Characterization of the steady state concentrations and pharmacogenetics of ritonavir boosted atazanavir in Kenyan HIV positive patients

George A Mugendi

U80/51877/2017

B. Pharm, M. Pharm (Clinical Pharmacy)

Supervisors

Dr Nasser Nyamweya, Professor Faith Okalebo

School of Pharmacy, University of Nairobi

Professor Jashvant D. Unadkat

School of Pharmacy, University of Washington

A thesis submitted in partial fulfilment of the requirements for the Doctor of Philosophy in Clinical Pharmacy of the University of Nairobi

November 2020

UNIVERSITY OF NAIROBI: DECLARATION OF ORIGINALITY FORM

NAME: George Arthur Mugendi – U80/51877/2017

COLLEGE: College of Health Sciences

SCHOOL: School of Pharmacy

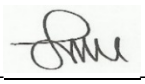
DEPARTMENT: Department of Pharmaceutics and Pharmacy Practice

COURSE: Doctor of Philososphy (Clinical Pharmacy)

TITLE OF WORK: CHARACTERIZATION OF THE STEADY STATE CONCENTRATIONS AND PHARMACOGENETICS OF RITONAVIR BOOSTED ATAZANAVIR IN KENYAN HIV POSITIVE PATIENTS

Declaration

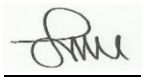
1. I understand what plagiarism is and I am aware of the University's policy in this regard.
2. I declare that this thesis is my original work and has not been submitted for examination, award of a degree or publication. Where other people's work or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi requirements.
3. I have not sought or used the services of any professional agencies to produce this work.
4. I have not allowed, and shall not allow anyone to copy my work with the intention of passing it off as their own.
5. I understand that any false claim in respect of this work shall result in disciplinary action, in accordance with the University Plagiarism Policy.

Signature  Date 22/11/2020

DECLARATION

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

George A. Mugendi (U80/51877/2017), M. Pharm. (Clinical Pharmacy)

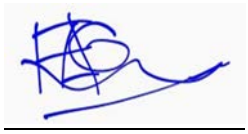
Signature  Date 22/11/2020

This thesis has been submitted for review with our approval as university supervisors.

Nasser Nyamweya, PhD

Signature  Date 23/11/2020

Faith Okalebo, PhD

Signature  Date 23/11/2020

Jashvant Unadkat, PhD

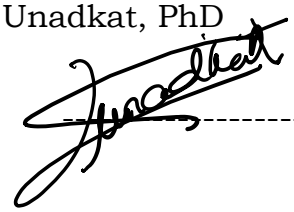
Signature  Date –11/23/2020

Table of Contents

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND ACRONYMS

AIDS	Acquired immunodeficiency syndrome
ALT	Alanine amino transferase
ARV	Antiretroviral
ATV	Atazanavir
AUC	Area under the curve
ART	Antiretroviral therapy
BMI	Body mass index
cART	Combined antiretroviral therapy
CCC	Comprehensive care centre
CD4	Cluster of Differentiation 4
Cl	Clearance
Cmax	Maximum concentration
Cmin	Minimum concentration
CRF	Case report forms
CYP	Cytochrome
DAIDS	Division of AIDS
DNA	Deoxyribonucleic acid
K-EDTA	Potassium Ethylene Diamine Tetra acetic Acid
EFV	Efavirenz
eGFR	Estimated Glomerular Filtration Rate
EMEA	European Medical Agency
ERC	Ethics and Research review committee
FDA	Food and Drug Administration
FDC	Fixed Dose Combination
HAART	Highly Active Antiretroviral Therapy
HIV	Human immunodeficiency virus
HPLC UV	High Performance Liquid Chromatography with Ultraviolet detection
KAIS	Kenya Aids Indicator Survey
KDHS	Kenya Demographic and Health Survey
KNH	Kenyatta National Hospital
LPV/r	Lopinavir/ritonavir

MRP	Multidrug Resistance Associated Proteins
NASCOP	National AIDS and STI Control Program
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NVP	Nevirapine
OATP	Organic Anion Transporting Polypeptide
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
PI	Protease Inhibitor
RNA	Ribonucleic Acid
SDG	Sustainable Development Goals
SNP	Single Nucleotide Polymorphisms
sSA	Sub Saharan Africa
TDM	Therapeutic Drug Monitoring
$t_{1/2}$	Half life
UGT1A1	UDP glucuronosyltransferase 1 family, polypeptide A1
UoN	University of Nairobi
ULN	Upper Limit Normal
Vd	Apparent volume of distribution
WHO	World Health Organization

LIST OF OPERATIONAL DEFINITIONS

Allele	One of two or more alternative forms of a gene that arise by mutation and are found at the same place on a chromosome
Gene	Is the functional and physical unit of heredity passed from parent to offspring
Genotype	The entire complement of genes in an organism
Mutation	Permanent change of the nucleotide sequence of the genome of an organism
Phenotype	Observable physical or biochemical characteristics of an organism as determined by both genetic makeup and environmental influences
Polymorphism	Natural variation in a gene, DNA sequence or chromosomes that have no adverse reactions on the individual and occur with fairly high frequency in the general population.
Single nucleotide polymorphism (SNP)	Genetic variations with a frequency of greater than 1% in the population. Can also be described as differences in DNA sequence that give rise to different phenotypic forms.
Hyperbilirubinemia	Elevation of the serum bilirubin concentrations above the upper limit normal by predetermined levels
C minimum	The plasma concentration of a drug sampled prior to the next dose
C maximum	The plasma concentrations of a drug sampled upon completion of the absorption phase and prior to start of elimination

ABSTRACT

Background

Atazanavir is a protease inhibitor currently recommended for use as a second line agent in Human Immunodeficiency Virus (HIV) infected patients who have failed therapy on a first line regimen. The pharmacokinetics of atazanavir are highly variable and may be influenced by factors such as age, sex, weight and genetics. In Kenya, there is a paucity of local HIV patient data on atazanavir pharmacokinetics, the influence of cytochrome P450 subfamily 3A5 (CYP3A5) genetic polymorphisms on its disposition and the prevalence of hyperbilirubinemia in patients using the drug.

