Characterization of epidemiological, nutritional, immunological and virological and genetic diversity parameters in HIV-infected individuals in Addis Ababa, Ethiopia

By

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DECLARATION

I, the undersigned, declare that the thesis submitted to the Department of Microbial, Cellular, and Molecular Biology, Addis Ababa University for the Degree of Doctor of Philosophy (PhD) in Biology (Biomedical Sciences) is my own work and has not been submitted at another university. The materials obtained from other sources are duly acknowledged in the thesis.

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Signed on June, 2021, Department of Microbial, Cellular, and Molecular Biology, College of Natural and Computational Sciences, Addis Ababa University, Arat Kilo.

We here by confirm this work has been done under our supervision

Beyene Petros (Prof.) Signature: _____Date_____

Adane Mihret (Assoc. Prof.) Signature: _____Date _____

DEDICATION

This work is dedicated to my mother Tiruye Teshale amd my father Adal Eshetie for their love and support. It would have been great if it was with your presence. My dedication also goes to my brothers Shimelash Adal and Sintayehu Adal for their dedicated support.

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Table of content

List of Figures

List of Tables

Acronyms and Abbreviations

AAHAPCO = Addis Ababa HIV/AIDS Prevention and Control Office

ABCA1 = ATP binding cassette subfamily A member 1

ADCC = Antibody dependent cellular cytotoxicity

AIDS = Acquired immune deficiency syndrome

ALERT = Leprosy Rehabilitation and Training Centre

ANC = Antenatal clinic

ANOVA = Analysis of variance

APOA, B, C & E = Apolipoprotein A, B, C & E, respectively

ART = Antiretriviral therapy

ARVs = Antiretrovirals

ATP = Adenosine triphosphate/ Adult Treatment Panel

BCC = Behavioural change communication

BMI = Body mass index

CD4+ T cells = Human T cells expressing CD4 antigens

CD8+ T cells = Human T cells expressing CD8 antigens

CDC = Americal Centre for Diseases Control and Prevention

CETP = Cholesterylester transport protein

CRF = circuilating recombinant forms

CSA = Central Statistical Agency

CTLs = Cytotoxic T lymphocytes

CVD = Cardiovascular disease

DNA = Deoxyribonucleic acid

EDHS = Ethiopian demographic and health survey

EDTA = Ethylene-diamine-teraacetic acid

EFY = Ethiopian fiscal year

EID = Early infant diagnosis

EPHA = Ethiopian Public Health Association

EPHI = Ethiopian Public Health Institute

EPHIA = Ethiopian population based HIV impact assessment

FFA = Free fatty acid

FHAPCO = Federal HIV/AIDS Prevention and Control Office

FMOE = Federal Ministry of Education

FMOH = Federal Ministry of Health

FSWs = Female sex workers

HC = Hydroxycholesterol

HDL = High density lipoprotein

HDL-C = High density lipoprotein cholesterol

HLA = Human leucocyte antigen

HTS = HIV testing services

IDUs = Intraveinous drug users

IFG = Impaired fasting glucose

IFN = Interferon

IGA = Income generating activities

IL = Interleukin

IQR = Inter quartile range

IRERC = Institutional Research Ethics Review committee

ISGs = Interferon-stimulated genes

IVA = Iterative viral assembly

KIRs = Killer-cell immunoglobulin-like receptors

KP = Key population

KPP = Key and priority population

LDL = Low density lipoprotein

LDL-C = Low density lipoprotein cholesterol

LDLR = LDL receptor

LRP = Low density receptor related protein

LTNPs = Long-term non-progressors

LTR = Long terminal repeat

MARPs = Most at risk populations

MEGA = Molecular Evolutionary Genetic Analysis

MHC = Major histocompatibility complex

MIP = Macrophage inhibitory protein

MIP-1α and MIP-1β = macrophage inflammatory protein-1α or 1β

MTCT = Mother-to-Child-Transmission

NCEP = National Cholesterol Education Program

NGS = Next generation sequencing

N-J = Neighbor-joining

NRE = Negative regulatory elements

NPV = Negative predictive value

NSI = Non-syncytium inducing

OSSHD = Organization for Social Services, Health and Development

PBMCs = Peripheral blood mononuclear cells

PBS = primer binding site

PCR = Polymerase chain reaction

PEPFAR = President's Emergency Plan for AIDS Relief

PIs = Protease inhibitors

PLHIV = people living with HIV

PMTCT = Prevention of Mother to Child transmission

PPs = Priority populations

PPV = Positive predictive value

PSI/E = Population Services International, Ethiopia

RANTES = β-chemokines regulated on activation, normal T expressed and secreted

RCT = Reverse cholesterol transport

RNA = Ribonucleic acid

RRE = RNA response element

RNH = RNA ribonuclease H

SDF-1 = α-chemokine stromal differentiating factor 1

SIV = Simian immunodeficiency virus

SNNP = Sothern Nations, Nationalities and Peoples

SNP = Single nucleotide polymorphism

STI = sexually transmitted infections

TCR = T cell receptor

TG = Triglyceride

TNF = Tumor necrosis factor

UNODC = United Nations Office on Drug and Crime

URFs = Unique recombinant forms

VLDL-C = Very low density lipoprotein cholesterol

VLDLR = VLDL receptor

VLS = Viral load suppression

WHO = World Health Organization

Abstract

HIV prevalence in Addis Ababa is relatively high among the high risk population. The aim of the present study was to investigate epidemiological, nutritional, immunological, and virological factors and HIV-1 diversity in antiretroviral naïve HIV-infected adults in Addis Ababa. To optimize study site and participant selection, a sytematic review of the existing baseline information was done to determine the prevalence of HIV/AIDS, and the predisposing risk factors in Addis Ababa. Data relevant for the systematic review were collected from online databases. In addition, survey and surveillance reports, performance and project assessment findings were also collected and analysed. From February to August 2013, this cross-sectional study was conducted on samples of 594 HIV-1 infected ART-naïve adult study participants, from four hospitals in Addis Ababa. CD4+ T cell count, HIV RNA load, fasting serum glucose, hemoglobin, fasting serum triglyceride and total cholesterol concentrations were determined. HIV-1 RNA amplification and sequencing was performed on 60 plasma samples, and assembled using the iterative viral assembler method. REGA subtyping tool was used for scanning of recombination and subtyping. Prediction of coreceptor usage was performed using online tools and phylogenetic analysis done using MEGA. The Spearman correlation, chi-square, Mann-Whitney and Kruskal-Wallis, independent t tests; ANOVA and regression analyses were used for data analysis. The prevalence of HIV in Addis Ababa appears to have stabilized at around 3%, but was higher in MARPs. The prevalence of undernutrition, excess weight, obesity and hypercholesterolemia were 15.1%, 22.1%, 5.4% and 16.6%, respectively. The hypercholesterolemia prevalence was higher in females (18.9%) than in males (11.0%) ($p < 0.05$). The median CD4+ T cell count was

357 cells/mm^3 (IQR = 248-537). Detectable HIV RNA load of 4.23 ± 0.83 log copies/mL was found for a sample of 500 study participants. Serum total cholesterol and CD4+ T cell count were significantly correlated with HIV RNA load ($p < 0.01$). Having lower concentration of serum total cholesterol was an independent predictor of higher HIV RNA load ($p < 0.05$). The immune status was better in females than males in the presence of higher serum total cholesterol ($p<0.05$). Genome sequencing of the HIV isolates identified 81.7% subtype C, 1.7% subtype A1, and 10% C/A1 and 5.0% C/A1/D recombinants. PhenoSeq results to determine coreceptor usage from the 50 genome sequences were 88.0% and 12.0% that correspond to R5 and CXCR4, respectively. Furthermore, subtypes HIV-1 C and A1; CRFs C/A1 and C/A1/D, and R5-viruses were dominant. Therefore, nutritional abnormalities in the ART naïve HIV-infected study participants indicate the need for targeted nutritional programs and regular lipid level monitoring as an integral part of HIV/AIDS care. The more than 14% WHO clinical stage III/IV patients detected among the study participants, categorized as ART naïve, showed the need to revise the patient care policy on ART-naïve status of HIV positive individuals. The study also indicated the need for further indepth studies on HIV-1 subtype diversity, CRFs and its phenotypic distribution for a better understanding of the epidemiology of HIV/AIDS. Such indepth characterization of HIV-1 sub-type diversity will be required for possible future use of maraviroc, the co-receptor antagonist, in HIV/AIDS treatment.

Key words: HIV/AIDS, malnutrition, lipid abnormalities, ART naïve, CD4+ T cell count, HIV RNA load, total cholesterol, genetic diversity, Addis Ababa

1. Introduction

1.1. Human immunodeficiency virus

Human immunodeficiency virus (HIV) is a ribonucleic acid (RNA) virus, which is a member of the Lentivruses of family Retroviridae where replication of HIV undergoes two genetic metamorphoses from RNA to deoxyribonucleic acid (DNA) and back to RNA (Elliott *et al*., 1997). Like all retroviruses, HIV is diploid and contains two molecules of the single-stranded plus-sense (positive) RNA. HIV-1 that is now dominant worldwide was discovered in 1983 (Barre-Sinoussi *et al*., 1983; Gallo *et al*., 1984).

The HIV genome consists of nine genes (**Table 1; Figure 1**). The HIV genome is flanked by the characteristic long terminal repeat (LTR) sequences. These LTR sequences contain transcription initiation (5' LTR) and termination (3' LTR). The 3' end is defined by the adjacent primer binding site (PBS) for lysyl transfer RNA (tRNA) that primes reverse transcription of minus stranded DNA (Coffin, 1990). LTR sequences are also involved in proviral integration into the cellular DNA (Hahn, 1994). The U3 region makes up the viral promoter and binding site for host cell transcription factor. The R facilitates the beginning of all viral RNA transcripts, and it form a stable stem-loop structure called trans-activation response element (TAR) that can interact with the transcriptional activator protein *tat* to promote high levels of gene transcription and expression.

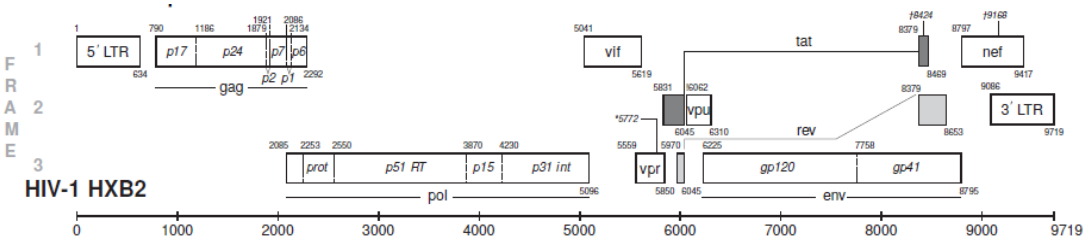

Figure 1. HIV genome map (adapted from Foley *et al.*, 2016).

HIV gene expression is divided into temporal phases, an early regulatory phase and a late regulatory phase. In early phase, multiply spliced messenger RNAs (mRNAs) is produced that encode the viral regulatory proteins tat, rev and nef. As expression levels of these early regulatory proteins rise, their synthesis is inhibited and expression of the messages encoding structural and late regulatory proteins is induced. This switch is caused by an accumulation of rev protein, of which accumulation of the rev protein then causes a switch to the production of unspliced and incompletely spliced RNA transcripts and the synthesis of structural proteins because RNA interacts with RNA Response element (RRE) found in unspliced/incompletely spliced mRNA. This bimodal expression pattern is a characteristic feature of complex retroviruses and is probably important for their ability to establish a persistent infection and cause a protracted disease course (Haseltine and Wong-Staal, 1988; Hahn, 1994). Mutations of all other accessory genes change viral growth rates, infectivity, and/or cytopathicity, but not destroy replication competence. The absence of ability to destroy replication competence led to the initial classification of *vif*, *nef*, *vpr*, *vpu* and *vpx* as non essential accessory genes (Hahn, 1994).

Two spacer peptides are present within p55 encoding *gag* gene: P2, located between CA and NC, and P1, located between NC and p6 where the arrangement is MA, CA, P2, NC, p1, p6, respectively, from amine to carboxyl terminus (Freed, 1998). The mRNA that can direct *gag* synthesis is the full length unspliced viral RNA. The myristylated *gag* precursor p55 gives rise to the matrix protein (MA, p17), the caspid protein (CA, p24), the nucleocapsid proteins (NC, p7/p9) and p6 by protease cleavage (Pal *el al*., 1990). The

proteins then polymerize and associate with viral RNA to form the viral core particle and facilitate virion budding (Young, 1994; McClure and Dalgleish, 1998). The virus particle is comprised of an inner core that contains two copies of the viral genomic RNA and associated lysyl tRNA molecule, along with mature *gag* and *pol* protein products required for early steps of replication (Hahn, 1994; Young, 1994).

Pol sequence does not include an initiation codon and thus cannot be translated independently. *Pol* is always synthesized as a fusion protein with *gag*. The virally encoded PR then cleaves the *pol* polypeptide away from the *gag* and further digests it to separate the *pol* components. The *pol* gene encodes for protease (PR, p10), reverse transcriptase (RT, p51), ribonuclease H (RNH, p15) and integrase (IN, p31). RT is responsible for reverse transcription of RNA to DNA. The RNH has the ability to degrade RNA in the context of a DNA-RNA hybrid, which takes place within a viral nucleoprotein complex. The minus single stranded DNA which remains single after RNA degradation is used as a template for the plus stranded and makes the double stranded proviral DNA and form complexes with nucleoproteins (Vartanian and Wain-Hobson, 1994). The IN protein recognizes linear viral DNA ends (the so-called att sites) for accomplishment of integration. The choice of host integration site appears random with regard to the DNA sequences but strongly depends on accessibility of the local chromatin. As increased accessibility of chromatin correlates with increased transcriptional activity, retroviruses may thus preferentially integrate in the vicinity of actively expressed genes (Coffin, 1990; Young, 1994).

The *env* gene encodes for the gp160, which further cleaved into the two *env* proteins, gp120 and gp41 (Peeters *et al.*, 2000). The amino-terminal portion of gp120 is located entirely exterior to the cell membrane and is noncovalently associated with carboxyl terminus of the gp120 that is anchored to the viral membrane. The conserved gp120 surfaces involved in binding to its three minimally polymorphic ligands, gp41, CD4, and chemokine receptors, each exhibit particular problems with respect to the elicitation of or sensitivity to the neutralizing antibodies (Wyatt and Sodroski, 1998). Mulligan *et al.* (1990) indicated that *env* glycoprotein of HIV are central to $CD4^+$ cells, receptor binding, viral entry and syncytium formation, and cytopathic effects (Emerman and Malin, 1998).

HIV-1 *tat* produces a large increase in transcriptional initiation as well as overcoming a block to transcriptional elongation. The HIV-1 *tat* can interact with cellular transcription factors to increase the steady state level of the β-polymerase (Srivastava *et al.*, 2001). The *rev* product suppresses the production of the *nef* gene and itself by preventing accumulation of spliced forms of RNA from which both proteins are made. It is generally agreed that *rev* mediates export of these mRNAs to the cytoplasm. If *nef* protein accumulates first, then virus replication may be suppressed for prolonged periods. If *rev* accumulates first, abundant or controlled replication may occur (Haseltine, 1988; Haseltine and Wong-Staal, 1988).

Nef product suppresses the synthesis of viral RNAs by decreasing the rate of RNAs initiation via its protein products which act on a section of the LTR called negative regulatory element (NRE) which in turn sends a message down regulating viral

replication by inhibiting the production of structural genes (Schoub, 1994; Peeters *et al.*, 2000). Down regulation of CD4 has been observed in cells that express the *nef* gene. *Nef* also removes preexisting CD4 from the cell surface by recruiting CD4 into clathrin coated pits, and ultimately into degradative lysosomes. It is also reported that it down regulates expression of major histocompatibility complex I (Emerman and Malin, 1998; Yoon *et al*., 2001). This effect might conceivably benefit the virus by facilitating virion assembly and budding in the absence of the immune surveillance of the host, or by preventing re-infection of the cell.

Table 1. The nine HIV genes and the proteins encoded by these genes (adapted from Foley *et al.*, 2016).

Name	Size	Function	Localization
Gag			
MA	p17	membrane anchoring; env interaction; nuclear transport of viral core (myristylated protein)	virion
CA	p24	core capsid	virion
NC	p7	nucleocapsid, binds RNA	virion
	p6	binds Vpr	virion
Pol			
Protease (PR)	p15	Gag/Pol cleavage and maturation	virion
Reverse Transcriptase (RT)	p66, p51	reverse transcription, RNAse H activity	virion
RNase H	p15		virion
Integrase (IN)	p31	DNA provirus integration	virion
Env	gp120/gp41	external viral glycoproteins bind to CD4 and secondary receptors	plasma membrane, virion envelope
Tat	p16/p14	viral transcriptional transactivator	primarily in nucleolus/nucleus
Rev	p19	RNA transport, stability and utilization factor (phosphoprotein)	primarily in nuleolus/nucleus shuttling between nucleolus and cytoplasm
Vif	p23	promotes virion maturation and infectivity	cytoplasm (cytosol, membranes), virion
Vpr	p10-15	promotes nuclear localization of preintegration complex, inhibits cell division, arrests infected cells at G2/M	virion nucleus (nuclear membrane?)
Vpu	p16	promotes extracellular release of viral particles; degrades CD4 in the ER; (phosphoprotein only in HIV-1 and SIVcpz)	integral membrane protein
Nef	p27-p25	CD4 and class I downregulation (myristylated protein)	plasma membrane, cytoplasm, (virion?)
Vpx	p12-16	Vpr homolog present in HIV-2 and some SIVs, absent in HIV-1	virion (nucleus?)
Tev	p28	tripartite tat-env-rev protein (also named Tnv)	primarily in nucleolus/nucleus

1.2. Epidemiology of HIV

The Joint United Nations Program on HIV/AIDS report (UNAIDS, 2018) indicated that ~36.9 million people live with HIV globally. Out of which ~66% are in sub-Saharan Africa. The adult (15-59) HIV prevalence in Ethiopia is 0.9%, with varying burden by sex, age, and other demographic characteristics, across sub-regions and population groups. The urban HIV prevalence (2.9%) is seven times higher than the prevalence in rural settings (0.4%), women (1.2%) having twice higher HIV prevalence than men (0.6%) (CSA and ICF, 2016). According to HIV related estimates and projections for Ethiopia (EPHI, 2018a), there are 610,335 HIV infected people with adult prevalence of HIV 0.96%, mainly infected by subtype C (Abebe *et al.,* 2000; Kassu *et al.,* 2007). The Ethiopian demographic and health survey (EDHS) 2016 report (CSA and ICF, 2016) shows Gambella region (4.8%) and Addis Ababa (3.4%) to have the highest HIV prevalence rates while Somali (<0.1%), and Southern Nations, Nationalities and peoples (SNNP, 0.4%) regional states have the lowest.

Surveys and assessment conducted in Addis Ababa such as Ethiopian demographic and health survey (EDHS, 2006, 2012, 2016), and Ethiopian population based HIV impact assessment (EPHIA, 2018) showed that prevalence of HIV is 4.7%, 5.2%, 3.4% and 3.1%, respectively. The Ethiopian population based HIV impact assessment (EPHIA) showed that prevalence of HIV is 3.0% in urban settings (EPHI, 2018b). This relatively high prevalence of HIV in Addis Ababa initiated us to look into the magnitude of the HIV prevalence, who and why they are affected, the availability and utilization of services for the most affected groups, and the gaps and challenges to address the problem.

Therefore, systematic review was carried out aiming in determining the prevalence of HIV and mortality rate by AIDS, predisposing risk factors, identification of hotspot areas, key and priority populations, availability and utilization of services, and challenges and gaps to be addressed for prevention and control of HIV epidemic in Addis Ababa.

Phylogenetic analyses of numerous strains of HIV-1, isolated from diverse geographic origins, have revealed that they can be subdivided into types, groups, subtypes, sub-subtypes and circulation recombinant forms (CRFs). Subtyping is done based on phylogenetic analysis of *env* and *gag* sequences (Simon *et al*., 1998), where there is about 30% nucleotide sequence divergence between the subtypes in the *env* and 14% in the *gag* genes and 13% in the total genome (Peeters *et al.*, 2000).

A virus isolated as a specific classification should fulfill defined criteria. The identified strains with no direct epidemiological linkage must be found in at least three individuals, at least three near full-length genomic sequences should be available, and the subtype should resemble each other with no recombination with other existing subtype through out the genome, a subtype should have consistent clustering and all parts of the genome should be approximately equidistant from one another (Robertson *et al*., 2000). Group M includes the viruses that dominate the global epidemic which is further divided into subtypes, subsubtypes and CRFs. The diversity of HIV-1 has given rise to a large number of variants, including nine subtypes (A–D, F–H, J–K), six sub-subtypes (A1–A4, F1–F2), multiple CRFs and thousands of URFs (Triques *et al.*, 2000). Group O contains a pool of highly divergent but genetically related viruses with its epicenter restricted to West and

Central Africa. Group N, the least spreading of the viruses, is represented by only a handful of viruses identified in Cameroon (Mauclere *et al.,* 1997; Peeters *et al*., 1997; Ayouba *et al*., 2000). A human isolate closely related to SIVgor was isolated and named Group P (Plantier *et al.*, 2009).

All known representatives of what was initially described as subtype E appear in fact to be recombinants of the subtypes A and E, and are now designed (CRF01-AE). CRF04-cpx viruses correspond to the previously described *env* subtype I viruses that are complex recombinant viruses involving at least four subtypes (Mboup *et al*., 1999). Recombinant forms of the virus will continue to appear as long as there is transmission of the different subtypes of HIV-1 across the globe (Peeters, 2000). The highest degree of genetic diversity in HIV-1 is observed in Africa where all subtypes and groups can be observed. In Africa, all the known HIV-1 genetic subtypes including groups N and O are present (McClure and Dalgleish, 1998).

The predominant viral forms in the global epidemic are subtypes A and C, followed by subtype B and the recombinants CRF01-AE and CRF02-AG (McCutchan *et al.,* 1999; Nicole *et al.,* 2000; Peeters *et al.,* 2000). The greatest genetic diversity of HIV-1 has been found in Africa, especially Central Africa. The HIV-1 subtypes are unevenly distributed in the world and the distribution of subtypes is always in state change. In South and East Africa subtype C predominates (Heyndrickx *et al.,* 1998; Hussein *et al.,* 2000). In West and West Central Africa, the majority of viruses are CRF02-AG (Montavon *et al.,* 2000). A/J and A/G recombinants are reported in Yaoundi, Cameroon (Tscherning-Casper *et al*.,

2000). In North America, Europe and Australia, subtype B is by far the most common. However, various other group M subtypes, and even group O viruses, have been reported in the US (Brodine *et al.,* 1999; Womack *et al.,* 2001) and several European countries (Heyndrickx *et al.,* 2000; Lasky *et al.,* 1997). In South America, subtype B predominates, but subtypes F and C are also found (Janini *et al.,* 1998; Russell *et al.,* 2000). The subtype F has a wide spread in South America, Africa, and some regions of Europe (Op de Coul *et al.*, 2000). Different subtypes circulate in Asia, subtype C predominates in Ethiopia, India and china, and CRF01-AE is predominant in Southeast Asia (Anderson *et al*., 1999).

The first two positive sera in Ethiopia were detected in 1984 collection (Tsega *et al*., 1988). The first AIDS cases were diagnosed in Addis Ababa in 1986 (Lester *et al*., 1988). The HIV-1 subtype C is dominant in Ethiopia (Abebe *et al*., 2000). However, subtypes, A and D have also been reported sporadically (Abebe *et al*., 1997; Hussein *et al*., 2000). In Kenya and Uganda, subtype A and D are dominating. In Djibouti subtypes A and C are reported (Louwagie *et al*., 1995). Therefore, there is a high occurence of influx of these subtypes from these countries. The first HIV-1 subtype C sequence in Ethiopia was reported in 1991 (Ayehunie *et al*., 1991) followed by partial *gag* and *env* sequences by Ayehunie *et al*. in 1993. The first full length Ethiopian subtype C sequence was reported in 1996 from a 1986 Ethiopian sample (Salminen *et al*., 1996) and the other full length Ethiopian sequence documenting the first evidence of the subtype A/C recombinant (Sherefa *et al*., 1998). Since there is high dymamism of the HIV tramsmission and epidemiology, there is need of constant surveillances and studies.

1.3. HIV life cycle

HIV infects $CD4^+$ T cells and other antigen presenting cells such as monocytes, macrophages, Langerhans cells, Kupffers cells and microglial cells that have CD4 marker (Vartanian and Wain-Hobson, 1994). The entry of HIV into cells is through fusion of viral and cellular membrane and endocytosis (Young, 1994). The α and β-chemokines receptors CXCR4 and CCR5, respectively, have been identified as the principal coreceptors for T-cell line tropic or syncytium inducing (SI) and macrophage tropic or non-syncytium inducing (NSI) HIV-1 isolates, respectively (Berger *et al*., 1999).

The integrated provirus may remain quite latent in resting lymphocytes. Activated $CD4^+$ T-cells provide a comparable replicative substrate for HIV strains through production of RNA transcripts and proteins, leading to the synthesis of new virions (Holguin *et al*., 2000). Thus, the replication of HIV depends on an intimate interplay between host transcriptional activators such as sp1 and NF-κB, and viral regulatory genes, such as *tat* and *rev* (Weiss, 1994). In the course of HIV-1 infection, HIV replication and $CD4^+$ T cell activation work in feedback loop that the one increase also increase the other (Orendi *et al*., 1998).

The HIV life cycle (**Figure 2)** is summarized in the following series of events: (1) the infection process begins when *env* gp120 binds CD4 and interacts with coreceptors; and fusion reaction induced by *env* gp41 occurs between the lipid bilayer of the virion and the host cell plasma membrane, releasing the viral core into the cytoplasm. (2) Uncoating is taken place and during uncoating CA is lost while at least some MA, as well as NC, the

pol-encoded enzymes IN, RT and the viral protein R (*vpr*) are thought to be retained as part of a high molecular weight complex. (3) During uncoating, reverse transcription of the HIV RNA to generate a double-stranded DNA is largely completed in the presence of lysyl tRNA primer. (4) The high molecular weight complex now referred to as a preintegration complex is transported across the nuclear membrane by expense of adenosine triphospahe (ATP). (5) Integration of the HIV DNA into the host cell chromosome in the nucleus is catalyzed by IN. (6) The integrated provirus serves as the template for the synthesis of the viral RNAs, which are transported to the cytoplasm. (7) The *env* glycoprotein, *gag* and the *gag-pol* polyproteins precursors are synthesized and transported to the plasma membrane. (8) During or after transported, the *gag* precursor, recruits two copies of the single stranded viral RNA genomes, interacts with the *gag-pol* precursor, and assembles into structures visible by electron microscopy as dense patches lining the inner face of the plasma membrane. (9) The assembled *gag* protein complex induces membrane curvature, leading to the formation of a bud. (10) During budding, the viral *env* glycoprotein is incorporated into the nascent particles. (11) Budding is completed as the particle pinches off from the plasma membrane. (12) During budding, the viral PR cleaves the *gag* and the *pol* proteins. PR cleavage leads to core condensation and the generation of a mature, infectious virion, which is now capable of initiating a new round of infection (Taylor *et al.*, 2008).

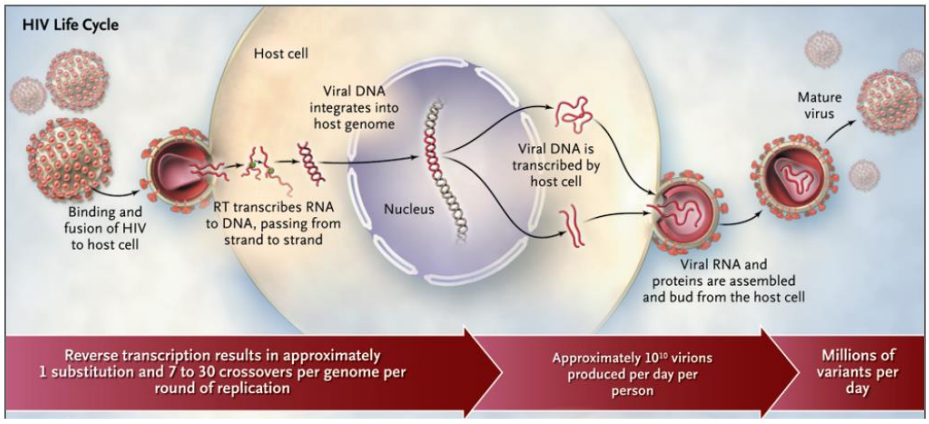

Figure 2. The life cycle and evolution of diversity of HIV-1 by point mutations and recombinations during reverse trascription (adapted from Taylor *et al.*, 2008).

