INTRONIC POLYMORPHISM IN *ALB* GENE AMONG HIV-1 INFECTEDANTIRETROVIRAL TREATMENT ART-NAIVE AND ART-EXPERIENCED INJECTION SUBSTANCE USERS IN MOMBASA COUNTY, KENYA

ERICK JUMA BARASA

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August, 2020

DECLARATION

This thesis is my original work prepared with no other than the indicated sources and support and has not been presented elsewhere for a degree or any other award.

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Erick JumaBarasa

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CERTIFICATION

The undersigned certify that they have read and hereby recommended for acceptance of MasindeMuliro University of Science and Technology a thesis entitled "**Intronic polymorphism in *ALB* gene among HIV-1 infected antiretroviral treatment ART-naive and ART-experienced injection substance users in Mombasa County, Kenya**".

Sign……..Date…….

Tom Were, PhD
Department of Medical Laboratory Sciences
MasindeMuliro University of Science and Technology

Sign……...Date……

Nathan Shaviya, PhD
Department of Medical Laboratory Sciences
MasindeMuliro University of Science and Technology

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DEDICATION

I dedicate this thesis to the family of Mr David BarasaWanyama

ACKNOWLEDGMENTS

I would like to express my sincere thanks to the study participants for consenting to the study. To my supervisors, Dr. Tom Were and Dr. Nathan Shaviya, I am short of words to express my appreciation since your effort and guidance are inexpressible. In addition, I acknowledge the clinical, laboratory staffs and management of Bomu Hospital for their support during sample collection period. To my friends, classmates, parents and my fiancé Nancy thank you for standing by me. Finally, my appreciation goes to God for his guidance and provision during my study period.

ABSTRACT

Injection substance use is both a social and public health concern. Globally, the co-burden of human immunodeficiency virus and injection substance use (HIV-ISU) is high especially in coastal urban and peri-urban region of Sub-Saharan Africa. Although, antiretroviral treatment (ART) has increased the quality of life in HIV-1 infected patients, injection substance use is detrimental to HIV-1 infected individuals.Injection substance user accelerate disease development ininjection substance users (ISUs)as well as the general population. Reduced circulation albumin levels is among the pathophysiological pattern of deregulated marker in injection substance user HIV-1 infected individuals. This is attributed to multi-interaction of injection substances, HIV-1 infection,and ARTas well as host genetics. The albumin gene expression is modulated through several mechanisms including intronic consensus elements. Thus, the current study investigated intron VII (C/G) polymorphism in the *ALB* gene (rs1445776009) and its associationwith circulating albumin levels, adiposity markers, immunosuppression and high density HIV-1 viremia. This cross sectional case control study was conducted at Bomu hospital Mombasa County, Kenya. The study participants were recruited using purposive and snowball sampling. A total of 155 ISUs were recruited into the study comprising of case (ART-experienced, n=93) and control (ART-naive, n=62). Apre-tested questionnaire was used to collect demographic and substance use profiles of study participants.Anthropometric measurements were taken by well-trained clinician. CD4 T cells were enumerated using an automated BD FACSCalibur flow cytometer, HIV-1 RNA copies were determined usingmechanized Abbott m2000 System and circulating albumin was measured usingprogrammed clinical chemistry analyzer (Roche COBAS® 6000).Intron VII rs1445776009 of the *ALB* gene was genotyped using polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP). Genotype frequency was similar, with wildtype, CC and heterozygous, CG genotype carriage most and least prevalent, respectively in the case (40.3 vs 26.9), control (59.7 vs 19.3) as well as general population (50.3 vs 23.9); (P=0.074). The alleles frequency was in line with Hard Weinberg equilibrium for both the ART-experienced, (P=0.178), ART-naive, (P=0.288) and the overall study population, (P=0.096). The mutant GG, genotype relative to wildtype CC, genotype carriage wasassociated with high odds of havinghypoalbuminemia(OR, 1.933; 95% CI, 1.524-4.664; P=0.033), underweight(OR, 2.412; 95% CI, 1.124-5.782; P=0.026), immunosuppression (OR, 3.036; 95% CI, 1.957-9.633; P=0.021) and high density HIVviremia (OR, 1.836; 95% CI, 1.134-6.298; P=0.016), in the ART-experienced ISUs. These findingssuggest that injection substance use and ARTmay modulate the *ALB*gene expression and subsequently influence HIV-1 disease outcomes. Indicating that,rs1445776009 polymorphism can be a good surrogate marker of disease outcomes in HIV-1 infected ISUs.

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LIST OF ABBREVIATIONS AND ACRONYMS

AIDS	Acquired immunodeficiency syndrome
***ALB* gene**	Albumin gene
ART	Antiretroviral treatment
BMI	Body mass index
CD4+	Cluster of differentiation
CTL	Cytotoxic T lymphocyte
EDTA	Ethylenediaminetetraacetic acid
HAART	Highly active antiretroviral therapy
HBV	Hepatitis B virus
HCV	Hepatitis c virus
HIV	Human immunodeficiency virus
HAS	Human albumin
ISUs	Injectable substance user
NACC	National aids control council
NCBI	National Centre for biotechnology information
PCR	Polymerase chain reaction
PWID	People who inject drugs
RFLP	Restriction fragment length polymorphism
SNPs	Single nucleotide polymorphisms

SPSS	Statistical package for social science
TC	Total cholesterol
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
TG	Triacylglycerol
UNAIDS	United nation acquired immunodeficiency syndrome
UNODC	United nation office for drugs and crime
WHO	World health organization

CHAPTER ONE: INTRODUCTION

1.1. Background information

Injection substance use is an essential factor of public health concern, since its among key causes of both morbidity and mortality worldwide(WHO, 2019). Recent reports indicate that injection substance use is on the increase especially in urban and peri-urban centres.This is attributed to socio-economic pressure that is facing several countries worldwide (Kurth et al., 2015; UNDOC, 2018, 2019a; WHO, 2019a). Globally, substance use has developedto be the mostcommunal and well-being crisis, especially among youths. Recent statistics have reported an ascendingtendency in the use of tobacco, alcohol, bhang, , cocaine, opium as well as heroin (Gounder *et al*., 2017; Guise*et al*, 2015; Syvertsen *et al*., 2016). In Kenya, Nairobi, Mombasa and Kisumu town have been identified as important transit points for substance use(Mathers *et al*., 2008; Musyoki *et al*., 2015; Syvertsen *et al*., 2015). Mombasa town in the coastal region of Kenya has reported the highest prevalence of injection substance users (ISUs)(Brodish *et al*., 2011; Musyoki *et al*., 2018; Syvertsen *et al*., 2015). The commonly used substances includes heroin, cocaine as well asbenzodiazepines and amphetamine-like substances(NACADA, 2019; NASCOP, 2012).

Injection substance use is linked with high odds of Human immunodeficiency virus type 1 (HIV-1) infection. For instance, in 2018, 13% of HIV infection globally was attributed to injection substance use(WHO, 2019b).In Sub-Saharan Africa, lack of substances uses surveillance systems has been a key problem in the estimation of narcotic and injection substance use. However, in 2017, at least 27 countries in tropical Africa reported on

injection substance use(UNDOC, 2018). Nevertheless, it was estimated that 6% to 42% of HIV-1 infection in Africa was attributed to injection substance use(Mathers et al., 2008). In Kenya, the prevalence of HIV-1 infection in ISUs is approximately 18% compared to 4.9% in the general population,indicating that ISUs are about four times at risk of getting HIV-1(Brodish *et al*., 2011; Guise *et al*., 2015; Syvertsen *et al*., 2015). In Mombasa County, HIV-ISUs is approximately19% implying that they are at highrisk of getting HIV-1 relative to the general population (4.6%).

HIV-1 infected injection substance users suffer marked decrease in serum albumin levels(Were *et al*., 2014). Reduced serum albumin could be as results of HIV-1 infection and injection substance use. For instance, low circulating albumin has been demonstrated in HIV-1 infected individuals alone. Similarly, decreased plasma albumin has been reported in the HIV-1 infected ART-experienced individuals(Carvallo *et al.*, 2017; Devadas *et al*., 2016; Rao *et al*., 2019; Shaheduzzaman *et al*., 2002). Implying that HIV infection and ART use could be influencing circulating albumin levels. As such, low serum albumin have been also associated with both injection and non- injection substance uses such as heroin and alcohol (Leboffe *et al*., 2017; Sun *et al*., 2019). Apart from HIV-1 infection, ART and substance use, host genetics have shown influence on *ALB* gene expression and hence regulating serum albumin(Bannon *et al*., 2005; Mash *et al*., 2007; Zhou *et al*., 2015).Low serum albumin imply that less transportation of drug molecules to various tissues hence leads to rapid disease progression. Taken together, the findings appear to indicate that a complex interaction of multiple factors in HIV-1 infected ISUs could influence human albumin gene expression. However, the complex interaction of

multifactor with host genetics on influence of encoded albumin protein is not well documented especially in the context in injection substance user, HIV-1 infected ART-experienced and ART-naive.

Apart from reduced serum albumin levels, HIV-1infected ISUs also sufferdisturbance in thenutrition status markers such as body mass index, mid upper arm circumference, waists and hip circumference and plasma lipid panel such as triacylglycerol (TG), cholesterols, low density lipoproteins and high density lipoprotein (Chen *et al*., 2018; Malapati *et al*., 2014; Matoga *et al.*, 2017; Scheffler *et al*., 2018). For instance, low BMI <18.5 kg/m^2 (underweight) has been reported in HIV-1infected ISUs. Circulating lipid profile such triacylglycerol (TG), cholesterols, low density lipoproteins have shown to increase and decrease in high density lipoprotein in HIV-1 infected ISUs. In addition, HIV-1infected ISUs also present withlow CD4+ cells count (immunosuppression) andhigh viral load (high density HIV viremia)(Pralhadrao *et al*., 2016; Sudfeld *et al*., 2013;Rao *et al*., 2019). The derangement in both nutrition markers and clinical presentation in HIV-ISUs may be attributed to either or combination of injection substance use, HIV-1 infection andART(Golub, 2000; Riddler *et al*., 2003; Sudfeld *et al*., 2013). Further, the alteration could be due to host genetics that influence body response to invading pathogen and foreign particles that is manifested as disturbance of both nutritional markers as well as lipid profile.

ALB gene is an essential gene that is almost constitutively expressed by a majority of cells such as hepatocytes and adiposity cells(Spinella *et al*., 2016; X. Wang *et al*.,

2003).The human albumin gene codes for serum albumin, akey proteins that perform a myriad of physiological functions in the body (Moman & Varacallo, 2019). For instance, albumin a product of *ALB* gene is involved in regulating oncotic pressure, transportation of hydrophobic and body metabolites such as dietary lipids and bilirubin to the liver for metabolism. Albumin has also been demonstrated to be a surrogate for monitoring HIV-1 progression, even though its specificity and sensitivity are low (Mehta *et al*., 2006; Olawumi & Olatunji, 2006).Therefore, optimal serum albumin levels is required to ensure adequate transport hydrophobic and drugs molecules to the liver for metabolism and other tissues and maintenance of body fluids oncotic pressure (Fasano *et al*., 2005; Moman & Varacallo, 2019; Spinella *et al*., 2016, 2016; Bocedi *et al*., 2004).This imply that reduced serum albumin lead to low transportation of ARTs to tissues hence leads to disease progression. While high circulating albumin mean high drug dosage hence increasing cost of disease management.

There arenearlyeighty-three genetic variants of the *ALB* gene which have been characterised (The Albumin Website, 2020). Of these, only few polymorphisms have been established to be associated with reduced serum albumin levels, of which many are of exon type. In contrast, little has been done on the association of intronic variations with *ALB* gene expression, especially in HIV-ISUs. However, previous studies have shown that introns play integral roles in gene expression through direct and indirect influence on transcriptional process(Rose, 2019). In addition, introns are essential in post-translation processes such as splicing process using splicing regulatory protein such as hnRNP transcriptional (Abebrese *et al*., 2017; Caridi *et al*., 2012; Cooper, 2010; Gallegos

& Rose, 2017). As such, several intronic single nucleotide polymorphisms(SNPs) have been characterized in the human albumin gene and have shown that intronic SNPs deregulates *ALB*gene expression(Minchiotti *et al*., 2008b). For instance, novel intron III (c.270+1G>T) and intron II, (c.138-2 A>G) have respectively been associated with serum albumin levels (Anna & Monika, 2018; Caridi et al., 2012, 2016a). Likewise,intron VII is a key component in the alternative splicing of the gene (Sorek & Ast, 2003). To date, no single study has evaluated the influence of intronic SNPs in HIV-1 disease outcome especially in HIV-1 infected ISUs. Thus, the current study determined intron VII polymorphism rs1445776009 in the human *ALB* gene and its association with serum albumin levels,underweight, CD4+T cell count and HIV-1 RNA viral load in HIV-1 infected ART-experiencedand ART-naiveISUs in Mombasa County, Kenya.

1.2. Statement of the problem

Injection substance use is among the most important social and public health concerns worldwide. Regional, the problem ofintravenous substance use is increasing, especially in Sub-Saharan Africa in countries along the coastal region including Kenya(UNDOC, 2019). The possible reason could be that the coastal region is a major drug transhipment and consuming market (UNDOC, 2019a).Even though,ART use have tremendously increased the quality of life in HIV-1 infected, HIV-1injection substance users may not meet similarbenefits as a result of poly-substance use, risk behaviours and non-adherence to ART resulting into transmission, retransmission as well as increasing cost HIV-1 disease management(Brodish *et al.*, 2011; Vlahov *et al*., 2010; Beckerleg *et al*., 2005; Musyoki *et al*., 2015).

Clinically, HIV-1infected injection substance users presents with reduced serum albumin levels(Quach *et al*., 2008; Souza *et al*., 2017; Were *et al*., 2014). Apart from reduced circulating serum albumin, HIV-1 infected injection substance users also presents with underweight, low CD4+ T cell count and High density HIV-1 RNA viremia.Assessment of HIV-1 infection progression majorly relay on CD4+ T cell and HIV-1 RNA copies, which are expensive and slow. Serum albumin levels have demonstrated to be cheap and faster method of prognosis of HIV-1 infection. However, it is not known whether disturbance in circulating serum albumin is attributed to a composite factor that include interaction of injection substance use, HIV-1, ARTor due tohost genetics.

Serum Albumin, encoded by the *ALB* gene, play a significant role in thetransportation of small hydrophobic and drug molecules to the liver for metabolism (Moman & Varacallo, 2019; Spinella *et al*., 2016). Therefore, alteration in circulating albumin may influence key body physiological and metabolicprocesses thus influencingHIV-1disease outcomes. For instance, increased serum albumin levels require high dosage for effective efficacy of ARThence increasing cost of management. On other hand, decreased serum albumin level may lead to less transportation of drug molecules to various immunological tissue, leading to increased progression of HIV-1 infection. In addition, decrease serum albumin levels may influence transportation of hydrophobic molecules either to the liver for emulsification or to adipose tissues for storage resulting in increase of plasma lipid profile such as total cholesterol in the circulation.

The albumin gene expression is modulated through several mechanismsintronic consensus elements included(Rose, 2019). The role of intronic variations in modulating the *ALB* gene expression has been demonstrated. However, the role of rs1445776009 intron VII variant in regulating *ALB* gene expression has not been reported. Additionally, it is not known whether the complex interaction of injection substance use, HIV-1, ART or due to host geneticsinfluences human *ALB* gene expression and subsequent disease outcomes. Therefore, the current study sought to determiners1445776009 intronic polymorphism in the *ALB* gene and its associationwith albumin levels, underweight, CD4+ T cell count and high density HIV-1 RNA viremiain HIV-1 infected ART-naive and ART-experienced ISUs in Mombasa County, Kenya.

1.3. Objective of the study

1.3.1. Broad objective

To determine the rs1445776009 intronic polymorphism in the *ALB* gene in injection substance use HIV-1 infected antiretroviral treatment experienced and ART-naive in Mombasa County, Kenya.

1.3.2. Specific objectives

i. To determine the distribution of rs1445776009 genotypes and alleles in injection substance users HIV-1 infected ART-experienced and ART-naiveindividuals
ii. To evaluate the association between rs1445776009 genotypes and hypoalbuminemia,adiposity markers in injection substance use HIV-1 infected ART-experienced and ART-naiveindividual

iii. To evaluate the association between rs1445776009 genotypes andimmunosuppression, high density HIV-1 RNA viremiain the injection substance user HIV-1 infected ART-experienced and ART-naive

1.4. Null hypothesis

i. There is no difference in the distribution of rs1445776009 genotypes and alleles in injection substance users HIV-1 infected ART-experienced and ART-naive individuals

ii. There is noassociation between rs1445776009 genotypes and hypoalbuminemia, adiposity markers in injection substance use HIV-1 infected ART-experienced and ART-naive individual.

iii. There is no association between rs1445776009 genotypes and immunosuppression, high density HIV-1 RNA viremia in the injection substance user HIV-1 infected ART-experienced and ART-naive study participants

1.5. Justification of the study

Injection substance use is still a globalsocial and public health problem(WHO, 2019). In Kenya, major towns especially those in the coastal region have recorded a markedly increased injection substance users(Beckerleg *et al*., 2005; Syvertsen *et al*., 2015). High prevalence of ISUs is attributed to the fact that the coastal towns are the major drug transhipment points and drug consuming market (UNDOC, 2019; Musyoki *et al*., 2018). Injection substance use is associated with high odds of contracting HIV-1 infection. For instance, 13% of HIV-1 infection casesare attributed to injection substance use, globally,

while in Kenya, it reported three times in injection substance user than in the general population (WHO, 2019). The HIV-1ISUs suffer markedly decreased serum albumin levels (Sudfeld *et al.*, 2013; Were *et al.*, 2014).Circulating albumin encoded by human*ALB* gene is key in HIV-1ISUs managementof the disease. It is easy and cheaper to determine its level as compare to determination of HIV-1 RNA copies and reduced CD4+ T cells. Reduced serum albumin levels couldsuggest HIV-1 disease progression. Serum albumin levels have been deregulated amongpoly-substance use, HIV-1 infected ARTs use individuals (Adedeji et al., 2019; Caridi et al., 2016b; Leboffe et al., 2017). However, it is not known whether the complex interaction of HIV-1, ART, ISUs and host genetics influence *ALB* gene expression in HIV-1 infected ISUs. Therefore, it is very essential to determine the rs1445776009 intronic SNP in the *ALB* gene as a marker in HIV-1 infected ISUS use and its relation with hypoalbuminemia, underweight,immunosuppression and high density HIV viremiain HIV-1 infected ART-experienced and ART-naive ISUs, since it will be. The findings of this study are key in the management of HIV-1 infected ISUs.This could be attributed to the fact that it provide information on intron VII polymorphism (rs1445776009) in the *ALB* gene as specific marker for management of HIV-1 disease.

