderson, 2014; Schneider et al., 2016). Example of this type of mutations are those selected by AZT and D4T (Naeger et al., 2002). The AZT and D4T are both thymidine nucleoside analogs and share common resistance mutations known as the thymidine analog mutations (TAMs) as well as an insertion at position 69 (Wensing et al., 2017). The TAMs may develop by one of two distinct pathways: the TAM 1 pathway (including mutations M41L, L210W, and T215Y) or the TAM 2 pathway (including mutations D67N, K70R and K 219E/Q) (Chaplin et al., 2011; El-Khatib et al., 2011). Once RT acquires TAM complex mutations with high resistance to thymidine analogs, it could also develop further mutations like T69 insertion complex and become resistant to a variety of NRTIs resulting to cross-resistance to all members of NRTI class (Iyidogan
and Anderson, 2014; Suraj and Prasanna, 2015). The TAMs are involved in resistance to all NRTIs, except Lamivudine (3TC). However, the degree of cross-resistance depends on the NRTI considered and the number of TAMs on the virus. Some interactions exist between the different mechanisms of resistance for example the M184V/I mutations, selected by 3TC and emtricitabine (FTC), delay the appearance of TAMs and increase the *in vitro* susceptibility to AZT and D4T. The mutations to this group are summarized in table 2.1

**Table 2.1: Resistance mutations to NRTIs** ([https://hivdb.stanford.edu/hivdb/by-sequences](https://hivdb.stanford.edu/hivdb/by-sequences)).

<table>
<thead>
<tr>
<th></th>
<th>Non-TAMs</th>
<th>TAMs</th>
<th>MDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>POS</td>
<td>184 65 70 74 115</td>
<td>41 67 70 210 215 219</td>
<td>69 151</td>
</tr>
<tr>
<td>CON</td>
<td>M K K L Y</td>
<td>M D K L T K</td>
<td>T Q</td>
</tr>
<tr>
<td>3TC</td>
<td>VI R</td>
<td></td>
<td>Ins M</td>
</tr>
<tr>
<td>FTC</td>
<td>VI R</td>
<td></td>
<td>Ins M</td>
</tr>
<tr>
<td>ABC</td>
<td>VI R E VI F</td>
<td>L W W FY</td>
<td>Ins M</td>
</tr>
<tr>
<td>DDI#</td>
<td>VI R E VI</td>
<td>L W FY</td>
<td>Ins M</td>
</tr>
<tr>
<td>TDF</td>
<td>*** R E F</td>
<td>L R W FY</td>
<td>Ins M</td>
</tr>
<tr>
<td>D4T#</td>
<td>*** R E</td>
<td>L N R W FY QE</td>
<td>Ins M</td>
</tr>
<tr>
<td>ZDV</td>
<td>*** * *</td>
<td>L N R W FY QE</td>
<td>Ins M</td>
</tr>
</tbody>
</table>

**Legend:** Mutations in **Bold/underline:** High-level reduced susceptibility or virological response. **Bold:** reduced susceptibility or virological response. **Plain text:** reduced susceptibility in combination with other NRTI-resistance mutations. **Asterisk:** increased susceptibility. #: no longer recommended. **Abbreviations:** ABC; Abacavir, Cons; Consensus AA, FTC; Emtricitabine, 3TC; Lamivudine, MDR; Multidrug resistance mutations, Pos; AA position, TDF; Tenofovir, AZT; Zidovudine, D4T; Stavudine, DDI; Didanosine, TAMs; Thymidine analog mutations.
2.6.2.1.4 Resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs)

Mutations responsible for NNRTI resistance are situated in the hydrophobic pocket targeted by NNRTI and thus changing the conformation of the dNTP binding pocket. These changes reduce the affinity of the drug to bind to the target site. There are four essential groups of mutations involved in the resistance to NNRTIs (WHO, 2001). These mutations are found in clusters between codons 100-108 and 179-190 and confer broad-based cross-resistance to all available drugs in this class (Wensing et al., 2017). Since the drugs bind to the same site on RT, mutations that confer resistance to 1 drug in this class generally confer cross-resistance to most other NNRTIs (Table 2.2). In addition, mutations in the NNRTI binding pocket may interfere with NRTI resistance. The most commonly selected mutations are Y181C and Y188C, followed by K103N (Ren and Stammers, 2008).

Table 2.2: Resistance mutation to NNRTIs (hivdb.stanford.edu, Wensing et al., 2017)

<table>
<thead>
<tr>
<th>Codon</th>
<th>V</th>
<th>A</th>
<th>L</th>
<th>K</th>
<th>K</th>
<th>V</th>
<th>V</th>
<th>E</th>
<th>V</th>
<th>Y</th>
<th>Y</th>
<th>G</th>
<th>H</th>
<th>P</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos</td>
<td>99</td>
<td>98</td>
<td>100</td>
<td>101</td>
<td>103</td>
<td>106</td>
<td>108</td>
<td>138</td>
<td>179</td>
<td>181</td>
<td>188</td>
<td>190</td>
<td>221</td>
<td>225</td>
<td>227</td>
</tr>
<tr>
<td>EFV</td>
<td>I</td>
<td>EP</td>
<td>NS</td>
<td>AM</td>
<td>I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETR</td>
<td>I</td>
<td>G</td>
<td>EP</td>
<td>NS</td>
<td>AM</td>
<td>I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPV</td>
<td>I</td>
<td>EP</td>
<td>NS</td>
<td>AM</td>
<td>I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NVP</td>
<td>I</td>
<td>EP</td>
<td>NS</td>
<td>AM</td>
<td>I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legend: Bold/underline: High-level reduced susceptibility or virological response. Bold: reduced susceptibility or virological response. Plain text: reduced susceptibility in combination with other NNRTI-resistance mutations. Asterisk: increased susceptibility. Abbreviations: EFV; Efavirenz, ETR; Etravirine, NVP; Nevirapine, RPV; Rilpivirine
2.6.2.2 Resistance to protease inhibitors (PIs)

Resistance to PIs results from the step-wise accumulation of mutations within the enzyme that leads to the reduced binding affinity of the inhibitors while maintaining the natural substrate binding interactions (Wensing et al., 2010; Iyidogan and Anderson, 2014). Typically, there is a substitution in the substrate-binding cleft of the viral protease resulting in enlargement of the catalytic site. This enlargement leads to decreased binding to the inhibitor and therefore causing a decrease in drug sensitivity which leads to loss of viral fitness (Iyidogan and Anderson, 2014). This is considered primary or major mutations. Secondary or minor mutations may also occur at the neighboring non-active site pocket residues that compensate for the impaired protease activity via increasing the activity and/or the stability of the protein. Secondary mutations in conjunction with major mutations will improve the replication of viruses. Another potential mechanism of resistance is through an amino acid insertion. Such insertions may lead to decrease in PIs susceptibility and slightly improve viral replication. Nevertheless, it can only contribute to PIs resistance when in combination with other mutations in protease or gag protein (Wensing et al., 2010). Due to the high binding capacity, PI requires more than one mutation to exert drug resistance to protease mutations (Table 2.3).
**Table 2.3: Mutation resistance to protease inhibitors** (Wensing et al., 2017)

<table>
<thead>
<tr>
<th>Cons</th>
<th>L</th>
<th>V</th>
<th>I</th>
<th>G</th>
<th>K</th>
<th>L</th>
<th>D</th>
<th>V</th>
<th>L</th>
<th>E</th>
<th>M</th>
<th>K</th>
<th>M</th>
<th>G</th>
<th>G</th>
<th>I</th>
<th>F</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos</td>
<td>10</td>
<td>11</td>
<td>13</td>
<td>16</td>
<td>20</td>
<td>24</td>
<td>30</td>
<td>32</td>
<td>33</td>
<td>34</td>
<td>36</td>
<td>43</td>
<td>46</td>
<td>47</td>
<td>48</td>
<td>50</td>
<td>53</td>
<td>54</td>
</tr>
<tr>
<td>ATV/r</td>
<td>IFVC</td>
<td>E</td>
<td>RMTV</td>
<td>I</td>
<td>I</td>
<td>IFV</td>
<td>Q</td>
<td>ILV</td>
<td>IL</td>
<td>V</td>
<td>VM</td>
<td>L</td>
<td>L</td>
<td>Y</td>
<td>LVMTA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRV/r</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>F</td>
<td>YA</td>
<td>V</td>
<td>ML</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPV/r</td>
<td>FIRV</td>
<td>I</td>
<td>IL</td>
<td>V</td>
<td>V</td>
<td>LVM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDV/r</td>
<td>IRV</td>
<td>MR</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>IL</td>
<td>V</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPV/r</td>
<td>IFRV</td>
<td>MR</td>
<td>I</td>
<td>I</td>
<td>IL</td>
<td>V</td>
<td>VM</td>
<td>V</td>
<td>L</td>
<td>VLAMTS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NFV</td>
<td>FI</td>
<td>N</td>
<td>I</td>
<td>I</td>
<td>IL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SQV/r</td>
<td>IRV</td>
<td>I</td>
<td>V</td>
<td>V</td>
<td>VL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPV/r</td>
<td>V</td>
<td>V</td>
<td>MR</td>
<td>F</td>
<td>I</td>
<td>T</td>
<td>L</td>
<td>V</td>
<td>AMV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cons</th>
<th>Q</th>
<th>D</th>
<th>I</th>
<th>L</th>
<th>I</th>
<th>H</th>
<th>A</th>
<th>G</th>
<th>T</th>
<th>L</th>
<th>V</th>
<th>V</th>
<th>I</th>
<th>I</th>
<th>N</th>
<th>L</th>
<th>L</th>
<th>L</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos</td>
<td>58</td>
<td>60</td>
<td>62</td>
<td>63</td>
<td>64</td>
<td>69</td>
<td>71</td>
<td>73</td>
<td>74</td>
<td>76</td>
<td>82</td>
<td>84</td>
<td>85</td>
<td>88</td>
<td>89</td>
<td>90</td>
<td>93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATV/r</td>
<td>E</td>
<td>V</td>
<td>LMV</td>
<td>VTIL</td>
<td>CSTA</td>
<td>ATFI</td>
<td>V</td>
<td>V</td>
<td>S</td>
<td>V</td>
<td>M</td>
<td>LM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRV/r</td>
<td>P</td>
<td>V</td>
<td>V</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPV/r</td>
<td>S</td>
<td>V</td>
<td>AFST</td>
<td>V</td>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDV/r</td>
<td>VT</td>
<td>SA</td>
<td>V</td>
<td>I</td>
<td>AFT</td>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPV/r</td>
<td>P</td>
<td>VT</td>
<td>S</td>
<td>V</td>
<td>ATFS</td>
<td>V</td>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NFV</td>
<td>VT</td>
<td>I</td>
<td>AFTS</td>
<td>V</td>
<td>DS</td>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SQV/r</td>
<td>V</td>
<td>VT</td>
<td>S</td>
<td>I</td>
<td>AFTS</td>
<td>V</td>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPV/r</td>
<td>E</td>
<td>K</td>
<td>P</td>
<td>L</td>
<td>D</td>
<td>V</td>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Legend:** **Bold/underline:** High-level reduced susceptibility or virological response. Bold: reduced susceptibility or virological response. Plain text: reduced susceptibility in combination with other PI-resistance mutations. **Abbreviations:** /r; Administered with ritonavir for pharmacokinetic boosting. ATV/r; Atazanavir/r, Cons; Consensus AA, DRV/r; Darunavir/r, FPV/r; Fosamprenavir/r, IDV/r; Indinavir/r, LPV/r; Lopinavir/r, NFV; nelfinavir, Pos; AA position, SQV/r; Saquinavir/r, TPV/r; Tipranavir/r.
2.6.2.3 Resistance to integrase inhibitors (INI)

Integrase inhibitors bind to the active site of the integrase enzyme and prevent it from functioning properly. Drug-resistant mutations against INIs are determined via drug selection and viral fitness studies. Several residues D64, C65, T66, H67, E92, N120, F121, D116 Q62, I141, P142, Y143, I151, E152, N155, K156, and K159 are the targets of INI on which they bind and oppose strand transfer. Thus, any mutation at the level of these residues could modify the affinity of the enzyme for the inhibitors and impede their proper fixation, thus preventing their inhibition function. Significant cross-resistance has been observed among the INIs (Arts and Hazuda, 2012) (Table 2.4).

Table 2.4: Resistance mutation to integrase inhibitors (Wensing et al., 2017)

<table>
<thead>
<tr>
<th>Cons</th>
<th>T</th>
<th>L</th>
<th>E</th>
<th>T</th>
<th>F</th>
<th>E</th>
<th>G</th>
<th>Y</th>
<th>S</th>
<th>Q</th>
<th>N</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos</td>
<td>66</td>
<td>74</td>
<td>92</td>
<td>97</td>
<td>121</td>
<td>138</td>
<td>140</td>
<td>143</td>
<td>147</td>
<td>148</td>
<td>155</td>
<td>263</td>
</tr>
<tr>
<td>DTG</td>
<td>Y</td>
<td>AK</td>
<td>AS</td>
<td></td>
<td></td>
<td></td>
<td>HKR</td>
<td>H</td>
<td>H</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EVG</td>
<td>IAK</td>
<td>QG</td>
<td>A</td>
<td>Y</td>
<td>G</td>
<td></td>
<td>HKR</td>
<td>H</td>
<td>H</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAL</td>
<td>M</td>
<td>Q</td>
<td>A</td>
<td>Y</td>
<td>AK</td>
<td>AS</td>
<td>RHC</td>
<td>HKR</td>
<td>H</td>
<td>H</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Legend:** Mutations in **Bold/underline:** High-level reduced susceptibility or virological response. **Bold:** reduced susceptibility or virological response. **Plain text:** reduced susceptibility in combination with other INI-resistance mutations. **Abbreviations:** Cons; Consensus AA, DTG; Dolutegravir, EVG; Elvitegravir, RAL; Raltegravir, Pos; AA position.

2.6.2.4 Resistance to fusion or entry inhibitors

Drug-resistant mutations against fusion inhibitors are mediated by amino acid substitutions within the first heptad repeat region (HR1) of gp41 at amino acids 36 to 45 (G36D, I37T, V38A, V38M, N42T, N42D, N43K) (Lobritz et al., 2010; Arts and Hazuda, 2012). Any mutation at the level of these residues significantly reduced the
binding affinity of the enzyme for the inhibitors and impedes their proper fixation, thus preventing their inhibition function. (Arts and Hazuda, 2012) (Table 2.5). The impact on the loss of susceptibility to fusion inhibitors caused by changes at different positions varies considerably, although it appears that changes in simple amino acids may be responsible for high-level resistance to enfuvirtide in some cases (Lobritz et al., 2010; Iyidogan and Anderson, 2014). However, these mutations do not confer cross-resistance to other entry inhibitors such as attachment inhibitors or co-receptor inhibitors (Arts and Hazuda, 2012).

**Table 2.5: Resistance mutation to fusion inhibitors** (Wensing et al., 2017)

<table>
<thead>
<tr>
<th>Cons</th>
<th>G</th>
<th>I</th>
<th>V</th>
<th>Q</th>
<th>Q</th>
<th>N</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos</td>
<td>36</td>
<td>37</td>
<td>38</td>
<td>39</td>
<td>40</td>
<td>42</td>
<td>43</td>
</tr>
<tr>
<td>Enfuvirtide</td>
<td>DS</td>
<td>V</td>
<td>AME</td>
<td>R</td>
<td>H</td>
<td>T</td>
<td>D</td>
</tr>
</tbody>
</table>

**2.6.2.5 Resistance to co-receptor inhibitors (CCR5)**

Potential mechanisms of resistance include; tropism switching for CXCR4 instead of CCR5 (gp120 has increased affinity for CCR5). Utilization of inhibitor to the entry receptor occurs at a faster rate thus inhibiting the entry step (Iyidogan and Anderson, 2014). It is suggested that most CCR5 antagonist-resistant strains preserve the use of the CCR5 co-receptor and that multiple mutations within different regions of gp120 (V3, V2 and constant regions 2 and 4) account for the drug-resistant phenotype (Lobritz et al., 2010; Arts and Hazuda, 2012).
2.7 HIV and hepatotoxicity

Hepatotoxicity (liver disease) is often characterized by the elevation in serum hepatic enzymes (Bello et al., 2014). Potential causes of hepatotoxicity, especially among HIV-infected patients, include medication-related (ART, non steroidal anti-inflammatory drugs and herbal remedies), comorbidity infection with other viruses (hepatitis A, B and C), alcohol, non-alcoholic fatty liver disease, and less common causes include, opportunistic infections such as Cytomegalovirus, mycobacteria, parasitic infection, or lymphoma, autoimmune hepatitis and primary biliary cirrhosis (Kress, 2005; Price and Thio, 2010; Pandit et al., 2012; Ayelagbe et al., 2014; Bello et al., 2014). In the era of ART, 1–54.0% of patients are shown to have hepatotoxicity which accounts for the discontinuation of ART (Barve et al., 2010; Lucien et al., 2010; Osakunor et al., 2015; Wambani et al., 2015; Wenderlein et al., 2016).

2.7.1 Antiretroviral therapy and hepatotoxicity

Prevention and management of hepatotoxicity have emerged as a major issue for HIV/AIDS treatment and care (Kontorinis and Dieterich, 2003; Fokunang et al., 2010; Wenderlein et al., 2016). In the era of ART, hepatotoxicity has emerged as an important cause of death among persons with HIV accounting for 14-18% (Soriano et al., 2008; Price and Thio, 2010; Bello et al., 2014). Virtually, hepatotoxicity has been described in association with all major classes of ARV medication (Kress, 2005; Soriano et al., 2008; Wenderlein et al., 2016) which challenge the management of HIV infected patients to a greater extent. Even though all classes of ARVs have been linked with an increase in serum transaminases, NNRTIs (NVP and EFZ) and PIs (Ritonavir, Nelfinavir, Indinavir, and Saquinavir) are commonly associated with liver enzyme elevations while NRTIs; (AZT > 3TC > TDF > DDI >D4T being the most
associated with severe hepatotoxicity) (Sulkowski et al., 2000; Kontorinis and Dieterich, 2003; Nunez, 2006; Bello et al., 2014).

The severity of hepatotoxicity ranges from the absence of symptoms to liver decompensation which leads to liver failure and death (Kress, 2005; Nunez, 2006). Although there has been broad variability in the criteria used in clinical studies to categorize the severity of hepatotoxicity, hepatotoxicity is classified using elevated levels of transaminases above the upper limits of normal (ULN). Severe hepatic injury is defined as >5 ULN AST and/or ALT levels during antiretroviral treatment (Nunez, 2006; Fokunang et al., 2010) and has been reported to occur in 5 to 10% of HIV patients on HAART for a period of over six months (Soriano et al., 2008; Wambani et al., 2015).