1.4. Causes of genetic variation in HIV

The rate of evolution of many region of a genome is a function of the spontaneous mutation rate, positive and negative selection pressures, numbers of generations per unit of time upon which selection can act, and recombination. Factors such as codon usage bias, methylation of CpG dinucleotides and nucleotide composition bias are examples of selective pressures that can contribute to the evolution rate (Kuiken *et al*., 2000).

1.4.1. Mutation

The remarkable diversity of the virus that causes AIDS makes it an especially challenging virus to study. This diversification occurs when the virus leaves one host, enters a new host, and rapidly diversifies its genome in attempt to adapt its new environment. One of the most important mechanisms for producing new types of RNA viruses is mutation, mostly in the form of point mutation, but also including deletion and rearrangement (Haseltine and Wong-Staal, 1988). The HIV diversity is mainly due to

accumulation of point mutations introduced durinf reverse transcription by the error prone HIV-1 reverse transcriptase (Bonhoeffer *et al*., 1995; Mansky, 1998). This is because most DNA dependent RNA polymerases and RNA dependent DNA polymerase appear to almost lack 3'-5' exonuclease-proofreading activities. Furthermore, about 10^{10} virions are produced each day through its high rate of replication that results in increasing rate of error introduction (Perelson *et al*., 1996).

Despite the rapid evolution rate of lentiviruses, many elements in the lentiviral genome have been observed conserved overtime. It is clear that the regions of conservation of protein sequences represent functional domains of the proteins. Within proteins, catalytic and/or functional domains are highly conserved (Kuiken *et al*., 2000). In addition, the primer binding sites as well as regulatory sequences are conserved along the genome. Subsequent nucleotide sequence comparisons confirmed that sequence differences among different viral genomes are not evenly distributed throughout and is not the property of the entire genome. The changes in conserved genes are synonymous nucleotide changes resulting in silent mutations. The evolution of HIV isolates demonstrates the extreme plasticity of the HIV genome with regard to its genetic and biologic properties: cell tropism, virulence, and antigenicity (Wang-Staal, 1990; McClure and Dalgleish, 1998).

HIV-1 genome exhibits extensive nucleotide sequences variation with blood cells of infected individuals (Pang *et al.*, 1991). The diversity of HIV-1 is mainly caused by mutations that affect the gene encoding the gp120. An amino acid sequence of gp120 from a number of different HIV-1 isolates has revealed five discontinuous regions (V1-

V5) that contain highly variable amino acid residues interspersed with five relatively conserved regions designated C1-C5. The most antigenetically dominant domain on the envelope of HIV-1, the V3 loop of gp120, induces neutralizing antibodies.

The evolution of V3 sequences is apparently host dependent, rapid, and independent of the level of antigen expression (Wolfs *et al*., 1990). Because this region of the virus is highly variable, it ensures an endless source of antibody escape and drug-resistant variants, which may lead disease progression even in the presence of neutralizing antibodies (Young, 1994). Therefore, these viruses may have biological and immunological properties, which differ substantially from viruses, which contain a V3 region similar to the consensus. The conserved residues in the V3 loop are GPGX motif, the two-cysteine residues, and the N-linked glycosylation site.

Mutational and functional analysis of the *env* glycoprotein shows that the conserved regions encode functions such as CD4 recognition, membrane anchorage and membrane to membrane fusion (Haseltine, 1988). Although Gly-Pro-Gly (GPG) motif in the V3-loop lies at the tip of the loop, the three residues are highly conserved and are essential for the *in vitro* tropism of HIV (Young, 1994). But it is known that the cellular and neutralization abilities of antibodies can be affected by the number of charges and glycosylation in the V3-loop. The conformation of V3 domain and the antibody response directed against this epitope may vary from one variant to another which means that the absence of high titer antibodies to the important functional domains, the CD4 binding region and the fusion region, is the possible consequence of their being sequestered by

tertiary folding of the *env* glycoprotein. This variation leads to further delay in the development of a universal prophylactic vaccine and therapeutics to come (Lenz *et al.*, 2001).

1.4.2. Recombination among subtypes

Recombination is when the genome of the virus originates from separate parental viruses. More is known about recombinants through new techniques that allow amplifying and sequencing the whole genome. Recombinants can only be detected if full-length sequencing of the genome is available (Delwart *et al.,* 1993; Heyndrickx *et al.,* 1998). This has led to the reclassification of viruses that were previously known as recombinants. The studies then should be how the modified virus behaves in the culture to know the implications of naturally occurring recombination.

The highly recombinogenic nature of RT enzyme is known as far as the individual are infected by genetically diverse viruses (Hu *et al.,* 1990). Recombination requires the simultaneous infection of a cell with two different proviruses, allowing the encapsidation of one RNA transcript from each provirus into a heterozygous virion. After the subsequent infection of a new cell, the RT, by jumping back and forth between the two RNA templates, will generate a newly synthesized retroviral DNA sequence that is recombinant between the two parental genomes (Goodrich and Duesberg, 1990; Hu *et al.,* 1990; Stuhlmann and Berg, 1992). Mosaic viruses are supported by the fact that discrete breakpoints can be identified between the genomic regions with different phylogenetic associations (Carr *et al.,* 1998; Kuiken *et al.,* 2000). As the epidemic spreads, and as

subtyped previously isolated to populations in specific regions more into new populations, the probability for new recombinant viruses increases.

1.5. HIV temporal and spatial variation

Virus in-patients early in the infection are relatively homogenous and then diverged with time, more consistently at its non-synonymous sites. Within the constraints of the current dataset, it is concluded that the virus appears continually accumulate changes in its amino acid sequences throughout the incubation period or well into CD4 T cell decline during the progression to disease (Shankarappa *et al*., 1998). The dynamic interaction between viral diversity and the human immune system suggests the existence of an antigen diversity threshold, below which the immune system is able to regulate viral population growth but above which the virus population induces the collapse of the $CD4^+$ lymphocyte population. This suggests that antigenic diversity is the cause, not a consequence, of immunodeficiency disease (Nowak *et al*., 1991).

Tissue specific variant populations and distributional differences of variants in the mucosa and peripherl blood mononuclear cells (PBMCs) suggest that selective pressures on the two virus pools are different. The mechanism by which HIV-1 subtypes are selectively transmitted and subsequently undergo clearance, sequestration, and evolution in different tissue compartments will be paramount importance for understanding early immune responses against sexually transmitted HIV-1 and ultimately designing globally effective therapeutics and vaccine (Poss *et al*., 1995).

Evidence for viral compartmentalization was suggested by earlier studies that noted a weak correlation between viral RNA level in mucosa and in blood, lack of association between culturability of virus in mucosa and viral RNA level in blood, discordant distribution of viral phenotypes, and differences in the virus load response to antiretroviral therapy (Coombs *et al*., 1998; Kovacs *et al*., 2001). Significant genotypic differences are found in cell free virus from matched blood plasma and vaginal secretions (Adal *et al.,* 2005). Moreover, drug resistance-associated mutations appear in plasma virus several months before appearing in vaginal virus. These findings indicate the cellular replication of HIV-1 occurs in vaginal secretions and can result in a virus population with important differences from that in blood (Ellerbrock *et al*., 2001).

Tissue HIV-1 is present at high levels in genital tract secretions during acute primary infection stage and is followed by reduced levels during the subsequent period of clinical latency by increased levels during late-stage disease (Wahl *et al*., 1999). A similar diversity was observed in virus from cells present in cervical secretions (Overbaugh *et al*., 1999). It has been reported that the virus transmitted to an individual by sexual contact is a minor component of the virus population in the donor blood (Zhu *et al*., 1993) and that variants found in the genital secretions of the infected women are different from those in the peripheral blood (Poss *et al*., 1995; Overbaugh *et al*., 1996). The compartmentalization of blood and semen infection was supported by genetic analysis of several infectious HIV clones isolated from semen cells and peripheral blood cells of another donor not on antiretroviral therapy (Kiessling *et al*., 1998).

Temporal structure in the mucosal compartment could result from successive migrations of virions not well represented in either PBMC or plasma viral sequences. Alternatively, the presence of significant temporal phylogenetic structure could reflect tissue specific evolution of the mucosal variants. The premise that phylogenetic structure arose by independent migration events and subsequent viral proliferation is consistent with the biology of memory cells homing to specific tissue compartments (Mackay *et al*., 1992a; Mackay *et al*., 1992b).

1.6. Selection and HIV immune control mechanisms

The diversity of viral quasispecies is shaped by a combination of mutation, recombination and selection forces. The main selective forces that have been proposed to derive HIV diversity are the immune response, cell tropism and random activation of infected cells (Bonhoeffer *et al*., 1995). Early in the infection the immune system will respond strongly against common viral strains and hence favor rare mutants there by providing a strong positive selection pressure for diversification. HIV infects many different cell types and tissues in the body with the notion that most viral diversity in the V3 region is caused by adaptation for various cell tropisms.

Stimulation of cells induce a complex wave of events leading to contextual changes in gene expression, the consequences of which depend entirely on the cell type, be it proliferation, secretions, cell death, differentiation or a myriad of other responses (Woodgett, 1996). Activation of the HIV genome for integration requires viral and host cell encoded protein factors. If the infected cell is not activated, integration of the HIV

genome into the host genome is inefficient. Furthermore, activation of the cell is required for the integrated HIV genome to be transcribed into either genomic or mRNA (Berger *et al.*, 1999). The expression of viral genes requires the collaborative activities of the host-cell transcription machine (RNA polymerase and transcription factors sp1 and NF-κB) and viral regulatory proteins *tat* and *rev*. The concentration gradients for such regulatory factors are thought to vary depending on the growth cycle of the cell (Haseltine, 1988; Coffin, 1990; Young, 1994).

The accelerated rate of disease progression has been related to chronic immune stimulation due to pathogenic microbes, inducing parasites (Gilks, 1993; Bentwich *et al*., 1995). It is found that a greater systemic HIV-1 heterogeneity in HIV/TB patients than in HIV patients (Collins *et al*., 2000). Malarial antigen increased HIV-1 replication by increasing viral mRNA expression and by activating LTR directed viral transcription through the production of cytokines (Xiao *et al*., 1998; Hoffman *et al*., 1999). Sexually transmitted infections (STIs) increase HIV shedding, thereby increasing the likelihood that genital secretions contain higher viral load of the HIV required for transmission (Hitchcock and Fransen, 1999; Lawn *et al*., 2000). Data suggest that treatment of STI and malaria concurrent infection significantly reduced high viral loads unless there are other infections untreated in semen and blood, respectively (Hoffman *et al*., 1999).

1.6.1. Role of HLA and $CD8^+$ cells in HIV immune control

During the acute stage of infection, HIV-1 replicates very rapidly leading to a viral load of several million copies/mL of blood accompanied by a marked drop in the number of circulating $CD4^+$ T cells (Tindall and Cooper, 1991). The viral load decreases to a median viral setpoint that is approximately 10^2-10^3-fold lower than the peak of viremia (Lyles *et al.*, 2000; **Figure 3**). Although attempts to correlate the breadth of the cytotoxic T lymphocytes (CTL) antiviral response and control of HIV-1 infection *in vivo* have been equivocal (Masemola *et al.*, 2004; Liu *et al.*, 2007), accumulating evidence support the beneficial role of CTL responses (Ramduth *et al.*, 2005; Honeyborne *et al.*, 2007).

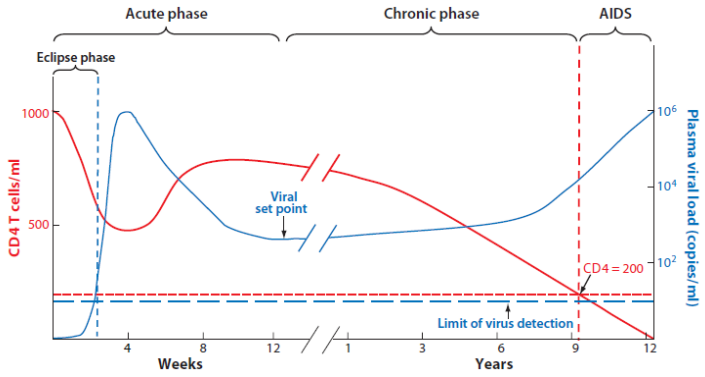

Figure 3. Dynamics of peripheral blood CD4 T cell counts and plasma viral load during a typical course of HIV infection (Source: Bashirova *et al.*, 2011).

The evidences for protective role of CTL responses are, first, their presence during SIV infection leads to decreased viral replication and slower disease progression, and administration of monoclonal antibodies specifically depleting $CD8^+$ T cells abrogated the decline in viremia in rhesus macaques (Schmitz *et al.*, 1999). Second, resolution of acute viremia/drop in viral load is coincident with a major expansion of HIV-specific $CD8^+$ T cell responses to viral peptides presented by HLA molecules even if differences exist in their antiviral effectiveness based on their HLA restriction, epitope specificity,

functional epitope avidity and targeted viral protein (Koup *et al.,* 1994; Goulder and Watkins, 2008). Third, during primary and chronic infection, immunologic pressure mediated by SIV- and HIV-specific $CD8^+$ T cells is often manifested by viral escape mutation (Goulder *et al.,* 1997). Finally, there are strong correlations between the expressions of certain HLA class I alleles, lack of escape mutations, and nonprogressive HIV infection before the individual reaches to AIDS stage (Carrington and O'Brien, 2003). Ultimately, the $CD8^+$ T-cell response to HIV in most infected patients is insufficient to maintain HIV viral load and disease progression. After infection with HIV-1, the clinical course is highly variable between different human hosts and AIDS typically develops within 8-10 years. Some individuals referred to as long-term non-progressors (LTNPs), however, display no signs of disease progression although they have been infected with HIV-1 for more than 10 years.

Variation at the HLA class I locus has a stronger influence on HIV-1 disease outcome than that of any other genetic locus identified so far (Fellay *et al.,* 2007). HLA-B has been the primary focus of research, relative to HLA-A and HLA-C, because the strongest genetic (Martin *et al.,* 2002; Martin *et al.,* 2007) and functional (Kiepiela *et al.,* 2007) associations with HIV infection outcomes have involved this locus. In particular, HLA alleles B*27, B*57 and HLA-B*58:01 that are over-represented among HIV-1 infected LTNPs confer particularly strong protection against HIV (O'Brien *et al.,* 2001; Goulder and Watkins, 2008). In contrast to the beneficial outcomes associated with the protective HLA-restricted Gag-specific $CD8^+$ T cell responses, some other alleles [B*35(Px), B*5802 and B*18:01] are associated with rapid AIDS progression through mechanisms

that are not yet clear (Leslie *et al.,* 2008; Kiepiela *et al.,* 2004). Gag HLA-C-restricted $CD8^+$ T cell responses appear to contribute little or even negatively to viral control *in vivo* (Kiepiela *et al.,* 2007). However, Thomas *et al*. (2009) showed that high HLA-C expression is more effective in control of HIV-1.

Protective HLA molecules bind fewer peptides, which delete fewer self-reactive T cells in the thymus, giving virus-specific T cells broader fine specificity (Kosmrlj *et al.,* 2010). This could reduce virus options to escape by mutation (Fischer *et al.,* 2010). Broad T-cell responses to many HIV epitopes appear a major determinant of the HIV set point and appear to be more likely to contain HIV infection (Koup *et al.,* 1994). Possessing heterologous HLA alleles, and thus possibly allowing the individual to present broader arrays of epitopes, has also been associated with slower disease progression (Carrington *et al.,* 1999). This indicates that the intrinsic ability to present fewer or specific viral epitopes could affect clinical markers of disease progression and puts forth the epitope repertoire as a mechanistic component of the multi-faceted HIV-specific CTL response with the broader T cell specificity to limit escape (Rolland *et al.,* 2008).

HIV-specific $CD8^+$ T cells may also display different differentiation status and activation profiles (Papagno *et al.,* 2004). It has been suggested that these phenotypic differences are associated with divergent functional antiviral capacities of virus specific T cells (Almeida *et al.,* 2009). Besides a skewed maturation process from effector memory to effector cells (Betts *et al.,* 2006), significant differences in the ability of polyfunctional HIV-1 specific $CD8^+$ T cells that are able to proliferate and secrete up to five different

effector functions upon *in vitro* peptide stimulation (IFN-γ, IL-2, TNF-α, MIP-1β and CD107α) have better antiviral activity between progressors versus nonprogressors (Horton *et al.,* 2006; Precopio *et al.,* 2007).

$CD8^+$ T cell responses restricted by the protective alleles are reported to be polyfunctional with effective viral control when compared to $CD8^+$ T cell responses restricted by HLA-A and HLA-C alleles (Harari *et al.,* 2007; Kiepiela *et al.,* 2007). Available data have also suggested that the functional profile of $CD8^+$ T cells is largely a consequence of the duration and level of antigen load, with prolonged continuous exposure to high levels of antigen resulting in exhausted $CD8^+$ T cells characterized by a monofunctional effector profile that results in less antiviral activity and disease progression (Streeck *et al.,* 2008). Differences between antigen-specific $CD8^+$ T cells with respect to up-regulation of programmed death-1 (PD-1) that is used as a marker for activation and susceptibility to apoptosis (Petrovas *et al.,* 2006; Day *et al.,* 2006), and downmodulation of the IL-7 receptor (CD127) in chronic viral infection that is used as a marker for a lack of transition into memory T cells (Kaech *et al.,* 2003; Wherry *et al.,* 2004) have been demonstrated between progressors and nonprogressors. Both markers have been linked to the accumulation of functional impairments of virus-specific $CD8^+$ T cells from early to chronic stages of infection (Petrovas *et al.,* 2007; Zhang *et al.,* 2007).

LTNPs possess HIV-specific $CD8^+$ CTLs restricted by HLA-B*27 or HLA-B*57/*58:01 that can continue to proliferate throughout chronic infection, whereas the majority of HIV-specific $CD8^+$ CTLs restricted by other HLA alleles lose their proliferative capacity

(Migueles *et al.,* 2002; Horton *et al.,* 2006). Proliferative ability is linked to upregulation of perforins and granzymes, and is associated with the cytotoxic capabilities of virus-specific $CD8^+$ CTLs (Migueles *et al.,* 2002). HLA-B*27 and HLA-B*57 restricted HIV-specific $CD8^+$ CTLs possess an additional feature in that they evade suppression mediated by Treg cells by killing them. This provides another contributatory mechanism for why *HLA-B*27* and *HLA-B*57* allele groups are associated with delayed HIV-1 disease progression (Elahi *et al.,* 2011).

1.6.2. Role of HLA and NK cells in HIV immune control

NK cells are a subset of lymphocytes of the innate immune system that are important in the elimination of virus infected and tumor cells (Hamerman *et al.,* 2005). NK cells express a diverse repertoire of activating and inhibitory receptors that are encoded by multiple genes (Parham, 2005). HLA class I molecules can also modulate the innate response by serving as ligands for the genetically polymorphic killer cell immunoglobulin-like receptors (KIRs) expressed on NK cells. KIRs recognize particular peptide-MHC class I complexes (Sanjanwala *et al.,* 2008).

Although down-regulations in HLA class I molecules can 'disinhibit' NK activity that result in destruction of the infected cells, retention of HLA-C expression on HIV-1–infected targets allows them to remain invisible to most NK cells (Cohen *et al.,* 1999). Under normal conditions, the inhibitory signals are dominant and prevent the destruction of normal cells by NK cells. However, in response to viral infections, the expression pattern of NK cell receptor ligands is altered dramatically on infected cells (Bonaparte and Barker, 2004). These changes allow NK cells to recognize infected cells by

triggering the activating receptors and/or through the loss of a strong inhibitory signal to highly expressed receptors resulting from HLA-B downregulation on HIV-1-infected cells. Thus, the delicate balance between activation and inhibition that controls NK cell function is tipped toward activation, resulting in the lysis of the target cell.

This information may also help to explain the well-established observation that *HLA-B57* is associated with slower HIV disease progression, whereas *HLA-B35* is associated with more rapid progression (Goulder and Watkins, 2004). In HIV-1 infected individuals expressing both HLA-B*57 and highly expressed KIR3DL1, the virus may be cornered between two potent antiviral killers: $CD8^+$ T cells and KIR3DL1 NK cells that react to any downregulation of HLA-B*57 with strong 'disinhibition'. Thus, these new data point toward important synergistic interactions between HIV-specific $CD8^+$ T cell activity and NK activity that are of significance in HIV pathogenesis.

1.7. HIV immune response evasion mechanisms

HIV can avoid an immune response through Nef based down regulation and presence of escape and compensatory mutations that help the viruses to escape the humoral and/or cell-mediated arms of immunity.

1.7.1. The role of Nef in immune evasion

The Nef protein uses the endocytic sorting machinery to misdirect MHC class I molecules away from the cell surface by associating with the cytoplasmic domain of HLA-A and HLA-B molecules (Williams *et al.,* 2002; Schwartz *et al.,* 1996), which

makes infected cells less vulnerable to T cell–mediated recognition and lysis (Collins *et al.,* 1998). HLA C is not down-regulated by HIV-1 Nef from the surface of infected cells to the same extent that HLA-A and HLA-B molecules are (Cohen *et al.,* 1999; Collins *et al.,* 1998). Thus, NK cell receptors that bind HLA-C might be preferentially important in recognition of infected cells.

Nef can target MHC class I molecules early in the folding pathway when expressed in T cells by preferentially binding newly synthesized hypophosphorylated H-chains of MHC class I molecules. This may in part be responsible for the more pronounced effects on slow maturing MHC class I molecules, leading to their accumulation within the trans-Golgi network and diversion to lysosomes (Kasper *et al.,* 2005). Due to down-regulation of the HLA type I the control ability of CTLs will be compromised.

1.7.2. Escape and compensatory mutations

The HIV genome is found to be diverse and with the range of strains. One of the most important mechanisms for producing new strains of retroviruses is mutation (Bonhoeffer *et al*., 1995) and the role of $CD8^+$ lymphocyte responses in driving viral evolution (Allen *et al.,* 2005; Liu *et al.,* 2006).

Sequence alterations can occur at anchor positions of the epitope and reduce or abrogate peptide binding to the restricting MHC class I molecule. Alternatively, amino acid changes within or immediately adjacent to $CD8^+$ T-cell epitopes can interfere with intracellular antigen processing via altered proteasomal cleavage or transporter associated

with antigen processing (TAP) transport (Allen *et al.,* 2004; Draenert *et al.,* 2004), or directly alter the structural interaction between the epitope of MHC class I complex and the T cell reptor (TCR) of the corresponding $CD8^+$ T cells (Price *et al.,* 2004).

Mutations involving amino acids which are important for TCR recognition, in contrast, have no effect on presentation of the epitope to CTL but rather lead to diminished, or sometimes even complete loss of, recognition of the epitope-specific CTL, although it has been shown that new CTL recognizing the mutated epitope can be generated (Allen *et al.,* 2005; Bailey *et al.,* 2006). There is influence of HLA allele subtypes on TCR selection and extensive TCR diversity is not a prerequisite to prevent allowable viral mutations (Yu *et al.,* 2007). Variant peptides can act as antagonists for T cells responsive to the viral epitope, thus allowing both mutant and wild-type viruses to survive (Lichterfeld *et al.,* 2007; Thananchai *et al.,* 2007; McMichael, 2007). It has been shown that CTL escape mutations can be stable after transmission to a new host even if the recipient expresses different HLA alleles. This may be because HIV-1 is accumulating mutations contributing to fitness of the virus and the human immune system fails to control viremia (Leslie *et al.,* 2005; Goepfert *et al.,* 2008). This means that transmission of HIV containing a given escape mutation to a new recipient may result in reversion of the mutation, depending on the fitness cost and the recipient's immune response.

The escape and compensatory mutations are accumulating over time during acute as well as chronic phase of infection and often undermine immune control by $CD8^+$ T cells. They are generally associated with rapid progression to AIDS (Crawford *et al.,* 2007). This

strengthens the suggestion that evasion of the CTL response through mutation of the virus is a significant mechanism of viral persistence (Phillip *et al.,* 1991). Escape mutations also have a fitness cost expressed in terms of weak replication ability of the virus despite continuous immune pressure on the wild-type virus that will favour the outgrowth of the attenuated escape variant. The escape mutation imparts a fitness cost on the virus and the virus rapidly mutates back to wild-type if transmitted to individuals with different HLA type that results in positive selection of the escape mutant (Goulder *et al.,* 2001). Reversion is observed in lesser extent even for rapidly reverting variants and explained either by mutant acquisition exceeding reversion rate or by selection of compensatory mutations slowing or halting reversion altogether (Peyerl *et al.,* 2004).

1.7.3. HIV mutations and neutralizing antibodies

The persistence of HIV infection through the long latency period of AIDS demonstrates HIV has ability to avoid being eliminated by the host immune response. Immune evasion has frequently been attributed to two viral mechanisms: latent cellular infection, and rapid mutation. As a retrovirus, HIV can persist integrated in the genome of a cell, in a completely latent fashion; since latently infected cells display no viral antigens, they will not be removed by an immune response. It has been suggested that AIDS latency is a period of predominantly latent and low-level cellular infection (Fauci, 1988). However, more data indicates that substantial populations of free virus and actively infected T4 cells may be present during the latent period (Ho *et al.,* 1989; Coombs *et al.,* 1989).

Antibody-dependent cellular cytotoxicity (ADCC) is of considerable interest as an immune response that may facilitate the control of HIV infection. ADCC responses to

either gp140 Env protein or HIV peptide pools were common in HIV-positive subjects when NK cells from the HIV-positive subject were used. ADCC responses to whole gp140 Env protein were strongly associated with a slower decline in CD4 T-cell loss when healthy donor NK cells were used as effectors (Chung *et al*., 2011).

Neutralizing antibody as part of the humoral responses against autologous HIV-1 were reported first by Weiss *et al.* (1986), and several later studies have suggested that its appearance is slow to develop and of low titer (Burton, 1997; Parren *et al.,* 1999). The antibody responses to these variants exert a selective pressure that drives continuous evolution of neutralization escape mutants. Neutralizing antibody responses account for extensive variation in the envelope gene that is observed in the early months after primary HIV-1 infection. It comprised of multiple clonal responses with neutralizing activity directed against several epitopes on gp120 variable loops, the CD4-binding site and the co-receptor binding site. Amino-acid sequence variation, the masking of otherwise vulnerable regions on the envelope glycoproteins with sugars called glycans and conformational flexibility of HIV-1 envelope glycoproteins are the defence mechanisms of HIV-1 against antibody neutralization (Kwong *et al.,* 2002; Wei *et al.,* 2003). Many HIV infected patients produce neutralizing antibodies (NAbs), and a small fraction makes extremely potent NAbs with broad crossreactivity (Binley *et al.,* 2008; Doria-Rose *et al.,* 2009; Simek *et al.,* 2009). Many of the new NAbs are more potent than the original prototype HIV NAbs and many recognize novel epitopes on *Env* glycoprotein gp120, illuminating new targets for vaccine design (Walker *et al.,* 2009; Wu *et al.,* 2010). Analysis of neutralization by the full complement of anti-HIV NAbs now available

reveals that certain combinations of antibodies should offer markedly more favourable coverage of the enormous diversity of global circulating viruses than others, and these combinations might be sought in active or passive immunization regimes.

1.8. ART and HIV potential cure

Combinations of strategies are used to prevent and cure HIV/AIDS (**Figure 4**). Since the approval of zidovudine (AZT) in 1987, over 25 antiretroviral agents in six classes that include nonnucleoside reverse transcriptase inhibitors (NNRTIs), nucleoside reverse transcriptase inhibitors (NRTIs), protease inhibitors (PIs), CCR5 antagonists, fusion inhibitors, and integrase strand transfer inhibitors (INSTIs) have been approved to treat HIV infection. Until recently, a first-line regimen for ART-naive individuals consisted of two NRTIs plus an NNRTI, a ritonavir-boosted protease inhibitor (PI/r), or an INSTI. The regimen selection is based on virologic efficacy, potential for adverse effects, pill burden and dosing frequency, drug–drug interaction potential, resistance test results, comorbid conditions, social status and cost. Current antiviral treatments can reduce HIV-associated morbidity and mortality, and prevent HIV transmission. With prolonged virologic suppression, improved clinical outcomes and longer survival, patients will be exposed to antiretroviral agents for decades. Therefore, maximizing the safety and tolerability of ART, as well well as monitoring of drug resistance is at a high priority.