1.6. Significance of the study

Thestudy is among the few studies that have shade light and provided further information on the burden of injection substance use in HIV-1 infected individuals in Kenya. In addition, the study is essential in the monitoring of HIV-1 infection managements in theintravenous substance users as it reports not only on multifaceted interaction of factor

but also host genetics influence on diseases outcome. For instance, human *ALB* gene encode for serum albumin which is key in transportation of drug molecules such ART. Over expression of the gene suggest high dosage ART is required for effective efficacy this increase cost of HIV-1 disease management. While down regulation of the gene imply that less ART drug molecules will be transported hence low efficacy which in turn increase disease progression. Since, rs1445776009 intronic SNP influence *ALB* gene expression, therefore it is important to be included as marker for HIV-1 disease management as it more sensitive and specific as compared to CD4 T cell count and HIV-1 RNA viral load.

1.7. Limitation of the study

1. Self-reported substance use and availability of scar were not efficient since could lead to bias during recruitment.
2. Even though, HIV-1 infected ISUs reported using substances, urine samples were not available for toxicological analysis of concentration of both specific substance use as well as ART.
3. No information on mode of transmission, duration of HIV-1 infection and ART adherence of study participants, of which both have impact on disease outcome.

1.8. Conceptual framework

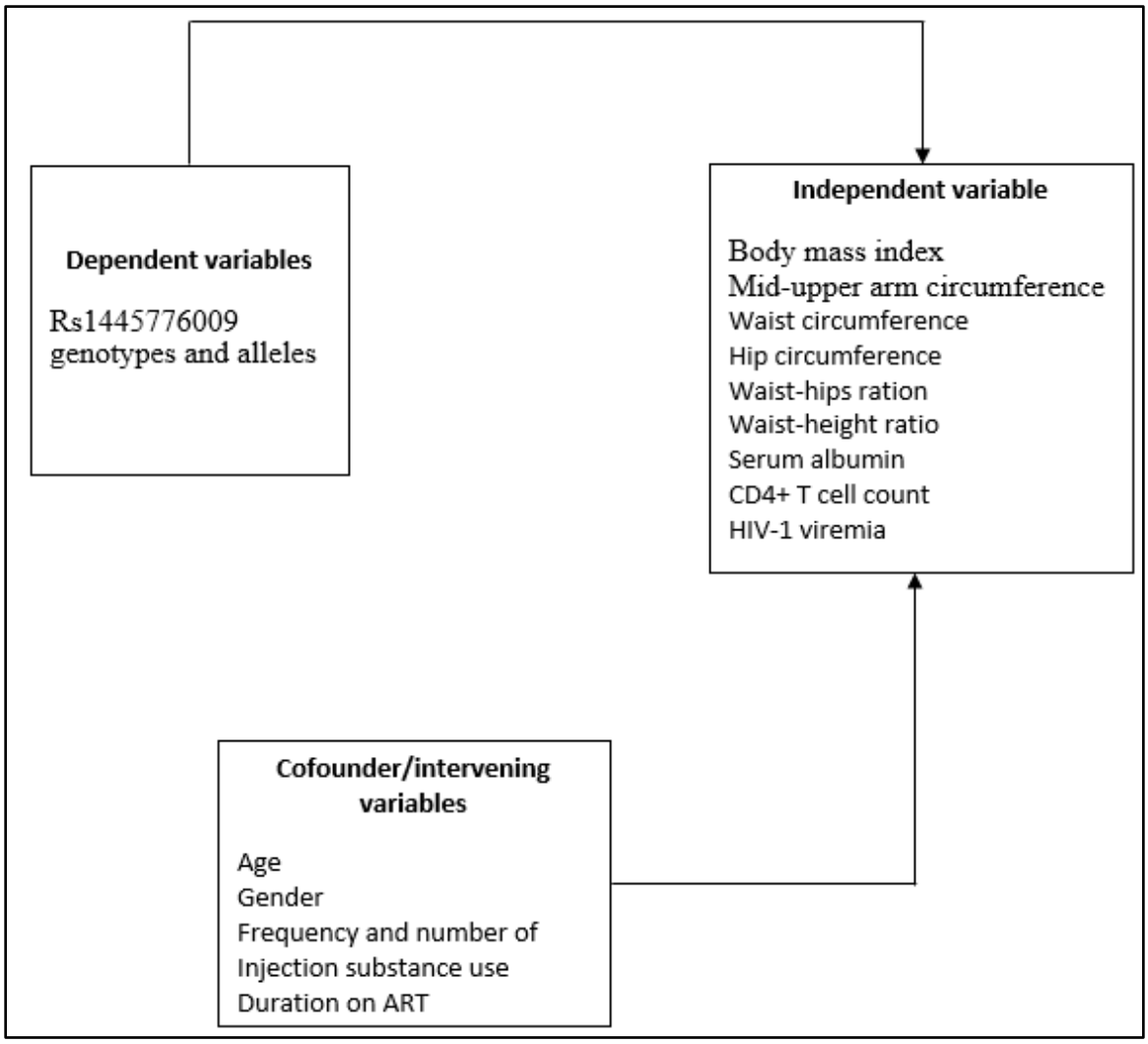

Figure 1: 2: Conceptual frame work

The figure above shows relationship between dependent, independent and intervening variables. Rs1445776009 genotypes and alleles influence in HIV-1 disease outcome in the HIV-1 injection substances users. The disease outcomes can also be influenced by intervening variables.

CHAPTER TWO: LITERATURE REVIEW

2.1. Burden of substance use

Substance use refers to the use of illegal drug and other substance for the purpose to alter physiological functions of which may have detrimental effect on self, society or both (UNDOC, 2019a). Substance use is categorized into injection such as the injection of heroin and non-injection substances such as consumption of alcohol (UNDOC, 2020). Non-injection substances are those illegal drugs administered through inhalation, oral ingestion and application on the skin. While injection substance use involves use of illegal drugs through parenteral route(Mathers *et al*., 2008). Approximately 271 million (5.6%) of the world population in the age bracket of 15-64 year at least once used illicit substance.Estimated 11 million of the global population under substance use; are injection substance user (UNDOC, 2019b). In Sub-Saharan Africa, little information exist on substance use, though it is likely that about 3%-42% of the Sub-Saharan Africapopulation in the aged between 15-64 year at least once used illicit substance (Nelson, 2016). In Kenya, substance use is estimated be 18.2% of the Kenyan population age 15-65 year (NACADA, 2019). Substance use is of great concern of public health sector, since it has detrimental effect on both nutrition status and immune system(WHO, 2019b). For instance, substance use is associated with both underweight, reduced serum albumin levels and weakens the immune system among users(Aslan & Sanlier, 2016; Golub, 2000; Monoarul Haque, 2014; Sanli *et al*., 2015).

2.2. HIV-1 infection in injection substance user

Injection substance users (ISUs) are the most susceptible groups to HIV infection. Studies have revealed that ISUs are highly susceptible to contracting HIV infection relative to the general population (UNAIDS, 2018). For instance, one out of ten incidences of HIV infection is associated withinjection substance use globally (AIDS Alliance., 2016). In Africa, it is approximated that 3%-40% of HIV-1 infection is as a result of injection substance use (UNAIDS, 2018). Increased risk of HIV infection among ISUs, is attributed to the fact that injection substance use is prevalent among the population with the minimum access to HIV mitigation measures such as treatment and prevention. That is ascribed to the fact that injection substance use is often criminalized and stigmatized (UNAIDS, 2016). WHO and UN agencies have suggested various mitigation measures such as needle programs and substance use therapy from an angle of evidence-based (WHO, 2018). However, the criminalization of substance use and stigma against injection substance use has highly resulted in a progressive increase in HIV infection (UNAIDS, 2015).

2.3. Pathogenesis of HIV-1 infection

The pathogenesis of HIV-1 infection and the progression to AIDS are as result of the infecting virus and the host's immune response to theHIV-1 virus. The balance between these two components determines the different diseases outcome, from development of AIDS to long-term survival. HIV cannot survive outside the bloodstream or lymphatic tissue. Furthermore, virus is easily inactivated by the exposure to common detergents and disinfectants. Thus, virus transmission requires the exposer to infected blood or

secretions in the presence of skin damage, for instance by needles or sharp tools, or abrasions in mucosal tissues within sexual intercourses (Suligoi et al., 2010). Transmission of HIV is highly dependent on the biologic properties of the virus, its concentration in the infected body fluid, and host susceptibility. HIV is mainly replicating into the infected cells, which are the main vehicles of virus transmission (Martin & Sattentau, 2009). In the case of infection acquired through hetero-sexual intercourse, the cervix mucosa is the first tissue being infected (Lekkerkerker et al., 2006). Here, dendritic cells and CD4+ lymphocytes can be infected through receptor dependent mechanisms and allow virus spreading to regional lymph nodes and subsequently into the bloodstream (Embretson et al., 1993). In particular, virus particles can be found within follicular dendritic cells, macrophages, and activated CD4+ T-cells, which are the main targets of infection (Fiebig et al., 2003).

The onset of viremia in plasma is a critical time point in the natural history of HIV-1 infection because it indicates that infected individual has acquired the potential of transmitting the infection (Ling et al., 2000)and provides the first chance to diagnose the infection in the blood sample. HIV-1 RNA plasmaviremia levels rapidly and predictably increase (Nguyen & Busch, 2000).These high levels of HIV-1 viremia are normally short-lived, since the host generates humoral and cellular immune responses that partly control viral replication. Over the following weeks, viremia declines by several orders of magnitude until it reaches a lower steady level or drops under detection level. Several factors associated with innate and acquired antiviral immunity can influence viral replication and the establishment of a viral setpoint during this phase

of infection. However, the role of the virus specific cell-mediated immune response, in particular, of the specific CD8+ T-cell cytotoxic activity, seems to be central in the initial control of virus replication at this stage of the infection, before the appearance of anti-HIV binding and/or neutralizing antibodies(Allen et al., 2000). The time period in which the infection is present, but antibodies are not detectable, yet, can be referred as the serological “window period”. However, in rare occasions infected individuals could result seronegative over 3 months after virus trans-mission, indicating that in some circumstances the generation of HIV-specific antibodies may require a longer period(Plucinski et al., 2019).Ranging from few days to few weeks since expo-sure to HIV, most of the infected individuals present symptoms as fever, maculopapular rash, oral ulcers, lymphadenopathy, arthralgia, pharyngitis, malaise, weight loss and myalgia These clinical features are heterogeneous and it has been reported that individuals who display more severe chronic symptoms in the course of acute infection tend to progress more rapidly to AIDS (Alexaki et al., 2008). The symptomatic phase of acute HIV-1 infection lasts be-tween 7 and 10 days, and rarely longer than 14 days. During acute HIV-1 infection, the number of CD4+ T-cells dramatically declines, in association with high viremia levels, before the onset of antiviral immune response (Gupta, 1993). When specific immune response has been elicited, HIV viremia drops and CD4+ T-cells raise again, although to levels lower than those present before infection, suggesting the persistence of virus-associated pathogenic effects. Few weeks after the onset of acute infection, most of the infected individuals enter into a clinical asymptomatic period, generally associated with the drop of HIV viremia levels and absence of symptoms. This event reflects primarily the antiviral action exerted by both innate and adaptive

immune responses (Pedersen et al., 1989). In particular, antibodies specifically bind to HIV antigens, determining the prevention of cell infection favoring the elimination of infected cells by a mechanism known as Antibody-Dependent Cellular Cytotoxicity (ADCC), mediated by T-lymphocytes and natural killer cells (Lekkerkerker et al., 2006). In addition, HIV-specific T-lymphocytes recognize virus antigens on the surface of infected cells and promote their elimination by antigen-specific cytotoxic mechanisms (Bangham, 2009). In the course of asymptomatic phase, HIV continuously replicates in the body compartments, counteracting antiviral immunity and inducing a state of chronic systemic inflammation. Thus, virus replication keeps occurring in the lymphoid compartment, and transitory peaks of HIV-viremia can be detected in plasma (Ford et al., 2009). The asymptomatic period, HIV-1 associated pathogenic effects persist and induce a slow but progressive loss of CD4+ lymphocytes and impairment of the immune system (Mehandru et al., 2004). The progression of the disease is characterized by the destruction of the lymphoid tissue, which is a consequence of the virus replication and of the chronic activation of the cells of immune system. This leads to an increase of virus diffusion to surrounding CD4+ T-cells and favors HIV-1 spread within local, regional and whole lymphoid environment. In addition, HIV infection is associated with an extensive replication in the gut lamina propria and submucosa and in draining lymph nodes, with local depletion of CD4+ T-cells (Mehandru et al., 2004). The further progression of the disease depends on the capacity of the host to contain virus replication and to reconstitute the pool of memory T-cells within the mucosa associated lymphoid tissue or lymph nodes. In absence of virus containment, the destruction of the lymphoid system proceeds and CD4+ T-cell number continues to

drop to levels (< 200 cells/µl) which determine the risk of onset of opportunistic infections by bacteria, viruses, fungi and parasites, and tumors, as a consequence of a serious impairment of the immune system, hence leading to death(Mehandru et al., 2004).

2.4. Effects of substance use on the HIV-1 disease outcome

Injection substance use has significant detrimental consequences in HIV-1 infected ISUs(Li *et al*., 2016; Lowry *et al*., 2014). For instance, high prevalence of underweight has been reported in HIV-1 infected injection substance users incomparison to population infected with HIV-1 alone(McIlwraith *et al*., 2014; Quach *et al*., 2008). High prevalence of underweight could be as a result of substance use which deprive injection substances users appetite for food, disrupt absorption of micro and macro nutrients leading to malnourished (Monoarul Haque, 2014).A part from underweight, low serum albumin has been reported in injection substance users. The possible reason could be that psychoactive substances have down regulatory effect on function of the liver in HIV-1 infected ISUs(Sanli *et al*., 2015). In addition,injection substances has been associated with decreased proinflammatory and proimmune cytokine responses such as IFN-g and IL-2 which are critical in resistance to most infections. In addition, injection substance use results in increase in anti-inflammmatory and immunosuppressive cytokines responses like IL-4 and IL-5 that accelerates disease progression.This imply that injection substance use affect immunomodulation, hence influencing HIV-1 disease outcome(Graham *et al*., 2007). Beside immunity, injection substance use is associated with high HIV-1 RNA viral copies among HIV-1 infected ISUs(Peterson *et al*., 1992).

2.5.Effects of ART use in HIV-1 infected ISUs

Introduction of the ART(a combination of antiretroviral drugs) in the clinical management of HIV-1 infection has reduced both disease aggressiveness and mortality(WHO, 2020). Even though, ART have improved quality of life, HIV-1 infected ISUs may not meet similar benefit due to non-adherence and change in behaviour which greatly predispose them to further and other infections(Budambula *et al*., 2018; Musyoki *et al*., 2015; Pengpid *et al*., 2019; Shukla *et al*., 2016). In line with serum albumin levels, ART have been shown to increase serum albumin levelsduring phase I of introduction(Feigl *et al*., 2016). However, chronic use of ART has been associated with reduced serum albumin levels(Avery *et al*., 2013a). This has been attributed to the fact that ART causes liver damage which impact negatively to *ALB* gene expression(den Brinker *et al*., 2000; Neff *et al*., 2006). Further, low serum albumin is as a results of injection substance use that cause hepatotoxicity which regresses synthetic function of the liver, hence deregulate *ALB* gene expression(Perlemuter *et al*., 2003; Sanli *et al*., 2015). Besides, serum albumin levels as a marker of nutrition status, underweight has been reported among HIV-1 infected ART-experienced(Liu *et al*., 2011; Zemede *et al*., 2019). Moreover, ART influence lipid panel in the serum(Gatechompol et al., 2019; Malapati *et al.*, 2014; Riddler *et al*., 2003). HIV-1 infected individual with no ART normally presents with elevated triacylglycerol (TG) and low cholesterol, low density lipoprotein and high density lipoprotein (Dronda, 2004). However, specific ART regimens appear to alter different lipid profiles (Gatechompol *et al*., 2019; Matoga *et al*., 2017; Riddler *et al*., 2003). In addition, ART causes an upsurge in the CD4+ T cell and

reduction in HIV-1 RNA copies in HIV-1 infected individuals. This transition is due development of less toxic ART, enhanced adherence and controlling of co-infection(Gilks *et al*., 2006; The lancet. HIV, 2017).

2.6. Serum albumin levels and Lipid profiles in HIV-1 infected ISUs

Albumin is one of the major plasma circulating proteins. The protein which is encoded by the *ALB* gene, is mainly involved in the upkeep of osmotic pressure through binding of small molecules such as fatty acids and xenobiotic substances such as drugs, nutrients and some endogenous substances such as electrolytes and bilirubin (Anguizola *et al*., 2013; Avery*et al*., 2013). Studies have indicated low albumin levels in HIV-1 infected individualis linked to the severity of the infection and subsequently has been proposed as a cheap biomarker for assessing the progression HIV-1 infection(Seve *et al*., 2006). Implying that reduced serum albumin is associated with poor HIV-1 disease outcome. In the contest of substance use, studies have indicated, that both non-injection and injection substance use influence circulating levels of albumin. For example of non-ISUs; alcoholic and cigarette smoking in non-injection substance usersis associated with low albumin levels as compared to normal individuals(Kuteesa *et al*., 2019). On the other hand, ISUs; amphetamine is associated with increased synthesis of liver proteins, albumin included in injection substance users(Hobkirk *et al*, 2016). In the context of HIV-1 infected heroin uses, low albumin serum levels have been reported in the ART-experienced relative to the ART-naiveISUs (Sudfeld *et al*., 2013; Were *et al*., 2014). It has been demonstrated that multiple substances use have vital roles in modulating the expression of the *ALB* gene, and thus has a key role in influencing circulating albumin levels(Rhodes & Crabbe, 2005; Sanli *et al*., 2015). However, the mechanism of *ALB* gene

modulation is not well elaborated, though it is speculated that substance use may alter the structure of the *ALB* gene and subsequently its expression.