2.7.2 Mechanisms of hepatotoxicity

The pathophysiologic mechanisms underlying ARV related hepatotoxicity are not fully understood. Postulated hepatotoxic mechanisms include direct drug toxicity, immune-mediated hypersensitivity reaction, mitochondrial toxicity (associated with lactic acidosis or lipodystrophy syndrome, and steatohepatitis, underlying liver disease) and other metabolic abnormalities (Nunez, 2006).

2.7.2.1 Direct or drug-related toxicity

Drugs including ARVs (especially Nevirapine) are metabolized in the liver through the cytochrome pathways and may cause liver toxicity through enzyme polymorphisms (Pandit et al., 2012). These idiosyncratic polymorphisms of the enzymatic complexes lead to the development of hepatotoxicity in certain individuals (Nunez, 2006). They may also cause oxidant stress and cell injury that initiates
additional liver injury that leads to hepatotoxicity (Maiuri et al., 2015; Wambani et al., 2015).

2.7.2.2 Hypersensitivity reactions

Hypersensitivity reactions have been observed with all NNRTIs, the NRTI (Abacavir) and PI (Amprenavir, Fosamprenavir) (Spengler et al., 2002). Hypersensitivity reactions are due to idiosyncratic reactions of the host. These immune-mediated drug reactions may cause the production of neoantigens as a result of the covalent bonds formed between the liver proteins and the drug metabolites which then result to liver damage (Nunez, 2006; Pandit et al., 2012; Wambani et al., 2015). Clinical features that may suggest an acute, immune-mediated hypersensitivity response include fever, skin rash and eosinophilia. Hepatic histopathology can vary considerably and includes hepatocellular necrosis, cholestasis, tissue eosinophilia, parenchyma and periportal infiltrates with a predominance of lymphocytes and plasma cells (Spengler et al., 2002; Kontorinis and Dieterich, 2003).

2.7.2.3 Mitochondrial toxicity

Mitochondria play an important role in energy production, glucose and fat metabolism (Soriano et al., 2008). The ability of NRTIs (DDI, D4T, AZT, 3TC, ABC, TDF) and PIs (ritonavir) to inhibit mitochondrial DNA synthesis by inhibiting the human mitochondrial DNA polymerase-γ result in mitochondrial toxicity (Spengler et al., 2002). Depletion of mitochondrial DNA impairs the cellular respiratory chain and eventually inhibits pyruvate and fatty acid oxidation pathways. The main feature of the hepatic lesion is the accumulation of microvesicular steatosis in liver cells which may be associated with lactic acidosis and liver failure (Soriano et al., 2008). In addition, interruption of electron transfer in the mitochondrial respiratory chain results
in the release of reactive oxygen species that causes hepatic tissue damage by initiating lipid peroxidation of accumulated fatty acids and causes damage to mitochondrial and nuclear DNAs (Kontorinis and Dieterich, 2003; Nunez, 2006; Wambani et al., 2015). The release of cytokines that result in inflammation and injury of hepatocytes may further induce necrotic cell death that will aggravate the liver injury (Jaeschke et al., 2002; Grattagliano et al., 2009). Ultimately the loss of mitochondrial integrity results in the releases of cytochrome C into the cytosol, which activates caspases and thus triggers apoptosis in hepatocytes (Spengler et al., 2002; Pandit et al., 2012).

2.7.2.4 Metabolic abnormalities

HAART may cause certain metabolic abnormalities in the metabolism of both lipids and glucose, such as lipodystrophy syndrome, insulin resistance which leads to steatosis that may cause hyper transaminasemia that will later contribute to the development of liver toxicity (Spengler et al., 2002; Nunez, 2006).

2.7.3 Diagnosis of hepatotoxicity

Hepatotoxicity can be confirmed by the liver enzymes in the serum. The liver uses enzymes to get rid of the waste generated in the body and to break down drugs, alcohol, and medications (www.nlm.nih.gov). When the liver is stressed or damaged by various infections it produces an increased level of these enzymes. The liver enzyme level is often assessed to validate its functionality (Wondemagegn et al., 2013). These enzymes include aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP], Gamma-glutamyltransferase (GGT), L-lactate dehydrogenase (LD) test among others. The level of liver deformity
determines the types of liver disease and helps to decide whether the disease is acute or chronic and whether cirrhosis and hepatic failure are present (Hyder et al., 2012).

2.7.3.1 Alanine aminotransferase (serum glutamic pyruvic transaminase)

This is an enzyme found primarily in the liver which is released into the blood as a result of liver damage. The enzyme catalyzes the transfer of an amino group from alanine to α-ketoglutarate forming glutamate and pyruvate. The pyruvate produced is reduced to lactate by lactate dehydrogenase (LDH) and reduced nicotinamide adenine dinucleotide (NADH)

$$\text{L-Alanine} + \alpha\text{Ketoglutarate} \xrightarrow{\text{ALT}} \text{L-Glutamate} + \text{Pyruvate}$$

Pyruvate + NADH+H$ \xrightarrow{\text{LDH}}$ Lactate + NAD (www.spinreact.com)

The rate of decrease in the concentration of NADH, measured photometrically, is proportional to the concentration of ALT present in the sample. The serum ALT has been regarded as a reliable and sensitive marker of liver disease because ALT is primarily localized in the liver (Thapa and Walia, 2007).

2.7.3.2 Aspartate aminotransferase (serum glutamic oxalacetic transaminase)

This is an enzyme present in body tissues like the heart, skeletal muscle, kidney, brain, and liver (Thapa and Walia, 2007). The enzyme is released into the blood when either of these organs is damaged. This enzyme catalyzes the reversible transfer of an amino group from aspartate to α ketoglutarate forming glutamate and oxalacetate. The oxalacetate produced is reduced to malate by malate dehydrogenase (MDH) and NADH in the following equation:

$$\text{L-Aspartate} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{AST}} \text{L-Glutamate and oxalacetate}$$

Oxalacetate + NADH+H$ \xrightarrow{\text{MAD}}$ L-Malate + NAD (www.spinreact.com)
The rate of decrease in the concentration of NADH, measured photometrically, is proportional to the concentration of AST present in the sample.

2.7.3.3 Alkaline phosphatase

It is an enzyme found principally in the liver and bones. ALP catalyzes the hydrolysis of p-nitrophenyl phosphate (pNPP), liberating p-nitrophenol and phosphate, according to the following reaction

\[
pNPP + H_2O \xrightleftharpoons{\text{ALP}} \rightarrow p\text{-Nitrophenol} + \text{Phosphate}
\]

The rate of p-nitrophenol formation, measured photometrically, is proportional to the concentration of ALP present in the sample (www.spinreact.com).

When liver cells are damaged or bone is rapidly growing (either normal or abnormal growth), large amounts of ALP are often released into the blood (Thapa and Walia, 2007). A rise in ALP levels can lead to liver damage if gamma-glutamyl transpeptidase levels are also elevated. Since the chemical structure of the enzyme differs depending upon its origin, high levels of ALP requires that further tests be done to identify the subfractions or isoenzymes in other to determine if the elevation could be due to the liver or bone damage (Patil et al., 2013).

2.8 Cytokines in response to HIV infection

The principal damage caused by HIV infection is the weakening of the cellular immune system which causes severe immunodepression which leads to opportunistic infections in the infected individual (Tasca et al., 2012). Several studies have uncovered relationships between cytokine levels and the severity of the disease. The interaction between cytokines and HIV expression is multifaceted. A number of
studies conducted indicate that HIV infection causes dysregulation of the cytokine profile both in vivo and in vitro (Spear et al., 2005; Tasca et al., 2012). HIV infection is associated with a switch from a predominantly Th1 to Th2 which is indicative of HIV disease progression and replication that leads to HIV-associated immune deficiency (Vishwanath et al., 2011; Keating et al., 2011).

It has been shown that tumor necrosis factor alpha (TNF-α) plays a critical role in the origin and progression of HIV disease as such there is a positive correlation between HIV-1 viremia and TNF-α levels (Vishwanath et al., 2011). Thus regulating its release serves as a potential means of therapy for HIV. TNF-α induce other pro-inflammatory cytokines such as IL-6 and IL-8, which aid in the up-regulation of viral replication (Tudela et al., 2014) and stimulate the production of anti-inflammatory cytokine IL-10, which prevents further inflammation by TNF-α inhibition (Keating et al., 2011). As such IL-10 increases significantly as HIV-1 patient’s progress to the advanced stages of the disease thus reducing immune activation by inhibiting virus replication (Deng et al., 2015). In addition IL-10 regulates the immune response by inhibiting the proliferation of certain immune cells and promoting the proliferation of others by reducing the production of inflammatory cytokines; and promoting the secretion of antibodies.

On the other hand interferon gamma (IFN-γ) is capable of inhibiting viral replication and cell proliferation in a non-specific manner. IFN-γ secretion correlates positively with the patient’s T cells count and negatively with HIV-1 plasma viral load (Kedzierska and Crowe, 2001). Interleukin–6 is usually associated with disease progression as such are elevated in HIV infection (De Medeiros et al., 2016). Interleukin 2 leads to immunosuppressive effects that lead to the development
of opportunistic infections. IL-2 production is related to CD4⁺ T cells count and clinical status of the patients. Thus IL-2 is significantly decreased in patients with CD4⁺ T cells count of <200 cells/mm³ compared to those with higher CD4⁺ T cells count (Kedzierska and Crowe, 2001).

IL-17 production plays a key role in inflammatory responses that may lead to the serious tissue damage, as such IL-17 production is associated with the pathogenesis of several autoimmune and chronic inflammatory diseases (Falivene et al., 2015; Lyadova and Panteleev, 2015). However, Th17 cells that characterize the production of IL-17 are highly susceptible to HIV and are depleted with the use of HAART. The use of HAART prevents HIV replication and thus inhibites the production of Th17 cells (Ndhlovu et al., 2008; Hammerich et al., 2010).

2.8.1 Cytokines and hepatotoxicity

Plasma cytokine levels have been postulated to change dramatically over the course of HIV (De Medeiros et al., 2016). The liver consists of several cell types that usually produce minimal levels of cytokines under normal circumstances. However, when the liver cells (predominantly Kupffer cells) become activated cytokine production increases dramatically (Tilg, 2001; Crane et al., 2012).

Cytokines are known to coordinate a complex array of numerous processes and biochemical reactions that lead to liver damage (Neuman, 2003). An imbalance in cytokine production when these cells are activated by the presence of disease-causing microorganisms or substances plays a vital role in the development of liver damage (Crane et al., 2012).
Research data from both animal models and patients with hepatitis B and C suggest that pro-inflammatory and anti-inflammatory cytokines are involved in the development of liver injury (hepatotoxicity) through the activation of the necrotic and apoptosis pathways (Tilg, 2001; Zhang and Wang, 2006; Liu, 2009; Neuman et al., 2015). The apoptosis pathway is apparently the major mechanism contributing to acute and chronic liver injury (Guicciardi and Gores 2005).

Although different cytokines can cause hepatocyte injury, many studies in patients and animal models have strongly suggested that TNF-α and IL-6 are involved in the induction of apoptosis which triggers the destruction of the liver. This has lead to the pathogenesis of many chronic liver diseases such as viral hepatitis, alcoholic liver disease, fulminant liver failure among others (Tilg, 2001; Cosgrove et al., 2009; Maiuri et al., 2015; Seki and Schwabe 2015). These cytokines have shown to be higher among individuals with more advanced liver disease (Neuman et al., 2015).
CHAPTER THREE: MATERIALS AND METHODS

3.1 Study sites

This study was carried out in the Northwest region (NWR) of Cameroon which ranks 2nd (6.3%) in HIV prevalence in Cameroon and first in the Anglophone regions (INS, 2012). Patients were recruited from 5 HIV treatment clinics of; Bafut District Hospital, Santa District Hospital, Bali District Hospital, Ndop District Hospital, and the Bamenda Regional Hospital (Figure 3.1). The sampled sites better represent the different backgrounds of individuals from the entire NWR of Cameroon. Bafut, Santa, Bali, and Ndop are in rural areas while Bamenda is from urban settings.
Map of the Northwest Region (NWR)

Map of Cameroon showing the NWR

Scale: 0 100 200km

Figure 3.1: Map of the Northwest region (Bamenda) showing the study sites (RTG, 2013 unpublished)
3.2 Study design

This study was a longitudinal study involving newly diagnosed HIV positive individuals visiting any of the five HIV treatment clinics in the NWR (Bafut, Santa, Bali, and Ndop District Hospital, and the Bamenda Regional Hospital). Ethical clearance was obtained from the Cameroon National Ethics Committee (N02016/01/685/CF/CNERSH/SP; Appendix III). Before executing the study, the aim, procedure, benefits, and risk of the study (Appendix IV and V) were explained in a language (Pidgin English, English or French) best understood by the participants before signing of the informed consent (Appendix VI and VII). Samples were collected at baseline prior to ART initiation (D0) and again after one (D30) and six months (D180) intervals of follow up after treatment (Figure 3.2).

![Figure 3.2: Schematic representation of the study design](image-url)
3.3 Sampling technique

The region was first stratified into urban and rural and random picking of sites from each group was done using a random number generation method.

3.4 Sample population and size determination

The minimum acceptable sample size was calculated using the Lorenz formula as stated below (Fokunang et al., 2010).

\[ N = \frac{(Z_1^2 \alpha^2)P(1-P)}{\epsilon^2} \]

Where, \( Z_1 - \alpha = \) the normal distribution value = 1.96

\( P = \) Relative prevalence of HIV in the region = 6.3% (INS, 2012)

\( \epsilon = \) precision (sampling error) = 0.05

\[ N = \frac{(1.96^2)(0.063)(1-0.063)}{(0.05)^2} = 91. \]

Considering an attrition rate of 17% (Rougemont et al., 2009) by the 6th month a total of 120 subjects were recruited for the study. Based on the total population of 1,921,229 of the NWR; Bamenda, Ndop, Bali, Bafut and Santa make up 18.4%, 10.9%, 2.9%, 3.2% and 3.7% of this population respectively (RTG, 2013 unpublished), as such 57, 33, 9, 10 and 11 patients were recruited respectively from Bamenda, Ndop, Bali, Bafut, and Santa.

3.5 Inclusion and exclusion criteria

Inclusion criteria:

1. Confirmed HIV newly-infected patients and not yet on treatment,

2. Acceptance to participate in the study after having signed an informed consent form,
3. Male or female ≥ 18 years and residing within the study areas.

**Exclusion criteria:**

1. Refusal to participate in the study
2. HIV infected but already on treatment
3. Patients with signs of toxicity like hepatitis B and C positive patients
4. Looking severely sick

**3.6 Demographic data**

A structured questionnaire (Appendix VIII) was administered to consenting participants in order to collect demographic data (weight, age, sex, level of education, income), personal daily habit (alcohol consumption, cigarette smoking), ART status (history of ARV use before enrolment, date of drug initiation and type of ARV), pregnancy state (for females), information on compounding situations like (use of other drugs such as anti TB, paracetamol, herbal concoction, previous pregnancies for females) and the name of ARV to be initiated. The WHO stage classification was obtained from CD4+ T cells counts at baseline. Alcohol intake was classified following WHO operational definitions as no alcohol intake: for those patients who never had a habit of drinking alcohol, mild intake: those who have stopped drinking within the past 6 months, moderate alcohol intake: drinking weekly and less than 5 drinks per occasion and heavy alcohol intake: drinking weekly and >5 drinks per occasion (Wondemagegn et al., 2013). Smoking histories were classified based on the number of cigarette sticks consumed. Participants were asked about the mean number of cigarette sticks smoked per day over the previous 6 months and were classified into none, mild (<10 sticks/day), moderate (10-20sticks /day), and heavy (daily smokers with >20 sticks /day) (Jang et al., 2012).
3.7 Sample collection

Eight ml of venous blood was collected in uniquely coded tubes. Of this 3ml and 5ml were transferred into dry and ethylene diamine tetraacetic acid (EDTA) tubes respectively. Blood was separated and serum transported in an ice-box to Santa district hospital for liver enzymes determination while plasma was stored at -80°C and later transferred in an ice-box to “Centre International de Reference Chantal Biya” (CIRCB) Yaounde where it was kept at -20°C for subsequent analysis of cytokine profile, genetic diversity and drug resistance. For the transaminases and cytokine profile, the same procedure was repeated at months one and six.

3.8 Hepatitis B and hepatitis C screening

Serological status on Hepatitis B and C of the samples was performed using rapid HBV and HCV Diaspot strips respectively (Jakarta, Indonesia) according to the manufacturer’s procedure. In brief, the test strip was removed from the pouch and immersed in plasma for about 10-15 seconds. The strip was then placed on a non-absorbent surface for 15 minutes (mins). A negative result was recorded when only the control band (C) shows a red line, a positive result when there were two distinct read lines on the C and test (T) regions and an invalid test when the C line fails to appear (http://sam-techdiagnostics.com/pdfs/HCV/HIV.pdf).

3.9 Measurement of liver function enzymes (ALT, AST, and ALP)

Measurement of ALT, AST, and ALP was done using the SPINREACT commercial kits (Ctra Santa Coloma, Spain) as described by manufactures’ manual and guided by the controls (www.spinreact.com) using the Urit 3300 machine (Diamond Diagnostics, USA).
3.9.1 Alanine aminotransferase (ALT)

The ALT reagent (Appendix IX: A) was used to measure ALT by an enzymatic rate method. ALT catalyzes the reversible transfer of an amino group from L-alanine to α-ketoglutarate forming glutamate and pyruvate. The pyruvate formed is reduced by NADH to form L-lactate and NAD+. The rate of NADH oxidation is directly proportional to the ALT catalytic activity which is determined by measuring the absorbance at 340 nm. The value was recorded as calculated by the machine and expressed in U/L. The normal reference value was up to 32U/L for female and up to 40U/L for male (www.spinreact.com).

3.9.2 Aspartate aminotransferase (AST)

AST reagent (Appendix IX: B) was used to measure AST activity enzymatically. AST present in the sample catalyzed the reversible transfer of an amino group from L-aspartate to α-ketoglutarate forming glutamate and oxalacetate. The oxalacetate reacts with NADH and is reduced to malate by malate dehydrogenase. The rate of the NADH oxidation is directly proportional to the AST and absorbance measured at 340 nm. The machine calculates and expresses the AST activity in U/L. The normal reference value was up to 31U/L for female and up to 38U/L for male (www.spinreact.com).

