# HIV-1 GENETIC DIVERSITY, TROPISM AND DRUG RESISTANCE MUTATIONS AMONG HIV INFECTED PATIENTS ATTENDING COMPREHENSIVE CARE CLINIC IN KISII TEACHING AND REFERRAL HOSPITAL, KENYA

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# HIV-1 Genetic Diversity, Tropism and Drug Resistance Mutations among HIV Infected Patients Attending Comprehensive Care Clinic in Kisii Teaching and Referral Hospital, Kenya

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**A Thesis Submitted in Partial Fulfillment for the Requirements of the Degree of Doctor of Philosophy in Medical Virology of the Jomo Kenyatta University of Agriculture and Technology**

**2021**

## DECLARATION

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

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## DEDICATION

I dedicate this work to my dear husband Dr. Anthony Kebira and our lovely children, Januel, Janeel, Joylean and Janviele who have always been my pillars of moral support.

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ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| **3TC** | Lamivudine |
| **ABC** | Abacavir |
| **ADR** | Acquired drug resistance |
| **AIDS** | Acquired immunodeficiency syndrome |
| **AIS** | International AIDS Society |
| **ART** | antiretroviral therapy |
| **AZT** | Zidovudine |
| **CCC** | Comprehensive Care Clinic |
| **CCR5** | Chemokine receptor 5 |
| **cDNA** | Complementary DNA |
| **CD** | Cluster of Differentiation |
| **COMESA** | Common Market for Eastern and Southern Africa |
| **CRFs** | Circulating recombinant forms |
| **CxCR4** | chemokine receptor 4 |
| **D4T** | Stavudine (dideoxy-4-thymidine) |
| **ddC** | Zalcitabine (2′-3′-dideoxycytidine) |
| **DDI** | Didanosine |
| **DNA** | Deoxyribonucleic acid |
| **DR** | Drug resistance |
| **EDTA** | Ethylenediamine tetra-acetic |
| **EFV** | Efavirence |

| | |
|---|---|
| **EVG** | Eltegravir |
| **FTC** | Emtricitabine |
| **Gag** | Group antigen gene |
| ***Gp*** | Glycoprotein |
| **HAART** | Highly active antiretroviral therapy |
| **HIV** | Human immunodeficiency Virus |
| **HIV *en*** | Human immunodeficiency virus envelope |
| **HIV *pol*** | Human immunodeficiency virus polymerase |
| **ICAM-1** | Intercellular adhesion molecular 1 |
| **INSTIs** | Integrase strand transfer Inhibitors |
| **JKUAT** | Jomo Kenyatta University of Agriculture and technology |
| **KAIS** | Kenya AIDS Indicator Survey |
| **KEMRI** | Kenya Medical Research Institute |
| **KTRH** | Kisii Teaching and Referral Hospital |
| **LTR** | long terminal repeat |
| **NAMs** | Nucleotide analog mutations |
| **NASCOP** | National AIDS & STI control Program |
| **NNRTI** | Non-Nucleoside Reverse Transcriptase Inhibitors |
| **NRTIS** | Nucleoside Reverse Transcriptase Inhibitors |
| **NVP** | Nevirapine |
| **PCR** | polymerase chain reaction |
| **PDR** | pre-treatment drug resistance |
| **RAL** | Raltegravir |

| | |
|---|---|
| **RNA** | Ribonucleic acid |
| **RT** | Reverse transcriptase |
| **STIs** | Sexually Transmitted Infections |
| **TAMs** | thymidine analog mutations |
| **TDF** | Tenofovir |
| **TDR** | Transmitted drug resistance |
| **UCF** | Unique circulating forms |
| **UV** | ultra violet |
| ***Vif*** | Viral infection gene |
| **VL** | Viral Load |
| **WHO** | World Health Organization |

## ABSTRACT

Since the initiation of Kenya's nationwide free antiretroviral therapy (ART) in 2003 and 3 by 5 initiatives the availability of highly active antiretroviral therapy (HAART) has increased dramatically. This initiative has since increased the survival levels of the infected individuals. However, this benefit is often limited by development of drug resistance, leading to treatment failure. Effectiveness of this program requires constant surveillance monitoring and evaluation not only on treatment efficacy but also on circulating viral strains. This exercise is more often poorly implemented or non-existent in most of developing countries. This study aimed at determining HIV-1 genetic diversity, viral tropism, acquired and transmitted drug resistance among treatment naive and experienced HIV infected patients attending Kisii Level Five Hospital, Kenya. Cross sectional and prospective cohort study designs were used. Consecutive sampling was used to select participants. A total of 226 participants consisting of both drug naïve (113) and experienced (113) were consented to participate in this study. Venous blood samples were drawn, CD4 and viral load was estimated using Facs calibur flow cytometry and NucliSens EasyQ HIV monitor respectively. Genotyping of HIV was determined at baseline and at 12$^{th}$ month of follow up. Viral cDNA was extracted using QIAamp ® DSP Virus Spin Kit (Qiagen) and specific HIV-1 genes *env C2V3*, *Integrase* and HIV-*pol RT* were amplified in nested PCR using specific primers and directly sequenced. The generated sequences were phylogenetically analysed. HIV-1 Drug resistance was determined using Stanford HIV drug resistance database and confirmed by International AIDS Society (IAS) algorithm. Viral tropism was predicted using *in-silico* Geno2pheno $_{[coreceptor]}$ with a false positive rate of 15%. A total of 448 sequences were generated (both drug naïve and experienced); HIV-1 *env*-C2V3 (164), pol-*RT* (99) and *pol*-integrase (129). Phylogenetic analysis revealed that majority of the study subjects were infected with HIV-1 A1 (71%), followed by HIV-1 C (14.3%),HIV-1 D (12.5%),HIV-1 G (1.6%),HIV-1 A2 (0.4%) pure subtypes and (0.2%) each for A2U and 02A1 CRFs. Most drug resistance mutations were NNRTIs (4, 6.8%) followed by NRTI (1, 1.5%). Drug associated mutation K238T 4.5% (n=3) was the most predominant followed by V106A, M184V, G190A and V118I (1.4%). From pooled 284 Kenyan HIV *integrase* sequences that were analysed for drug resistance, no major mutations conferring resistance to integrase inhibitors were detected. Prevalence of 69% acquired drug resistance was detected. Thirty patients had NRTI resistance mutations with NAMs; K65R, TAMs (23%) and M184V (NRTI). In addition, 46 patients had NNRTIs resistance with V106A as the most common mutation followed by 227L, K103N, Y181C, T238N, V190I, V108I, 230L and 179D.From 164 HIV-1 *env* gene sequences analysed, 75.6% were CCR5 tropic, 23.2% were CXCR4 tropic while 1.2% were duo tropic. This study showed that viral strains in the study population harbour transmitted intermediate drug resistance with those on treatment having significantly high levels of resistance. The detection of high level of circulating R5 strains and no *integrase*-associated mutations suggests the likelihood of a successful implementation and use of CCR5 antagonists and INSTIs in Kenya where HIV-1 A1 is the most predominant. Furthermore,

transmitted intermediate drug resistance, natural occurring polymorphisms and accumulative drug resistance will in future pose a challenge to the program. This therefore warrants monitoring treatment among drug experienced individuals to combat underlying pre-treatment drug resistance and failure.

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background information

At the end of 2018, approximately 37.9 million people were living with HIV-1 worldwide with 1.5 Million from Kenya (UNAIDS, 2018). Since the start of HIV epidemic, 74.9 million have been infected with 32 million recorded deaths. With recorded 1.7million new HIV infections by end of 2018, World Health Organization (WHO) has adopted ambitious 90 90 90% HIV prevention strategy with aim of ending AIDS epidemic by 2025 (UNAIDS, 2014). It is expected that by the year 2020, 90% of all people living with HIV will know their HIV status, 90% of all people with diagnosed HIV infection will receive sustained antiretroviral therapy(ART) and 90% of all people receiving ART will have viral suppression (UNAIDS, 2019). At end of 2018, the number of people on (ART) in eastern and southern Africa have significantly increased since 2015 from (10.3) million to 13.8 by 2018 (UNAIDS, 2018). In working towards achievement of 90% of people on treatment, public health approach has since been adopted as recommended by WHO on initiation of HIV treatment (Monleau *et al.,* 2011). The approach has been revised to current "test and treat all" irrespective of CD4 count or upon diagnosis (UNAIDS, 2018).

Following the 90 90 90% HIV prevention strategy, patients initiated on ART treatment has since increased dramatically over the last 14 years reaching 1,121,938 by end of 2017, including 1,035,615 adults and 86,323 children (KAIS, 2018). This treatment program has since been guided by clinical disease progression and viral load monitoring (Puthanakit *et al.,* 2010). By the end of 2019, findings from Kenya indicates that people living with HIV who knew their HIV positive status were 79.4% , those on treatment 95.7% while those who had achieved viral suppression were 88.4% (MOH, 2018). These findings show an overall good progress nationally towards achievement of the new HIV prevention strategy as confirmed by HIV prevalence of 4.6% (MOH, 2019). However, this may not be the trend in some counties like Kisii which has shown to be among the counties with highest incidence

of HIV (NASCOP, 2018). This is challenge especially in Kenyan settings where we do not have a routine personalized treatment monitoring including provision of genotypic drug resistance, may limit the achievement of this new HIV prevention strategy (Boulle *et al.,* 2007).

In cases; where there is poor response to treatment, the virus may develop drug associated mutation due to drug pressure. HIV drug resistance more often develops due to its error prone nature of HIV replication resulting in a high mutation rate in combination with the ongoing presence of drug-selective pressures (Rusine *et al.,* 2013). Human Immunodeficiency Virus Drug Resistant (HIVDR) strains that emerge after treatment initiation (referred to as acquired or secondary HIVDR) can subsequently be transmitted to previously uninfected patients (referred to as transmitted or primary HIVDR) (Bennett *et al.,* 2009). Transmitted HIVDR increases the risk of virological therapy failure (Wittkop *et al.,* 2011) and compromises the efficacy of first-line ART regimens. This is important in a context of limited treatment options (Rusine *et al.,* 2013).

Despite advances in ART that have revolutionized HIV disease management, effective control of the HIV infection pandemic remains elusive. By virtue of Africa harbouring diverse HIV-1 subtypes, Kenya is among African countries with some of these HIV subtypes. Even though the HIV cases are going down with current intervention, the circulating subtypes may be increasing in their diversity following mixed up or super infections leading formation of viral recombinants (Andrews & Rowland-Jones, 2017).

It is expected that diverse viral strains, may pose an impact on treatment response, vaccine design, replication fitness and viral tropism (Easterbrook *et al.,* 2010). The existing subtype-specific differences in virological and immunological characteristics have been observed in faster rate of CD4 decline in subtype D infected patients, impact on viral load, viral tropism, syncytia formation and fitness, and immune response (Santoro *et al.,* 2013). Resistance pathways in different subtypes may affect drug cross-resistance and the potential use of specific second-line regimens (Kantor *et al.,* 2002).

With increasing trend in pre-treatment drug resistance in HIV-1, even newer drugs patients on treatment to be monitored. The risk of emerging resistance to newer classes of ARV drugs is unknown. However, as countries with high levels of NNRTI resistance modify their first-line ART regimens including the newly introduced, it is expected that resistance will invariably emerge which there is a need to closely monitored (WHO, 2017). Prior to the introduction of the revised implementation of dolutegravir, there is a need to evaluate its efficacy prior to its implementation. This study was therefore focused on determining the possible underlying drug resistance to dolutegravir prior to its implementation. In addition, the reported high HIV incidence in Kisii County is wanting hence demanding the need to determine the circulating HIV-1 strains and their spread in the county.

## 1.2 Statement of the Problem

By end of 2019, national HIV prevalence in Kenya was 4.9% with estimated people living with HIV at 1.5 million with that of Kisii County being 8.1% prevalence with disease burden of a total of 63,715 people living with HIV (NASCOP, 2020). Kisii County had 6,086 new infections by the end of 2017, making it among the highest counties with high incidence of HIV infection (NASCOP and Kisii County, 2018). With this rate of infection, there are underlying drivers of HIV epidemic in this region. The HIV related deaths by end of 2018 was 634 people with those under ART coverage being 79% (NASCOP, 2020). This disease burden had an impact on the workforce including stigma which have impacted negatively on their families and county economy due to loss of man-hours and man power. By working towards achievement of 95-95-95 strategy, WHO recommends treat all (UNAIDS, 2014). According to this county, patients on virological suppression were below expectation at 62% achievement (NASCOP, 2020). The low virological suppression level could be associated with either poor drug adherence or development of HIV drug resistance that will lead to treatment failure. It is evident that HIV drug resistance will develop in ART-patients even with the optimal adherence and adequate clinical monitoring. This is due to the biological characteristics of HIV-1.Varying HIV Drug resistance (DR) among people taking ART has been reported in Africa with resistance ranging between 5% and 24% (Rehle *et al.,* 2010). Among individuals in national ART

programs with confirmed virological failure, this has ranged from 44% to 100% (Wallis *et al.,* 2010).

In resource-limited settings, it may not be feasible to perform individual drug resistance testing (Puthanakit *et al.,* 2010). In addition, virological monitoring is still not feasible for most patients on ART due to the absence of adequate laboratory facilities and high cost. Viral load monitoring after initiation of ART is usually not available through treatment programmes in resource-limited settings (Chasombat *et al.,* 2009). Furthermore, early warning indicators, a strategy meant to monitor drug resistance or poor patient response to treatment is also non-existence (WHO, 2016). For example, national AIDS programme provides antiretroviral drugs for HIV infected patients and virological monitoring at every 6 months to a facility that is not yet achieved 100% county coverage. Hence, majority of patients are diagnosed with treatment and virological failure that will lead to fast disease progression. In these individuals, eventually, drug resistance-associated mutations may have occurred as a result of persistent viral replication under drug pressure (Puthanakit *et al.,* 2010).

It has been shown that, the level of resistance to antiretroviral drugs differs among HIV variants (Lessells *et al.,* 2012). Indeed, there is limited knowledge of resistance mutations in non-B subtypes of HIV 1 and their clinical relevance, despite the fact that more than 90% of patients with HIV-1 infection worldwide have non–subtype B variants. Most reports on drug resistance have subtype B infections. Both enzymatic and virological data indicate that naturally occurring polymorphisms among different HIV subtypes can influence HIV-1 susceptibility to antiretroviral drugs and the propensity of HIV to acquire certain resistance mutations (Nyamache *et al*., 2012) This study was conducted to monitor the transmission dynamics of HIV-1 virus in Kisii region where there has been reported increase in prostitution and populations from various institutions and businesses.

### 1.3 Justification of the study

With the rollout of ART, development of HIV-1 drug resistance is an increasing public health concern in sub-Saharan Africa. However, such data is limited in Kenya where HIV-1 drug resistance testing is not routinely performed. Kisii Teaching and

Referral hospital, serves both Kisii and Nyamira counties and surrounding counties. It is recommended that patients who experience failure of NNRTI-based regimens should switch to second-line regimen based on resistance testing.

With already treatment failures due to developed drug resistance, there is need for information about HIV-1 drug resistance mutations which is therefore crucial for predicting second line regimen or development of novel effective drugs. Currently there are no existing systems to monitor mutations causing drug resistance among patients failing therapy. There is thus a need to assess virological outcomes in routine care settings in order to evaluate the effectiveness of current antiretroviral programs. It is therefore necessary that HIV drug resistance genotyping be conducted at population level for surveillance and monitoring (Nasir, 2017). The Kisii region being an agricultural area with a lot of businesses in urban centres, there is a likelihood of having diverse populations including those from other counties hence the need to assess the transmission dynamics of these viral strains among the people living in Kisii County. It will be appropriate to elucidate the current circulating HIV subtypes in the county in order to guide control or prevention measures.

### 1.4 Research questions

i. What are the current HIV-1 subtypes circulating among the studied population?
ii. What is the prevalence of transmitted HIV-1 drug resistance mutations among drug naive patients in the population studied?
iii. What are the acquired HIV-1 drug resistance mutations in the study population?
iv. What is the prevalence of HIV-1 Integrase gene associated drug resistance mutations prior to the roll out of integrase inhibitors by Kenyan HIV/AIDS treatment program in KTRH CCC?
v. What are the HIV-1 tropism of circulating HIV strains in patients attending Comprehensive Care Clinic in Kisii Teaching and Referral Hospital?

### 1.5 Broad Objective

To determine HIV-1 genetic diversity, viral tropism, and drug resistances mutations among HIV infected patients attending Kisii Teaching and Referral Hospital.

### 1.5.1 Specific objective

i. To determine the genetic diversity of HIV-1 among HIV infected patients attending Kisii Teaching and Referral Hospital Comprehensive Care Clinic, Kisii County.
ii. To determine the prevalence of transmitted drug resistance mutations among HIV-1 infected patients attending Kisii Teaching and Referral Hospital.
iii. To determine the acquired drug resistance mutations among HIV-1 infected patients attending Kisii Teaching and Referral Hospital.
iv. To determine HIV-1 Integrase gene drug associated mutations prior to the roll out of integrase inhibitors by Kenyan HIV/AIDS treatment program in KTRH CCC
v. To determine the viral tropism among circulating HIV-1 strains in patients attending Kisii Teaching and Referral Hospital.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Virology of HIV-1

The HIV belongs to the retroviridae family and lentivirinae genus. The virion in spherical in shape, enveloped and its diameter is ranging between 100-150nm, containing two of positive sense, single stranded RNA molecules. The HIV-1 viral particles have a diameter of 100 nm and are surrounded by a lipoprotein membrane. Each viral particle contains 72 glycoprotein complexes, which are integrated into this lipid membrane, and are each composed of trimmers of an external glycoprotein *gp*120 and a trans membrane spanning protein *gp*41 (Figure 2.1) (Checkley *et al.,* 2011). The bonding between *gp*120 and *gp*41 is not strong and therefore *gp*120 may be shed spontaneously within the local environment. Glycoprotein *gp*120 may also be detected in the serum (Checkley *et al.,* 2011) as well as within the lymphatic tissue of HIV-infected patients (Estes, 2013).

During the process of budding, the virus may also incorporate different host proteins from the membrane of the host cell into its lipoprotein layer, such as HLA Class I and II proteins, or adhesion proteins such as ICAM-1also known as CD54 that may facilitate adhesion to other target cells. The matrix protein *p17* is anchored to the inside of the viral lipoprotein membrane (Checkley *et al.,* 2011). The *p24* core antigen contains two copies of HIV-1 RNA. The HIV-1 RNA is part of a protein nucleic acid complex, which is composed of the nucleoprotein *p7* and the reverse transcriptase *p66* (RT). The viral particle contains all the enzymatic equipment that is necessary for replication: a reverse transcriptase (RT), an integrase *p*32 and a protease *p*11 (Menendez, 2010) (Figure 2.1).

HIV genome is approximately 9.6 kilobases in length, contains 9 different genes encoding 15 proteins (Doms & Moore, 2000). The major genes include; 5'gag-pol-env-3', encoding major structural proteins as well as essential enzymes (Mushahwar *et al.,* 2006). The HIV virus is spiked. It has a pair of RNA strands within the

matrix of the virus. The soft surface of the virus has the *gp*120 and *gp*41 proteins (Checkley *et al.,* 2011) (Figure 2. 1).