Blood lipid profile; a pattern of lipids in the blood, including high-density lipoprotein cholesterol (HDL-C), triacylglycerol (TG), low-density lipoprotein cholesterol (LDL-C) total cholesterol (TC)are reported to be significantly altered during HIV-1 infection(Aronson, 2009). For instance, HIV-1ART-naive patients showed a decrease in serum TC, LDL-C and HDL-C levels. HAAT has been associated with increased in TC, TG, LDL-C, and minimal change in HDL-C and TGs (Malapati *et al*., 2014; Denue *et al*., 2013; Riddler *et al*., 2003). HIV-1 infection has been demonstrated to increase circulating TG levels and in return reduces circulating levels of lipoproteins and cholesterols.

Albumin protein which displays extraordinary depot for a variety of ligands such as fatty acid and drugs to the liver for metabolism is key in the regulation of lipid profiles and drugs in circulation. For instance, low serum albumin levels in circulation has been associated with low lipid profiles among the ART-naive, while a significant increase in albumin among concentration has been reported ART- experienced HIV-1 patients(Denue *et al*., 2013; Riddler *et al*., 2003; Bocedi *et al*., 2004)

2.7. Association between serum albumin levelsand immunosuppression, high density HIV-1 viremia.

The adaptive immune reaction mainly involves cellular mediated immunity by the T lymphocytes. These cells are classified into (Th)-cells, also called CD4 + T-cells and

cytotoxic T lymphocytes (CTLs), which are also called CD8+ T-cells (Kovacs et al., 2001). The antigens present on MHC II molecules activate CD4+ T-cells and induce progression of B-cells into memory B-cells, plasma cells and stimulates macrophages and CTLs (Barré -Sinoussi *et al*., 2013). Research has established that the CD4 glycoprotein expressed on the Th-cells is a receptor for HIV 120gp. Thus, HIV bind and enter into the CD4+ T-cells and incorporates its DNA into host DNA, which it uses to replicate. Later, the viral elements are freed into the circulation where they attack new cells. It has been suggested that HIV infection destroys CD4+ T cells via three major means; through direct killing of the disease-ridden cells, progressive apoptosis of the diseased cells and indirect killing of attacked cells by CTLs.

HIV plasma genomic RNA may be quantified by viral load capacity which is essential for the analysis and prediction of HIV-1 infection. The number of HIV particle in plasma can predict extend at which the infection destroys the immune system. Also, plasma viral loads have been used to monitor the efficiency of ARV drugs, whereby, the capacity of an ARV end or reverse advancement of HIV-1 infection to AIDS correlates with its capability to overwhelm viremia(Coffin & Swanstrom, 2013). Consequently, the WHO has recommended viral load monitoring as a criterion for monitoring response to ARV treatment and diagnosis of failed treatment in HIV-1 infected individuals. Treatment failure is indicated by the presence of >1000 copies/ml of viral loads in blood. As a result, it is it has been emphasised that viral load is to be determined within six months following ART-initiation and at least a year to detect any treatment failure. Moreover, routine checking of HIV-1 viral load during HIV-1 infection is preferred to immunologic

and clinical monitoring since it offers an accurate and early signal of ART failure (Petersen *et al*., 2014). CD4+ T cell and viral load numbers have been recommended aspotentdiagnostic markers of HIV disease development(Graham *et al*., 2007). However, both markers have been widely used in 1st World countries owing to high technology and cost which is unaffordable in developing countries. Low levels of serum albumin is associated with increased death rates in acute and chronic conditions such cancer (Seve *et al*., 2006; Vymetalkova *et al*., 2015). Studies have reported on low albumin levels which are affiliated with fast HIV-1 infection advancement to AIDS and subsequent increase in viral load and reduced CD4 count (Mehta *et al.*, 2006). Circulating albumin levels may substantiate to be essential, inexpensive and available supportive marker for determining the rigorousness of HIV infection, prior-treatment examination, clinical monitoring ARTresponse as well as prediction of survival (Sudfeld *et al*., 2013).

2.8. The Human albumin gene (*ALB* gene)

The human albumin gene also known as PRO0883, PRO0903, and PRO1341. Cytogenetically, *ALB* gene is locatedon sub-centromere region of 4q13.3 and is made up of 17245bp that runs from putative "Cap site to first poly (A). The gene is characterised by the many 14 intervening intron and 15 exon. Intron VII which is made up of 1,293bp is contained in domain 2 subdomain A and genotypic variation occurs between 8803-8806 bp(Homo sapiens Annotation Release 109, GRCh38.p12) (Minchiotti *et al*., 2008). The gene encodes for albumin a major circulating protein. The core function of circulating albumin is to sustain colloid osmotic pressure and also acts as carrier for variety hydrophobic xenobiotic substances. The xenobiotic substances comprise both

endogenous such as hormones, electrolytes, bilirubin, and metabolites while exogenous substances include fatty acids, drugs, and toxins (J. A. Anguizola *et al*, 2013; Avery *et al*, 2013; Otagiri, 2005; Michelis *et al*, 2016).

ALB gene is essential in the synthesis of albumin protein. However, it has been reported that there exist some polymorphisms in the gene which mainly influence the*ALB* gene expression (Minchiotti *et al*., 2008). For instance, intronic and intron-exon junction variation of the gene has been associated with analbumineminic through influencing mRNA synthesis and in turn posttranscriptional processing which has significance influenceon the amount of mRNA available for translation (Caridi et al., 2016b). However, no single study has linked genotypic variation in intron VII of the *ALB* gene with albumin levels in circulation especially in HIV-ISUs ART-experienced and ART-naive.

2.9.Selection strategy for the human ALB gene intron VII rs1445776009 SNP for genotyping

The selection of human *ALB* gene was based on the fact that the gene encodes for serum albumin is major transporter of xenobiotic such drug molecules. Previous genetic study in South African bantoid population showed that the frequency of wildtype, C genotype was (64%) and mutant, G genotype (36%) was an added advantage (Moolman *et al*., 1991). Additionally, putative function of the SNP in splicing process and location of the SNP in the *ALB* gene and intronic site for binding of transcription factor are also added advantage(Marques *et al*; 2016).

2.10. Association between *ALB* gene andserum albumin levels in HIV-1 infected ISUs

ALB gene is among the genes that are constitutively expressed. The expression of the *ALB* gene is vital since its product is significant as a drug and fatty acids carrier and as a biomarker for monitoring a number of disease development such as HIV infection(Mehta *et al*., 2006). Despite, *ALB* gene being vital, its expression is poorly understood. Unlike the other genes, which are regulated by binding of the regulatory factor on the promoter and enhancer site. *ALB* gene has other factors such as ALU elements, polyA signal, and motif which are distributed in the introns as well as exon and are believed to be principal determinant that influence gene expression. For instance, the ALU element in the intron VII acts as promoter and enhancer where regulatory factors bind hence influence gene expression (Cooper, 2010; Häsler & Strub, 2006). Additionally, it is believed that the ALU elements helped in the splicing of pre-mRNA transcripts hence are important in gene expression. Furthermore, various studies have shown that intronhas transcription sites that is bind by transcription factors hence influence the post-transcriptional process of the *ALB* gene (Minchiotti *et al.*, 2008).

Previous studies have demonstrated associations between alterations in the *ALB* gene and availability of clinical condition such as bisalbuminemia, hypoalbuminemia, and analbuminemia. Besides, about 83 genotypic variants that have been identified, including those of intron VII, approximately twelve genotypic variations of clinical significance have been reported in NCBI (Minchiotti *et al*., 2008). It is not clear whether the rs1445776009 variation influences the gene expression. Besides, there is limited data

linking intron *ALB*gene polymorphism in HIV-1 infected ISUs with clinical markers. In fact, to date, no study has linked polymorphism in the *ALB* gene especially intron VII variation with clinical markers such as CD4+ T cell count, viral load, albumin levels and adiposity markers in HIV-1 infected ISUs. Therefore, this study determined intron VII polymorphism of the *ALB* gene and associated it with albumin level, adiposity markers, CD4+ T cells and viral load in HIV-1 positive ISUs ART-naive and -experience.

CHAPTER THREE: MATERIALS AND METHODS

3.1. Study site

The study was conducted at Bomu Hospital in Mombasa County, coastal Kenya. Mombasa County has a total area of 294.7 km^2. The land has an area of 229.7 Km^2 and water covers an area of 65Km^2. The latitudes $3^0$56' and $4^0$10' south of the equator and the longitudes $39^0$34^{0,} and $39^0$46^0 East of the equator. It is a city and the country's oldest (circa 900 AD) and second-largest city, after the capital Nairobi. The county is a metropolitan with an approximated population of 1.208 million people and poverty index of 37.6%.The population comprise of male 610 257 and female 598,046. The population density of 5495 people per square kilometre with an average of 4 people per house hold. The Mijikenda are majority (35%) which are followed by Kamba and those of kikuyu, EmbuMeru association (GEMA) origin(KNBS, 2009). The county hosts a variety of tourist attractions sites such as Fort Jesus, water sports, white sandy beaches, historical sites, Mombasa marine national park, and cultural sites. Besides, it has several world-class tourist hotels hence making it suitable for tourist attraction on the East Africa coast. Apart from tourism, other major economic activities include; fishing and farming for coconuts, sugarcane, cashew nuts, livestock, and sisal. Tourism attraction is the main revenue-generating activity for the county government of Mombasa. Furthermore, tourism is identified as a key economic factor that has fuels risk behaviour that has bridged HIV transmission to the general population. Mombasa County has the high HIV prevalence of 7.4 %, nearly one and half times that of the overallprevalence in Kenya (5.6%) (NACC., 2016). In addition, the county also has the highest prevalence of ISUs in the county (UNDOC, 2018).

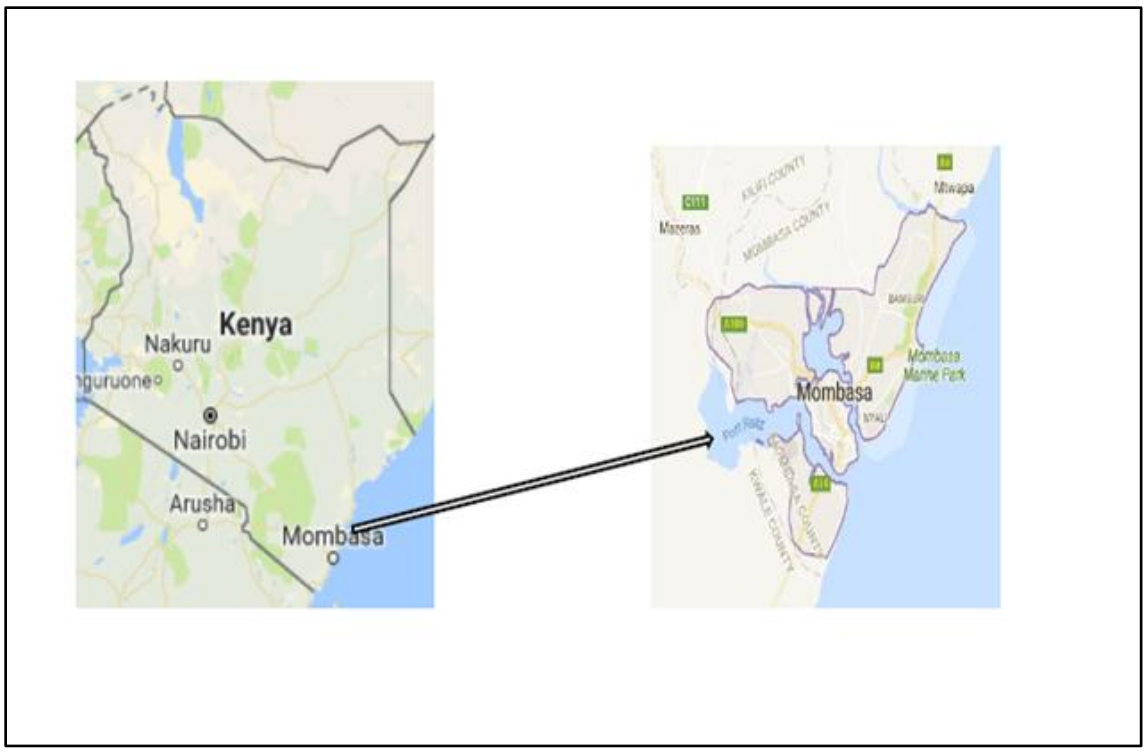

Figure 1: Map of Mombasa County, Kenya. (Google map)

3.2. Study design

A cross-sectional, case control study design was used in genotyping the *ALB* geners1445776009SNP in theART-experienced and ART-naiveISUs residents of Mombasa County, Kenya.

3.3. Study population

The study population constituted adult, HIV-1 infected ISUs. The ISUs were defined as individuals reporting injecting any illicit substances for the past two weeks, and showing evidence of injection needle-stick scars according to United Nations Office on Drugs and Crime (UNODC) registry (UNODC, 2014). Injection substance users were further stratified into ART-experienced HIV-1 (n=93), case and ART-naive, control (n=62).

3.3.1. Inclusion criteria

Only consenting adults aged ≥18 years testing HIV-1 positive for both ART- experienced and ART-naive and exhibited needle-scars or reported injecting any illicit substances at least two weeks before recruitment as categorized under UNODC registry were enrolled to the study (UNODC, 2016)

3.3.2. Exclusion criteria

Those individuals who refused to consent to the study, under 18-year individuals, without HIV-1 and those individuals with HIV-1 non-ISUs were not enrolled in the study. HIV-1 patients presenting co-morbidities including TB, HBV, HCV, diabetes and hypertension were also excluded from recruitment. Furthermore, those individuals on methadone rehabilitation treatment and other medication were excluded from the study.

3.4. Study variables

3.4.1. Dependent variable

This comprised of the human intron VII *ALB* gene rs1445776009 genotypes and alleles influence on gene expression.

3.4.2. Independent variable

The independent variables included the disease outcomes; anthropometric measurements which predicted the nutrition status such as body mass index, mid-upper arm circumference, waist circumference, hip circumference, waist-hip ratio and waist-height

ratio, laboratory markers such serum albumin levels and lipid profiles and finally clinical markers such as CD4+ T cell count and HIV-1 RNAviremia.

3.4. 3. Intervening variable

The intervening variable comprised ofage, gender, frequency and number of injection substance use as well as duration on ART influences on diseases outcomes.

3.5. Sampling design and strategy

ISUs were recruited via respondent-driven sampling (RDS), snowball and targeted street outreach (TSO) sampling technique which targeted HIV infected ART-experienced and ART-naive ISUs among residents of Mombasa County, Kenya (Rudolph *et al.*, 2015). One potential ISUs was purposively identified who later on recruited others. A total of 155 HIV-1 ISUs (62 ART-naive(control) and 93 ART-experienced ISUs (case)) were involved in the study.

3.6. Sample size determination

The Wayne (2016) formula (ni = {p1 (1- p1) +p2 (1- p2)} (Z/E) 2) was used to calculate the sample sizes. In a study of the human *ALB* rs1445776009 SNP among bantoid in Sub-Saharan Africa, the frequency of the C allelewas 64%. Therefore, this value (64%) was entered into the formula as P1(Moolman *et al*, 1991). In addition, the frequency of G allele (36%) was entered into the formula as p2 (Moolman *et al*, 1991).Ni = {0.64(1-0.64) +0.36 (1-0.36)} (1.96/0.2)2 = 62**,** ni+ni=122, ni =Is the sample size for study population; P1- frequency of major allele =64%; P2- frequency of minor allele=36%; Z -

Standard normal variants for the chosen (confidence level 95%) E- Percentage allowable margin of error = 20%.; 20% attrition error= ˜ 155

3.7. Data and information collection of study participants

3.7.1. Demographic and substance use profiles of study participants

A pre-tested well-structured questionnaire was used for collection of socio-demographic features (gender and age, history of drug use (type of drug, route of administration, frequency of substance used, and durationon injection substance use)) and antiretroviral therapy during the recruitment.

3.7.2. Anthropometric measurements

Anthropometric data (weight, (kg); and height, (m)) were collected using mechanical weighing and height scale (RGZ-160) machine by a well-trained clinicianduring the recruitment. Based on the obtained weight and height of the study participants, individual BMI, (kg/m^2) were electronically calculated and recorded. The baseline BMI was classified based on World Health Organization adult nutritional status as underweight ($BMI<18.5\ kg/m^2$), normal body weight ($BMI\geq18.5\ kg/m^2$) and overweight ($BMI\geq25.0\ kg/m^2$) (WHO, 2019).Middle upper arm circumference (MUAC) was measured midway, Hip circumference (HC) was assessed around the maximum circumference of the buttocks and amid the tip of acromion and olecranon process, Waist circumference (WC) was measured at smallest diameter between the iliac crest and low rib. MUAC, WC, HC were all estimated to the nearest 0.1 (cm). While waist-to-hip ratio (WHR) was calculated as WC (cm)/HC (cm) and waist height ratio (WHR) as height (cm)/ WC (cm).