Objectives

We optimized and verified a high-performance liquid chromatographic method with ultra-violet detection (HPLC-UV) for the determination of plasma concentrations of atazanavir. We determined the prevalence of single nucleotide polymorphisms in CYP3A5 and uridine diphosphate glucuronosyltransferase 1 family, polypeptide A1 (UGT1A1) enzymes. Thereafter, we examined whether polymorphisms in CYP3A5 enzymes were associated with atazanavir steady-state plasma concentrations. We determined the prevalence and risk factors of hyperbilirubinemia and investigated the effect of UGT1A1 polymorphisms on bilirubin levels and the risk of hyperbilirubinemia.

Methods

We conducted a cross sectional study enrolling 110 male and female HIV positive patients on ritonavir boosted atazanavir over two months from the Kenyatta National Hospital. Participants were 18 years and older and provided written informed consent. We abstracted data from patient files, conducted interviews and drew five blood samples: one at 30 minutes before the morning dose then 2 and 4 hours after the morning dose. The two additional samples were for genotyping and for determination of plasma bilirubin levels respectively. Atazanavir quantification was achieved by high performance liquid chromatography with ultraviolet detection at 261nm. Genetic analysis was performed by fast real time polymerase chain reaction for CYP3A5 and UGT1A1 single nucleotide polymorphisms.

Statistical analysis was done in STATA version 13.1 and R i3.8.6 version 3.3.1 with the level of significance set at p value ≤ 0.05. Ethical approval was granted by the Kenyatta National Hospital and University of Nairobi Ethics and Research Review Committee (KNH-ERC/A/110).

Results

Optimization and verification of a HPLC-UV method for the quantification of plasma atazanavir levels was achieved. The coefficient of determination was > 0.99 and was linear between 100 -10000 ng/mL. The precision (% relative standard deviation) was 8,8% (SD 6.2) and the accuracy ranged from 91.3 -

120.8%. The limit of detection was 40 ng/mL whereas the limit of quantitation was 120ng/mL.

The prevalence of single nucleotide polymorphisms was as follows: CYP3A5*3 TT wild type genotype - 24%, heterozygous TC genotype - 70%, CC homozygous genotype -6%; CYP3A5*6 CC wild type genotype - 2.7%, heterozygous CT genotype - 97.3%; CYP3A5*7 AA wild type genotype – 1.8%, heterozygous A/- genotype – 95.5%, -/- variant genotype - 2.7%; UGT1A1 CC genotype 16.4%, heterozygous CT 83.6%.

A small proportion of participants had subtherapeutic plasma trough concentrations of atazanavir (13%) whereas 39% had supratherapeutic levels. CYP3A5*6 was found to influence the trough plasma concentrations of atazanavir (adjusted estimate 2151.1 ng/mL, 95% CI 275.5, 4026.8). The prevalence of grades III and IV hyperbilirubinemia among the participants was 12.7 and 1.8%. Hyperbilirubinemia of any grade was present in 57.3% of the participants

Risk factors for hyperbilirubinemia were high atazanavir trough concentrations (aOR 1.001, 95% CI 1.001, 1.002), UGT1A1 heterozygosity (aOR 7.01, 95% CI 1.86, 34.01) and a positive history of alcohol use (aOR 0.18, 95% CI 0.03, 0.78).

Conclusions

CYP3A5 and UGT1A1 polymorphisms exist in this population. A significant proportion of participants had supratherapeutic trough plasma concentrations of atazanavir. CYP3A5 polymorphisms have an influence on the trough

concentrations of atazanavir. Hyperbilirubinemia is prevalent in patients on atazanavir and is influenced by UGT1A1 status, trough plasma concentrations of atazanavir pharmacokinetics and alcohol use.

1.0 INTRODUCTION

1.1 Background on the HIV epidemic and atazanavir use

The United Nations Program on the HIV/Acquired Immune Deficiency Syndrome (AIDS) (UNAIDS) global AIDS update report released in May 2016 estimates that as of 2015, there were between 34-39.8 million people living with the HIV and AIDS globally. Further, there were 2.1 million people newly infected with HIV in the same year (2015). Most of the people living with HIV/AIDS live in sub-Saharan Africa (sSA) and the figure at 2015 was 19.1 million, with the largest number of new infections (960,000) again occurring in the same region (1).

Kenya is listed as one of the six HIV "high burden" countries in Africa. The national HIV prevalence stands at 6.0 % (with varying levels across the counties); this translates to approximately 1.6 million people living with HIV/AIDS as of 2013. Women are disproportionately affected, with a prevalence of 7.6% compared to 5.6% for men (2).

Most strategic plans are committed to ending new HIV infections by the year 2030 (1,3,4). One of the targets of Sustainable Development Goal (SDG) 3 is to attain zero new HIV infections by that timeline. The UNAIDS has ambitious much publicized 90/90/90 target: 90% of people (children, adolescents and adults) living with HIV know their status, 90% of people living with HIV who know their status are receiving treatment and 90% of people on treatment are virally suppressed (4).

In Kenya, there is a 78% national antiretroviral therapy (ART) coverage among adults and 42% among children with 34% of the population deemed as virally suppressed. By 2014, 64% of eligible males and 61% of eligible females were on ART (CD4 target then was 500) (3). A key consideration in scaling up treatment is the effectiveness of antiretrovirals (ARVs) used in the management of HIV. The Kenyan HIV treatment guidelines together with World Health Organization (WHO) guidelines provide information to clinicians on the appropriate regimens to use for each patient (5,6). Therapeutic options are classified as either first line or second line in most cases, with the choice depending on the clinical response of the patient.

Atazanavir (ATV) is a protease inhibitor (PI) that was approved for use in June 2003 by the United States Food and Drugs Authority (FDA) and thereafter by the European Medical Agency (EMEA) in 2004. Its advantage over other PIs was that it was the first to be dosed once only daily with a lower risk of gastrointestinal discomfort and dyslipidemias. It is available as three dosage forms: a 300 mg capsule with 100 mg of ritonavir (r) as a pharmacokinetic booster; 200 mg; and 400 mg of the unboosted ATV with the dose limit of 800 mg (permissible dose range is between 200-800 mg) (7,8). ATV is prescribed as a second line option to those who have failed treatment on recommended first line agents usually in combination with r as ATV/r 300/100 mg.

The drug is metabolized by cytochrome enzymes CYP3A4/5. Both ATV and r inhibit these two metabolizing enzymes to varying extents: r is a potent inhibitor

and inactivator of CYP3A4 whereas ATV is a mild inhibitor. ATV inhibits UGT1A1 resulting in an increase in the concentration of unconjugated bilirubin, more commonly called hyperbilirubinemia. CYP3A5 and UGT1A1 genetic mutations can result in enzymes with low to nil capacity to metabolize ATV and unconjugated bilirubin respectively and this is hypothesized to predispose patients to hyperbilirubinemia through increased plasma concentrations of atazanavir.