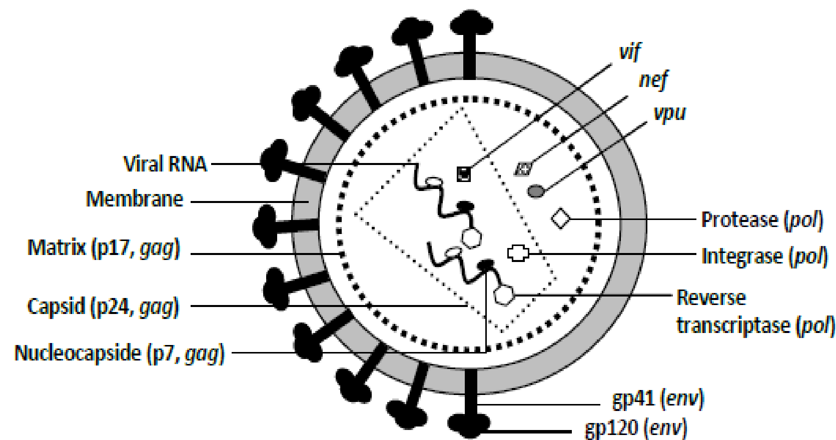


**Figure 2.1: The structure of HIV virion** (Rajarapu, 2014)

## 2.2 HIV Replication cycle

To effectively infect a target cell, HIV must introduce its genetic material into the cytoplasm of the cell (Waldmann *et al.,* 2001). During HIV infection, the virus only infects cells baring CD4+ marker. These cells could include; monocytes/ macrophages, dendritic cells, oligodendrocytes, astrocytes and neurone which could also provide reservoirs for it. During infection, the virus binds on CD4 receptor and viral gp120. This interaction promotes a conformational change in gp120, which results in exposure of the bridging sheet, composed of four anti-parallel ß-strands from the V1/V2 and C4 regions of gp120. The bridging sheet and the V3 region interact with the co receptor, either CCR5 or CxCR4 leading to a conformational change in gp41. Consequently, a fusion peptide in gp41 is inserted in the host cell membrane. The formed six-helix bundle of gp41 will bring cellular membranes together finally allowing fusion of gp41 and CD4 receptors to occur (Checkley *et al.,* 2011; Veesler & Johnson, 2012). With internalization of this virus, it can now remain infective up to four days. The virus then penetrates into the cell and uncoats, at this stage, HIV release integrase enzyme which is involved in integrating HIV

DNA into host cell nucleus. At this stage, the virus may either undergo latency or continue to reverse transcription step. Once the capsid is delivered to the cytoplasm, +ssRNA is reverse transcribed into double stranded (ds) DNA by reverse transcriptase and synthesis of several copies of HIV genome and shorter strands of mRNA. This mRNA is later used as a blue print/template to make long chains of HIV proteins. Thereafter, viral long chains of HIV are cut by protease and the new viral virions assembled. Finally, these new virions mature and then are pushed out from the host cell by budding off to infect new cells (Veesler & Johnson, 2012) (Figure 2.2)

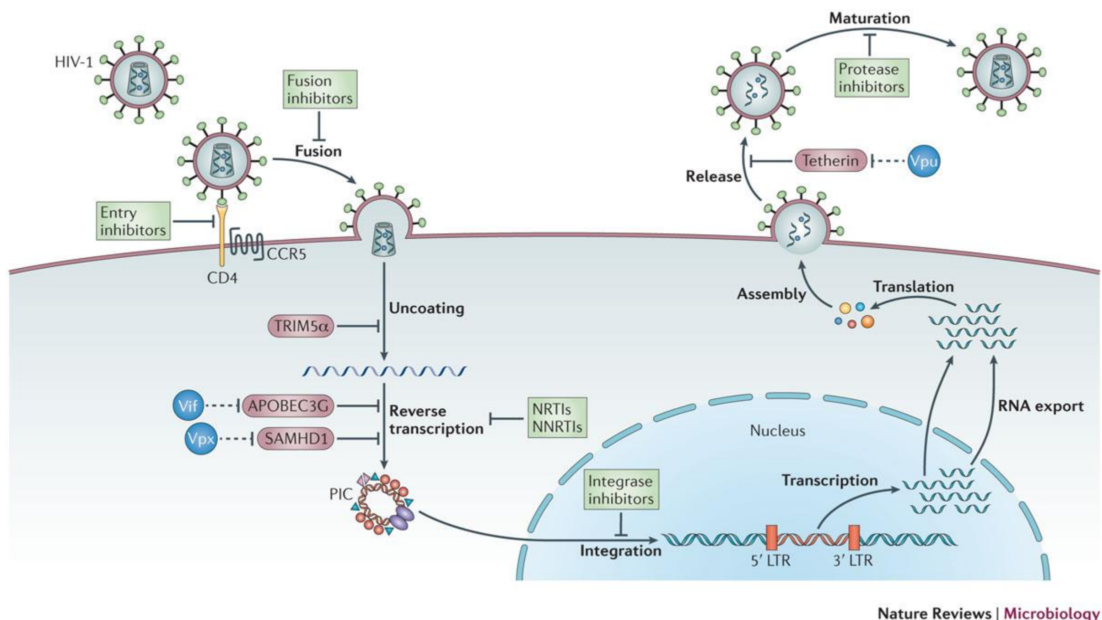


**Figure 2.2: HIV Replication Cycle** (Barré-Sinoussi, 2013)

## 2.3 The HIV-1 genetic diversity

HIV exists into two major types; HIV-1 and HIV-2. The HIV–1 strains are divided into four phylogenetically distinct groups designated M (Major), N (non-M, non-O), O (Outlier) and P (pending the identification of further human cases) (Plantier, 2009; Feng *et al.,* 2014). Group M, which is the major group, comprises most HIV-1 strains that have continued to cause AIDS (Acquired Immune Deficiency Syndrome)

pandemic worldwide. In this group, the full-length genome sequencing shows that group M HIV-1 viruses can be further subdivided into at least nine different, non-recombinant, subtypes (A, B, C, D, F, G, H, J, and K) which are approximately equidistantly related with intra-subtype divergence up to 20% and inter-subtype divergence between 25% to 35%, for the e*nv* amino acid sequences (Achkar *et al.,* 2004). The HIV-1 subtypes A, B, D and F can also be further phylogenetically divided into A (A1-A7), D (D1-D3) and F (F1-F2). In mixed infections, these viral subtypes have led to the formation of viral recombination during viral assembling leading to the formation of viral strains that are recombinant of more than one subtype, circulating recombinant forms (CRFs). Now, we have about 101 CRFs worldwide with several unique recombinant forms (URFs) (Recordon-Pinson *et al.,* 2018) (Figure 2.3).

The HIV 2 which is more confined to West African countries with up 1-2million people having been infected (Nyamweya *et al.,* 2013). Genetically, this virus is genetically divided into nine groups A-I with only A and B circulating. To date, only a single circulating recombinant form (CRF), (CRFO1_AB) has been described as well as a single unique recombinant forms (Visseaux *et al.,* 2016). With elusive information regarding the circulating subtypes, its postulated that HIV-2 group A could be divided into distinct subtypes of diverse geographical origins (Visseaux *et al.,* 2016) (Figure 2.3).

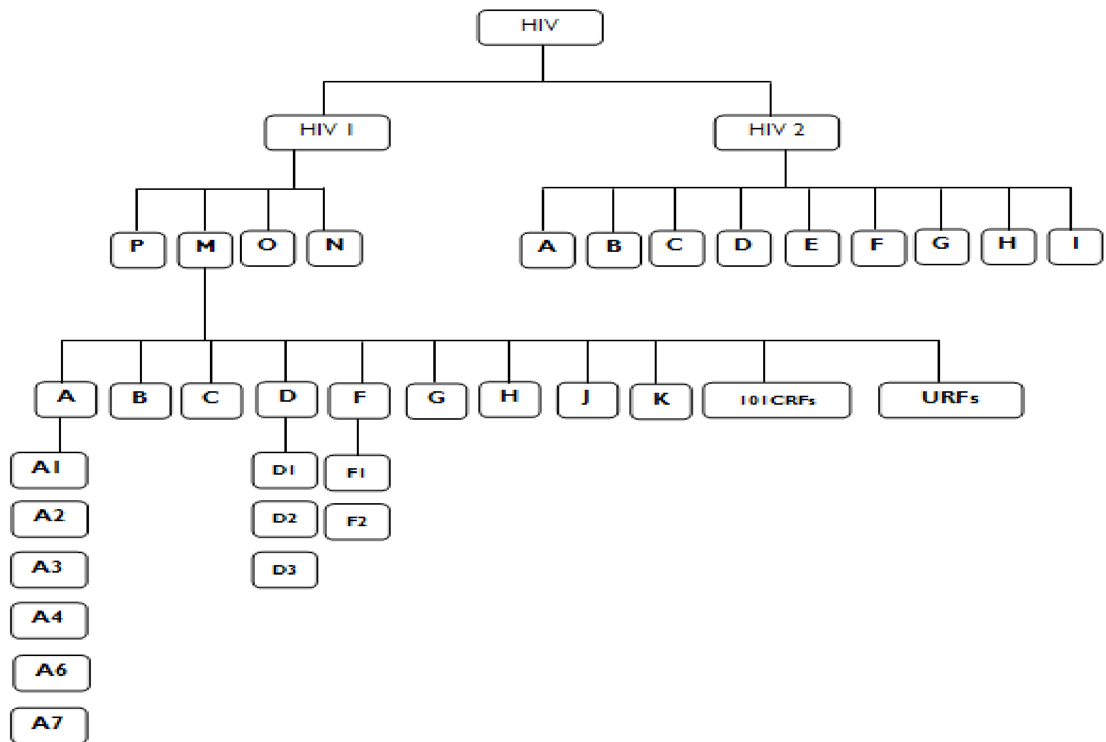


**Figure 2.3: HIV-1 updated classification proposal; groups, subtypes and sub-subtypes** (Desire *et al.,* 2018)**. (Modified)**

### 2.4 Global Distribution of HIV-1 subtypes

Following mixed infections and population migration leading to diverse sexual networks, the intermixture of HIV-1 variants circulating within a certain region have provided an opportunity leading to development viral recombination with duly infected individual. So far, by 2019, over one hundred and one circulating recombinants forms (CRFs) and numerous unique mosaic strains of HIV-1 have been identified (Zhang *et al.,* 2019). However, based on this, HIV-1 subtypes have been distributed evenly around the world with some subtypes predominating in certain regions or another (Lihana *et al.,* 2006).

HIV-2 has remained strictly in West Africa with some proportions of it been detected in some parts of Europe; (Visseaux *et al*., 2016), India and the United States of America (Visseaux *et al.,* 2016). HIV-2 has also been documented to be less infectious than HIV-1 (Nyamweya *et al.,* 2013) it is comprised of at least nine groups (formerly referred to as subtypes; A to I) of which groups A and D are

presently circulating (Visseaux *et al.,* 2016). HIV-2 subtype data is still limited and only a few recombinants have been described (Visseaux *et al.,* 2016).

In Europe, HIV-1 subtype B has continuously remained predominant even other subtypes have also been detected although in low proportions. HIV subtype B is predominant in homosexuals, while a variety of subtypes are found in a relatively small numbers of people infected through heterosexual contact in Europe and the countries of the former Soviet Union (Ortiz *et al.,* 2000). However, in recent years, all group M subtypes as well as O strains have been isolated in these regions, with exception of the N group, mainly in immigrants or in travellers to endemically infected areas (Ortiz *et al.,* 2000; Rangel *et al.,* 2009). Indonesia, the Philippines, and Taiwan have also been predominated with HIV subtype B (Ortiz *et al.,* 2000). In Thailand, Cambodia and Vietnam are predominated by CRF01_AE and a minority of subtype B strains that facilitating the sustained emergence of recombinants between the two (Chung *et al.,* 2018). The CRFA1_AE predominates in China, Myanmar (Burma), Malaysia, and southeast China (Zhou *et al.,* 2014; Chung *et al.,* 2018; Xuan *et al.,* 2018). In the Americas (North, South and Central), as well as in Australia, New Zealand, and Japan, subtypes B is most common. However, other subtypes have also been detected. Subtype F occurs in Romania, and subtype C is found in a small proportion in Brazil (McCutchan *et al.,* 2000).

Nevertheless, several subtypes circulating in Asia have been referred as “hotbed” for circulating recombinant viruses with several CRFs (Pang *et al.,* 2012). The circulating HIV subtypes in South East Asia are subtypes B and CRF01_AE, while in India the predominant subtype is C (Shama *et al.,* 2019) with small numbers of A and B infections (McCutchan *et al.,* 2000). Subtype A predominates Russia and other Soviet Union countries although CRF02_AG being common in in Kyrgyzstan (Aibekova *et al.,* 2018).The predominantly circulating strains in South America have been HIV-1 subtypes B and F (Cevallos *et al.,* 2017). However, other subtypes like HIV subtype C have been reported with increasing trend especially in Brazil and CRFs have been found to circulate, although at low levels (Avanzi *et al.,* 2017).

The greatest HIV-1 subtype diversity has remained to be witnessed in Africa especially West Africa countries where HIV-2 is also thought to have originated from. However, in other parts of Africa, the viruses have been found to be diverse (Abongwa *et al.,* 2019). Nevertheless, Africa, all subtypes have been found with subtypes A and C being the most predominant (Lihana *et al.,* 2012). Subtype C has remained predominant in southern Africa while A in East Africa even though subtypes D and C viruses circulates too in south Africa (Gounder *et al.,* 2017).

An increase in HIV-1 subtype C and a decrease in D have been reported in recent past in Kenya with extensive inter-subtypes recombinants. In Uganda, there has been a report on increase intersubtype recombination up 46% based on full length sequences analysis (Madhavi *et al.,* 2017). Studies in Tanzania have continuously shown that some parts of southern Tanzania like Mbeya to be predominated with HIV-1 subtype C (Hoelscher *et al.,* 2001). This probably reflects introduction from southern African countries where subtype C is the predominant type (Baar *et al.,* 2000). Other HIV-1 subtypes A and D have been detected in other parts of Tanzania even though HIV subtype A1 predominates in large proportion of Tanzania in relation to subtype C (Billings *et al.,* 2017).

Recent data suggest a significant increase in the prevalence of recombinants in some countries ( Gounder *et al.,* 2017; Sharma *et al.,* 2017; Neogi *et al.,* 2017)) and several newly discovered CRFs/ URFs especially in some parts of Asia and Africa. This suggests that coinfection or super infection by divergent HIV-1 strains has become more common in regions where multiple subtypes co-circulate (Gounder *et al.,* 2017; Feng *et al.,* 2017).

### 2.4.1 The HIV subtypes circulating in Kenya

Almost all circulating HIV-1 strains belong to group M and have been characterized according to HIV nomenclature. From the HIV database, majority of sequences deposited belong to subtype A (66%), with significant components of subtype D (14.1%), subtype C (5.8%) and four CRF derived of these circulating subtypes. The detected viral subtypes and CRFs confirms the existence of confirming the diverse nature of circulating HIV-1 subtypes and recombinants (Lihana *et al.,* 2006;

Nyamache *et al.,* 2013).Therefore, previous studies have confirmed that HIV-1 subtype A remains the most predominant subtypes in the country (Nyamache *et al.,* 2013; Abongwa *et al.,* 2019) In addition, following mixed infections, recombinant forms have also been detected, AID (6.2%), followed by AIC 2.0% and lastly A2D 1.1% with other unique and rare recombinants forms (Dowling *et al.,* 2002).

Other HIV-1 subtypes have also been detected in the country. For instance, low proportion of HIV-1 subtype; G have been reported (Hassan *et al.,* 2018; Carr *et al.,* 1998), recombinants between A1, A2, and D; A1 and D; A1 and G; A1 and C; A1, C, and D have also been detected (Dowling *et al.,* 2002). Of recent past, an increase in HIV-1 subtype C has been reported together with an extensive intersubtypes' recombination (Gounder *et al.,* 2017). The neighbourhood countries have also been an influencing on HIV-1 genetic diversity in the country based on population migration (Abongwa *et al.,* 2019). For instance, Northern part of Kenya which borders Ethiopia that is predominated by HIV-1 subtype C has influenced high circulation of this subtype in the urban centres across borders of Moyale (Khamadi *et al.,* 2005). The commercial activity and eastern African road and rail transport has also impacted some regions of Kenya with diverse HIV-1 subtype A, D, C and small proportions of other subtypes like G in the country (Nyamache *et al.,* 2013). Even though a wide analysis of the HIV genetic diversity has been conducted and more so on urban centres, predominance of HIV-1 subtype A has remained since. Most of the CRFs and URFs have also been detected and indicating the nature or suggested mixed infections (Lihana *et al.,* 2006).

## 2.5 Antiretroviral drugs

Upon diagnosis of HIV infections, the patients are initiated on first-line drugs; two Nucleoside Reverse Transcriptase Inhibitors (NRTIs), (3TC (/FTC) +AZT/TDF) and one Non-Nucleoside Reverse Transcriptase Inhibitor, (NNRTI), (EFV/NVP). The patients on treatment are then monitored with viral loads counts (VL) with those with virological failure (≥1000 copies/ml), current regimen changed and followed with viral loads at three months interval as other issues associated with failure are addressed (Gilks *et al.,* 2006; Puthanakit *et al.,* 2010; MOH Kenya, 2018). With

available resources, it is recommended by WHO that viral load should be monitored routinely at every 6 months or in a targeted approach to confirm treatment failure (Gilks *et al.,* 2006; WHO, 2016). For poor resource settings, NNRTI-based regimen is recommended by WHO based on its efficacy, tolerability and low cost (Puthanakit *et al.,* 2010). Routine testing of viral Load and CD4 counts during treatment is required for easy monitoring of HIV drug. In most developing countries, this monitoring of patients under treatment is not possible due their economic situations and reliability of them on donor funding. Following this, CD4 and viral loads counts are monitored with persistent increase in viral load above 1,000 particles/millilitre used as indicator of underlying treatment failure. A switch to second-line therapy will therefore be recommended when viral load is elevated on two consecutive measurements and drug resistance evaluated to guide it (Clumeck *et al.,* 2014). However, in most Kenyan HIV care clinics not all are accessible to viral load evaluation or monitoring (Mwau *et al.,* 2018).

Nevertheless, genotyping of HIV drug resistance is neither a routine point of care leaving viral load as the only option to rely on switching treatment. In this case patients' viral load is monitored after every 3 months till suppression of viral load before increasing the period to 6 months (Mwau *et al.,* 2018). Drug resistance testing is therefore a key in patient's management with transmitted drug resistance being used in evaluating the efficacy of the currently used first-line drugs. Switching treatment failure patients from first-line to second-line is aimed at fully suppression of HIV replication Therefore, it is expected new drugs should comprise at least two, but preferably three, fully active drugs. This means, the patient is put on two NRTIs with NNRTIs being switched with PIs (Ruzicka *et al.,* 2019).

### 2.5.1 Nucleoside Reverse Transcriptase Inhibitors (NRTIs)

During treatment, guideline indicate that the patient should be put on one of either zidovudine (Azidothymidine (AZT), Lamivudine (3TC), Didanosine (ddI), Zalcitabine (ddC), Stavudine (d4T), Abacavir (ABC) ) as nucleoside analogues as chain terminator. These drugs comprise a base (thymidine in the case of AZT) that

attaches to a ribose sugar in which the normal 3' hydroxyl is replaced by an azido group during elongation of the growing DNA chain (Zdanowicz, 2006).

Both nucleoside and non-nucleotide reverse transcriptase (RT) inhibitors must enter the cell and become phosphorylated in order to act as synthetic substrates for RT. Both of these classes of agents have confirmed to prevent infection of susceptible cells with no effect on cells that already harbor HIV. HIV drug resistance to NRTI's can either occur by formation of mutations on the active sites of drugs leading to their reduced incorporation of the NRTI into the growing DNA chain. While some of these mutations arise in the actual catalytic site of RT, or are close in proximal to the active catalytic site of RT (Zdanowicz, 2006).

### 2.5.2 Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

The NNRTIs includes; nevirapine, donavirine, delavirdine and efavirenz as drugs that are structurally different from NRTIs. This class of drugs unlike NRTI do not require phosphorylation to be activated however, they act directly inhibit the RT enzyme by binding to a pocket residue of the enzyme hence inhibiting DNA polymerization by inducing allosteric changes to HIV RT at its active site. This change decreases the RT and nucleoside binding affinity leading to the prevention of viral replication (Zdanowicz, 2006).

Drugs of this class are often limited in their action by rapid development of viral drug resistance. These drugs have low genetic barrier hence single mutation like K103N or V106A could render the entire class ineffective. As a result, the NNRTIs are often used in combination with other drugs but not as monotherapy. Drug resistance often occurs mainly through mutation of hydrophobic RT residues within the binding pocket of these drugs (Zdanowicz, 2006). Since these drugs essentially bind at the same position on RT, mutations on this position will eventually render the entire class ineffective This limitation better explains why this class of drugs has got high rates of cross resistance (Brenner *et al.,* 2003).