Underweight based on MUAC was set at cut off <24.5cm and based on WHR was <0.5. Central obesity as based on WC was set at cut off male ≥ 94cm and female ≥ 80cm and as based on WHR, male ≥ 0.88 and female ≥ 0.86(WHO, 2019d)

3.7.3. Collection of blood sample

Well trained phlebotomists obtained 3ml of blood fromeach participants. The phlebotomist introduced himself/ herself gently to the patient, then identified the right hand. A patient vein was identifiedby the application of tourniquet on the upper arm. Once the vein was more conspicuous when a patient made a tight fist. The venepuncture site was sterilized with 70% methanol and air dried for half a minute to avoid contamination which could lead to lysis of blood. A sterile, non-reusable phlebotomy needle was inserted along the line of the vein, with the bevel of the needle facing directly upwards and steadily 3mL of blood drown from media cubital vein. After collection of required volume the tourniquet was released and the fist was opened. Then the needle was removed, and the patient was instructed to press a piece of dry cotton firmly on the venepuncture site. The patient continued pressing on the puncture site with the arm raised until the bleeding stopped. The blood was transferred into a well-labelled EDTA-containing tube with the patient's details, date and time of collection. The blood was well mixed steadily by flip-flopping the tube 5 times and was stored in the fridge at -20 $C^{o.}$

3.7.4. HIV-1 sero-testing

Each study participant was counselled before being tested of HIV-1 infection status at a time of recruitment. Rapid immune -chromatographic tests, Determine^{TM} (Abbot

Laboratories, Tokyo, Japan) and Unigold^{TM}(Trinity Biotech Plc, Bray, Ireland) used to HIV-1 status. Based on the Kenyan national HIV testing algorithm (NASCOP, 2015), ISUs who tested positive for both Determine and Unigold were confirmed infected with HIV.

3.7.5. CD4 T cell enumeration

CD4 T cells baseline were enumeratedinprogrammed technique using the BD FACSCalibur flow cytometer (Becton-Dickinson™, Franklin Lakes, USA). In summary, 2.5.0 µl of EDTA blood specimen was pipetted in a tube and 1.0 µl RBC lysis buffer were thenadded. The mixture was incubated for 3 minutes, after then the cells were washed. Fluorescent-tagged antibodies (anti-CD3, anti-CD4 and anti-CD45) was added and incubated for 15 minutes. This was followed withsamples washing and the CD4+ T cells enumeration on the flow cytometer. Immunosuppression was based on WHO immunological staging of HIV, of which patients with CD4 T cell count <500 cells/µl were considered immunosuppressed (WHO, 2019e)

3.7.6. HIV-1 RNA copies determination.

HIV-1 copies were determined using the Abbott m2000SP System with an automated sample extraction, amplification and detection system (Abbott Molecular Inc., Illinois, U.S.A). The RNA was extracted from 0.1 mL blood samples using the 0.1mL plasma RNA extraction and master mix addition protocol of the Abbott m2000SP sample preparation system. The master mix containing the viral RNA was then automated transferred to the Abbott m2000rt instrument (Abbott Molecular Inc., Illinois, USA) for

viral load detection using the program for 0.1 mL RNA amplification. The RNA was then reverse transcribed into cDNA and amplified using HIV-1 and internal control primers;forward primer, (5′-TGGCATGGGTACCAGCACA-3′), and the reverse primer, (5′-CTGGCTACTATTTCTTTTGCTA-3′), were chosen to amplify a 199-bp fragment in a region of HIV-1 pol gene. Real time PCR technology used in the Abbott real time (RT) detection system uses two probes: a fluorescent-tagged longer fragment complementary to the target sequence and a quencher molecule bound onto a shorter fragment. Fluorescence emission of the HIV-1 probe will be proportional to the amount of HIV-1 target sequence in the sample. The fluorescence counts were then converted into viral loads by the analyzer. High density HIV-1 viremia ≤ 1000 HIV-1 RNA, copies/ml this was based on WHO (WHO, 2015, 2016).

3.7.7. Determination of serum albumin levels

The baseline serum albumin levels was determined using automated clinical chemistry analyser according to the manufacturer's instructions and reported in g/l (Roche Cobas 6001 (Roche COBAS® 6001, Lausanne, Switzerland). Briefly, the machine work on the cationic features, albumin displays at acidic pH (Citrate buffer: 95 mmol/L, pH 4.1) that enable it bind bromcresol green (0.075 M) (an anionic dye), at absorbance of 628 nm and room temperature with composition of NaCl 9%, 50 mL as a salt to form a blue-green complex. The intensity of the color is directly proportional to the albumin concentration in the sample that is determined photometrically.The hypoalbuminemia was defined based on previous established reference range of clinical biochemical parameters among adults of East African population in Rwandan population<32g/l (Gahutu & Wane, 2006).

3.7.8. Determination of lipid profiles

Lipid profiles of study participants were measured using an automated Hitachi analyzeraccording to the manufacturer's instructions (Roche COBAS® 101, CANADA). The reaction conditions are established by the Instrument Settings. The Total Cholesterol, Direct HDL, Precipitated HDL, Triglycerides, and LDL reagents, R1 and R2 contain the following components: R1 Cyclodextrin/Buffer, supplied as a solution, ready to use. 0.5 mmol/l α-cyclodextrin 0.5 g/l dextran sulfate 7.0 mg/ml magnesium sulfate (MgSO4) 0.3 g/l EMSE 10 mmol/l MOPS (3-morpholino-propane sulfonic acid) buffer, pH 7.0) unspecified preservative. R2 Buffer/PEG-enzyme/4-Aminophenazone, is supplied as a lyophilized mixture and is reconstituted with diluent supplied in the reagent kit. R2 contains the following approximate concentrations after reconstitution: > 1 kU/l PEG cholesterol esterase (EC 3.1.1.13; Pseudomonas species; 25oC) > 5.6 kU/l PEG cholesterol oxidase (EC 1.1.3.6; Pseudomonas species; 25oC) > 30 kU/l peroxidase (EC 1.11.1.7; horseradish; 25oC) 0.5 g/l 4-aminophenazone 10 mmol/l MOPS (3-morpholino-propane sulfonic acid) buffer, pH 7.0 Detergent and preservative. In summary, Place a 100 ul aliquot of sample into the disposable sample cups on the instrument carousel using a disposable polyethylene transfer pipet. Arrange the samples on the carousel in the order in which they are to be analyzed, as determined from the download file. Place the quality control samples into their assigned positions on the instrument and begin the analysis. The results are printed on the Hitachi printout and also sent in real time to computer text files on a dedicated computer. A separate file is created for each sample. The reference range for total cholesterol (TC) >20year75 -169 mg/dL and <21year 100 -199 mg/dL.

High density lipoprotein(HDL) >40 mg/dL and low density lipoprotein (LDL) normal <100 mg/dL(Johnson & Dowe, 2014).

3.8. Genotyping of ALB gene in HIV-1 ISUs

3.8.1. DNA extraction

RBC in the blood was lysed by hypotonic buffer $(NH3)_2CO_3$, NH_3Cl and himedia) without lysis of lymphocyte. 3ml of lyse buffer was added to the specimen, vortexed for 5 minutes and centrifuged at 2000 *g* for 5 minutes. ~1ml of centrifuged solution was left after discarding supernatant; that prevented loss of cell. 3ml of RBC lysed buffer was added to pellets and well mixed by inverting, vortexing, inverting and centrifuging. The step was repeated three times till a white pellet and a clear supernatant was formed. The supernatant was discarded after last wash, then pellets were re-suspended in 500 µl PBS, 400 µl cell lysis buffer (10 mMTris-HCl, 10 mM EDTA, 50m M NaCl, 10% SDS, pH 7.5) and 10 µl proteinase K (10 mg/ml stock; Himedia) was added. The pelleted sample was dissolved by vortexing and incubated for 2 h at 56°C in a water bath (CW-30G; Jeio Tech) for lysis. The same volume of phenol (equilibrated with Tris, pH 8) was added, mixed gently by inverting for 60 seconds. After centrifugation at 10,000 *g* (at 4°C) for 5 minutes, an aqueous top layer was moved to a fresh tube having the same capacities (1:1) of chloroform and phenol: isoamyl alcohol (24:1). The tube was thoroughly mixed by centrifugation for 10 min at 10,000 *g* (at 4°C) and inverting for 1 min. The supernatant was then transferred to a fresh tube, and 10 µl of 10 mg/ml RNase (Fermentas, Thermo Scientific) was added. The sample was incubated at 37°C for 30 minutes before an equal volume of chloroform: isoamyl alcohol (24:1) was added and mixed by inverting the tube

for 1 minute and centrifuging at 10,000 *g* (at 4°C) for 10 minutes. The supernatant was transferred to a fresh tube, and twice the volume of absolute alcohol (Merck) was added and inverted gently 3 times and chilled at -20°C, followed by centrifugation at 10,000 *g*ats (4°C) for 20 minutes. The supernatant was discarded, 250 µl 70% ethanol was added, and the pellet was tapped gently, followed by centrifugation at 10,000 rpm for 10 minutes and decant the supernatant gently. The pellet was air-dried in laminar airflow, and the dried pellet was re-suspended in 50 µl 1× TE buffer and frozen at −20°C or −80°C for storage(Dhaliwal, 2020).

3.8.2. DNA purification and amplification

The amplicons were cleaned using a viral DNA Purification Kit as per the company's protocol (Wizard® Genomic DNA Purification Kit). 80µl of PBI Buffer was pipetted to the PCR product and mixed thoroughly. The mixture was transferred into 2 mL tube and spanned for 2 minute and the filtrate was discarded. 0.8 ml of PE Buffer was pipetted into 2 mL tube, spanned at 1800rpm for 60 seconds and the filtrate cast-off. DNA elution was performed by the addition of 30 µL ofEB Buffer (10 mMTris -Cl, pH 8.5) to the spin column in a fresh 1.5 ml micro-centrifuge tube, followed by centrifugation for 1 minute(Dhaliwal, 2020).

The single nucleotide polymorphism (SNP) was amplified using PCR as previously described(Moolman et al., 1991). The reaction was conducted in a total volume of 50 µLcomprising 25µL of PCR master mix which constituted (5 µL of PCR Buffer, 1 µL of dNTPs (10 mM), 1 µL of Forward primer (50 pmol), 1 µL of reverse primer (50 pmol), 1 µL Taq DNA polymerase (5U/µL, 5 µL of Template DNA, 1 µL of $MgCl_2$ (50 mM) and

10 µL of deionized water), 1 µL of Forward primer (50 pmol), 1 µL of reverse primer (50 pmol, 5 µL of Template DNA and finally 18 µL of deionized water. PCR amplification of DNA segments, containing intronic rs1445776009 was performed using the following primers; 5'-*ALB* gene forward primer, 5'-GTAGGTGGACTTGGAGAAGG-3' and 3'-*ALB*gene reverse primer, 5'-GATATACTTGGCAAGGTCCG-3'. (Syngene, Frederik,MD, USA).A genomic portion of 674 bp containing two Hae III sites was amplified. The PCR cycles were performed as follows: 94 °C for 5 min, with 30 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s and final extension 72 °C for 10 minutes (Dhaliwal, 2020; Moolman *et al*., 1991).

3.8.3. Restriction fragment length polymorphism

The PCR amplified DNA fragments were incubated with *Hae III* restriction enzymes which recognized the restriction sites. The restriction enzymes and their respective sites were identified using SNPs(New England Biolabs, Ipswich, USA). The restricted fragments were resolved in 2% agarose gel. PCR products (10 µl) of *ALB* gene which targeted nucleotides between 8477-9496bp (1019 bp) were digested in a 30-µl reaction volume overnight with the time saver protocol comprised of 1.5 U of *Hae III* at 37 °C, 1 µg DNA template, 10X NEB buffer. The PCR genomic fragments (674 bp) were digested by restriction enzyme *Hae III* (). The reaction was stopped by incubating the digests at 65 °C for 20 minutes. The genotypes were compared in terms of fragment length through agarose gel electrophoresis.

3.9. Data management and analysis

Each study questionnaire had a coded study identity that was uniquely associated with each study participant clinical and laboratory tests. Biological samples were coded in line with the study participant's codes. Socio-demographic, laboratory and clinical information was entered, cleaned and stored in spreadsheet password-protected computer, dated and time-stamped with an electronic signature for tracking purpose in case of any changes.

Statistical analysis was accomplished by Statistical Package for the Social Sciences (SPSS) version 21.0 (SPSS Inc, Chicago, USA). Data was categorised into continuous data examples were age, height, MUAC, weight, BMI, waist circumference and hip circumference. Discrete datawere gender, CD4 T cell count and HIV-1 RNA viral copies, and categorical data were nutrition status and immune status. Continuous data weresummarized as median, and interquartile ranges (IQR) and categorical data were summarized as the proportion (frequency and percentage (n, %) andboth were presented in tables. The frequency of genotypes and alleles was summarized as proportion and presented as graphs.

Mann Whitney U test was used to compare Continuous data such age, height, MUAC, weight, BMI, waist circumference and hip circumference. While kruskal Wallis was used to compared continuous data discrete data across the genotypes. However, Chi-squire compared discrete and categorical data between the clinical groups and presented in tables

Genotypes frequencies were compared across the group using hard -Weinberg equilibrium to determine linkage disequilibrium. Hardy-Weinberg equilibrium was determined using the Online Encyclopedia for Genetic Epidemiology studies software at www.oege.org/software/hwe-mr-calc.shtml.

Binary logistic regression analyses were used to determine the associations of genotypes with the hypoalbuminemia, underweight, immunosuppression and high density HIV-1 RNA viremia. In these regression models, we controlled for age, gender, duration on ART as well as frequency and duration of substance injection in the ART-experienced. However, in the ART-naive, we controlled all intervening variable except duration on ART.$P<0.05$ was considered statistically significant.

3.10. Ethical consideration

Ethical approval was sought from MMUST Institutional Ethical Review Committee (IREC). The participants were assigned code number during the study for identification. Each participant appended a signature on a written informed consent prior to enrolment in the study. Data that was obtained was cleaned entered and kept in a password secured computer spreadsheet. The data was only accessed by the principal investigator and co-investigators. The study participants experienced mild pain during the blood collection process 'venepuncture', however, it was minimized by using well trained and experienced phlebotomists. ART treatmentART-naive study participants were initiated into ART therapy according to the National guidelines for HIV treatment, supportive and palliative

care at Compressive Care centre clinic at Bomu Hospital and Coast General Referral Hospital. All participants benefitted from free health education on risk of acquiringinfections commonly associated with injection substance use and Sexual transmitted infection (STI).

CHAPTER FOUR: RESULTS

4.1. Demographic, anthropometric and substance use profiles of ISUs

Evaluation of demographic, anthropometric and substance use profiles of the injection substance users(ISUs) are summarized in Table 4.1.The study participants (n=155) comprised of case ART-experienced, (n=93) and control ART-naive, (n=62). Among the ART-experienced individuals 38 (40.9%) were males and 55 (59.1%) were females. Likewise, of the 62 ART-naive controls, 31 (50.0%) were males while 31 (50.0%) were females, (P=0.262).Age was comparable between the ART-experienced and ART-naive ISUs (median, 30.6; IQR, 6.6 years vs median, 30.6; IQR, 6.4 years); (P= 0.685). The body height was also similar between the study group (cases median, 1.7; IQR 0.1 m and control median, 1.7; IQR 0.1 m; P=0.387).

Bodyweight was significantly lowin the ART-experienced(median, 53.0; IQR, 6.5kg) relative to the ART-naive(median, 54.0; IQR, 8.3 kg)ISUs;P=0.045). In addition, body mass index (BMI), was consistent with bodyweight with a significantly low in theART-experienced (median, 18.4; IQR 2.3 kgm^2 relative to the ART-naive (median, 18.4; IQR, 2.4 kgm^2 ISUs;**(**P=0.036). The frequency of underweight was significantly high in theART-experienced (46.2%) relative to the ART-naive(32.3%) individuals; (P=0.042).

The Mid upper arm circumference (MUAC) was also significantly lowin the ART-experienced (median, 25.0; IQR 3.0cm) relative to the ART-naive (median, 24.0; IQR 2.8cm) ISUs; (P=0.002). In addition, frequency of underweight based on MUAC was highin theART-experienced (37.6%) relative to the ART-naive(22.6%) ISUs; (P=0.036). Likewise, Waist circumference (WC) was significantly lowamong the ART-

experienced(median, 72.0; IQR 9.5cm compared to the ART-naive(median, 76.0; IQR 9.5cm) ISUs; *P*=0.006. The prevalenceof the central obesity based on WC were similar between the ART-experiencedand (5.4%) ART-naive(8.1%) ISUs; (*P*=0.506). The hip circumference (HC) was significantly lowin the ART-experienced (median, 89.0; IQR 6.0cm) relative to the ART-naive(median, 91.0; IQR 10.5cm ISUs; (*P*=0.041). However, the waist hip ratio (WHR) was comparable between the ART-experienced (median, 0.83; IQR 0.1) and the ART-naive(median, 0.84; IQR 0.1) ISUs; (*P*=0.175). Central obesity based on WHR was also similar between the ART-experienced (32.3%) and the ART-naive (25.8%) ISUs; (*P*=0.324). The waist to height ratio (WHtR) was significantly low in the ART-experienced (median, 0.44; IQR 0.1) compared to the naive (median, 0.45; IQR 0.1) ISUs; (*P*=0.324). The rate ofunderweight based on WHtRwas comparable between the ART-experienced (25.8%) and the ART-naive(22.5%) ISUs; (*P*=0.032).

Heroin was the most prevalent reported injection substances use in both the ART-experienced (71.0%) and the ART-naive (75.8%) ISUs; (*P*=0.619). Other injection substances use included Cocaine, diazepam, and flunitrazepam. Besides, injection substances use,non-injection substances were also reported. Cigarette was the most used non-injection substances with a majorityin the ART-experienced (72.0%) relative to theART-naive ISUs (67.7%), ISUs; (*P*=0.567). Alcohol, cannabinoids and *Catha edulis* (khat) were among other non-injection substance use with a high rate in the ART-experiencedalcohol (54.8%) andcannabinoids (52.7%) relative to the ART-naivealcohol (48.4%) andcannabinoids (50.0%) ISUs;(*P*=0.744; *P*=0.384).