1.2 Study problem

The risk of ATV/r induced hyperbilirubinemia (defined as Grade III - >2.5 times the Upper Limit Normal (ULN), and Grade IV - >5 times the ULN) in different populations is predicted to lie between 20% and 52% (9). This risk is driven primarily by elevated plasma concentrations of ATV which we hypothesize could be due to single nucleotide polymorphisms in CYP3A5 and UGT1A1 leading to expression of non-functional alleles *3/6/7 and *28 respectively. Hyperbilirubinemia could potentially lead to reduced adherence to ATV and result in treatment discontinuation when patients suspect that the drug is causing them harm (10).

Genetic polymorphisms in CYP3A5 and UGT1A1 enzymes have been studied to a greater extent in Caucasian and Asian populations compared to Africans. Data from diverse indigenous African populations suggest that there is considerable variability in the CYP3A5 gene, with East Africa termed the most heterogeneous region (11). A scrutiny of the ethnic groups sampled in those studies indicates

that none were drawn from Kenya, and as such, the inferences drawn cannot be directly applied to the diverse Kenyan populace (12).

There have been ongoing debates on whether the dose of ATV should be reduced from the current 300 mg to 200 mg (with r), but there is no consensus yet (13). A recent study among Thai patients showed similarity of outcomes (viral suppression at 48 weeks of follow up) of the 200 mg compared to 300 mg dose of ATV with r (14). Currently, we do not have data on the steady-state plasma concentrations of atazanavir in Kenyan patients.

We therefore propose to do a preliminary study to determine the prevalence of genetic polymorphisms of the ATV drug metabolizing enzymes CYP3A5 and UGT1A1 and then determine their influence on ritonavir boosted ATV plasma concentrations and the risk of hyperbilirubinemia among HIV positive Kenyan patients.

1.3 Research questions

Among Kenyan HIV positive patients enrolled for care at the Kenyatta National Hospital (KNH);

1. What was the prevalence of the CYP3A5 and UGT1A1*28 single nucleotide polymorphisms?
2. Did the steady state plasma levels of atazanavir lie within the recommended therapeutic range of 150 - 800 ng/ml?
3. What is the influence of CYP3A5 polymorphisms on the steady state plasma concentrations of atazanavir?

4. What was the prevalence and risk factors of hyperbilirubinemia?
5. What was the effect of UGT1A1*28 polymorphisms on the bilirubin levels and risk of hyperbilirubinemia?

1.4 Hypothesis

We hypothesized that CYP3A5 non expressers (CYP3A5*3/*6/*7) single nucleotide polymorphisms would have higher plasma concentrations of atazanavir at steady state and a higher prevalence of hyperbilirubinemia. This higher prevalence of hyperbilirubinemia would be exacerbated by the presence of UGT1A1*28

1.5 Broad objective

To determine the steady state plasma concentrations of atazanavir, the prevalence of single nucleotide polymorphisms in CYP3A5, UGT1A1 and hyperbilirubinemia and its risk factors in Kenyan HIV positive patients at the KNH.

1.6 Specific objectives

Among Kenyan HIV positive patients enrolled for care at the Kenyatta National Hospital, we intended to;

1. Optimize and verify a high-performance liquid chromatographic method for the determination of plasma concentrations of atazanavir.
2. Determine the prevalence of CYP3A5 (*1, *3, *5 & *7) and UGT1A1*28 single nucleotide polymorphisms.

3. Examine the influence of genetic polymorphisms of CYP3A5 on the steady state plasma levels of atazanavir.
4. Determine the prevalence and risk factors of hyperbilirubinemia.
5. Examine the influence of UGT1A1*28 on bilirubin levels and risk of hyperbilirubinemia.

2.0 LITERATURE REVIEW

2.1 Pharmacology of atazanavir

Atazanavir is a PI antiretroviral agent. As a class, PIs inhibit the enzyme aspartyl protease that is involved in the proteolytic cleavage of HIV gag and pol polypeptides leading to the release of immature or non-infectious virions. These polypeptides are precursors for structural (p7, p9, p17, and p24) and enzymatic (protease, integrase and reverse transcriptase) parts of the virus. Enzyme inhibition results in the release of non-infectious virions (15).

ATV is an azapeptide PI designed with a C2 symmetrical structure (Figure 2.1) and it has activity against both HIV-1 and HIV-2 as well as wild type and mutant strains (16).

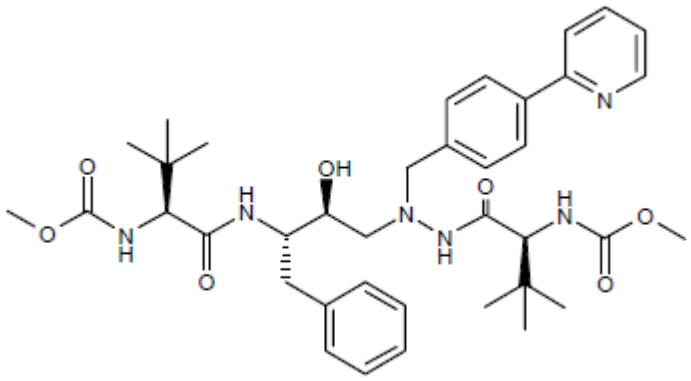


Figure 2.1: Chemical structure of atazanavir (17)

A one compartment model with a first order absorption rate constant best describes the pharmacokinetics of ATV (18,19). Following oral administration, ATV is rapidly absorbed (absorption rate constant is 0.405^{-hr} with a lag time of 0.88 h) attaining peak concentrations 2 hours post dose. The rate of absorption

is influenced by the presence of food and gastric pH. The oral bioavailability of ATV (F_{Oral}) is between 60-70% and is greatly affected by food. Increases in ATV area under the plasma concentration-time curve (AUC) by as much as 70% after a light meal and 35% after a high fat meal have been recorded.

The oral clearance (CL) of ATV is 12.9 L/h in the absence of ritonavir (RTV), whereas in its presence, CL is reduced by as much as 46% to 7.0 L/h. The elimination half-life increases from 4.6 h to 8.8 h when co-administered with RTV. The drug has an apparent volume of distribution (Vd) of 83L (20). The mean clearance of atazanavir is reduced by about 9% in females compared to men, though the mechanism underlying such as variability based on sex has not been fully understood (21).