### 2.5.3 Protease Inhibitors

Proteolytic cleavage of viral polypeptide precursors into mature enzymes and structural proteins is critical step in HIV replication cycle. This proteolytic activity is absolutely required for the production of mature, infectious virions. These enzymes offer a unique and attractive target for therapeutic intervention (Zdanowicz, 2006). Protease inhibitors therefore act by fitting and binding at the catalytic site of protease enzyme leading to blocking of the viral enzyme activity or prevention of dimerization of the enzyme (Yang *et al.,* 2012). These drugs include; saquinavir, indinavir, ritonavir, nelfinavir and amprenavir. Protease inhibitors are considered as second line drugs due to their high genetic barrier (Zdanowicz, 2006). The development of protease Inhibitors (PI) resistance is believed to be a stepwise process. Drug resistance to this class of drugs will require multiple mutations in order to overcome drug activity (Fonseca *et al.,* 2007).

### 2.5.4 Fusion Inhibitors

The HIV infection especially at the point of entry of HIV has formed substantially target for therapeutic intervention. Fusion inhibitors act by binding either *gp*41, CCR5 or CXCR4 receptors hence blocking the fusion or entry of HIV into the host cells during infection. The fusion inhibitors drugs include; Enfuvirtide, vicriviroc, maraviroc, plerixafor and aplaviroc (Moore *et al.,* 2003; Greenberg & Cammack, 2004; Zdanowicz, 2006).

However, HIV-1 has been found to develop drug resistance against enfuvirtide by development of mutations at amino acid positions on gp41 between residues 36 and 45 positions or changes on changes on gp120 (Zdanowicz 2006; Westby *et al.,* 2007). However, for CCR5 or CXCR4 antagonist resistance could either occur due to viral switching receptors or mutations on CCR5 or CXCR4 receptors (Dorr *et al.,* 2005; Anastassopoulou *et al.,* 2009).

### 2.5.5 Integrase inhibitors

The HIV-1 integrase enzyme is essential important during viral replication and has become another target for antiretroviral therapy (Charpentier *et al.,* 2008). Integrase strand-transfer inhibitors (INSTIs) is another class of drugs that target integrase protein and have been found to be effective against resistance viruses to other drugs (Nyamache *et al.,* 2012). This integrase enzyme has two catalytic functions; first it removes a dinucleotide from each $3^{-}$ end of viral DNA (the $3^{-}$ processing reaction), and second, it mediates the transfer of the proviral DNA strand in the host nucleus and covalently links the $3^{-}$ ends into the host DNA (the strand transfer reaction) (Bray *et al.,* 2016). Intergrase inhitors includes; raltegravir (RAL), elvitegravir (EVG), and dolutegravir (DTG) (Markowitz *et al.,* 2007). Their mode of action is by binding to catalytic Pre-Integration Complex (PIC), hence blocking the strand transfer of HIV complimentary DNA (cDNA) to cellular DNA by removing a dinucleotide from the $3^{1}$ ends before integration (Evering & Markowitz*,* 2008).

Development of drug resistance to integrase is through occurrence of mutations on integrase active site near the amino acid residues that coordinate the essential magnesium cofactors (De Enda *et al.,* 2013). For instance, resistance to raltegravir (RAL) arises from three major independent pathways or sets of mutations as defined by primary/signature mutations at positions Y143, Q148, and N155 (Mbisa *et al.,* 2011). With low genetic barrier, these INSTIs do experience a cross resistance following their shared similar inhibition mechanism (Cohn *et al.,* 2015)

## 2.6 The HIV-1 Drug Resistance

The HIV-1 ‘s rapid replication rate, throughout its course of infection and error prone on its reverse transcriptase enzyme by lack of 3”exonuclease enzyme for proof reading are the main rreasons for frequent occurrence of copying errors in the transcription of viral genetic information (Sanjuán & Domingo-Calap, 2016). Nevertheless, the virus could also develop mutations due to pressures exerted either by immune response or antiretroviral drugs (Nowak *et al.,* 1990). Therefore, viral drug resistance in HIV largely occurs due to changes (mutations) in the genetic material that codes for the HIV-1 reverse transcriptase (RT), integrase, envelope, and

protease enzymes. It can also occur on the viral gp41 gene and envelope as targeted by drug and immune response (Brumme *et al.,* 2003).

Most mutations resulting from this could either be lethal (by reducing its replication fitness) or neutral to the virus or may confer resistance to the drugs leading to treatment failure (De Luca, 2006). All current antiretroviral (ARV) drugs, including newer classes, are therefore at risk of becoming partially or fully inactive due to the emergence of drug-resistant virus.

In case where individuals are on treatment, the mutations that develop in HIV during its replication are thus referred to as acquired drug resistance while among drug naïve individuals without any history of drug exposure are referred to as transmitted drug resistance (Buckheit Jr, 2004). Nevertheless, for the pre-treatment drug resistance, these mutations are often detected ARV drug-naive people initiating ART or people with prior ARV drug exposure initiating or reinitiating first-line ART. PDR is either transmitted or acquired drug resistance, or both (Gupta *et al.,* 2018). PDR may have been transmitted at the time of infection (TDR), or it may be acquired by virtue of prior ARV drug exposure (for instance in women exposed to ARV drugs for the prevention of mother-to-child transmission of HIV, in people who have received pre-exposure prophylaxis, or in individuals reinitiating first-line ART after a period of treatment interruption without documented virological failure) (Ayalew *et al.,* 2016).

Previous studies conducted in Kenya among patient failing treatment with prevalence of acquired drug resistance ranging from 41% to 91% (Kassaye *et al.,* 2007; Hassan *et al.,* 2011; Koigi *et al.,* 2014) including as high as 99% in Zambia (Seu *et al.,* 2015). Pretreatment drug resistance is more than two-fold higher among people starting first-line ART with prior ARV drug exposure, compared to ARV drug-naive individuals (WHO, 2017). From recent surveys on levels of pre-treatment drug resistance to efaverenz and nevirapine, the levels have already reached 10% from survey data from six of the 11 countries; (Argentina, Guatemala, Namibia, Nicaragua, Uganda and Zimbabwe (WHO, 2017). With continued ART scale-up,

this group is likely to represent an increasing proportion of people initiating treatment who may not be receiving effective treatment.

Therefore, this survey data indicates that NNRTI resistance in people starting ART may be at levels that should trigger public health action (WHO, 2017). Kenya like most countries have not yet conducted surveys on pre-treatment drug resistance (PDR) yet studies have shown an increasing trend in levels of TDR up to 13% (Hassan *et al.,* 2013). The NNRTIs are known to be the backbone of recommended first-line ART regimen. Therefore, their efficacy should be preserved if possible (Ngo *et al.,* 2018). However, studies have reported NNRTI resistance among people retained on ART to range from 4% to 28%, while among people with unsuppressed viral load on first-line NNRTI regimens; ranging from 47% to 90% A (De Luca *et al.,* 2017). This speaks to the need to scale up viral load testing, promptly switch individuals with confirmed virological failure to second-line treatment, and those failing not necessarily due to drug resistance drug adherence strengthened in countries with lower levels of acquired drug resistance (ADR) (Girón-Callejas *et al.,* 2019).

## CHAPTER THREE

## MATERIALS AND METHODS

### 3.1 Study site

This study was carried out at Kisii Teaching and Referral Hospital (KTRH) in Kisii County. Kisii County is one of the forty-seven Counties of Kenya. It borders Nyamira County to the North East, Narok County to the South and Homa Bay and Migori Counties to the West. The County lies between latitude 00 30’ and 100 South and longitude 340 38’ and 350 East. It covers an area of 1,302km2, a population of 1,152,282 people as per the 2009 census. Kisii County has a population of about 1,161,269 categorized with a population growth rate of about 21%  Nyanza Region (KCHASP, 2018) (Figure 3.1)

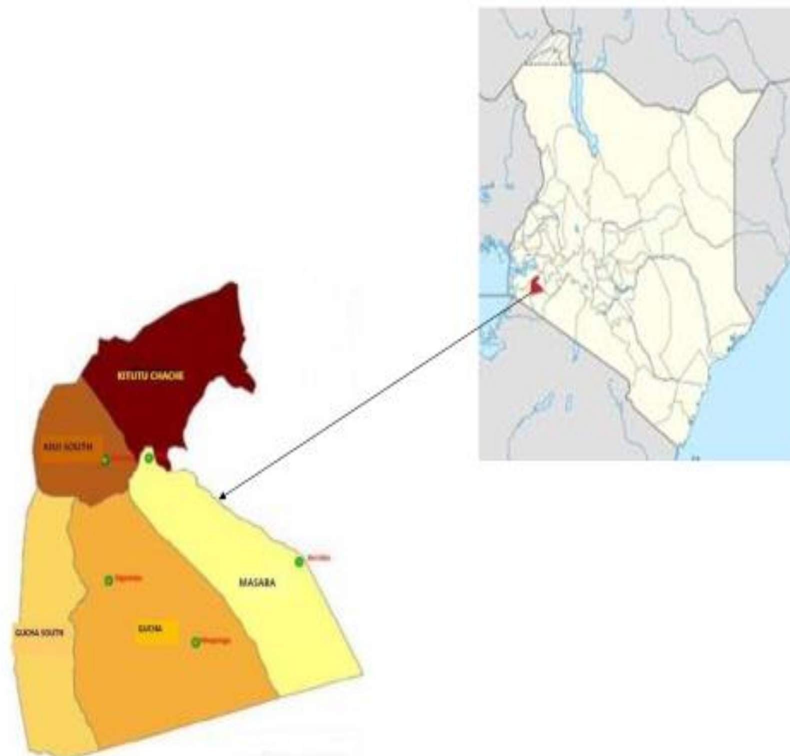


**Figure 3.1: Map showing Kisii County (Mokua *et al.,* 2019)**

### 3.2 Study population

This study was conducted among HIV infected individuals both male and female aged above 18 years. These individuals consisted of both drug naive and those on first line drug treatment who were attending HIV care clinic of KTRH during the period between October 2017 and August 2019.

### 3.3 Study design

This was a two methods study consisting of a cross sectional and longitudinal/prospective cohort study designs. For transmitted drug resistance estimation, a descriptive cross-sectional design was adopted and participants recruited from among newly diagnosed HIV infected patients prior to their linkage to Kisii teaching and Referral Hospital (KTRH) HIV treatment program. For acquired drug resistance, a cohort study design was used and participants recruited among HIV infected patients attending KTRH HIV care clinics and monitored for a period of twelve months.

### 3.4 Sample size calculation:

Using Naing (2003) method, the sample size was calculated as;

$$N=\frac{Z^2 P\ (1-P)}{D^2}$$

**Where:**

**N**= Minimum sample size required

**Z**= 1.96 Standard Error

**P**= HIV Prevalence in Kisii County (8%) (KAIS, 2015).

**D**= 0.05 the inverse of 95% confidence limit (degree of accuracy required)

$$N= \frac{1.96^2 \times 0.08\ (1-0.08)}{0.05^2} = 113 \text{ samples}$$

Therefore, the minimum sample size was 113

### 3.5 Inclusion criteria

#### 3.5.1 For acquired drug resistance, individuals

i. Willing to participate in the study by giving consent/ assent
ii. Aged 18years and above
iii. Starting ART treatment

#### 3.5.2 For transmitted drug resistance, individuals

i. Aged 18 years and above
ii. Newly diagnosed with HIV
iii. Has lived in Kisii county for a minimum of a year

### 3.6 Exclusion criteria

#### 3.6.1 For acquired drug resistance individuals

i. Individuals on second line treatment
ii. Those with virological failure (≥1,000 copies/ml)
iii. Individuals without full mental capacity

#### 3.6.2 For transmitted drug resistance individuals

i. Record or self-report of previous positive HIV diagnosis
ii. Previous exposure to ARV drugs
iii. Any indication of AIDS like symptoms
iv. For women, more than one pregnancy
v. Individuals without full mental capacity

### 3.7 Sampling design

A consecutive sampling procedure was used in recruiting patients. This recruitment was conducted among HIV patients attending HIV comprehensive clinic at KTRH and Hospital VCT centre for the newly diagnosed patients.

### 3.8 Demographic data

A structured questionnaire (appendix i) was administered during consenting and recruitment of study participants (Appendix ii). The questionnaire was used to collect data on; gender, age and history of ART.

### 3.9 Collection of blood samples

Venous blood was collected for analysis. Briefly, using a sterile syringe and a needle, approximately, five millilitres of venous blood was collected into Ethylenediaminetetraacetic acid (EDTA) and another in a plain tube . Blood in EDTA tube was immediately used for CD4/CD8 estimation analysis while for the plain tube, was separated into vials for viral load and drug resistance genotyping. The samples were then packaged properly and transported in a cool box to centre for virus research (CVR) in Kenya Medical Research Institute (KEMRI) Nairobi, for subsequent analysis. The remnants of the samples after laboratory analysis were disposed off by incineration.

### 3.10 The CD4 T –lymphocyte count

The lymphocyte subsets were analysed using a FACSCalibur flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, California) with three monoclonal antibodies (BD Tritest CD4-FITC/CD8-PE/CD3-PerCP reagent) according to the manufacturer's instructions (Bosire *et al.,* 2013). Immunological failure was defined as failure to achieve a CD4 gain of at least 50 cells above baseline $6^{th}$ month post therapy levels or having an absolute CD4 count of less than 100 cells/mm3 after one year of therapy (Rusine *et al.,* 2013).

### 3.11 The HIV viral load detection

Plasma samples were collected at; baseline month zero, $6^{th}$ and $12^{th}$ months follow up and subjected to viral load tests using an automated m2000 Abbott Real Time HIV-1 assay system following manufacturer's protocol (Abbott Laboratories, Abbott Park, Illinois). Briefly, internal control RNA was added to 200 ml of plasma and loaded onto the m2000sp instrument for RNA extraction. The limits of detection

were ranged between 40 and 10,000,000 HIV-1 RNA copies. The undetectable VL (viral load) was reported as 39 copies of HIV RNA/mL while 10,000,001 copies of HIV RNA/mL as the reportable upper limit. This test served as both qualitative purposes for final diagnosis of acute HIV infection and quantitative for disease staging and ART enrolment (Srinivasulas *et al.,* 2011). Virological treatment failure was defined as a confirmed VL.1000 copies/mL at month 12 (Rusine *et al.,* 2013).

### 3.12 Nucleic acid (DNA) Extraction

Viral cDNA was extracted from whole blood using QIAamp ® DSP Virus Spin Qiagen Kit (Qiagen Inc., Basel, Switzerland) protocol according to the manufacturer's instructions (Clausen *et al.,* 2007). Briefly, 25 µl of Qiagen protease (QP) was pipetted into a 1.5 ml eppendorf tube LT. Then an equal volume of 200 µl of blood and buffer AL (containing a carrier RNA) were added to the Qiagen protease reagent in the test tube. It was then mixed by pulse-vortexing for 15 seconds and incubated at 56°C for 15 minutes in a heating block. After incubation, the mixture was briefly centrifuged to remove any drops from the inside of the lid and 250 µl of absolute ethanol added and pulse vortexing for 15 seconds and incubated for 5 minutes at room temperature(15-25°C).Centrifugation was done in order to remove all drops from inside the lid. All the lysate was carefully transferred onto the QIAamp MinElute column in a wash tube (WT) without wetting the rim and centrifuged at 6000 x g for 1 minute and the filtrate discarded.

The QIAamp MinElute column was placed into a clean 2 ml collection tube and 500 µl of wash buffer (Buffer AW1) added and centrifuged at 6000 x g for 1 minute. The wash tube containing the filtrate was discarded and replaced with a clean 2ml wash tube and 500 µl of wash buffer (Buffer AW2) added and centrifuged at 6000 x g for 1 minute and the filtrate discarded. Five hundred microliters of absolute ethanol (96-100%) was added without wetting the rim and centrifuged at 6000 x g for 1 minute, and the filtrate discarded. The QIAamp MinElute was placed in a clean 2ml wash tube (WT) and centrifuged at full speed (20,000 x g; for 3 minutes to dry the membrane. QIAamp MinElute was placed in a new 2ml wash tube (WT) to air dry by leaving the lid open and incubated at 56°C in a heating block for 3 minutes. The

wash tube containing the filtrate was discarded and the QIAamp MinElute column placed in an elution tube (ET). DNA was eluted with a 120µl of elution buffer (Buffer AVE) for 5minutes. This was then centrifuged at full speed (20,000 x g; 14,000 rpm) for 1 minute to elute the viral DNA. Extracted DNA was kept at -80$^{o}$C till use (Burchard *et al.,* 2014).

### 3.13 DNA Quantification by Qubit 3.30 Fluorometer

The DNA quantification was done according to manufacturer's instructions (Qiagen Company Netherlands) (Dash *et al.,* 2020). Briefly, into three different 0.5ml eppendorf tubes containing 10 µl of upper limit DNA buffer, lower limit buffer controls and test eppendorf tube with test 10 µl DNA were prepared. To each tube, 190 µl of working solution was then added to make a final volume of 200 µl, which was incubated at room temperature for two minutes. Finally, the quantification of DNA was measured in ng/ µl and concentration determined based on lower and upper limit of the controls reads (Figure 3.2) (Sathiamoorthy *et al.,* 2018).

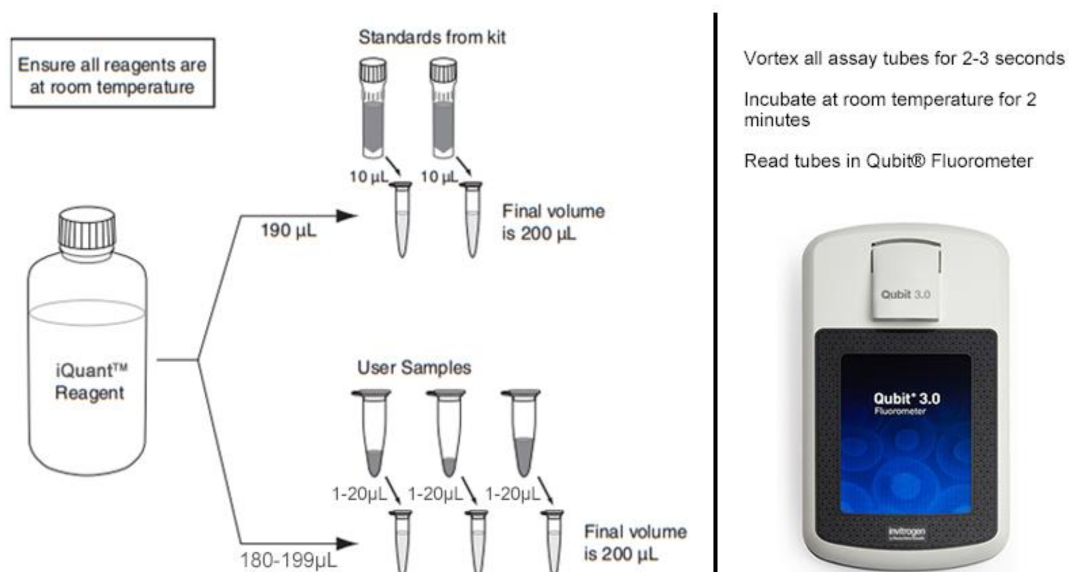


**Figure 3.2: A flow chart of Viral Quantification** (Sathiamoorthy *et al.,* 2018)

### 3.14 Amplification of HIV-1-*pol* gene

A part of HIV-1 *pol* gene reverse transcriptase of 692bp (corresponding to 2265–3180 nt in HIV-1 $_{HXB2}$) was amplified using the following PCR conditions; master mix 12.5 µl, 1ng of DNA 5 µl, water 6.5 µl and both 1µM each of forward and reverse primers 0.5µl each; primers RT18 (5'-GGAAACCAAAAATGATAGGGGGAATTGGAGG-3') and KS104 (5'-TGACTTGCCCAATTTAGTTTTCCCACTAA-3') in the first round and KS101 (5'-GTAGGACCTACACCTGTTCAACATAATTGGAAG-3') and KS102 (5'-CCCATCCAAAGAAATGGAGGAGGTTCTTTCTGATG-3') in the second round using the same PCR conditions. Amplification was achieved using 1 cycle of 95°C for 10 min and 35 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for one minutes, with a final extension of 72°C for 10 minutes (Nyamache *et al.,* 2011; Lin *et al.,* 2017).