Injection substance use was further categorized based on the period for which an individual had been using injectionsubstances and the frequency of injection. Durationof injection was further categorized into those who had used for more than a year and those who had used less than a year. The majority of individuals reporting injecting substances for more than one year were generally high relative to those who injected for less than one year in both the ART-experienced(87.1%) vs (27.4%) and the ART-naive (72.6%) vs (12.9%) ISUs; (P=0.024). The frequency of injection was further categorized according to thosewho were injecting substances for more than once a day and once a day. In addition, individual who were injecting more than once a day were high compared to thoseinjecting once a day between the ART-experienced (80.6%) vs (40.9%) and ART-naive (59.1%) vs (19.4%) ISUs; (P<0.001).

Table 4.1: Demographic, anthropometric and substance use profiles for ISUs

Characteristics	HIV-1[+] ART [-], n=62	HIV-1[+] ART [+], n=93	*P*
Male/female, n (%)	31 (50.0)/31 (50.0)	38 (40.9)/55 (59.1)	0.262
Age, years	30.6 (6.4)	30.6 (6.6)	0.685
Height, m	1.7 (0.1)	1.7 (0.1)	0.387
Weight, kg	54.0 (8.3)	53.0 (6.5)	**0.045**
BMI, kgm^2	18.4 (2.4)	17.6 (2.3)	**0.036**
Nutrition status, n (%)			
Overweight	1 (1.6)	1 (1.1)	
Normal	41 (66.1)	49 (52.7)	**0.042**
Underweight	20 (32.3)	43 (46.2)	
MUAC, cm	25.0 (3.0)	24.0 (2.8)	**0.002**
Normal	47 (75.8)	58 (62.4)	**0.038**
Underweight	14 (22.6)	35 (37.6)	
WC, cm	76.5 (9.5)	72.0 (9.5)	**0.006**
Central obesity	5 (8.1)	5 (5.4)	0.506
Normal	57 (91.9)	88 (94.6)	
HC, cm	91.0 (10.5)	89.0 (6.0)	**0.041**
WHR	0.84 (0.1)	0.83 (0.1)	0.175
Central Obesity	20 (32.3)	24 (25.8)	0.324
Normal	42 (67.7)	69 (74.2)	
WHtR	0.45 (0.1)	0.44 (0.1)	**0.032**
Normal	14 (22.5)	24 (25.8)	**0.023**
Underweight	48 (77.5)	69 (74.2)	
Injection substances, n (%)			
Heroin	47 (75.8)	66 (71.0)	0.619
Others	15 (24.2)	27 (29.0)	0.508
Non-injection substances, n (%)			
Bhang	31 (50.0)	49 (52.7)	0.744
Cigarettes	42 (67.7)	67 (72.0)	0.567
Alcohol	30 (48.4)	51 (54.8)	0.384
Duration of injection, n (%)			
≥1 year	45 (72.6)	81 (87.1)	**0.024**
<1 year	17 (27.4)	12 (12.9)	
Frequency of injection, n (%)			
≤1 times/day	50 (80.6)	55 (59.1)	**0.001**
>1 times/day	12 (19.4)	38 (40.9)	

Data shown are median, and interquartile range (IQR) for continuous variables and numbers (n) and proportion (%) of study subjects for categorical variables. ISU, injection substance use, HIV-1[+], human immunodeficiency virus type HIV-1. ART [-], anti-retroviral treatment ART-naive. ART [+], anti-retroviral treatment ART-experienced. BMI; body mass index; overweight ≥25kgm^2, normal 18.5-24.9 kgm^2 and underweight >18.5 kgm^2, MUAC; mid-upper arm circumference; normal ≥24.5cm, underweight >24.5cm, WC; waist circumference; central obesity (M/F:≥94/≥80cm), underweight >24.5cm HC; hip circumference, WHR; waist hip ration; Central obesity (M/F; ≥ 0.88/≥ 86cm), normal (M/F; ≤0.88/≤ 86cm) and WHtR; waist height ratio; normal ≥0.5 ,underweight <0.5. Values in bold are significant *P*-values at cut-off <0.05.

4.2. Clinical and laboratory findings of the study participants

Clinical profile and laboratory feature of the study participants are summarized in Table 4.2. The CD4+ T cells count was significantly low in the ART-experienced (median, 398.0; IQR 427.0 cells/μl) relative to theART-naive (median, 518.5; IQR 468.5 cells/μl) ISUs: (P = 0.022). Based on WHO immunological staging of HIV-1 infection, a majority of theART-experienced HIV-1 patientswere in phase II, III and IV (case, I (38.7), II (18.3%), III (23.7) IV (19.4%) compared to the ART-naive(I (53.2%), II (16.1%) III (16.1%) IV (14.5%) ISUs; (P=0.042). However, HIV-1 RNA copies were significantly low in theART-experienced(median, 2.6; IQR 2.3 copies/ml) relative to the ART-naive(median, 2.2; IQR 2.1 copies/ml) ISUs; (P = 0.036).Furthermore, the frequency of high density HIV-1 viremia (≥1000 HIV RNA copies/ml) was high in the ART-experienced(60.2.8%) compared to the ART-naive(48.4 %/) ISUs; (P = 0.047).

Serum albumin levels were significantly low in theART-experienced (median, 29.0; IQR 12.5 g/l) compared to the ART-naive (median, 33.5; IQR 9.5 g/l)ISUs; (P=0.023). The rate of hypoalbuminemiawas high in the ART-experienced (41.9%) relative to the ART-naive (17.7%ISUs; (P = 0.019).

The plasma lipid profiles were comparable between theART-experienced and the ART-naive ISUs. Total cholesterol (TC), (median, 4.2; IQR 1.6mmol/l vs, median, 4.3; IQR 1.9mmol/l); (P=0.988),High density lipoprotein (HDL),(median, 1.0; IQR 0.8mmol/l vsmedian, 1.1; IQR 0.7mmol/l); (P=0.891) and low density lipoprotein (LDL), (median, 1.8; IQR 1.2mmol/l, vsmedian, 2.1; IQR 1.9mmol/l); (P=0.496),triacylglycerol (TG),

(median, 1.4; IQR 0.3mmol/l, median, 1.2; IQR 0.8mmol/l);(P=0.117) and very low density lipoprotein (VLDL), ISUs (median, 0.28 IQR 0.1mmol/l, median, 0.24; IQR 0.2mmol/l); (P=0.106), respectively. TC/HDL ratio and LDL/HDL ratio were also compared between the -experience and ART-naive ISUs (TC/HDL ratio, median, 1.9; IQR 0.9mmol/l vsmedian, 1.8; IQR 0.7mmol/l); (P=0.478) and (TC/HDL ratio, median, 4.1; IQR 1.5mmol/l vs median 3.9; IQR 1.0mmol/l); (P=0.595), respectively.

Table 4.2: Clinical and laboratory findings of the study participants.

Characteristic	HIV-1[+] ART [-], n=62	HIV-1[+] ART [+], n=93	*P*
CD4+ T cells/μl	518.5 (468.5)	398.0 (427.0)	**0.022**
WHO Immunological staging of HIV, n (%)			
Stage IV	9 (14.5)	18 (19.4)	
Stage III	10 (16.1)	22 (23.7)	**0.042**
Stage II	10 (16.1)	17 (18.3)	
Stage I	33 (53.2)	36 (38.7)	
Log_{10} HIV-1 RNA, copies/ml	2.2 (2.1)	2.6 (2.3)	**0.036**
High	30 (48.4)	56 (60.2)	**0.047**
Low	31 (50.6)	37 (39.8	
Albumin, g/l	33.5 (9.5)	29.0 (12.5)	**0.023**
Normal	19 (30.6)	23 (24.7)	**0.019**
Hypoalbuminemia	11 (17.7)	39 (41.9)	
TC, mmol/l	4.3 (1.9)	4.2 (1.6)	0.988
HDL, mmol/l	1.1 (0.7)	1.0 (0.8)	0.891
LDL, mmol/l	2.1 (1.9)	1.8 (1.2)	0.496
TG, mmol/l	1.2 (0.8)	1.4 (0.3)	0.117
VLDL, mmol/l	0.24 (0.2)	0.28 (0.1)	0.106
TC/HDL ratio	1.8 (0.7)	1.9 (0.9)	0.478
LDL/HDL ratio	3.9 (1.0)	4.1 (1.5)	0.595

Data shown are median, and interquartile range (IQR) for continuous variables and numbers (n) and proportion (%) of study subjects for categorical variables, human immunodeficiency virus type-1. ART [-], anti-retroviral treatment ART-naive. ART [+], anti-retroviral treatment ART-experienced, CD4 T cell count, cluster of differentiation; WHO immunological staging of HIV; stage IV <200/mm^3, stage III 200-349/mm^3, stage II 350-499/mm^3 stage I ≥500/mm^3, high density ≥1000 HIV RNA copies/ml and viral suppression<1000 HIV RNA copies/ml. albumin; normal ≥32 g/lHypoalbuminemia<32 g/l, TC, total cholesterol, HDL, high-density lipoprotein, LDL, low-density lipoprotein, TG, triacylglycerol, VLDL, very low-density lipoprotein. Values in bold are significant *P*-values at cut-off 0.05.

4.3: Distribution rs1445776009 genotypes and alleles in the study case and control

Distribution of rs1445776009 genotypes and alleles in the ART-experienced and ART-naiveindividuals are summarized in Figure 4.1A. There were no significant difference in the frequency of genotypes between theART-experienced(CC, 40.3%; CG, 26.9%; GG, 29.0%) and ART-naive (CC, 59.7%, CG, 19.4%, GG, 21.0%) ISUs; [a]*P*=0.074). Based on major and minor allele model, the distribution of alleles in the ART-experienced and ART-naive individuals are summarized in Figure 4.1B. The C(major) relative to G, (minor) allele was dominant in both experienced (57.5% vs42.5%) and ART-naive (69.4% vs 30.6%), individuals. The frequency of alleles in the ART-experienced([d]HWE,*P*=0.178)ART-naive[c]HWE, *P*=0.288) as well as overall study population (ARTART-experienced and ART-naivecombined) ([b]HWE, *P*=0.096) ISUs was consistent with the Hardy-Weinberg equilibrium (HWE).

The gel electrophoresis of the *Hae III* digest of the PCR amplicons are shown in Figure 4.2; heterozygous, three band of 418 b, 347b, and 256b; 2; homozygous mutants; two bands of 347b and 256b and 3; homozygous wildtype, band of 418b.

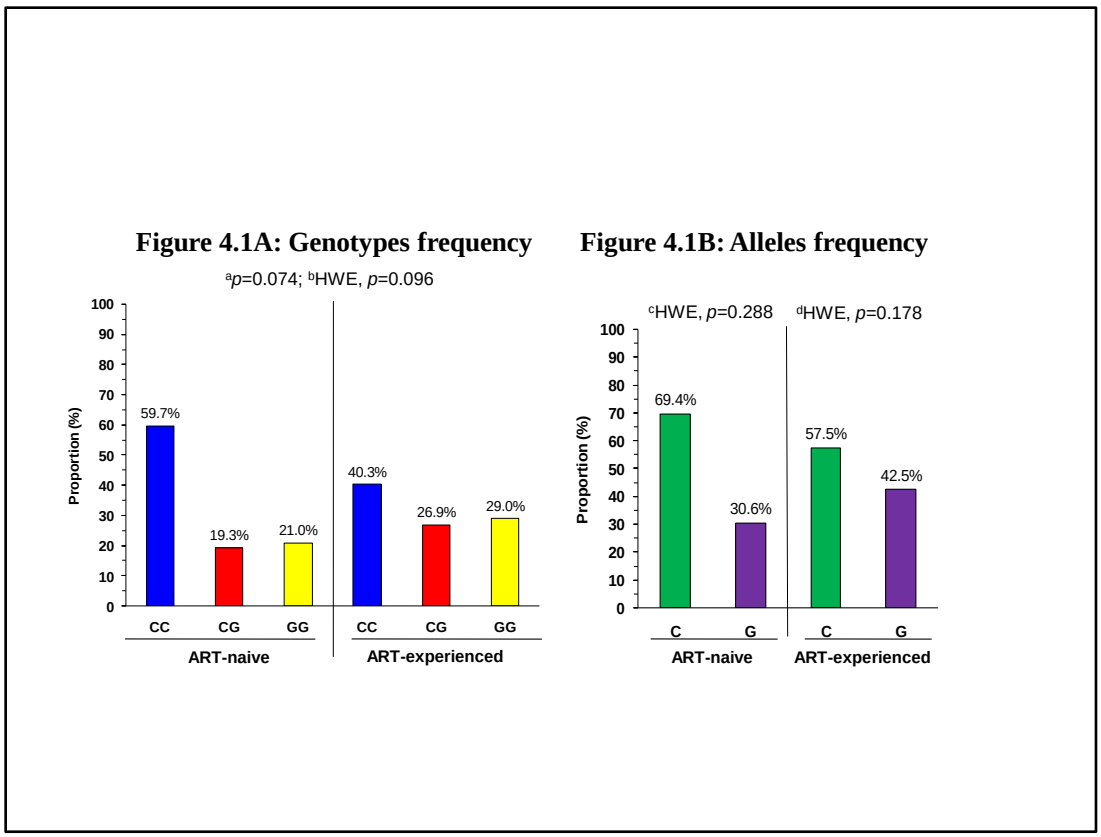

Figure 4. 1: Distribution rs1445776009 genotypes and alleles in study population.
Data shown are proportion (%) of study participants for genotypes and alleles. HIV-1[+], human immunodeficiency virus type-1. ART [-], anti-retroviral treatmentART-naive. ART [+], anti-retroviral treatmentART-experienced, CC, Homozygous wildtype, CG, Heterozygous and GG, Homozygous mutant. HWE, Hardy Weinberg equilibrium, was calculated using the formulae $p^2 + 2pq + q^2 = 1$. [a]p, overall genotypes distribution, [b]HWE, overall Hardy Weinberg equilibrium,[c]HWE, Hardy Weinberg equilibrium for the allele in ART-naive, [d]HWE Hardy Weinberg equilibrium for the allele in the experienced. Significant *P*-values at a cut –off $P \leq 0.05$.

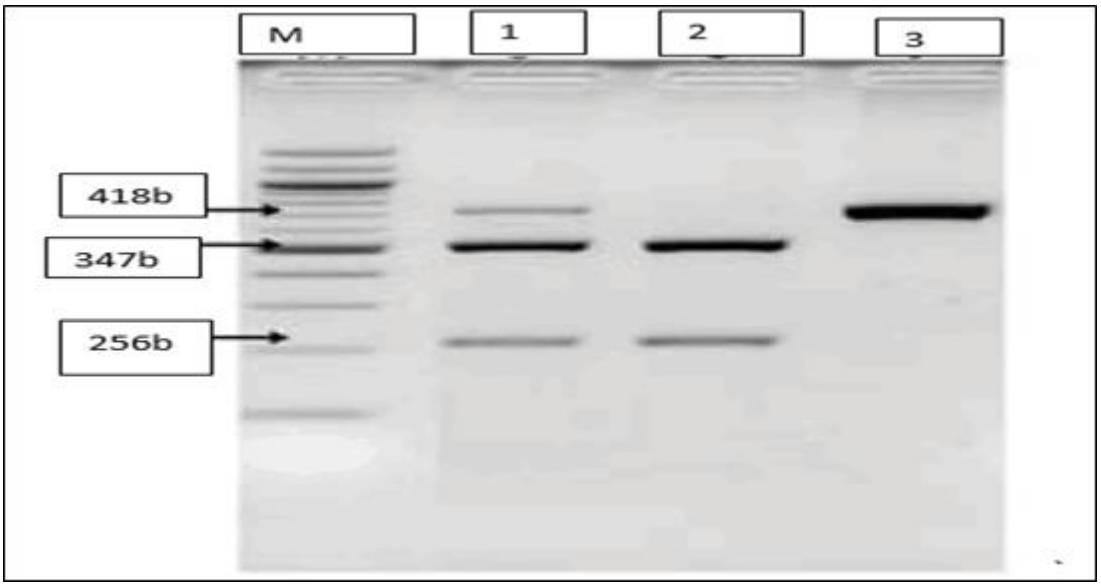

Figure 4. 2:Gel electrophoresis of the Hae III digest of the PCR amplicons

The figureabove shows RFLP analysis of the *Hae III* digest of the PCR amplicons that targeting locus rs1445776009 of the *ALB* gene separated on a 2% agarose gel. Lane M: Marker Ladder of 100bp, (1) Heterozygous (CG) shows three bands of sizes 418, 347 and 256 bp. (2) Homozygous mutant (GG), shows two bands of sizes 347 and 256bp and (3) Homozygous Wild-type (CC) shows one band of 418bp.

4.4. Serum albumin levels and adiposity markers in rs1445776009 genotypes in the ART-experienced ISUs.

Distribution of albumin levels and adiposity markers in rs1445776009genotypes in the ART-experienced ISUs study is summarized in Table 4.3. Albumin levels were significantly different cross the CC, (median, 30.0; IQR 9.5g/l), CG, (median, 29.0; IQR 13.3g/l) andGG, (median, 17.0; IQR 14.5g/l), genotypes; (P=0.020). In addition, the frequency of Hypoalbuminemia was also significantly different across the CC (22.0%), CG (25.9%), GG (32.0%), genotypes;(P=0.042).