An extensive reduction of the reservoir to below the limit of detection of the current assays is not sufficient to achieve a long-term remission and result in the persistence of integrated proviruses despite therapy. Presence of a select HIV-infected population of

memory CD4+ T cells and the expanding proviruses have been shown to be a source for residual viremia during ART, and they may be a source for viral rebound after interrupting ART. The latent HIV viruses can reactivate and rebound within weeks in a majority of HIV-1-infected patients once ART is stopped (Solomon and Sax, 2015). However, treating patients during primary HIV infection does reduce the viral load and global viral genetic diversity, and result in loss of virus fitness in the population due to the selection of viral strains, even if there is accumulation of drug resistance mutations in the target genes to almost all classes of ARTs (Iyidogan and Anderson, 2014; Arenas, 2015). It also reduces the size and diversity of the reservoir and may minimize the number of cells that are capable of proliferation and harbor intact proviruses. This restricted pool of variants will allow more focused targeting by therapeutic vaccines and other immune approaches, such as those inducing CTL responses to eliminate infected cells after reactivation with latency reversing agents. Therefore, there is a growing interest in approaches that will provide a cure or remission for HIV infection.

Advances in understanding the basic biology of HIV have provided a framework to conceptualise novel approaches to curing the disease. These include genetic engineering technologies to either protect CD4+ T cells from future infection or deliver probes to inactivate integrated HIV DNA in the cell. Use of genetically engineered T^{+} cells to provide long-term immune surveillance or to activate latently infected cells and destroy them and any associated HIV virions before they proliferate are being investigated (De Crignis and Mahmoudi, 2014). Another potential approach for an HIV cure is a “shock and kill” strategy. This approach uses latency-reversing agents that, firstly, induce

latently infected cells to produce virus ("shock") and then strive to clear these virus-producing cells ("kill") through an enhancement of host antiviral immunity (Barouch and Deeks, 2014). The latency-reversing agents mainly include histone deacetylase inhibitors, methylation inhibitors, and cytokines like interleukin-7 (Jiang *et al.*, 2015). The immunologic strategies for killing the virus-producing cells include therapeutic vaccines, monoclonal antibodies, and immune checkpoint inhibitors (Katlama *et al.*, 2013; Barouch and Deeks, 2014). Whether these strategies can either singly or in combination result in a true cure or what the field has termed a "functional" cure (that is, the ability to maintain viral suppression without antiretrovirals (ARVs) with no risk of sexual or perinatal transmission) remains to be determined (De Crignis and Mahmoudi, 2014).

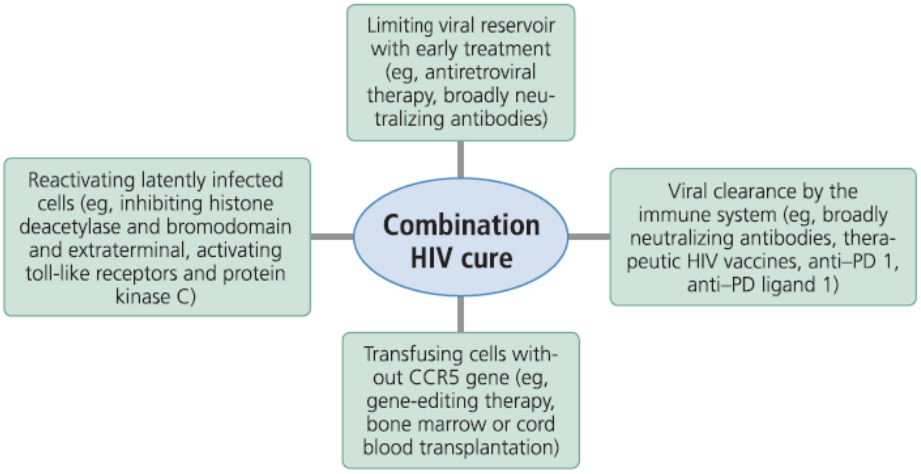

Figure 4. Different HIV cure Strategies currently used in human studies that may need to be combined in order to achieve HIV remission. CCR5, CC chemokine receptor 5; PD, Programmed Cell Death (Adapted from Ananworanich, 2015).

1.9. Prospective HIV vaccine and protection correlates

Interventions to reduce HIV acquisition - large scale circumcision programs, pre- and postexposure prophylaxis with tenofovir-based regimens for high risk individuals, and the use of ARTs to prevent mother to child transmission (MTCT) have favorably influenced the trajectory of HIV infections. However, the need for a globally effective HIV vaccine is more compelling than ever.

HIV vaccine development has been challenging because of unclear immune correlates of protection, high genetic diversity, lack of a relevant animal model and little pharmaceutical interest. The presence of individuals who are highly exposed to HIV-1 but do not get infected and LTNP provide hope for a better understanding of correlates for protection that may lead to a more effective vaccine strategy. Highly exposed seronegative populations have been identified among intravenous drug users, children born to seropositive mothers, discordant couples and commercial sex workers.

Although most current viral vaccines are based on either live attenuated or whole-inactivated viruses, these approaches were considered unsafe for an HIV vaccine because of the danger of integration of the proviral DNA in the host chromosome. Therefore, candidate vaccines against HIV include protein or sub-unit vaccines, recombinant viral vector and DNA based vaccines. While more than 200 vaccine candidates (concluded or ongoing mostly Phase I or II) have been tested clinically since 1987, only six vaccine efficacy trials of Phase IIb and III (VAX004, VAX003, Step, Phambili, RV144 and HVTN 505) have been completed (**Table 2**). The majority of these trials involved

different prime-boost combinations, followed in frequency by proteins or peptides, poxvirus vectors, DNA vaccines, adenovirus vectors, and other concepts.

Table 2. Completed HIV-1 vaccine efficacy trials (adapted from Stephenson *et al.*, 2016).

Trial	Vaccine description	Immune responses observed	Efficacy outcome
VAX004	AIDSVAX B/B gp120 (MN and GNE8 subtype B) gp120 in alum	Non-neutralizing antibody response; ADCVI	No efficacy
VAX003	AIDSVAX B/E gp120 (subtype B MN and CRF01_AE CM244) gp120 in alum	Non-neutralizing antibody response	No efficacy
HVTN 502/Step Trial	Adenovirus type 5 Clade B gag/pol/nef	HIV-1 specific CD4+ and CD8+ responses	No efficacy, increased infection risk
HVTN 503 (Phambili trial)	Adenovirus type 5 Clade B gag/pol/nef	HIV-1 specific CD4+ and CD8+ responses	No efficacy, increased infection risk
RV144	ALVAC-HIV (recombinant canarypox vector)/vCP1521 and AIDSVAX B/E rgp120 in alum	Humoral and cellular immune responses; Non-neutralizing Ab to V1V2*, high ADCC, HIV-1 specific IgG3, FcγRIIC receptor * Correlate of protection	31.2% efficacy at 42 months, 60% efficacy at 12 months
HVTN 505	DNA Gag, Pol, and Nef from HIV-1 subtype B and Env from subtypes A, B, and C, and rAd5 subtype B Gag-Pol and Env A, B, and C	T-cell responses to HIV-1 potential T-cell epitopes; CD4+ HIV Gag responses	No efficacy

HVTN, HIV Vaccine Trials Network; ADCVI, antibody-dependent cell-mediated virus inhibition; ADCC, antibody-dependent cellular cytotoxicity.

The only modestly efficacious RV144 HIV vaccine trial conducted in Thailand evaluated the efficacy of an ALVAC-HIV prime expressing clade E *env* and clade B *gag* and *pol*, followed by an AIDSVAX clade B/E gp120 protein boost. The trial demonstrated the first year postimmunization efficacy of 60% and an overall 31% efficacy after 3 years in preventing HIV infection (Rerks-Ngarm *et al.*, 2009; Robb *et al.*, 2012). The immune-correlates study generate the hypotheses that V1V2 antibodies may have contributed to protection against HIV-1 infection, whereas high levels of Env-specific IgA antibodies may have mitigated the effects of protective antibodies. This analysis failed to identify neutralizing antibodies as a potential correlate, turning the attention to a potential role of non-neutralizing antibodies, probably to those involved in mediating ADCC (Alpert *et al.*, 2012; Wren and Kent, 2011). Protection correlated with antibodies against the V1/V2

region of gp120, particularly those antibodies of the IgG3 subclass that mediate potent ADCC (Yates *et al.*, 2014).

A number of studies have identified potential epitopes for NAbs and are directed to five general HIV-1 Env glycoprotein targets that include the gp41 membrane proximal external region (MPER), the gp120 variable loop 1/2 (V1V2), the gp120 variable loop 3 glycan (V3), the CD4 binding site (CD4bs) on gp120, and a conformational combined gp41-gp120 set of epitopes (Mascola and Haynes, 2013; Falkowska *et al.*, 2014; Scharf *et al.*, 2014; **Figure 5**). The most important challenge is which of these epitopes need to be targeted by future vaccines. The further question is whether vaccines should focus on individual epitopes or a combination of multiple epitopes. The focus should also be on the breadth, magnitude, and durability and other characteristics that make NAbs significantly neutralizing and useful. Future studies should also focus on elucidating the right sequence of somatic hypermutations to derive effectively NAbs. Vaccines need to be effective in the long term and offer continuous protection. With regard to stimulation of cytotoxic T cells, the exact mechanism for the production of effective CD8 T cell responses needs to be researched and understood.

HIV candidate vaccines evaluated to date have either failed or have shown very modest and debated efficacy. Unlike other vaccines that use a sequence of immunizations with three identical immunogens, an HIV vaccine may require a sequence of immunizations with different immunogens to guide antibody responses from naive B cells to mature and produce bNAbs and improve the durability of vaccine-induced immune responses

because of the protracted evolutionary pathways that bNAbs undergo in HIV-1-infected persons. Meanwhile, new vaccine concepts including native-like envelope trimers, nanoparticle, DNA based vaccines, nonreplicating viral vector-based vaccines, mosaic vaccines, passive immunization, and mRNA vaccine design strategies have entered the clinic and new insights have been obtained in basic research that will ultimately help to guide rational vaccine design (Burton *et al.*, 2019; Jones *et al.*, 2020).

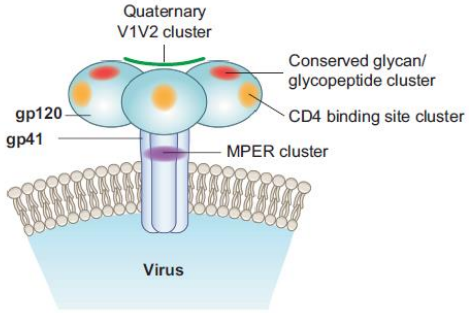

Figure 5. Schematic diagram of a trimeric structure in the lipid bilayer of the virus envelope glycoprotein complex of HIV-1. The major broadly neutralization-sensitive epitopes on gp41 and on gp120 are labeled (adapted from Chiodi and Weiss, 2014).

1.10. HIV/AIDS prevention and control in Ethiopia

HIV/AIDS prevention and control programs are implemented based on the national strategic plan that intends to achieve the three 90 targets by 2020 through targeted social mobilization and HIV testing, linkage to care, quality HIV treatment, and virtual elimination of MTCT, envisioning ending AIDS by 2030 (FHAPCO, 2014). HIV transmission interventions include behavioural, biomedical and structural components. There are various behavioural, socio-cultural and structural predisposing risk factors that drive the epidemic. The current HIV program is applying targeted approaches. Cognizant of that, Ethiopia has identified key and priority populations (KPPs) based on HIV prevalence rate in specific groups. The recognized key population (KP) group in Ethiopia

are the female sex workers (FSWs). Ethiopia's efforts to reach most-at-risk populations (MARPs) with HIV prevention, treatment and care services are inadequate. In addition, systems for linkage of identified HIV-positive clients to care and treatment services are not effective. Since the positive testing yield is often low for provider intiated testing and counseling (PITC), case-based-surveillance (CBS), index-case-testing (ICT) and partner-notification-services (PNS) are important tools for targeted HIV testing for new case finding. The status of the three 90's for Addis Ababa is below the national urban average. Status of the three 90's in Addis Ababa for the age group 0-64 years is lower than the national average which is: 65.2 % for the 1st 90, 63.3 % for the 2nd 90 and 58.2% of all people living with HIV (PLHIV) had viral load suppression (VLS) with viral load level of <1000 copies/ml (EPHI, 2018b).

1.11. Consequences of genetic variation of HIV

HIV diversity is caused by mainly accumulation of point mutations (Bonhoeffer *et al*., 1995; Mansky, 1998). The high rate of replication also amplified it (Perelson *et al*., 1996). The number of charges and glycosylation in the V3-loop can affect cellular and neutralization abilities of antibodies (Pollakis *et al.,* 2001). The development of medical interventions gets much more difficult because of the high genetic diversity of the variants within an individual overtime and the emergence of recombinants. This may also enable HIV both to escape from the immune response and to develop resistance to antivirals. In addition, it makes difficult development of diagnostics, vaccine(s) and therapeutics (Batra *et al*., 2000; Lenz *et al.,* 2001; McMichael *et al*., 2007). The study of

genetic variation, subtyping and circulating recombinant forms (CRFs) would be necessary for monitoring transmission and its epidemiology.

Biological properties of subtypes may differ in tissue tropism, virulence and transmissibility (Tscherning *et al*., 1998). HIV-1 subtype C infected Ethiopian patients harbor low frequency of syncytium inducing (SI) viruses (Abebe *et al*., 1999). There is correlation between progression to disease and the viral phenotype (Moore *et al.,* 2004; Nabatov *et al.,* 2004). HIV-1 strains of Non-syncytium inducing (NSI) use primarily CCR5 computing with β-chemokines like macrophage inflammatory protein-1α or 1β (MIP-1α and MIP-1β) receptor, and regulated on activation, normal T expressed and secreted (RANTES), while SI strains use CXCR4 in comptetition with α-chemokines like stromal differentiating factor 1 (SDF-1) (Chowdhury *et al*., 1995). Different bioinformatics algorithms for prediction of coreceptor usage are developed (Lengauer *et al.,* 2007; Cashin *et al.,* 2015). The combinations of the net charge of V3 and presence of lysine and argenine amino acids at positions 11/24/25 are also used to determine the phenotype (Adal *et al*., 2005).

The homozygous delta32 deletion results in resistant to HIV infection that makes CCR5 one of the therapeutic targets. Nevertheless, there is reduced expression of CCR5 in those individuals who are heterozygous for the deletion and have slow decreases in the CD4 T cell count and slower progression to the disease (Liu *et al*., 1996; Huang *et al*., 1996; de Roda *et al*., 1997). Maraviroc has been used with minimized side effects and positive results as an anti-inflammatory ART in cognitive impairment and liver steatosis (Spudich and Ances, 2013; Xu *et al.,* 2014). Therefore, generating information in Ethiopian setup

on the type of the viral strain dominantly detected and CCR5 antagonist is important to determine the possibility of use in our context.

1.12. Nutritional status and lipid abnormalities in HIV/AIDS

In addition to the genetic and biological nature of the virirus, nutritional status of HIV infected individuals is also very important factor is disease progression. Independent of HIV infection, poor nutritional status can impair immunity and increases infections (Lawn *et al.,* 2008). HIV impairs nutritional status and weakens the immune system through vomiting, reduction of nutrient intake due to nausea, anorexia, abdominal pain and HIV-induced enteropathy. Lack of food safety, lack of hygienic care, food insecurity and dehydration due to diarrhea could compromise the nutritional status. In addition, liver and kidney disease leading to altered use, maldigestion and malabsorption due to infection, impaired storage and excretion of nutrients, medication-related side effects, increased requirements for both micro- and macro- nutrients and are also factors that affect nutritional status (Macallan, 1999; Obi *et al.,* 2010; Kosmiski, 2011). Increased susceptibility to AIDS-related illnesses, higher mortality rates, disease progression, and suboptimal response to HIV drugs have been shown to be associated with undernutrition in HIV-infected individuals (Paton *et al.,* 2006; Marazzi *et al.,* 2008). On the other hand, excess weight raised the prevalences to metabolic complications such as dyslipidemia, diabetes, insulin resistance, lipodystrophy and hypertension (Wilson *et al.,* 2002; Fontaine *et al.,* 2003; Feeney and Mallon, 2011). Increased dyslipidemia in HIV/AIDS patients also increases atherosclerosis and cardiovascular disease (CVD) risks (Armstrong *et al.,* 2011; Pefura *et al.,* 2011).

Dyslipidemia is common in HIV patients receiving ART than in patients not on therapy (Carr *et al.,* 1999; Segerer *et al.,* 1999). ART causes raised levels of total cholesterol (TC), low density lipoprotein (LDL) cholesterol (LDL-C) and triglycerides (TG), and variable effects on high density lipoprotein (HDL) cholesterol (HDL-C) levels (Grunfeld, 2010). HDL-C level increased and TG level decreased with exposure to non-nucleotide reverse transcriptase inhibitor (NNRTI) based therapy (Young *et al.*, 2005). Protease inhibitors (PIs) are associated with an increased risk of atherogenic lipid abnormalities (Giannarelli *et al.,* 2011). Impaired peripheral free fatty acid (FFA) trapping in adipose tissue, impaired reverse cholesterol transport (RCT) by HIV nef protein that causes degradation of ATP binding cassette subfamily A member 1 (ABCA1), increased apolipoprotein B (APOB) and inhibition of its degradation, increased FFAs influx to the circulation that cause abnormal signaling mediated by increased inflammatory cytokines that impaired clearance of TG from the circulation due to reduced lipase activity, decreased HDL-C, increased LDL-C and VLDL, and increased oxidative stress and lipid peroxidation could cause HIV/ART-related dyslipidemia (Eren *et al.,* 2012; Helleberg *et al.,* 2012; Feeney *et al.,* 2013). Furthermore, PIs interfere with the normal postprandial metabolism of FFAs by reducing catabolism of sterol-regulatory element binding protein 1 (SREBP1) in the adipose tissue and liver (Chattopadhyay and Aldous, 2016). The PI-associated dyslipidemia could also be by inhibition of the function of host proteins due to sequence and structural similarity with HIV protease (Carr *et al.,* 1999).

Dyslipidemia does not appear in every HIV-positive individual who take the same ART regimens may due to difference in playing of genetics and immunologic factors (Grunfeld, 2010; Chang *et al.,* 2010). Genome-wide-based association studies and candidate genes have identified single nucleotide polymorphisms (SNPs) that could account for a significant portion of modulation in blood lipid levels and the variation such as APOA, APOB, APOC and APOE, SREBPs, lipases, 3-hydroxy-3-methylglutaryl-coenzyme-A reductase (HMGCR), phospholipid transfer protein (PLTP), sterol-regulatory-element-binding-factor cleavage activating protein (SCAP), lecithin-cholesterol acyltransferase (LCAT), cholesterylester transport protein (CETP), ABCA1, microsomal triglyceride transfer protein (MTTP), LDL receptor (LDLR), low density receptor related protein (LRP) and very low density lipoprotein (VLDL) receptor (VLDLR) (Rotger *et al.,* 2009; Veloso *et al.,* 2010; De Andrade *et al.,* 2011).

Viral entry, uncoating, replication, protein synthesis, assembly, budding and infectivity are facilitated by lipids (Lorizate and Kräusslich, 2011; Mazzon and Mercer, 2014). Regulation of the cellular cholesterol balance (Negredo *et al.,* 2008; Musiol *et al.,* 2013) and endocrine-immunity interaction (Klein, 2000; Hel *et al.,* 2010; Beagley and Gockel, 2003) are also important in replication of viruses. Viruses use membrane microdomains where receptors and/or coreceptors and viral cholesterol-rich region are localized called lipid rafts to infect the target cells (Brügger *et al.,* 2007; Morrow *et al.,* 2010). Nef impairs cholesterol efflux (van't *et al.,* 2005; Mujawar *et al.,* 2006), and inhibits activity of the ATP binding cassette transporter A1 (ABCA1) (Fitzgerald *et al.,* 2010). Nef also induces genes involved in cholesterol biosynthesis (van't *et al.,* 2005) and facilitate

cholesterol delivery to lipid rafts (Arora *et al.,* 2002). Depletion of cellular cholesterol by stimulation of cholesterol efflux through activation of ABCA1 or inhibiting its biosynthesis or suppress HIV-1 infection and replication *in vitro* (Chukkapalli *et al.,* 2012; Petersen *et al.,* 2014).

The innate immune system produces interferons (IFNs) during viral infection *in vivo* that are involved in up-regulation of interferon-stimulated genes (ISGs). Production of oxysterols is dependent upon some of the ISGs (Blanc *et al.,* 2011). Cholesterol-25-hydroxylase (Ch25h) as one of the antiviral ISGs that can convert cholesterol to 25-hydroxycholesterol (25-HC) inhibits viral entry by blocking membrane fusion between virus and cell (Holmes *et al.,* 2011; Liu *et al.,* 2013). In addition, 25-HC controls sterol biosynthesis by promotion of down-regulation of the enzymes involved in sterol biosynthesis (Brown and Goldstein, 2009) and feedback inhibition (Cyster *et al.,* 2014).

Due to the differences in endocrine-immune interactions, females are less susceptible than males to infections (Klein, 2000). In support to this, studies showed that plasma HIV-1 RNA levels in men are higher than in women (Sterling *et al.,* 2001; Gandhi *et al.,* 2002); and female rhesus macaques was protected from transmission of simian immunodeficiency virus (SIV) by treatment with estrogen through thickening of the genital tract mucosal tissue (Hel *et al.,* 2010). *In vitro* study also showed that beta-estradiol inhibited HIV-1 replication in primary human peripheral blood lymphocytes (Zhang *et al.,* 2008) by inhibiting of the target cell infection (Rodriguez-Garcia *et al.,* 2013).

Antiretroviral treatments mainly PIs are associated with abnormal lipid metabolism and redistribution such as lipodystrophy, including lipoatrophy (wasting) and lipohypertrophy (deposition), and dyslipidemia characterized by abnormal changes in lipid profile of people with HIV infection (Sattler, 2008; Grunfeld, 2010; Troll, 2011). The lipid profile change could be characterized by increased level of TG, TC, LDL-c, and decreased HDL-c (El-Sadr *et al.,* 2005). Some antiretroviral drugs, such as PIs (Anastos *et al.,* 2007) and stavudine Gallant *et al.,* 2004) increase the blood levels of LDL-c, TC, and TGs with variable effects on levels of HDL-c. Nevirapine (NVP) use is associated with increased in LDL-c (Vander *et al.,* 2001), whereas increases in TC and TG are observed with use of efivinavir, particularly with longer duration of therapy. The mechanism for lipid abnormality is not clear but with the possibility caused by many factors such as HIV infection itself, the antiretroviral agents, host genetics, and changes in body composition along age (Carr *et al.*, 1998; Jones *et al.*, 2005; Young *et al.,* 2005; Rasheed *et al.,* 2008).

Socio-economic changes like urbanization and changing lifestyles cause increase in non-infectious health disorders and nutritional impairment in sub-Saharan Africa (Fontaine *et al.,* 2003; Crum-Cianflone *et al.,* 2008). High rates of dyslipidemia in HIV-infected individuals from developed world have been documented in number of studies both on and off ART (Riddler *et al.,* 2003). Few studies from resource limited settings have shown elevated TG, low-to-normal TC and LDL, and decreased HDL among ART-naïve individuals (Buchacz *et al.,* 2008; Fourie *et al.,* 2010). The prevalence of hypercholesterolemia, hypertriglyceridemia and low HDL-C was 10–27%, 23–40% and

19–27%, respectively, depending on the antiretroviral regimen (Friis-Møller *et al.*, 2003). The information from other parts of the world on undernutrition, excess weight and lipid abnormalities cannot be extrapolated to the Ethiopian context directly due to socio-demographic, economic and genetic differences among populations.

Initiation of ART in HIV-infected people is recommended at any CD4+ T cell count regardless of the World Health Organization (WHO) clinical stages, giving priority to WHO clinical stages III/IV or CD4 cell count $\leq$ 350 cells/mm^3 (WHO, 2015). The recommendation for the time to initiate ART varies among countries. In Ethiopia, as per the Ethiopian Federal HIV/AIDS Prevention and Control Office of Ministry of Health recommendations, initiation of ART should be considered if CD4+ T cell count is $\leq$500 cells/mm^3 or are with WHO clinical stages III/IV (FHAPCO, 2014). Although CD4+ T cell count and viral load testing remains a challenge for resource-constrained countries, WHO continues to recommend CD4+ T cell count and viral load as the main laboratory tests for making decisions about disease progression and when to start ART (WHO, 2015).

In the absence of CD4+ T cell count and viral load, markers such as low BMI, absolute lymphocyte count (ALC), blood hemoglobin (HB), erythrocyte sedimentation rate (ESR), absolute eosinophilic count (AEC), elevated serum ß2 microglobulin, C-reactive protein (CRP), serum albumin, total protein, total cholesterol, high density lipoproteins (HDL) and low density lipoprotein (LDL) are potential prediction markers of CD4+ T-cell count (Mata-Marín *et al.*, 2010; Owiredu *et al.*, 2011; Azzoni *et al.*, 2012; Ramana, 2013).

2. Hypothesis, objectives and significance of the study

2.1. Hypothesis

Malnutrition, lipid abnormalities, and sex differences affect HIV RNA load and CD4+ T cell count in HIV infected ART naïve individuals.

2.2. Objectives

2.2.1. General objective

- To determine factors associated with malnutrition, lipid abnormalities, HIV RNA load and CD4+ T cell count; and HIV diversity in antiretroviral naïve HIV-infected adults in Addis Ababa;

2.2.2. Specific objectives

i. A systematic review to determine the prevalence of HIV, predisposing risk factors, identification of hotspot areas, key and priority populations, availability and utilization of services, and challenges and gaps to be addressed for prevention and control of HIV epidemic;

ii. To explore the prevalences of lipid abnormalities and malnutrition, and to identify factors associated with these abnormalities in ART naïve HIV-infected individuals;

iii. To investigate the association of serum total cholesterol and sex with HIV disease progression in ART naïve study participants; and

iv. To determine the HIV-1 subtype(s), CRFs and the dominant phenotypic tropism in the study participants.

2.3. Significance of the study

Undernutrition, hypercholesterolemia, hypertriglyceridemia and excess weight were variably prevalent in this ART naïve HIV-infected study population. This implies the need for HIV/AIDS care through targeted nutritional programs. Lipid levels in patients on or off ART are needed to be monitored regularly. In addition, positive change in lifestyle, improvement on household income and nutritional treatment to reduce morbidity and mortality are necessary interventions in HIV/AIDs patient management. The study is also important to understand the association of serum total cholesterol and sex to CD4+ T cell count and HIV RNA load *in vivo.* It also could contribute in selecting alternative markers for monitoring HIV disease progression and ART eligibility among HIV-infected ART naïve individuals in absence of CD4+ T cell count and HIV RNA load in resource limited settings. This will be important to provide a basis for therapeutic strategies to control HIV-1 infection and replication, to improve laboratory testing strategies and effective patient management in HIV infection in the absence of the standard assays.

The high proportion of HIV-1 subtype C, and reports of HIV-1 subtype A1, CRFs C/A1 and C/A1/D in Addis Ababa suggests the importance of continuous studies on HIV genetic variation, subtypes and CRFs to understand HIV/AIDS epidemiology, vaccine and therapeutic designs, and detection of genetic determinants. Furthermore, if the use of the co-receptor antagonist maraviroc is planned in Ethiopia, the finding on dominance of R5-tropic viruses has important implication. Identifying predisposing risk factors and high risk populations, knowing the HIV burdon and distribution, designing effective interventions for efficient case finding and strong health service are vital to achieve the three 90 targets.