### 3.13.1 Amplification of HIV-integrase gene

A part of the HIV-1 *pol* gene covering 288 bp of the integrase (corresponding to 4493–4780 nt in HIV-1 $_{HXB2}$) was amplified using the following PCR conditions; master mix 12.5 µl, 1ng of DNA 5 µl, water 6.5 µl and both 1µM forward and reverse primers 0.5µl each; primers Unipol5 ($5^1$TGGGTACCAGCACACAAAGGAATAGGAGGAAA-$3^1$) and Unipol6 ($5^1$CCACAGCTGATCTCTGGCCTTCTCTG TAATAGACC-$3^1$) in the first round and Unipol1 ($5^1$-AGTGGATTCATAGAAAGCAGAAGT-$3^1$) and Unipol2 ($5^1$CCCCTATTCCTTCCCCTTCTTTTAAAA-$3^1$) in the second round using same conditions. Amplification conditions were similar, with a hot start at 95°C followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 45°C for 30 seconds, and extension at 72°C for 1 minute and a final extension of 72°C for 7 minutes (Khamadi *et al.,* 2005; Nyamache *et al.,* 2012).

### 3.13.2 Amplification of HIV-envelope C2V3 gene

A part of the HIV-1 group M *env* gene covering the C2V3 region of 550bp (corresponding to 6975–7520 nt in HIV-1 $_{HXB2}$) was amplified by nested polymerase

chain reaction (PCR) using conditions; master mix 12.5 µl, 1ng of DNA 5 µl, water 6.5 µl and both 1µM forward and reverse primers 0.5µl each; M5 (5$^{l}$CCAATTCCCATACATTATTGTGCCCCAGCTGG-3$^{1}$) and M10 (5$^{1}$CCAATTGTCCCTCATATCTCCTCCTCCAGG-3$^{1}$) in the first round and M3 (5$^{1}$-GTCAGCACAGTACAATGCACACATGG-3$^{1}$) and M8 (5$^{1}$-TCCTTGGATGGGA GGGGCATACATTGC-3$^{1}$) in the second round (Nyamache *et al.,* 2013). Amplification was done in both first and second PCR using the same conditions: one cycle of 95°C for 10 minutes and 35 cycles of 95°C for 30seconds,annealing temperature of 55°C for 30 seconds, and 72°C for 1minutes with a final extension of 72°C for 10 minutes (Lengauer *et al.,* 2007; Nyamache *et al.,* 2013).

### 3.14.3 Analysis of PCR products by gel electrophoresis

Immediately after the 2$^{nd}$ PCR ended, these products were loaded for visualization using UV transilluminator (UVP, San Gabriel, A, USA) after staining the amplified DNA with ethidium bromide 3 µL. The products were then allowed to separate for 45 minutes at 100 volts. The expected average molecular weight for *reverse transcriptase* gene (692bp), *envelope* 550bp, and *intergrase gene* 288bp (Khamadi *et al.,* 2005; Lihana *et al.,* 2009a; Nyamache *et al.,* 2012; 2013).

### 3.14.4 Sequencing PCR and amplicons purification

The potential drug resistant mutations in the target viral genes were identified by analysing sequences. Sequencing was done through the following process; sequencing the purified PCR products using the Big Dye terminator v3.1 cycle sequencing kit (Applied Biosystems). Briefly, from the 2$^{nd}$ PCR purified products, direct sequencing was performed using specific primers of nested second PCR. The sequencing PCR was carried out with reaction mixture of 20 µL containing 3 µL of DNA, 1.5µL 5X sequence buffer, 2.0 µL BigDye, 10.5 µL of distilled water, and 1.5µL of forward and reverse primers. The amplification was then carried out in a GeneAmp 9700 thermal cycler (MJ Research, Inc.) in a reaction (denature for 5minutes at 96°C, and again for 10seconds at 96°C, anneal at 50˚C for 5seconds, final extension 60˚C 4minutes for 25 cycles). The products were finally purified by

use of QIAquick® PCR purification kit (Qiagen GmbH, Hilden Germany), from the purified products in a pellet, 20 μL of template suppressor reagent was added and heated at 95% for 3minutes then the contents were directly sequenced using an automated ABI 3100 sequencer (Applied Bio systems, Foster City, CA) (Khamadi *et al.,* 2005; Nyamache *et al.,* 2013) .

### 3.15 Analysis of DNA sequences

Generated sequences were assembled and edited with Seqman™II v7.0 and Bio Edit v7.0 and aligned using MAFFT v6. The subtypes and recombinant forms were determined by phylogenetic analysis. This Phylogenetic analysis was performed with neighbour-joining method, based on Kimura´s two-parameter distances, using MEGA 7.0. The HIV-1 Drug resistance was determined using Stanford HIV drug resistance database and confirmed by International AIDS Society (IAS) algorithm. Co-receptor usage was done using *in-silico* viral tropism with online tools: webPSSM http://indra.mullins.microbiol.washington.edu/, CoRseqV3-C, ds Kernel, Geno2Pheno with false positive 5% [coreceptor] http://coreceptor bioinf.mpi-inf.mpg.de/, and Kernel http://genome.ulaval.ca/hiv-dskernel software (Nyamache *et al.,* 2013).

### 3.16 Data analysis

To determine if the HIV subtypes had any difference in co-receptor usage, two sample T test was used. One-way ANOVA was used to determine difference in co-receptor usage across used *insilico* tools with their means homogenized across subsets using Tukey's HSD test. A 2 tailed Pearson correlation test was used to determine impact of CD4 on co-receptor usage across the four *insilico* tools used (geno2pheno, WEB-PSSM, ds Kernel and CORseq tools), in both categorized and uncategorized data. A 2 tailed Pearson's correlation test was used to determine treatment impact on co-receptor usage using unstandardized coefficient.

### 3.17 Ethical considerations, Care and protection

This study was approved by the Kenyatta University Ethics Review Committee with approval number PKU/716/E73 (appendix III). In this study, patients benefited from free prior tests on; CD4 counts, viral load and drug resistance testing. Those who were found failing treatment by persistent increase in viral load above 1000copies/ml, a class switch and second line drugs were suggested for them. The participants' samples were labelled using unique identification numbers and records were kept in the lock and key drawers.

# CHAPTER FOUR

# RESULTS

## 4.1 Demographic characteristics of HIV patients visiting Kisii Teaching and referral Hospital Comprehensive Care Clinic for treatment management

A total of 226 participants took part in the study. They consisted 113 drug experienced and 113 drug naïve participants. For drug experienced participants, 68 (60.2%) were female while 45 (39.8%) were male. Out of 113 drug experienced patients 54 (47.8%) were married with 3 (2.7%) as widowers. The levels of education showed that most had college level of education with almost none without any formal of education. These participants age ranged between 18 and 68 years (Table 4.1). In addition, for those who participated in transmitted drug resistance arm, 47 (41.6%) were males while 66 (58.4%) were females. Their age ranged between 18 and 75 years. Out of 113 HIV-1 naïve participants sampled, 40 (35.4%) were aged between 18 and 30 years (Table 4.1).

**Table 4.1: Demographic characteristic of HIV infected patients seeking treatment at Kisii Teaching and Referral Hospital**

| Patients' Characteristics | | |
|---|---|---|
| **Gender** | Experienced n (%)113 | Naïve n (%) 113 |
| **Male** | 45(38.8) | 47(41.6) |
| **Female** | 68(60.2) | 66(58.4) |
| **Marital Status** | | |
| **Single** | 41(36.3) | 43(38.1) |
| **Married** | 54(47.8) | 52(46) |
| **Divorced** | 5(4.4) | 2(1.8) |
| **Separated** | 4(3.5) | 1(0.9) |
| **Widow** | 6(5.3) | 10(8.8) |
| **Widower** | 3(2.7) | 5(4.4) |
| **Age (years)** | | |
| **Range** | 18-68 | 18-75 |
| **mean(±SD)** | 33.5 (SD: 13.04) | 35.09 (SD: 10.87) |
| **18-30** | 33 (29.2) | 40(35.4) |
| **31-40** | 34 (30.1) | 37(32.7) |
| **41-50** | 30(26.5) | 25(22.1) |
| **51-6 0** | 11(9.7) | 7(6.2) |
| **>60** | 5(4.0) | 4(3.5) |
| **Education levels** | | |
| **Primary level** | 12 (10.6) | 17(15) |
| **Secondary** | 37 (33.6) | 36 (32.7) |
| **Tertially/ college** | 45 (39.8) | 42 (38.1) |
| **Universities** | 18 (15.9) | 16 (14.2) |
| **None** | 1 (0.9) | 2 (1.8) |

## 4.2 The CD4 counts

From the blood samples of 74 participants that were successfully analysed for CD4 count, their mean 730.5 (SD-190.9) cells/mm3 was obtained with most of the participants 73 (98.6%) having their CD4 count above 301cells/mm3.

In addition, from the longitudinal study of 90 participants who were successfully analysed, the CD4 count estimation at $6^{th}$ and $12^{th}$ months intervals respectively. The mean CD4 count at baseline was 418.23 (SD=186.5) and 627.3 (SD=174.3) at $12^{th}$ month interval. Most participant 38 (42.2%) had their CD4 count ranging between 201-300 cells /mm3 (Table 4.2). From the patients on treatment, eight patients had virological failure (>1000cps/ml). A comparison on gender and age in relation with the CD4 counts was done. From the analysis, neither gender ($p$=0.576) nor the age ($p$=0.844) had any significant relationship with the levels of CD4 counts (Table 4.2).

**Table 4.2: Immunological and virological characteristic of HIV infected patients visiting Kisii Teaching & Referral Hospital HIV care clinic**

| | Drug naïve n=74 | | | Drug experienced n=90 | | |
|---|---|---|---|---|---|---|
| | **ALL** | **F** | **M** | **ALL** | **F** | **M** |
| **Gender** | n=74 (%) | n(44) | n(30) | n= 90(%) | n(49) | n(41) |
| **Mean age (years)** | | 30 | 38 | 33.54(SD: 13.04) | 30.5 | 40.2 |
| **CD4 Counts (Cells/mm3)** | 730.5 (SD-190.9) | | | | | |
| **Mean (SD)** | | | | | | |
| **<200** | 1(1.4) | 1 | 0 | 13(14.4) | 5 | 8 |
| **201-300** | 6(8.4) | 3 | 3 | 38 (42.2) | 24 | 14 |
| **301-400** | 18 (23.6) | 13 | 5 | 11(12.1) | 7 | 4 |
| **401-500** | 23(31.9) | 14 | 9 | 16(18) | 7 | 9 |
| **>500** | 26(34.7) | 14 | 15 | 12(13.3) | 6 | 6 |
| **Baseline** | 35.09 (SD:10.9) | 713.2 (SD212.6) | 751.5 (SD 161.6) | 418.23 (SD=186.5) | | |
| **6th month** | | | | 616.2 (SD=230.9 | | |
| **12th Month** | | | | 627.3(SD 174.3) | 634.6(SD 176.9) | 622.1 (SD 175.7) |
| **Mean Viral Load (cps/ml)** | | | | | | |
| **Baseline** | | | | 406.5(SD=44.7) | | |
| **6th Month** | | | | 526.8(SD=52.3) | | |
| **12th month** | | | | 446.5 SD=56.6) | | |
| **Virological failure (>1000cps/ml)** | | | | 8 | 3 | 5 |

**KEY**: F: Female, M: Male

### 4.3 The HIV-1 Genetic Diversity

A total of 448 sequences were generated from this study consisting; *env-C2V3* 164(36.6%), HIV-*pol-RT* 155(34.6%), HIV-pol *integrase* 129(28.8%). Most of the study subjects were infected with HIV-1 A1 (71%) subtypes, followed by HIV-1 subtype C (14.2%),HIV-1 subtype D (12.5%), and low proportions HIV-1 subtype G (1.5%), A2 (0.4%) and CRFs; A2U and 02A1 each (0.2%) (Table 4.3).

**Table 4.3: HIV-1 genetic diversity based on HIV (*RT, env* & *integrase* genes) among HIV infected patients visiting Kisii Teaching & Referral Hospital for treatment**

| HIV-1 Subtypes | *env* n=164 (%) Naïve/exp | Drug naïve (RT) n=74(%) | Drug exp (RT) n=81(%) | Integrase n=129(%) Naïve/exp | Total sequences n(448) | (%) |
|---|---|---|---|---|---|---|
| A1 | 113(68.9) | 48 (64.8) | 50 (61.7) | 106(82) | 317 | 71 |
| D | 19(11.6) | 10 (13.5) | 17(21) | 10(8) | 56 | 12.5 |
| C | 25(15.2) | 15(20.3) | 11(13.7) | 13(10) | 64 | 14.2 |
| G | 5(3.1) | 1(1.4) | 1(1.2) | N | 7 | 1.5 |
| A2U | 0(0) | 0(0) | 1(1.2) | N | 1 | 0.2 |
| A2 | 2(1.2) | 0(0) | 0(0) | N | 2 | 0.4 |
| 02A1 | 0(0) | 0(0) | 1(1.2) | N | 1 | 0.2 |

N: None

#### 4.3.1 Phylogenetic analysis of the reverse transcriptase gene

Alignment of 155 sequences with references from Los Alamos database and phylogenetic analysis revealed nine main clusters (Figure 4.1 and 4.2). The distribution of HIV-1 subtypes was; A1 98(63.3%), D 27(17.4%), C 26(16.8%), G 2(1.3%), A2 1(0.6%) and A2U 1 (0.6%) at bootstrap values above 75% (Figure 4.1).

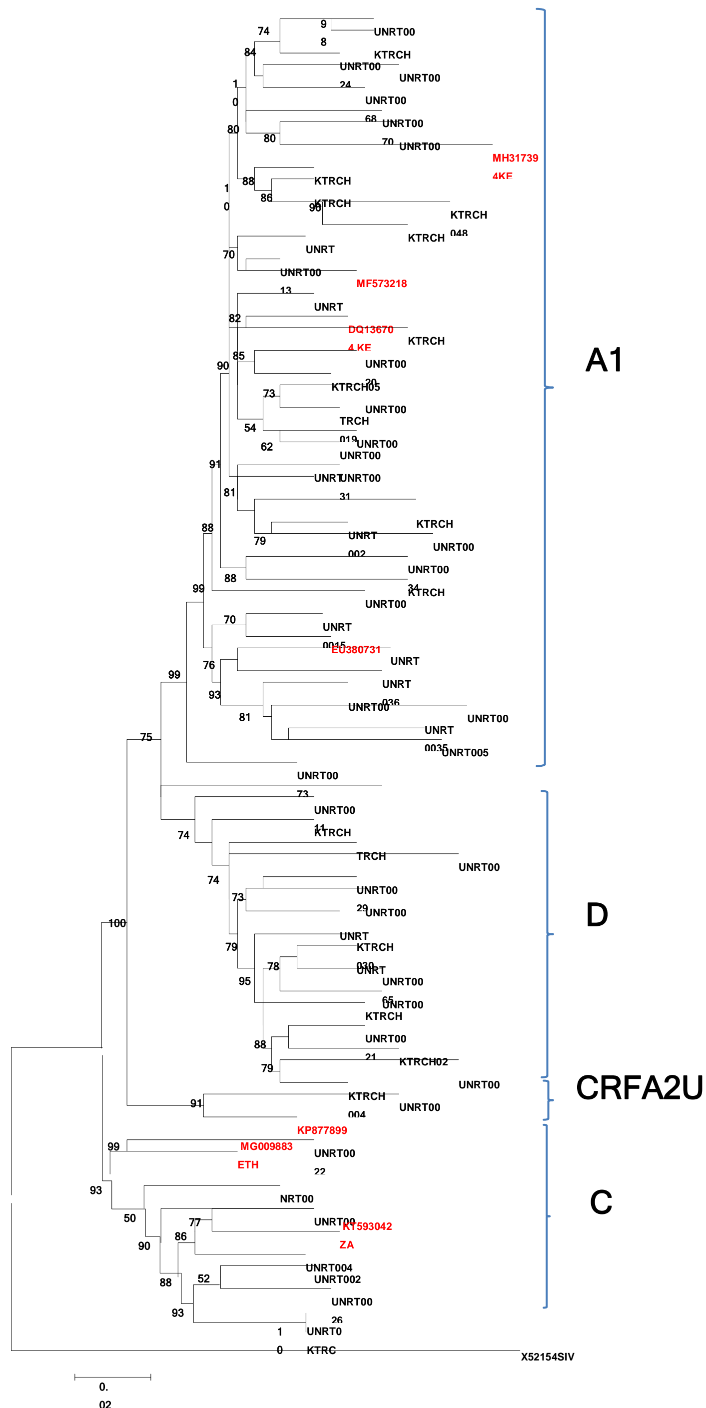


**Figure 4.1: Phylogenetic tree of RT sequences generated from sampled drug experienced participants from Kisii.** Neighbor-joining method based on 1,000 bootstrap replicates was used. The sequences from Kisii are shown in black while reference sequences are in red represented by accessory numbers.

Consequently, from the second phylogenetic tree (Figure 4.2) analysis, the detected HIV-1 subtypes A, A2, D C and CRF02A1 were aligned with references sequences from neighborhood region (Figure 4.2).

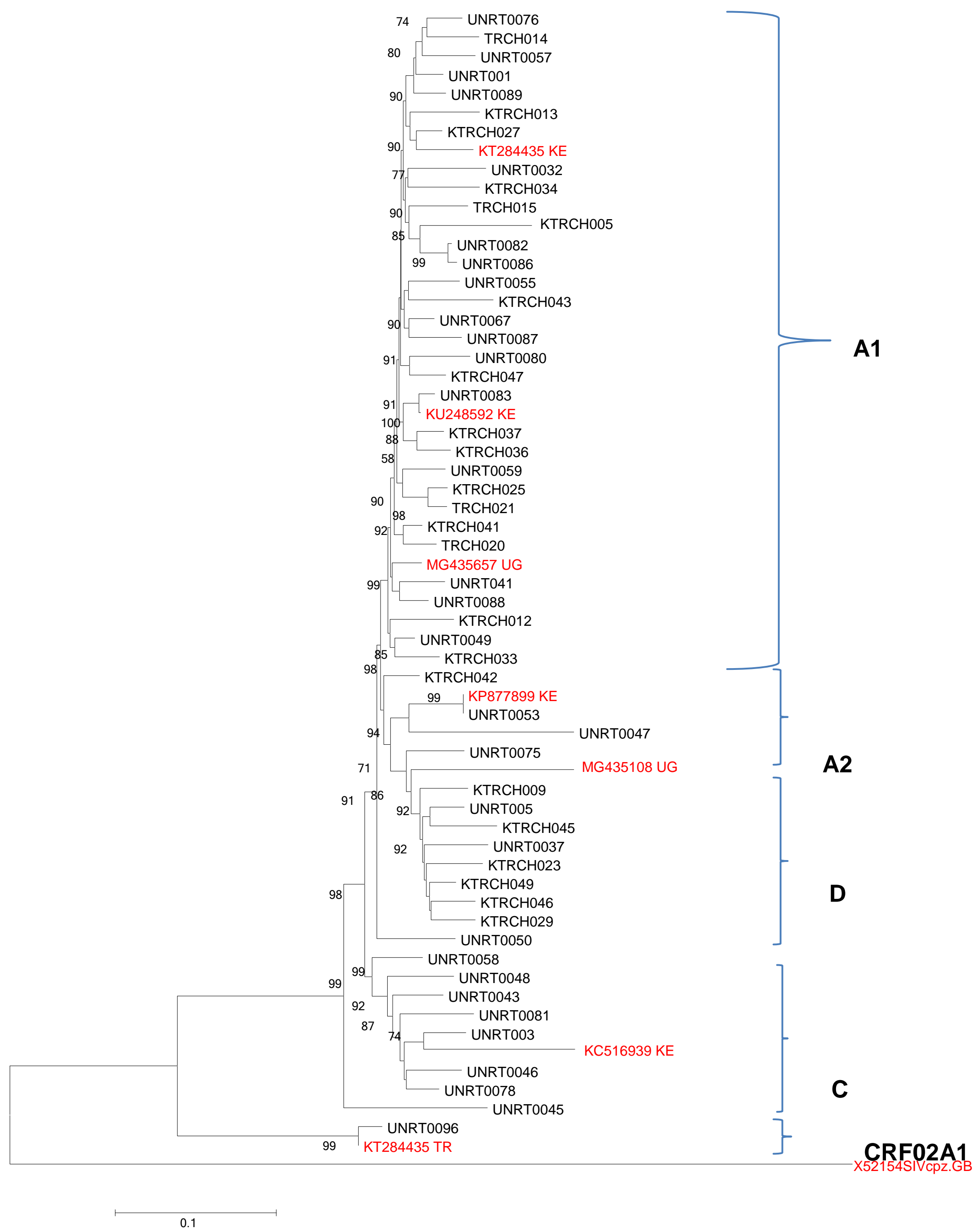


**Figure 4.2: Phylogenetic tree of RT sequences generated from sampled drug naive participants from Kisii**. Neighbor-joining method based on 1,000 bootstrap

replicates was used. The sequences from Kisii are shown in black while reference sequences are represented by accessory numbers

### 4.3.2 HIV-1 genetic diversity based on *env* gene among HIV infected individuals attending Comprehensive Care Clinic in Kisii Teaching and Referral Hospital

From a total of 164 *env* sequences phylogenetically analysed, seven clusters were obtained. The analysis revealed HIV-1 subtypes A1 (68.9%), C (15.2%), D (11.6%) and A2 (1.2%) (Figures 4.3-4.4). From the phylogenetic analysis, it revealed HIV-1 subtypes that clustered with references sequences from neighborhood countries (Figure 4.3).