3.9.3 Alkaline phosphatase (ALP)

The ALP reagent (Appendix IX: C) was used to measure ALP activity by a standardized kinetic method. ALP catalyzes the hydrolysis of p-nitrophenyl phosphate into phosphate and p-nitrophenol. The p-nitrophenol released is directly proportional to the catalytic activity of ALP and is determined by measuring the absorbance at 405
nm. The machine calculated and expressed the activity in U/L. The normal reference value was 98 - 279 U/L (www.spinreact.com).

Hepatotoxicity was graded based on sex and age according to the criteria set by the AIDS Clinical Trials Group (ACTG) as follows: grade 1 (1.25–2.5 × ULN), grade 2 (2.51–5.0 × ULN), grade 3(5.1–10 × ULN), and grade 4 (>10 × ULN). Severe hepatotoxicity was defined as grade 3 or 4 hepatotoxicity. If the AST and ALT grades were discordant, the higher of the two grades was used for classification (Sulkowski et al., 2000; Fokunang et al., 2010; Mugusi et al., 2012).

3.10 Cytokine measurement

Cytokines were measured by the Cytometric Bead Array (CBA) flow cytometry using the Human Th1/Th2/Th17 CBA kit (BD Biosciences, California) as described by Williams et al., (2013). This allowed for the detection of IL-2, IL-6, IL-10, IL-17A, TNF-α, and IFN-γ. Briefly, the samples were thawed, diluted with assay diluents (1:2 v/v) and added to the prepared cytokine standards, capture beads and the phycoerythrin detection reagent. The CBA analysis was then performed according to the manufacturer’s instruction with the FCAP Array software using the BD FACS Canto™ II flow cytometer (BD Biosciences, USA).

3.11 HIV subtyping and drug resistance determination

3.11.1 Viral RNA Extraction

Viral RNA was extracted using QIAamp® Viral RNA Mini Kit (QIAGEN, USA) according to the manufacturers’ instructions and immediately stored at –20°C for subsequent cDNA synthesis (www.qiagen.com/resources/download).
3.11.2 Reverse Transcription- Polymerase Chain Reaction (RT-PCR)

The extracted viral RNA was then transcribed to cDNA using in-house RT-polymerase chain reaction (RT-PCR) protocol using a one-step kit (SuperScript™ One-Step RT-PCR System, Invitrogen, USA) in a reaction tube containing for each sample; 25 µl 2X reaction mix (Mg^{2+} buffer and dNTPs), 8 µl MgSO_{4} (5 mM), 3 µl DNAse- and RNAse-free water, 0.75 µl sense primer (10 µM stock; BS: 5’GAC AGG CTA ATT TTT TAG GG 3’ located at 2075-2094gag), 0.75 µl antisense primer (10 µM stock; GIO2: 5’ TTT CCC CAT ATT ACT ATG CTT 3’ located at 3683-3703pol ), 1 µl RNase OUT (40 U/ µl; Invitrogen, USA), 1.5 µl RT-Taq (SuperScript One-Step RT-PCR reverse transcriptase and Platinum Taq DNA polymerase; Life Technologies, USA) and 10 µl of viral RNA. The RT-PCR was run under the following thermal cycling conditions; Initial step or cDNA synthesis: (1 cycle; 50°C for 30mins), denaturation: (1 cycle, 94°C for 2mins), amplification: 40 cycles (consisting of denaturation: 95°C for 30s, annealing: 52°C for 30s, extension: 72°C for 1min), final elongation: 1 cycle 72°C for 10mins and 1 cycle 4°C for 30 mins. For each PCR reaction, positive and negative controls were used to ensure the effectiveness of the reaction and the absence of cross-contamination respectively. The reaction was run in the AB 2720 thermocycler (Applied Biosystems, USA)

3.11.3 Agarose gel electrophoresis

It was used to verify the amplified amplicon obtained from RT-PCR reaction. A 1% agarose (Merck, Germany) was prepared and 1.5 µl ethidium bromide added to it. Three µl of low DNA mass ladder was loaded into the first wells. Five µl amplicons from each PCR reaction (cDNA) were added to 1.5µl of loading buffer and loaded into subsequent wells. The samples were electrophoresed at 90 volts for 45 minutes.
The gel was viewed for the presence of the amplified product under UV light with the help of a UVP transilluminator (Analytik Jena, Germany) (Figure 3.3).

<table>
<thead>
<tr>
<th>№</th>
<th>Sample ID</th>
<th>Gel Picture</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>+ve Control</td>
<td><img src="image" alt="Gel Picture" /></td>
</tr>
<tr>
<td>02</td>
<td>B001</td>
<td></td>
</tr>
<tr>
<td>03</td>
<td>B003</td>
<td></td>
</tr>
<tr>
<td>04</td>
<td>B004</td>
<td></td>
</tr>
<tr>
<td>05</td>
<td>B005</td>
<td></td>
</tr>
<tr>
<td>06</td>
<td>B006</td>
<td></td>
</tr>
<tr>
<td>07</td>
<td>-ve Control</td>
<td></td>
</tr>
</tbody>
</table>

**Legend:** MW- Molecular weight marker, 01- positive control, 07-negative control

**Figure 3.3:** Verification of amplified RT-PCR product on 1% agarose gel

**3.11.4 Semi-nested PCR**

This was done with 2 µl amplicons from the RT-PCR in a reaction containing: 45 µl Platinum™ PCR supermix high fidelity (H₂O, Buffer TAQ [10X], MgCl₂ [25 mM], dNTPs [12.5 mM], TAQ Gold) (Invitrogen, USA), 1.5 µl sense primer (10 µM stock; BS {5’GAC AGG CTA ATT TTT TAG GG }3’ located at 2075-2094gag), 1.5 µl antisense primer (10 µM stock; TAK 3{5’ GGC TCT TGA TAA ATT TGA TAT GT 3’} located at 3561-3583 pol). The Nested PCR was run under the following conditions; Initial denaturation (1 cycle, 93°C for 12 mins), amplification: 40 cycles (denaturation: 94°C for 30s, annealing: 53°C for 45s, extension: 72°C for 2 mins), final elongation: 1 cycle 72°C for 10mins, 1 cycle 4°C for 30 mins and 10°C for infinity. The expected cDNA is about 1510 bp (position 2075 [gag] to 3583 [pol]) in
length. For each reaction, positive and negative controls were used to ensure the effectiveness of the reaction and the absence of contamination respectively. The reaction was run in the AB 2720 thermocycler (Applied Biosystems, USA). Amplification of the amplicon product was verified using 1% agarose gel electrophoresis (Figure 3.4).

<table>
<thead>
<tr>
<th>N°</th>
<th>Sample ID</th>
<th>Gel Picture</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>+ve Control</td>
<td><img src="image" alt="Gel Picture" /></td>
</tr>
<tr>
<td>02</td>
<td>B001</td>
<td></td>
</tr>
<tr>
<td>03</td>
<td>B003</td>
<td></td>
</tr>
<tr>
<td>04</td>
<td>B004</td>
<td></td>
</tr>
<tr>
<td>05</td>
<td>B005</td>
<td></td>
</tr>
<tr>
<td>06</td>
<td>B006</td>
<td></td>
</tr>
<tr>
<td>07</td>
<td>-ve Control</td>
<td></td>
</tr>
</tbody>
</table>

**Legend:** MW- Molecular weight marker, 01- positive control, 07-negative control

**Figure 3.4:** Verification of amplified semi-nested PCR product on 1% agarose gel

### 3.11.5 Purification of PCR products

Semi-nested PCR products were purified using labeled Amicon 100 ® Microcon Ultra Pure 0.5 ml filters (Centrifugal Filters Devices, Millipore, USA) to eliminate all elements (dNTPs, primers, salts) that might have a negative influence during sequencing. Each filter was hydrated with 250 μl RNase-DNase free water and 50 μl of water added to each amplification product. All the amplification products were then transferred into the corresponding filter and the tubes centrifuged for 12 mins at 14000g. New Eppendorf tubes were labeled and the filters transferred into them. Approximately 5-15 μl of DNase-free and RNase-free water was added (depending on
the concentration of the cDNA) and allowed for 2 to 3 mins at room temperature. The filters were then inverted, centrifuged for 2 mins at 1000g and the purified product was stored at -20°C.

3.11.6 Sequence PCR

The purified products were sequenced in sense and antisense orientations in an automated sequencer (Applied Biosystems, Japan) using eight different overlapping sequence-specific primers (B, F, SEQ1, SEQ2, SEQ3, SEQ4, SEQ5 and TAK 3). The used primers nucleotide bases were; 5’ AGC AGA CCA GAG CCA ACA GC 3’ (2140-2159 gag), 5’ CCA TCC ATT CCT GGC TTT AAT 3’ (2582-2602 pol), 5’ CAG GAA TGG ATG GCC CAA AA3’ (2590-2609 pol), 5’ TTG TAC AGA AAT GGA AAA GGA AGG 3’ (2660-2683 pol), 5’ CCC TGT GGA AAG CAC ATT GTA 3’ (2985-3004 pol with an insertion) 5’ GCT TCC ACA GGG ATG GAA A 3’ (2993-3011 pol), 5’ CTA TTA AGT CTT TTG ATG GGT CA 3’ (3506-3528 pol), and 5’ GGC TCT TGA TAA ATT TGA TAT GT 3’(3561-3583 pol). The primers for the sequencing reaction were prepared based on the number of samples. Each sample contained 8 different primers-mix (3.2 µl of each primer [1 pmol], 4.8 µl H2O, 3 µl of Big Dye Terminator and 5 µl Big Dye Buffer [Applied Biosystems, USA]). For each primer, 4 µl of purified cDNA was added for a total volume of 20 µl. The sequencing reaction conditions were as follows: 35 cycles (96°C for 10s; 55°C for 10s; 60°C for 4 mins), 1 cycle (4°C for 30 mins). The reaction was run in the AB 2720 thermocycler (Applied Biosystems, USA).

3.11.7 Sequence products purification

The sequencing product was purified using Sephadex-50 resin (Merck, Germany) through gel filtration chromatography in order to eliminate unincorporated
dideoxynucleotides (ddNTPs), excess primers and salts. Dry Sephadex G-50 Resin was loaded in a 96-well Multiscreen HV plate (Merck, Germany) and transferred to a 96-well microtitre plate. Each well was hydrated with 300 μl of DNAase and RNAase free water and incubated at room temperature for 3 hours. The plate was centrifuged at 12000g for 8 mins. The samples were transferred to the 96-well microtiter plate. A new 96 wells plate to collect the purified sample was then used as support to the 96-well microtiter plate. The plates were centrifuged at 12000g for 8 mins.

3.11.8 Preparation of the optical plate for sequencing

An aliquot of formamide (Sigma-Aldrich, USA) was thawed and 13 μl pipetted into the labeled optical plate column (every 8 wells) to be used. In the case of odd number samples, formamide was used to fill the last column. Six μl of the purified sequence reaction was added into the corresponding optical plate. The mixture was denatured in the AB2720 thermocycler (Applied Biosystems, USA) under the following condition: 95° C for 2 mins followed by 4°C for 2 mins.

3.11.9 Reading the sequences on the genetic analyzer

The plate was then transferred to the Abbott Applied Biosystem (ABI) Prism 3130 genetic analyzer (Applied Biosystems, USA) and the result read. The device also allows the reading and correction of the sequences obtained by comparison with a wild-type sequence. The sequence obtained was copied and transferred to the database as a FASTA file and the results analyzed.

3.12 Data analysis

The clinical assessment and laboratory results were recorded and double-checked using Microsoft Excel. Hepatotoxicity, HIV subtypes and drug resistance mutation were analyzed using the Statistical Package for Social Sciences (SPSS) version 23.
(Armonk, USA.) while cytokine data was done using Graph pad prism 6 software (San Diego, USA) version 16.0. The data were summarised and presented in the form of tables, histograms, and graphs. Data were categorized and classified into different categories of age groups, sex, body mass index (BMI), level of education, baseline CD4+ T cells count, monthly income, alcohol consumption, cigarette or tobacco intake, WHO stages, year of diagnosis and type of HAART being administered. Categorical variables were expressed as frequencies and proportions and compared using Chi-square test while continuous variables (age, BMI, CD4, ALT, AST, and ALP) were expressed as means ± SEM and compared to the different treatment durations using the analysis of covariance (ANCOVA). The fixed covariates considered to be possible risk factors for the liver elevated enzyme (LEE) at baseline parameters were explored using univariate logistic regression with unadjusted odds ratios and adjusted odds ratios for multivariate analyses to identify risk factors associated with severe hepatotoxicity. The variables included in the multivariate model were those with a p-value, < 0.1 in the univariable logistic regression. Mean cytokine differences between the two groups were determined using Mann-Whitney (U) unpaired t-test. Regression analysis to determine the relationship between disease progression (CD4+ T cell count) and liver function tests were carried out using SPSS.

HIV-1 pol gene subtyping was done by phylogeny and the Stanford HIV-1 Drug resistance database (Stanford University, USA), rapid subtyping tools available at http://hivdb.stanford.edu/. Any discrepancies in HIV-1 subtype and CRF identification between the two methods was confirmed using recombinant identification program (RIP 3.0) available at (http://www.hiv.lanl.gov/content/sequence/RIP/RIP.html, 2017).
The Phylogeny was analyzed using MEGA vs. 7. The sequences were aligned using ClustalW, and phylogenetic analysis performed using the neighbor-joining method of MEGA-vs.7 software with a Bootstrap value of 1000 replicates. The reference sequences were obtained from the Los Alamos database and aligned with test sequences.

The level of transmitted drug resistance and patient-specific resistance-associated mutations (RAMs) were identified using the Stanford HIVDR Interpretation Program. The level of significance was set at p<0.05.
CHAPTER FOUR: RESULTS

4.1 Study population

Of the 120 clients recruited, 20 (16.7%) were excluded. Of this, 7 (5.8%) patients died from HIV associated complications, 5 (4.2%) patients took other drugs, 3 (2.5%) relocated to another area, 2 (1.7%) promised to come but did not show up (Figure 4.1).

![Flow chart showing the study population](image)

**Figure 4.1: Flow chart showing the study population**

4.2 Socio-demographic and clinical characteristics of the study population

Of the 100 HIV-1 infected patients participating in this study, the mean (age range) of the patients was 36.53(18-61) years. The patients were predominantly within the age range of 30-40 years; 43(43.0%). There were females; 53(53.0%), came from the urban area; 55(55%) had attained primary education; 51(51%), with a monthly
income of $90\,US$; 77(77%), with CD4$^+$ T cells count of <200 cells/mm$^3$; 57(57.0%), WHO clinical stage 2; 45(45%) and 64 (64.0%) of them placed on TDF+3TC+EFV regimen (Table 4.1).

Table 4.1: Socio-demographic and clinical characteristics of the study population by drug type

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Indicators (%)</th>
<th>Frequency (%) of drug type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TDF+3TC+EFV(64)</td>
<td>AZT+3TC+EFV(22)</td>
</tr>
<tr>
<td>Age range (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30 years(27)</td>
<td>17(63.0)</td>
<td>6(22.2)</td>
</tr>
<tr>
<td>40 years(43)</td>
<td>29(67.4)</td>
<td>5(11.6)</td>
</tr>
<tr>
<td>&gt;40 years(30)</td>
<td>17(56.7)</td>
<td>11(36.7)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female(53)</td>
<td>29(54.7)</td>
<td>15(28.3)</td>
</tr>
<tr>
<td>Male(47)</td>
<td>35(74.5)</td>
<td>7(14.9)</td>
</tr>
<tr>
<td>Site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban(55)</td>
<td>38(69.1)</td>
<td>11(20.0)</td>
</tr>
<tr>
<td>Rural(45)</td>
<td>25(55.6)</td>
<td>11(24.4)</td>
</tr>
<tr>
<td>Level of education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None(5)</td>
<td>2(40.0)</td>
<td>3(60.0)</td>
</tr>
<tr>
<td>Primary(51)</td>
<td>29(56.9)</td>
<td>14(27.5)</td>
</tr>
<tr>
<td>Secondary(38)</td>
<td>27(71.1)</td>
<td>5(13.2)</td>
</tr>
<tr>
<td>Tertiary(6)</td>
<td>6(100.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low(47)</td>
<td>32(68.1)</td>
<td>8(17.0)</td>
</tr>
<tr>
<td>Normal(31)</td>
<td>15(73.7)</td>
<td>11(50.0)</td>
</tr>
<tr>
<td>Overweight(7)</td>
<td>6(85.7)</td>
<td>1(14.2)</td>
</tr>
<tr>
<td>Obese(15)</td>
<td>11(73.4)</td>
<td>2(13.3)</td>
</tr>
<tr>
<td>Monthly Income $&lt;US 90S$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;77(77)</td>
<td>47(61.5)</td>
<td>18(23.1)</td>
</tr>
<tr>
<td>$&lt;US 90S$ (23)</td>
<td>16(72.7)</td>
<td>4(18.2)</td>
</tr>
<tr>
<td>Alcohol intake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes(56)</td>
<td>48(75.0)</td>
<td>18(81.8)</td>
</tr>
<tr>
<td>No(44)</td>
<td>16(25.0)</td>
<td>4(18.2)</td>
</tr>
<tr>
<td>Cigarette or Tobacco intake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No(73)</td>
<td>46(63.0)</td>
<td>16(21.9)</td>
</tr>
<tr>
<td>Yes(27)</td>
<td>17(63.0)</td>
<td>6(22.2)</td>
</tr>
<tr>
<td>Year of diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1 Year(84)</td>
<td>55(65.5)</td>
<td>16(19.0)</td>
</tr>
<tr>
<td>1-3 Years(4)</td>
<td>3(75.0)</td>
<td>1(25.0)</td>
</tr>
<tr>
<td>&gt;3 Years(12)</td>
<td>6(50.0)</td>
<td>5(41.7)</td>
</tr>
<tr>
<td>CD4 class (cells/mm$^3$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200(57)</td>
<td>35(61.0)</td>
<td>12(21.1)</td>
</tr>
<tr>
<td>&gt;200(43)</td>
<td>29(67.4)</td>
<td>10(23.3)</td>
</tr>
<tr>
<td>WHO clinical staging</td>
<td></td>
<td></td>
</tr>
<tr>
<td>stage 1(14)</td>
<td>7(50.0)</td>
<td>6(42.9)</td>
</tr>
<tr>
<td>stage 2(45)</td>
<td>36(80.0)</td>
<td>2(4.4)</td>
</tr>
<tr>
<td>stage 3(37)</td>
<td>20(54.1)</td>
<td>13(35.1)</td>
</tr>
<tr>
<td>stage 4(4)</td>
<td>12(50.0)</td>
<td>12(50.0)</td>
</tr>
</tbody>
</table>

Legend: 3TC; Lamivudine, AZT; Zidovudine, EFV; Efavirenz, NVP; Nevirapine, TDF; Tenofovir
At baseline, the mean CD4\(^+\) T cells counts (cells/mm\(^3\)) was 191.7 cells/mm\(^3\) while mean ALT, AST and ALP were 25.8(U/L), 26.7(U/L) and 90.8(U/L) respectively (Table 4.2).