3. Materials and methods

3.1. Study design and population

The study period for this cross-sectional study was from February to August 2013. The study participants were HIV-infected adults aged ≥18 years at St. Paul, Leprosy Rehabilitation and Training Centre (ALERT), Yekatit-12 and Zewditu Memorial Hospitals in Addis Ababa, Ethiopia. A total of 594 study participants who were ART naïve and provided informed consent to participate in the study were recruited. The study participants were recruited among those who were already enrolled or came to be enrolled in ART care centers of our study sites in a consecutive way. Because using randomization could make getting the 594 study participants with the stringent inclusion and exclusion criteria difficult to achieve, we use only purposive consecutive recruitment. They were enrolled by anti-retroviral treatment nurses under close supervision of the investigator. Patients in immediate intensive care requirement, with cognitive impairment using drugs with possible interference with serum lipid levels, and pregnant women were not included in the study.

Clinical data, socio-demographic and anthropometric were collected from patient medical records. The data from records were triangulated by the data collected by structured questionnaire on the day of blood sample collection (Annex 1).

3.2. Methods used for the systematic review of HIV/AIDS situation

This study was designed and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) tool (Moher *et al.*, 2015). Analytic methods and inclusion criteria were specified and documented in advance. Thus, the eligibility criteria included all studies carried out in Ethiopia, including Addis Ababa, from 2005 to April 2019, written in English. The studies addressed: HIV/AIDS prevalence; predisposing behavioural, biological and socio-demographic risk factors; identified hotspots; identified most-at-risk populations (MARPS) as key and priority populations; addressed intervention strategies such as behavioural, biomedical and structural intervention and availability of services; and identified gaps that could be challenges and opportunities for prevention and control of HIV/AIDS.

The documents relevant to be reviewed to address HIV situation in Addis Ababa were collected from online databases Google scholar and Pubmed for published works. In addition to published works, unpublished survey and surveillance reports, performance reports and project assessment findings, and mapping results were collected from Federal Health Ministry (FHM), Ethiopian Public Health Institute (EPHI), Ethiopian Public Health Association (EPHA), Organization for Social Services, Health and Development (OSSHD), Federal HIV/AIDS Prevention and Control Office (FHAPCO), Addis Ababa HIV/AIDS prevention and Control Office (AAHAPCO), Addis Ababa Health Bureau (AAHB), Population Services International/Ethiopia (PSI/E), American Centre for Disease Control and Prevention (CDC) and World Health Organization (WHO).

The following terms and phrases were used for searching the documents as needed: HIV, AIDS, prevalence, highly active antiretroviral therapy, HAART, antiretroviral therapy, ART, compliance, adherence, resistance, predisposing factors, behavioural, biological, socioeconomic, most-at-risk populations (MARPs), khat, alcohol, drug use, knowledge, attitude, practice, KAP, behavioural change; condom use, abstinence, faithfulness, stigma, discrimination, HIV counseling and testing, voluntary counseling and testing, HCT, VCT, prevention of mother to child transmission and PMTCT. A total of 241 documents were collected, among which only 109 were relevant and used for the quick review.

The quick review form was developed to collect all necessary information of the source document with full citation, main findings, and to match to specific objectives as indicated in the objectives. After quick review, 41 documents with relevant information were selected and used. Quantitative data were collected from surveys and performance reports. Based on a closer and in-depth review of quantitative data, the raw information was categorized under exclusive thematic areas based on the specific objectives in order to make the data presentation easier (Figure 6). Furthermore, the quantitative information was summarized in Tables (Table 3 and Annex 6) and Figures.

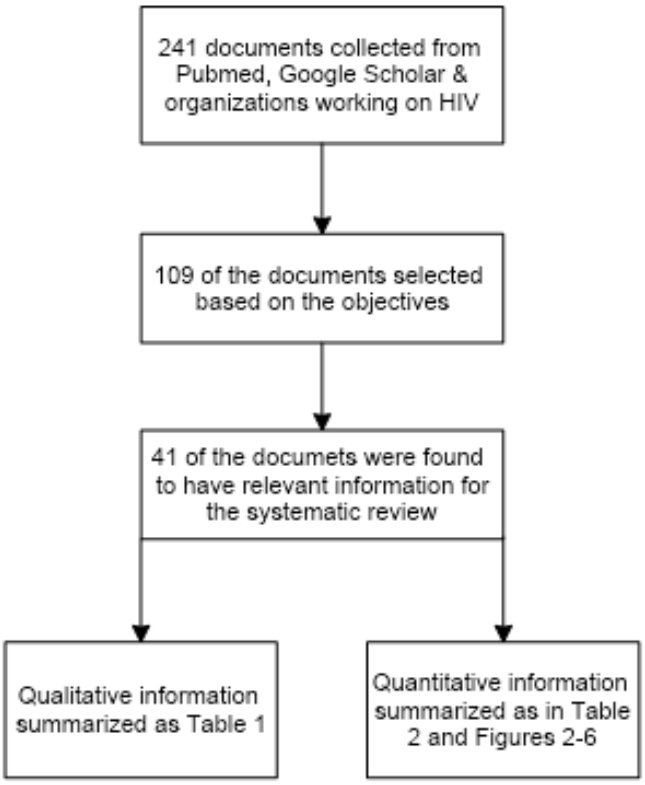

Figure 6. The flow chart used for collection of qualitative and quantitative data, March 2019, Addis Ababa, Ethiopia.

3.3. Anthropometric measurements

Height to the nearest 1mm and weight to the nearest 100gm were measured. Individual's body weight divided by the square of their height (kg/m^2) was done to determine body mass index (BMI). The protein-energy nutritional status was determined using WHO-established BMI cut offs [thinness or acute undernutrition (BMI <18.5 kg/m^2), normal (BMI = 18.5–24.9 kg/m^2), overweight (BMI = 25-29.9 kg/m^2) and obese (BMI ≥30). BMI ≥25 kg/m^2 was determined as excess weight (WHO, 1995).

3.4. Haematological and biochemical assays

Blood samples collected using EDTA vacutainer tubes were used to determine hemoglobin concentrations and CD4+ cells count. Automated FACS counter (Becton and Dickinson, San Jose, CA, USA) was used to determine the count of CD4+ cells from whole blood. Sysmex-21 automated blood analyzer by noncyanide method (Sysmex, KX-21N, Kobe, Japan) was used to determine hemoglobin concentration. Enzymatic colorimetric method (Human diagnostics, HumanStar 180, Wiesbaden, Germany) was used to determine fasting total cholesterol, glucose, and triglycerides levels from serum. All of these haematological and biochemical assays were done in the study sites. Hemoglobin concentration <12 g/dL for women and <13 g/dL for men were considered anemic based on WHO 1994 reference value (WHO, 1994). Metabolic abnormalities were defined according to the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) criteria of USA (2002). Hypercholesterolemia and hypertriglycerdemia were defined by serum total cholesterol ≥200 mg/dL and triglycerides ≥150 mg/dL, respectively. In addition, hyperglycemia, impaired fasting glucose (IFG) and diabetes were defined as fasting glucose level >110-, 110-125- and ≥126 mg/dL, respectively.

3.5. HIV RNA load determination

Ethylenediaminetetraacetic acid (EDTA) vacutainer tubes were used to collect whole blood. Plasma was separated and stored at -80˚C. The HIV ribonucleic acid (RNA) load in 200 µL plasma was determined by Abbott RealTime HIV-1 assay at Ethiopian Public Health Institute (Abbott Molecular Inc., Des Plaines, IL, USA).

3.6. HIV-1 RNA amplification and sequencing

The 60 samples with detectable viral load for HIV sequencing were selected based on CD4+ T cell count ranges <200, 200-350, 350-500 and >500 by considering the role immunity as selection force and cause of evolution of the virus. RNA was extracted and purified from the plasma samples with detectable HIV RNA load at University College of London (UCL). A template library was created by reverse transcribing the extracted RNA and producing the complementary deoxynucleic acid (cDNA) through processing by the polymerase chain reaction-amplicon (PCR-amplicon) method. Next generation sequencing (NGS) was performed using Illumina MiSeq through a PCR method with four overlapping amplicons spanning the whole genome (Gall *et al.*, 2012).

3.7. Genome assembly

Quality control checks, trimming of the raw reads, assembly of consensus sequences and mapping of reads onto the consensus for variant calling were done. Reads that were low quality and below a minimum length (50 bases) were removed and the rest aligned to both human and HIV genomes. HIV raw reads that aligned with the human genome were discarded. After then, NGS raw reads were assembled into genomes using the iterative viral assembler (IVA). The contigs produced were aligned with full length HIV genomes from an HIV sequence database in order to select reference to fill gaps. Using the draft genomes constructed, gap filling was done by aligning the good quality reads onto the draft genome by replacing bases from the reference. Gap filling was repeated for a maximum of 10 times to get the expected consensus genome (Hunt *et al.,* 2015).

3.8. Assembled sequence analysis

Sequence analysis was done to identify CRFs, subtypes, tropism and resistance mutations. Scanning of recombination and subtyping were done by the REGA subtyping tool v3.0. The V3 loop sequence from the whole genome was derived by gene cutter. Prediction of coreceptor usage was carried out using PhenoSeq (Cashin *et al.,* 2015) and Geno2Pheno clonal–model (Lengauer *et al.,* 2007). In the V3 loop, predicted positive charges <5.0 and ≥5.0 suggest that the viruses are macrophage tropic and T cell tropic, respectively (Adal *et al*., 2005). The presence of signature amino acids, lysine and arginine, at positions 11, 24, and 25 in the V3 loop are used to identify SI and NSI phenotypes (Chesebro *et al*., 1992). A false positive rate (FPR) below 10% was considered as indicator of X4-tropic strains in Geno2Pheno analysis. Howeer, PhenoSeq identifies the virus as non-X4 using or X4 using. Clustal W was used for sequence alignment. Phylogenetic and molecular evolutionary analyses (MEGA) version 6 was used to generate phylogenetic tree (Tamura *et al.,* 2013) in presence of reference sequences from A, C and D subtypes and recombinants of these subtypes. A tree with 1000 bootstrap replications was generated using maximum likelihood with the neighbor-joining (N-J) methods. Stanford HIV Drug Resistance Database was used to detect resistance mutations (Gifford *et al.,* 2009).

3.9. Data quality assurance

The questionnaire for the data collection was standardized through pilot study. The clinicians and technical individuals involved for data collection were trained by the

principal investigator (PI). The laboratory data was generated with calibilated tools. The The data collection process was supervised and reviewed by the PI to clarify any discrepancies and to correct it in time. The data entry was assured through double entry. Further cleaning of the data was done by using SPSS package after entry.

3.10. Data analysis

Data generated through laboratory tests and questionnaire were analyzed using GraphPad Prism version 5.03 (GraphPad software, California, USA) and STATA software version 11.0 (Stata Corp, College station, Texas, USA). The descriptive results were presented as frequency counts, percentages, median with inter quartile range (IQR) and mean ± standard deviation (SD). The analysis of variance (ANOVA) and independent t-test were used to compare means. Spearman rank order correlation between log viral load, BMI, CD4+ T cell count, hemoglobin level and total cholesterol was done. Pearson chi-square and Fisher's exact test to test the associations of categorical variables were applied. Pearson chi-square for trend was also carried out. The Kruskal-Wallis and Mann-Whitney tests were used to compare categories with non-normal distribution. Spearman correlation of serum triglycerides and total cholesterol levels with CD4+ T cell count were done. Diagnostic performance of low serum total cholesterol, anemia, and total cholesterol or anemia together for predicting CD4+ T cell count or WHO clinical stage, and HIV RNA load categories were tested using Spearman rank order correlation. Risk factors for increase of HIV RNA load were identified using univariate (for crude coefficient, β) and then multivariate linear regression analysis after adjusting for potential confounders (for adjusted coefficient, β). In addition, independent variables which were identified as

statistically significant by chi-square test were analyzed using univariate (for crude odds ratio, COR) and then for multivariate logistic regression analysis after adjusting for potential confounders (for adjusted odds ratio, AOR). Level of significance $\alpha < 0.05$ with confidence interval of 95% was considered.

3.11. Ethical consideration

The study was ethically cleared by the National Ethical Review Committee, Ministry of Science and Technology with reference number 3.10/004/2015 and the Institutional Research Ethics Review Committee (IRERC) of participating Institutions (Annex 2 and 3, 4, 5). Study participants considered were those who gave written consent.

4. Results

4.1. HIV situation in Addis Ababa

4.1.1. HIV prevalence

Surveys and assessments conducted in Addis Ababa such as EDHS (CSA and ICF, 2005; CSA and ICF, 2011; CSA and ICF, 2016), and EPHIA assessment (EPHI, 2018b) showed prevalence of HIV to be 4.7%, 5.2%, 3.4% and 3.1%, respectively (Table 3). Around 104,851 PLHIV live in Addis Ababa, contributing nearly 17.7% of the PLHIV population in the country, while the city contributes 3.5% to the total population of the country (EPHI, 2018a).

Table 3. HIV prevalence in Addis Ababa from EDHS and EPHIA (CSA and ICF, 2005; CSA and ICF, 2011; CSA and ICF, 2016; EPHI, 2018b).

Studies	% HIV prevalence		
	Total	Women	Men
EDHS 2005	4.7	6.1	3.0
EDHS 2011	5.2	6.0	4.3
EDHS 2016	3.4	4.2	2.2
EPHIA 2017	3.1	-	-

Prevalences of HIV in Addis Ababa from Antenatal care (ANC)-based surveillance of 2005-2014 were in the range of lowest in 2012 (4.4%) to the highest in 2005 (12.1%). The prevalence was relatively higher in 2014 (5.5%) than the prevalence in 2012 (4.4%). In addition, the prevalence from prevention of mother to child (PMTCT) surveillance report of 2016 (1.8%) was lower than the prevalence from ANC surveillance report of 2014 (Figure 7).

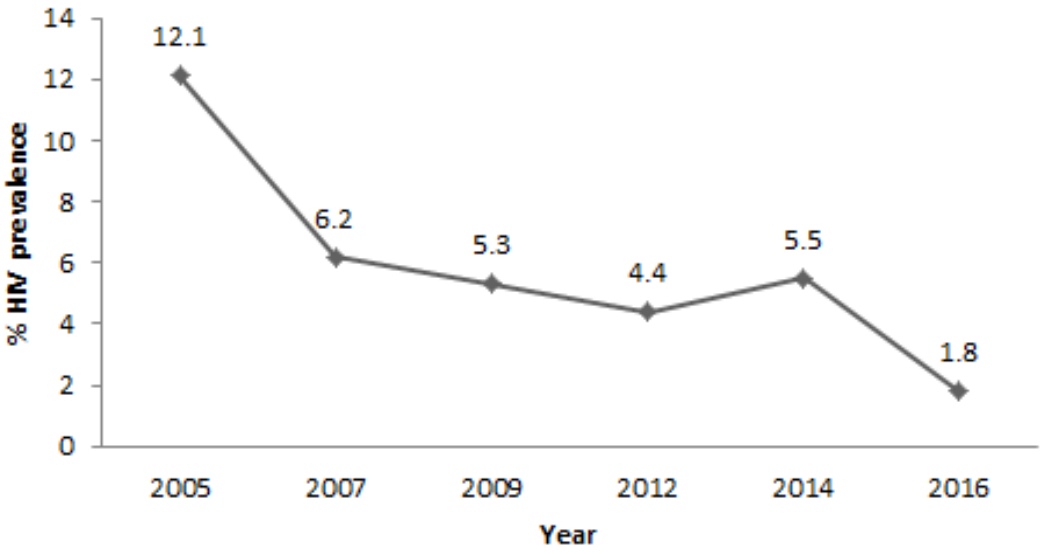

Figure 7. HIV prevalence in Addis Ababa, ANC 2005-2014 and PMTCT 2016 (EPHI, 2011; EPHI, 2014; EPHI, 2015; EPHI, 2017b).

4.1.2. Hotspot areas of HIV transmission

The most common hotspots in Addis Ababa are areas where bars, groceries, pensions, guest houses, hotels, brothels, massage houses, khat houses, shisha houses, night clubs, drinking establishments and tourist frequented settings are concentrated. Condominiums were also mentioned as hotspot areas because sex workers commonly rent condos and are becoming centres of sexual transactions. There are various behavioural, biological and socio-economic predisposing risk factors that drive the epidemic in these hotspot areas in particular and the general population in general (Lakew *et.al.,* 2015; AAHAPCO, 2017).

4.1.3. Factors involved in driving the epidemic

Some of the gaps and challenges in the HIV prevention and control are weak monitoring of the quality of interventions, limited linkage of positive clients, lost to follow up, long turnaround time of viral load (VL) and EID tests, limited index-case-testings, limited effort in preventing substance abuse, inconsistent supply of test kits and condom, financial shortage, limited man power and coordination, data quality problem, and gap in use of program data or research findings.

4.1.3.1. Behavioural factors

Low comprehensive knowledge about HIV/AIDS and substance abuse such as alcohol, khat and shisha; gender based violence including rape; sex with multiple partners; practices of unsafe sex and inconsistent condom use; and dissatisfaction with sexual life in marriage are among major predisposing behavioural risk factors for the spread of HIV

(FMOE, 2012; AAHAPCO, 2017). According to study conducted by OSSHD, 72.5% of the intraveinous drug users (IDUs) in Addis Ababa had the habit of reusing needle and syringe (Deyessa *et al.,* 2018). In addition, early sexual debut, peer influence of young girls to engage in transactional sex, virginity selling, unfaithfulness in marriage, and boyfriend/girlfriend sharing were identified as risk factors for HIV transmission (FMOE, 2012; Cherie *et al.,* 2012; AAHAPCO, 2017). In other studies, the percentage of men who had sex with non-marital, non-cohabiting partners is highest in Addis Ababa (26%) compared to the national average (16%). In Addis Ababa, the highest mean number of lifetime sexual partners reported by men is 5.2; and 72.4% of women and 41.8% of men reported using male condom during last sexual intercourse with non-regular partners (CSA and ICF, 2016).

4.1.3.2. Biological factors

From the total HIV positive couples in Addis Ababa, 4.3% were found to be discordant (CSA and ICF, 2016) and the HIV-negative partners in discordant couples have the highest risk of acquiring HIV (PEPFAR, 2018). According to Kassa *et al.* (2018), the proportion of disclosure of HIV/AIDS diagnosis in HIV-infected children is low, with almost one in ten HIV exposed infants reported as HIV positive, and only 2 and 4% babies, among the HIV exposed, were reported as HIV positive by 6 and 18 months, respectively. Furthermore, other reports (Klaus *et al.,* 2015; Endalamaw *et al.,* 2018) have shown low utilization of early infant diagnosis (EID) services, which correlated with being from the rural residence, home delivery, lack of understanding of the efficacy of ART, negative religious influences, and mixed infant feeding practices, which would increase the risk of HIV transmission to children.

4.1.3.3. Socio-economic factors

Multiple reports (Menna *et al.,* 2014; CSA and ICF, 2016; AAHAPCO, 2017) have shown various socioeconomic factors contributing to the high HIV epidemic in the City of Addis Ababa. Among the factors, high concentration of FSWs as means of livelihood; low socio-economic status; increasing sexual practices in massage houses; practice of intergenerational sex; large number of establishments like bars, hotels, restaurants, pastries, day and night clubs, brothels, pensions, local drink houses, and guest houses; engagement of gate-keepers, brokers and hotel owners in facilitating young girls to have transactional sex; growing number of construction and industry sites leading to increasing daily laborers from all parts of the country; living in groups to share house rent; high presence of movie houses that show pornographies; virtual appointments for dating and sexual relation; presence of naked dancing and call girls service; the role of cosmetic and cloth shops in drug distribution; increasing number of migration and visitors; cultural change and moral deterioration are the predisposing risk factors. In addition, the absence of recreational centres for youth, divorce and widowhood are reported as aggravating factors for the spread of HIV in the City.

4.1.4. Key and priority populations

MARPs Survey showed the prevalence of HIV infection to be as high as 23% in self-identifying FSWs and 4.5% in truck drivers (EPHA *et al.*, 2013); 4.2% in prison settings (UNODC and Prision Adminstration, 2014) and 5.7% HIV among mobile workers

(Lakew *et.al.,* 2015). About 15.5% of drivers have misconceptions about HIV prevention methods (EPHA *et al.,* 2013).

According to estimates, there are about 200,000 FSWs in Ethiopia (EPHA *et al.,* 2013). The majority of FSWs (57.5 %) are 24 years and younger, and about 14% are 19 years or younger (PSI/E, 2016). MARPs study (EPHA *et al.,* 2013) also showed that the size of FSWs in Addis Ababa was estimated to be 10,267. HIV prevalence in FSWs is four times higher than the general population.

A total of 4,068 IDUs are estimated to be located in Addis Ababa (Demissie *et al.,* 2018). The majority (72.5%) of the IDUs from Addis Ababa had the habit of reusing needle and syringe. Of the 177 Addis Ababa residents who claimed to have tested for HIV, 70 (39.5%) disclosed as HIV positive (Deyessa *et al.,* 2018). In addition, the prevalence of HIV among IDUs in Addis Ababa is 6%, and 40% of IDUs reported ever-sharing needles. Furthermore, among HIV-positive IDUs, 60% reported sharing a needle the last time they injected (Demissie *et al.,* 2018). Male IDUs are higher in number than female users at a ratio of 9:1 and 3/4 of the IDUs were below the age of 35 years (Deyessa *et al.,* 2018).

In Addis Ababa, following identifying FSWs as KP, various priority populations were also identified. The priority populations (PPs) are divorced and widowed persons; HIV-negative partners in discordant couples; long-distance truckers and taxi drivers and their assistants; paying clients and non-paying ('Balukas') of sex workers; individual engaged

in transactional sex including sugar daddies and mummies, and waitresses; daily labourers in constructions and factories; IDUs; brokers, managers and workers in bars, groceries, pensions, guest houses, hotels, local drink houses, massage houses and shisha houses; and vulnerable adolescents and youth (immigrants from all parts of the country, migration returnees, house maids, street children, higher education institution and night school students) (FMOE, 2012; AAHAPCO, 2017; PEPFAR, 2018; FHAPCO, 2018).

4.1.5. HIV transmission interventions

4.1.5.1. Behavioral interventions

Behavioural change communication (BCC), conducting peer and outreach education, transmitting messages using mini-media and mass-media, condom promotion, and life skill trainings are the common behavioural interventions. The national average performance of condom distribution to MARPs group is 43.9% of the plan while for Addis Ababa it was 28.9% of their plan that is far below the national average. Likewise, the proportion of condom distributed to MARPs is very low, only 18.4% of the total condom distributed in the city (FHAPCO, 2010 EFY; FMOH, 2018; Figure 8).

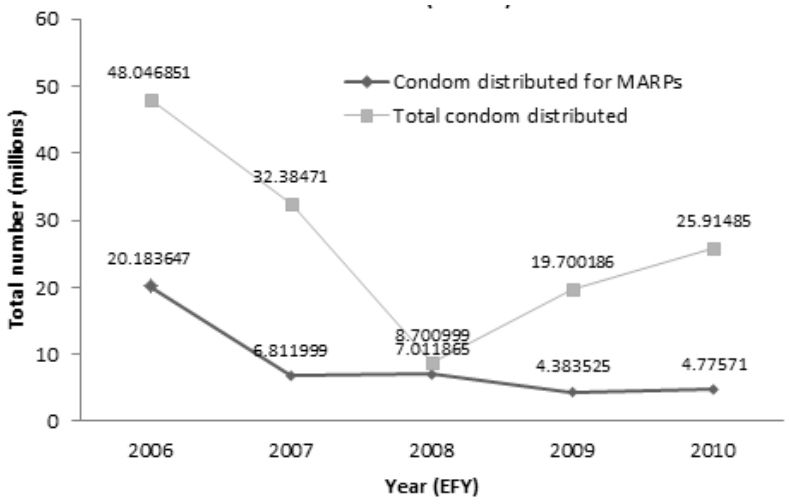

Figure 8. Total condom distributed and condom distributed for MARPs, 2006-2010 EFY (FHAPCO, 2010 EFY). EFY= Ethiopian fiscal year.

4.1.5.2. Structural interventions

Structural interventions aiming to reduce vulnerability or ensuring service accessibility are being implemented including provision of economic strengthening, mapping and identification of hotspot areas and risky target groups, drop-in-centres (DICs), gender based violence and referral linkage (FHAPCO, 2010 EFY; FMOH, 2018). Findings indicated that economic strengthening interventions are diminishing in scale (Figure 9).

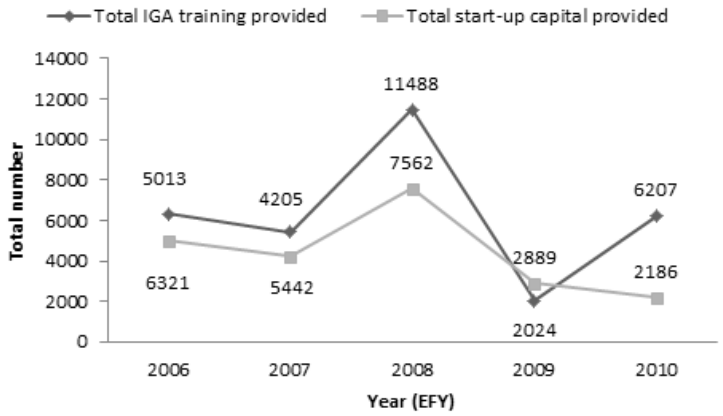

Figure 9. Total number of individuals who were provided IGA training and start-up capital from 2006-2010 EFY; IGA = income generating activities (FHAPCO, 2010 EFY). EFY= Ethiopian fiscal year.

4.1.5.3. Biomedical interventions

Biomedical interventions services are distribution of condom, HIV testing, sexually transmitted infection (STI) screening and treatment, ART, PMTCT and family planning, and ART post-exposure prophylaxis. In addition, ART pre-exposure prophylaxis for FSWs and discordant couples is at piloting stage. More than 10% of the BCC beneficiaries/FSWs had never been tested for HIV (PSI/E, 2016). Some parents are refusing to give consent for their children to access HIV testing services (HTS) and ART services (Biadgilign *et al.*, 2011).

Behavioral, socio-economic and biomedical factors contributed to discontinuation ART. Heavy pill burden, fear of stigma and discrimination, cost and access to transportation, medication side effects, economic problems in the household, long travel due to distance from ART clinics, long waiting times, alcohol drinking, smoking, being with baseline CD4 <200 cells/mm^3, having mental illness, being bed ridden functional status, and dissatisfaction with healthcare services were risk factors for ART discontinuation. Males were reported to be most affected by discontinuation from being away from home (Gesesew *et al.,* 2016; Gesesew *et al.,* 2017a & 2017b). More than 6% of HIV positive FSWs who started ART reported discontinuation of treatment for more than seven days in the three months prior to the assessment (PSI/E, 2016). With the introduction of appointment spacing, some patients complain of lack of storage space for the six-month supply of ARTs, poor storage conditions for their medicines, and preference of frequent follow up. On the other hand, health workers are also concerned about adherence given

the less frequent contact of PLHIV with the health services (Bezabhe *et al.,* 2014; Tiruneh and Wilson, 2016).

The HIV care and treatment service coverage indicated ART coverage to be 74.6%, and viral load testing coverage, about 60% with 87.5% viral suppression among those who received viral load testing (FHAPCO, 2010 EFY). The national average for the first, second and third 90's for urban Ethiopia is 72%, 71% and 70.1%, respectively. VLS among 15-64 years of age HIV-positives in urban areas is close to the target (70.1%) but varies by age, sex and region. VLS is distinctly lower at 48.2% in youth 15-24 compared to the adult above 25 years of age. The status of the three 90's for Addis Ababa is below the national urban average. Status of the three 90's in Addis Ababa for the age group 0-64 years is lower than the national average which is: 65.2 % for the 1st 90, 63.3 % for the 2nd 90 and 58.2% of all PLHIV had VLS with viral load level of <1000 copies/ml (EPHI, 2018b). In Addis Ababa, the total number of clients on ART were 94,240 and 3,616 were newly enrolled during the reporting period. The retention at 12 months was 87% (FHAPCO, 2010 EFY; Figure 10).