ATV steady state concentrations are achieved between 4 and 8 days of starting RTV boosted therapy (15,16). ATV is highly bound to both alpha 1 acid glycoprotein and albumin in nearly identical proportions (89 vs 86% respectively) (17,20). The recommended range for the trough plasma concentrations of ATV is between 150 – 800 ng/mL (13). Other have extended the upper limit to 850ng/mL in some investigations. The median trough concentrations among treatment experienced patients has been reported as 402 ng/mL (22,23).

2.2 Metabolism of atazanavir

ATV undergoes phase I metabolism catalyzed by CYP 3A4 predominantly and CYP3A5 through oxidation (8,14,15). A small fraction (7%) of the drug is excreted unchanged. The metabolites then undergo phase II reactions and are excreted

thereafter in feces and in urine (minimal, 7%). Three metabolites with anti-HIV activity are formed. The drug is also a substrate for membrane transporters that include P-glycoprotein, multidrug resistance associated proteins (MRPs) and organic anion transporting polypeptide (OATP) (26). Metabolites of ATV have been characterized in some populations and the pathway is illustrated in figure 2.2 (24,25). ATV also inhibits CYP3A isoforms, but to a lesser extent than ritonavir (27).

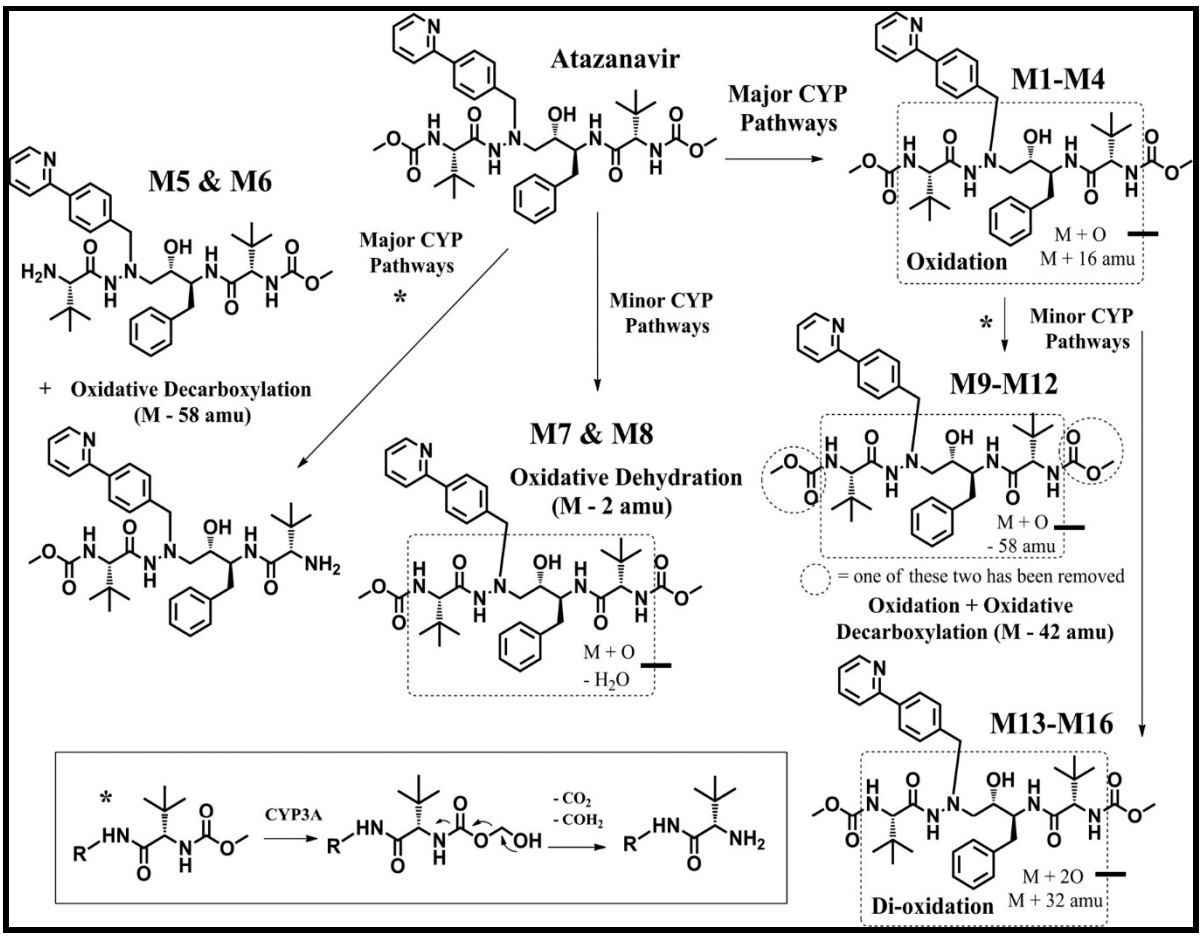


Figure 2.2: Metabolic pathways of atazanavir (25). ***Adapted from Wempe et al. (2011) Atazanavir metabolism according to CYP3A5 status: an in vitro -in vivo assessment, Drug Metabolism and Disposition. 2011;39(3):522–7, 39; 522-27***

2.3 Pharmacogenetics of atazanavir metabolism

CYP3A4/5 are the main enzymes involved in the metabolism of ATV and may be responsible for much of the inter-ethnic and inter-population differences in disposition of drugs dependent on this pathway for metabolism (28). CYP3A5 is expressed in polymorphic fashion, whereas CYP3A4 is not. There are four CYP3A5 alleles that determine the inter-ethnic differences in expression of proteins; CYP3A5*1 is the expresser phenotype whereas CYP3A5*3, CYP3A5*6 (both due to defective alternative splicing) and CYP3A5*7 (due to a frame shift mutation) are the non or low expresser phenotypes (29).

The prevalence of CYP3A5 SNPs from a diverse sample in Africans identified CYP3A5*1, CYP3A5*3 and CYP3A5*6 in all samples genotyped in the ranges of 4-81, 4-81 and 4-33% respectively. The frequency of CYP3A5 expression in East Africa was projected to be 36%, which was lower than other regions of sub Saharan Africa (12).