**Table 4.2: Clinical and hepatic markers at baseline**

<table>
<thead>
<tr>
<th>Factors</th>
<th>Range</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m(^2))</td>
<td>11.5-48.3</td>
<td>27.1</td>
<td>1.3</td>
</tr>
<tr>
<td>CD4(^+) counts(cells/mm(^3))</td>
<td>8-498</td>
<td>197.1</td>
<td>13.6</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>2.7-40.0</td>
<td>25.8</td>
<td>1.1</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>6.9-37.8</td>
<td>26.7</td>
<td>1.6</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>74.1-187.6</td>
<td>90.8</td>
<td>3.4</td>
</tr>
</tbody>
</table>

**Legend:** ALP: Alkaline phosphatase, ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: Body mass index, SEM: standard error of the mean.

4.3: Variation of liver biochemical markers

4.3.1 Variation of mean ALT, AST and ALP with respect to duration of treatment

In table 4.3, mean ALT, AST and ALP increased significantly with increase in treatment duration at D30 and D180; ALT (F=16.8; p= 0.001), AST (F=11.3; p= 0.001) and ALP (F=7.8; p= 0.001). Mean ALT values were lower than mean AST values with a significant positive correlation between ALT and AST (r=0.76, p= 0.000) and ALT and ALP (r=0.53, p= 0.000) and an insignificant positive correlation between AST and ALP (r=0.12, p= 0.26).
Table 4.3: Variation of ALT, AST and ALP during the study duration

<table>
<thead>
<tr>
<th>Indicator (U/L)</th>
<th>time</th>
<th>Mean</th>
<th>SEM</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>D 0</td>
<td>24.47</td>
<td>13.01</td>
<td>16.83</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>D 30</td>
<td>69.65</td>
<td>13.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D180</td>
<td>130.79</td>
<td>13.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>D 0</td>
<td>27.066</td>
<td>17.58</td>
<td>11.29</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>D 30</td>
<td>75.126</td>
<td>17.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D180</td>
<td>144.55</td>
<td>17.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALP</td>
<td>D 0</td>
<td>92.3</td>
<td>20.535</td>
<td>7.83</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>D 30</td>
<td>134.39</td>
<td>20.535</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D180</td>
<td>206.04</td>
<td>20.535</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Legend:** ALT; alanine aminotransferase, AST; aspartate aminotransferase, ALP; alkaline phosphatase, D; day, SEM; Standard error of mean

4.3.2 Variation of mean ALT, AST and ALP with respect to drug type

Mean values of ALT, AST and ALP were highest in patients who took AZT+3TC+NVP regimen compared to TDF+3TC+EFV and AZT +3TC+EFV at D30 and D180 (Table 4.4). However, no significant interaction was seen between treatment duration and type of the drug. ALT (F=2.27; p= 0.06), AST (F=0.98; p= 0.42) and ALP (F=0.23; p= 0.92).
Table 4.4: Variation of ALT (U/L), AST (U/L), and ALP (U/L) with drug type and duration

<table>
<thead>
<tr>
<th>Indicator (U/L)</th>
<th>Time</th>
<th>HAART taken</th>
<th>Mean</th>
<th>SEM</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>D 0</td>
<td>TDF+3TC+ EFV</td>
<td>27.12</td>
<td>13.4</td>
<td>2.27</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AZT+3TC+EFV</td>
<td>25.12</td>
<td>22.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AZT+3TC+ NVP</td>
<td>21.17</td>
<td>28.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D 30</td>
<td>TDF+3TC+ EFV</td>
<td>74.14</td>
<td>13.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AZT+3TC+EFV</td>
<td>51.22</td>
<td>22.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AZT+3TC+ NVP</td>
<td>83.62</td>
<td>28.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D 180</td>
<td>TDF+3TC+ EFV</td>
<td>116.93</td>
<td>13.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AZT+3TC+EFV</td>
<td>65.76</td>
<td>22.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AZT+3TC+ NVP</td>
<td>209.69</td>
<td>28.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>D 0</td>
<td>TDF+3TC+ EFV</td>
<td>26.51</td>
<td>18.11</td>
<td>0.98</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AZT+3TC+EFV</td>
<td>26.89</td>
<td>30.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AZT+3TC+ NVP</td>
<td>27.79</td>
<td>38.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D 30</td>
<td>TDF+3TC+ EFV</td>
<td>101.4</td>
<td>18.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AZT+3TC+EFV</td>
<td>62.31</td>
<td>30.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AZT+3TC+ NVP</td>
<td>121.67</td>
<td>38.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D 180</td>
<td>TDF+3TC+ EFV</td>
<td>143.67</td>
<td>18.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AZT+3TC+EFV</td>
<td>92.72</td>
<td>30.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AZT+3TC+ NVP</td>
<td>197.25</td>
<td>38.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALP</td>
<td>D 0</td>
<td>TDF+3TC+ EFV</td>
<td>87.03</td>
<td>21.16</td>
<td>0.23</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AZT+3TC+EFV</td>
<td>105.97</td>
<td>36.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AZT+3TC+ NVP</td>
<td>84.11</td>
<td>45.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D 30</td>
<td>TDF+3TC+ EFV</td>
<td>134.74</td>
<td>21.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AZT+3TC+EFV</td>
<td>130.99</td>
<td>36.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AZT+3TC+ NVP</td>
<td>137.43</td>
<td>45.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D 180</td>
<td>TDF+3TC+ EFV</td>
<td>190.28</td>
<td>21.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AZT+3TC+EFV</td>
<td>189.01</td>
<td>36.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AZT+3TC+ NVP</td>
<td>238.82</td>
<td>45.23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legend: 3TC; Lamivudine, ALP; alkaline phosphatase, ALT; alanine aminotransferase, AST; aspartate aminotransferase, AZT; Zidovudine, EFV; Efavirenz, NVP; Nevirapine, SEM; Standard error of mean, TDF; Tenofovir
4.4 Risk factors associated with severe hepatotoxicity

4.4.1 Prevalence of hepatotoxicity

At baseline Day (D) 0, all the patients 100(100%) did not present with hepatotoxicity (grade 0) as classified using ALT, AST and ALP values. Elevated ALT was seen in 30(30%) and 46(46%) patients at D30 and D180 respectively. Majority of the patients presented with grade 1 followed by grade 2 hepatotoxicity compared to grade 3 and 4 at D30 and D180 (Figure 4.2).

![Figure 4.2: Prevalence (%) of hepatotoxicity by ALT relating to treatment duration](image)

$\chi^2 = 61.48 \quad P=0.000$

A similar trend was seen with AST during which the prevalence of hepatotoxicity increased significantly ($p= 0.0001$) with an increase in treatment duration. A total of 34 (34%) and 43(43%) patients presented with elevated AST at D30 and D180 with the majority of patients also presented with grade 1 and 2 hepatotoxicity (Figure 4.3).
$\chi^2 = 55.24 \quad P=0.000$

Figure 4.3: Prevalence (%) of hepatotoxicity by AST with regard to treatment duration

As concerns ALP, 11(11%) and 19(19%) patients presented with hepatotoxicity at D30 and D180 respectively. This difference was statistically significant ($p=0.002$). Most of the patients presented with grade 1 hepatotoxicity followed by grade 2 hepatotoxicity with 1 patient presenting with grade 3 hepatotoxicity and none with grade 4 hepatotoxicity (Figure 4.4).

$\chi^2 = 21.0 \quad P=0.002$

Figure 4.4: Prevalence (%) of hepatotoxicity by ALP regarding treatment duration
Using a higher grade of either ALT, AST or ALP, 37(37%) and 49(49%) patients presented with hepatotoxicity at D30 and D180 respectively. Of this, 22(22%) and 21(21%) patients had mild-to-moderate (grades 1 and 2) hepatotoxicity at D30 and D180 while 15(15%) and 28(28%) patients had severe hepatotoxicity (grades 3 and 4) at D30 and D180 respectively (Figure 4.5). Thus, hepatotoxicity increases with an increase in treatment duration.

\[ \chi^2 = 68.18 \quad P=0.000 \]

**Figure 4.5: Prevalence (%) of hepatotoxicity within the study duration**

### 4.4.2 Risk factors associated with severe hepatotoxicity at D180

Assessing risk ratios of severe hepatotoxicity occurrence at 95% confidence interval (CI) showed that all levels of hepatotoxicity severity occurred with respect to ALT and AST elevations, whereas grade 4 was not observed with ALP. In the univariate analyses, severe hepatotoxicity was significantly (p< 0.05) high in the age group <30 years 5(22.7%), in males 19(40.4%), patients with low monthly 26 (33.8%) and in patients with low BMI (<18.5kg/m²) 18(38.3%). Furthermore, HAART treatment
showed a significant differentiating characteristic (p=0.01) between those presenting with severe hepatotoxicity. Patients on AZT + 3TC + NVP drugs were 8(53.4%) more likely to have severe hepatotoxicity at 95% CI (2.86; 0.90-9.04, P=0.01). The mean (SEM) CD4\(^+\) T cells count of those with severe hepatotoxicity was 168.0 (25.3) cells/mm\(^3\), 20(35.1%) patients with CD4\(^+\) T cells count of >200 cells/μl presented with a high level of severe hepatotoxicity. Nevertheless, the difference was not significant (p=0.06). Even though the prevalence of severe hepatotoxicity was high in those who consumed alcohol 17(30.4%) and cigarette or tobacco smoking 11(40.7%), the association only showed a trend (p=0.07) and (p=0.09) respectively. Level of education (p=0.89), the year of diagnosis (p=0.91) and WHO clinical stages (p=0.27) were not predictive for the development of severe hepatotoxicity in the univariate analyses. However, patients with a tertiary level of education 2(33.3%), patients who were diagnosed >3 years before drug initiation 4(33.3%), and patients presenting with WHO stage 4; 2(50.0%), had a higher prevalence of severe hepatotoxicity (Table 4.5).
Table 4.5: Cox proportional univariate analysis for baseline characteristics of patients

<table>
<thead>
<tr>
<th>Characteristics of study population</th>
<th>Indicator</th>
<th>Univariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No n(%)</td>
<td>Yes n(%)</td>
</tr>
<tr>
<td>Age group (Years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;40</td>
<td>14(51.9)</td>
<td>13(48.1)</td>
</tr>
<tr>
<td>30-40</td>
<td>33(76.7)</td>
<td>10(23.3)</td>
</tr>
<tr>
<td>&lt;30</td>
<td>25(83.3)</td>
<td>5(16.7)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>28(59.6)</td>
<td>19(40.4)</td>
</tr>
<tr>
<td>Female</td>
<td>44(83.0)</td>
<td>9(17.0)</td>
</tr>
<tr>
<td>Body mass index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>26(83.9)</td>
<td>5(16.1)</td>
</tr>
<tr>
<td>Low</td>
<td>29(61.7)</td>
<td>18(38.3)</td>
</tr>
<tr>
<td>Overweight</td>
<td>6(83.7)</td>
<td>1(14.3)</td>
</tr>
<tr>
<td>Obese</td>
<td>11(73.3)</td>
<td>4(26.7)</td>
</tr>
<tr>
<td>Level of education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>4(80.0)</td>
<td>1(20.0)</td>
</tr>
<tr>
<td>Primary</td>
<td>38(74.5)</td>
<td>13(25.5)</td>
</tr>
<tr>
<td>Secondary</td>
<td>26(68.4)</td>
<td>12(31.6)</td>
</tr>
<tr>
<td>Tertiary</td>
<td>3(66.7)</td>
<td>2(33.3)</td>
</tr>
<tr>
<td>Monthly income</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; US 90$</td>
<td>51(66.2)</td>
<td>26(33.8)</td>
</tr>
<tr>
<td>&gt; US 90$</td>
<td>21(91.3)</td>
<td>2(8.7)</td>
</tr>
<tr>
<td>Alcohol intake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>33(75)</td>
<td>11(25)</td>
</tr>
<tr>
<td>Yes</td>
<td>39(69.6)</td>
<td>17(30.4)</td>
</tr>
<tr>
<td>Cigarette or tobacco intake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>56(76.7)</td>
<td>17(23.3)</td>
</tr>
<tr>
<td>Yes</td>
<td>16(59.3)</td>
<td>11(40.7)</td>
</tr>
<tr>
<td>Year of diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1 Year</td>
<td>61(72.6)</td>
<td>23(27.4)</td>
</tr>
<tr>
<td>1-3 Years</td>
<td>3(75.0)</td>
<td>1(25.0)</td>
</tr>
<tr>
<td>&gt;3 Years</td>
<td>8(66.7)</td>
<td>4(33.3)</td>
</tr>
<tr>
<td>CD4 count (cells/mm3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>37(64.9)</td>
<td>20(35.1)</td>
</tr>
<tr>
<td>&gt;200</td>
<td>35(81.4)</td>
<td>8(18.6)</td>
</tr>
<tr>
<td>WHO stages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>stage 1</td>
<td>8(57.1)</td>
<td>6(42.9)</td>
</tr>
<tr>
<td>stage 2</td>
<td>32(71.1)</td>
<td>13(28.9)</td>
</tr>
<tr>
<td>stage 3</td>
<td>30(81.1)</td>
<td>7(18.9)</td>
</tr>
<tr>
<td>stage 4</td>
<td>2(50.0)</td>
<td>2(50.0)</td>
</tr>
<tr>
<td>ARV regimen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDF+3TC+EFV</td>
<td>46(73.0)</td>
<td>18(27.0)</td>
</tr>
<tr>
<td>AZT+3TC+EFV</td>
<td>20(90.9)</td>
<td>2(9.1)</td>
</tr>
<tr>
<td>AZT+3TC+NVP</td>
<td>6(42.9)</td>
<td>8(57.1)</td>
</tr>
</tbody>
</table>

Legend: 3TC; Lamivudine, AZT; Zidovudine, EFV; Efavirenz, NVP; Nevirapine, TDF; Tenofovir

All socio-demographic variables that showed significance (p<0.05) or a trend (p=0.05-0.09) in the univariate analysis were used for the multivariate analysis. Patients <30 years old were at risk of developing severe hepatotoxicity, the risk became higher
0.16(0.04-0.72) after controlling for the other variables. Low BMI at baseline was also found to be a significant predictor of severe hepatotoxicity in this study. At the multivariate analysis, the risk of developing severe hepatotoxicity in patients with low BMI decreased to 0.33 (10%) (AOR=0.33, 95% CI=0.11-.99) after controlling for the other variables. Furthermore, patients with a monthly income of <US $90 had 0.18 risk of severe hepatotoxicity (OR=0.18, 95% CI=0.04-0.86) but after controlling for the other variables, the risk decreased to 0.14(22.2%). This data reveals that patients who took AZT+3TC+NVP regimen had 2.86 higher risk of developing severe hepatotoxicity than those patients who were on other regimens (OR=2.86, 95% CI=0.90-9.04). After adjusting for the rest of the variables in the multivariate model, the risk became lower (OR=2.44, 95% CI=0.58-10.26). Even though alcohol intake, cigarette or tobacco intake and CD4+ T cells count showed a trend in univariate analysis indicating an insignificant higher risk for developing severe hepatotoxicity, these factors were not predictors of severe hepatotoxicity. However, the risk to develop hepatotoxicity was higher in those who consumed alcohol and had low CD4+ T cells count (Table 4.6).
Table 4.6: Cox proportional multivariate analysis for baseline characteristics of patients with or without severe hepatotoxicity

<table>
<thead>
<tr>
<th>Characteristics of study population</th>
<th>Indicators</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OR(95%CI)</td>
<td>P</td>
</tr>
<tr>
<td>Age group (Years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;40</td>
<td>Male</td>
<td>1</td>
<td>0.02</td>
</tr>
<tr>
<td>30-40</td>
<td>Male</td>
<td>0.40(0.11-1.47)</td>
<td>0.32(0.08-1.24)</td>
</tr>
<tr>
<td>&lt;30</td>
<td>Male</td>
<td>0.15(0.03-0.65)</td>
<td>0.16(0.04-0.72)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>1</td>
<td>0.04</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>0.26(0.895.28)</td>
<td>1.78(0.58-5.49)</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td>1</td>
<td>0.02</td>
</tr>
<tr>
<td>Low</td>
<td></td>
<td>0.28(0.04-3.35)</td>
<td>0.33(0.11-0.99)</td>
</tr>
<tr>
<td>Overweight</td>
<td></td>
<td>0.30(0.11-0.78)</td>
<td>0.35(0.21-4.59)</td>
</tr>
<tr>
<td>Monthly income</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;US 90$</td>
<td></td>
<td>1</td>
<td>0.01</td>
</tr>
<tr>
<td>&gt;US 90$</td>
<td></td>
<td>0.18(0.04-0.86)</td>
<td>0.14(0.030.79)</td>
</tr>
<tr>
<td>Alcohol intake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>1</td>
<td>0.07</td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td>0.40(0.11-1.47)</td>
<td>0.42(0.09-1.64)</td>
</tr>
<tr>
<td>Cigarette or tobacco intake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>1</td>
<td>0.09</td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td>2.26(0.885.80)</td>
<td>1.68(0.51-5.60)</td>
</tr>
<tr>
<td>CD4 Count values</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td></td>
<td>1</td>
<td>0.06</td>
</tr>
<tr>
<td>&gt;200</td>
<td></td>
<td>0.42(0.16-1.08)</td>
<td>0.65(0.34-2.37)</td>
</tr>
<tr>
<td>ARV regimen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDF+3TC+EFV</td>
<td></td>
<td>1</td>
<td>0.01</td>
</tr>
<tr>
<td>AZT+3TC+EFV</td>
<td></td>
<td>0.25(0.05-1.18)</td>
<td>0.18(0.030.99)</td>
</tr>
<tr>
<td>TDF+3TC+NVP</td>
<td></td>
<td>2.86(0.90-9.04)</td>
<td>2.44(0.5810.26)</td>
</tr>
</tbody>
</table>