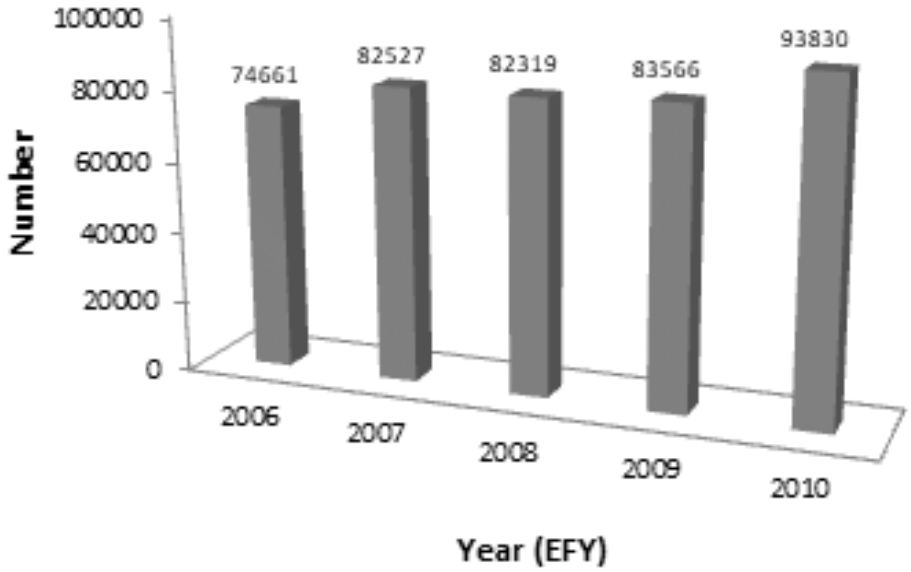

Figure 10. Number of individuals on ART (cumulative), 2006-2011 EFY (FHAPCO, 2010 EFY). EFY= Ethiopian fiscal year.

The Addis Ababa Mortality Surveillance Program using burial surveillance with verbal autopsy method (EPHA/CDC, 2012) to identify AIDS and other causes of death showed that HIV/AIDS mortality is higher among females (12.1%) as compared to males (9.5%). Relatively, higher proportion of death due to HIV/AIDS (13.2%) was observed in the age group of 30-49 years (EPHI, 2017a). In Addis Ababa from 2007-2010, an overall declining trends of AIDS related mortality was observed. However, starting 2010 onwards it seems stabilized (Figure 11).

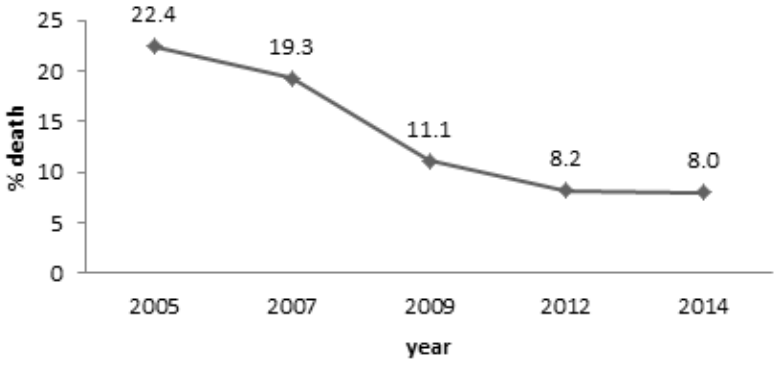

Figure 11. Percentage of Death due to AIDS in Addis Ababa, 2007-2011(EPHA/CDC, 2012).

4.2. Characteristics of the ART naïve HIV positive study participants

Among ART naïve study participants, 423 (71.2%) were women **(Table 4)**. The study participants' median age was 34 (IQR = 32.0-35.0) years. The median ages of women and were men 32 and 37, respectively, where men were older ($p < 0.001$). Seventy-nine percent of the study participants had primary or above primary level of education; and <2 dollars earning per day was reported in 69.9% of the study participants. Clinically, 14.4% were at WHO clinical stages III/IV. Majority of the study participants (~83%) had CD4+ T cell count ≥200 cells/mm^3 with median of 357 cells/mm^3 (IQR = 248-537). The study participants at AIDS stage with WHO clinical stages III/IV or CD4+ T cell count <200 cells/mm^3 were 25.9%. Detectable HIV RNA load were found for 500 study participants with mean ± SD of 4.23 ± 0.83 log copies/mL. From the total study participants, 84 (14.1%) were found to have HIV RNA load below detectable limit (<150 copies/mL) and it was not done for 10 (1.7%) due to sample limitation.

The nutritional status of the study participants was undernourished (15.1%), overweight (16.7%) and obese (5.4%). The overall hyperglycemia in the the random fasting glucose level of the study population was 13.6% that includes impaired fasting glucose (IFG, 7.5%) state and diabetic (6.1%). The hypercholesterolemia prevalence was 16.7%. In addition, the prevalence of anemia was 11.2% in the overall study population, 12.8% in men and 10.6% in women ($p > 0.05$).

Table 4. Characteristics of ART naïve study participants, February-September 2013, Addis Ababa, Ethiopia.

Variables	Number	% (95% CI)
Sex		
Male	171	28.8 (22.0-35.6)
Female	423	71.2 (66.9-75.5)
Age		
18-29	170	28.7 (21.9-35.5)
30-39	266	44.7 (38.7-50.7)
40-79	157	26.5 (19.6-33.4)
Education		
No formal	124	21.0 (13.8-28.2)
Primary	219	37.1 (30.7-43.5)
Secondary	188	31.8 (25.1-38.5)
Tertiary	60	10.1 (2.5-17.7)
Marital status		
Never married	109	18.4 (11.1-25.7)
Married/living with partner	293	49.3 (43.6-55.0)
Divorced/widowed/separated	192	32.3 (25.7-38.9)
Income per day (dollar)		
<1	162	34.5 (27.2-41.8)
1-2	171	36.4 (29.2-43.6)
>2	137	29.1 (21.5-36.7)
BMI (kg/m^2)		
<18.5	87	15.1 (7.6-22.6)
18.5-24.9	362	62.8 (57.8-67.8)
25.0-29.9	96	16.7 (9.2-24.2)
≥30	31	5.4 (-2.6-13.4)
WHO clinical stage		
Stage 1	328	55.6 (50.2-61.0)
Stage 2	177	30.0 (23.2-36.8)
Stage III/IV	85	14.4 (6.9-21.9)
CD4+ T cell count (cells/mm3)		
<200	102	17.2 (9.9-24.5)
200-349	182	30.6 (23.8-37.3)
350-499	144	24.2 (17.2-31.2)
≥500	166	28.0 (21.2-34.8)
Hemoglobin level (g/dl)		
Non-anemic	507	88.8 (86.1-91.5)
Anemic	64	11.2 (3.5-18.9)
Fasting glucose conc. (mg/dL)		
Normal (<110)	413	84.4 (80.9-87.9)
Impaired fasting glucose (IFG =110-125)	36	7.5 (-1.1-16.1)
Diabetic (≥126)	29	6.1 (-2.6-14.8)
Cholesterol level (mg/dl)		
<200	472	83.3 (79.9-86.7)
≥200	95	16.7 (9.2-24.2)
Triglycerides level (mg/dl)		
<150	401	70.2 (65.7-74.7)
≥150	170	29.8 (22.9-36.7)
HIV RNA load (copies/mL)		
<10000	427	45.2(39.2-51.2)
≥10000	157	54.8(49.3-60.3)
HIV RNA load in log copies/mL (mean ± standard deviation)	500	4.23 ± 0.83

4.3. Prevalence of undernutrition and excess weight

The overall median BMI was 21.9 kg/m^2 (IQR = 21.5-22.2). It was found to be 21.5 kg/m^2 in men and 21.9 kg/m^2 in women. The undernutrition prevalence was 14.8% in women, 15.8% in men and 15.1% in overall study participants. The excess weight (overweight or obese) prevalence was 22.1% (95% CI: 11.9-31.4) that includes 5.4% (95% CI: -2.6-13.4) obesity and 16.7% (95% CI: 9.2-24.2) overweight in the overall study participants. Excess weight was 23.8% in women and 17.6% in men. On the other hand, obesity prevalence was 6.1% in women and 3.6% in men study participants. The median BMI, the prevalences of undernutrition, excess weight and obesity were not significantly different between women and men ($p > 0.05$).

4.4. Factors associated with undernutrition and excess weight

The associations of all independent variables with undernutrition and excess weight were analyzed (**Table 5; Table 6**). Variables that have significant associations (age categories, being anemic/normal and WHO clinical stages for undernutrition; and age categories, income, WHO clinical stages, education, and hypercholesterolemia/normal for excess weight) in chi-square test or univariate binomial logistic regression analysis were considered to multivariate binomial logistic regression analysis ($p <0.05$).

Table 5. Variables associated (chi-square) with malnutrition among HIV-infected ART naïve study participants, February-September 2013, Addis Ababa, Ethiopia.

Variables	Undernutrition (BMI < 18.5)		Excess weight (BMI ≥25)	
	<18.5 N (%)	≥ 1.8.5 N (%)	≥25 N (%)	<25 N (%)
Age				
18-29	35(40.2)	129(26.4)[c]	17(13.5)	147(32.7)[a]
30-39	38(43.7)	221(45.3)	62(49.2)	197(43.9)
40-79	14(16.1)	138(28.3)	47(37.3)	105(23.4)
Education				
no formal			12(9.4)	104(23.3)[b]
Primary	-	-	43(33.9)	169(37.9)
Secondary			54(42.5)	132(29.6)
Tertiary			18(14.2)	41(9.2)
Income per day (dollar)				
<1	-	-	23(23.9)	133(36.8)[c]
1-2			35(36.5)	132(36.6)
>2			38(39.6)	96(26.6)
WHO clinical stages				
Stage I	35(40.7)	284(58.3)[a]	88(69.3)	231(51.8)[c]
Stage II	25(29.1)	147(30.2)	30(23.6)	142(31.8)
Stage III/IV	26(30.2)	56(11.5)	9(7.1)	73(16.4)
Hemoglobin level (g/dL)				
Non-anemic	71(81.6)	424(91.0)[b]	-	-
Anemic	16(18.4)	42(9.0)		
Cholesterol level (mg/dL)				
<200	-	-	81(67.5)	374(87.2)[a]
≥200			39(32.5)	55(12.8)

Note: a, b, c refers to p value <0.001, <0.01 and <0.05, respectively.

WHO clinical stages III/IV and age categories were found to be associated with undernutrition ($p < 0.05$); and WHO clinical stages III/IV, age categories and hypercholesterolemia/normal were found to be associated with excess weight ($p < 0.05$) (**Table 6**). The undernutrition prevalence decreases significantly as age increases in age 30-39 by 1.85 times (AOR = 0.54; 95% CI: 0.32-0.91) and 40-79 by 2.86 times (AOR = 0.35; 95% CI: 0.18-0.70) in comparison to age 18-29. However, a significant risk factor to undernutrition was WHO clinical stages III/IV (AOR = 3.54; 95% CI: 1.92-6.51).

Increase in age was found to be a risk factor for excess weight in ages 30-39 (AOR = 3.23; 95% CI: 1.38-7.55) and 40-79 (AOR = 3.64; 95% CI: 1.47-8.98) in comparison to

ages 18-29. However, excess weight decreases significantly at WHO clinical stages III/IV by 3.13 times (AOR = 0.32; 95% CI: 0.10-0.98). Total cholesterol concentration ≥200 mg/dL was also found to be a risk factor to excess weight (AOR = 3.94; 95% CI: 2.03-7.77). Furthermore, only older ages 40-79 is found a risk factor for obesity (AOR = 0.041; 95% CI: 1.06-14.70).

Table 6. Variables associated with malnutrition among HIV-infected ART-naïve study participants, February-September 2013, Addis Ababa, Ethiopia.

Variables	Undernutrition (BMI<18.5)		Excess weight (BMI≥25)	
	COR	AOR	COR	AOR
Age				
18-29	1.00	1.00	1.00	1.00
30-39	0.63(0.38-0.99)[b]	0.54(0.32-0.91)[c]	2.72(1.53-4.85)[b]	3.23(1.38-7.55)[b]
40-79	0.37(0.19-0.73)[b]	0.35(0.18-0.70)[b]	3.30(1.80-6.04)[a]	3.64(1.47-8.98)[b]
Education				
no formal			1.00	1.00
Primary	-	-	2.21(1.11-4.37)[c]	1.73(0.65-4.60)
Secondary			3.55(1.80-6.97)[a]	2.29(0.83-6.31)
Tertiary			3.80(1.68-8.60)[b]	2.24(0.66-7.64)
Income per day (dollar)				
<1	-	-	1.00	1.00
1-2			1.53(0.86-2.73)	1.38(0.64-2.94)
>2			2.29(1.28-4.09)[b]	1.92(0.85-4.33)
WHO clinical stages				
Stage I	1.00	1.00	1.00	1.00
Stage II	1.38(0.80-2.39)	1.35(0.77-2.37)	0.55(0.35-0.88)[c]	0.63(0.33-1.22)
Stage III/IV	3.77(2.10-6.75)[a]	3.54(1.92-6.51)[a]	0.32(0.16-0.67)[b]	0.32(0.10-0.98)[c]
Hemoglobin level (g/dL)				
Non-anemic	1.00	1.00	-	-
Anemic	2.27(1.21-4.26)[c]	1.84(0.94-3.58)		
Cholesterol level (mg/dL)				
<200	-	-	1.00	1.00
≥200			3.27(2.04-5.27)[a]	3.97(2.03-7.77)[a]

Note: a, b, c refers to p value <0.001, <0.01 and <0.05, respectively.

The excess weight prevalence in ages 18-29, 30-39 and 40-79 were 13.5%, 49.2% and 37.3%, respectively, that showed an increase in trend in overall study participants ($p < 0.001$) (**Figure 12A)**. This trend was maintained in women but not men. The excess weight prevalence decreased in overall, women and men with the progress of WHO clinical stages ($p < 0.05$) (**Figure 12B**). The undernutrition prevalence in ages 18-29, 30-39 and 40-79 which were 40.2%, 43.7% and 16.1%, respectively, showed a decrease of trend in overall study participants ($p < 0.05$) (**Figure 12C)**. This trend was also maintained in women but not in men. Interestingly, undernutrition prevalence increased in men with progress of WHO clinical stages, but, it decreased in women and overall study participants with progress of WHO clinical stages ($p < 0.01$) (**Figure 12D**).

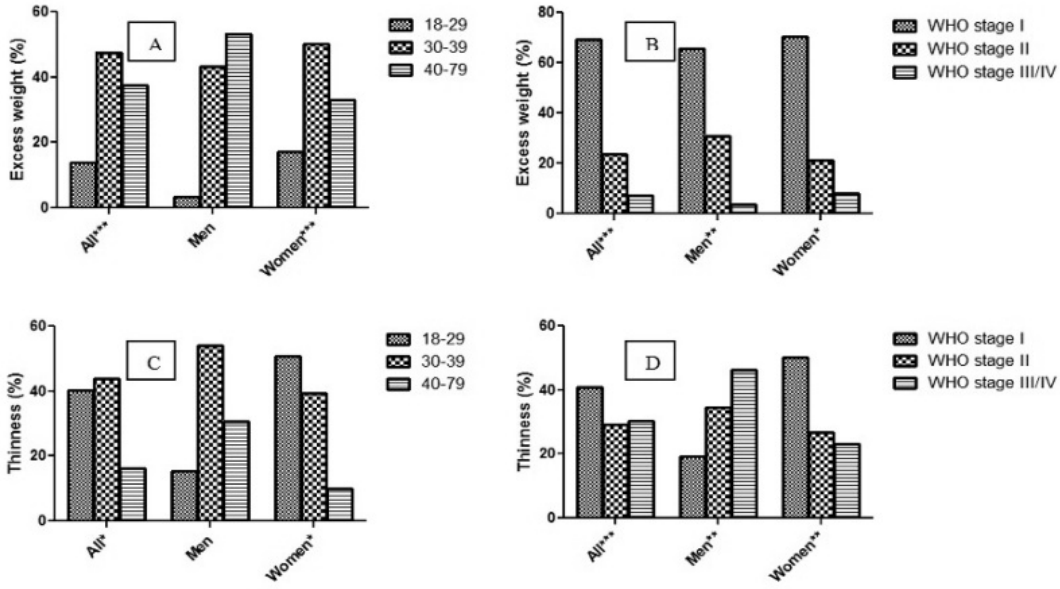

Figure 12. Prevalence of excess weight and thinness (undernutrition) among adults aged ≥18.The prevalences are presented by sex and age categories (A and C); and by sex and WHO clinical stages (B and D) among HIV-infected ART naïve study participants, February to September 2013, Addis Ababa, Ethiopia. ***, ** and * refer to significant increasing linear trend at $p < 0.001$, $p<0.01$ and $p<0.05$, respectively.

4.5. The prevalence of hypercholesterolemia and hypertriglyceridemia

The hypercholesterolemia prevalence was 16.6% (95% CI: 9.1-24.1) in overall study participants (11.0% in men and 18.9% in women), which was significantly higher in women than in men (p = 0.019). Being female, older age and higher triglycerides level were found significant risk factors for increment of serum total cholesterol level (**Figure 13** and **Table 14**). Furthermore, the median serum total cholesterol of the study participants was 150.0. The median serum total cholesterol between men and women; and triglycerides level between <150- and ≥150 mg/dL were significantly different (p <0.001). In addition, the median serum total cholesterol were found significantly different between ages 18-29 and 40-79 (p <0.001), and 30-39 and 40-79 (p = 0.036). However, there was no significant difference in serum total cholesterol between ages 18-29 and 30-39 (p = 0.067). Significant Spearman correlation between serum total cholesterol levels and CD4+ T cell count was found (r = 0.210, p<0.001).

The hypertriglyceridemia prevalence was 28.0% in women, 34.2% in men and 29.8% in overall study participants. There was no significant difference in hypertriglyceridemia prevalence in women and men (p = 0.144). The median triglycerides were 121.0 in women 128.0 in men and 122.0 in overall study participants. There was no significant difference between median triglycerides level of men and women (p = 0.113). No significant Spearman negative correlation between triglycerides level and CD4+ T cell count (r = -0.007, p > 0.05).

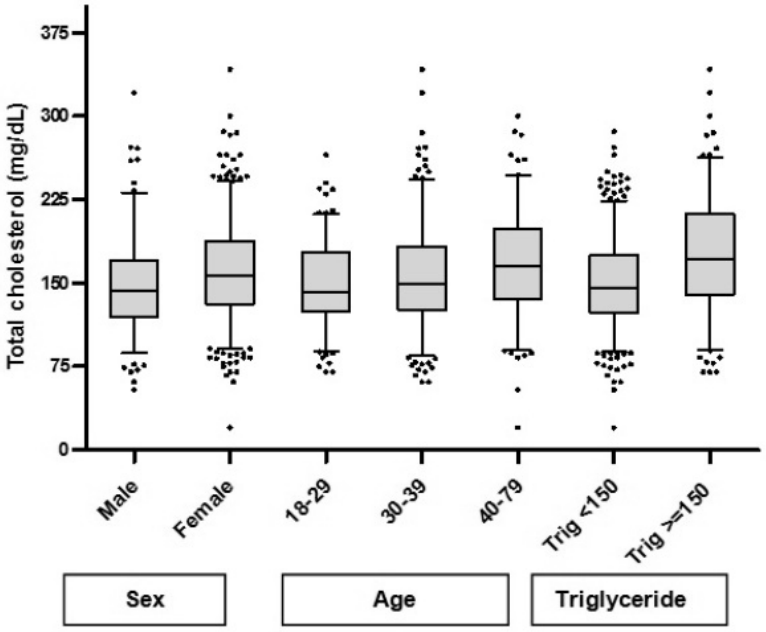

Figure 13. Serum total cholesterol level by sex, age and triglycerides level categories, February-September 2013, Addis Ababa, Ethiopia.

4.6. Risk factors for hypercholesterolemia and hypertriglyceridemia

Median serum total cholesterol were significantly different along age, sex, BMI, ≥200 CD4+ T cells/mm^3/AIDS, WHO clinical stages, being anemic/normal and hypertriglyceridemia/normal (**Table 7)**. The serum total cholesterol level showed an increasing trend in men in comparison to women, along age, BMI and triglycerides level. In contrast, total serum cholesterol level decreased with disease progression as shown by CD4+ T cell count, hemoglobin level and WHO clinical stages ($p < 0.01$). On the otherhand, median triglyceride level were significantly different BMI, along age, fasting glucose and hypercholesterolemia/normal ($p<0.01$). The triglyceride level showed an increasing trend along increasing of BMI, age, fasting glucose level and serum total cholesterol ($p < 0.05$).

Table 7. Median comparison of lipid-profiles in different groups among HIV-infected ART-naïve study participants, February-September 2013, Addis Ababa, Ethiopia.

Variables	Total cholesterol Median (IQR)	p value	Triglycerides Median (IQR)	p value
Sex				
Male	143.5(119.0-170.0)	<0.001		
Female	156.0(131.0-187.0)		-	-
Age				
18-29	142.0(124.5-177.0)		111.0 (77.5-150.0)	
30-39	145.0(126.5-182.5)	0.002	121.0 (92.5-156.5)	
40-79	165.0(135.5-199.0)		135.5 (101.3-190.5)	<0.001
BMI (Kg/m^2)				
<18.5	144.0(120.0-176.0)		120.0 (87.0-165.0)	
18-24.9	149.0(127.8-179.3)	<0.001	118.5 (86.8-153.3)	<0.001
≥25	172.5(140.0-213.0)		139.0 (107.3-179.5)	
WHO clinical stage				
Stage I	161.0(135.0-192.5)	<0.001		
Stage II	147.0(127.0-180.8)		-	-
Stage III/IV	135.0(112.0-162.5)			
CD4+ cell count ($cells/mm^3$)				-
<200	156.0(132.0-186.0)	<0.001	-	
≥200	133.5(115.3-163.3)			
Hemoglobin conc. (g/dL)				
Non-anemic	152.0(131.0-183.0)	<0.001	-	-
Anemic	127.0(106.5-159.5)			
Fasting glucose conc. (mg/dL)				
Normal (<110)	-	-	120.0(88.0-154.0)	0.028
IFG (110-125)			132.0(106.0-159.0)	
Diabetic (≥126)			154.0(98.5-206.5)	

Note: a, b, c refers to p value <0.001, <0.01 and <0.05, respectively.

All the variables which showed difference in median serum total cholesterol level (sex, age, BMI, WHO clinical stages, being HIV-positive/AIDS, being anemic/normal, and hypertriglyceridemia/normal) were found to be significantly associated factors with hypercholesterolemia (**Table 8**). In addition, age, fasting glucose and hypercholesterolemia/normal were also found associated with hypertriglyceridemia ($p < 0.05$).

The results of multivariate binomial logistic regression analysis (**Table 8**) indicated risk factors for hypercholesterolemia ($p < 0.05$). The independent risk factors for

hypercholesterolemia were found being female (AOR = 2.18, 95% CI = 1.07-4.45) in comparison with male, ages 30-39 (AOR = 2.13; 95% CI = 1.05-4.42) and 40-79 (AOR = 2.52; 95% CI = 1.17-5.64) in comparison with age 18-29, and hypertriglyceridemia (AOR = 4.80, 95% CI = 2.57-8.97) in comparison to the normal category of triglycerides level. In addition, diabetes (AOR = 3.46, 95% CI = 1.57-7.65) in comparison with normal fasting glucose levels, hypercholesterolemia (AOR = 3.34, 95% CI = 1.97-5.67) in comparison with normal serum total cholesterol level were found to be significant independent risk factors for hypertriglyceridemia.

Table 8. Variables associated with total cholesterol among HIV-infected ART-naïve study participants, February-September 2013, Addis Ababa, Ethiopia.

	Total cholesterol ≥200	
Variables	**COR (95% CI)**	**AOR (95% CI)**
Sex		
Male	1.00	1.00
Female	1.89(1.09-3.28)[c]	2.18(1.07-4.45)[c]
Age		
18-29	1.00	1.00
30-39	2.05(1.10-3.82)[c]	2.13(1.05-4.32)[c]
40-79	3.22(1.69-6.15)[a]	2.52(1.17-5.64)[c]
BMI (Kg/m3)		
<18.5	1.00	1.00
18-24.9	1.08(0.52-2.24)	0.72(0.32-1.59)
≥25	3.51(1.64-7.54)[a]	1.82(0.77-4.30)
WHO clinical stages		
Stage I	1.00	1.00
Stage II	0.60(0.36-1.00)	0.69(0.39-1.24)
Stage III/IV	0.20(0.07-0.56)[b]	0.36(0.11-1.11)
CD4+ cell count (cells/mm^3)		
<200	1.00	1.00
≥200	0.35(0.15-0.77)[c]	0.55(0.23-1.32)
Hemoglobin conc. (g/dL)		
Non-anemic	1.00	1.00
Anemic	0.24(0.07-0.80)[c]	0.41(0.12-1.39)
Triglycerides level (mg/dl)		
<150	1.00	1.00
≥150	3.48(2.21-5.47)[a]	4.80(2.57-8.97)[a]

Note: a, b, c refers to p value <0.001, <0.01 and <0.05, respectively.

4.7. Independent predictors of HIV RNA load

The mean HIV RNA load was found significantly higher in men, and in study participants with serum total cholesterol <200 mg/dL. In addition, the mean HIV RNA load was found to be different between CD4+ T cell count and/or WHO clinical stages, and anemic or normal ($p < 0.05$) **(Table 9)**.

The association of each independent variable with dependent variable (log HIV RNA load) was analyzed. Sex, CD4+ T cell count and/or WHO clinical stage categories, being anemic or normal, and being hypercholesterolemia or normal were found significantly associated with HIV RNA load ($p < 0.05$; **Table 8)**.

The multivariate linear regression analysis showed that sex, CD4+ T cell count and/or WHO clinical stages, being anemic or normal, being hypercholesterolemia or normal were found to be independent predictors of HIV RNA load ($p < 0.05$) (**Table 9)**. Women are at lower risk of having higher HIV RNA load in comparison to men. Having higher total cholesterol was found to be associated with reduced HIV RNA load in comparison to those with lower serum total cholesterol. In addition, study participants with CD4+ T cell count <500 cells/mm^3 and/or stages III/IV and who are anemic were found to be risk factors for increase of HIV RNA load. CD4+ T cell count <500 and/or WHO clinical stages, and anemia were found to be significantly associated with the three HIV RNA categories. In addition, sex and low serum total cholesterol were found to be associated significantly with HIV RNA ≥10,000 and ≥40,000 copies/mL. However, sex and low

serum total cholesterol were not found to be associated with HIV RNA ≥100,000 copies/mL (p>0.05).

Table 9. Association of variables with HIV RNA load among ART-naïve study participants, February-September 2013, Addis Ababa, Ethiopia.

Variables	Log HIV RNA load (Mean ± SD)	Univariate, β (95% CI)	Multivariate, β (95% CI)
Sex			
Male	4.49 ± 0.76[a]	Ref.	Ref.
Female	4.11 ± 0.84	-0.38 (-0.54, -0.23)[a]	-0.24 (-0.40, -0.07)[b]
CD4+ T cell count and/or stages III/IV			
≥500 and/or not stages III/IV	3.73 ± 0.82[a]	Ref.	Ref.
<500 and/or stages III/IV	4.36 ± 0.79	0.63 (0.46, 0.80)[a]	0.56 (0.38, 0.73)[a]
Hemoglobin conc. (g/dL)			
Non-anemic	4.18 ± 0.81[c]	Ref.	Ref.
Anemic	4.68 ± 0.79	0.50 (0.27, 0.73)[a]	0.41 (0.18, 0.64)[b]
Total cholesterol conc. (mg/dL)			
<200	4.31 ± 0.82[c]	Ref.	Ref.
≥200	3.85 ± 0.78	-0.46 (-0.67, -0.25)[a]	-0.25 (-0.45, -0.04)[c]
Variables	**≥10000, AOR (95% CI)**	**≥40000, AOR (95% CI)**	**≥100000, AOR (95% CI)**
Sex			
Male	1.00	1.00	1.00
Female	0.51(0.33-0.79)	0.52(0.34-0.79)	0.64(0.41-1.08)*
CD4+ T cell count and/or stages III/IV			
≥500 and/or not stages III/IV	1.00	1.00	1.00
<500 and/or stages III/IV	4.44(2.86-6.90)	5.29(2.90-9.64)	5.36(2.26-12.70)
Hemoglobin conc. (g/dL)			
Non-anemic	1.00	1.00	1.00
Anemic	2.85(1.44-5.66)	2.72(1.52-4.85)	2.46(1.34-4.51)
Total cholesterol conc. (mg/dL)			
≥200	1.00	1.00	1.00
<200	2.22(1.32-3.74)	2.65(1.36-5.17)	2.35(0.97-5.69)*

Note: ANOVA = analysis of variance; SD = standard deviation; and CI = confidence interval; β = represents coefficients of univariate and multivariate linear regression. Note: AOR = Adjusted odds ratio; * = p not statistically significant. a, b, c refers to p value <0.001, <0.01 and <0.05, respectively.