In a study of healthy volunteers, ATV oral clearance was 1.39 – fold faster and the C_{min} nearly half in CYP3A5 expressers compared with non-expressers. These differences were however absent in African Americans in that study, but this could have been due to a small sample size in the race strata hence lack of power to statistically detect a difference (30). A follow up study found that the ratio of M1 and M2 phase I metabolites was nearly twice as large in CYP3A5 expressers compared to non-expressers, but more importantly, the differences in the ration of M1 to M2 metabolites were similar in African Americans and non-African

Americans (25). Patients on ritonavir boosted atazanavir and having CYP3A5 non-expresser functionality, there is a possibility of observing exaggerated plasma concentrations.

2.4 UGT1A1 pharmacogenetics and its effect on bilirubin concentrations

Atazanavir is known to inhibit the enzyme uridine diphosphate glucuronosyltransferase (UGT) 1A1 thereby blocking the glucuronidation of bilirubin (31). A polymorphism in UGT1A1 gene, specifically a promoter tandem TA repeat, is strongly associated with inter-individual differences in the plasma concentrations of indirect bilirubin (26). Promoters with 7 TA repeats ($A(TA)_7TAA$ (UGT1A1*28 allele) are reported to have up to 70% less activity than the promoter with 6 TA repeats. This polymorphism is more prevalent in persons of African descent at 43% compared to the Japanese (11%) (32).

Individuals who are homozygous for this mutation (UGT1A1*28) experienced more than 2 episodes of hyperbilirubinemia when on therapy with ATV, with 1.6 – 2.8 times higher levels of indirect bilirubin compared to heterozygotes and non-expressers (32,33). Hyperbilirubinemia can result in reduced adherence and treatment discontinuation(10).

The effect of decreased function UGT1A1 status on treatment discontinuation was examined in 121 Swiss HIV Cohort study participants who were on ritonavir boosted atazanavir. Participants with UGT1A1 decreased function alleles (*28/*28 or *28/*37) were at increased risk of all cause ATV/r discontinuation. Participants carrying two non-functional alleles had higher discontinuation rates

compared to those with one or two functioning alleles (63%, 24% and 15% respectively) (34). In the ACTG protocol A5257 study, treatment discontinuation among patients on ATV/r was strongly associated with rs887829 TT (35). The rs88729 variant is in very high linkage disequilibrium with the promoter TA repeat ($r^2 = 0.99$), where the C allele is in linkage with the $(TA)_5$ and $(TA)_6$ whereas the T allele is in linkage with the $(TA)_7$ and $(TA)_8$ (10).

2.5 Side effect profile of atazanavir

Atazanavir is known to cause a rise in the unconjugated bilirubin levels through mechanisms that have been alluded to - the competitive inhibition of the enzyme UGT1A1. This side effect is more common among individuals who have Gilbert's syndrome, which is an inherited cause of unconjugated hyperbilurbinemia. Its frequency in the general population is 3 -10% (8, 19).

The incidence of grade 3 and 4 hyperbilurbinemia, defined as elevations in serum bilirubin levels by 2.5- and 5-times upper limit normal respectively, may be as high as 45-49% among patients using ATV. A cohort study of 1150 HIV positive patients followed for about 9 years found that exposure to ATV conferred a higher risk of hyperbilirubinemia in comparison to exposure to other ARVs. Persistent elevations above Grade 4 can warrant a dosage adjustment or a discontinuation. (36).

Other side effects associated with the use of ATV include dyslipidemias, nephrolithiasis and cholelithiasis. A retrospective review of therapeutic drug monitoring (TDM) data in Italy found that 26 and 45 patients developed

hypercholesterolemia and hypertriglyceridemia respectively from a sample of 240. Eleven patients experienced nephrolithiasis and all patients with abnormalities had higher plasma concentrations of ATV compared to those without (13).

2.6 Risk factors for hyperbilirubinemia in persons living with HIV

Risk for grade 3 or 4 hyperbilirubinemia has been found to be higher in persons using ATV compared to non-users. This was demonstrated in a cohort of Canadian HIV patients on follow. Age at the point of enrollment into the study and use of ritonavir were also associated with increased risk of developing hyperbilirubinemia. Ritonavir as earlier mentioned increases the plasma concentrations of ATV and is hence correlated with hyperbilirubinemia (36).

There are conflicting results on the impact of sex on hyperbilirubinemia. Some researchers have reported a protective effect in women(37), while others have demonstrated that the female sex is a risk factor for hyperbilirubinemia due to higher plasma concentrations of ATV and bilirubin levels of 1.3mg/dL (38).

Some factors are known to worsen hyperbilirubinemia in a situation where there is ATV use. These include CD4 and abnormal bilirubin at baseline (36) as well as the UGT1A1 nonfunctional variant (39). Locally generated data on the risk factors for hyperbilirubinemia is lacking.

2.7 Guidelines on use and dosing of atazanavir

According to the newly released ART guidelines by the World Health Organization (WHO), ATV is recommended for use as second line treatment in adults,

adolescent and pregnant women failing first line therapy. The drug is also recommended as an alternative second line in children 3 to less than 10 years of age (6). The Kenyan HIV treatment guidelines concur with the WHO recommendations (40).

In adults, ATV is given orally as a 300 mg with 100 mg RTV as a pharmacokinetic booster. Alternatively, unboosted ATV (recommended dose 400 mg) is also available meaning that RTV is not co-administered. The recommended therapeutic window for ATV is 150 ng/ml to 800 ng/ml (13). A recent study on therapeutic drug monitoring reported that 43.9% of patients on ritonavir boosted atazanavir had peak concentrations that were above the recommended limit. A direct association was also established between the C_{min} concentrations and the degree of hyperbilirubinemia. Thirty six percent of patients on unboosted ATV had sub therapeutic concentrations (13). These two scenarios lend credence to the theory that the optimal dosing regimen for ATV is yet to be ascertained.

3.0 METHODS

3.1 Study design

This was a pre-dominantly a cross sectional study, though other study designs were employed:

i. A laboratory based experimental study to validate the HPLC method
ii. A longitudinal study over 6 hours to collect blood samples for the determination of plasma concentrations of atazanavir.
iii. Cross sectional study to determine the prevalence of genotypic variants of CYP3A5, UGT1A1and the prevalence of hyperbilirubinemia and its risk factors

3.2 Study area

The study was done at the Comprehensive Care Centre (CCC) of the Kenyatta National Hospital (KNH) situated in Nairobi. KNH is the largest teaching and referral hospital in Eastern Africa. The CCC is a clinical unit that provides specialist outpatient services to HIV/AIDS patients on HAART. Patients visit the clinic for medication refills during which their progress is monitored. Patients were drawn from Nairobi and the neighbouring counties. KNH had 339 patients on ATV based regimens (as of September 2017).