Legend: 3TC; Lamivudine, AZT; Zidovudine, EFV; Efavirenz, NVP; Nevirapine, TDF; Tenofovir

4.5 Inflammatory markers and hepatotoxicity

4.5.1 Correlation of CD4 T-cell counts with ALT and AST at D0

In figure 4.6, CD4⁺ T cells count varies with levels of ALT and AST. A significantly inverse regression was observed between CD4⁺ T cells count and ALT (R=-0.27, p=0.001) and AST (R=-0.25, p=0.001).
**Legend:** ALT; alanine aminotransferase, AST; aspartate aminotransferase

**Figure 4.6:** Relationship between CD4⁺ T-cell counts with ALT and AST at D0

### 4.5.2 Comparison of mean cytokines between patients with and without hepatotoxicity

Mean plasma concentrations of TNF-α (p = 0.95), IFN-γ (p = 0.54), IL-17A (p = 0.04), IL-6 (p = 0.02), and IL-2 (p = 0.42), were higher in HIV-1 patients presenting with hepatotoxicity compared to those without hepatotoxicity at D30 with a significant difference was seen in mean IL-17A and IL-6 cytokines (Figure 4.7).
On the other hand, mean plasma concentrations of TNF-α (p =0.09), IL-17A (p =0.03), IL-10 (p =0.36), IL-6 (p =0.03), and IL-2 (p =0.11) cytokines increased in HIV-1 patients presenting with hepatotoxicity compared to those without hepatotoxicity at D180 (Figure 4.8). However a significant difference was seen in mean IL-17A and IL 6 cytokines.
Figure 4.8: Mean cytokine variation between patients presented with and without hepatotoxicity at D180

4.6 HIV-1 genetic diversity

4.6.1 Demographic of patients

Of the 100 patients enrolled in the study, 94 samples were sequenced. Of these 62 (62%) were sequenced in the required protease and reverse transcriptase coding
regions while 19 (19%) were sequenced in required reverse transcriptase coding regions giving a successfully sequencing performance of 81%. The DNA sequences of the HIV-1 protease-reverse transcriptase sequences were submitted to GenBank under the following accession numbers: MK061035-MK061115 (Appendix X). The participants were aged between 18-61 years, with a median age of 36.9 years and 55.5% (45) were female. The mean (range) of CD4\(^+\) T cells count was 194.3(8–498) cells/mm\(^3\) and a greater part 59.3 % (48) of the participants had CD4\(^+\) T cells count of <200 cells/mm\(^3\). The majority of the patients were residents in an Urban setting 54.3% (44). WHO clinical stages varied from stage 1 to stage 4 with the majority of patients 48.1% (39) classified as WHO clinical stage 2 (Table 4.7).
Table 4.7: Descriptive characteristics of study participants (n=81)

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Variable (n)</th>
<th>Female (%)</th>
<th>Male (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n=45</td>
<td>n=36</td>
</tr>
<tr>
<td>Site</td>
<td>Rural (37)</td>
<td>24 (53.3)</td>
<td>13 (36.1)</td>
</tr>
<tr>
<td></td>
<td>Urban (44)</td>
<td>21 (48.7)</td>
<td>23 (63.9)</td>
</tr>
<tr>
<td>Age in years</td>
<td>Mean ± SEM</td>
<td>36.31±1.41</td>
<td>37.39±1.51</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>18-56</td>
<td>20-61</td>
</tr>
<tr>
<td></td>
<td>&lt;30 (17)</td>
<td>12 (70.6)</td>
<td>5 (29.4)</td>
</tr>
<tr>
<td></td>
<td>30-40 (37)</td>
<td>18 (48.8)</td>
<td>19 (51.2)</td>
</tr>
<tr>
<td></td>
<td>&gt;40 (27)</td>
<td>15 (55.6)</td>
<td>12 (44.4)</td>
</tr>
<tr>
<td>CD4 Classification cells/ μL</td>
<td>Mean ± SEM</td>
<td>207.36±21.27</td>
<td>177.97±20.84</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>8-489</td>
<td>31-498</td>
</tr>
<tr>
<td></td>
<td>&lt;200 (48)</td>
<td>23 (49.9)</td>
<td>25 (52.1)</td>
</tr>
<tr>
<td></td>
<td>200-350 (19)</td>
<td>13 (68.4)</td>
<td>6 (31.6)</td>
</tr>
<tr>
<td></td>
<td>350-500 (14)</td>
<td>9 (64.3)</td>
<td>5 (35.7)</td>
</tr>
<tr>
<td>WHO stage</td>
<td>1 (10)</td>
<td>5 (50.0)</td>
<td>5 (50.0)</td>
</tr>
<tr>
<td></td>
<td>2 (39)</td>
<td>18 (46.2)</td>
<td>21 (53.8)</td>
</tr>
<tr>
<td></td>
<td>3 (28)</td>
<td>19 (67.9)</td>
<td>9 (32.1)</td>
</tr>
<tr>
<td></td>
<td>4 (4)</td>
<td>3 (75)</td>
<td>1 (25)</td>
</tr>
</tbody>
</table>

4.6.2 Phylogenetic analysis

Phylogenetic analysis of the 81 sequences revealed a high HIV-1 group M isolates genetic diversity with four subtypes and five CRFs. The subtypes were F2 (6: 7.4%), G (4: 4.9%), D (3: 3.7%), and A1 (2: 2.4%), and the circulating recombinant forms were CRF02_AG (61: 75.3%), CRF22_01A1(2: 2.4%), CRF06_cpx(1: 1.2%), CRF09_cpx(1: 1.2%) and CRF11_cpx(1: 1.2%) as shown in figure 4.9. This tree also revealed 3 groups of closely related clusters (B045 and B077, B007 and B101, B062 and B0119).
Sample sequences have black shapes. The reference sequences are not colored and were taken from the Los Alamos HIV database. Some references were omitted to enable better visualization of the sample sequences.

Figure 4.9: A phylogenetic tree of HIV-1 pol gene
4.6.3 HIV-1 genotypes and hepatotoxicity

After data nominalization using the natural log, the prevalence of hepatotoxicity did not show any significant difference among HIV subtypes (p= 0.35). The prevalence of hepatotoxicity was insignificantly (p=0.6) high 70.3% (26/37) among individuals harboring CRF02_AG virus (Figure 4.10).

![Figure 4.10: Prevalence (%) of hepatotoxicity by HIV subtypes](image)

However, when comparing non-CRF02_AG and CRF02_AG subtypes, non-CRF02_AG infected subjects recorded a higher prevalence of 55.0% (11/20) compared to CRF02_AG with a prevalence of 4.26% (26/61). This difference was however not significant (p= 0.40).

4.7 Prevalence of transmitted drug resistance

The prevalence of transmitted drug resistance was 11.1% (9/81). Of the resistance mutations observed, 8.6% (7/81) confers resistance to NRTI, 4.9% (4/81) to NNRTI
and 1.2% (1/81) to PI. The distribution of NRTI resistance-associated mutations also included the thymidine analog mutations (TAMs): M41L, D67N, K70R, T215F, and K219Q that occurred in 6.2% (5/81) patients. In this study, the most common mutations were K219Q (2.5%; 2/81) and E138A (2.5%; 2/81) which confers resistance to NRTIs and NNRTIs respectively. Singleton mutations that were associated with NRTI included: T215TA, M184MV, M184V, I54IFV, D67N, K70R, K70T, and M41ML; while that of, NNRTI was K103N, V108I, V179E, and Y181C and for PI was I54IFV. Dual-class DRM involving both NRTI and NNRTI was observed in three patients (3.7%). One patient was detected with HIV-1 variant harboring multi RAMs in both NRTI (D67N, K70R, M184V, T215F, K219Q) and NNRTI (V108I, V179E, Y181C) (Table 4.6).

The RAMs linked to ARV drug susceptibility and the mutation net drug score, showed four resistant phenotypes, namely high level, intermediate level, low level, and potential low-level resistance. Majority of the RAMs were associated with low-level resistance 36.9% (17/46) followed by potential low-level resistance 30.4% (14/46). NRTIs resistance rate was highest for D4T (8/10; 80%) while for NNRTI it was same and highest for EFV and NVP (4/5; 80%) (Table 4.8).
Table 4.8: Demographic and immunologic characteristics of patients with TDR and predicted ARV drug resistance

<table>
<thead>
<tr>
<th>GenBank ID</th>
<th>Site</th>
<th>Gender</th>
<th>WHO stage</th>
<th>HIV subtype</th>
<th>Drug class resistance mutation</th>
<th>*Resistance-associated Mutation predicted ARV drug resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NRTI</td>
<td>NNRTI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>K219Q</td>
<td>None</td>
</tr>
<tr>
<td>MK061058</td>
<td>Urban</td>
<td>Male</td>
<td>4</td>
<td>CRF02_AG</td>
<td>T215TA</td>
<td>None</td>
</tr>
<tr>
<td>MK061064</td>
<td>Rural</td>
<td>Male</td>
<td>2</td>
<td>CRF02_AG</td>
<td>M41ML</td>
<td>None</td>
</tr>
<tr>
<td>MK061100</td>
<td>Rural</td>
<td>Female</td>
<td>3</td>
<td>CRF02_AG</td>
<td>K65E</td>
<td>None</td>
</tr>
<tr>
<td>MK061051</td>
<td>Rural</td>
<td>Female</td>
<td>2</td>
<td>CRF02_AG</td>
<td>None</td>
<td>E138A</td>
</tr>
<tr>
<td>MK061069</td>
<td>Urban</td>
<td>Male</td>
<td>2</td>
<td>CRF02_AG</td>
<td>K70T</td>
<td>E138A</td>
</tr>
<tr>
<td>MK061076</td>
<td>Urban</td>
<td>Male</td>
<td>2</td>
<td>CRF02_AG</td>
<td>M184MV</td>
<td>K103N</td>
</tr>
<tr>
<td>MK061065</td>
<td>Urban</td>
<td>Male</td>
<td>2</td>
<td>CRF02_AG</td>
<td>D67N, K70R,</td>
<td>V108I,</td>
</tr>
<tr>
<td>MK061090</td>
<td>Urban</td>
<td>Female</td>
<td>4</td>
<td>CRF02_AG</td>
<td>M184V, T215F,</td>
<td>V179E,</td>
</tr>
<tr>
<td>MK061080</td>
<td>Rural</td>
<td>Female</td>
<td>3</td>
<td>CRF22_01A1</td>
<td>K219Q</td>
<td>Y181C</td>
</tr>
</tbody>
</table>

NB: The **bold and underlined** are drugs used in this study.
Legend: 3TC; Lamivudine, ABC; Abacavir, ATV/r; Atazanavir/r, AZT; Zidovudine, D4T; Stavudine, DDI; Didanosine, EFV; Efavirenz, ETR; Etravirine, FPV/r; Fosamprenavir/r, FTC; Emtricitabine, IDV/r ; Indinavir/r, LPV/r; Lopinavir/r, NFV; nelfinavir, NNRTI; Non-nucleoside reverse transcriptase inhibitor, NRTI; Nucleoside reverse transcriptase inhibitor, NVP; Nevirapine, PI; protease inhibitors, RPV; Rilpivirine, SQV/r; Saquinavir/r, TDF; Tenofovir, TPV/r; Tipranavir/r

a = Potential low-level resistance (mutation net drug score (10–14)).

b = Low-level resistance (mutation net drug score of 15–30).

c = Intermediate resistance (mutation net drug score of 31–59).

d = High-level resistance (mutation net drug score of ≥60).

4.7.2 Assessing the presence of TDR and prevalence of hepatotoxicity at end of D180

A total of 37 (45.7%) patients presented with hepatotoxicity at Day 180 (Table 4.9). Of this, 18.9% (7/37) had TDR. The hepatotoxicity grades of the patients with TDR were as follows; grade 1(n=1), grade 2 (n=2), grade 3 (n=2) and grade 4 (n=2). This difference was significant P=0.04.

Table 4.9: Assessing the presence of TDR and prevalence of hepatotoxicity at end of D180

<table>
<thead>
<tr>
<th>Hepatotoxicity</th>
<th>Drug-resistance mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (%)</td>
</tr>
<tr>
<td>No</td>
<td>42(58.3)</td>
</tr>
<tr>
<td>Yes</td>
<td>30(41.7)</td>
</tr>
</tbody>
</table>

χ² = 3.87

p= 0.040
CHAPTER FIVE: DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

5.1 Discussion

Hepatotoxicity linked to HAART is expressed by elevation of liver-associated enzymes such as ALT, AST, and ALP (Ngala et al., 2015). These liver associated enzymes are measures of liver homeostasis and abnormal levels of these enzymes can be classified as either hepatocellular (increase in AST and ALT), cholestatic (increase in ALP), or mixed with hepatocellular hepatotoxicity being the most common type (Mata-Marín et al., 2009; Fokunang et al., 2010). Elevations of these enzymes have been associated with all classes of ART (NRTI, NNRTI, and PI). However, not all drugs within each class have similar effects on the liver thus the extent to which each drug of HAART contributes to hepatotoxicity varies (Lucien et al., 2010; Wondemagegn et al., 2013; Wu et al., 2015; Abongwa et al., 2017).

5.1.1a Variation of liver biochemical markers in relation to the duration of treatment

Data from this study showed a significant positive linear relationship between elevated ALT, AST and ALP levels with an increase in the duration of treatment irrespective of the type of drugs. Similar results have been reported elsewhere (Ayelagbe et al., 2014; Bello et al., 2014; Ngala et al., 2015). These liver enzymes elevation could be as a result of mitochondrial damage, direct drug toxicity or hypersensitivity reactions (Ofotokun et al., 2007; Navarro et al., 2014; Shiferaw et al., 2016). Nevertheless, it may also be associated with other variety of factors such as alcohol, opportunistic infections and concomitant medications (Nagu et al., 2012; Ayelagbe et al., 2014) since in the course of interaction with these patients, some of
them confirmed the use of traditional or herbal concoctions as alternative sources of therapies. As indicated in the treatment guideline all these patients took Trimethoprim-sulfamethoxazole for the prevention of opportunistic infections (MPH, 2015). Trimethoprim-sulfamethoxazole has shown to cause hepatotoxicity by idiosyncratic liver injury or hypersensitivity reaction (Lucien et al., 2010; Navarro et al., 2014; Ngala et al., 2015; Abongwa et al., 2017).

A higher AST value seen compared to ALT value is similar to previous studies in the same country (Lucien et al., 2010; Abongwa et al., 2017). This is probably because ALT is an enzyme present only in liver cells while AST is a mitochondrial enzyme that is also present in liver and other body organs like brain, pancreas, heart, kidneys, lungs, and skeletal muscles (www.verywellhealth.com/liver-enzymes) as such if any of these tissues are damaged, AST will be released into the bloodstream. Considering that the AST/ALT ratio is useful in differentiating between causes of hepatotoxicity, the ratio of AST and ALT in this study was less than 2 indicating that hepatotoxicity in these patients was induced by HAART and not alcohol (Nyblom et al., 2004; Thapa and Walia, 2007).

5.1.1b Variation of liver biochemical markers in relation to drug type

In this study, mean levels of ALT and AST increased significantly in NVP-based HAART compared to non-NVP-based HAART similar to previous studies (Soriano et al., 2008; Sterling et al., 2008). However, this is contrary to studies by Gisolf et al., (2000) and Wu et al., (2015) which state that the use of ritonavir and zidovudine respectively were found to be severely hepatotoxic. The mechanism of hepatic injury with Nevirapine is associated with hypersensitivity reactions (Sulkowski et al., 2002; Chu et al., 2010). Furthermore, Nevirapine is a potent inducer of Cytochrome P450
isoenzymes CYP2B6 that favor the production of a toxic intermediate such as reactive oxygen species (ROS) that might cause hepatic therapeutic failure and injury (Hedrich et al., 2016).

5.1.2 Risk factors associated with severe hepatotoxicity

Results from this study like in previous studies in other parts of Cameroon (Fokunang et al., 2010; Lucien et al., 2010) and elsewhere (Wambani et al., 2015; Wenderlein et al., 2016) showed a high prevalence of hepatotoxicity in HIV patients on HAART (13%-53%). The values from this study were higher than the 13% to 32% range in previous studies (Wondemagegn et al., 2013; Tseng et al., 2014; Wenderlein et al., 2016) and lower compared to the 53% as reported by Lucien et al., (2010) in the Littoral region of Cameroon. The difference in the rate of hepatotoxicity reported in these studies compared to other studies is probably due to differences in the population characteristics (patients with hepatitis B or C, patients on anti TB drugs among others), definitions of hepatotoxicity (use of baseline ALT or AST values with reference to the normal range), frequency of patient monitoring, type of HAART and other drugs, since HIV patients often ingest a cocktail of drugs in association with the HAART regimen to control other illnesses.

The high prevalence of hepatotoxicity 37(37%) recorded as early as one month during the course of treatment has also been reported in other studies (Mugusi et al., 2012; Wenderlein et al., 2016). Of the 37 patients presenting with hepatotoxicity at D30, 17(44.7%) did not present with hepatotoxicity at D180. This is a clear indication that some liver diseases are often associated with HIV infection other than HAART. Nevertheless, it has been reported that the hepatotoxic effects of HAART may resolve with time when the organ gets used to the drug in some patients (Bello et al., 2014).
As such, it is evident that hepatotoxicity in HIV patients is multifactorial (Ayelagbe et al., 2014) and can be attributed to mitochondrial toxicity, hypersensitivity reaction, or other AIDS-related opportunistic diseases caused by bacteria (Cryptosporidium parvum, Microsporidium, Mycobacterium avium complex), fungi (Candida albicans, Aspergillus fumigates, Cryptococcus neoformans, Histoplasma capsulatum, Coccidioides immitis), protozoa (Toxoplasma gondii, Leishmania species), Helminth (Strongyloides stercoralis), viruses (Cytomegalovirus, Herpes simplex virus, Human herpesvirus 8, Varicella-zoster virus, Epstein-Barr virus, Adenovirus). These opportunistic infections stimulate an immunological response in the hepatic phagocytes which account for liver enzyme elevation (Price and Thio, 2010; Ayelagbe et al., 2014). Thus, since HIV patients present with different ailments, there is high probability that mechanisms of hepatotoxicity from HAART may result from the interaction between HAART and non antiretroviral drugs (such as antibiotics, antifungal, antiviral and herbal concoctions) used as alternative therapies in combating the different diseases (Hamza et al., 2014; Wenderlein et al., 2016; Abongwa et al., 2017). Thus the incidence rates of hepatotoxicity during HAART may vary across different populations and with different drug combinations (Navarro et al., 2014; Osakunor et al., 2015; Wambani et al., 2015). Moreover, the high prevalence of hepatotoxicity seen at D180 can also be attributed to the fact at D180 there is an increase in CD4+ T cells count with the continuous use of HAART. A gradual increase in CD4+ T cells count usually leads to weight gain from the baseline which increases the level of transaminases (Mugusi et al., 2012; Tseng et al., 2014).