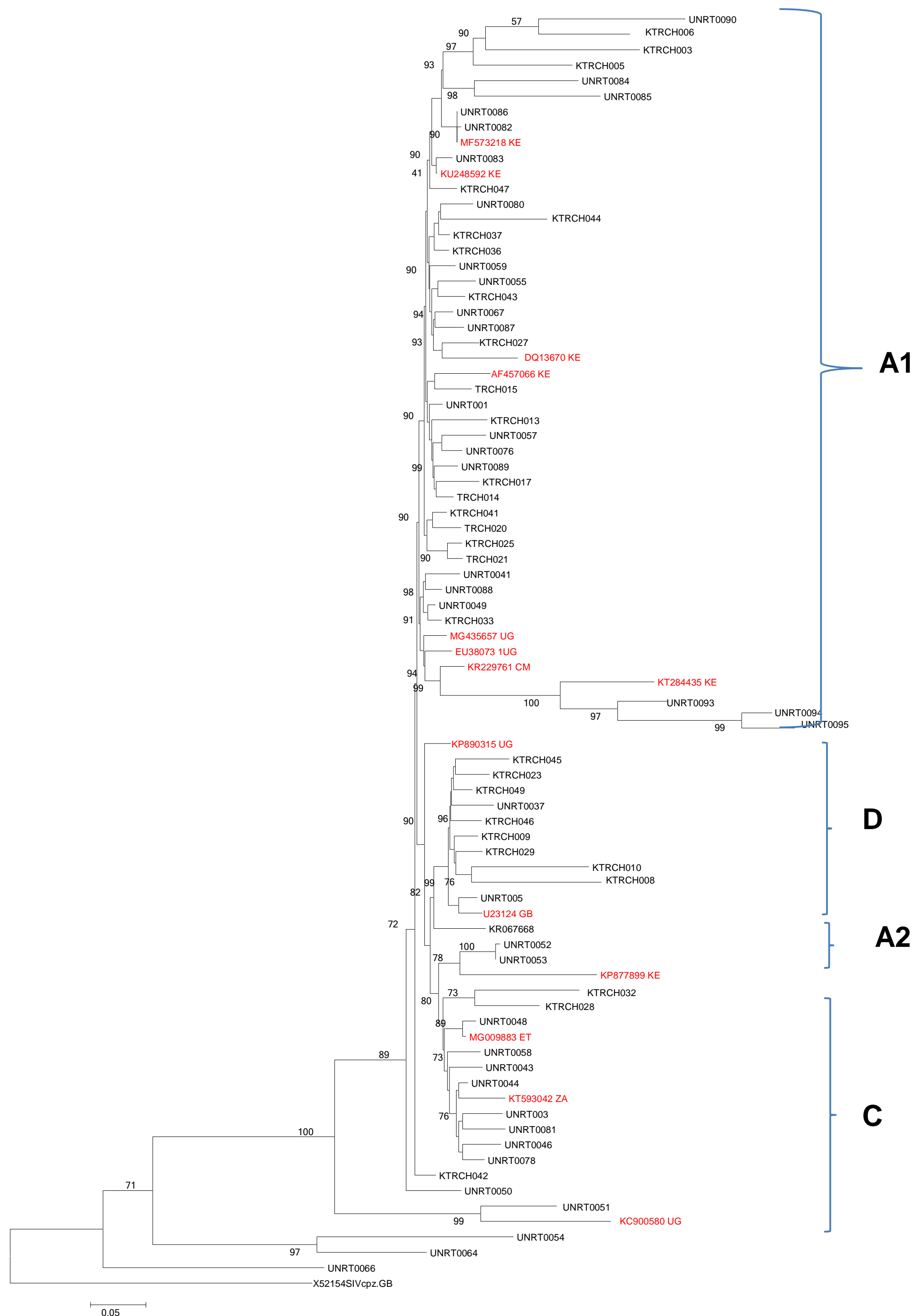


**Figure 4.3: Phylogenetic tree of HIV-1 C2V3 sequences generated from drug experienced study participants from Kisii.** Neighbor-joining method based on 1,000 bootstrap replicates was used. The sequences from Kisii are shown in black while references sequences in red represented by accessory numbers.

Alignment and phylogenetic analysis clustered the sequences into three groups at over 70% bootstrap values. From the phylogenetic analysis, the revealed HIV-1 subtypes were clustered with reference sequences (Figure 4.4).

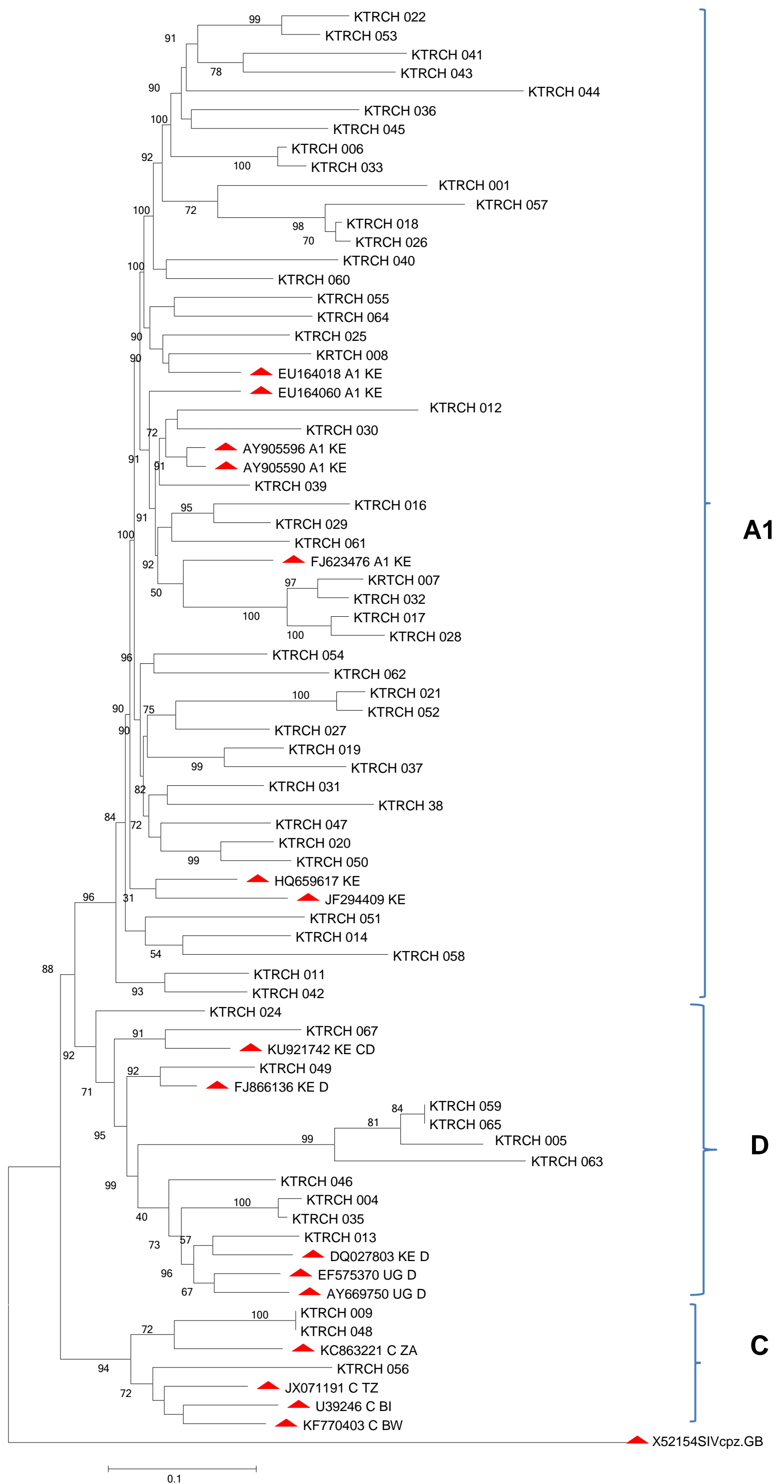


**Figure 4.4: Phylogenetic analysis of HIV-1 C2V3 sequences from drug naïve study participants from Kisii.** Neighbor-joining method based on 1,000 bootstrap

replicates was used.The sequences from Kisii are shown in black while references are in red symbol represented by accessory numbers.

### 4.3.3 HIV genetic diversity based on integrase gene among HIV infected individuals attending Comprehensive Care Clinic in Kisii Teaching and Referral Hospital

A total of 129 integrase sequences, were generated, aligned and phylogenetically analysed. The distribution of HIV-1 subtypes were; A1 (82%), C (10%) and D (8%) (Figures: 4.6 and 4.7).  The analysis showed three clusters at bootstrap values above 70%. This consisted for HIV-1 subtype A1, C and D clustering with references sequences obtained from African Neighbor countries (Figure 4.5).

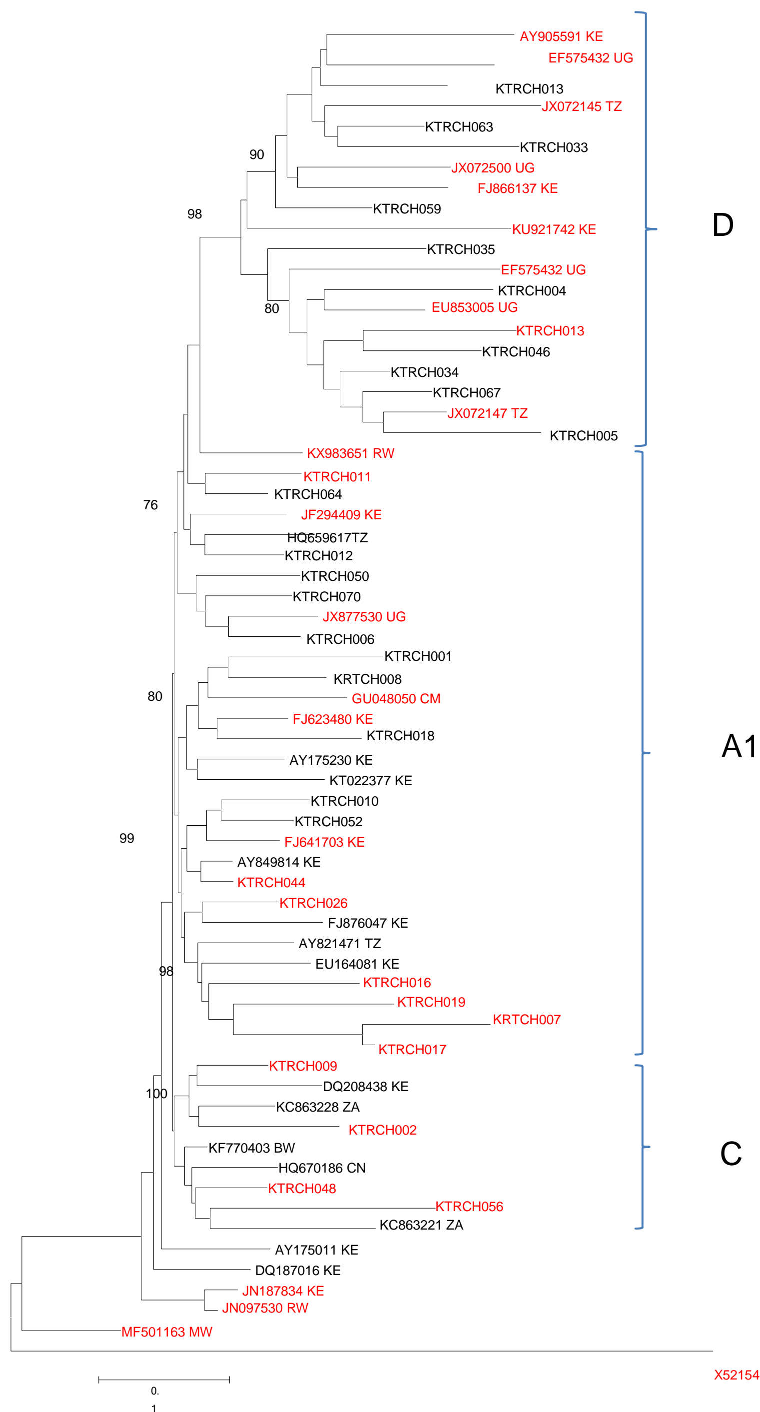


**Figure 4.5: Phylogenetic tree of the HIV -1 pol -integrase sequences of samples collected from study participants from Kisii.** Neighbor-joining method based on 1,000 bootstrap replicates was used. Kisii sequences are shown in black while the references are in red represented by accessory numbers.

From these analyses, the sequences aligned into four clusters of HIV subtype A1, C and D with reference sequences at above 70% bootstrap values (Figure 4.6).

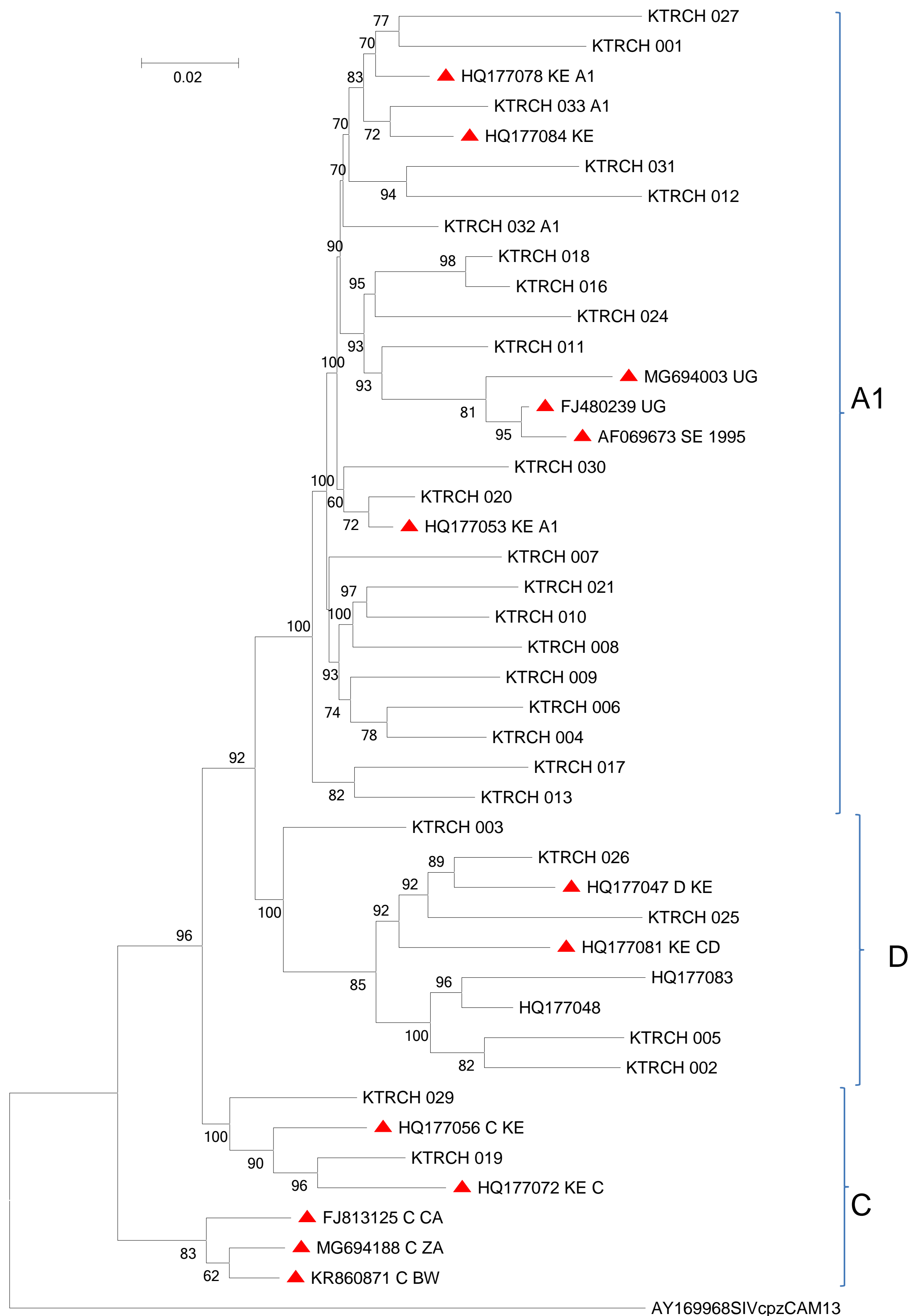


**Figure 4.6: Phylogenetic tree of the HIV -1 pol -integrase sequences of samples collected from Kisii study participants.** Neighbor-joining method based on 1,000 bootstrap replicates

## 4.4 Transmitted HIV-1 drug resistance among drug naïve individuals seeking treatment at Kisii Teaching and Referral Hospital

A total of 74 samples were analysed for transmitted drug resistance in this study. Overall TDR prevalence of 6(8.1%) was detected. The distribution of class specific TDRMs in the six persons were quantified showing diverse grades of predicted drug resistance ranging from potential low to high-level resistance (Table 4.4). Low to high level Nevirapine (NVP)/Efavirenz (EFV) and Lamivudine (3TC) resistant mutations were detected. Four (6.7%) had one class mutation; NNRTI TDR and one (1.4%) person with NRTI TDR mutations. Class specific transmitted drug resistance (TDR) prevalence was 4(6.8%) (NNRTI) and 1(1.7%) (NRTI) drugs. In total six TDRM were observed in reverse transcriptase gene. The dominant TDRM in the RT gene was K238T 3 (50%) and 1(1.7%) each for V106A, M184V, G190A and V118I mutations were detected (Table 4.4).