BMI was lowest in GG, (median, 17.3; IQR 2.4 kg/m^2) compared to CC, (median, 18.8; IQR 1.9 kg/m^2) and CG, (median, 18.7; IQR 2.2 kg/m^2), genotypes carriage; (P=0.030). Furthermore, the frequency of underweight was highest in GG, (51.9%) in relation to CC, (41.5%) and CG, (44%), genotypes carriage; (P=0.001). Waist circumference was comparable cross the genotypes CC, (median, 74.0; IQR 10.0cm), CG, (median, 71.0; IQR 10.0cm) and GG, (median, 72.0; IQR 10.0cm) genotypes carriage; (P=0.719). The rate of central obesity was prevalent in CG, (12.0%),relative CC, (4.9%), and GG, (0.0%), genotypes carriage; (P=0.001). Hip circumference was comparable across the genotypes CC, (median, 90.0; IQR 5.8 cm),CG, (median, 87.0; IQR 6.5 cm) and GG, (median, 89.0; IQR 7.0 cm); (P=0.462). In addition, MUAC was not significant across the genotypes CC, (median, 24.0; IQR 3.0 cm), CG, (median24.0; IQR 1.5), GG, (median, 24.0; IQR 3.0), genotypes carriage; (P=0.726). The frequencies of underweight based on MUAC was significantly different across CC, (56.1%), CG, (68.0%) and GG, (66.7%) genotypes carriage; (P=0.017). Waist to hip ratio was relatively similar across CC, (median, 0.8; IQR 0.1), CC, (median, 0.9; IQR 0.1) and GG, (median, 0.8; IQR 0.1), genotypes carriage; (P=0.458). Based on waist to hip ratio, central obesity was significantly different across the CC, (29.3%),CG, (32.0%) and GG, (14.8%), genotypes carriage; (P=0.001). Waist to hip ratio was comparable across the CC, (median, 0.45; IQR 0.1) CG, (median, 0.43; IQR 0.1) and GG, (median, 0.44; IQR 0.1); (P=0.458). The frequency of underweight based on waist to hip ratio was significant different across the CC, (31.7%) CG, (20.0%), GG, (22.2%), genotypes carriage; (P=0.001)

Plasma lipid profiles were compared across the genotypes. The levels of total cholesterol were as followsCC, (median, 4.6; IQR 1.9 mmol/l),CG, (median, 4.3; IQR 0.6 mmol/l), GG, (median, 4.0; IQR 3.5 mmol/l), in the genotypes carriage; (P=0.914). The levels of triacylglycerols were;CC, (median, 1.5; IQR 0.6 mmol/l),CG, (median, 1.4; IQR 0.6 mmol/l), and GG, (median, 1.3; IQR 1.1 mmol/l), in the genotype carriage; (P=0.426). High density lipoprotein levels across the genotypes CC, (median, 1.2; IQR 0.9 mmol/l)CG, (median, 1.0; IQR 0.5 mmol/l and(median, 0.8; IQR 1.0 mmol/l); (P=0.559). Low density lipoprotein levels in, CC, (median, 2.1; IQR 1.7 mmol/l), CG, (median, 1.8; IQR 0.4 mmol/l) and GG, (median, 1.8; IQR 2.3 mmol/l), genotype carriage; (P=0.860). Very low density lipoprotein levels in CC, (median, 1.3; IQR 0.8 mmol/l) CG, (median, 0.3; IQR 0.1 mmol/l) and GG, (median, 0.12; IQR 0.1 mmol/l), genotype carriage; (P=0.308). Total cholesterol to high density lipoprotein ratio CC, (median, 2.3; IQR 3.5), CG, (median, 1.3; IQR 0.8) and GG, (median, 1.5; IQR 0.8), genotypes carriage; (P=0.258). Low density lipoprotein to high density lipoprotein ratio relative to CC, (median, 4.2; IQR 12.5), CG, (median, 3.9; IQR 1.7) and GG, (median, 4.2; IQR 2.7), genotypes carriage. (P=0.363).

Table 4.3: Serum albumin levels and adiposity markers in rs1445776009 genotypes in theART-experienced ISUs.

	HIV-1[+] ART [+], n=93			
Markers	CC, n=41	CG, n=25	GG, n=27	*P*
Albumin	30.0 (9.5)	29.0 (13.3)	25.0 (14.5)	**0.020**
Normal	16 (39.0)	10 (40.0)	13 (48.1)	**0.042**
Hypoalbuminemia	9 (22.0)	7 (25.9)	7 (32.0)	
BMI	18.8 (1.9)	18.7 (2.2)	18.3 (2.4)	0.683
Overweight	1 (2.4)	0 (0.0)	0 (0.0)	
Normal	23 (56.1)	14 (56.0)	13 (48.1)	**0.001**
Underweight	17 (41.5)	11 (44.0)	14 (51.9)	
WC, cm	74.0 (10.0)	71.0 (10.0)	72.0 (10.0)	0.719
Central obesity	2 (4.9)	3 (12.0)	0 (0.0)	**0.001**
Normal	39 (95.1)	22 (88.0)	27 (100)	
HC, cm	90.0 (5.8)	87.0 (6.5)	89.0 (7.0)	0.462
MUAC, cm	24.0 (3.0)	24.0 (1.5)	24.0 (3.0)	0.726
Normal	18 (43.9)	8 (32.0)	9 (33.3)	**0.017**
Underweight	23 (56.1)	17 (66.7)	18 (68.0)	
WHR	0.8 (0.1)	0.9 (0.1)	0.8 (0.1)	0.439
Central obesity	12 (29.3)	8 (32.0)	4 (14.8)	**0.001**
Normal	29 (70.7)	17 (68.0)	23 (85.2)	
WHtR	0.45 (0.1)	0.43 (0.1)	0.44 (0.1)	0.458
Underweight	13 (31.7)	5 (20.0)	6 (22.2)	**0.001**
Normal	28 (68.3)	20 (80.0)	21 (77.8)	
TC, mmol/l	4.0 (3.5)	4.6 (1.9)	4.3 (0.6)	0.914
TG, mmol/l	1.3 (1.1)	1.5 (0.6)	1.4 (0.6)	0.426
HDL, mmol/l	0.8 (1.0)	1.2 (0.9)	1.0 (0.5)	0.559
LDL, mmol/l	1.8 (2.3)	2.1 (1.7)	1.8 (0.4)	0.860
VLDL, mmol/l	0.12 (0.1)	1.3 (0.8)	0.3 (0.1)	0.308
TC/HDL ratio	2.3 (3.5)	1.7 (1.1)	1.5 (0.8)	0.258
LDL/HDL ratio	4.2 (12.5)	3.9 (1.7)	4.2 (2.7)	0.363

Data shown are median, and interquartile range (IQR) for continuous variables and numbers (n) and proportion (%) of study subjects for categorical variables. ISU, injection substance use, HIV-1[+], human immunodeficiency virus type HIV-1. ART [+], anti-retroviral treatmentART-experienced. CC, Homozygous wild type; CG, Heterozygous and GG, Homozygous mutants; BMI; body mass index; overweight ≥25 kgm^2, normal 18.5-24.5 kgm^2 and underweight >18.5 kgm^2, MUAC; mid-upper arm circumference; normal ≥24.5 cm, underweight >24.5cm, WC; waist circumference; central obesity (M/F;≥94/≥80cm), underweight >24.5cm HC; hip circumference, WHR; waist hip ration; Central obesity (M/F; ≥ 0.88/≥ 86), normal (M/F; ≤0.88/≤ 86) and WHtR; waist height ratio; normal ≥0.5 ,underweight <0.5; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triacylglycerols and VLDL, very low-density lipoprotein. Values in bold are significant *P*-values at cut-off <0.05.

4.5. Serum Albumin levels and adiposity markers in rs1445776009genotypes in theART-naiveISUs

Distribution of albumin levels and adiposity markers in rs1445776009genotypes in the ART-experienced ISUs study is summarized in Table 4.4. Albumin levels were significantly different cross the CC, (median, 37.0; IQR 9.9 g/l), CG, (median, 37.0; IQR 9.9 g/l) and GG, (median, 29.5; IQR 16.8 g/l), genotype carriage; (P=0.580). In addition, the rate of Hypoalbuminemia was also significantly high in GG (32.0%) as compared to CC (22.0%) and CG (25.9%), genotypes; (P=0.042).

BMI was lowest in GG, (median, 17.3; IQR 2.4 kg/m^2) compared to CC, (median, 18.8; IQR 1.9 kg/m^2), CG, (median, 18.7; IQR 2.2 kg/m^2), genotypes carriage; (P=0.068). Furthermore, the frequency of underweight was highest in CG, 7 (58.3%) and CC, 8 (21.6%) and CG,5 (38.5%), genotypes carriage; (P=0.048). Waist circumference was comparable cross the genotypes CC, (median, 78.0; IQR 10.0cm), CG, (median, 73.0; IQR 9.0cm) and GG, (median, 74.0; IQR 10.5cm) genotypes carriage; (P=0.597). The individuals who presented with central obesity were prevalent in CG, 3(8.3%), relative CC, 1 (8.1%), and GG, 1 (7.7%), genotypes carriage; (P=0.998). Hip circumference was comparable across the CC, (median, 92.0; IQR 9.8 cm), CG, (median, 89.0; IQR 8.1 cm) and GG, (median, 91.0; IQR 10.0 cm) genotypes; (P=0.462). In addition, MUAC was not significant across the genotypes CC, (median, 25.0; IQR 4.0 cm), CG, (median 24.5; IQR 3.cm), GG, (median, 26.0; IQR 3.3), genotypes carriage; (P=0.459). The majority of underweight based on MUAC was comparable across the CC, 29(78.4%), CG, 9(75.0%) and GG, 4 (30.8%), genotypes carriage; (P=0.699). Waist to hip ratio was relatively

similar across CC, (median, 0.8; IQR 0.1), CC, (median, 0.9; IQR 0.1) and GG, (median, 0.8; IQR 0.1), genotypes carriage; (P=0.757). Based on waist to hip ratio, central obesity was comparable across the CC, (35.1%), CG, (41.7%) and GG, (15.4%), genotypes carriage; (P=0.319). Waist to hip ratio was comparable across the CC, (median, 0.46; IQR 0.2) CG, (median, 0.45; IQR 0.1) and GG, (median, 0.45; IQR 0.1), genotypes; (P=0.444). The frequency of underweight based on waist to hight ratio was comparable across the CC, 17 (45.9%) CG, 4 (33.3%), GG, 2 (15.4%), genotypes carriage; (P=0.143).

Plasma lipid profiles were compared across the genotypes. Total cholesterol levels for the CC, (median, 3.9; IQR 2.5 mmol/l), CG, (median, 5.5; IQR 3.3 mmol/l), GG, (median, 3.5; IQR 2.6 mmol/l), genotypes carriage; (P=0.489). Triacylglycerol levelsfor the CC, (median, 1.2; IQR 0.8 mmol/l), CG, (median, 1.1; IQR 1.1 mmol/l), and GG, (median, 0.8; IQR 0.7 mmol/l), genotype carriage; (P=0.559). High density lipoprotein levels for the CC, (median, 1.0; IQR 0.8mmol/l) CG, (median, 1.6; IQR 0.9mmol/l and (median, 0.9; IQR 0.8mmol/l); (P=0.732). Low density lipoprotein levels for the, CC, (median, 1.8; IQR 1.9mmol/l), CG, (median, 3.1; IQR 1.7mmol/l) and GG, (median, 1.9; IQR 1.6mmol/l), genotype carriage; (P=0.736). Very low density lipoprotein levels for the CC, (median, 0.24; IQR 0.8 mmol/l) CG, (median, 0.23; IQR 0.2mmol/l) and GG, (median, 0.16; IQR 0.7mmol/l), genotype carriage; (P=0.222). Total cholesterol to high density lipoprotein ratio for the CC, (median, 4.2; IQR 1.0), CG, (median, 3.8; IQR 1.6) and GG, (median, 1.7; IQR 1.0), genotypes carriage; (P=0.439). Low density lipoprotein

to high density lipoprotein ratio relative to CC, (median, 1.5; IQR 0.9), CG, (median, 2.0; IQR 1.1) and GG, (median, 1.7; IQR 1.0), genotypes carriage (*P*=0.439).

Table 4. 4: Serum albumin levels and adiposity markers in rs1445776009 genotypes in the ART-naive ISUs.

Markers	HIV-1[+] ART [-], n=62			
	CC, n=37	CG, n=12	GG, n=13	*P*
Albumin, g/l	37.0 (9.9)	30.0 (16.8)	29.5 (12.3)	0.580
Normal	11 (29.7)	2 (16.7)	6 (46.2)	0.156
Hypoalbuminemia	6 (16.2)	4 (33.3)	1 (7.7)	
BMI, kg/m^2	19.6 (2.2)	18.7 (2.6)	18.1 (1.7)	0.068
Overweight	1 (2.7)	0 (0.0)	0 (0.0)	
Normal	28 (75.7)	5 (41.7)	8 (6.5)	**0.048**
Underweight	8 (21.6)	7 (58.3)	5 (38.5)	
WC cm	78.0 (10.3)	73.0 (9.3)	74.0 (10.5)	0.597
Central obesity	3 (8.1)	1 (8.3)	1 (7.7)	0.998
Normal	34 (91.9)	11 (91.7)	12 (92.3)	
HC, cm	92.0 (9.8)	89.0 (8.1)	91.0 (10.0)	0.630
MUAC, cm	25.0 (4.0)	24.5 (3.3)	26.0 (3.3)	0.459
Normal	7 (18.9)	5 (25.0)	9 (69.2)	0.699
Underweight	29 (78.4)	9 (75.0)	4 (30.8)	
WHR	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)	0.757
Central obesity	13 (35.1)	5 (41.7)	2 (15.4)	0.319
Normal	24 (64.9)	7 (58.3)	11 (84.6)	
WHtR	0.46 (0.2)	0.45 (0.1)	0.45 (0.1)	0.444
Underweight	17 (45.9)	4 (33.3)	2 (15.4)	0.143
Normal	20 (54.1)	8 (66.7)	11 (84.6)	
TC, mmol/l	3.9 (2.5)	5.5 (3.3)	3.5 (2.6)	0.489
TG, mmol/l	1.2 (0.8)	1.1 (1.1)	0.8 (0.7)	0.559
HDL, mmol/l	1.0 (0.8)	1.6 (0.9)	0.9 (0.8)	0.732
LDL, mmol/l	1.8 (1.9)	3.1 (1.7)	1.9 (1.6)	0.736
VLDL, mmol/l	0.24 (0.8)	0.23 (0.2)	0.16 (0.7)	0.222
TC/HDL ratio	4.2 (1.0)	3.8 (1.6)	1.7 (1.0)	0.439
LDL/HDL ratio	1.5 (0.9)	2.0 (1.1)	1.7 (1.0)	0.439

Data shown are median, and interquartile range (IQR) for continuous variables and numbers (n) and proportion (%) of study subjects for categorical variables. ISU, injection substance use, HIV-1[+], human immunodeficiency virus type HIV-1. ART [-], anti-retroviral treatment ART-naive. CC, Homozygous wild type; CG, Heterozygous and GG, Homozygous mutants; BMI; body mass index; overweight ≥25 kgm^2, normal 18.5-24.5 kgm^2 and underweight >18.5 kgm^2, MUAC; mid-upper arm circumference; normal ≥24.5 cm, underweight >24.5cm, WC; waist circumference; central obesity (M/F;≥94/≥80cm), underweight >24.5cm HC; hip circumference, WHR; waist hip ration; Central obesity (M/F; ≥ 0.88/≥ 86), normal (M/F; ≤0.88/≤ 86) and WHtR; waist height ratio; normal ≥0.5, underweight <0.5; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, triacylglycerols and VLDL, very low density lipoprotein. Values in bold are significant *P*-values at cut-off <0.05.

4.6. Association between rs1445776009 genotypes and Hypoalbuminemia, adiposity markers in the ART-experiencedISUs.

The association between rs1445776009genotypes andhypoalbuminemia, adiposity marker in the ART-experienced ISUs is summarized in Table 4.6. The heterozygous, CG genotype in relation to wildtype, CC genotypeswere not associated with hypoalbuminemia (OR, 9.680; 95% CI, 0.668-140.285; *P*=0.096), underweight based on BMI, MUAC and WHtR (OR, 4.814; 95% CI, 1.068-3.706; (*P*=0.071), (OR, 1.204; 95% CI, 0.248-5.848; *P*=0.818) and (OR, 1.568; 95% CI, 0.855-15.149; *P*=0.345) and central obesity based on WC and WHR (OR, 0.894; 95% CI, 0.072-9.992; *P*=0.846) and (OR, 0.795; 95% CI, 0.193-3.286; *P*=0.752).

In addition, mutant, GG genotypes relative to wildtype, CC genotype not associated with underweight based on MUAC , (OR, 1.880; 95% CI, 0.413-8.548; *P*=0.414) and central obesity based both WC (OR, 0.786; 95% CI, 0.065-9.568; *P*=0.850) and WHR (OR, 3.340; 95% CI, 0.611-18.263; *P*=0.164). However, the genotype associated with hypoalbuminemia, (OR, 1.933; 95% CI, 1.252-4.664; *P*=0.033) and underweight based on both BMI (OR, 2.412; 95% CI, 1.214-5.782; *P*=0.026) and WHtR; OR, 7.472; 95% CI, 1.216-15.906; *P*=0.030)

Table 4.5: Association between rs1445776009 genotypes and hypoalbuminemia, adiposity markers in the ART-experienced ISUs.

Genotype	Markers	HIV-1[+] ART [-], n=93		
		OR	95% CI	*P*
CG	Hypoalbuminemia	9.680	0.668-14.285	0.096
	BMI, kg/m2	1.614	1.068-3.706	0.071
	MUAC, cm	1.204	0.248-5.848	0.818
	WC, cm	0.894	0.072-9.992	0.846
	WHR	0.795	0.193-3.286	0.752
	WHtR	1.568	0.855-15.149	0.345
GG	Hypoalbuminemia	1.933	1.252-4.664	**0.033**
	BMI, kg/m^2	2.412	1.124-5.782	**0.026**
	MUAC, cm	1.880	0.413-8.548	0.414
	WC, cm	0.786	0.065-9.568	0.850
	WHR	3.340	0.611-18.263	0.164
	WHtR	7.472	1.216-45.906	**0.030**

Data shown are Odd ratio (OR), 95%CI, at 95% confidence interval of the subject. ISU, injection substance use; HIV-1[+], human immunodeficiency virus type-1; ART [+], anti-retroviral treatmentART-experienced; CG, Heterozygous; GG, Homozygous mutants; hypoalbumnemia (albumin<32 g/l), BMI, Body mass index, underweight <18.5 kg/m^2, MUAC, mid-upper arm circumference, underweight <24.5cm; WC, Waist circumference, central obesity (M/F; ≥94/≥80 cm); WHR, Waist to hip ratio, central obesity (M/F; ≥0.88/ ≥86) and, WHtR, Waist to height ratio, underweight <0.5. Values in bold are significant *P*-values at a cut-off of $P<0.05$.