It has been reported that mild-moderate (grade 1 and 2) hepatotoxicity is of limited clinical relevance and often ignored by most investigators since mild-moderate hepatotoxicity resolves whether HAART is continued or not (Bello et al., 2014;
In this study, the high prevalence of severe hepatotoxicity at D30 (15%) and D180 (28%), is higher than the 6-12% reported elsewhere (Sulkowski et al., 2002; Wambani et al., 2015; Wenderlein et al., 2016) and in Cameroon (Fokunang et al., 2010). However, this high prevalence of severe hepatotoxicity seen in this study ties with a previous retrospective review of adults living with AIDS in Boston, where researchers confirmed that long time use of HAART is associated with a high rate of severe hepatotoxicity regardless of drug class or combination (Luma et al., 2012). As such it is necessary to assess the risk factors associated with severe hepatotoxicity after D180 of treatment.

Various conflicting risk factors for the development of severe hepatotoxicity have been described in other studies and include; HIV itself, age, gender, race, hepatitis B and C co-infection, increase in CD4+ T cells count after the start of ART, higher baseline levels of ALT and AST, opportunistic infections, cirrhosis, most antiretroviral, anti-tuberculosis, lipid-lowering drugs, alcohol, metabolic syndrome (Chu et al., 2010; Nagu et al., 2012; Wondemagegn et al., 2013; Abongwa et al., 2017).

Age was found to be a predictor of severe hepatotoxicity contrary to another study by Wondemagegn et al., (2013). Findings from this study revealed that the prevalence of severe hepatotoxicity was significantly high in patients <30 years contrary to studies by Fokunang et al., (2010), Lucien et al., (2010), Luma et al., (2012), and Abongwa et al., (2017) who found that those patients >30 years were at risk of developing hepatotoxicity. These differences in age can be attributed to the difference in the study design (characteristics of the population, duration of treatment, and definitions of severe hepatotoxicity) in the different studies. The most probable reason for the high prevalence in this group can be attributed to their lifestyle. This group is an
active age group and constitute people with high social activities such as high alcohol intake and cigarette smoking that have shown to increase the level of liver enzymes (Jang et al., 2012; Park et al., 2013). Secondly, there is a high probability that this group of individuals might also take other drugs as well as herbal concoctions to get healed faster to meet up with their economic and social demands.

Observations from this study demonstrate an important relation between BMI and serum activity rates of liver enzymes. This data suggest that body weight may be the major factor in determining the serum level of liver enzymes. Low BMI was found to be an independent predictor of severe hepatotoxicity. This finding confirms the findings of Wambani et al., (2015) and Wenderlein et al., (2016), that good nutritional status at the start of HAART is protective against early hepatotoxicity. However, other studies show that BMI had no association with liver enzyme levels (Adams et al., 2008; Park et al., 2013). Previous studies in Pakistani (Qureshi et al., 2006) and India (Tasneem et al., 2015) indicated that increase in serum ALT and AST levels are associated with an increase in BMI as a result of an increase in dietary fat content (Tasneem et al., 2015). High level of dietary fat causes higher levels of oxidative stress and lipid peroxidation which lead to mitochondrial DNA damage and thus hepatocellular injury (Mattar et al., 2005; Wambani et al., 2015). Furthermore, it has been reported that there exists a positive association between alcohol consumption and BMI with liver enzymes (Adams et al., 2008; Tasneem et al., 2015). This reflects the high prevalence of hepatotoxicity seen at D180.

Monthly income has been shown to play an important role in the life of individuals. A positive correlation (r=0.69; p=0.05) seen between monthly income and BMI is a clear indication that people with low monthly income presented a high prevalence of
severe hepatotoxicity. In this study low monthly income was another significant predictor of severe hepatotoxicity in the multivariate model. Mostly, this group of persons also present with low BMI at baseline and an increase BMI at D180. Increase in BMI results to accumulation of dietary fat in the liver that leads to liver injury. In addition, lower monthly income is associated with poor quality of life as patients with low monthly income might go in for traditional or herbal and substandard drugs during treatment (Oguntibeju, 2012).

There was sampling bias in terms of drug administration as the majority of the patients (64%) were placed on TDF+3TC+EFV and the least (14%) on AZT+3TC+NVP. Drugs were administered based on the patients CD4$^+$T cells count, weight, hemoglobin values, and the WHO clinical stage. The development of hepatotoxicity is common with all classes of ART (Ofotokun et al., 2007; Soriano et al., 2008; Price and Thio, 2010; Wambani et al., 2015; Wenderlein et al., 2016) and is of great concern as it necessitates cessation or modification of treatment. However, the risk varies depending on the HAART combination and other patient-related factors such as alcohol and/or cigarette consumption, CD4$^+$ T cells levels, BMI (Wambani et al., 2015). In this study, the prevalence of severe hepatotoxicity was highest among patients who took the NVP-based regimen. It has been reported that NVP causes elevated liver enzyme level by mitochondrial damage or hypersensitivity reaction (Chu et al., 2010; Tseng et al., 2014; Hamza et al., 2014; Hedrich et al., 2016; Wenderlein et al., 2016). From this data, it was also realized that those who took AZT+3TC+ NVP presented with elevated ALP. This confirms the fact that the use of NVP is also associated with cholestatic liver enzyme elevations.
Although gender showed a trend in univariate analysis, no association was found in the multivariate analysis. Results from this study were similar to those of Nagu et al., (2012), Bello et al., (2014), and Abongwa et al., (2017) and contradict those of Sulkowski et al., (2002), Chu et al., (2010), and Wondemagegn et al., (2013). The reason for this gender discrepancy is not clear given that the courses of HAART metabolism in humans are not sex-dependent (Wondemagegn et al., 2013). Thus, this high prevalence could be due to other social habits that are common in males than females. In this study, more males than females were found to consume alcohol (59.4% vs. 41.6%) and cigarettes (92.9% vs. 7.1%). These two factors increase liver function enzymes Park et al., 2013; Wondemagegn et al., 2013; Alsalhen and Abdalsalam, 2014). In addition, high alcohol consumption rate accounts for excess weight gain that leads to high BMI which has been associated with a greater risk of elevated transaminases (Tasneem et al., 2015). The high hepatotoxicity prevalence in male gender can also be due to hormonal effects on drug metabolism. These differences, however, warrant further study.

Alcohol consumption is a common practice in the adult populations and virtually constitutes a behavioral norm that cut across all boundaries of gender, race, age and economic strata (Priya and Venkatalakshmi, 2013). Alcohol consumption is one of the factors most frequently associated with elevated liver enzyme levels (Park et al., 2013; Wondemagegn et al., 2013). Alcohol use and abuse occur frequently in those with HIV/AIDS (Barve et al., 2010) and has been linked to poor response to HIV treatment, as well as the rapid progression of HIV disease (McCance-Katz et al., 2013). Thus, alcohol-HAART interactions are essential for the development of hepatotoxicity in HIV patients. Studies have shown that alcohol abuse is associated with severe hepatotoxicity in patients on HAART (Nunez 2006; Barve et al., 2010).
Although the prevalence of severe hepatotoxicity was higher in those who took alcohol, it’s intake was not a significant risk factor of severe hepatotoxicity in this study similar to results reported by Wambani et al., (2015). However, alcohol intake has previously been reported as an independent risk factor for hepatotoxicity in HIV infected persons (Barve et al., 2010; Hamza et al., 2014; Subramaniyan and Middha, 2016) and non-HIV patients (Priya and Venkatalakshmi, 2013). Alcohol and HAART-induced liver disease share similar mechanisms of hepatic injury. This includes altered metabolism cytokines, dysfunction of some cell components and mitochondria toxicity (Barve et al., 2010; Wondemagegn et al., 2013; Subramaniyan and Middha, 2016). This result is also apparent as more men and patients with overweight BMI presented with a higher prevalence of severe hepatotoxicity at D180. Thus it is most likely that alcohol consumption may lead to liver enzyme elevation (Suter, 2005; Adams et al., 2008). However, since the AST/ALT ratio at D180 was <2 it is highly suggestive that HAART is the etiology of the liver injury and not alcohol (Salaspuro, 1987; Nyblom et al., 2004) in this study. Nevertheless, this result should be interpreted with caution since measurements of blood alcohol was not done and there were inconsistencies regarding the definitions of alcohol consumption taking into account the participants took different types of alcohol (imported and locally produced).

Smoking is a common addiction of present times (Alsalhen and Abdalsalam, 2014; Kumar et al., 2015) and causes series of adverse effects on organs that have no direct contact with the smoke due to the over 4000 harmful toxic chemical substances (nitrogen oxide, nicotine, tar, hydrogen cyanide, carbon monoxide and free radicals such as superoxide, hydrogen peroxide, among others) that it produces (Abdul-Razaq and Ahmed, 2013; Kumar et al., 2015). Thus, smoking is one of the basic causes of
many diseases such as cardiovascular diseases, respiratory disorders, cancer, peptic ulcers, gastroesophageal reflux disease, male impotence and infertility, blindness, hearing loss, bone matrix loss, and hepatotoxicity (El-Zayadi, 2006; Abdul-Razaq and Ahmed, 2013; Alsalhen and Abdalsalam, 2014). In this study, an insignificant rise in serum ALT, AST and ALP activity was recorded in cigarette smokers when compared to nonsmokers. These results are consistent with known facts from other studies (Abdul-Razaq and Ahmed, 2013; Elameen and Abdrabo, 2013; Alsalhen and Abdalsalam, 2014; Kumar et al., 2015) while other studies contradict this effect (Jang et al., 2012; Dass et al., 2013).

Alteration in liver enzymes in smokers can either be direct or indirect immunological effects due to the toxic chemical substances produced by the cigarettes (El-Zayadi, 2006; Elameen and Abdrabo, 2013). These toxic chemical substances induce oxidative stress on hepatocytes that damage the biological cell membrane of the liver, thereby increasing the severity of hepatic lesions (Abdul-Razaq and Ahmed, 2013; Alsalhen and Abdalsalam, 2014; Kumar et al., 2015). In addition, nicotine produced during smoking has shown to increased the production of pro-inflammatory cytokines (IL-1, IL-6, TNF-α) that have been involved in liver cell injury (El-Zayadi, 2006; Liu, 2009; Wannamethee and Shaper, 2010; Abdul-Razaq and Ahmed, 2013; Elameen and Abdrabo, 2013). Moreover, smoking has shown to further affect the metabolic effects of the liver in detoxifying alcohol and medications (Wannamethee and Shaper, 2010; Jang et al., 2012; Elameen and Abdrabo, 2013; Hamza et al., 2014).

Evaluation of CD4+ T cells count level is a vital tool in the management of HIV-1 infection (Kang et al., 2012). Interestingly, in our study low CD4+ T cells count was a minor risk factor for liver toxicity since low CD4+ T cells count showed a trend with
hepatotoxicity only in the univariate analysis. Results from this data also revealed that the high CD4+ T cells count after D180 (470.57 cells/mm³) is associated with a higher risk of hepatotoxicity in the adjusted multivariate analysis though it was insignificant. This is similar to a report by Wambani et al., (2015) which states that an increase in CD4+ T cells count from baseline has a higher chance of hepatotoxicity in HIV and HBV/HCV co-infection. Since the use of HAART has shown to increase CD4+ T cells count and BMI, therefore, there is a high probability that increases in CD4+ T cells count will lead to elevated ALT and AST (Nagu et al., 2012). This result attests to the fact that the use of NVP-based regimen associated with high CD4+ T cells count is a risk factor to the development of severe hepatotoxicity (Hamza et al., 2014; Wambani et al., 2015).

5.1.3 Inflammatory markers associated with hepatotoxicity

While infection of hepatocyte cell lines is thought to be CD4+ T-cell independent, as most primary hepatocyte cell lines do not express CD4+ (Denue et al., 2013), evaluation of CD4+ T cells count level is a vital tool in the management of HIV-1 infection. With the progressive loss of CD4+ T cells count in HIV infection, the dysfunction in the T cells compartment is reflected by cytokine expression (Deng et al., 2015). The negative correlation between CD4+ T cells count and transaminase levels is probably due to the fact that a reduction in CD4+ lymphocytes results in an increased protein (for example lipopolysaccharide) turnover which acts as a pro-inflammatory and pro-fibrotic substance on the liver resulting to a rise in the liver enzymes (Shiferaw et al., 2016).

Th17 cells are a subset of CD4+ T-helper cells characterized by the production of cytokine IL-17 (Hammerich et al., 2010). These Th17 cells are highly susceptible to
HIV and thus, are depleted with the use of HAART (Ndhlovu et al., 2008). On the contrary, higher mean IL-17 was recorded at the end of the study. Studies in human liver disease (chronic HBV and HCV infections) indicate a close correlation between liver inflammation and activation of Th17 cells in relation to liver damage (Hammerich et al., 2010). It has been reported that IL-17 also up-regulates the production of TNF-α and IL-6, which stimulate hepatocytes signaling responses resulting in liver diseases and injuries (Galun and Axelrod, 2002; Cosgrove et al., 2009; Deng et al., 2015). In addition, the high level of IL-17 seems to be potentially disadvantageous for the patient in terms of antiviral defense and liver disease progression, since higher levels of IL-17 are associated with higher viral plasma load and increased levels of serum transaminases (Rowan et al., 2008; Hammerich et al., 2010). In addition, a review study by Njoku (2014) demonstrates that high levels of IL-17 have been detected in the sera from patients with idiosyncratic liver injury. Besides in a mice model, administration of recombinant IL-17 causes elevated plasma transaminases which lead to liver injury. This strongly indicates that IL-17 cells are involved in liver injury (Kobayashi et al., 2009). Thus, this result indicates that IL-17A is associated with hepatotoxicity.

Other studies have also demonstrated the importance of IL-6 during the course of liver infection (Neuman, 2003; Liu, 2009; Neuman et al., 2015). In this study a significantly high level of IL-6 was seen in hepatotoxicity patients compared to non-hepatotoxicity patients at D30 and D180 similar to a study by Neuman et al., (2015). In addition, decreased plasma levels of IL-6 at D30 and an increase by D180 can also be attributed to hepatotoxicity since the prevalence of hepatotoxicity was highest at D180. It has been reported that IL-6 plays an important role in inflammatory signaling
in liver regeneration following liver injury (Galun and Axelrod, 2002; Knight et al., 2005).

An insignificant increase in TNF-α values seen in participants with hepatotoxicity is similar to previous reports (Zhang and Wang, 2006; Liu, 2009; Neuman et al., 2015). This is most probably because TNF-α which regulates viral replication is involved in the induction of apoptosis which triggers the destruction of the liver and thus hepatic failure (Liu, 2009). Secondly, TNF-α also regulates the secretion of IL-6 which is involved in liver regeneration and repair (Neuman, 2003; Liu, 2009). Although this study did not demonstrate any significant changes in TNF-α levels between participants with and without hepatotoxicity, the high levels of TNF-α in people with hepatotoxicity is supportive of the fact that TNF-α may also induce hepatotoxicity since TNF-α is a prominent known cytokine player in liver injury and regeneration (Galun and Axelrod, 2002). Beside, TNF-α has induced liver injury in the rat model of alcoholic liver disease (Kawaratani et al., 2013). Further investigation is necessary to determine if TNF-α is expressed on hepatic cells considering that TNF-α is an inflammatory cytokine that triggers the production of IL-6 that has been shown to be significantly higher in patients with hepatotoxicity (Neuman, 2003; Hileman et al., 2015).

Although the difference was not significant, increased levels of IL-10 in patients with hepatotoxicity is indicative that IL-10 plays a major role in chronic liver disease as the liver is the main source of IL-10 production (Zhang and Wang, 2006; Kawaratani et al., 2013). Furthermore, the positive correlation observed between IL-10 and AST in patients with chronic hepatitis B (CHB) is a clear indication that IL-10 can also be secreted in patients with HAART-induced hepatotoxicity (Lian et al., 2014). As such,
future studies using a larger population with longer treatment duration needs to be
carried out to confirm these findings.

This study also reveals that IL-2 increases insignificantly in participants with
hepatotoxicity compared to those without hepatotoxicity at D30 and D180. During
treatment, viral replication is significantly inhibited (Meira et al., 2008; Tudela et al.,
2014) and thus subsequent reduction of IL-2. As such the high prevalence of
hepatotoxicity at D180 might contribute to the observed high level of IL-2. Hence,
there is a probability that IL-2 can affect hepatic cells implying that its detection
might be useful in the management of HAART-induced hepatotoxicity.

5.1.4 HIV-1 genetic diversity

Periodic updating of HIV-1 reference sequences is fundamental in improving
subtypes characterization in the context of effective epidemiological surveillance. The
extreme variability and the high evolution rate of HIV-1 favor the development of
ARV resistant HIV (Santoro and Perno, 2013; Saber et al., 2016). Thus, HIV-1
diversity is one of the greatest challenges among the many challenges in achieving an
effective and preventive vaccine development (Santoro and Perno, 2013; Saber et al.,
2016; Patino-Galindo et al., 2017). Data from this study showed a wide genetic
diverse population among HIV-1 group M and these include 4 pure subtypes and 5
CRFs despite the low HIV prevalence (3.4%) in Cameroon
(www.cameroononline.org). Similar distributions of HIV-1 subtypes have also been
described in other towns such as Douala, Yaounde and Bertoua in Cameroon
(Ragupathy et al., 2011; Ceccarelli et al., 2012; Agyingi et al., 2014; Courtney et al.,
2016; Teto et al., 2017) and other African countries like Nigeria (Chaplin et al., 2011)
and Kenya (Onywera et al., 2017). This broad genetic diversity is most probably due
to the fact that this region is located around the central Cameroon, where almost all HIV groups and subtypes have been found (Ndembi et al., 2008; Courtney et al., 2016).