4.8. Independent predictors of CD4+ T cell count and/or WHO clinical stages

The median values of CD4+ T cell count were found to be [385(364-409) and 195(164-249), p<0.001] for HIV RNA load <10,000- and ≥10,000 copies/mL, [296(263-323) and 386(364-414), p<0.001] for men and women, [258(206-313) and 366(354-389), p<0.001] for being anemic and non-anemic, and [352(326-365) and 457(368-502), p<0.001] for serum total cholesterol <200- and ≥200 mg/dL, respectively.

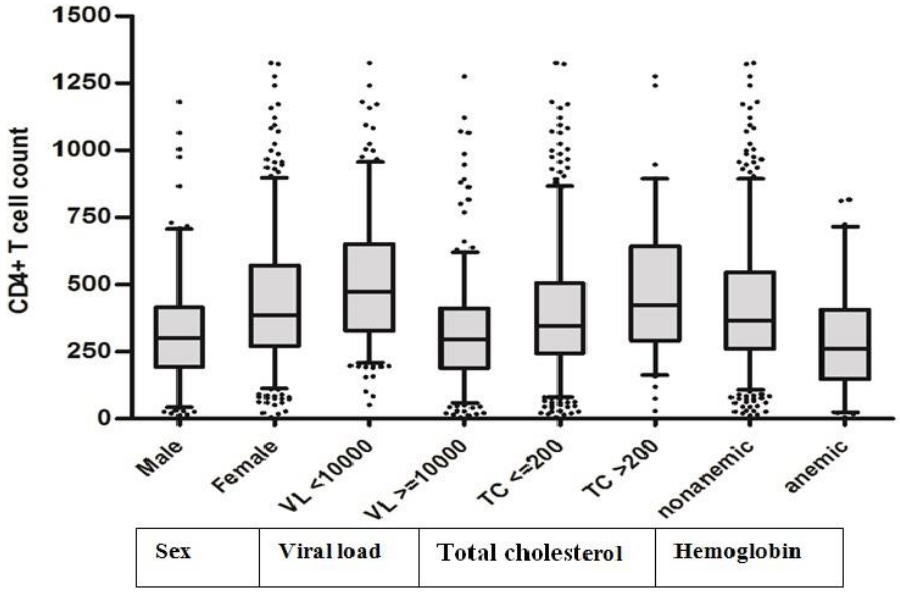

Figure 14. CD4+ T cell count by sex, HIV RNA load, serum total cholesterol (TC) and hemoglobin, February-September 2013, Addis Ababa, Ethiopia.

As indicated in **Table 10,** sex, HIV RNA load, hemoglobin and serum total cholesterol levels were found to be associated with all the three categories of CD4+ T cell count and/or WHO clinical stages ($p < 0.05$). Multivariate binomial logistic regression analysis showed sex and HIV RNA load ≥10,000 to be independently associated with CD4+ T cell count and/or WHO clinical stages. In addition, anemia and low serum total cholesterol were found to be independently associated with <500 and/or stages III/IV ($p<0.05$). However, anemia and low serum total cholesterol were not significantly associated with <350 and/or stages III/IV, and <500 and/or stages III/IV ($p>0.05$).

Table 10. Association of variables with CD4+ T cell count among ART-naïve study participants, February-September 2013, Addis Ababa, Ethiopia.

Variables	Overall [Number (%)]	<200 or stage III/IV [Number (%)]	<350 or stage III/IV [Number (%)]	<500 or stage III/IV [Number (%)]
Sex				
Male	171(28.8)	64(41.6) [a]	111(35.5) [a]	147(33.7) [a]
Female	423(71.2)	90(58.4)	202(64.5)	289(66.3)
HIV RNA load (copies/mL)				
<10000	264(45.2)	27(17.9) [a]	84(27.4) [a]	149(34.8) [a]
≥10000	320(54.8)	124(82.1)	223(72.6)	279(65.2)
Hemoglobin conc. (g/dL)				
Non-anemic	507(88.8)	117(78.5) [a]	252(83.7) [a]	362(86.6) [b]
Anemic	64(11.2)	32(21.5)	49(16.3)	56(13.4)
Total cholesterol conc. (mg/dL)				
≥200	95(16.6)	9(6.2)	34(11.4) [a]	56(13.4) [b]
<200	476(83.4)	136(93.8) [a]	264(88.6)	362(86.6)

Variables	<200 and/or stages III/IV, AOR (95% CI)	<350 and/or stages III/IV, AOR (95% CI)	<500 and/or stages III/IV, AOR (95% CI)
Sex			
Male	1.00	1.00	1.00
Female	0.58(0.36-0.95)	0.60(0.36-0.99)	0.38(0.19-0.73)
HIV RNA load (copies/mL)			
<10000	1.00	1.00	1.00
≥10000	3.89(2.34-6.46)	4.09(2.80-6.00)	4.49(2.69-7.49)
Hemoglobin conc. (g/dL)			
Non-anemic	1.00	1.00	1.00
Anemic	2.39(1.28-4.46)	1.73(0.86-3.56)*	1.67(0.65-4.28)*
Total cholesterol conc. (mg/dL)			
≥200	1.00	1.00	1.00
<200	2.14(1.01-4.59)	1.53(0.90-2.54)*	1.38(0.75-2.56)*

Note: a, b refers to p value <0.001 and <0.01, respectively. * refers to p value not significant (p>0.05).

4.9. Variables correlated with viral load, and CD4+ count or WHO clinical stages

As indicated in **Figure 15** below, body mass index (r = -0.170, p <0.01), CD4+ T cell count (r = -0.412, p <0.01) and total cholesterol concentrations (r = -0.249, p <0.01) were found significantly correlated with HIV RNA load. However, hemoglobin concentrations were not significantly correlated with HIV RNA load (r = -0.084, p >0.05).

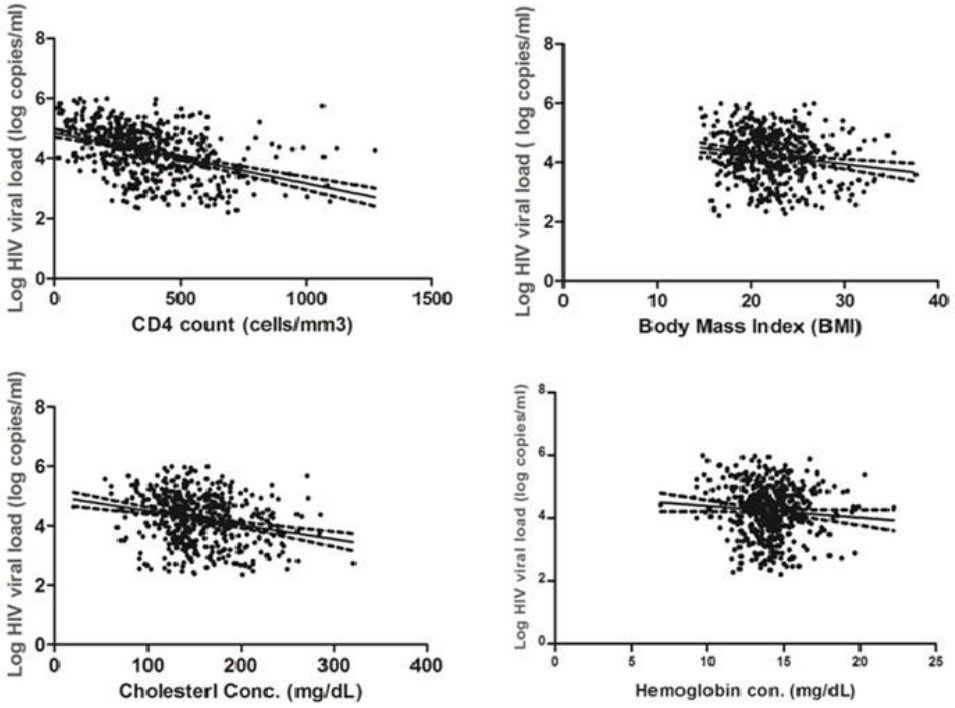

Figure 15. Significant Pearson correlation ($p < 0.01$) of log HIV RNA load with CD4+ T cell, BMI, total cholesterol and hemoglobin level of HIV-infected ART naïve study participants, February-September 2013, Addis Ababa, Ethiopia.

Anemia, low serum total cholesterol and the combination of the two markers were found significant but with small Spearman correlation ($r < 0.3$, $p < 0.05$). Furthermore, CD4+ T cell count and/or WHO clinical stages, and HIV RNA load categories were also found to have significant moderate correlation between them ($0.3 < r < 0.5$, $p < 0.01$; **Table 11**).

Table 11. Correlation of alternative biomarkers in reference with CD4+ T-cell count and/or WHO clinical stages, and HIV RNA load categories among ART-naïve study participants, February-September 2013, Addis Ababa, Ethiopia.

Variables	CD4+ T cell count and/or WHO clinical stages III/IV							
	<200 and/or stages III/IV		<350 and/or stages III/IV		<500 and/or stages III/IV		Total CD4+ T cell count	
	r	p	r	p	r	p	r	p
HIV RNA load ≥10000	0.307	<0.001	0.377	<0.001	0.362	<0.001	-0.432	<0.001
Anemia	0.199	<0.001	0.173	<0.001	0.121	<0.01	-0.187	<0.001
Cholesterol <200	0.154	<0.001	0.147	<0.001	0.146	<0.001	-0.146	<0.001
Anemia and/or cholesterol <200	0.147	<0.001	0.144	<0.001	0.157	<0.001	-0.146	<0.001
Variables	**HIV RNA load**							
	≥10000		≥40000		≥100000		Total HIV RNA load	
	r	P	r	P	r	p	r	p
<500 and/or stages III/IV	0.362	<0.001	0.311	<0.001	0.319	<0.001	0.333	<0.001
Anemia	0.179	<0.001	0.196	<0.001	0.168	<0.001	0.214	<0.001
Cholesterol <200	0.192	<0.001	0.176	<0.001	0.120	<0.01	0.185	<0.001
Anemia and/or cholesterol <200	0.198	<0.001	0.190	<0.001	0.115	<0.01	0.194	<0.001

Note: Classification of Correlation Co-efficient (r):- Up to 0.1: Trivial Correlation; 0.1-0.3: Small Correlation; 0.3-0.5: Moderate Correlation; 0.5-0.7: Large Correlation 0.7-0.9: very Large Correlation; 0.9- 1.0: Nearly Perfect correlation; 1: Perfect correlation.

4.10. Diagnostic performance of alternative markers to HIV disease progression

Diagnostic performance (specificity, sensitivity and predictive values) of <500 and/or stages III/IV, HIV RNA load ≥10,000, lower serum total cholesterol, anemia, and combination of anemia and lower serum total cholesterol were done to determine disease progression as shown in **Table 12.** <500 and/or stages III/IV and HIV RNA load ≥10,000 had a better specificity and sensitivity to determine disease progression. The combination of anemia and lower serum total cholesterol and low **s**erum total cholesterol alone showed low specificity but high sensitivity of >80.0%. Lower serum total cholesterol was better than HIV RNA load ≥10,000 and <500 and/or stages III/IV in its sensitivity, but low in its specificity. Anemia was found to be with a poor diagnostic performance when used alone. It had low sensitivity even if it had high specificity. High negative predictive values (NPV) and low positive predictive values (PPV) were observed in <200 and/or

stage III/IV. PPV value improved but NPV reduced in <350 and/or stages III/IV, and <500 and/or stages III/IV. However, it was the opposite of the CD4+ T cell count and/or WHO clinical stages that happened to the NPV and PPV in HIV RNA load (**Table 12**).

Table 12. Diagnostic performance of alternative biomarkers in reference to CD4+ T-cell count and/or WHO clinical stages, and HIV RNA load categories among ART-naïve study participants, February-September 2013, Addis Ababa, Ethiopia.

	Sensitivity	Specificity	PPV	NPV
Variables		**<200 and/or stages III/IV**		
HIV RNA load ≥10,000	82.1	54.7	38.8	89.8
Anemia	21.5	92.4	50.0	76.9
Cholesterol <200	93.8	20.2	28.6	90.5
Anemia and/or cholesterol <200	85.1	24.5	28.3	82.4
		<350 and/or stages III/IV		
HIV RNA load ≥10,000	72.6	65.3	70.1	68.1
Anemia	16.2	94.4	76.6	50.0
Cholesterol <200	88.6	22.6	55.8	64.2
Anemia and/or cholesterol <200	81.8	26.6	55.7	56.5
		<500 and/or stages III/IV		
HIV RNA load ≥10,000	65.2	73.7	87.2	43.6
Anemia	13.4	94.8	87.5	28.6
Cholesterol <200	86.6	25.5	76.1	41.1
Anemia and/or cholesterol <200	80.5	29.1	75.8	35.1
		HIV RNA load ≥10,000		
<500 and/or stages III/IV	87.2	43.6	65.2	73.7
Anemia	16.1	94.8	79.4	47.8
Cholesterol <200	89.9	24.7	58.9	67.0
Anemia and/or cholesterol <200	84.1	29.9	59.3	60.8
		HIV RNA load ≥40,000		
<500 and/or stages III/IV	91.6	35.5	40.9	89.7
Anemia	20.2	93.1	58.7	70.7
Cholesterol <200	93.5	22.4	36.8	87.6
Anemia and/or cholesterol <200	86.9	26.7	36.6	80.8
		HIV RNA load ≥100,000		
<500 and/or stages III/IV	92.0	30.6	21.5	94.9
Anemia	22.7	91.2	34.9	84.9
Cholesterol <200	93.8	18.9	19.3	93.6
Anemia and/or cholesterol <200	87.0	24.2	19.2	90.0

Note: PPV = positive predictive value, NPV = negative predictive value

4.11. Characteristics of study participants for subtyping and CRFs

A total of 60 study participants, 41 (68.3%) women and 19 (31.7%) men, were selected among the 594 study participants. In addition, 21 (35.0%) samples had >500 CD4+ T cell count and WHO clinical stages 1-2; 12 (20.0%) samples had 350-500 CD4+ T cell counts and WHO clinical stages 1-2; 14 (23.3%) samples had 200-349 CD4+ T cell count and WHO clinical stages 1-4; and 13 (21.7%) samples had <200 CD4+ T cell counts and fell under the WHO clinical stages 1-4. The the study participants' median age was 34.5 years (IQR: 30.0-40.0). Seven (11.7%) of the study participants were at WHO clinical stage III/IV and 13 (21.7%) were at AIDS stage with CD4+ T cell count <200 cells/uL. WHO clinical stages were not associated with HIV-1 tropism ($p > 0.05$), whereas, the four groups of CD4+ T cell counts and HIV RNA load were associated with viral tropism ($p < 0.05$). In all 60 study participants, the median viral load was 8,704.00 copies/mL (IQR: 3280.75-106955.50 copies/mL). And, the median CD4+ T cell count was 368.5 cells/uL (IQR: 251.25 - 571.75 cells/uL). The median values of HIV RNA load and CD4+ T cell count between phenotypes determined by Geno2Pheno were compared. It is found that [CXCR4 median 107,267.00 copies/mL (IQR: 74,976.50-475,570.00 copies/mL); R5 median 8,734.00 copies/mL (IQR: 5,092.50-106,646.00 copies/mL); $p = 0.003$], and [CXCR4 median 209.00 cells/uL (IQR: 126.00-288.50 cells/uL); R5 median 364.00 cells/uL (IQR: 261.50-525.00 cells/uL); $p = 0.003$] (Table 13).

Table 13. Summary of demographic, hematological and virological characteristics of only for the HIV subtyped HIV-infected ART naïve study participants, February-September 2013, Addis Ababa, Ethiopia.

Variables	Number (%)
Sex	
Male	19(31.7)
Female	41(68.3)
Age (years)	
18-29	9(15.0)
30-39	34(56.7)
40-79	17(28.3)
WHO clinical stage	
Stage 1	27(45.0)
Stage 2	26(43.3)
Stage III/IV	7(11.7)
CD4+ cell count (cells/mm^3)	
<200	13(21.7)
≥200	47(78.3)
HIV RNA load (copies/mL)	
<10000	33(55.0)
≥10000	27(45.0)

4.12. Subtyping and unique recombinant forms (URFs)

Among the total 60 HIV whole genomes sequenced, 49 were subtype C (81.7%); 1 was subtype A1 (1.7%); 6 were recombinant C/A1 (10%); and 3 were recombinant C/A1/D (5.0%). One of the sequences could not be assigned to a subtype by REGA due to poor sequence quality (Figure 16 & 17; Table 2).

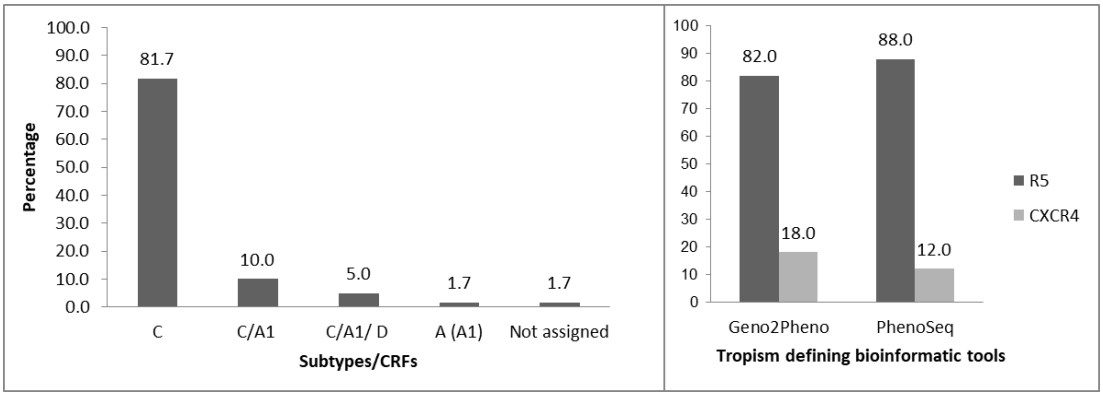

Figure 16. Percentage of subtypes, circulating recombinant forms from a total of 60 samples sequenced, and R5 and CXCR4 coreceptor uses from the total of 50 sequenced and analyzed for tropism.

For the nine recombinants, the presence of recombination and recombinant breakpoints were detected as shown in Figure 17. The genome of subtype C was the dominant sequence in the recombinants. However, both subtypes A1 and C were dominant in SP-090. The recombination breakpoints were dominantly on *pol* in subtype A1 and C recombinants; and on *env* and accessory genes when subtype D recombine with subtypes A1 and/or C.

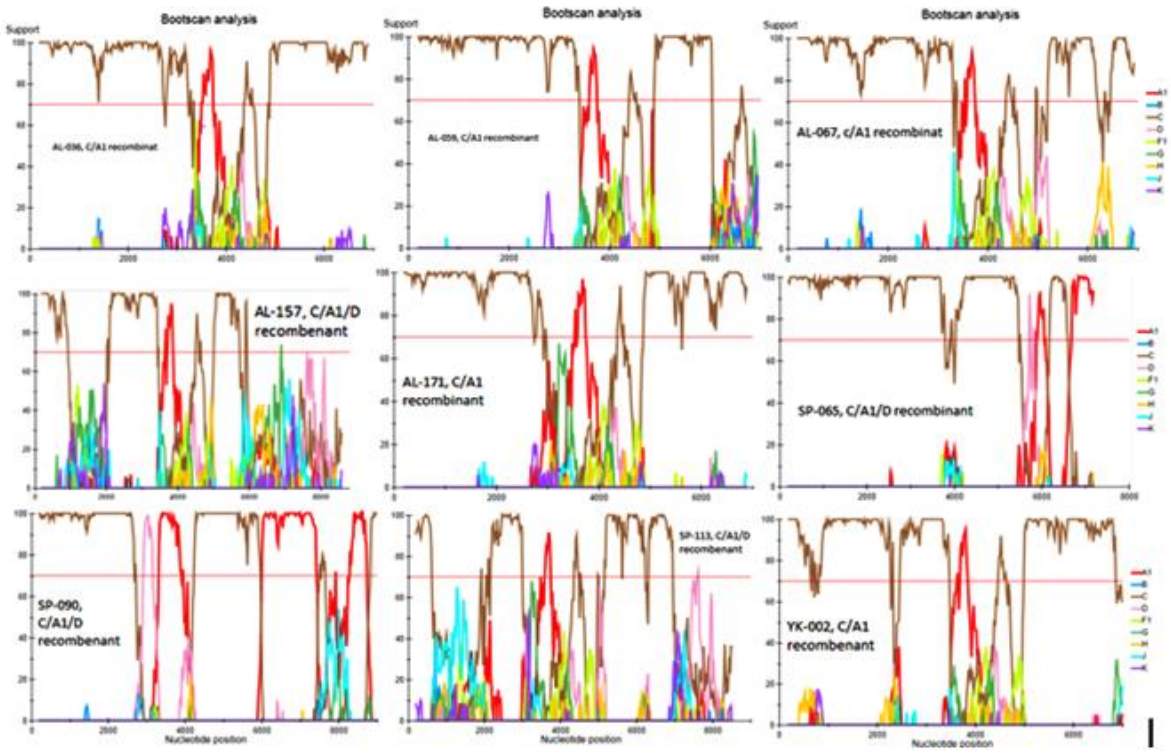

Figure 17. Bootscan analysis of 9 recombinants generated by performing with window size 400 base pairs and step size 20 base pairs carried for all the 60 whole genome consensus sequences. The cut value for recombination is considered at 70%.

All viruses of 56 sequences cluster with subtype C, except for the virus from subject AL-062 (subtype A1) and Sp-065 and SP-090 (A/C recombinants). The branch length between sequences of plasma was not the same as indicated in the tree (Figure 18).

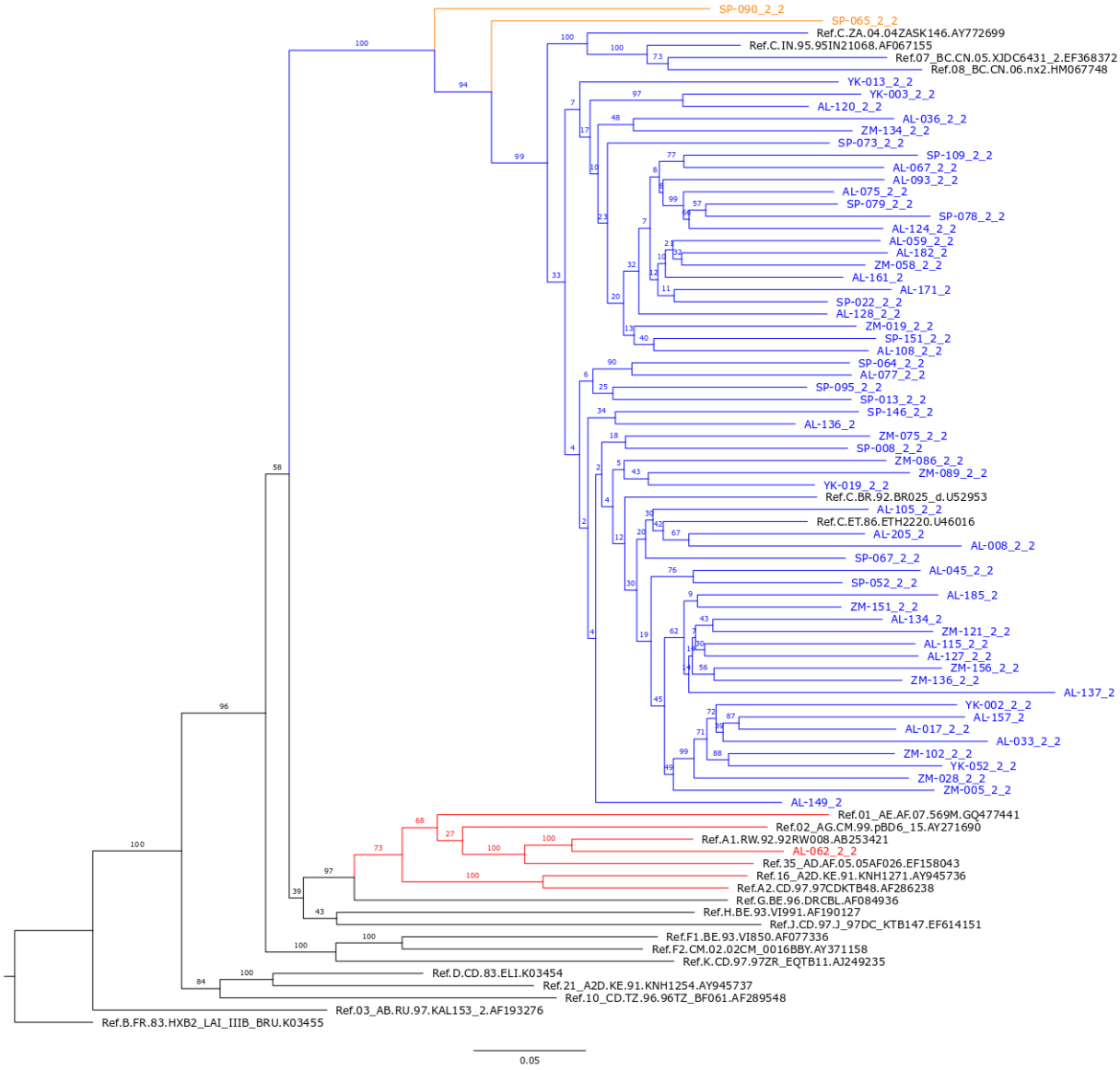

Figure 18. Neighbor-joining tree to show the evolutionary relationship and the distance of the the HIV-1 genome sequences. Sixty sequences from plasma samples, Subtupe A1, subtype C, and AC CRFs as reference sequences from the Los Alamos database were used. Colour-coded maximum likelihood tree with bootstrap values refers to blue are samples aligning with subtype C references, red with subtype A, the two in orange are A/C recombinants.The scale bar represents a genetic distance of 5%.

4.13. Tropism and glycosylation sites in V3 loop of the viral isolates

Among the total 60 HIV genomes sequenced, 10 (16.7%) did not have good sequence in V3 loop to determine tropism. From the 50 sequences where tropism was determined by PhenoSeq, 6 (12.0%) and 44 (88.0%) were X4 and R5, respectively. In addition, phenotyping by Geno2Pheno showed that 9 (18.0%) and 41 (82.0%) were X4 and R5, respectively. There was no positively charged amino acid lysine (K) or arginine (R) at position 11. In ZM-028, lysine (K) was recorded at position 24 with net positive charge of +5. Lysine was recorded at position 25 with the net positive charge of +5 in AL-108 and in SP-065 with net positive charge of +6. Therefore, AL-108, SP-065 and ZM-028 carried X4 tropic viruses. The other possible X4 phenotype virus with net positive charge of +5 was identified in SP-064 (Figure 16, Table 14). The the most dominant potential N-linked glycosylation NNNT motif in 35 (70%) at the beginning of the loop was identified. Additional glycosylation sites including GNNT in 8 (16%), SNNT in 4 (8%), GNNI in 1 (2%), NNNR in 1 (2%) and QNNT in 1 (2%) were identified as (Table 14).

Table 14. HIV-1 *env* V3 loop consensus sequences, co-receptor use, and subtypes and circulating recombinant forms of HIV-infected ART naïve study participants in Addis Ababa, Ethiopia, February-August 2013.