4.7. Association between rs1445776009 genotypes andhypoalbuminemia, adiposity markers in theART-naive ISUs.

The association between rs1445776009genotypes andhypoalbuminemia and adiposity marker in the ART-experienced ISUs is summarized in Table 4.6. The heterozygous, CG genotype in relation to wildtype, CC genotypeswere not associated with hypoalbuminemia (OR, 1.123; 95% CI, 0.289-4.367; $P=0.867$), underweight based on BMI, MUAC and WHtR (OR, 0.623; 95% CI, 0.225-1.726; $P=0.362$), (OR, 0.609; 95% CI, 0.209-1.775; $P=0.364$) and (OR, 0.490; 95% CI, 0.126-1.910; $P=0.304$) and central

obesity based on WC (OR, 0.425; 95% CI, 0.058-3.113; *P*=0.400) and WHR and (OR, 0.441; 95% CI, 0.107-1.822; *P*=0.258)

Similarly, mutant, GG genotypes relative to wildtype, CC genotype did not associated with hypoalbuminemia (OR, 0.712; 95% CI, 0.164-3.090; *P*=0.650), underweight based on BMI, MUAC and WHtR (OR, 0.679; 95% CI, 0.222-2.081; *P*=0.499), (OR, 0.807; 95% CI, 0.282-2.309; *P*=0.690) and WHtR (OR, 1.116; 95% CI, 0.229-5.432; *P*=0.892) and central obesity based on WC (OR, 0.437; 95% CI, 0.602-3.469; *P*=0.569) and WHR and (OR, 0.401; 95% CI, 0.086-1.861; *P*=0.243).

Table 4. 6: Association between rs1445776009 genotypes and hypoalbuminemia and adiposity markers in the ART-naive ISUs.

Genotype	Marker	HIV-1[+] ART [+], n=62		
		OR	95% CI	*P*
CG	Hyoalbuminemia	1.123	0.289-4.367	0.867
	BMI, kg/m^2	0.623	0.225-1.726	0.362
	MUAC, cm	0.609	0.209-1.775	0.364
	WC, cm	0.425	0.058-3.113	0.400
	WHR	0.441	0.107-1.822	0.258
	WHtR	0.490	0.126-1.910	0.304
GG	Hyoalbuminemia	0.712	0.164-3.090	0.650
	BMI, kg/m^2	0.679	0.222-2.081	0.499
	MUAC, cm	0.807	0.282-2.309	0.690
	WC, cm	0.437	0.602-3.469	0.569
	WHR	0.401	0.086-1.861	0.243
	WHtR	1.116	0.229-5.432	0.892

Data shown are Odd ratio (OR), 95%CI, at 95% confidence interval of the subject. ISU, injection substance use; HIV-1[+], human immunodeficiency virus type-1; ART [-], anti-retroviral treatmentART-naive; CG, Heterozygous; GG, Homozygous mutants; hypoalbuminemia (albumin<32 g/l), BMI, Body mass index, underweight <18.5 kg/m^2, MUAC, mid-upper arm circumference, underweight <24.5cm; WC, Waist circumference, central obesity (M/F; ≥94/≥80 cm); WHR, Waist to hip ratio, central obesity (M/F; ≥0.88/ ≥86) and, WHtR, Waist to height ratio, underweight <0.5. Values in bold are significant P-values at a cut-off of *P*<0.05.

4.8. Association between rs1445776009 genotypes and immunosuppression and high density HIV-1 viremia in the ART-experienced ISUs.

The association between rs1445776009genotypes with immunosuppression and high density HIV-1 viremia in the ART-experienced ISUs is summarized in Table4.8. The heterozygous, CG genotype in relation to wildtype, CC genotype did not associated with both immunosuppression (OR, 2.750; 95% CI, 0.849-8.913; *P*=0.092) andhigh density HIV-1 viremia, (OR, 1.644; 95% CI, 0.515-5.252; *P*=0.401). However, mutant, GGgenotypes relative to wildtype, CC genotype associated with both immunosuppression (OR, 3.036; 95% CI, 1.957-9.633; *P*=0.024) and high density HIV-1 viremia, (OR, 1.836; 95% CI, 1.134 -1.286; *P*=0.016).

Table 4. 7: Association between rs1445776009 genotypes with immunosuppression and high density HIV-1 viremia in the ART-experienced ISUs.

Genotypes	Clinical characteristics	HIV-1[+] ART [+], n=93		
		OR	95% CI	*P*
CG	Immunosuppression	2.750	0.849-8.913	0.092
	High density HIV-1 viremia	1.644	0.515-5.252	0.401
GG	Immunosuppression	3.036	1.957-9.633	**0.021**
	High density HIV-1 viremia	1.836	1.134 -9.286	**0.016**

Data shown are OR, odds ratio; 95%CI, at 95% confidence interval; HIV-1[+], human immunodeficiency virus type-1; ART [+], anti-retroviral treatment ART-experienced; CG, Heterozygous; GG, Homozygous mutants;Immunosuppression <500CD4+ T cell cells/μl and High density HIV-1 viremia>1000 HIV-1 RNA copies/ml. Values in bold are significant *P*-values at a cut-off of <0.05.

4.9. Association between rs1445776009 genotypes with CD4+ T cells counts and viral load in the ART-naive ISUs.

The association between rs1445776009genotypes with immunosuppression and high density HIV-1 viremia in the ART-experienced ISUs is summarized in Table 4.9. The heterozygous, CG genotypes in relation to wildtype, CC genotypeswere not associated with both immunosuppression (OR, 1.631; 95% CI, 0.407-6.538; *P*=0.490) andhigh density HIV-1 viremia, (OR, 1.306; 95% CI, 0.515-5.252; *P*=0.698). In addition, mutant, GG genotypes relative to wildtype, CC genotype was not associated with both immunosuppression (OR, 1.721; 95% CI0.453-6.545; *P*=0.426) and high density HIV-1 viremia, (OR, 2.88; 95% CI, 0.711-11.724; *P*=0.138).

Table 4. 8: Association of rs1445776009 genotypes Immunosuppression and High density HIV-1 viremia in the ART-naive ISUs

	Clinical characteristic	HIV-1[+] ART [-], n=62		
		OR	95% CI	*P*
CG	Immunosuppression	1.631	0.407-6.538	0.490
	High density HIV-1 viremia	1.306	0.339-5.032	0.698
GG	Immunosuppression	1.721	0.453-6.545	0.426
	High density HIV-1 viremia	2.88	0.711-11.724	0.138

Data shown are OR, odds ratio; 95%CI, at 95% confidence interval; HIV-1[+], human immunodeficiency virus type-1; ART [-], anti-retroviral treatment ART-naive; CG, Heterozygous; GG, Homozygous mutants; Immunosuppression <500 CD4+ T cell cells/µl and High density HIV-1 viremia>1000 HIV-1 RNA copies/ml. Values in bold are significant *P*-values at a cut-off of <0.05.

CHAPTER FIVE: DISCUSSION

5.1. Demographic, anthropometric and substance use profilesof ISUs

Evaluation of demographic and anthropometric data revealed that weight and BMI were significantly lowin ART-experiencedas compared ART-naive ISUs. A part from low weight and BMI, HIV-1 infected ART-experienced ISUs also presented with reduced MUAC and waist to height ratio relative to theART-naivestudy participants. Besides, a majority of underweight study participants were in ART-experienced relative to the ART-naive ISUs group. This finding implies that ART could be a factor contributing to underweight. In line with this study findings, HIV-1 alone has been associated with reduced MUAC and underweight in HIV-1 infected ISUs. For instance, a survey carried out in Kenya by WHO in HIV-1 positiveART-naivepregnant women reported reduced MUAC and underweight (<21cm) (Liu *et al*., 2011; WHO, 2019). Also, ART has been associated with reduced MUAC and underweight at phase 1 of the introduction to ART(Petersen et al., 2014). This has been associated with the metabolic complication of the ART which results in mal-absorption of nutrients resulting in muscle wasting and general weight loss (Feigl *et al*., 2016; Zemede *et al*., 2019). Contrary to this finding, previous studies have shown an increase in BMI upon initiation on ART(Chen *et al*., 2018; Jiang *et al*., 2019; Kim *et al*., 2017; Martinez *et al*., 2016). For instance, an institutional-based retrospective study reported an increase in BMI upon second-line ART initiation for the first two years (Baraki *et al*., 2019). Furthermore, a majority of ART-experienced ISUs were in WHO stage III and IV. This could imply that they were at worse weight gain state in comparisonto patients at the low WHO stage. The potentialcause could be HIV enteropathy,unsolved chronic diarrhoea, mal-absorption,

high fever associated with the late stage of the diseasedevelopment, non-adherence and ART defaulting. Taken together, these factors may lead to leads to increased energy loss and muscle wasting (Feigl *et al*., 2016; Martinez *et al*., 2016; Nooka & Ghorpade, 2017; Deribe *et al*., 2008; Pengpid *et al*., 2019; Shukla *et al*., 2016). In HIV-1 alone, underweight has been a common phenomenon due to metabolic complications hence leading to micronutrient deficiencies such as selenium, zinc, vitamins A, B12 and vitamin D which generally have been associated with undernourishment and subsequent underweight (Adhikari *et al*., 2016; Deshwal & Arora, 2019; Phiri *et al*., 2019).

Waist andhip circumference are key anthropometric measurements for central obesity. WC and HC were low in ART-experienced relative to ART-naive. The current study revealed a low proportion of central obesity in ART-experienced relative to ART-naive. This findings imply that ART could be a key factor contributing to low central obesity among ART-experienced. Previous studies have alsoreported an increase in proportion of central obesity among HIV-1 patients. For instance, a cross-sectional study in Copenhagen in United State of America comorbidity among people living with HIV-1 verse HIV-1 negative reported significantly high rate of central obesity in HIV-1 positive relative to the control group (Gelpi *et al*., 2018). Low central obesity in the ART-experienced ISUs could also be attributed to fat redistribution syndromes amplified by the parallel interconnected synergistic interaction between HIV-1 infection, substance abuse and ART(Castro *et al*., 2016; Martinez *et al*., 2016).

Apart from the anthropometric measurement of the study participants, the current study also investigated substance use profiles of the study subject. This study found heroin and cocaine to be the most injected illicit substances. The current study is in line with a longitudinal study carried out among people who inject drugs in Kenya(PWID) Kenya which reported heroin and cocaine as key ISUs along the coast of Kenya (Guise *et al*., 2015). Also, cross-sectional clinical laboratory study conducted in coastal region of Kenya among HIV-1 infected ISUs and a cross-sectional descriptive studyconducted in HIV-1 infected and uninfected intravenous substance users at coastal, Kenya reported a high prevalence of heroin and cocaine use (Budambula *et al*., 2018; Were *et al*., 2014a). Furthermore, UNDOC has also reported heroin and cocaine to be the most prevalent injected illicit substances globally (UNDOC, 2019a). The substances are highly addictive and dependency hence they are likely to be the most dominant substances among ISUs (Sun *et al*., 2019; Syvertsen *et al*., 2015). Other substances injected by study participants were diazepam and flunitrazepam. Besides injection substance use, this study found that cigarettes, bhang, and alcohol were commonly used non-injection substances The findings are also consistent with UNODC world drug report of 2019 which found bhang, alcohol, and cigarettes to be the most abused non-injection substances (Kuteesa *et al*., 2019, 2019; UNDOC, 2019a). Other abused non-injection substances by study participants included brown sugar, flunitrazepam, and khat.In terms of duration of injection substances use, most of the study participants were on injection substance use for more than one year. Furthermore, theART-experienced ISUs had significantly more individuals who had injected for longer durations than in theART-naive. This study also revealed that most of the study participants injected more than once a day with the ART-

experienced injecting more frequently than the ART-naive ISUs. Therefore, the current study could imply that the ART-experienced group was adversely affected relative to ART-naive.

5.2. Clinical and laboratory characteristics of study participants

CD4+ T cell countwas significantly low (<500 CD4+ T cell/ml) in ART-experienced relative to ART-naive ISUs. However, HIV-1 RNA, copies were significantly high (>1000 HIV-1 RNA, copies/ul) in the ART-experienced and ART-naive ISUs.ART is supposed to reduce disease adversity in HIV-1 patients. However, these findings suggest that theART-experiencedISUs are highly affected relative to theART-naive ISUs. This could be due to the fact that most of the ART-experienced participants were in stages II, III and IV while the ART-naive were mainly in stage I of WHO immunological staging of HIV-1 infection(WHO, 2019e). In addition, the ART-experienced ISUs had been enrolled for a longer duration, some may have defaulted ART even though information on defaulting was not collected. Furthermore, some of the ART-experiencedwere probably at the chronic phase of the disease progression, though information on duration of HIV-1 infection was not also collected and therefore, appear severely affected.

Further, the ART-experienced had low circulating albumin were compared to the ART-naive ISUs. Consistent with low albumin levels in the ART-experienced participants, the current study also found a high rate of hypoalbuminemia. This finding implies that ART could be lowing the albumin level in theART-experienced ISUs. The findings were consistent with a study conducted in this population and reported a high serum albumin

levels in the ART-naive compared to ART-experienced ISUs (Were *et al*., 2014). Contrary to this finding, previous studies have reported elevated serum albumin levels in ART ART-experienced HIV-1 patients (Carter, 2006; Chong *et al*., 2015; Ronit, 2017), although these studies only focused on HIV-1 infected patients. In as much as initiation on HAART is associated with elevated albumin, prolonged use of HAART could lead to liverhepatoxicity which in turn suppresses the production of circulating albumin (Inductivo-Yu & Bonacini, 2008). This situation is further compounded by substance use that has a direct and adverse effect on the liver. Thus, low serum albumin levels in the experienced could be due to hepatoxicity emanating from both ART and injection substance use.

Plasma lipid panels were similar between ART-experienced and ART-naive ISUs. Even though HIV-1 infection alone has been linked to increase in total cholesterol levels, triacylglycerol, low-density lipoprotein and a decrease in high-density lipoprotein levels in HIV-1 infected alone(Denue *et al.*, 2013). This has been associated with cardiovascular disorders among others (Matoga *et al*., 2017; Phalane *et al*., 2019). Besides HIV-1 infection, some ART such as lopinavir have been associated with hyperlipidemia(Gelpi *et al*., 2018; Matoga *et al*., 2017), while others ARTs such, rilpivirine have been associated with normal levels of lipid profile in HIV-1 infected individuals(Gatechompol *et al*., 2019). Further,variations in lipid profiles could be as a results of substance use such as cannabinoids, mate tea, and chocolate which are associated with elevated lipid pane among HIV-1 ISUs (Scheffler *et al*., 2018; Souza *et al*., 2017).

5.3. Distribution of human intron VII *ALB*gene rs1445776009 genotypes and alleles in study population.

The current study analyzed rs1445776009 genotypes and alleles distribution in the study groups. Thefrequency of the genotype and allele between the ART-experienced and –naive was similar. A majority of the study participants were homozygous wild type, with C, alleleprevalent in both ART-experienced and ART-naive study participants. The current study is consistent with other studies that have reported on the frequency of the rs1445776009 genotypes and alleles in African populations such as the San and Bantu of South Africa (Moolman, *et al*., 1991). Analysis of the human intron VII *ALB* gene rs1445776009 alleles revealed consistency with the Hardy-Weinberg equilibrium, Implying that the rs1445776009genotype and allele frequencies are equal in this population, which possibly could suggest that it is a discrete non-overlapping population where there is no random mating (Wang *et al* 2017). Furthermore, the study found low heterozygosity in the study population, which is generally associated with inbreeding in a population (Ceballos *et al*., 2018; Rosenberg & Jakobsson, 2008). Since this study was carried out in Mombasa County where the inhabitants are mainly of Mijikenda sub-tribe then it is possible that majority of the study participants were of theMijikenda Bantu ethnic group. This study is consistent with previous studies; a case-control and a prospective hospital-based in the community adjacent to the current study site among children of <13 years and -14 year, respectively. Both studies reported a high homozygosity and low heterozygosity among several markers including rs1445776009 marker that were associated with bacteriemia and severe malaria (Lyons *et al*., 2009; Ndila *et al*., 2018). Similarly, low heterozygosity in this population has been reported in

the ADIPOQ gene(Shaviya *et al*., 2020). These observation could be attributed to high incidences of incest and marriage between close relatives in this population (Parkins, 2019).

5.4. Association between rs1445776009 genotypes and hypoalbuminemia, adiposity markers

The study found that themutant, GG genotype carriageare associated with high odds of having low serum albumin levels (<32g/l)relative to wildtype, CC genotype among HIV-1 infectedART-experienced ISUs. The result suggest GG, genotype carrier, HIV-1 positive ART-experiencedISUs are highly associated withhypoalbumnemia. The possible reason could be due complex interaction of ART, injection substances and HIV-1.The findings are concordant with studies that have reported variation in serum albumin levels depending on polymorphisms in the *ALB* gene including rs1445776009 (Minchiotti *et al*., 2008). In addition, studies have reported underweight and reduced serum albumin levels in the HIV-1infected individual alone (Dao *et al*., 2011; Dusingize *et al*., 2012). Furthermore,injection substance and ART use have been shown to influence various gene expression and subsequently affect both structure and quantity of encoded protein (Bannon *et al*., 2005; Mash *et al*., 2007; Rhodes & Crabbe, 2005; Sanli *et al*., 2015; Vahey *et al*., 2007). In contraryto this findings, previous studies in HIV-1 infected alone have shown an increase in serum albumin levels uponinitiation on ART. However, prolonged ART use leads to liver toxicity which reduces synthetic functionality of the liver (Rodrigues *et al*., 2019; Vahey *et al*., 2007). However, it's important to note that very few studies have analyzed albumin levels to the level of gene polymorphism in HIV-

1 infected ISUs patients. The current study is the first one to describe rs1445776009 in HIV-1 infected ISUs.