The recombinant CRF02_AG which predominates (75.3%) in this study falls within the 48.6-80% range of previous studies conducted in Cameroon and other countries in West Africa, Central Africa and Europe (Powell et al., 2010; Ragupathy et al., 2011; Veras et al., 2011; Ceccarelli et al., 2012; Negedu-Momoh et al., 2014; Teto et al., 2017). The high prevalence of CRF02_AG suggests that this viral strain may be well adapted in the Cameroonian population due to a founder effect from the parent strains subtype A and G, or may have some biological advantages such as a higher replicative fitness relative to parental subtype A and G and modification of tropism over other co-circulating recombinants strains in our country (Ragupathy et al., 2011; Veras et al., 2011; Agyingi et al., 2014). Thus, the high prevalence of CRF02_AG species is suggestive that CRF02_AG will certainly aid in the design of an effective vaccine.

Compared with other studies from different towns in Cameroon, the prevalent subtypes after CRF02_AG were F2, D, and G contrary to the G, F2, and D (Ndembi et al., 2008) and D, F2, G subtypes (Courtney et al., 2016). The difference in prevalence may be explained by the difference in the HIV-1 epidemic in different geographical regions and the migration of populations. Three complex strains; CRF06_cpx, CRF09_cpx and CRF11_cpx were also found in this study. This complex mosaic virus has also been found in other continents like Europe and Australia and likewise in some West African countries such as Ivory Coast, Niger, Mali and Senegal (Peeters and Sharp, 2000; Negedu-Momoh et al., 2014).
In this study, HIV-1 subtypes that were closely related were also identified. Similar cluster phenomenon has been observed in previous studies carried out elsewhere (Smith et al., 2009; Weng et al., 2014; Parczewski et al., 2015; Patino-Galindo et al., 2017). These clusters suggested that these patients might have been infected recently with the HIV-1 virus. It is worth noting that Cameroon, as well as other countries, is currently implementing the test and treat policy where all patients tested positive for HIV are placed on treatment irrespective of their CD4+ T cells count or WHO clinical stage. However, it could also be possible that the transmission of related viral sequences may correlate with shared social or risk-behaviour patterns such as scarification, intravenous drug use or HIV transmission between men having sex with men among others (Weng et al., 2014; Parczewski et al., 2015; Patino-Galindo et al., 2017). Several studies have shown that resistance mutations with minimal impact on viral fitness may be transmitted serially (Weng et al., 2014; Steegen et al., 2016).

While having clinical implications, the potential benefit in identifying these clusters in public health requires further investigation to ascertain this fact. This will alleviate ongoing transmission originating from recently infected individuals or tracing back the potential index case (Smith et al., 2009; Weng et al., 2014). Identifying HIV clusters are important in helping public health officials to identify areas where interventions might be useful in preventing further spread of the infection. Thus, the presence of clustering is an important contribution to the spread of the resistant virus and requires further investigation with a wider population.

This study was the first to assess the impact of HIV subtypes on hepatotoxicity. HIV subtypes alongside other risk factors associated with hepatotoxicity can help curb the incidence of hepatotoxicity in our settings. The result of this study revealed that hepatotoxicity is more common in patients with CRF02_AG. This high prevalence
may be due to sampling bias in favor of CRF02_AG since CRF02_AG accounted for more than half of the total HIV-1 subtypes. In addition, categorizing the data as CRF02_AG and non-CRF02_AG showed that hepatotoxicity was insignificantly higher in the non-CRF02_AG subtypes. As such it is required that such studies be carried with equal HIV-1 subtype populations.

5.1.5 Transmitted drug resistance

Cameroon like other poor resource-limited countries is moving towards reaching the 90-90-90 universal access to HIV prevention, care, and treatment for those in need and at high risk of infection (Konou et al., 2015; Sidibe et al., 2016). This has led to the widespread use of ART in Cameroon and other countries (Billong, et al., 2016; Teto et al., 2017).

HIV isolates containing resistance mutations in drug-naïve individuals have been previously described in Cameroon. Our study revealed a moderate level (11.1%) of TDR as classified by WHO (Steegen et al., 2016). This prevalence falls within the global TDR estimates range of 8–44% in developing countries (Ragupathy et al., 2011; Xiaobai et al., 2014; Ferreira et al., 2017; Onywer a et al., 2017). In Cameroon, this value is higher compared to the 7.3-10.8% range (Ndembi et al., 2008; Teto et al., 2017; Mbunkah et al., 2018; Tchouwa et al., 2018) and lower than the 13.9- 24% range (Burda et al., 2010; Agyingi et al., 2014; Zoufaly et al., 2014) as documented in previous studies. The moderate prevalence can be attributed to poor drug adherence, loss of follow-up, pharmacy stock-outs, lack of proper patient retention in care and the use of ARV drugs to prevent mother to child transmission or as pre and post-exposure prophylaxis (Billong et al., 2013; Fokam et al., 2015; Parczewski et al., 2015; Steegen et al., 2016; Sallam et al., 2017; Teto et al., 2017). The use of HAART
in HIV prevention has led to a high rate of acquired drug resistance and possible onward transmission of the resistant strains to newly infected individuals. In addition, it can also be associated with mutations that occur within the HIV genome (Onywera et al., 2017; Teto et al., 2017). This moderate level of TDR in naïve patients suggests that drug resistance viruses are transmitted in this region and thus, might create a major threat to the success of HIV treatment programs by limiting the selection of effective first-line therapeutic options. In addition, it may further decrease the efficacy of pre-exposure prophylaxis (PrEP) and post-exposure prophylaxis (PEP) as well as facilitate the onward transmission of DRM in the viral population. This, therefore, highlights the need for drug resistance testing before drug initiation in HIV-1 infected patients.

The overall prevalence of mutations associated with NRTIs, NNRTIs, and PIs was 8.6%, 4.9%, and 1.2% respectively. This result is contrary to results from other towns of Cameroon and elsewhere which state that resistance is more frequent with NNRTI mutations (De Luca et al., 2017; Fokam et al., 2018; Tchouwa et al., 2018) but similar to studies carried out by Vergne et al., (2006) and Mbunkah et al., (2018) in Cameroon. However, the high prevalence of NRTIs seen in this study could be associated with the shift from monotherapy NNRTI to HAART for all pregnant and breastfeeding women living with HIV-1 regardless of CD4+ T cells count or WHO clinical stage (Abdissa et al., 2014; MPH, 2015; Machnowska et al., 2017). In addition, it might be due to the fact that NRTI has been in use for quite a long time (Vergne et al., 2006; MPH, 2015).

The most common NRTIs DRM recorded was K219Q and this confers resistance to AZT and D4T (Chaplin et al., 2011; Wensing et al., 2017). Previous studies in
Cameroon and elsewhere have recorded M184V as the most prevalent mutation (Burda et al., 2010; Negedu-Momoh et al., 2014; Zoufaly et al., 2014; Teto et al., 2017; Wensing et al., 2017). In drug-experienced patients, K219Q mutation usually develops in patients receiving incompletely suppressive AZT, and D4T regimens (Elmi Abar et al., 2012; Teto et al., 2017; Wensing et al., 2017) and this will lead to onward transmission. As such the high prevalence of this RAM is most probably because of the use of AZT and D4T since 2002 in our setting.

In addition, a prevalence of 6.2% (5/81) patients with 5 of the 6 known TAMs (M41ML, D67N, K70T/R, T215TA/F, and K219Q) was recorded in this study. The high prevalence of TAMs among drug naïve patients may be explained by the extensive use of thymidine analogs (AZT and D4T) since 2002 (WHO, 2002). TAMs induce resistance by excision of the drug incorporated into viral DNA (Wensing et al., 2017). This recorded high prevalence of TAMs, is a public health concern as this might lead to cross-resistance between all NRTI analogs (Elmi Abar et al., 2012; Negedu-Momoh et al., 2014; Teto et al., 2017; Wensing et al., 2017). However, the level of resistance observed depends on the types and number of TAMs in the infected individual.

The 4.9% prevalence of NNRTIs was lower than the >10% prevalence in a survey carried out in Africa (De Luca et al., 2017). Four (K103N, V108I, V179E, Y181C) of the 5 NNRTI resistance-associated mutations confer resistance to EFV and NVP by reducing the binding affinity of these drugs to the viral target (Chaplin et al., 2011; De Luca et al., 2017; Wensing et al., 2017). It is worth mentioning that these drugs are the only two drugs currently being used in Cameroon (MPH, 2015). The data from this study showed that E138A (2.5%; 2/81) was the most common NNRTI mutation.
Similar results have recently been reported in Cameroon (Tchouwa et al., 2018) and elsewhere (Kleyn et al., 2014; Parczewski et al., 2015; Theys et al., 2016). E138A is a polymorphic mutation which usually occurs in <5% of ARV-naive patients (Theys et al., 2016; Tchouwa et al., 2018). E138A mutation is weakly selected in patients receiving second-generation NNRTIs (ETR and RPV) which are drugs not being used in Cameroon and other countries in Sub Saharan Africa (Kleyn et al., 2014; MPH, 2015; Tchouwa et al., 2018). However, this high prevalence of E138A may limit the use of RPV or ETR-based ART in patients who develop resistance to first-generation NNRTIs (Kleyn et al., 2014; Wensing et al., 2017). In other words, the increasing frequency of E138A mutation makes the use of RPV or ETR not appropriate as salvage therapy in Cameroon considering that 4 of the 5 RAMs cause resistance to EFV and NVP.

In this study, a low prevalence of PI mutations recorded is similar with reports from other countries in Central and West Africa (Ndembi et al., 2008; MPH, 2015, National AIDS control committee, 2018). It, however, contradicts the high prevalence in developed countries, where PI regimens are readily available (Ndembi et al., 2008). In comparison to previous studies carried out in other regions of Cameroon, this prevalence is lower than the 1.3-2.9% range (Vergne et al., 2006; Ndembi et al., 2008; Agyingi et al., 2014; Mbunkah et al., 2018) and higher than the 0.2-1.04% recorded (Vessiere et al., 2006; Wittkop et al., 2011) The low prevalence of PI mutations seen in this study reflects the limited use of PIs in Cameroon as only 4.3% of infected persons are on second and third line treat (National AIDS control committee, 2018). Secondly, studies have shown that PI mutations do not exist as natural polymorphisms (that is, have a high genetic barrier to resistance) and this class
of ARVs is not easily transmitted as compared to NNRTIs and NRTIs (Ndembi et al., 2008; Parczewski et al., 2015; Onywera et al., 2017).

The presence of dual-class resistance against NRTIs and NNRTIs (3.7%; 3/81), confer resistance to most of the ARVs recommended as first-line therapy in most countries including Cameroon (Elmi Abar et al., 2012; Agyingi et al., 2014; MPH, 2015; Wensing et al., 2017). This value is lower than 3.2% reported by Agyingi et al., (2014) and higher than the 9.5% prevalence reported by Burda et al., (2010) in studies carried out in other regions in Cameroon. This multi-class resistance will lead to increased risk of treatment failure to the first-line drugs that are currently being used in our setting (Wensing et al., 2017). Since their contribution to virological failure could be assessed, all patients with dual-class resistance were switched to second-line treatment.

Overall, 10 major mutations which could confer resistance to NRTIs, 5 major mutations to NNRTIs and one major mutation associated with PIs (Table 4.8) were identified. The mutation patterns observed in this study were similar to those obtained in other studies with most patients (6/9) presenting with singleton mutations (Ndembi et al., 2008; Agyingi et al., 2014; Andrade et al., 2017). The antiretroviral drug susceptibility with mutation net drug score showed five phenotypes, namely; high, intermediate, low, potential low-level resistance or susceptible.

Majority of the resistance associated mutations (RAM) were associated with potential low-level resistance followed by low-level resistance. In this study, most of the TDR mutations were associated with predicted resistance level to DDI followed by D4T. This accounts for the reason why these drugs are no longer recommended for use in our setting (http://hivdb.stanford.edu; MPH, 2015). The likely explanation for the
high spread of D4T and DDI mutants is due to the suboptimal use of D4T and DDI based therapy at the beginning of ART scale up.

Three of the 9 patients with TDR were switched to second-line treatment, indicating that the current first-line protocol used in Cameroon is still effective. These patients were found to harbor multi-drug class (NRTI and NNRTI) mutations. In addition, the high prevalence of 77.8% (7/9) of hepatotoxicity seen in patients with RAM, therefore, indicates that the current treatment guideline for first-line treatment is still effective.

Although the prevalence of TDR is moderate (5-15%) in this study, the use of ARV in the prevention of mother to child transmission (PMTCT) and the prevention of new HIV-1 infections depicts a public health problem. As such, the threat of drug resistance deserves careful attention from clinicians and public health officials advocating for ARV use to control HIV epidemic.

Since 2012, most countries including Cameroon have been implementing the WHO’s PMTCT antiretroviral guidelines which recommend the initiation of lifelong combination ART for all pregnant and breastfeeding women living with HIV-1 regardless of CD4+ T cells count or WHO clinical stage (MPH, 2015; Machnowska et al., 2017). This has improved maternal health by reducing the morbidity and mortality of HIV-1 positive women and has also reduced the rate of MTCT to about <2% (Caceres et al., 2015a; Machnowska et al., 2017; Ruperez et al., 2018). However, the use of ARV for the PMTCT can also lead to increased risk of the emergence of HIV-1 drug resistance due to suboptimal adherence, poor retention to care, inadequate monitoring and loss of follow-up seen among these women after the lactation period (Billong et al., 2016; Andrade et al., 2017; Machnowska et al., 2017). In line with
this, the mutations identified in this study could confer possible resistance to pediatric HAART options (AZT, 3TC, ABC/ EFV, NVP /LPV/r). Therefore, policies need to be put in place to prevent the spread of the drug-resistant virus in order to minimize the rate of treatment failure and mortality.

The use of TDF plus FTC as PrEP/PEP has been demonstrated to be efficacious for the prevention of HIV acquisition (Caceres et al., 2015a; Andrade et al., 2017; Dimitrov et al., 2018). However there is a high probability that PrEP/PEP does not eliminate HIV risk completely and this might lead to the development of DRM (Parikh and Mellors, 2016; Dimitrov et al., 2018; Ruperez et al., 2018). Resistance associated with PrEP/PEP usually occurs when the person concerned acquires HIV during implementation since PrEP/PEP were not designed for treatment (Caceres et al., 2015b; Ruperez et al., 2018). Development of resistance to PrEP/PEP can potentially limit the use of first-line treatment. Transmission of HIV during implementation can also occur when PrEP/PEP is administered during the acute HIV syndrome phase (window period) or as a result of poor drug adherence (Caceres et al., 2015b). As such it is of prime importance to build delivery systems that will reliably check the HIV status within a shorter window period, and a thorough assessment of acute seroconversion symptoms, for those eligible for PrEP/PEP. This can be attained by the use of rapid HIV antibody test followed by an HIV RNA test to identify HIV infection in the window phase. Taking into consideration that some of the mutations seen in this study can also affect the current PrEP/PEP protocol (MPH, 2015), the use of these drugs on a larger scale will decrease the effectiveness of these regimens for both prevention and treatment. As such DRM genotyping is necessary before drug initiation.
5.1.6 Study limitations

The present study is limited by a number of factors.

i. The short period of follow up only provides data on possible hepatotoxicity within the six months of treatment. In addition, the protocol did not allow us to establish the treatment type balance ratio at recruitment. Likewise, information on the number of non-HAART regimens used by participants could not be determined.

ii. No information on the possible means of HIV transmission was obtained during the recruitment of participants.

iii. The HIV-RNA viral load could not be determined at baseline and D180 after treatment.

iv. Lastly, patients with TDR were not followed up to assess the outcome of their response to treatment to determine if the resistance mutations present will be observed later in the course of treatment.

5.2 Conclusions

i. Mean ALT, AST, and ALP increased significantly with an increase in treatment duration and with the highest level seen among patients who took AZT+3TC+NVP.

ii. The findings indicate a prevalence of hepatotoxicity to be 37(37%) and 49 (49%) with 15% and 28% patients presenting with severe hepatotoxicity at D30 and D180 respectively. This study revealed significant findings that age group < 30years, low baseline BMI, low monthly income and use of AZT+3TC+ NVP regimens are independent risk factors for developing severe hepatotoxicity.
iii. Mean IL-6 and IL-17A play a significant role in the pathophysiology of hepatotoxicity.

iv. This study confirmed previous findings that CRF02_AG subtype is still the most predominant subtype and patients harboring CRF02_AG subtype recorded the highest prevalence of hepatotoxicity.

v. The study revealed moderate levels (11.1%) of transmitted drug resistance mutation. Of this, 77.9% (7/9) patients presented with hepatotoxicity.

5.3 Recommendations

i. Given the relatively high prevalence of HAART-related hepatotoxicity, frequent monitoring of transaminases is important during HAART follow-up.

ii. The use of NVP based regimen should not be recommended for patients <30 years and with low baseline BMI 18 kg/m².

iii. We suggest that IL-6 and IL-17 measurement should be incorporated as a valuable tool during HAART initiation and follow up.

iv. Continuous assessment is needed to monitor the evolving HIV diverse strains and its potential impact of drug resistance on ART programs.

v. There is a need for routine HIVDR surveillance in Cameroon.