**Table 4.4: Prevalence of transmitted HIV-1 drug resistance mutations among drug naïve individuals visiting Kisii Teaching and referral Hospital HIV care clinic**

| TDR Mutations | n=74 (%) | Drugs Affected | | |
|---|---|---|---|---|
| | | **NNRTI** | **NRTI** | **PI** |
| None | 68 (91.9) | - | - | - |
| K238T | 3(4) | NVP , EFV | - | - |
| V106A/M | 1(1.4) | NVP , EFV | - | - |
| V118I | 1(1.4) | NVP , EFV | - | - |
| M184V,G190A | 1(1.4) | | 3TC | - |
| | | NVP , EFV | - | - |
| **Overall TDR Prevalence** | **6 (8.1%)** | | | |

**NVP:** nevirapine; **3TC:** lamivudine; **EFV:** efaverenz; **NRTI:** Nucleoside reverse transcriptase inhibitors; **NNRTI**: Non-Nucleoside Reverse Transcriptase Inhibitors

## 4.5 Acquired HIV Drug Resistance among drug experienced patients visiting Kisii Teaching and referral Hospital HIV care clinic

### 4.5.1 Prevalence of NRTI Mutations among drug experienced patients

From the recruited 113 participants, 80% (n=90) of the participants were successfully monitored till the end of the study. All 81 participants whose genotypic drug resistance was successfully analysed, 8(9.9%) had a virological failure. These patients were therefore advised for further three months follow up for persistent virological failure before being advised to switch to second line supported by developed drug resistance mutations. Drug combinations of these patients were; EFV/TDF/3TC 24(29.6%), EFV/AZT/3TC 21(25.9%), EFV/DDI/3TC 1(1.2%) for Efaverenz combinations while NVP/TDF/3TC 9(11.1%), NVP/DDI/ABC 12(14.8%) and NVP/AZT/3TC 14(17.3%). Those who had virological failure were further followed for another three months for persistent virological failure supported by detected drug associated mutations. Of the 81 patients whose drug resistance were analysed, 56(69%) had developed drug resistance mutations. The most common NRTI mutation M184V 12(23.5%) was detected in 56 patients. Mutations K70R 10(17.9%) was second common detected thymidine analogue mutation (TAM) followed by T215Y 8(14.3%) and D67N 7(12.5%) mutations occurring among patients who were on AZT and DDI treatment, respectively. For Nucleoside analogue mutations (NAMs), K65R mutation was detected in 5(8.9%) patients. These mutations occurred among patients who were either on AZT or DDI. There is no patient that was detected with both NAMs and TAMs mutational pathways. In addition, other mutations; V179I 12(14.8%), K238T 9(11%), Y115F 4 (4.9%), 69N 4(4.9%), 100I 2(2.5%), L74V 2(2.5%), V75M 2(2.5%) and 1.25% each for F77L, 62V, 77L, F221L, Q151M were also detected (Table 4.5).

**Table 4.5: Frequency of NRTIs drug resistance mutations among patients on HAART treatment attending Kisii Teaching and referral Hospital HIV care clinic**

| ARV Regiments | n /81 | NAMs | TAMs | | | | | OTHERS | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **ARV regimens** | | **K65 R** | **M184 V** | **T215 Y** | **K70 R** | **D67 N** | **K219 E** | **K238 T** | **Y115 F** | **V179 I** | **69 N** | **77 L** | **100 I** | **L74 V** | **F221 L** | **V75 M** | **Q151 M** |
| EFV/TDF/3TC | 24 | | 1 | 1 | - | - | - | 3 | - | - | - | - | - | - | - | - | - |
| EFV/AZT/3TC | 21 | 2 | 4 | 3 | 5 | 4 | 6 | 1 | 2 | 6 | 1 | 1 | 2 | - | - | - | - |
| EFV/DDI/3TC | 1 | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Subtotal** | **46** | **3** | **5** | **4** | **5** | **4** | **6** | **4** | **2** | **6** | **1** | **1** | **2** | | | | |
| NVP/TDF/3TC | 9 | | 2 | 2 | 1 | 1 | | 2 | - | - | - | - | - | 1 | - | 2 | 1 |
| NVP/AZT/3TC | 14 | 1 | 1 | 1 | 1 | | | 2 | | 1 | - | - | - | - | - | - | - |
| NVP/DDI/ABC | 12 | 1 | 4 | 1 | 3 | 1 | 3 | 1 | 2 | 3 | 3 | 1 | - | 1 | 1 | - | - |
| **Subtotal** | **35** | **2** | **7** | **4** | **5** | **2** | **3** | **5** | **2** | **4** | **3** | **1** | - | - | - | - | - |
| **Total** | **81** | **5** | **12** | **8** | **10** | **6** | **9** | **9** | **4** | **10** | **4** | **2** | **2** | **2** | **1** | **2** | **1** |

**Key:** ABC: abacavir, 3TC: lamivudine, TDF: Tenofovir, DDI: Didanosine, AZT: Zidovudine, EFV: Efavirenz, NVP: Nevirapine

### 4.5.2 Prevalence of NNRTI mutations among drug experienced patients visiting Kisii Teaching and referral Hospital HIV care clinic

From the analysed samples, non-nucleoside reverse transcriptase inhibitors (NNRTI) mutational profiles were also detected. From the 56 samples, mutation K103N was the most common NNRTI mutations detected 10(12.3%) and frequently occurring among patients on EFV 5(60%) than NVP 2(40%) drug combination (Table 4.6).

### 4.5.2 Prevalence of NNRTI mutations among drug experienced patients visiting Kisii Teaching and referral Hospital HIV care clinic

From the analysed samples, non-nucleoside reverse transcriptase inhibitors (NNRTI) mutational profiles were also detected. From the 56 samples, mutation K103N was the most common NNRTI mutations detected 10(12.3%) and frequently occurring among patients on EFV 5(60%) than NVP 2(40%) drug combination (Table 4.6).

**Table 4.6: Frequency of NNRTIs drug resistance mutations among patients on HAART treatment attending Kisii Teaching and referral Hospital HIV care clinic**

| ARV Regiments NNRTI /NRTI | n/81 | K103 N | E138 A | Y181 C | V75 I | V190 I | A98 G | K101 E | F122 L | Y188 H | V108I I | V106 A | V118I I | T238 N | 230 L | 90 I | 236 L | M41 L | 227 L | 179 D | 219 R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| EFV/TDF/3TC | 24 | 4 | 3 | 1 | - | 1 | - | - | - | - | - | 1 | - | - | - | 1 | - | - | 1 | - | 1 |
| EFV/AZT/3TC | 21 | 1 | - | 4 | - | 1 | 3 | - | 4 | 1 | 1 | 1 | 2 | 6 | 2 | - | 2 | 1 | 4 | 3 | - |
| EFV/DDI/3TC | 1 | 2 | - | - | - | - | - | - | - | 1 | - | 1 | - | - | - | - | - | - | - | - | - |
| NVP/TDF/3TC | 9 | 1 | 1 | - | 1 | 1 | | | 1 | - | - | - | - | - | - | - | - | - | 1 | - | - |
| NVP/AZT/3TC | 14 | 1 | - | 1 | | 1 | | 1 | 2 | - | - | - | - | - | 1 | - | - | - | 2 | - | - |
| NVP/DDI/ABC | 12 | 1 | 1 | 2 | 1 | 1 | 1 | - | 1 | - | 4 | 1 | - | - | 1 | 1 | 1 | - | 3 | - | - |
| **Total** | **81** | **10** | **5** | **8** | **2** | **5** | **4** | **1** | **8** | **2** | **5** | **4** | **2** | **6** | **4** | **2** | **3** | **1** | **11** | **3** | **1** |

**Key:** ABC: abacavir, 3TC: lamivudine, TDF: Tenofovir, DDI: Didanosine, AZT: Zidovudine, EFV: Efavirenz, NVP: Nevirapine

From the 12 months patients' monitoring, 8 (9.9%) of them had virological failure (viral load >1000 copies per ml). It was suggested that these patients be monitored for further 3 months for any viral load persistent above 1000 copies before advising for treatment switch to second line. These patients were infected with viral strains detected with drug resistance mutations. Five of these patients were infected with viral strains that had TAMs mutations with two of them having both TAMs and NAMs K65R mutations. The other three patients were infected with viral strains with K103N mutations (Table 4.7).

**Table 4.7: Occurrence of drug resistance mutations among virological failure patients on HAART treatment at 12 months attending Kisii Teaching and referral Hospital HIV care clinic**

| Viral Load cps/ml | NRTI associated mutations (TAMs/NAMs) | NNRTI | Others mutations |
|---|---|---|---|
| **1200** | M184V, K70R, K219Q/E, | - | |
| **1300** | K65R, M184V, K238T, | - | |
| **1010** | M184V, T215I, K70R, D67I, 219E,69N, | A98G, V108I ,Y181C, | |
| **1100** | - | K103N | |
| **1001** | 238T | K103N | |
| **1130** | M184V, K70R, T215V, D67I, K219E,69N | 90I, V108I,Y181C,236L, | |
| **1200** | K65R, M184V, Y115F,D67N, V75M, Q151M | V106A,V108I, F227L | |
| **1001** | M184V,T215Y,V75M | K103N | V118I, V132I |

## 4.6 HIV-1 Integrase Drug Resistance associated mutations among HIV infected individuals attending Kisii Teaching and Referral Hospital Comprehensive Care Clinic

HIV-1 drug resistance was defined as the presence of one or more resistance-related mutations based on consensus B subtypes (http:// hivdb.stanford.edu/) guided by consensus mutation guidelines of the International AIDS Society-USA. From the analysis, no primary mutations associated with reduced susceptibility to the integrase inhibitors Raltegravir and Elvitegravir were detected. However, about 28(22%) of the analysed integrase sequences had HIV-1 integrase drug associated mutations with reduced susceptibility to Raltegravir INSTIs (Table 4.8).

**Table 4.8: Frequency of HIV-1 integrase drug resistance associated mutations among HIV infected patients visiting Kisii Teaching and referral hospital Comprehensive Care Clinic**

| Integrase Mutations | Frequency 129(n) | % |
|---|---|---|
| **M50I** | 12 | 9.3 |
| **E157Q** | 1 | 0.8 |
| **L74M/I** | 1 | 0.8 |
| **T97A** | 2 | 1.6 |
| **G140S/A/C** | 2 | 1.6 |
| **S153YG** | 1 | 0.8 |
| **E92G/V/Q** | 3 | 2.3 |
| **125A** | 1 | 0.8 |
| **135V** | 1 | 0.8 |
| **288N** | 1 | 0.8 |
| **113V** | 2 | 1.6 |
| **D286F** | 1 | 0.8 |
| **Overall Prevalence** | **28** | **22** |
| **No drug associated mutations** | **101** | **78** |

### 4.7 HIV-1 tropism among HIV infected Patients attending Kisii teaching and Referral Hospital Comprehensive care Clinic for treatment

A total of 203 of samples were analysed and HIV C2V3 gene amplified, with 81% (n-164) successively analysed. Averagely from 164 HIV-1 *env* gene sequences that were analysed, 123 (75.6%) were CCR5 tropic, 39 (23.2%) were CXCR4 tropic while 2(1.2%) were duo R5/X4 (Table 4.9).

There was no significant difference in the viral tropism across circulating HIV subtypes in the sampled population (Table 4.9). Treatment with ARVs had no significant impact on co-receptor usage (Y=-0.507-0.324 geno2+0.520 PSSM +0.167ds-Kernel+0.083 coRseq). In addition, CD4 counts had no impact on viral tropism across the used tools, except for Web-PSSM and ds Kernel tools in uncategorized CD 4 counts category (Table 4.9).

**Table 4.9: HIV-1 tropism and influence of CD4, HIV-1 subtypes among patients seeking treatment in Kisii Teaching and referral Hospital HIV care clinic**

| HIV-1 Tropism | | R5 | R5/X4 | X4 | Female | Male | *p*= value |
|---|---|---|---|---|---|---|---|
| **T cell count** | | 124 | 2 | 38 | 85 | 79 | |
| | | | | | 652.7 | 656.7 | |
| **CD4 Mean** | 593.2 | | | | 600.6 | 589.9 | |
| **counts (SD)** | (SD=220.9) | | | | (SD=221. 8 | (SD=217.5) | |
| **≥500** | 108 | | | | | | 0.212 |
| **≥ 400-499** | 26 | | | | | | 0.061 |
| **≥ 201-399** | 25 | | | | | | 0.78 |
| **≥ 200** | 7 | | | | | | 0.319 |
| **Age (mean Range)** | | | | | 30.9 (18-69) | 38.8 (18-75) | |
| **HIV-1 subtypes** | | | | | | | |
| **A1 (113)** | 39 | 22 | | 17 | | | 0.012 |
| **Non A1 (51)** | 10 | 4 | | 6 | | | 0.052 |
| **Treatment** | | | | | | | |
| **Drug experienced** | 81 | | | | | | 0.37 |
| **naive** | 83 | | | | | | 0.37 |
| **Prediction algorithm** | | | | | | | |
| ***in-silico tools*** | | | | | | | |
| **Geno2pheno** | 124 | 84 | 2 | 38 | | | *p*=0.653 |
| **Web-PSSM** | 126 | 86 | 2 | 36 | | | |
| **ds-Kernel** | 123 | 84 | 3 | 38 | | | |
| **CoRSeqV3** | 118 | 78 | 2 | 44 | | | *p*=0.653 |

From the predictive tools used in analyzing viral tropism, there was no significant difference across the applied tools in predicting viral tropism $p = 0.653$ (Figure 4.7).

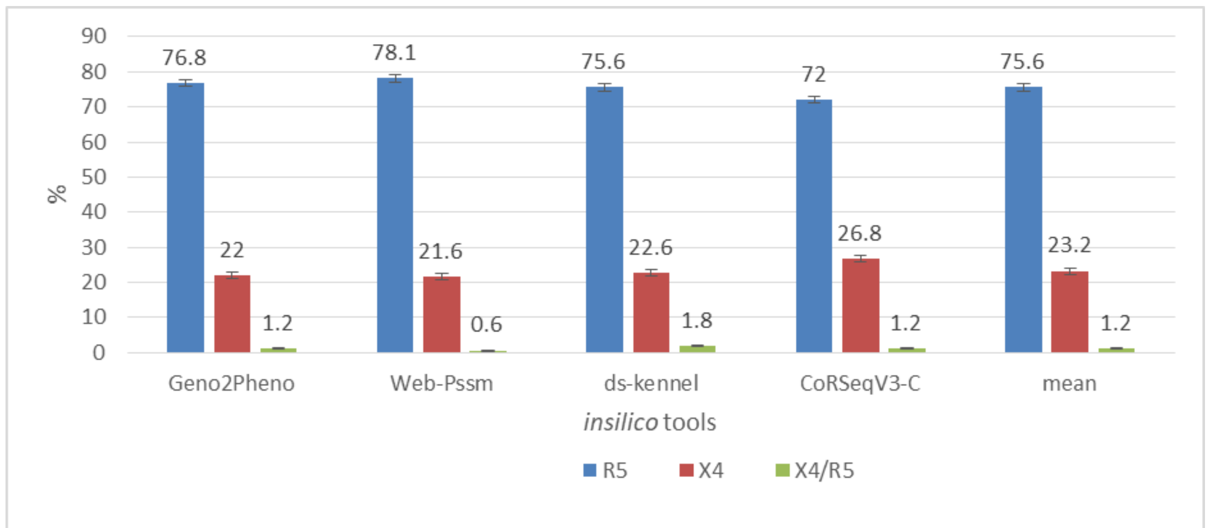


**Figure 4.7: *In silico* viral co-receptor tropism prediction of HIV-1 sequences using Geno2pheno $_{\text{(coreceptor)}}$ tool with a false positive rate of 15% .**

# CHAPTER FIVE

# DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Discussion

### 5.1.1 Distribution of HIV-1 subtypes in the studied population in Kisii County

The circulating HIV subtypes among HIV infected populations of Kisii County are divergent. In comparison to the rest of the regions of the country mainly Nairobi, Coast, Western, Northern parts of Kenya and partly in Rift valley and Nyanza, similar findings have been reported (Songok *et al.,* 2003; Khamadi *et al.,* 2005; Lihana *et al,* 2009a Nyamache *et al.,* 2013).

From the phylogenetic analysis, majority of the Kisii sequences were either related to East Africa reference sequence or Kenyan origin. These data therefore suggest that the HIV-1 epidemic in Kisii is likely to be predominantly of Kenyan and partly East African origin. From the analysis, the region was confirmed to have divergent circulating strains of HIV-1 similarly to what is shown in Eastern African countries (Billings *et al.,* 2017). HIV-1 subtypes A1, C and D therefore remains as the main subtypes causing epidemics in East Africa (Billings *et al.,* 2017). HIV -1 subtype A1 prevailed as the most frequent strain in circulation in this region (Figures 4.1-4.6). HIV-1 subtypes C, D and G were low in proportion with HIV-1 subtype, A2 and recombinant mosaic of CRFA2U occurring marginally. In this region, very low proportions of CRFs were detected showing low or non-independent transmission networks. This finding was consistent with previous studies conducted in other parts of country including East African countries where HIV-1 subtype A and D have remained predominant strains driving HIV epidemics in Kenya, Tanzania and Uganda ( Songok *et al.,* 2003; Khamadi *et al.,* 2005; Somi *et al.,* 2008; Ndembi *et al.,* 2009; Lihana *et al,* 2008; Ndembi *et al.,* 2011; Nyamache *et al.,* 2013; Hassan *et al.,* 2013; Lee *et al.,* 2014; Onywera *et al.,* 2017).

HIV-1 subtype A1 strains in this analysis clustered with reference sequences from within Kenya and its neighbourhood countries of East Africa; Uganda, Tanzania, and Rwanda indicative of transmission dynamics of the virus within Kenya from East African origin. Transmission of these viral strains could be linked to sexual networks by population migrating from one region of the country to another either on business, work or among area residents (Grabowski *et al.,* 2020). Kisii region has been reported to have high incidence of HIV infection (KCHASP, 2018). Drivers of HIV epidemics in this region have been linked to increasing levels of prostitution by sexual workers constituting either (female, male and transgender), casual heterosexual sex, some socio-cultural practices like “house entering” where widows exchange sexual partners, casual laborers, soapstone carvers, banana/sugarcane sellers, adolescents and young women (cross generational sex) and by virtue of HIV infected people getting stigmatized and discriminated (KCHASP, 2018).

Transmission of these infections has also been suggested to be associated with alcohol use as a risk factor. It has been reported that Kisii region has a lot of outlets of alcohol and residents often get drunk, which risk them to engage in risk sexual behavior following their impaired judgment (Wamalwa *et al.,* 2007). Some HIV-1 subtype A sequences suggested genetic relatedness with reference sequences from Uganda, Rwanda and Tanzania which could be associated with an extensive transport and commerce networks by road through Kisii to Uganda a region that has been noted to have high incidence of HIV infections (KCHASP, 2018). However, for those that clustered with reference sequences outside East Africa, including Sweden and Cameroon, this could be linked to Kenyans who travelled or migrated to these countries or residents from these countries could have been infected while visiting Kisii areas as tourist or business persons.

This study confirms the HIV-1 subtype C strains could be increasingly being spread in Kisii region. Compared with previous studies, the levels of HIV-1 subtype C has been reportedly shown higher increase than that of HIV-1 subtype D in the recent past (Tovanabutra *et al.,* 2010; Nyamache *et al.,* 2013). This trend has been shown to increase in proportion in Kenya like the rest of Sub Saharan Africa countries and beyond. Owing to its rapid replication and transmission efficiency compared to the

rest of subtypes, HIV-1 subtype C is dominant in Sub Saharan region (Nyamache *et al.,* 2013;'Cavalli *et al.,* 2018).

From the phylogenetic relatedness of sequences from this study, most of them were clustered with those from Kenyan origin indicative of local transmission (Lessells *et al.,* 2012). By virtual that HIV-1 subtype C is most dominant in Sub Saharan Africa, HIV-1 subtype C sequences in this study were clustered with reference sequences from Ethiopia, Rwanda, Uganda, Malawi, Zambia and Botswana hence confirming its continuous rapid spread across these regions including Canada (Faria *et al.,* 2019). This spread could be linked to commercial activities and population migration following common market for eastern and southern Africa (COMESA) activities and tourism. Following the predominance of HIV subtype C in most of sub Saharan African countries like Rwanda, it has been suggested that these countries could be the main net exporters of HIV subtype C in the Central and East Africa region with Uganda acting as the main source of HIV subtype A1 and D (Faria *et al.,* 2018). The fact that these sequences tends to intermingle with those obtained from the neighbourhood countries, this suggests that they could be having a common evolutionary origin (Khamadi *et al.,* 2005).