Addition to reduced serum albumin, GG genotype also was associated with low BMI (18.5kg/m^2) in theART-experiencedISUs. The possible reason could be due both injection substance use, HIV-1 infection and ART complex interaction. Low BMI could be as result of fatty change of the liver due to poor nutrition, loss of micro proteins the urine and low serum albumin associated with drug toxicity in the liver (Zemede *et al*., 2019). Both BMI and albumin are important predictors HIV-1 outcomes (Bhamidipati *et al*., 2011; Jiang *et al*., 2019; Ronit, 2017). Previous studies have shown that patients presenting with low BMI often have low circulating albumin levels and vice versa (Chen *et al*., 2018; Chong *et al*., 2015; Feigl *et al*., 2016). Since GG, genotype carriage was demonstrated to be associated with high odds of presenting with low serum albumin levels and low BMI, then it follows that patients with these genotypes are likely to be associated with both hypoalbuminemiaand underweight.

5.5. Association between rs1445776009 genotype andimmunosuppression, high density HIV-1 viremia.

The current study revealed that mutant, GG, genotype relative to wildtype, CC genotypes were associated with high odds of being immunosuppressed (CD4+ T cell count <500/µl) and having high density viremia (>1000 HIV-1 RNA copies/ml of blood) in theART-experienced ISUs. The possible reason could be due to low serum albumin levels which act as a carrier for transportation of ARTdrug components to various tissues to boost

immunological tissues in fightingamplification of the HIV-1 virus. In addition, since majority of the HIV-1 ART-experienced participants were underweight, then it imply that they were malnourished, leading depletion CD4+ T cellscount and high density HIV-1 RNA copies. This is consistent with previous studies, where reduced serum albumin levels, low CD+ T cell count and high density HIV-1 RNA viremia have been report in the ART-experienced alone (Koethe & Heimburger, 2010; Olawumi & Olatunji, 2006b; Sundaram *et al*., 2009; Tabarsi *et al*., 2012). For instance, a cross sectional hospital based study at JLN Medical College and Hospital among 200 ART-experienced patients, reported significant positive correlation between serum albumin levels and CD4+ count, $P<0.001$ (Pralhadrao *et al*., 2016). In contrary, this findings, ART have been shown to increase both serum albumin levels and CD4+ T cells count low HIV-1 RNA copies in HIV-1 alone(Petersen *et al*., 2014), however, prolonged use of ART affects gene expression in liver and other immunological cell such as macrophages responsible for fighting HIV-1 infection, leading to low serum albumin levels , low CD+ T cells and high viral load(Adedeji *et al*., 2019; Rodrigues *et al*., 2019). Furthermore, substance use have been found to stimulate replication of HIV-1 RNA copies as demonstrated in vitro cocainein cytomegalovirus-stimulated peripheral blood mononuclear cells study(Peterson *et al*., 1992). Since GG, genotype carriage associated with high odds of being, underweight, hypoalbuminemia, immunosuppressed and having high density HIV-1 viremia, then, it follows that patients with GG, genotype carrier HIV-1 ART-experienced ISUs are likely to be at poor state of disease progression. Taken together, injection substance use, HIV-1 infection, ART as well as host genetics appears to influence gene expression leading to altered circulating albumin in both HIV-1 infected ART-

experienced ISUs. This study shows that mutant, GG genotype carriage are at poor state of disease outcome as compared to other genotypes carriage.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions of the study

The current study demonstrated that the ART-experienced ISUs presented with low circulating albumin levels, (<32g/l) and low BMI, (<18.5kg/m^2), low CD4+ count(<500 copies/ml) and high density HIV-1 RNA copies (>1000 HIV-1 RNA copies/ul).

Thefrequencyof the human intron VII *ALB* gene rs1445776009genotypes and alleles was similar in the ART-experienced,ART-naive largely dominated by CC, genotypes (wildtype) and C, alleles (major). In addition, allele's frequency deviated from hardy Weinberg equilibrium with low heterozygosity.This could imply that there is inbreeding in this study population.

The findings indicated that mutant, GG genotypes relative to wildtype, CC genotypes was associated with underweight, hypoalbuminemia, immunosuppression and high density HIV-1 viremia in the ART-experienced ISUs. Thus GG genotype carrier in ART- experienced ISUs are at poor state of disease progression unlike CC and CG genotype carriage.

6.2. Recommendations

The analysis of rs1445776009 intron VII variation in the human *ALB* gene should be includedas basis for conducting genetic studies in HIV-infection ISUs in

understanding a complex interaction of the host genetic factor,injection substance use, HIV-1 infection and ART influence on HIV-1 disease outcome.

Serum albumin levels can be used as a diagnostic marker ofdisease developmentin the HIV-1infectedISUs as it has been demonstrated that serum albumin levels regresses as the disease severity of disease progresses.

ALB gene can be used as surrogates of disease outcomes in HIV-1 infection ISUs since it influence HIV-1 disease outcome.

6.3. Recommendations for further research

A prospective study design, using additional polymorphisms using gene sequencing other than RFLPs should be studied in predicting possible disease outcomes of the patients other than just focusing on a single SNP.

Other disease markers such as liver functional test, renal function test, inflammation and anaemia, should be studiedin predicting disease outcome among HIV-1 infected ISUs since HIV-1 infection influence functionality of various systems and organs.

Invitro study could be carried out to provide a mechanism on how injection substances, HIV-1 infection, and ARTinteract with *ALB* gene and the subsequent effect on the disease outcome.

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APPENDIX I: INFORMED CONSENT FORM

English Version

Title: HIV infection amongst Injection Drug Users in Mombasa, Kenya.
Dear participant:
You are invited to take part in this research study because you have a history of intravenous drug use. This form tells you why this research study is being done, please read then you can decide if you want to join this study or not. The Investigators in this study are from MasindeMuliro University. A study team will be working closely with the investigators and the study will run for 3 years.
The purpose of this study is to determine the factors associated with HIV infection among injection drug users and non-drug users. If you choose to participate in this study, the team will require 3ml of blood (HIV voluntary testing and Complete Blood Count). No drug or chemical will be introduced into your body.
You can decide whether to take part in this study or not. You are free to say yes or no. If you say no, your regular medical care will not change. Even if you join this study, you do not have to stay in it, you may stop at any time. It is important to note that there is no financial benefit for participating in this study at the same time there will be no cost implications for you. Participation in this study is important as the findings of the study have the potential of being used to lobby for funding for antiretroviral drugs (ART) and primary healthcare for drug users.
The risks in this study include possible discomfort due to questions on health and personal behaviour/history. Also, discomfort may be experienced while a blood sample is being obtained. Every effort will be made to keep your study records confidential but we cannot guarantee it. No funds have been set aside to pay any costs if you are harmed because of this study. If you think that you were harmed because of this study, contact the Principle or co-Investigator.
By signing my name below, I confirm the following:
I have read (or been read to) this entire consent document. All of my questions have been answered to my satisfaction. The study's purpose, procedures, risks, and possible benefits have been explained to me. I agree to let the study team use and share the health information and other information gathered for this study. I voluntarily agree to participate in this research study. I agree to follow the study procedures as directed. I have been told that I can withdraw from the study at any time.
Participant's Name..... Signature.....Date.....
Principal Investigator..... Signature.....Date..... Or the supervisor
Note: Below are some of the key contacts
Principle investigator – Erick Barasa 0710238293; Co-Investigator – Dr. Tom Were 0720326127;

Kiswahili Version

MadaYaUtafiti: Uambukizopamojawavirusivya HIV katiyawatumiajiwamihadaratikwakujidunganawasiotumiwamadawayakulevya, Mombasa Kenya

Kwakomhusika:
Unaalikwakushirikikwenyeutafitikwasababuukonahistoriayautumiziajiwamihadaratikwakujidungashindano. Fomuhiiinakuelezeakwasababuganiutafitihuuunafanywa. Tafadhali soma fomuhiihalafuuamuekamautashirikikwenyeutafitihuu. Watafitiniwanatoka Chuo kikuu cha MasindeMulirona Chuo cha utalamu cha Mombasa. Timuyautafitiitafanyakazikwakaribunawatafitiwakuu, nautafitimwenyeweutachukuamudawamiakamiwili.
Nia hasayautafitihuunikutathimini au kuamuasababuzinazohusishwanauambukizopamojawavirusivya HIV nakifuakikuukatiyawatumiajiwamihadaratikwakujidunga.
Ukichaguakushirikikwenyeutafitihuu, hiitimuyawatafitiitahitajikiasi cha mililita 3 zadamukutokakwako (kwaajiliyaupimajiwahiyariwavirusivya HIV nahesabuyakiwango cha damu). Hakunadawaamakemikalizozotezitakazoekwakwamwiliwako.
Unawezakutoauamuziwakushirikikwenyeutafitihuu au la, piaukohurukusemandioama la. Ukisema la matibabuyakoyakawaidahayataathirika. Si lazimakubakikamamshirikiunawezaukakatizakushirikiwakatiwowote. Ni muhimukufahamukwambahakunafaidazakifedhakwakushirikikwenyeutafitihuu.
Wakatihuohuohautagaramikakivyovyotekifedhakwakushiriki.
Kushirikikatikautafitihuunimuhimukwasababu, uvumbuziamamajibuyautafitihuuyatasaidiakupigania au kushawishimisaadayakifedhakwadawazakuvunjamakaliyavirusinapiaafyayamsingikwawatumiajiwamihadarati.
Hatarizinazoambatananakushirikikatikautafitihuunikamausumbufukutokananamaswaliya kiafyanayakibinafsihasatabianahistoriayako.
Kadhalikautahisiusumbufuhasawakatiwakutolewadamu.
Juhudizotezitafanywakwaajiliyakuhifadhihabarinajumbezakozotekwanjiayausiriwahaliyajuu. Lakinihatuezikuhakikishahili. Hakunafedhaambazozimehifadhiwakwaajiliyakukufidiaendapoutadhurikakutokananautafitihuu. Kama unafikiriakwambaulidhurikakutokananautafitihuuwasilianamtafitimkuu.
Kwakuwekasahihijinalangunathibitishayafuatayo:
1) nimesoma (amanimesomewa) karatasihiiyakutoaidhiniyakukubali, namaswaliyanguyoteyamejibiwananimeridhika; 2) nia, mitindo, hataripamojanafaidazinazoambatananautafitihuuzimeelezwakwangu; 3) nakubalinakuruhusutimuyautafitikutumianakugawahabarizakiafyaamaainayoyoteyahabarizitakazokusanywakutokananautafitihuu; 4) nimekubalikwahiyarikushirikikwenyeutafitihuu. Nakubalikufuatamitindoyautafitihuu; 5) nimeelezwakwambaninawezakukomakushirikiwakatiwowote.
Jina la mshiriki..... Sahihi..... Tarehe.....
Mtafitimkuu/Msaidizi..... Sahihi..... Tarehe.....

Zaidi; wasilianana wafuatao
Mtafitimkuu – Erick Barasa 0710238293; Mtafitimsaidisi – Dr. Tom Were 0720326127;

APPENDIX II: STUDY QUESTIONNAIRE

English Version

Title: HIV infection amongst injection Drug Users in Mombasa, Kenya.

Study participant code _____ Study participant's residence_____
Interview date_____/_____/_____

1. Age _____ (Years) Birth date_____/_____/_____
2. Gender: ☐ Male ☐ Female
3. Do you inject drugs?
   ☐Yes ☐No
4. If Yes in number 3 name them _____ _____ _____ _____

5. How long have you injected drugs? _____ (Months)

6. How many times per day do you inject drugs_____?
7. Apart from injecting drugs, do you use any non-injecting drug?
   Yes No
8. If yes in number 3 name the drugs _____ _____ _____ _____
9. Do you know your HIV status?
   ☐Yes ☐No
10. If yes in question 9, are you on antiretroviral therapy?
    ☐Yes ☐No

11. How long have you been on antiretroviral therapy?

Kiswahili version

MadaYaUtafiti:
UambukizopamojawavirusivyaukimwinaKifuakikuukatiyawatumiajiwamihadaratikwakujidunga, Mombasa Kenya

Nambari ya siri ya mhusika _____ Eneoanapoishimhusika_____
Sikuyakutahiniwa_____/_____/_____
1. Umri_____ (Miaka) Tareheyakuzaliwa_____/_____/_____
2. Jinsia: ☐ Mme Mke ☐
3. Je unajidungamadawayakulevya?
Ndi☐ La☐
4. kamaunajitungadawayakulevyayataje --------- -------- ----------
5. Umetumiamadawahayakwamudagani? _____ (Miezi)
6. Jeunajidungamarangapikwasiku?
7. Kandonamadawayakujitunga,
Jeunatumiamadawayakulevyayoyoteambayosiyakujitunga?
8. Yatajemadawayakulevyaunayotumia kyasioyakujitungaambayounatumia ---------- ---------- ----------
9. Je unajuahaliyakokuhusukirusi cha ukimwi?
☐Yes ☐No
10. Je unatumiamadawayakupunguzamakaliyakirusi cha ukimwi?
Ndiola☐ ☐
11 kamandivyonambari10,umetumiakwamudaupi?

APPENDIX IV: SCHOOL OF GRADUATE STUDY APPROVAL

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY (MMUST)

Tel: 056-30870
Fax: 056-30153
E-mail: directordps@mmust.ac.ke
Website: www.mmust.ac.ke

P.O Box 190
Kakamega – 50100
Kenya

Directorate of Postgraduate Studies

Ref: MMU/COR: 509099

8th May, 2019

Erick Juma Barasa,
SBM/G/01-54525/2017,
P.O. Box 190-50100,
KAKAMEGA.

Dear Mr. Barasa,

RE: APPROVAL OF PROPOSAL

I am pleased to inform you that the Directorate of Postgraduate Studies has considered and approved your masters proposal entitled: ***"Intronic Polymorphisms in ALB Gene Among HIV-1 Infected Antiretroviral Treatment -Naive and -Experienced Injection Substance Users in Mombasa County, Kenya"*** and appointed the following as supervisors:

1. Dr. Tom Were - SPHBST, MMUST
2. Dr.Nathan Shaviya - SPHBST, MMUST

You are required to submit through your supervisor(s) progress reports every three months to the Director Postgraduate Studies. Such reports should be copied to the following: Chairman, School of Public Health, Biomedical Sciences and Technology Graduate Studies Committee and Chairman, Medical Laboratory Sciences Department. Kindly adhere to research ethics consideration in conducting research.

It is the policy and regulations of the University that you observe a deadline of two years from the date of registration to complete your master's thesis. Do not hesitate to consult this office in case of any problem encountered in the course of your work.

We wish you the best in your research and hope the study will make original contribution to knowledge.

Yours Sincerely,

DEAN
SCHOOL OF GRADUATE STUDIES
MASINDE MULIRO UNIVERSITY
OF SCIENCE & TECHNOLOGY

Dr. Consolata Ngala
ASSOCIATE DEAN, DIRECTORATE OF POSTGRADUATE STUDIES

APPENDIX V: MMUST ETHICAL CLEARENCE

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY
Tel: 056-31375
Fax: 056-30153
E-mail: ierc@mmust.ac.ke
Website: www.mmust.ac.ke
P. O. Box 190-50100
Kakamega, Kenya

Institutional Ethics Review Committee (IERC)

Ref: MMU/COR: 403012 vol2 (17) **Date:** 6th June, 2019

Erick Juma Barasa
Masinde Muliro University of Science and Technology
P.O. Box 190-50100
KAKAMEGA

Dear Mr. Juma

RE: Intronic polymorphisms in ALB gene among HIV-1 infected antiretroviral –Naïve and experienced injection substance users in Mombasa County ,Kenya- MMUST/IERC/35/19

Thank you for submitting your proposal entitled as above for initial review. This is to inform you, that the committee conducted the initial review and approved (with minor changes) the above Referenced application for one year.

This approval is valid from **6th June, 2019 through to 6th June, 2020**. Please note that authorization to conduct this study will automatically expire on **6th June, 2020**. If you plan to continue with data collection or analysis beyond this date please submit an application for continuing approval to the MMUST IERC by **6th May, 2020.**

Approval for continuation of the study will be subject to submission and review of an annual report that must reach the MMUST IERC secretariat by **6th May, 2020**. You are required to submit any amendments to this protocol and any other information pertinent to human participation in this study to MMUST IERC prior to implementation.

Please note that any unanticipated problems or adverse effects/events resulting from the conduct of this study must be reported to **MMUST IERC**. Also note that you are required to seek for research permit from **NACOSTI** prior to the initiation of the study.

Yours faithfully,

Dr. Gordon Nguka (PhD)
Chairman, Institutional Ethics Review Committee

Copy to:
- The Secretary, National Bio-Ethics Committee
- Vice Chancellor
- DVC (PR&I)
- DVC (A & F)

APPENDIX VI: KU ETHICAL CLEARANCE

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Fax: 8711242/8711575
Email: kuerc.chairman@ku.ac.ke
kuerc.secretary@ku.ac.ke
Website: www.ku.ac.ke

P. O. Box 43844,
Nairobi, 00100
Tel: 8710901/12

Our Ref: KU/R/COMM/51/32-4

Date: June 6th, 2012

Valentine Budambula
School of Public Health,
Kenyatta University
P.O. Box 43844, Nairobi.

Dear Ms. **Valentine**

APPLICATION NUMBER PKU019/I16 of 2012 - **'HIV/Pulmonary TB co-infection amongst intravenous drug users in Mombasa, Kenya. Version 4.**

1. IDENTIFICATION OF PROTOCOL

The application before the committee is with a research topic 'HIV/**Pulmonary TB co-infection amongst intravenous drug users in Mombasa, Kenya'**, Version 4. Dated 19th May, 2012.

2. APPLICANT

Valentine Budambula
School of Public Health,
Kenyatta University
P.O. Box 43844, Nairobi.

3. SITE

Mombasa County, Kenya.

4. DECISION REACHED.

The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines, and is of the view that against the following elements of review,

i. Scientific design and conduct of study,
ii. Recruitment of research participant,
iii. Care and protection of research participants,
iv. Protection of research participant's confidentiality,
v. Informed consent process,
vi. Community considerations.

AND APPROVED that the research may proceed for a period of ONE year from 6th June, 2012.