Env name	V3 HIV aligned sequences	charge	**Coreceptor** usage		Subtype/recombinant
			PhenoSeq	Geno2Pheno	
	* 11 24 25 *				
AL-008	CTRPSNNTRK S IRIGPGQAFYAT G D VTGDIRQAHC	+3	R5	R5	Subtype C
AL-017	CTRPNNNTRE S IRIGPGQTFYAT G A IIGDIRQAHC	+2	R5	R5	Subtype C
AL-045	CTRPGNNTRE S IRIGPGQAFYAT G D IIGDIRQAYC	+1	R5	R5	Subtype C
AL-062	CTRPSNNTRT S IRIGPGQAFFAT G D IIGDIRQAHC	+2	R5	R5	Subtype A (A1)
AL-075	CIRPNNNTRK S MRIGPGQTFYAT G G IIGDIRAAYC	+4	R5	R5	Subtype C
AL-077	CTRPNNNTRK S VRIGPGQTFYAT G D IIGDIRQAYC	+3	R5	R5	Subtype C
AL-105	CTRPNNNTRK S IRIGPGQTFYAT G E IIGDIREAHC	+2	R5	R5	Subtype C
AL-108	CTRPNNNTIK S MRIGPGQTFYAT G K IVGNIRQAHC	+5	CXCR4	CXCR4	Subtype C
AL-115	CTRPNNNTRR S IRIGPGQAFYAT E D VIGDIRQAYC	+2	R5	CXCR4	Subtype C
AL-124	CIRPGNNTRK S IRIGPGQTFYAT G D IIGNPRKAYC	+4	R5	R5	Subtype C
AL-127	CTRPNNNTRQ S IRIGPGQTFYAT G E IIGDIRQAHC	+2	R5	R5	Subtype C
AL-128	CTRPNNNTRK S MRIGPGQTFYAT - D IIGDIRQAHC	+3	R5	R5	Subtype C
AL-134	CVRPNNNTRK S IRIGPGQAFYAT G A IIGDIRQAYC	+4	R5	R5	Subtype C
Al-136	CTRPNNNTRK S VRIGPGQAFFAT G D IIGDIRQAHC	+3	R5	R5	Subtype C
AL-137	CMRPGNNTRT S VRIGPGQTFYAT G D IVGDIKQAHC	+2	R5	R5	Subtype C
AL-149	CARPNNNTRK S VSVGPGQAIYAT G D IIGDIRQAHC	+2	CXCR4	R5	Subtype C
AL-161	CTRPNNNTRK S VRIGPGQTFYAT G A IIGEIRQAHC	+4	R5	R5	Subtype C
AL-182	CTRPNNNTRK S IRIGPGQTFYAT - D IIGDIRQAHC	+3	R5	R5	Subtype C
AL-185	CTRPNNNTRQ S IRIGPGQTFYAT G E IIGDIRQAHC	+2	R5	R5	Subtype C
AL-205	CTRPNNNTRE S IRIGPGQTFYAT G D IIGDIRQAHC	+1	R5	R5	Subtype C
SP-008	CIRPNNNRRK S VRIGPGQTFYAT G D IIGDIRAAFC	+4	R5	R5	Subtype C
SP-013	CTRPGNNTRK S VRIGPGQTFYAT G D IIGDIKQAHC	+3	R5	R5	Subtype C
SP-022	CTRPSNNTRK S VRIGPGQTFYAT G D IIGNIRQAYC	+4	R5	R5	Subtype C
SP-052	CTRPNNNTRE S IRIGPGQTFYAT G D IIGDIRQAYC	+1	R5	R5	Subtype C
SP-064	CTRPNNNTRK Y VRIGRGQVFHAT G E IIGDIRKAYC	+5	CXCR4	CXCR4	Subtype C
SP-065	CTRPNNNTRK S VRIGPGQTFYTT - K IIGNIRLAHC	+6	CXCR4	R5	Recombinant of C, A1
SP-067	CTRPGNNTRE S VRIGPGQAFYAT G E IIGDIRKAHC	+2	R5	R5	Subtype C
SP-073	CTRPNNNTRK S VRIGPGQTFFAT G E IIGNIRKAYC	+5	R5	R5	Subtype C
SP-078	CTRPGNNTRK S VRIGPGQTFYAT G D IIGDIRQAHC	+3	R5	R5	Not identified
SP-079	CTRPNNNTRK S VRIGPGQTFYAT G A IIGEIRQAHC	+5	R5	R5	Subtype C
SP-090	CTRPNNNTRK S VRIGPGQVFYAT G D IIGDIRQAHC	+3	R5	R5	Recombinant of C, A1, D
SP-095	CTRPNNNTRR S VRIGPGQTFYAT G E IIGDIKQAHC	+3	R5	R5	Subtype C
SP-109	CTRPGNNIRK S MRIGPGQAFYAT G D IIGDLRQAHC	+3	R5	CXCR4	Subtype C
SP-146	CTRPNNNTRQ S MRIGPGQAFYAM G D IIGDIRQAHC	+2	R5	R5	Subtype C
SP-151	CTRFNNNTRK S IRIGPGQAFYTA G E IIGEIRQAHC	+3	R5	R5	Subtype C
YK-003	CTRPGNNTRK S VRIGPGQTFYAT G A ----------	-	R5	CXCR4	Subtype C
YK-019	CTRPGNNTRR S VRIGPGQTFYAT G D IIGDIRQAHC	+3	R5	R5	Subtype C
YK-052	CTRPQNNTRR S VRIGPGQAFYTT G D IIGDIRQAHC	+3	R5	R5	Subtype C
ZM-005	CTRPNNNTRK S IRIGPGQAFYAR G D IIGDIRQAHC	+4	R5	CXCR4	Subtype C
ZM-019	CTRPNNNTRK S MRIGPGQVFYAT E D IIGDIRQAHC	+2	R5	CXCR4	Subtype C
ZM-028	CMRPNNNTRK S IRIGPGQTFYAT K D IIGNIRQAHC	+5	CXCR4	CXCR4	Subtype C
ZM-058	CTRPNNNTRE S VRIGPGQTFFAT G D IIGDIRQAHC	0	R5	R5	Subtype C
ZM-075	CTRPGNNTRR S VRIGPGQTFFAT G E IIGDIRQAYC	+3	R5	R5	Subtype C
ZM-086	CTRPNNNTRK S VRIGPGQTFYAT G D IIGNIRQAHC	+4	R5	R5	Subtype C
ZM-102	CTRPNNNTRT S IRIGPGQSFHAT G A ITGRIRQAHC	+4	CXCR4	R5	Subtype C
ZM-121	CTRPNNNTRK S VRIGPGQAFYAT G D IIGNIRQAYC	+4	R5	R5	Subtype C
ZM-134	CTRPNNNTRK S VRIGPGQTFYAT G D IIGNIRQAHC	+4	R5	R5	Subtype C
ZM-136	CERPNNNTRE S IRIGPGKTFYAT G E IIGDIRQAYC	+1	R5	R5	Subtype C
ZM-151	CTRHSNNTRK S IRIGPGQAFFAT G E VIGDIRLAHC	+3	R5	R5	Subtype C
ZM-156	CMRPNNNTRK S IRIGPGQAFFAT G A VTGDIRQAHC	+4	R5	CXCR4	Subtype C

4.14. Detection of secondary drug resistance responsible mutations

The presence of important mutations responsible for secondary drug resistance in these sequences was checked using the Stanford HIV Drug Resistance Database. However, no important resistance mutations were detected. This may be because of the combinations of the study participants are drug naïve and the sequenced samples are only sixty.

5. Discussion

The current HIV global program is applying targeted approaches so that both national and global targets are realized. Cognizant of that, Ethiopia has identified KPPs based on HIV prevalence rate in specific groups and their respective contexts. The recognized KP group in Ethiopia is the FSWs. FSWs are identified as KP in response to HIV epidemic as they are highly and consistently exposed to risky sexual practices which lead to HIV infection and transmission. Accordingly, evidence from Addis Ababa showed that HIV prevalence among FSWs is the highest compared to other risk groups. Also, the density of the FSWs population correlated closely with high PLHIV burden as reported by several studies (FMOE, 2012; PEPFAR, 2017; FHAPCO, 2018). The strategic HIV transmission interventions plan in Addis Ababa intends to achieve the three 90 targets by 2020 through targeted social mobilization and HIV testing, linkage to care, quality of HIV treatment, and virtual elimination of MTCT, envisioning ending AIDS by 2030 (FHAPCO, 2014).

Although EDHS (2006, 2011, 2016) findings indicated a relative decline of HIV prevalence between 2005 and 2016 in Addis Ababa, antenatal clinic (ANC)-based surveillance in 2014 (5.5%) and PMTCT that substituted ANC surveillance in 2016 (1.8%) revealed a significant variation in reduction between the two study findings, EPHI (2015) and EPHI (2017a), thus requiring cautious interpretation of the ANC and PMTCT surveillance comparisons.

As a result of the relatively better economic activities and social services, Addis Ababa attracts productive age groups from all over the country and the city also serves as a gateway to other parts of the world. This implies huge cultural exchange and exposure to complex sexual behaviours, practices and networking catalysed by the presence of various types of sex workers (AAHAPCO, 2017) leading to sexual mixing of KPPs with the general population.

Combination ART acheives sustained HIV viral suppression and contributes to improvement in the quality of life, and reductions in mortality, progression to AIDS, opportunistic infections (OIs), hospitalization, and decreased HIV transmission to uninfected persons (Gudina *et al.,* 2017). The stability of death from HIV/AIDS in Addis Ababa may be explained by better adherence, follow-up and access for care and treatment (EPHA and CDC, 2012).

The study revealed that noncompliance to medical instruction and poor adherence to ART use, which could foster emergence of drug resistance. In addition, limited availability of laboratory services such as HIV RNA load and drug resistance testing and monitoring due to lack of experienced health professionals, and weak infrastructure and health care system were identified to contribute to the delay in diagnosis of treatment failure (Misgena, 2011; Bernabas *et al.,* 2017). This is complicated by the high rate of transmitted and preexisting drug resistance mutations reported from Ethiopian HIV/AIDS patients (Telele *et al.,* 2018). The finding of HIV-positives on ART with high viral load in some studies (EPHI, 2018b) increases the risk of HIV transmission in the community.

Undernutrition is more common in developing countries, where HIV patients are often not diagnosed and/or commence ART until they have advanced disease (Girardi *et al.,* 2007; Gesesew *et al.,* 2017c). Among those who were found at AIDS stage in this study based on CD4+ T cell count (17.2%) and at WHO clinical stage III/IV (14.4%), 52.5% were enrolled for <1 year in ART care centers for free ART. The number of people diagnosed late with HIV is high depending on the criteria used both in developing and developed countries. For example, a late diagnosis of 24-43% was reported for CD4+ T cell count <200 cells/uL (Girardi *et al.,* 2007). This indicates that there are a suset of the population who do not get tested for HIV possibly for fear of stigmatization, and they make themselves available for test and treatment at late stage of the disease (Gesesew *et al.,* 2017c). This has negative implication in prevetion and control of HIV transmission.

The 15.1% average prevalence (15.8% in men and 14.8% in women) of undernutrition in Addis Ababa was much lower than the national average (36.6% in men age 15-59 and 26.9% in women age 15-49) (CSA and ICF, 2012). However, it is closer to that reported from Dilla University Referral Hospital (12.3%) (Hailemariam *et al.,* 2013), which is an urban centre like Addis Ababa. However, undernutrition prevalence in women on ART in Humera Hospital, which is more rural, was much higher (42.3%) (Hadgu *et al.,* 2013), than in Addis Ababa and Dilla, indicating a socio-economic disparity advantaging the urban setting compared to the rural, in Ethiopia, as it is true elsewere (Wilson *et al.,* 2002; Fontaine *et al.,* 2003). The lower prevalence of undernutrition in this study population in comparison with those studies on individuals on ART could be that undernourished HIV-infected individuals may have suboptimal response to HIV drugs

when they start ART at lower CD4+ T cell count or WHO clinical stages III/IV (Paton *et al.,* 2006; Marazzi *et al.,* 2008). The other possible reasons for low undernutrition prevalence in this study population could be dietary diversity, better household food security and nutritional care and support since there may be relatively better standard of living in Addis Abab in comparison to other parts of the country. In addition, most study participants in this study (82.8%) were with better immune status (CD4+ cell count ≥200 cells/mm^3) that could protect against undernutrition (Lawn *et al.,* 2008; Kosmiski, 2011; Marazzi *et al.,* 2008). Lipid based nutritional supplements for short duration (three months) improved gain of weight, lean body mass, and grip strength through lessening risk of metabolic complications and resource demand in patients with HIV starting ART. Besides, protein containing supplement supported immune recovery. Lipid based nutritional supplements given during the first three months of ART improves certain domains of quality of life than delayed supplementation as well (Olsen *et al.,* 2014; Tesfaye *et al.,* 2016).

The overall excess weight prevalence was 22.1% (23.8% in women and 17.6% in men) and obesity 5.4% (6.1% in women and 3.6% in men) in this study. The excess weight prevalence was 6% in women and 2% in men age 15-49 (CSA and ICF, 2012). In addition, the excessive weight prevalence surpassed undernutrition (22.1% vs 15.1%). Other studies also showed similar results (Morse and Kovacs, 2006; Crum-Cianflone *et al.,* 2008). This may be because AIDS has been linked to wasting syndrome (Macallan, 1999) that may lead to the change of feeding habit of HIV-infected individuals to a high-

calorie diet and avoidance of physical activity, which is known to cause increase in weight and lipid levels (Wilson *et al.,* 2002; Fontaine *et al.,* 2003).

Introduction of free ART service based on CD4+ cell count <200 cells/uL and/or WHO Stages III/IV (FHAPCO, 2007) left out HIV positive individuals included in the present study on the basis of their higher BMI and better median CD4+ cell count (357 cells/mm^3). This relative immunocompetence may have accounted for the relatively lower prevalence of undernutrition and the higher prevalence of excess weight (Crum-Cianflone *et al.,* 2008).

Older age resulted in increasing trend in excess weight and in a decreasing trend in undernutrition, and the trends in women study participants were maintained similar to Kroll *et al.* (2012). This may indicate that age and sex differences are important factors in determining nutritional status (Thielman *et al.,* 2018). The positive association of undernutrition and the negative association of excess weight with WHO clinical stages III/IV indicated that undernutrition appeared at the advanced stages of AIDS (Deyessa *et al.,* 2008). Similar to this, a study from Uganda had shown that HIV positive persons in WHO clinical stage IV were often characterized by severe wasting (Rawat *et al.,* 2010) that resulted in significant decrement of excess weight.

An increase in total cholesterol concentration, which is related to higher excess body weight, which also is a major risk factor for CVD morbidity and mortality in developing countries (Malaza *et al.,* 2012), was found to be an independent risk factor of excess

weight in this study. Similar to the findings of Daniyam and Iroezindu (2013), lipid abnormalities were also common in ART naïve HIV-infected patients even in the absence of host-related risk factors for dyslipidemia. In addition, this study confirmed that HIV disease and demographic characteristics influence lipid parameters in ART naïve patients.

Significantly low serum total cholesterol and non-statistically significant increase in triglyceride level was noted in patients with advanced HIV infection. Other studies in resource limited settings on ART naïve HIV-infected individuals showed that the prevalences of hypertriglyceridemia and TC hypercholesterolemia were 31% and 11.1%, respectively, in Burayu, a suburb of Addis Ababa (Abebe *et al.,* 2014); and serum hypertriglyceridemia and TC hypercholesterolemia occurred in 31.0% and 15.9%, respectively, comparable to the present study in Hawassa (Tadewos *et al.,* 2012). The serum TC hypercholesterolemia prevalence of 24.6% in ART naive HIV-infected individuals from Cameroon (Pefura *et al.,* 2011), which is higher than the prevalence in this study.

The significant TC difference in men and women found in the study may be due to biological and life style differences that affect the nutritional status (Thielman *et al.,* 2018). This may also affect the general lipid transport and metabolism since in HIV-infected individuals both on and off ART, the increased VLDL/LDL possesses a higher content of APOC3 that results in reduced lipoprotein lipase activity and less TG hydrolysis (Kersten, 2014). The increased VLDL/LDL results in increased activity of

CETP that exchanges cholesterylesters from HDL for TG (Subramanian and Chait, 2012). The stimulation of lipoprotein lipase and phospholipase A2 occurs and results in much smaller HDL with a reduced affinity for APOA1 that leads to renal excretion due to their small sizes (Baker *et al.,* 2010). This excretion leads to decreasing levels of HDL-C in circulation. In addition, the remnants of VLDL/LDL are rich in cholesterol esters and poorly recognized by both LRP and LDLR for their delayed clearance from the circulation. This may result increased CVD and in accelerated atherosclerosis risk in HIV-positive individuals (Packard, 2003; Williams and Chen, 2010).

The requirement for higher serum total cholesterol for efficient viral replication has been proven based on *in vitro* studies involving inhibition of depletion of cellular cholesterol content or cholesterol biosynthesis by stimulation of cholesterol efflux by ABCA1 that decreases virus entry and replication (Morrow *et al.,* 2010; Chukkapalli *et al.,* 2012; Petersen *et al.,* 2014). The role of cholesterol for increasing viral replication *in vitro* could be through employing lipid rafts rich in cholesterol to efficiently infect the cell (Brügger *et al.,* 2007; Morrow *et al.,* 2010). It has also been shown that HIV induces an increase of cholesterol in cells through Nef protein that induces genes involved in cholesterol biosynthesis and inhibits the activity of ABCA1 that impairs cholesterol efflux (Fitzgerald *et al.,* 2010; Mujawar *et al.,* 2006; van't *et al.,* 2005).

Evidence from *in vivo* studies that substantiate the findings of the present study showed that high serum total cholesterol lowers HIV RNA load by inhibition of HIV replication (Liu *et al.,* 2013; Cyster *et al.,* 2014). Besides, the effect of ART is found impaired in

hypocholesterolemic patients (Miguez *et al.,* 2010). The HIV replication inhibition in the presence of high serum total cholesterol may have been due to the production of oxysterols (Liu *et al.,* 2013; Cyster *et al.,* 2014). Viral infection induced IFNs up-regulate ISGs like cholesterol-25-hydroxylase and cause down-regulation of sterol biosynthesis to protect the cells (Blanc *et al.,* 2011). One of the oxysterols, 25-HC, inhibits viral entry by blocking membrane fusion (Holmes *et al.,* 2011; Liu *et al.,* 2013), feedback inhibition of sterol biosynthesis (Cyster *et al.,* 2014), and down-regulating sterol biosynthesis by inhibiting the enzymes involved (Brown and Goldstein, 2009).

HIV RNA load in this study was found to be higher in men than women. This may be explained by the report (Beagley and Gockel, 2003) that women had induced cell-mediated immune responses and produce higher antibody following either infection or vaccination. Based on studies on SIV infected female rhesus macaques, estrogen treatment protects against the transmission of SIV (Hel *et al.,* 2010; Brambilla *et al.,* 2010). Furthermore, in the hormone level fluctuation during menstrual cycle, higher immunity and high estrogen levels in the follicular phase may have protective effects against invading pathogens. This was shown by a study done with estrogen treatment that showed protection of female rhesus macaques against SIV transmission by thickening mucosal tissue (Hel *et al.,* 2010; Brambilla *et al.,* 2010). Similarly, HIV protective role for female hormone has been demonstrated in an *in vitro* whereby beta-estradiol inhibited replication of HIV-1 in human peripheral blood lymphocytes (Zhang *et al.,* 2008). The mechanism of hormonal inhibition was shown to be blocking of viral entry through higher expression of chemokines (Rodriguez-Garcia *et al.,* 2013). Also, significant sex

differences have also been reported in CD4 parameters and HIV RNA levels in HIV-infected children before the onset of puberty. This indicates that intrinsic genetic differences between female and male individuals, unrelated to sex steroid hormone levels, influence CD4 parameters and HIV RNA level in HIV-infected individuals (Ruel *et al.,* 2011). This may possibly be explained by the expression differences of important genes due to epigenetic differences of the two sexes (Ellegren and Parsch, 2007; Ober *et al.,* 2008).

The association of higher cholesterol levels with lower HIV RNA load, found in the present study, is supported by earlier reports that individuals with hypercholesterolemia have higher immune activation as evidenced by more circulating lymphocytes, phagocytic activity, CD8+ T cells, total T cells, immunoglobulin production, and migration of lymphocytes than from persons with lower cholesterol levels (Hannedouche *et al.,* 2011; Liu *et al.,* 2011; Muldoon *et al.,* 1997). This could be explained by the role of downstream oxysterol metabolites and intermediates in the cholesterol-biosynthetic pathway that have been found to influence immune system (Liu *et al.,* 2013; Cyster *et al.,* 2014).

In this study, predicting disease progression with high sensitivity (>80.0%) but low specificity were observed by low serum total cholesterol in combination with anemia. The combination predictor marker for RNA load and cholesterol was not done because the aim was to find a marker which could be available in poor set up. But, RNA load determination is still high in its cost. The effect of ART was found to be impaired in

hypocholesterolemic HIV-infected patients (Miguez *et al.,* 2010), this may signify the potential of serum total cholesterol use as ART efficacy predictive marker. The low sensitivities and specificities, the weak correlations, and the high fluctuation in PPVs and NPVs of this study may partly be explained by high background prevalence of other non-HIV-related causes. For instance, anemia cannot distinguish between early and advanced HIV disease progression under situation of high prevalence of infections and undernutrition (CSA and ICF, 2012; Brown *et al.,* 2004). In addition, majority of the study participants were enrolled for ART care and eligible ones immediately go to ART treatment when they are at WHO clinical stages III/IV and/or have CD4+ T cell count <200 cells/mm^3 according to the Ethiopian ART Giudeline used during the study period (FHAPCO, 2007).

Although subtype A1 and the CRFs, C/A1 and C/A1/D, were detected in the study, the clustering of all the HIV-1 recombinants with subtype C can be explained by the fact that the major sequences in the recombinants were that of genome subtype C. This finding is in agreement with other studies that showed the dominant HIV-1 subtype C variant circulating in Ethiopia (Abebe *et al.,* 2000; Kassu *et al.,* 2007; Mulu *et al.,* 2013). But, sporadic infections with A and D subtypes have also been reported (Abebe *et al*., 1997; Hussein *et al.,* 2000; Adal *et al.,* 2005). The detected CRFs are an indication of recombinations of the predominant subtypes A, C and D circulating in East African. The presence of such recombinants in this study is an indication of the possibility of influx of A and D subtypes from the neighboring countries (Louwagie *et al*., 1995; Janssens *et al*., 1997; Poss *et al*., 1997; Hu *et al*., 2000).

The dominant use of CCR5 coreceptor found in the present study is in agreement with other studies that showed subtype C to be associated with it, compared to other subtypes (Abebe *et al.*, 1999; Kalu *et al*., 2017a and b). This was further demonstrated that HIV-1 subtype C frequently uses CCR5 for cell entry even in advanced immunodeficiency (Bjorndal *et al.*, 1999; Cilliers *et al.*, 2003; Neogi *et al.*, 2010). NSI isolates preferential transmissibility compared with more pathogenic SI isolates may explain this finding. However, it is also possible that the primary immune response of an individual might be less efficient in eliminating NSI viruses than in eliminating SI viruses (Chesebro *et al*., 1992). An alternative explanation may be the differential expression of SDF-1 by competing with CXCR4, and MIP-1α, RANTES and MIP-1β that compete with HIV for access to cell surface CCR5 (Chowdhury *et al*., 1995; Ferbas *et al*., 2000). The difference detected in results of the coreceptor usage of subtype C in the bioinformatics tools used in this study is most likely due to the use of different statistical models to handle deletions, insertions and ambiguous positions (Ceresola *et al.*, 2015).

The R5 coreceptor usage determined in the present study by the HIV-1 subtype C viruses is associated with viral tropism. This indicates that individuals with lower CD4+ T cell counts and higher HIV RNA load would carry X4 using viruses, unlike R5 using viruses. The predicted phenotypes generated by bioinformatics tools based on signature amino acids and the net positive charge confirms the low prevalence of CXCR4 usage similar to previous studies on HIV-1 subtype C (Abebe *et al.*, 1999; Kalu *et al*., 2017a; Kalu *et al*., 2017b).

The positively charged signature amino acids at positions 11, 24 and 25 (Chesebro *et al.,* 1992); a net positive charge ≥5.0 (Adal *et al.,* 2005); and the V3 loop N-linked glycosylation site (Pollakis *et al.,* 2001), are predictive markers for T cell tropism of the isolated viruses. Consistent with the report of Chesebro *et al.* (1992), the less presence of positively charged amino acids at positions 11, 24 and 25 and the <5 net charge of the V3 loop indicated that majority of viruses detected in all study subjects were NSI viruses. This finding may have clinical relevance based on the circumstance that the CCR5-receptor antagonist maraviroc is decided to be used in Ethiopia.

The amino acid changes in the charged V3 loop that determines glycosylation differences and cellular tropism that result in escaping from its recognition by neutralizing antibodies in the V3 loop can be the result of different immune pressures or differences in coreceptor usage (Overbaugh *et al.,* 1999; Pollakis *et al.,* 2001). This may affect the transmission of the virus and disease progression in the presence of neutralizing antibodies (Moore *et al.,* 2004; Nabatov *et al.,* 2004).

The branch length differences in the phylogenetic tree among sequences indicate the high genetic diversity within the subtype C. These differences could also be responsible for viral escape to immunity. This is also responsible to challenges in the development of efficacious vaccine(s).

6. Limitations of the study

The limitations of the systematic review were the bad data quality in most sources used in the study, and limitations of the secondary information due to difference in study design and possible personal errors.

The results may overestimate excess weight and underestimate the burden of undernutrition because patients with more severe disease presentations, such as those with immediate intensive care requirement and cognitive impairment were not included. In addition, it was not possible to determine the causal relationship between risk and outcome variables since the study design was cross-sectional.

Screening for opportunistic infections was not done. Therefore, the role of opportunistic infections such as hepatitis B/C in determining the lipid abnormalities and nutritional status cannot be ascertained. Evidence for family history of dyslipidemia, depression, changes in mood, factors related to lifestyle (physical inactivity and smoking) and drug use that could interfere with serum lipid levels were not accounted for.

7. Conclusions

There is a relative decline and stabilization of HIV prevalence with time in Addis Ababa. However, it varies along different groups of the population within socio-demographic divide. There are many behavioural, biological and socio-economic factors identified that predisposed MARPs and the general population to HIV/AIDS.

Undernutrition was prevalent in HIV-infected ART naïve individuals and an overall increase in overweight, obesity, TC hypercholesterolemia and hypertriglyceridemia were found. The female sex and high serum total cholesterol were associated with higher CD4+ T cell count, lower HIV RNA load and WHO clinical stages.

Among the alternative markers tested, low serum total cholesterol alone and its combination with anemia could be used in predicting elevated HIV RNA load, low CD4+ T cell count and WHO clinical stages.

Although HIV/AIDS epidemic in Addis Ababa is dominated by HIV-1 subtype C R5-tropic viruses, HIV-1 subtype A1, and the unique recombinant forms C/A1 and C/A1/D were also identified. Hence, studies on HIV genetic variation, subtypes and CRFs, and phenotypes of viruses will be required for better understanding of HIV/AIDS epidemiology in Addis Ababa.

Detection of the dominance of R5-tropic viruses is a significant if the use of the co-receptor antagonist, maraviroc, would be considered to treat R5 HIV viruses in Ethiopia.

8. Recommendations

Although there is a decreasing trend, the relatively high HIV prevalence in Addis Ababa indicates that the Ministry of Health and other concerned bodies should work more on awareness creation, targeted and voluntary testing to prevent new HIV infections. In addition, the more than 14% WHO clinical stage III/IV and 17.2% CD4+ count <200 patients detected among the study participants, categorized as ART naïve, showed the need to revise the patient care policy on ART-naïves to increase tsting by reducing fear of discrimination and stigmatization.

HIV-infected patients should be screened for lipid disorders before commencement of ART. In addition, dyslipidemic individuals should be treated at the time of initiating ART to increase survival and to monitor possible rising trends. The study findings also indicated the need for nutritional therapy in malnourished HIV-infected individuals.

Further studies must be conducted to establish the causal relationship between higher CD4+ T cell count and reduction in HIV RNA load; and female sex and higher serum total cholesterol. This is because documentation of the relationship of sex and serum total cholesterol with HIV replication and immunity may provide a basis for therapeutic strategies to control HIV replication.