For the HIV-1 subtype D which is predominantly in Uganda (Hue *et al.,* 2012). These sequences suggested a genetic relatedness with reference sequences from Kenya, Tanzania, Uganda and Gabon. These sequences implied that they could be of East African origin with those outside East Africa like Gabon being linked to population migration within these countries.  From the suggested origins of HIV subtype D in East Africa, this could explain the observed dispersal spreading patterns of these viral strains (Faria *et al.,* 2018).

Circulating recombinant Forms (CRF) and Unique recombinant forms (URF) have continued to be detected in Kenyan epidemics including those containing HIV-1 sub-subtype A2 (Visawapoka *et al.,* 2006). Even though low proportion of CRF; 02A1 and A2U were detected in this study, this does not rule out circulation of recombinants based possible intra-transmission of these viral strains within Kenya borders. Nevertheless, these findings suggest independent infections even though

there could be more CRFs than reported here. Amplification of full HIV genome could have confirmed possible CRFs, a limitation that was experienced in this study based on the small amplified gene fragments.

HIV epidemic in Kisii region therefore appears to be mostly of local origin with low proportions from neighbourhood countries (Nyamache *et al.,* 2013). From the observation of Kisii region just like most urban settings in the country, HIV epidemic could be driven mainly by population behaviour of indulging in local brews that could influence them into high risk sexual behaviour and urban prostitution (Wamalwa *et al.,* 2005; KCHASP, 2018). Diversity of HIV-1 subtypes not only has an impact on vaccine design but there is a compelling evidence that variation in HIV-1 subtypes could also influence the development of drug resistance and susceptibility to certain antiretroviral drugs (Lessells *et al.,* 2012).

### 5.1.2 Prevalence of acquired drug resistance in the studied population in Kisii County

In the current study, a total of 56(69%) had developed drug resistance mutations at the end of 12 month follow up (Etta *et al.,* 2017). The M184V (14.8%) was the most predominant NRTI mutation followed by K70R which is a thymidine analogue mutation followed by K103N, a NNRTI mutation. These findings were similar to those previous obtained in Kenya and elsewhere (Koigi *et al.,* 2013, Etta *et al.,* 2017; Lin *et al.,* 2017). The high frequency detection of M184V could be associated with a wide use of lamivudine as a first-line drug (Kinyua *et al.,* 2018). Despite M184V mutation limiting the efficacy of lamivudine and emtricitabine (FTC), the patient are not switched to a different drug since it increases the virus susceptibility to AZT, TDF or D4T drugs combinations (Trivedi *et al.,* 2008). The detected K70R mutation, leads to intermediate level resistance to AZT or low level resistance to D4T, DDI, ABC and TDF and this mutation was also elevated as confirmed in this study. The highly frequent detected K103N mutation in this study was linked to the use of either NVP or EFV as NNRTI among the studied proportion (Chung *et al.,* 2020).

This implies that with development of this mutation these drugs NVP or EFV could be ineffective. These findings were similar to those previous obtained in the country

and other poor resource settings (van Sighem *et al.,* 2008; Liégeois *et al.,* 2012; Koigi *et al.,* 2014; Hassan *et al.,* 2014). From the present study, majority of patients (84%) were on thymidine, NNRTIs and lamivudine and this would be due to their low genetic barrier (Bangsberg *et al.,* 2006).

In addition, other mutations that have not yet been associated with any drug resistance were also detected. The occurrence of these mutations could be resulting either from compensatory or immune pressures or natural occurrence mutations during replication (Brockman *et al.,* 2012). This implies that majority of the individuals did not have mutations related to drug resistant in this study and this could suggest possible better drug adherence. Analysis of existence of TAMs and NAMs drug resistance mutations among drug experienced patients has often been used as a predictive measure to switch to second-line drugs (Villa *et al.,* 2018). In the present study, 5(6.2%) patients were infected with drug resistant viruses on a NAMs mutation pathway with K65R being detected. They were on AZT/3TC (3) and DDI/3TC (2) drug combinations.

This K65R mutation tends to limit the efficacy of DDI, TDF and ABC if not previously exposed (Vandamme *et al.,* 2011; Koigi *et al.,* 2014). In situations where K65R mutation occurs alone, it confers resistance to tenofovir (Miller *et al.,* 2004). This mutation also occurred in combination with M184V leading to reduction in susceptibility of the virus to TDF and EFV (Koigi *et al.,* 2014). For the TAMS pathway, the findings were consistent with other studies that have shown high levels of TAMs (Etta *et al.,* 2017). The known mutations M184V, T215, K70R and D67N mutations selected for TAMs were detected in 12 (14.8%) patients. The mutations occurred mostly in combination. The least being a combination observed was two TAMs mutations indicative of possible low tolerability of these drugs (Koigi *et al.,* 2014).

In this study, the occurrence of NNRTI mutations was varied depending on NVP and EFV regimen. Depending on either NVP or EFV drug combinations used, the virus developed, K103N, Y181C, V190I, E138A, K101E and V106A mutations in varied proportions (Table 4.8). Mutation V190A and Y181C were selected mostly among

patients who were on EFV than NVP where it causes low level of resistance and reduction in its efficacy respectively. These mutations also confer resistance to Etravirine which could have been used for either second or third-line regimen (Wainberg *et al.,* 2011). Other mutations; V179L, Y188L, F227C and M230L were also detected. These mutations similarly confer resistance to rilpivirine (Antinori *et al.,* 2002). In addition, mutations associated with etravirine; A98G, E138A, V179D, M230L and Y181C, or V106A, Y188C, F227C and M230L mutations associated with donavirine resistance were detected. Based on the detected high frequency of drug associated mutations known to confer resistance to NNRTIs this could better be explained by its low genetic barrier (Cohn *et al.,* 2015. What this implies is that with high level of detected NNRTI resistance mutations, efavirenz, Nevirapine, donavirine or rilpivirine may not be effective hence not prescribed (Antinori *et al.,* 2002).

It was observed from the present study that by the end of 12$^{th}$ month of monitoring, 8 (9.9%) patients had virological failure. These patients harbored viral strains that had equally developed drug resistance mutations (Table 4.7). From the detected TAMs and NAMs analysis and the viral loads, it suggests that these patients could be monitored for further three months and drugs resistance genotyped to confirm persistent and virological failure before guiding the switching them to second-line drugs (MOH, 2018).

### 5.1.3 Integrase mutations in HIV infected patients attending Kisii Teaching and referral hospital Care Clinic for treatment

In this study, there were no major mutations associated with reduced susceptibility to INSTIs dolutegravir, raltegravir and elvitegravir or bictegravir which were detected (Table 4.8). Since no INSTIs drugs have been rolled out Kisii County, this could be associated reasons for absence of major drug resistance mutations to this class of drugs. From these sequences, only polymorphic accessory mutations that are associated with reduced susceptibility to virus of Raltegravir and Eletegravir drugs were detected. These mutations occurred in low frequencies (>3%) that suggest that the INSTIs could be effective (Table 4.6). Based on high genetic barrier to resistance

in dolutegravir, detected mutations were only those associated with raltegravir and eletegravir (E92G (2.3%), T97A (1.6%), L74M (0.8%), G140s (1.6%), and E157Q (0.8%) that have a low genetic barrier. These mutations being polymorphic in drug sensitive HIV-1, they could naturally enhance viral fitness and virulence (Meixenberger *et al.,* 2017). This study confirms previous findings that detected these naturally occurring polymorphisms, suggesting possible adaptation to immune or drug pressures (Nyamache *et al.,* 2012). Drug associated mutations could occur even at positions that could be linked to drug resistance to either dolutegravir, raltegravir or eletegravir (Nyamache *et al.,* 2012). Polymorphic mutations were detected among the analysed sequences (Table 4.6). These mutations; 2(1.6%) T97A, 1(0.8%) L74M have been known to be associated with reduced susceptibility to RAL and EVT. However in combination with major mutation they cause high level of resistance to INSTIs. Since dolutegravir is now preferred regimen and salvage ART (WHO, 2019), dolutegravir resistance will evolve in patients in the future as the INSTIs are being rolled out for the management of HIV/AIDS. Based on this, development, continued surveillance of INSTI resistance in the country is very important.

### 5.1.4 Prevalence of Transmitted drug resistance mutations among drug naïve patients attending Kisii Teaching and referral hospital Care Clinic for treatment

This study reports intermediate level of TDR in Kisii county, a level that is higher than the estimates prevalence (7.4%) in East Africa (Gupta *et al.,* 2012) and overall weight TDR prevalence (5.6%) in the high HIV prevalence Sub Saharan Africa (Hamers *et al.,* 2011). Estimate levels of TDR worldwide range from 8-22% (Ndembi *et al.,* 2011; Yebra *et al.,* 2011; Onywera *et al.,* 2017). However, independent studies conducted in Kenya, have reported prevalence of TDR ranging between 1% and 13% (Lihana *et al.,* 2009a; Maman *et al.,* 2014; Chung *et al.,* 2016; Onywera *et al.,* 2017; Hassan *et al.,* 2018) and that of East Africa as 0-19% (Prince *et al.,* 2011; Ndembi *et al.,* 2011; Lee *et al.,* 2014; The study findings from this study shows TDR in the county since 2017 to 2018. The current scale up of the coverage and duration of ARV use in this region could have led to the observed prevalence

levels of TDR in Kisii County. With only 28% ARV coverage in Kisii County, this is among poorly ART coverage regions (NASCOP, 2020). Due to insufficient information especially after roll out of ARVs, it therefore remains unclear if the detected 8.1% could be part of reported increase trends of TDR in other parts of Kenya.

Reports from other parts of the country like Mombasa, have shown an increasing trend of TDR from 4.9% (2007/2009) (Hamers *et al.,* 2011) to 13.2% (2009/2010) (Sigalloff *et al.,* 2012). Similarly, to rest of East African countries like Uganda, the trends have been the same ranging 0-8.6% (Ndembi *et al.,* 2011; Lee *et al.,* 2014; Onywera *et al.,* 2017). Compared to other parts of the country, the recorded TDR levels were consistent with findings from parts of country; Nairobi (Chung *et al.,* 2016; Hassan *et al.,* 2018), Homabay (Maman *et al.,* 2014), Uganda (Ndembi *et al.,* 2011; Lee *et al.,* 2014). In addition, this data was also in contrast with low TDR levels reported in some parts of the country from previous studies conducted in Ndhiwa Homabay (Onywera *et al.,* 2017), Nairobi (Hamers *et al.,* 2011). This variation could be associated with difference HIV epidemics in these regions, behaviors and coverage of ARVs. It can be observed that, the recorded intermediate resistance in Kisii region could pose a challenge to ART program due to increased risk of virological failure associated with pre-treatment drug resistance (PDR) (Wittkop *et al.,* 2011).

Observed TDR mutations were non-complex, with a predominance of the common NNRTI-based K238T mutation. Other NNRTI mutations; V106A, V118I, G190A were also detected. These findings were consistent with previous studies that have recorded high levels of NNRTI associated mutations (Hassan *et al.,* 2018). This level of resistance has been attributed to the widespread use of NNRTI as first-line drugs and use of Nevirapine monotherapy in the prevention of mother to child transmission or Nevirapine tail in short-course triple ART PMTCT, which has been associated with increased risk of resistance due to low genetic barrier of NNRTI (Maman *et al.,* 2014; Richman *et al.,* 1994; Onywera *et al.,* 2017).

Contrary to other studies conducted in Kenya and East Africa countries, NRTI M184V mutation was detected (Somi *et al.,* 2008: Lihana *et al.,* 2009a; Sigallo *et al.,* 2012; Hassan *et al.,* 2013; Onywera *et al.,* 2017). The detected NRTI mutation may suggest there could be underlying change from the predominantly NNRTI to a combined RTI TDR epidemic, with majority of NRTI being M184V mutation in future (Onywera *et al.,* 2017). This M184V mutation is generally highly revertant. Despite that previous studies have suggested that an M184V mutation has a low fitness and hence transmitted (Wainberg *et al.,* 2011), it could be a minority variant (Onywera *et al.,* 2017).

### 5.1.5 HIV-1 tropism among circulating HIV strains in patients attending Kisii Teaching and Referral hospital comprehensive care clinic

The CCR5 antagonists are among the six different classes of drugs targeting HIV at different stages of HIV replication and infection (Arts *et al.,* 2012). The CCR5 antagonists like Maraviroc or Vicriviroc are effective drugs in treatment-experienced patients who have viremia with virus using either CCR5 receptor (R5 virus) or CXCR4 (X 4 viruses) (Nyamache *et al.,* 2012). Among the circulating viral strains detected in this study, majority of them were R5 strains, 72.2%. This data demonstrates high prevalence of R5 using strains among the studied patients seeking HIV care in Kisii Level Five teaching and referral hospital. This finding concurs with previous studies that have been conducted in Kenya and elsewhere (Hung *et al.,* 1999; Lihana *et al.,* 2009b; Nyamache *et al.,* 2013). The detected high levels of R5 strains confirms that majority of the patients were at their early stage of HIV infection (Schuitemaker *et al.,* 1992). Previous studies have suggested that R5-using variants are found predominantly during the early stages of HIV infection (Nyamache *et al.,* 2013). HIV-1 usually uses CCR5 and it may switch to CXCR4 with disease progression or treatment (Poveda *et al.,* 2007).

Previous studies have shown that a decrease in CD4 count could influence viral tropism especially in subtype D (Kitawi *et al.,* 2017). However, in this study, there was no significant relationship between CD4 counts and tropism. The study also analysed if there was any significant difference in CD4 counts between R5 and X4

variants (Table 4.9). There was no significant difference on the levels of CD4 counts. The R5 infected individuals had average CD4 count of 656 cells/mm3 against 631 cells/mm3 for X4-using variants. These finding concurs with previous studies that have shown no impact on CD4 count on viral tropism and detected high predominance of R5 using variants (Nyamache *et al.,* 2013). In this study, 27.8% were X4-using variants with an average 631,631cells/mm3 CD4 count. This suggests that, the X4-infected patients harbours predicted CCR5 inhibitors resistant strains to maraviroc or vicriviroc even though most of the infected individuals with these strains could be at stage 1 of AIDS (Table 4.4) (Lihana *et al.,* 2009b; Nyamache *et al.,* 2012).

In this study, treatment had no an influence on viral tropism. Even though this was a cross-sectional study, these findings confirm previous studies that have indicated that treatment may not influence co-receptor usage (Briz *et al.,* 2008). Previous studies have confirmed that different HIV-1 subtypes may vary in the co-receptor usage (Wolinsky *et al.,* 2004). This may pose a challenge during initiation of treatment especially with the newly introduced new class of drugs CCR5 antagonists. It will therefore be appropriate to screen for the co-receptors usage to guide either use of maraviroc or vicriviroc.

However, findings from this study indicate that there was no significant difference in co-receptor usage across circulating HIV-1 subtypes (Table 4.9). Despite these findings, this study had a number of limitations. First, a phenotypic assay was not conducted in the present study due to restricted availability of biosafety facility. However, Geno2pheno $_{\text{(coreceptor)}}$ with a false positive rate of 15% (co-receptor) was used based on 35 amino acids of the V3 region, an *insilico* tool which has been confirmed to provide good accuracy in tropism prediction (Nyamache *et al.,*2012). Secondly, population genotypic prediction system used in the current study may results in a misclassification of R5 using variants as X4-using variants. A more sensitive ultra-deep pyrosequencing could have brought multiple orders of magnitudes and even detect minor CXCR4 using variants.

### 5.2. Conclusions

i. HIV-1 subtype A1 remains the most predominant circulating HIV-1 subtype among HIV infected patients in Kisii region.
ii. The prevalence of transmitted drug resistance among drug naïve HIV infected individuals visiting Kisii Teaching and referral hospital is 8.1% an intermediate levels drug resistance.
iii. The study findings showed that HIV-1 drug resistance was high in the study population. The detected accumulated resistance strains show that emergence of HIV drug resistance will continue to be a big challenge in the future.
iv. No major mutations associated with INSTIs drug resistance were detected; however, naturally occurring polymorphisms were detected but at low frequency
v. This study confirms a high frequency of circulating HIV-1 R5 tropic strains among the studied population hence advocacy for CCR5 antagonist.

### 5.3 Recommendations

i. The genetic diversity of the circulating HIV-1 subtypes suggests the need for continuous monitoring of these viral strains that have high impact of antiretroviral treatment
ii. This study propose that the WHO-recommended programmatic actions for moderate TDR level be performed, including evaluation of early warning indicators and both regional and nationwide Pre-drug resistance(PDR) survey to determine the causes of TDR and advise on choice of first-line treatment in case of persistent high-level PDR.
iii. Presence of HIV-1 major drug mutations in this study calls for personalized patient treatment monitoring as well as regular appraisals of available regimens.
iv. The integrase inhibitors will be effective in Kenya and more especially in Kisii county where HIV-1 subtype A1 is still the most predominant. However, occurring polymorphisms may warrant further investigation among

drug experienced individuals on dolutegravir combination or integrase inhibitors treatment

v. Based on high frequency of R5 strains among the studied population, there is a potential benefit with the use of CCR5 antagonists as a therapeutic option in Kenya.

## 5.4 Further studies

i. There is need to evaluate the evolutionary trends of circulating HIV subtypes in Kisii county, sexual networks and viral dynamics within population in the county.

ii. There is a need to conduct a surveillance of pre-treatment drug resistance levels in this studied Kenyan population in order to validate its impact on the transmitted drug resistance.

iii. Further studies focusing on longitudinal studies and full-length genome analysis of viral variants in this region are recommended to optimize public health strategies for interventions.

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# APPENDICES

## Appendix I: Questionnaire

My name is Sepha Nyatichi Mabeya carrying out a research on hiv-1 genetic diversity, tropism, transmitted and acquired drug resistance mutations among HIV infected patients attending comphrensive care clinic in Kisii teaching and referral hospital, Kenya

Number/Nambali…………
Date/Tarehe…………

1. How old are you/Age /rika?

| | |
|---|---|
| 0-10 | 41-50 |
| 11-20 | 51-60 |
| 21-30 | 61-70 |
| 31-40 | 71-80 |

3. What is your residence?/Makao?

4. What is your marital status/ndoa?:

a) Married/nimeolewa

b) Separated/divorced/taraka

c) Single/sijaolewa

d) Other/Mengine

5. What is your occupation/Kazi

a) None/Hakuna

b) unskilled/hasiyenamaarifa

c) Skilled/Maarifa

d) Professional/gwiji

e) student/Mwanafunzi

d) Other/Mengin

7. Have you ever had a blood transfusion/kuekwadamu?

a) Yes/Ndio b) No/La

c) when/Lini

8. Are you on HAART treatment/Wanyua madawa ya virusi?

a) Yes/Ndio b) No/La

If yes,when did you start /kamaNdio,ulianzalini?

9What is your Education level/kisomochako

a) none/sinakisomo

b)Primary/shuleyamsingi

b) Secondary/shuleyaupili

c) Tertially/college/masomoyajuu

d) University/masomoyachuokiku

e) Other/Mengine

Thank you very much for the participation in this questionnaire!

**Appendix II: Informed Consent Form.**

**Informed Consent**

My name is Sepha Nyatichi Mabeya,I am a PhD student from Jomo Kenyatta University of Agriculture and Technology. I am conducting a study on ***"Hiv-1 Genetic Diversity, Tropism, Transmitted And Acquired Drug Resistance Mutations Among HIV Infected Patients Attending Compressive Care Clinic In Kisii Teaching And Referral Hospital, Kenya"***

The information will be used by the Ministry of Medical Services and Ministry of Public Health to improve the management of HIV in hospitals as well as in other regions of Kenya.