3.3 Target population

HIV positive patients on a regimen containing ATV/r (300/100 mg) were recruited for this study. These patients were recruited between October and November 2017 from the KNH CCC records of patients on follow up.

3.4 Inclusion and exclusion criteria

Inclusion criteria

Patients were considered for inclusion if they met the following criteria:

1. Males and females with a documented HIV infection
2. 18 years of age and older
3. Enrolled at the KNH CCC for care in September 2017 or earlier
4. Voluntarily provides written informed consent
5. On an ATV/r containing regimen for at least one month.

Exclusion criteria

Patients with the following characteristics were excluded from the study

1. Pregnant women or those with a documented positive pregnancy detection test and lactating women
2. Patients with an active opportunistic infection or a stage IV illness
3. Patients with a documented creatinine clearance of less than 60 mL/min
4. Patients who are not on an ATV/r containing regimen
5. Patients who take their doses of ATV/r in the evening.
6. Patients with a prior history of hyperbilirubinemia
7. Patients on any of the following medications known to interact with ATV; omeprazole/esomeprazole, lanzoprazole, rabeprazole, pantoprazole, rifampicin, rifabutin, cimetidine, ranitidine or famotidine.

3.5 Sample size determination

The hypothesis to be tested was that CYP3A5 non expressers (CYP3A5*3/*6/*7 SNPs) would have higher plasma concentrations of atazanavir at steady state and a higher prevalence of hyperbilirubinemia. This higher prevalence of hyperbilirubinemia would be exacerbated by the presence of UGT1A1*28 SNP.

The Kelsey *et al.* formula was used to determine the sample size for this study (41). This formula was selected because the main hypothesis of the study involved comparison of two groups and as such it was most suitable for determining a sample size that would achieve that objective.

The parameters used to calculate the sample size were adapted from the Gervasoni *et al.* study (13). Our primary endpoint was the minimum plasma concentrations of atazanavir at steady state.

$$N_1 = \frac{(Z_\alpha + Z_\beta)^2 pq(r+1)}{r(p_1 - p_2)^2}$$

$$n_1 = rn_2 \text{ where}$$

Z_α =1.96, Z_β =-0.80, n_1 is the number of exposed, n_2 is the number of unexposed, r is the ratio of unexposed to exposed, p_1 is the proportion of exposed with disease (0.88) and p_2 is the proportion of unexposed with disease (0.65).

Assuming that 88% of the participants had minimum trough ATV plasma concentrations exceeding 150 ng/mL, at an alpha of 0.05 and power of 80%, a sample size of 99 was sufficient to reject the null hypothesis that non expressers

with CYP3A5*3/*6/*7 single nucleotide polymorphisms had minimum plasma concentrations of atazanavir less than 150 ng/ml and thus a lower prevalence of hyperbiluribinemia. An adjustment for non-completeness of 10% was made bringing the sample size to 109.

3.6 Sampling method

A sampling frame was developed at the clinic listing all patients who were on ATV containing regimens. Potential participants were chosen by simple random sampling using a table of random numbers with replacement.

3.7 Participant recruitment strategy and consenting process

Pharmacy records were used to identify potential participants. From the records, the visit dates of potential participants were obtained. Based on random sampling techniques used, the participants were called and recruited ahead of their scheduled visits. CCC staff at counseling and the clinicians assisted with recruitment. Participants who were interested were led to a study clinician who took them through screening using the eligibility checklist (Appendix 1), consenting process (Appendix 2) and subsequent enrolment if they met the inclusion criteria.

3.8 Data collection

Data collection was done using a mix of patient interview, abstraction of information from patient files and laboratory analysis of blood samples.

3.8.1 Interview and abstraction of data from patient records

Study staff administered questionnaires to collect baseline demographics, locator information, adherence data and depression status. These tools are presented in the appendices (Appendix 1, Appendix 2, Appendix 3, Appendix 4) Case report forms (Appendix 1) were used to abstract data from medical records, including ART history, CD4 counts, co-morbidities, concurrent medicines, viral load, plasma atazanavir concentrations and liver function tests.

We used the 8 item Morisky Medication Adherence Scale (MMAS-8) (Appendix 2) to determine adherence. It is an interviewer administered questionnaire that assists in the assessment of adherence. It consists of eight questions, seven of which are answered in the affirmative or negative and one which is a Likert scale. The seven questions each attract a score of either zero or one depending on the response while the eighth question is scored from 0 – 4. The individual scores are then summed up and divided by four to finally classify patients as either having low, medium or high adherence (scores of <6, 6 to <8 and >8 respectively) (42).

The Patient Health Questionnaire 9 (PHQ 9) (Appendix 3) is a diagnostic screening tool for depression that can be self-administered. It consists of 9 symptoms that are based on the Diagnostic and Statistical Manual for Mental Disorders – IV (DSM - IV). For each symptom, there are 4 response options ranging from '0'= 'Not at all' to '3'='Nearly every day'. The total score can be anywhere from 0 to 27. Interpretation of the PHQ - 9 scores when applied as a

measure of severity is as follows: 1 – 4 = no depression, 5 – 9 = mild depression, 10 – 14 = moderate depression, 15 -19 = moderately severe depression and 20 - 27 = severe depression. For the diagnosis of major depression, 5 or more of the 9 symptoms must be present for more than half the days for the past 2 weeks and the patients must report a depressed mood, anhedonia or thoughts of being dead or harming oneself (43).

3.9 Blood sampling for pharmacokinetic and genotyping procedures

Blood samples were drawn for use in quantification of plasma drugs concentrations, determination of plasma bilirubin levels and for the genotyping assays. A sparse sampling approach was used. Three 4 mL blood samples were collected from each participant during the visit for drug quantitation. These were drawn by mid arm veno puncture and collected into well labelled K-EDTA venous blood collection tubes. The samples were drawn as follows; half an hour before the morning dose to correspond to the trough level then 2 and 4 hrs after the morning dose. Blood sampling was done at the CCC by a qualified phlebotomist. To obtain the trough levels sample, participants were requested to arrive at the facility early enough prior to taking their dose. To cater for inter-occasion variability, we attempted to resample a third of the participants, but only two consented to this procedure. Repeated sampling would have helped in the separate estimation of the components of intra-individual variability (44).