More work is needed to standardize measurement of alternative markers for use in HIV-positive individuals, as additional indicators of disease progression and to make decision on ART initiation. Besides, continuous surveillance studies to identify the HIV-1 subtypes, CRFs and phenotypes must be conducted to use the information as input for determining drug effectiveness.

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Annexes

Annex 1. Study participant demographic, socioeconomic, clinical and behavioural data collection questionnaire.

Directions for filling up the enclosed questionnaire:

- **Please mark "x" on the appropriate choice.**
- **Give the details as required in the space provided.**

Sec.	Questionnaire parameters	Coding categories
Section A: Identification		
A01.	**Hospital Card No.: _____**	
A02.	**Subject ID: _____**	
A03.	**Telephone address of the study subject: _____**	
A04.	**Date of interview**	**_____/_____/_____ E.C.** **dd mm yyyy**
A05.	**Study site/facility name and address**	**Facility Name _____**
A05.	**Visit type**	**Baseline 1** **6 months 2** **0thers_____**
Section B: study participant profile		
B01.	**Sex of the participant**	**Male 1** **Female 2**
B02.	**What is your birthday (age)?**	**date: _____/_____/ _____ E.C.** **dd mm yyyy** **age: _____**
B03.	**What is your place of residence?**	**Urban 1** **Rural 2** **Name of district _____**
B04.	**What is your level of education?**	**No education 0** **Primary 1** **Secondary 2** **Tertiary 3**
C05.	**What is your occupation?**	**Unemployed 0** **Casual labourer 1** **Peasant farmer 2** **Government employee 3** **Private employee 4** **Self employed 5** **other specify _____ 6**
B06.	**What is your marital status?**	**Never married 0** **Married 1** **Separated 2** **Divorced 3** **widow/widower 4** **Living with partner 5**
B07	**What is your ethnicity?**	**Amhara 1** **Oromo 2** **Tigraway 3** **Guragie 4** **Specify other (Affar, Gamo, Hadyia, Kembata, Nuwer, Anyiwak, Sidamo, Siltie, Welaita) _____ 5**
B08.	**What is your status of work?**	**Working full time 1** **Working part-time 2** **not working due to ill health/studying 3** **Others (specify): _____ 4**
B09.	**Number of individuals in the household?_____**	
B10.	**Estimated total monthly income of household? _____Ethiopian Birr**	
Section C: Individual's behaviour		

C01.	**Do you do physical exercise?**	**Never exercising 1** **Currently exercising 2** **Exercise in past but stopped ___yrs and ___months ago 3**
C02.	**Do you smoke cigarette?**	**Never smoked 1** **Currently smoking 2** **Smoked in past but stopped ___yrs and ___months ago 3**
C03.	**If currently/ smoked in past, how many cigarettes/pipes per day do/did you usually smoke (1 pack equals 20 cigarettes)?** _____	
C04.	**If you have smoking history, how long have/had you smoked? _____ years**	
C05.	**Do you drink alcohol?**	**Yes 1** **No 2** **Stopped now ≥ 6 months 3**
C06.	**How often do you drink?**	**Daily 1** **4-6 days per week 2** **2-3 days per week 3** **≤ 1 day in a week 4**
C07.	**Do you use soft drugs (e.g., khat, shisha, etc)?**	**Yes 1** **No 2** **Stopped now ≥ 6 months 3**

Section D: HIV clinical symptoms and test history (only filled by the clinician)			
D01.	**When did you think you are infected with the virus?**	**date: _____/_____/ _____** **dd mm yyyy**	
D02.	**When did you know your HIV status?**	**date: _____/_____/ _____** **dd mm yyyy** **Name of health facility: _____**	
D03.	**Clinical symptom screen (mark "x" on all that apply)**		
	o **Chronic cough**	o **Chronic diarrhea**	o **mental confusion**
	o **Weight loss**	o **Nausea/vomiting**	o **Stomatitis, dysphagia and/or odynophagia**
	o **Flu-like upper respiratory tract infection**	o **Rash**	o **General fatigue**
		o **Swelling lymph nodes**	o **STI symptoms**
D04.	**OPPORTUNISTIC ILLNESS (MARK "X" ON ALL THAT APPLY)**		
	E01. WHO STAGE 1 CONDITIONS	**E04. WHO STAGE 4 CONDITIONS**	
	o **Persistent generalized lymphadenopathy (PGL)**	o **Extrapulmonary tuberculosis**	
	E02. WHO STAGE 2 CONDITIONS	o **Atypical mycobacteriosis**	
	o **Minor mucocutaneous manifestations**	o **Cryptococcosis extrapulmonary**	
	o **Weight loss <10% of body weight**	o **Herpes simplex (mucocutaneous >1 month, visceral)**	
	o **Herpes zoster (shingles)**	o **HIV-related encephalopathy**	
	o **Recurrent urtis**	o **Lymphoma**	
	E03. WHO STAGE 3 CONDITIONS	o **Mycosis, disseminated (histoplasma, coccidioides)**	
	o **Oral candidiasis**	o **Salmonella septicaemia, non-typhoid**	
	o **Oral hairy leucoplakia**	o **HIV wasting syndrome**	
	o **Unexplained chronic diarrhea (>1 month)**	o **Candidiasis (esophagus, trachea, bronchi or lungs)**	
	o **Unexplained prolonged fever (>1 month)**	o **Cryptosporidiosis with diarrhea (>1 month duration)**	
	o **Weight loss >10% of body weight**	o **Cytomegalovirus (CMV) disease (other than liver, spleen, lymphnodes)**	
	o **Recurrent bacterial pneumonia**	o **Kaposi's sarcoma**	
	o **Other severe bacterial infections (*i.e.*, pyomyositis)**	o **Progressive multifocal leukoencephalopathy (PML)**	
	o **Pulmonary tuberculosis**	o **Toxoplasmosis of the brain**	
D05.	**WHO staging based on opportunistic illness**	**WHO stage 1 1** **WHO stage 2 2** **WHO stage 3 3** **WHO stage 4 4**	

D06-1.	**Did you take any prescription drug for opportunistic infections?**	**Yes 1** **No 2**
D06-2.	**If yes for D06-1, list any medications, nutrition supplements or vitamins that you are currently taking.** _____	
Section E. Anthropometric and other measurements		
E01.	**Height**	**_____ (cm)**
E02.	**Weight**	**_____ (kg)**
E03.	**Blood pressure**	**_____ (S/D mmHg)**
E04.	**Heart rate**	**_____ (B/M)**
E05.	**Respiratory rate**	**_____ (R/M)**
E06.	**Haemoglobin level**	**_____ (g/dL)**
E07.	**CD4 count**	**_____(cells/mm^3)**
E08.	**Glucose level**	**_____ (mg/dL)**
E09.	**Albumin**	**_____ (mmol/L)**
E10.	**Total cholesterol**	**_____ (mg/dL)**
E11.	**LDL cholesterol**	**_____ (mg/dL)**
E12.	**HDL cholesterol**	**_____ (mg/dL)**
Filled by: Date : _____/_____/ _____ Interviewer's Name: _____ Interviewer's final assessment: _____- _____ Interviewer's Signature: _____		Checked by: Date : _____/_____/ _____ Supervisor's Name: _____ Supervisor's final assessment_____ Supervisor's Signature:_____

Annex 2. Ethical clearance

በኢትዮጵያ ፌዴራላዊ ዲሞክራሲያዊ ሪፐብሊክ
የሳይንስና ቴክኖሎጂ ሚኒስቴር
The Federal Democratic Republic of Ethiopia
Ministry of Science and Technology

MoST
የሳይንስና ቴክኖሎጂ ሚኒስቴር

ቁጥር Ref.No. 3-10/287/2017
ቀን Date Dec 13, 2017

To: Armsuer Hansen Research Institute (AHRI)
Addis Ababa

Subject: Acceptance Letter of Renewal Request

Dear Sir/Madam /Mr./Mrs./Dr.

We are writing this letter in reference to your renewal request letter Ref No: AH/843/06/17 dated December 5, 2017.

After having in depth review of your request on "**Role of HLA Polymorphism in Driving HIV Variation and Influencing Disease Progression in HIV-infected Individuals in four Hospitals in Addis Ababa, Ethiopia**." the National Research Ethics Review Committee has accepted your renewal request for one year from (**December 13, 2017 – December 12, 2018.)**

This is, therefore, to notify that the ethical approval is renewed and your group can proceed in accordance to the latest approved document. Please ensure that you submit a biannual report and an annual renewal application 30 days prior to the expiry date. We are confident that you as PI of the project and your esteemed organization will monitor the ethical implication of the project as it is stipulated in the latest approved document.

With regards,

Awol Hussein Mohammed
Research Ethics Directorate
Acting Director

CC

- HE the Minister
- STI, Capacity Building/P/R/FP Director General
- Chairperson, NRERC
- Melaku Adal (PI)

Annex 3. Material transfer agreement

Ministry of Science and Technology, Federal Democratic Republic of Ethiopia
National Research Ethics Review Committee

Address: Tel. +251011-4-674353
P.O. Box 2490 Fax +251-011-4-660241
E-mail:nrerc2015@gmail.com
Addis Ababa –Ethiopia

Material Transfer Agreement

This Material Transfer Agreement (MTA) has been prepared for use by Armauer Hansen Research Institute (AHRI), Ethiopia and University College of London (UCL), Department of Microbiology and Virology, UK in all transfer of research material (samples, derivatives, and specimens) related to the protocol.

Provider: Armauer Hansen Research Institute (AHRI), Ethiopia

Recipient: University College of London (UCL), Department of Microbiology and Virology, UK

1. Provider agrees to transfer to recipient's designated (Dr. Kate El Bouzidi) the following research materials /specimen.

594 vials of blood plasma, and another 594 vials of peripheral blood mononuclear cells, collected from drug naïve HIV positive individuals.

The research material will only be used for research purposes as described in the protocol by recipient's investigator in designated laboratory for the research project described below, under suitable containment conditions. This research material will not be used for commercial purposes such as screening, production or sale for which a commercialization license may be required. Recipient agrees to comply with all National and International guidelines rules and regulations applicable to the Research Project and the handling of the Research Material.

a) Are the research materials of human origin?

Yes x No ☐

b) If yes, are they collected according to the details in the protocol and in adherence to National Research Ethics Review Committee (NRERC) and AHRI Ethics Review Committee recommendations and their approval?

Yes x No ☐

2. This research material and its derivatives will be used by recipient's investigator solely in connection with the following research project **("Role of HLA polymorphism in driving HIV variation and influencing disease progression in HIV-infected Ethiopians"**) described with specificity as HIV-HLA project with the following hypothesis and objectives:

Hypothesis: The HLA type I diversity in Ethiopian populations is distinct from other populations, which results in unique CTL escape HIV-1 subtype C mutants.

Objectives: We intend to conduct this study on samples collected from drug naive HIV-infected cohort to address the aims to:

- Describe the diversity of HLA class I sequences
- Sequence full-length HIV genome to determine autologous variants
- Integrate human and viral genetics with clinical and immunological data to determine the role of HLA class I polymorphism in driving HIV variation and disease progression

3. In all presentations or written publications concerning the research project, recipient will seek agreement of provider and acknowledge provider's contribution of this research material unless requested otherwise.

4. This research material represents a significant contribution on the part of provider and is considered proprietary to provider. Recipient therefore agrees to retain control over this research Material and further agrees not to transfer the research material to other people not under her/his direct supervision without advance written approval of provider. The research material will be disposed of as agreed upon per protocol at the end of completion of the project on January 30, 2020.

5. The provider does not take any responsibility for loss, damage, wastage or spoilage of the research material during or after shipment to the address provided by the recipient under conditions agreed to in the protocol on shipment of the samples. This research material is provided as a service to the research community. IT IS BEING SUPPLIED TO RECIPIENT WITH NO WARRANTIES, EXPRESS OR IMPLIED, INCLUDING ANY WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. Provider makes no representations that the use of the research material will not infringe any patent or proprietary right of third parties.

6. The recipient shall notify the provider in witting of any intention, improvement, modification discovery or development to the material or the information made by recipient or parties, collaborating with recipient, here in after referred to as "invention". Nothing in this agreement shall, however, be construed as conveying to the provider any rights under any patents or other intellectual property to such invention, other than as explicitly provided herein. At its option the provider shall be entitled to receive sample of any materials derived from the Materials for its own research and evaluation purposes only.

7. The under- signed provider and recipient expressly certify and affirm that the contents of any statements made here in are truthful and accurate.

8. Any additional terms (use an attached page if necessary):

9. The provider maintains, ownership right of the research material and its derivatives unless stated otherwise.

The provider will retain a copy (aliquot) of every sample sent abroad as much as possible for local research needs.

Note: the title of the thesis is modified to fit the data we have as "Characterization of epidemiological, nutritional, immunological and virological parameters, and HIV-1 genetic diversity in Addis Ababa, Ethiopia".

For Recipient
Recipient's Investigator
Dr. Kate El Bouzidi

Signature

Date: 26/11/17

Mailing Address for Material:
Department of Clinical Virology,
60 Whitfield Street, London, W1T 4EU

Email: k.elbouzidi@ucl.ac.uk

Duly Authorized
Dr Eleni Nastouli

Signature/Stamp

Date: 27/11/17

Mailing Address for Notices:
University College London, Department of Clinical Microbiology and Virology, 5th Floor Central, 250 Euston Road, London NW1 2PG

Tel: 020 3447 8987
Email: eleni.nastouli@nhs.net

For Provider

Provider's Investigator
Melaku Adal Eshetie
PhD Candidate

Signature

Date 05/12/2017

Mailing Address :
Armauer Hansen Research Institute (AHRI)
ALERT Center, Zenebework
Jimma Road
P.O.Box: 1005
Addis Ababa, Ethiopia
Tel: +251(0)983293246
Fax: +251(0)113211563
Email: melaku_adal@yahoo.com

Duly Authorized
Dr. Taye Tolera Balcha
Director General of AHRI

Signature/Stamp

Sponsored By the Governments of Ethiopia Norway & Sweden

Armauer Hansen Research Institute

Date ---Taye Tolera Balcha (Dr.) Director General Armauer Hansen Research Institute

Mailing Address for Notices:
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Fax: +251(0)113211563
Email: tayetolera@yahoo.com

Annex 4. Information for the participant

Dear participant:-

Background of the study:- Armauer Hansen Research Institute (AHRI/ALERT), Oxford University, and the School of Graduate Studies, Addis Ababa University (AAU) would like to carry out a collaborative study on HIV and HLA entitled "**Role of HLA polymorphism in driving HIV variation and influencing disease progression in HIV-infected Ethiopians.**" HLA molecules are involved in body's defense against pathogens like HIV. Some HLA molecules work better in HIV defense than others and some are known involved in viral control. Here a better understanding of HLA molecules may help to determine why some people progress more slowly to HIV/AIDS. Most studies of the interaction between the human immune response to HIV and changes in viral sequence selected to envade that immune response have been undertaken in B subtype infected Caucasians even if subtype C is the dominant in the world. This is one factor that hinders development of safe and effective multi-epitope vaccine. Since Ethiopia has one of the largest populations of HIV infected people in the world, studying the HLA type I polymorphism and HIV-1 evolution is an important input for HIV control and prevention.

Objective of the study:- The aims of the study is to assess associations of HLA class I alleles with viral load in chronically infected HAART naïve study subject whose HIV status is being followed by antiretroviral therapy care centres. HIV sequences also will be determined to correlate sequence variation with HLA allelic expression as evidence of ongoing immune selective pressure. In order to perform this, we will use different high-tech virological, immunological and molecular techniques. We hereby kindly request your participation in this study and help us to find the associations between HLA polymorphism and HIV diversity.

Methodology and confidentiality:- The study participants will be enrolled after informed consent based on the inclusion and exclusion criteria set. If you are volunteer to participate in this study and agree with the written informed consent, 20 mL of the venous blood in addition to routine blood work which would be done as part of the usual care at this clinic will be collected for different laboratory analysis. Furthermore, you may be asked to return to this care centre and provide a 20 mL to obtain a follow test to measure the levels of HIV in the blood and to test the CTL response to selected epitopes. Some of the laboratory procedures in this study may not able to be performed within this country. Therefore, blood samples would be shipped to laboratories outside Ethiopia. A portion of the sample may be stored and used for a study which is separate from this one in the future, provided those studies receive approval from the National Ethical Review Committee. HIV testing will be done with pre- and post-test counseling and you will be tested for TB using sputum smear if necessary. Pregnancy test will also be performed for female volunteer study subjects. Any personal information about you will be kept private except the knowledge of the physician and the investigator. If you are volunteer to participate in this study you will be asked to come to the health center following the doctor's instructions.

Risks associated to study:- You may feel some minor pain and discomforts, while blood is drawn from your vein. But this pain will disappear after a few hours. In all the steps, an experienced health professional will carry out the procedure with standard aseptic and sterile conditions. For any inconveniences related to these procedures, you will be provided appropriate medical care.

Benefits to the study participants:- Study participants enrolled in this study will have benefits. If the female study participant was found to be pregnant, guidance and counseling will be given before excluded from the study. The participant's health status will be followed during each visit. The viral load determination will be done for each patient so that you will get additional information to be treated properly

by your doctor. In case of TB drugs and ART need, you will get the standard care available and treated with proper guideline and counseling for treating patients.

Outcomes of the study:- Detection of the different HIV-1 escape variants associated with the specific HLA type I alleles and protective CD8 response *ex-vivo* in targeted dominant and subdominant CTL epitopes of infected populations may help to learn the mechanisms by which immune response against HIV-1 can be most effective. It can also help in the design, formulation and evaluation of effectiveness of multi-epitope candidate HIV vaccine that is most urgently needed.

Rights to refuse or withdraw:- You have full rights to refuse or withdraw from taking part in the study. Even if you do not want to take part in this study, you will still be able to be treated at this centre according to the usual standard of care and will not lose any benefits.

Do you understand what has been said to you? If you have any question you have the right to get proper explanation.

Contact person:- In case of any question regarding the study or related issues, you can contact the following individuals;

1. Melaku Adal; Tel. +251-0913-696751; P.O.Box 1005, AHRI/ALERT, Addis Ababa, Ethiopia; or melakuadal@yahoo.com
2. Dr. Abraham Aseffa; Tel. +251-0911-247525; P.O.Box 1005, AHRI/ALERT, Addis Ababa, Ethiopia; or aseffaa@gmail.com
3. Prof. Beyene Petros; Tel. +251-011-1239471; P.O.Box 1176, AAU, Addis Ababa, Ethiopia; or info@bio.aau.edu.et

Thank you for your kind cooperation and participation in this study.

Annex 5. Informed consent form for adults ≥ 18

Identification No: _____

AHRI/ALERT No: _____

Date: _____

I have been well informed that AHRI/ALERT, Oxford University, and the School of Graduate Studies, AAU would like to carry out a collaborative study on HIV and HLA entitled "**Role of HLA polymorphism in driving HIV variation and influencing disease progression in HIV-infected Ethiopians.**" In addition to routine blood work which would be done as part of my usual care at this clinic, I am aware that the study requires about 20 ml of the venous blood for laboratory investigation and asked if I volunteer to give these specimens. Furthermore, I understand that I may be asked to return to this care centre and provide 20 mL blood to obtain a follow test to measure the HIV viral load in blood and to test the CTL response to selected epitopes.

Please read, tick off each of the boxes and sign the form if you agree to take part in this study.

1.	I understand what this study is about and know how to contact the investigators if I want to.	☐
2.	A portion of the sample may be stored and used for the study.	☐
3.	I understand that portion of the stored sample may be used for other related future studies after approval by National Ethical Review Committee.	☐
4.	A portion of the sample stored would be shipped abroad for further analyses.	☐
5.	I understand that all the information given to the investigators and all test results will be kept private and confidential.	☐
6.	I understand that I will not get benefit financially from this study.	☐
7.	I understand that I am free to withdraw myself from this study if I want to.	☐
8.	I understand that if I refuse to take part in this study, that my care will not be affected.	☐

I have been given enough time to think over before I signed this informed consent. It is, therefore, with full understanding of the situation that I gave my informed consent to participate in this study.
The information was explained to me by: _____
Name of participant: _____ Signature:- _____
Name of the physician: _____ Signature:- _____

Annex 6. Summary information about the source materials used.

Documents	Type of document	Key findings	Objectives
EPHI, 2018a	Report	• HIV prevalence Table 3 and Figure 7	1
CSA and ICF, 2016	Survey	• HIV prevalence 3.4% in Addis Ababa • Men who had sex with non-cohabiting partners is highest in Addis Ababa (26%) than the national average (16%) • The mean number of lifetime sexual partners reported by men in Addis Ababa (5.2%) • Women reported using a condom during last sexual intercourse with non-regular partners 41.8% and men 72.4% • Discordant couples (4.3%)	1, 3
EPHI, 2018b	Survey	• HIV prevalence is 3.1% in Addis Ababa • VLS of whole country in urban areas is 70.1% (Female 71.7% and Male 66.8%), varies by age, sex, and region, • Status of the three 90's in Addis Ababa: 65.2 % for the 1st 90, 63.3 % for the 2nd 90 and 58.2% of all PLHIV	1, 5
CSA and ORC, 2005	Survey	• HIV prevalence is 4.7% in Addis Ababa	1, 3
CSA and ICF, 2011	Survey	• HIV prevalence is 5.2% in Addis Ababa	1, 3
EPHI, 2015	Report	• Figure 7 for HIV prevalence	1
EPHI, 2011	Report	• Figure 7 for HIV prevalence	1
EPHI, 2014	Report	• Figure 7 for HIV prevalence	1
EPHI, 2017	Report	• Figure 7 for HIV prevalence	1
FHAPCO, 2018	Report	• Behavioural, biomedical and structural interventions • ART coverage is 74.6%; viral load testing coverage ~60% with 87.5% VLS • In Addis Ababa, the total number on ART were 94,240 and 3,616 were newly enrolled; retention at 12 months 87% • Figures 8, 9, 10	1, 5
EPHA/CDC (2012)	Report	• Death related to HIV/AIDS in Figure 11	1
AAHAPCO, 2017	Synthesis	• Key drivers of the epidemic; hotspot areas; intervention strategies; challenges on intervention	2, 3, 4, 5
Lakew *et.al.*, 2015	Article	• 5.7% HIV-positives among mobile workers	1, 4

FMOE, 2012	Survey	• low level of knowledge, peer pressure, practices of unsafe sex, the proliferation of addictions (shisha, khat, alcohol) and substance abuse, gender–based violence were driving forces for the spread of the epidemic.	3, 4, 5
Deyessa *et al.*, 2018	Survey	• Male users dominated female users at a ratio of 9:1; 3/4 of the IDUs were below the age of 35 years • The estimated IDUs in Addis Ababa were 4,068 • The majority, 200 (72.5%) of the drug users from Addis Ababa had the habit of reusing needle and syringe • Of the 177 Addis Ababa residents who claimed to have tested for HIV, 70 (39.5%) disclosed as HIV positive	1, 3 ,4
Cherie *et al.*, 2012	Article	• Peer pressure is the most important factor associated with risky sexual behavior among school adolescents	3
Mirkuzie (2018)	Article	• 2% and 4% of the HIV exposed babies were HIV positive by 6 and 18 months, respectively • No prophylactic ART and mixed feeding were predictors for having an HIV positive antibody test at 18 months	5
Klaus *et al.*, 2015	Article	• The barriers to PMTCT completion: hopelessness and carelessness, lack of understanding of the efficacy of ARV, and negative religious influences.	3
Endalamaw *et al.*, 2018	Article	• Rural residence, home delivery, no ART prophylaxis and mixed feeding increased the risk of HIV transmission	3
Menna *et al.*, 2014	Article	• High knowledge of HIV/AIDS, attitude towards 'ABC' rules, being tested for HIV and chewing khat are factors associated with multiple sexual partnerships among secondary school students.	3
EPHA *et al.*, 2013	Report	• The estimated HIV prevalence among FSWs in towns was 23.0%,; 4.5% in truck drivers • ~15.5% of drivers have misconceptions about HIV prevention methods • 21 % of drivers accept that once they had unprotected sex with someone, there is no reason to use condoms • Divorced/Separated/Widowed have also high HIV prevalence	1, 3, 4
UNODC, 2014	Survey	• HIV prevalence 4.2% in prison settings	1, 4
PEPFAR, 2018	Strategic Plan	• There are about 200,000 FSWs in Ethiopia	1, 4
PSI/E, 2016	Research brief	• The majority of FSWs (57.5 %) are 24 years and younger, and about 14% are 19 years or younger • > 6% of HIV positive FSWs who started ART reported discontinuation of treatment for more than seven days in the three months prior to the assessment	1, 4, 5
Demissie *et al.*, 2018	Article	• The prevalence of HIV among IDUs was 6%	1, 3, 4

		• 40% of IDUs reported ever sharing needles; 56% reported sharing other injecting equipment; among HIV-positive IDUs, 60% reported sharing a needle the last time they injected. • Most of the IDUs were males (96%) with a mean age of 26 years.	
FHAPCO, 2018	National roadmap	• Key and priority populations	4
FMoH, 2018	Report	• Behavioural, biomedical and structural interventions	5
Biadgilign *et al.*, 2011	Article	• Parents refusing to give consent for their children to access HTS and ART services	5
Gesesew *et al.*, 2016	Article	• Males being away from home, drug addiction, fear of stigma & discrimination, distance from ART clinics, dependent on food supplies, mental problems, HIV negative partners; and baseline CD4 <200 cells/mm3 and WHO clinical stages 3 & 4 were factors of ART discontinuation.	5
Gesesew *et al.*, 2017a	Article	• Being rural dweller, illiteracy, marriage, alcohol use, smoking, having mental illness and being bed ridden functional status, having HIV positive partner and being co-infected with TB/HIV were factors for ART discontinuation.	5
Gesesew *et al.*, 2017b	Article	• ART discontinued adults were more likely to be females, tuberculosis/HIV co-infected, with immunological failure and no history of HIV testing.	5
Bezabhe *et al.*, 2014	Article	• Economic constraints, perceived stigma & discrimination, medication side effects, and dissatisfaction with healthcare services, disclosure of HIV status, social support, responsibility for raising children, improved health on ART, and receiving education and counseling were factors for patients being non-adherent and lost to follow-up	5
Tiruneh and Wilson, 2016	Article	• With the introduction of appointment spacing, some patients complain of lack of storage space for the six-month supply of ARTs, poor storage conditions for their medicines, and preference of frequent follow up. Health workers are also concerned about adherence given the less frequent contact of PLHIV with the health services	5
PEPFAR, 2016	Operation plan	• Key and priority populations	4
FHAPCO, 2014	Strategic plan	• HIV transmission interventions include behavioural, biomedical and structural components. • The plan intends to achieve the three 90 targets by 2020 through targeted social mobilization and HIV testing, linkage to care, quality of HIV treatment, and virtual elimination of MTCT, envisioning ending AIDS by 2030	5

Gudina *et al.*, 2017	Article	• Combination ART acheives sustained HIV viral suppression and contributes to improvement in the quality of life; and reductions in mortality, progression to AIDS, opportunistic infections (OIs), hospitalization, and decreased HIV transmission to uninfected persons	5
Misgena, 2011	Article	• Challenges related to HAART include lifelong therapy, failed treatment response, optimal time to start treatment and switching regimens, drug interaction, toxicity, cardiovascular risks, drug resistance, lost to follow-up, immune reconstitution inflammatory syndrome (IRIS), early mortality, challenges in viral load testing.	5
Bernabas *et al.*, 2017	Article	• Noncompliance to medical instruction and poor adherence fosters emergence of drug resistance. Limited availability of laboratory services such as HIV RNA load and drug resistance testing and monitoring due to lack of experience of health professionals, and weak infrastructure and health care system contribute to delay in diagnosis of treatment failure	5
Telele *et al.*, 2018	Article	• The high rate of transmitted and preexisting drug resistance mutations in Ethiopian patients are identified	5

Note: Objective representation of the agreed thematic areas, 1 = Determine the prevalence and incidence of HIV and mortality rate in the City; 2 = Identify the hot spot areas in the City; 3 = Establish factors involved in driving the epidemic in the city, through analysis of behavioural, biological, socio-economic and demographic data; 4 = Identify most-at-risk and priority population groups in the City Administration (sex workers, in-school youth, prisoners/inmates, discordant couples and IDUs); 5 = Quickly assess service availability, access and utilization for the identified most at risk/priority populations groups in the City Administration