**Procedures to be followed**

Participation in this study will require that I ask you some questions and approximate 5 ml of venous blood drawn from your arm veins by certified Medical laboratory technologist. This blood sample will then be tested for CD4/CD8 count, viral load and HIV drug resistance tests. The remnants of the sample if any will be disposed by incineration. Some demographic information regarding your age, sex, whether you are taking ARVs or not will also be obtained from you by administering a short questionnaire. You are reminded that you have a right to accept or decline to participate in this study. Whatever your decision, you will still get the same care and medical treatment in this clinic. Equally, your decision will not change the care you have been receiving in this clinic or any other at any particular time

Please remember the participation in this study is voluntarily. You are free to ask any questions related to the study at any time. You may refuse to respond to any questions and you may stop an interview at any time. You may also stop being in the study at any time without any consequences to the services you receive from this clinic or any other organization now or in the future.

**Discomforts and Risks**

Some of the questions you will be asked are on intimate subject and may be embarrassing or make you uncomfortable. If this happens, you may refuse to answer these questions if you so choose. You may also stop the interview at any time. The interview may add approximately half an hour to the time you wait before you receive your routine services.

**Benefits**

If you participate in this study you will help us to learn how to provide effective patient monitoring with aim of improving management of HIV infections. In this study, one is bound to benefit from free prior tests on; CD4 counts, viral load and drug resistance. Those found failing treatment by persistent increase in viral load above 1000pfu/µl, a class switch and second line drugs will be prescribed. Study participants will be followed up and adherence seminar and follow up using mobile phones.

**Confidentiality**

The interviews and examinations will be conducted in a private setting within the clinic. Your name will be coded on the questionnaire and kept in a locked cabinet for safe keeping at Kenya Medical Research Institute (KEMRI). Everything will be kept private and confidential.

**Contact Information**

If you have any questions you may contact Dr. Raphael Lihana On 0733735562 or Dr. Caroline Ngugi On 0722556790 or the Kenyatta University Ethical Review Committee Secretariat on chairman.kuerc@ku.ac.ke, secretary.kuerc@ku.ac.ke,secretariat.kuerc@ku.ac.ke

**Participant's statement**

The above information regarding my participation in the study is clear to me. I have been given a chance to ask questions and my questions have been answered to my satisfaction. My participation in this study is entirely voluntary. I understand that my records will be kept private and that I can leave the study at any time. I understand that I will still get the same care and medical treatment whether I decide to leave the study or not and my decision will not change the care that I will receive from the clinic today or that I will get from any other clinic at any other time.

Name of Participant………………………………………………………………………

Signature or Thumbprint ………………………Date ………………

**Investigators statement**

I, the undersigned, have explained to the volunteer in a language s/he understands, the procedures to be followed in the study and the risks and benefits involved

Name of Interviewer………………………………………………………………………

Signature or Thumbprint ………………………Date ………………

## Appendix III: Ethical Approval Letter

**KENYATTA UNIVERSITY**

**ETHICS REVIEW COMMITTEE**

**Moi Library 1st Floor, Office No. 25**

**Fax: 8711242/8711575**
**Email: chairman.kuerc@ku.ac.ke**
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**P. O. Box 43844,**
**Nairobi, 00100**

Tel: 8710901/12

Our Ref: KU/ERC/APPROVAL/VOL.1 (91)

Date: 18th October, 2017

**Sepha Nyatichi Mabeya**
**Jomo Kenyatta University of Agriculture & Technology**
**P.O Box 4471,**
**Nairobi**

Dear Sepha,

**APPLICATION NUMBER HIV-PKU/716/E73 GENETIC DIVERSITY, TROPISM, TRANSMITTED AND ACQUIRED DRUG RESISTANCE MUTATIONS AMONG HIV INFECTED PATIENTS ATTENDING COMPREHENSIVE CARE CLINIC IN KISII TEACHING AND REFERRAL HOSPITAL, KENYA**

**1. IDENTIFICATION OF PROTOCOL**

The application before the Committee is with a research topic **HIV-1 Genetic Diversity, Tropism, Transmitted and Acquired Drug Resistance Mutations Among HIV Infected Patients Attending Comprehensive Care Clinic in Kisii Teaching and Referral Hospital, Kenya** was received on 3rd July and discussed on 17th October, 2017.

**2. APPLICANT**

**Sepha Nyatichi Mabeya**

**3. SITE**

Kisii Teaching and Referral Hospital, Kenya

**4. DECISION**

The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines and **APPROVED that the research may proceed for a period of ONE year from 18th October, 2017.**

**5. ADVICE/CONDITIONS**

ix. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.

x. Serious and unexpected adverse events related to the conduct of the study are reported to this committee immediately they occur.

xi. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.

xii. Submit an electronic copy of the protocol to KUERC.

**When replying, kindly quote the application number above.**
**If you accept the decision reached and advice and conditions given please sign in the space provided below and return to KU-ERC a copy of the letter.**

**DR. TITUS KAHIGA**
**CHAIRMAN ETHICS REVIEW COMMITTEE**

I ...SEPHA NYATICHI MABEA... accept the advice given and will fulfill the conditions therein.

Signature............................................ Dated this day of...19/10/2017........ 2017.

cc.

DVC-Research Innovation and Outreach

## Appendix IV: Generated sequences

### General generated sequences

The generated sequences were deposited in the genebank with the following accession numbers.

### *HIV-1 env* C2V3 sequences

The envelope C2V3 sequences were deposited at the Genebank under accession numbers: MK 458778 – MK 458849

### HIV-1 integrase sequences

The *pol intergrase* sequences were deposited at the Genebank under accession numbers: MK 458850 – MK 458882

### HIV-1 *-pol* –RT sequences

The *pol intergrase* sequences were deposited at the Genebank under accession numbers: MK 458883 – MK 458933

## Appendix V: statistical analysis

Co-receptor usage

**One way ANOVA**

coreceptor usage

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 1.173 | 3 | .391 | .543 | .653 |
| Within Groups | 480.536 | 668 | .719 | | |
| Total | 481.708 | 671 | | | |

**coreceptor usage**

Tukey HSD[a]

| VAR00007 | N | Subset for alpha = 0.05 |
|---|---|---|
| | | 1 |
| web | 168 | 1.4345 |
| gen2 | 168 | 1.4643 |
| ds_Kernel | 168 | 1.4702 |
| CorS | 168 | 1.5476 |
| Sig. | | .613 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 168.000.

*To determine if the HIV subtypes have got any difference in co-receptor usage, two sampled T test was used and HIV-1 subtype A1 vs HIV-1 no A1 subtype was analysed. From the analysis, there was no significant influence of HIV-1 subtype against co-receptor usage.(geno2pheno (P-Value = 0.012), Web-PSSM (P-Value = 0.020), CoRseq (P-Value = 0.039) except for Ds Kernel (P-Value = 0.052),*

**samples are not of the same size (51 vs117)**

**Two-Sample T-Test and CI: geno2, subtype**

Two-sample T for geno2

| subtype | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| 0 | 51 | 2.353 | 0.483 | 0.068 |
| 1 | 117 | 2.154 | 0.407 | 0.038 |

Difference = mu (0) - mu (1)

Estimate for difference:  0.1991

95% CI for difference: (0.0452, 0.3530)

T-Test of difference = 0 (vs not =): T-Value = 2.57 ***P*-Value = 0.012** DF = 82

Ruling: There is significance difference in co-receptor usage across subtypes.

**Two-Sample T-Test and CI: pssm, subtype**

Two-sample T for pssm

| subtype | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| 0 | 51 | 2.333 | 0.476 | 0.067 |

| subtype | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| 1 | 117 | 2.154 | 0.385 | 0.036 |

Difference = mu (0) - mu (1)

Estimate for difference: 0.1795

95% CI for difference: (0.0290, 0.3299)

T-Test of difference = 0 (vs not =): T-Value = 2.37 P-Value = 0.020 DF = 79

**Two-Sample T-Test and CI: kernel, subtype**

Two-sample T for kernel

| subtype | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| 0 | 51 | 2.314 | 0.469 | 0.066 |
| 1 | 117 | 2.162 | 0.435 | 0.040 |

Difference = mu (0) - mu (1)

Estimate for difference: 0.1513

95% CI for difference: (-0.0016, 0.3042)

T-Test of difference = 0 (vs not =): T-Value = 1.97 P-Value = 0.052 DF = 89

**Two-Sample T-Test and CI: cor, subtype**

Two-sample T for cor

| subtype | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| 0 | 51 | 2.373 | 0.488 | 0.068 |
| 1 | 117 | 2.205 | 0.446 | 0.041 |

Difference = mu (0) - mu (1)

Estimate for difference: 0.1674

95% CI for difference: (0.0087, 0.3261)

T-Test of difference = 0 (vs not =): T-Value = 2.10 P-Value = 0.039 DF = 8

*Patient are diagnosed and treated immediately upon confirmation. Treatment for HIV patients was therefore analysed to confirm if influenced the co-receptors usage. A 2 tailed correlation Pearson analysis was conducted and impact of treatment on co-receptors usage determined. From the Pearson correlation analysis based on unstandardized coefficient, treatment had significant impact on co-receptor usage (Y=-0.507-0.324 geno2+0.520 pssm +0.167ds-kernel+0.083 coRs.*

**Using correlation**

**Correlations**

| | | treat | geno2 | pssm | kernel | coRs |
|---|---|---|---|---|---|---|
| treat | Pearson Correlation | 1 | .289** | .315** | .296** | .264** |
| | Sig. (2-tailed) | | .000 | .000 | .000 | .001 |
| | N | 168 | 168 | 168 | 168 | 168 |
| geno2 | Pearson Correlation | .289** | 1 | .953** | .682** | .698** |
| | Sig. (2-tailed) | .000 | | .000 | .000 | .000 |
| | N | 168 | 168 | 168 | 168 | 168 |
| pssm | Pearson Correlation | .315** | .953** | 1 | .654** | .643** |
| | Sig. (2-tailed) | .000 | .000 | | .000 | .000 |
| | N | 168 | 168 | 168 | 168 | 168 |
| kernel | Pearson Correlation | .296** | .682** | .654** | 1 | .690** |
| | Sig. (2-tailed) | .000 | .000 | .000 | | .000 |
| | N | 168 | 168 | 168 | 168 | 168 |
| coRs | Pearson Correlation | .264** | .698** | .643** | .690** | 1 |
| | Sig. (2-tailed) | .001 | .000 | .000 | .000 | |
| | N | 168 | 168 | 168 | 168 | 168 |

**Correlations**

| | | treat | geno2 | pssm | kernel | coRs |
|---|---|---|---|---|---|---|
| treat | Pearson Correlation | 1 | .289** | .315** | .296** | .264** |
| | Sig. (2-tailed) | | .000 | .000 | .000 | .001 |
| | N | 168 | 168 | 168 | 168 | 168 |
| geno2 | Pearson Correlation | .289** | 1 | .953** | .682** | .698** |
| | Sig. (2-tailed) | .000 | | .000 | .000 | .000 |
| | N | 168 | 168 | 168 | 168 | 168 |
| pssm | Pearson Correlation | .315** | .953** | 1 | .654** | .643** |
| | Sig. (2-tailed) | .000 | .000 | | .000 | .000 |
| | N | 168 | 168 | 168 | 168 | 168 |
| kernel | Pearson Correlation | .296** | .682** | .654** | 1 | .690** |
| | Sig. (2-tailed) | .000 | .000 | .000 | | .000 |
| | N | 168 | 168 | 168 | 168 | 168 |
| coRs | Pearson Correlation | .264** | .698** | .643** | .690** | 1 |
| | Sig. (2-tailed) | .001 | .000 | .000 | .000 | |
| | N | 168 | 168 | 168 | 168 | 168 |

**. Correlation is significant at the 0.01 level (2-tailed).

**By regression**

**Variables Entered/Removed[b]**

| Model | Variables Entered | Variables Removed | Method |
|---|---|---|---|
| 1 | coRs, pssm, kernel, geno2[a] | . | Enter |

a. All requested variables entered.

b. Dependent Variable: treat

**Model Summary**

| Model | R | R Square | Adjusted R Square | Std. Error of the Estimate |
|---|---|---|---|---|
| 1 | .347[a] | .121 | .099 | .47572 |

a. Predictors: (Constant), coRs, pssm, kernel, geno2

**ANOVA[b]**

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 5.058 | 4 | 1.265 | 5.588 | .000[a] |
| | Residual | 36.888 | 163 | .226 | | |
| | Total | 41.946 | 167 | | | |

a. Predictors: (Constant), coRs, pssm, kernel, geno2

b. Dependent Variable: treat

Using unstandardized coefficient.

**Coefficients[a]**

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. |
|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | |
| 1 | (Constant) | -.507 | .215 | | -2.358 | .020 |
| | geno2 | -.324 | .297 | -.284 | -1.090 | .277 |
| | pssm | .520 | .289 | .438 | 1.803 | .073 |
| | kernel | .167 | .123 | .150 | 1.355 | .177 |
| | coRs | .083 | .122 | .077 | .684 | .495 |

a. Dependent Variable: treat

**CD4 counts**

**Correlations**

| | | group | geno2 | pssm | kernel | coRs |
|---|---|---|---|---|---|---|
| group | Pearson Correlation | 1 | -.097 | -.145 | -.136 | -.077 |
| | Sig. (2-tailed) | | .212 | .061 | .078 | .319 |
| | N | 168 | 168 | 168 | 168 | 168 |
| geno2 | Pearson Correlation | -.097 | 1 | .953** | .682** | .698** |
| | Sig. (2-tailed) | .212 | | .000 | .000 | .000 |
| | N | 168 | 168 | 168 | 168 | 168 |
| pssm | Pearson Correlation | -.145 | .953** | 1 | .654** | .643** |
| | Sig. (2-tailed) | .061 | .000 | | .000 | .000 |
| | N | 168 | 168 | 168 | 168 | 168 |
| kernel | Pearson Correlation | -.136 | .682** | .654** | 1 | .690** |
| | Sig. (2-tailed) | .078 | .000 | .000 | | .000 |
| | N | 168 | 168 | 168 | 168 | 168 |
| coRs | Pearson Correlation | -.077 | .698** | .643** | .690** | 1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Sig. (2-tailed) | .319 | .000 | .000 | .000 | |
| | N | 168 | 168 | 168 | 168 | 168 |

**. Correlation is significant at the 0.01 level (2-tailed).

**Correlations**

| | | CD4 counts | geno2 | pssm | kernel | coRs |
|---|---|---|---|---|---|---|
| CD4 counts | Pearson Correlation | 1 | -.141 | -.167* | -.152* | -.136 |
| | Sig. (2-tailed) | | .068 | .030 | .050 | .079 |
| | N | 168 | 168 | 168 | 168 | 168 |
| geno2 | Pearson Correlation | -.141 | 1 | .953** | .682** | .698** |
| | Sig. (2-tailed) | .068 | | .000 | .000 | .000 |
| | N | 168 | 168 | 168 | 168 | 168 |
| pssm | Pearson Correlation | -.167* | .953** | 1 | .654** | .643** |
| | Sig. (2-tailed) | .030 | .000 | | .000 | .000 |
| | N | 168 | 168 | 168 | 168 | 168 |
| kernel | Pearson Correlation | -.152* | .682** | .654** | 1 | .690** |
| | Sig. (2-tailed) | .050 | .000 | .000 | | .000 |
| | N | 168 | 168 | 168 | 168 | 168 |
| coRs | Pearson Correlation | -.136 | .698** | .643** | .690** | 1 |
| | Sig. (2-tailed) | .079 | .000 | .000 | .000 | |
| | N | 168 | 168 | 168 | 168 | 168 |

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

*The CD4 counts were determined if they influenced the co-receptors usage across the used insilico tools. From a 2 tailed Pearson correlations test was used across the four insilico tools used (geno2pheno, WEB-PSSM, ds Kernel and CORseq tools), there was no significant impact of co-receptor usage on co-receptor usage in both categorized and uncategorized data except for WeB-PSSM and ds Kernel tools in uncategorized CD 4 counts category.*

# Appendix VI: Publications



***ORIGINAL ARTICLE***

## Characterization of HIV-1 Integrase Gene and Resistance Associated Mutations Prior to Roll out of Integrase Inhibitors by Kenyan National HIV-Treatment Program in Kenya

**Mabeya Sepha[1*], Nyamache Anthony[2], Ngugi Caroline[1], Nyerere Andrew[1], Lihana Raphael[3]**

**OPEN ACCESS**

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***ABSTRACT***

*BACKGROUND: Antiretroviral therapy containing an integrase strand transfer inhibitor plus two Nucleoside Reverse Transcriptase inhibitors has now been recommended for treatment of HIV-1-infected patients. This thus determined possible pre-existing integrase resistance-associated mutations in the integrase gene prior to introduction of integrase inhibitors combination therapy in Kenya.*

*METHODS: Drug experienced HIV patients were enrolled at Kisii Teaching and Referral in Kenya. Blood specimens from (33) patients were collected for direct sequencing of HIV-1 pol-integrase genes. Drug resistance mutations were interpreted according to the Stanford algorithm and phylogenetically analysed using insilico tools.*

*RESULTS: From pooled 188 Kenyan HIV integrase sequences that were analysed for drug resistance, no major mutations conferring resistance to integrase inhibitors were detected. However, polymorphic accessory mutations associated with reduced susceptibility of integrase inhibitors were observed in low frequency; M50I (12.2%), T97A (3.7%), S153YG, E92G (1.6%), G140S/A/C (1.1%) and E157Q (0.5%). Phylogenetic analysis (330 sequences revealed that HIV-1 subtype A1 accounted for majority of the infections 26 (78.8%) followed by D 5 (15.2%) and C 2 (6%).*

*CONCLUSION: The integrase inhibitors will be effective in Kenya where HIV-1 subtype A1 is still the most predominant. However, occurring polymorphisms may warrant further investigation among drug experienced individuals on dolutegravir combination or integrase inhibitor treatment*

*KEYWORDS: integrase, dolutegravir, mutations, resistance*

DOI: http://dx.doi.org/10.4314/ejhs.v30i1.6

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PREDOMINANCE OF CCR5 TROPISM IN NON-B HIV-1 SUBTYPES CIRCULATING IN KISII COUNTY, KENYA

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# PREDOMINANCE OF CCR5 TROPISM IN NON-B HIV-1 SUBTYPES CIRCULATING IN KISII COUNTY, KENYA

S. N. Mabeya, A. K. Nyamache, C. Ngugi, A. Nyerere and R. L. Wekesa

## ABSTRACT

***Introduction:*** **The chemokine receptors CCR5 and CXCR4 are considered as the main receptors during HIV infection, replication, transmission and subsequent AIDS progression. CCR5 antagonists are drugs designed to inhibit viral entry by binding to these chemokine receptors. However, characterisation of HIV-1 co-receptor usage before rolling out of CCR5/CXCR4 antagonists has not yet been done in the country.**

***Objective:*** **To determine the HIV-1 co receptor usage among HIV-1 infected individuals and predict possible use CCR5 antagonistic drugs**

***Design:*** **A cross sectional study**

***Setting:*** **Comprehensive HIV care clinics of Kisii Teaching & Referral Hospital, Kenya**

***Methods:*** **A total of seventy-two (72) blood samples were obtained from both drug naïve (32) and experienced (40) study participants. Viral DNA was extracted using QIAamp MinElute Virus kit and partial HIV-1 V3 region was amplified and directly sequenced. Coreceptor usage predicted using *insilico* Geno2pheno $_{(coreceptor)}$ with a false positive rate of 15%**

***Results:*** **Sixty-one individuals (77.8%) were infected with HIV-1 subtype A1, twelve (18.1%) HIV-1 subtype D and four (4.1%) were HIV-1 subtype C. CCR5-using variants were found in 52 (72.2%) while 20(27.8%) participants were infected with CXCR4–using variants. There was no significant difference in co - receptor usage a cross gender, HIV subtypes, disease staging or impact of treatment or CD 4 counts that was observed.**

***Conclusions and recommendation:*** **The detected high level of circulating R5 strains suggests the likelihood of a successful implementation and use of CCR5 antagonists in Kenya where HIV-1 A1 is the most predominant.**