After collection, blood samples were centrifuged at 1400 g for 10 minutes at +4 °C and the plasma stored at -20 °C prior to quantification. Plasma samples will

be archived at -80°C at the KNH CCC laboratory and the National Influenza laboratory in KEMRI, Nairobi for a maximum of 3 years after quantification. We plan to store the samples for 3 years primarily because we may require to re-analyze them at some point and secondly, we may develop other questions based on the initial study findings that could be answered through a new analysis of the stored samples.

One 4 ml blood sample per participant for use in the genotyping assays was drawn after sampling for the PK analyses from the antecubital fossa into a well labelled vacutainer. Whole blood was stored at -80 °C prior to sequencing.

An additional 2 ml of blood was collected in an anticoagulant free vacutainer and allowed to clot. Serum was used to determine the concentrations of conjugated, unconjugated and total bilirubin. These tests were performed within the KNH labs.

Plasma concentration of ATV were measured by a validated reverse phase high performance liquid chromatography (HPLC) method with ultra violet (UV) detection at 260 nm (45). Genotyping for CYP3A5 was done by polymerase chain reaction at the Kenya Medical Research Institute lab in Nairobi.

3.10 Genotyping

3.10.11 Materials and reagents

DNA extraction was performed using the PureLink® Genomic DNA Kits for purification of genomic DNA. The Kits contained Proteinase K (20 mg/mL in storage buffer), RNAse A (20 mg/mL in 50 mMTris-HCL, pH 8.0, 10 mM EDTA)

and buffers. There were several buffers; PureLink® Genomic Lysis/ Binding buffer, PureLink® Genomic Wash buffer 1, PureLink® genomic Wash buffer 2 and PureLink® Genomic Elution buffer (10 mMTris-HCL, pH 9.0, 0.1 mM EDTA).

Equipment used in the DNA extraction process included a a heat block, sterile microcentrifuge tubes (supplied with the kit), pipettes, spin columns, collection tubes (supplied with the kit), vortexing machine (Thermal Electron Corporation, Denley VibroMix) and a centrifuge (Biofuge Pico, Heraeus Instruments).

The reagents for genotyping consisted of four tubes of 20X TaqMan Drug Metabolism Genotyping Assay with assay ID of rs776746, rs10264272, rs76293380 and rs 887829 (c,-364 C>T: UGT1A1*80), 2X TaqMan Genotyping Master Mix and distilled water for PCR (dH2O). The TaqMan Drug Metabolism Genotyping assay consisted of 20X mix of unlabelled PCR primers and TaqMan MGB (minor groove binder) probes. The TaqMan MGB probes consisted of target-specific oligonucleotides with a reporter dye linked to the 5' -end of each probe (VIC dye for allele 1 probe and FAM dye for allele 2 probe). The probes also contained a non-fluorescent quencher (NFQ) at the 3'-end of the probe. The primers in the genotyping assay were for amplifying the sequence of interest and the two probes were for allele detection and allowed genotyping two possible variant alleles at the polymorphic site in a DNA target sequence. The minor groove binder was for increasing the melting temperature without increasing probe length. The 2X TaqMan Genotyping Master Mix contained AmpliTaq Gold DNA Polymerase to catalyze the reaction, dNTPs with dUTP as building blocks

for new DNA strands and to minimize PCR cross contamination respectively, passive Reference to normalize for differences in fluorescence levels and optimized buffer components (GeneAmp 10X PCR Buffer). The GeneAmp 10X PCR Buffer contained 500mM potassium chloride, 100mM Tris-HCL (pH 8.3 at room temperature), 15 mM magnesium chloride and 0.01% (w/v) gelatin. The equipment that were not supplied with the reagents included the 7500 Fast Real Time PCR machine (Applied Biosystems, Foster City, CA)

3.10.12 Protein and RNA digestion

The DNA was extracted from 200µL of packed cells using PureLink® Genomic DNA Kit as per the manufacturer's protocol (Life Technologies Carlsbad CA). All the proteins were lysed by the addition of the enzyme Proteinase K. This was followed by hydrolysis of RNA by the addition of 20µlof RNAse (20 mg/mL in 50 mMTris-HCL, pH 8.0, 10 mM EDTA). The contents were mixed well by vortexing and incubated at room temperature for 2 minutes. Two hundred microlitres of PureLink® Genomic Lysis/ Binding buffer was added and vortexed to obtain a homogenous solution, and incubated the lysate at 55^{o}C for ten minutes to promote protein digestion. The genomic DNA separated from cellular proteins into solution. To the lysate we added 200µL 96 – 100% ethanol to concentrate and desalt the genomic DNA in aqueous solution for downstream applications. The lysate was mixed well by vortexing for 5 seconds to obtain a homogenous solution.

3.10.13 Binding DNA

Six hundred and fifty microlitresof the lysate was transferred to sterile PureLink® Spin Columns in collection tubes. The genomic DNA was bound to the columns. The ethanol in the lysate also enhanced the binding of DNA to the column. The contents were centrifuged at 10,000 x g for 1 minute at room temperature. The impurities passed into the collection tubes while genomic DNA remained bound to the spin columns. The collection tubes were discarded and the spin columns placed into clean PureLink® Collection Tubes.

3.10.14 Washing DNA

Five hundred microlitres of wash buffer 1 prepared with ethanol was added to the spin columns and centrifuged the columns at room temperature at 10,000 x g for 1 minute. The collection tubes were discarded and the spin columns placed into clean PureLink® Collection tubes. The washing of genomic DNA allowed for removal of impurities, proteins and polysaccharides while the DNA remained bound to the column. Wash buffer 1 contained chaotropic salt whose purpose was to remove proteins and colored contaminants. Five hundred microlitres of wash buffer 2 prepared with ethanol was added to the spin columns and centrifuged the columns at maximum speed for 3 minutes at room temperature and discarded the collection tubes. The purpose for wash buffer 2 was to remove the salts leaving pure genomic DNA bound to the spin columns.