5.3.1 Areas for further research

i. Further research on hepatotoxicity should be carried out using another set of cytokines.

ii. Looking at the closeness of some species further investigations are needed to elucidate the presence of these clusters by using full genome sequencing.

iii. The presence of dual class mutations accentuates the need to study the evolution of mutation patterns in the context of newer antiretroviral regimens.
REFERENCES


and slow progressors show increased IL-6 and IL-10 levels in the pre-AIDS stage of HIV infection. *PloS One*, 11(15): e0156163


and Th17/Treg ratio at early HIV infection associate with protective HIV-specific CD8+ T-cell responses and disease progression. *Scientific reports*, **5**: 11511


https://www.cameroononline.org/cameroon-hiv-aids-prevalence-at-3-4-percent-survey. Accessed on 28th August 2018


http://www.hiv.lanl.gov/content/sequence/RIP/RIP.html. Accessed on 19th June 2017


Kantor, R., Katzenstein, D.A., Efron, B., Carvalho, A.P., Wynhoven, B., Cane, P., Clarke, J., Sunee, S.I., Marcelo, A.S., Snoeck, J., Pillay, C., Rudich, H., Rosangela,


idiosyncratic hepatotoxicity is driven by mitogen-activated protein kinases. *Toxicological Sciences*, **146**(2): 265-280


### APPENDICES

**Appendix I: First and second lines HIV treatments currently recommended in Cameroon**

*(Ministry of Public Health, 2015)*

<table>
<thead>
<tr>
<th>First-line regimen</th>
<th>Zidovudine + Lamivudine + Efavirenz</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2NRTI+1NNRTI)</td>
<td>Zidovudine + Lamivudine + Nevirapine</td>
</tr>
<tr>
<td></td>
<td>Tenofovir + lamivudine + Efavirenz</td>
</tr>
<tr>
<td></td>
<td>Tenofovir + Emtricitabine + Efavirenz</td>
</tr>
<tr>
<td>Special cases</td>
<td>Abacavir + Lamivudine + Nevirapine</td>
</tr>
<tr>
<td></td>
<td>Abacavir + Lamivudine + Efavirenz</td>
</tr>
<tr>
<td></td>
<td>Zidovudine/Abacavir + Lamivudine + Lopinavir/r or Atazanavir/r</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Second-line regimen</th>
<th>Zidovudine + Lamivudine + Atazanavir/r*</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2NRTI+1PI)</td>
<td>Zidovudine + Lamivudine + Lopinavir/r</td>
</tr>
<tr>
<td></td>
<td>Tenofovir + Lamivudine + Atazanavir/r*</td>
</tr>
<tr>
<td></td>
<td>Tenofovir + Lamivudine + Lopinavir/r</td>
</tr>
<tr>
<td></td>
<td>Abacavir + Didanosine + Lopinavir/r</td>
</tr>
</tbody>
</table>

*Atazanavir is the preferred choice because it is easy to take (one tablet), better digestive tolerance and better preservation of 2nd generation PI.
### Appendix II: List of amino acids

<table>
<thead>
<tr>
<th>3 Letter Code</th>
<th>Amino Acid Name</th>
<th>1 Letter Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>Alanine</td>
<td>A</td>
</tr>
<tr>
<td>Arg</td>
<td>Arginine</td>
<td>R</td>
</tr>
<tr>
<td>Asn</td>
<td>Asparagine</td>
<td>N</td>
</tr>
<tr>
<td>Asp</td>
<td>Aspartic acid</td>
<td>D</td>
</tr>
<tr>
<td>Cys</td>
<td>Cysteine</td>
<td>C</td>
</tr>
<tr>
<td>Glu</td>
<td>Glutamic acid</td>
<td>E</td>
</tr>
<tr>
<td>Gln</td>
<td>Glutamine</td>
<td>Q</td>
</tr>
<tr>
<td>Gly</td>
<td>Glycine</td>
<td>G</td>
</tr>
<tr>
<td>His</td>
<td>Histidine</td>
<td>H</td>
</tr>
<tr>
<td>Ile</td>
<td>Isoleucine</td>
<td>I</td>
</tr>
<tr>
<td>Leu</td>
<td>Leucine</td>
<td>L</td>
</tr>
<tr>
<td>Lys</td>
<td>Lysine</td>
<td>K</td>
</tr>
<tr>
<td>Met</td>
<td>Methionine</td>
<td>M</td>
</tr>
<tr>
<td>Phe</td>
<td>Phenylalanine</td>
<td>F</td>
</tr>
<tr>
<td>Pro</td>
<td>Proline</td>
<td>P</td>
</tr>
<tr>
<td>Ser</td>
<td>Serine</td>
<td>S</td>
</tr>
<tr>
<td>Thr</td>
<td>Threonine</td>
<td>T</td>
</tr>
<tr>
<td>Trp</td>
<td>Tryptophane</td>
<td>W</td>
</tr>
<tr>
<td>Tyr</td>
<td>Tyrosine</td>
<td>Y</td>
</tr>
<tr>
<td>Val</td>
<td>Valine</td>
<td>V</td>
</tr>
</tbody>
</table>
Appendix III: Ethical clearance

COMITE NATIONAL D’ETHIQUE DE LA RECHERCHE POUR LA SANTE HUMAINE

Arrêté N° 6977/A/MINSANTE/SES/SP/DROS/ du 18 avril 2012 portant création, organisation et fonctionnement des comités d’éthique de la recherche pour la santé humaine au sein des structures relevant du Ministère en charge de la santé publique

N° 2016/01-CE/CNERSH/SP

Yaoundé, le 05 janvier 2016

Cnethique_minsante@yahoo.fr

CLAIRANCE ETHIQUE

Le Comité National d’Ethique de la Recherche pour la Santé Humaine (CNERSH), en sa session ordinaire du 17 décembre 2015, a examiné le projet de recherche intitulé : « Evaluation of HIV-1 drug resistance, genetic diversity and cytokine profiles on hepatotoxicity among treated HIV patients in the Northwest Region of Cameroon » soumis par Mme LEM Edith ABONGWA, Investigateur Principal, étudiante à l’Université de Bamenda.

Le projet est d’un grand intérêt scientifique et social. L’objectif de cette étude est d’évaluer la résistance aux médicaments pour le VIH-1, la diversité génétique et le profil des cytokines sur l’hépatotoxicité chez les patients HIV positifs dans la région du nord-ouest du Cameroun. La procédure de l’étude est bien documentée et claire. Les risques liés au prélèvement de sang sont précisés ainsi que les mesures pour les éviter et les minimiser. La notice d’information et les différents formulaires de consentement, en français et en anglais, sont bien élaborés et simples à comprendre. Les mesures prises pour garantir la confidentialité des données collectées sont présentes dans le document. Les CVs des Investigateurs les décrit comme des personnes compétentes, capables de mener à bien cette étude. Pour toutes ces raisons, le Comité National d’Ethique approuve pour une durée d’un an, la mise en œuvre de la présente version du protocole.

Les Investigateurs sont responsables du respect scrupuleux du protocole approuvé et ne devraient y apporter aucun amendement aussi mineur soit-il, sans avis favorable du CNERSH. Les investigateurs sont appelés à collaborer pour toute descente du CNERSH pour le suivi de la mise en œuvre du protocole approuvé. Le rapport final du projet devra être soumis au CNERSH et aux autorités sanitaires du Cameroun.

La présente clairance peut être retirée en cas de non respect de la réglementation en vigueur et des recommandations susmentionnées.

En foy de quoi, la présente clairance éthique est délivrée pour servir de base ce que de droit.

Améliorations

- MINSANTE

N.B : cette clairance éthique ne vous dispense pas de l’autorisation administrative de recherche (AAR), exigée pour mener cette étude sur le territoire camerounais. Cette dernière vous sera délivrée par le Ministère de la Santé Publique.
Appendix IV: Information sheet (in English)

Title: Evaluation of genetic diversity, drug resistance mutational and cytokine patterns on hepatotoxicity markers among HIV patients in Northwest region Cameroon

Investigator: Lem Edith Abongwa, Ph.D. student

Introduction: This consent form explains the research study you are being asked to voluntarily participate. Please listen carefully and ask any question about the study before you agree to take part. You may ask questions at any time after joining the study.

We are doing a voluntary research study to know whether when a patient is on HAART that is taking medication to treat the HIV, the drug can cause some damage at the level of the liver, kidney and other organ particles like mitochondria. We will be testing your blood for these effects and we are doing this study because it is not known whether patients taking the medication will have these effects mentioned in our Cameroon population since we are different from other population in some countries where this test has also been done before.

The procedure of research project: If you agree to join this study, we will ask you information about your background and the type of antiretroviral that you are taking. It will take about 3-5 minutes to ask you these questions. You can skip any question that you do not want to answer. You can also stop answering the questions at any time.

We will also take about 8ml (about 1.5 teaspoons) of blood from you. Blood will be taken directly from your arm and sent to the laboratory within 24 hours. The blood will be analyzed for ARV toxic effects, genetic diversity, drug resistance, and cytokine profiles. Blood samples will be collected at initiation before you start taking ARV and also at one and six months following initiation to therapy.

Risks /Discomforts: if you join this study, there are some things that may cause you discomfort. You may feel a slight sting or pinch in your arm when the blood is collected. You may also get a small bruise where the needle went in. Some people may faint, but this is rare.
Benefit from this study: there is no direct benefit in financial reward however by participating in this study you will support research on the effect of taking this medication on the life of the patient, patient care and follow-up. You are also going to contribute to scientific knowledge for the promotion of health. Nevertheless, we may contact you and your health care provider if the research results carried out prove to be useful for your immediate medical follow-up.

Confidentiality: the fact learned in this study will be kept private to the extent allowed by law. Your answers and blood samples will only be labeled with the study code number. Any results from this study that are important to science will be published in magazines and only reported in general terms.

Voluntariness: your joining this research study is completely voluntary. You have the right to withdraw from the study at any time. You have the right to refuse any more blood test and to refuse to answer any more questions at any time.

Persons to Contact: if you have any questions concerning this study you may ask the following:

1. Research Student: Lem Edith Abongwa at 677951677, or email her at lemedith19@gmail.com
2. Research Director: Prof. Fokunang Charles at 670902446 or email him at charlesfokunang@yahoo.co.uk
3. National Ethics Committee for human health research at 243674339, or email at cnethique_minsante@yahoo.fr
Appendix V: Information sheet (in French)

Fiche de renseignements

Titre: Évaluation de la diversité génétique, la résistance aux médicaments contre le VIH-1 et le profil des cytokines, et les effect sur l'hépatotoxicité chez les patients VIH dans la région Nord-Ouest Cameroun.

Chercheur principal: Lem Edith Abongwa; étudiante en doctorat/PhD,

Introduction: Chers participants, dans le cadre de mes activités de recherches en doctoriales, il sera également question de savoir si la prise de médicament pour le VIH, en particularité la thérapie HAART, peut entraîner des dommages au niveau des organes comme le foie et les reins. Bien que des études de ce genre ont été réalisées dans d'autres pays, il sera important de le faire au Cameroun étant donné des différents genetiques et metaboliques qui peuvent avoir d’un pays a l’autre ou dans les groupes de la population.

Procédure De Projet De Recherche: Si vous acceptez d'adhérer à cette étude, nous vous demanderons des informations sur vos antécédents et le type d'antirétroviraux (ARV) que vous prenez. L'entretien pourra prendre environ 3-5 minutes et la reponse a une question est absolument volontaire a votre aise Vous pouvez également arrêter de répondre aux questions à tout moment.

Nous allons également prendre environ 8 ml (environ 1,5 cuilleres à café) de votre sang. Ce sang sera pris directement à partir de votre bras et envoyé au laboratoire dans les 24 heures. Le sang sera analysé pour des marqueurs de toxicite des ARV, résistance aux médicaments pour le VIH-1, la diversité génétique et le profil des cytokines. Des échantillons de sang seront prélevés au début de l'enquête (avant le debut de la prise des ARV), un mois après et au sixième mois après l'initiation de la thérapie.

Risques /malaises: Si vous participez à cette étude, il y a certains inconforts comme sentir un léger picotement ou pincer dans votre bras lorsque le sang y est prélevé. Vous pouvez également obtenir une petite contusion a l’endroit où l’aiguille est passéé. Certaines personnes peuvent s'évanouir, mais ceci est rare.
Bénéfices de cette étude: Il n'y a pas de bénéfice direct sous forme de récompense financière. Votre participation aidera à mieux comprendre la maladie étudiée pour l'élaboration de meilleures stratégies thérapeutiques pour améliorer les soins aux patients et leur suivi. Vous allez également contribuer à la connaissance scientifique et la promotion de la santé. Néanmoins, nous pouvons vous contacter si les résultats de la recherche effectuée s'avéreraient utile pour votre suivi médical immédiat.

La Confidentialité: Toute information dans cette étude sera maintenue privée conformément à la réglementation en vigueur. Vos réponses et vos échantillons de sang seront étiquetés uniquement avec le numéro de code de l'étude. Les résultats de cette étude qui sont importantes à la science seront publiés dans des revues scientifiques et en des termes anonymés.

Volontariat: votre adhésion à cette étude est entièrement volontaire. Vous avez le droit de vous retirer de l'étude à tout moment. Vous avez le droit de refuser tout test sanguin et reponse aux questions supplémentaires à tout moment.

Les personnes à contacter: si vous avez des questions concernant cette étude, vous pouvez demander les personnes suivantes:

1. Étudiante porteuse de la recherche: Lem Edith Abongwa à 677951677, e-mail: lemedith19@gmail.com
2. Directeur de la recherche: le Prof. Charles Fokunang à 670902446, e-mail: charlesfokunang@yahoo.co.uk
3. Comité national d'éthique de la research pour la santé humaine at 243 674 339, or email: cnethique_minsante@yahoo.fr
Appendix VI: Informed consent form (in English)

I (name) have been invited to participate in the study entitled; “Evaluation of genetic diversity, drug resistance mutational and cytokine patterns on hepatotoxicity markers among HIV patients in Northwest region Cameroon” to be carried out by LEM EDITH ABONGWA a PhD student in Kenyatta University, teaching in the University of Bamenda with Pr. Fokunang Charles and Dr. kebira Anthony as her research directors.

- I have read (it has been read to me) and understood the above the research information;
- I understood the objectives of the study;
- I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction;
- I understood the risks and the benefits in partaking in this study;
- I understand that I have the right to refuse to take part in the research activities at any time

I consent voluntarily to participate as a participant in this research and I will

- Answer all the questions to the best of my knowledge;
- Keep to the medical advice with respect to this study;
- Will give 8ml of blood as required three times;

I, patient with code number …………………………… accept to participate in this study for the period that need be.

Signature of subject……………………………………..Place……………….. Date …………………

Signature of investigator……………………………….. Place……………….. Date …………………
Appendix VII: Informed consent form (in French)

**Formulaire de consentement éclairé**

Je soussigné(e) --------------------------------- atteste avoir été invité(e) à participer à l'étude intitulée, Évaluation de la diversité génétique, la résistance aux médicaments contre le VIH-1 et le profil des cytokines, et les effect sur l'hépatotoxicité chez les patients VIH dans la région Nord-Ouest Cameroun faite par LEM EDITH ABONGWA, étudiante en Doctorat/PhD à ‘Kenyatta University’ sous l’encadrement de Pr. Fokunang Charles et Dr. Kebira Anthony. Elle est aussi une enseignante à l'Université de Bamenda.

• J'ai lu (il a été lu pour moi) et compris l'information de la recherche ci-dessus;
• J'ai bien compris les objectifs de l'étude;
• J'ai eu l'occasion de poser des questions à ce sujet et des questions que j'ai posées ont été répondues à ma satisfaction;
• J'ai bien compris les risques et les avantages de participation à cette étude;
• Je comprends que j'ai le droit de refuser de prendre part aux activités de recherche à tout moment;

Je consens volontairement à participer en tant que participant à cette recherche et je vais

➤ répondre à toutes les questions au meilleur de mes connaissances;
➤ tenir à l'avis médical à l'égard de cette étude;
➤ vais donner 8ml du sang trois fois comme exigé.

Moi, le patient avec le numéro de code ................................. accepte de participer à cette étude pour la période qu'il faudra.

Signature du participant........................................... Lieu ......................... Date ........

Signature de l’investigateur................................. Lieu ......................... Date........
Appendix VIII: Questionnaire

**TITLE:** Evaluation of genetic diversity, drug resistance mutational and cytokine patterns on hepatotoxicity markers among HIV patients in the Northwest region Cameroon

**Investigator:** Lem Edith Abongwa; Ph.D. student

Patients number................................. Sex, Male ☐ female ☐ If Female

Are you pregnant? Yes ☐ No ☐ If yes Parity-------- Gestation age--------

Age ................................. Height ................................. Weight .................................

Level of education, None ☐ Primary ☐ Secondary ☐ Tertiary ☐

Monthly income, <50,000frs ☐ 50,000-100,000frs ☐ 100,001-150,000frs ☐ 150000frs ☐

Do you drink alcohol (wine, beer, etc) Yes ☐ No ☐

If yes, how many bottles do you drink a day per week? ≤1 ☐ 1-7 ☐ > 7 ☐

Have you ever smoke Cigarette or Tobacco? Yes ☐ No ☐ If yes ☐

How many sticks do you take a day? Not smoking presently ☐ 1-20 ☐ >20 ☐

Have you done any of the following tests?

1. Hepatitis B: Yes ☐ No ☐ If yes, the year the test was done.............
   Result of test: Positive ☐ Negative ☐ I do not know ☐

2. Hepatitis C: Yes ☐ No ☐ If yes, the year the test was done.............
   Result: Positive ☐ Negative ☐ I do not know ☐
If negative, have you ever been vaccinated for Hepatitis B or C?  Yes  □  No  □

In which year were you diagnosed positive for HIV?  < 1 year  □  1-3 years  □  > 4 years  □

Have you ever taken ARV Before?  Yes  □  No  □  if yes, which type?

Herbal  □  Synthetic drug  □  Year of initiation………………

Name of ARV taken. ………………………………………………………………………. I do not know  □

Do you know the value of the following tests at the time you were diagnosed positive for HIV? If yes (ask to see)

  CD4⁺ T cells count:  Yes  □  No  □  If yes (value)………………

  WBC:  Yes  □  No  □  If yes (value)………………

  ALT:  Yes  □  No  □  If yes (value)………………

  AST:  Yes  □  No  □  If yes (value)………………

  Serum creatinine:  Yes  □  No  □  If yes (value)………………

  Viral load:  Yes  □  No  □  If yes (value)………………

Are you currently on any drug?  Yes  □  No  □  If yes tick all that is applicable

  TB drugs  □  Paracetamol  □

Orders (Specify for example herbal drugs) ______________________________________

WHO stage: I  □  II  □  III  □  IV  □

Name of ARV given. ………………………………………………………………………. 
Appendix IX: Composition of reagents (R) used for ALT, AST, and ALP measurement

A  Alanine aminotransferase

R1:  TRIS-Buffer (pH 7.5)  100 mmol/l
     L-Alanine  500 mmol/l
     Lactate dehydrogenase (LDH)  ≥ 1200 U/l
R2:  α-ketoglutarate  15 mmol/l
     NADH  0.18 mmol/l

B  Aspartate aminotransferase

R1:  TRIS-Puffer (pH 7.8)  80 mmol/l
     L-Aspartate  200 mmol/l
     Lactate dehydrogenase (LDH)  800 U/L
     Malate dehydrogenase (MDH)  600 U/L
R2:  α-ketoglutarate  12 mmol/l
     NADH  0.18 mmol/l

C  Alkaline phosphatase (ALP)

R1:  Diethanolamine (pH 10.4)  1.00 mol/l
     Magnesium chloride  0.50 mmol/l
R2:  p-Nitrophenylphosphate  10.0 mmol/l