3.10.15 Eluting DNA

The spin columns were placed in sterile 1.5-mL microcentrifuge tubes and added 100µL PureLink® Genomic Elution Buffer (10 mMTris-HCL, pH 9.0, 0.1 mM EDTA) to the spin columns. The contents were incubated at room temperature for 1 minute and centrifuged the columns at maximum speed for 1 minute at room temperature. The 1.5-mL microcentrifuge tube contained purified genomic DNA. The spin columns were removed and discarded. The extracted DNA was quantified using NanoDrop microvolume quantitation of nucleic acids technique (NanoDrop 2000c spectrophotometer, Thermal Fisher Scientific Inc) and stored in a freezer at -20^{o}C for further processing.

3.10.16 Real time PCR amplification

Real time PCR was conducted in three steps; preparation of the reaction mix, preparation of the reaction plate and performing the PCR.

3.10.17 Preparation of the reaction mix

The reaction mix consisted of 20X TaqMan Drug Metabolism Genotyping Assay, TaqMan Genotyping Master Mix and nuclease-free water. The reaction mix was prepared by wet DNA method and the total volume of each component needed for each assay was calculated as shown in Table 3.1

Table 3.1: Components of the reaction mix

Component	96 well plate (25 µL reaction)
2X TaqMan master mix	12.50 µL
20X TaqMan DME assay	1.25 µL
Working stock	
Nuclease free waster	
Total volume per well	13.75 µL

The 2X TaqMan Genotyping Master Mix was swirled gently to mix the contents. The 20X TaqMan Drug Metabolism Genotyping Assay was vortexed and centrifuged so as to mix properly. The required volumes of 2X TaqMan Genotyping Mix, 20X TaqMan Drug Metabolism Genotyping Assay and nuclease-free water were pipetted into sterile tubes, capped then vortexed briefly to mix the components. Air bubbles were eliminated from the solution by centrifuging the contents.

3.10.18 Preparation of the reaction plate

The reaction plate was prepared by the wet DNA method. Every DNA sample was diluted in nuclease free water to deliver 1-15 ng of DNA per well. The reaction mix was pipetted into each well of the reaction plate using volumes listed in Table 3.1. The reaction plate was covered with MicroAmp Optical Adhesive Film. The plate was gently swirled to spin down the contents and to eliminate air bubbles from the solutions. The MicroAmp Optical Adhesive Film was removed and into

each well of the plate, pipetted 11.25 µL of DNA samples diluted in nuclease free water, and included a well for a no template control to check for contamination. The reaction plate was again covered with MicroAmp Optical Adhesive Film and sealed with the plate with a MicroAmp Adhesive Film Applicator. The contents were swirled gently to eliminate air bubbles from the solutions.

3.10.19 Allelic discrimination – pre-read

The reaction plate was introduced into the reaction chamber of the Fast-Real Time PCR (Applied Biosystems, Foster City, CA) and the sequence detection software activated. Allelic discrimination pre-read test was performed in a total reaction mixture of 25µl.(4.25µl genomic DNA, 12.5µl TaqMan® Universal PCR Master Mix (NoAmperase UNG), 1.25µl TaqMan® Drug Metabolism Genotyping Assay Mix and 7µl DNAse free water). This was carried out at 60^{o}C for 1 minute.

3.10.20 Real time PCR amplification

The DNA amplification was done using 7500 Fast Sequence Detection System (Applied Biosystems, Foster City, CA). Instrumentation parameters consisted of initial hold cycle for 10 minutes at 95^{o}C, followed by 50 cycles of denaturation at 92^{o}C for 15 seconds and then annealing and extension at 60^{o}C for 1 minute and 30 seconds. Four negative controls were included in the reactions to detect contamination.

3.10.21 Allelic discrimination post read test

The Sequence Detection Software plotted Rn values using fluorescence measurements made during the plate read, based on the fluorescence signals from each well and then determined which alleles were in each sample. The general process consisted of creating and setting up a post read-PCR plate read document, performing a post-PCR plate read on a real-time PCR instrument, analyzing the experiment, making automatic or manual allele calls and verifying allele types. Each assay contained two allele specific probes and a primer that detected the specific SNP of interest. Increase in red dye (VIC) fluorescence indicated the presence of allele. Increase in blue dye (FAM) fluorescence indicated the presence of allele 2. The increase in green fluorescence indicated the presence of both allele 1 and allele 2. Sequence Detection Software was used to determine which genotypes were present using color codes of the alleles. The red dye (VIC) indicated the presence of homozygous wild-type allele, the blue dye (FAM) indicated the presence of homozygous variant allele while green indicated the presence of heterozygous allele. This was conducted at 60^{o}C for 1 minute.

3.11 Quality assurance

3.11.1 Quality assurance in the genetic laboratory

The following quality assurance measure were instituted in the Influenza Laboratory, KEMRI:

1 The laboratory was located in a section of the Public Health building that had been redesigned to cater for the performance of the daily

work. This ensured the quality, safety and efficacy of the service provided in addition to securing the health and safety of the laboratory personnel, patients and visitors.

2 The tests used in the laboratory were validated to ensure that they delivered the correct genotype under standard working conditions.

3 In every run using the real time PCR machine, it was mandatory to include four controls in the 96 well plate.

4 Procedures and processes in the laboratory were all done in accordance to standard operating procedures that were periodically reviewed and updated with older versions being removed from circulation and archived.

5 The laboratory periodically underwent internal audits by accrediting bodies.

6 The management of the laboratory reviewed the quality management systems every 12 months to ensure continued suitability and effectiveness.

7 The staff at the laboratory had requisite qualifications and also underwent regular trainings.

3.11.2 Quality assurance for the study

The case definition, the inclusion and exclusion criteria had been determined a priori. The inclusion and exclusion criteria were directly related to the study questions.

The instruments used were pre-tested to check on their reliability. Research assistants were trained on the research protocol, the tools and on the need to strictly adhere to the case definitions. Standard operating procedures were developed where need be.

The PI is a pharmacist with a masters degree in Clinical Pharmacy and a keen interest in specializing in pharmacometrics and pharmacogenetics. In summary, the PI was responsible for ensuring that the protocol was executed as planned, and he took part an active learning role in the genotyping, drug quantification and data analysis processes. Genotyping was done under the supervision of Prof. Wallace Bulimo who is the molecular biologist in charge of the National Influenza Laboratory in KEMRI. Quantification by HPLC-UV was done under the supervision of Dr. Hezekiah Chepkwony who is the current director of the National Quality Control Laboratory (NQCL). He is a qualified pharmaceutical analyst with a doctoral qualification. The supervisors are holders of doctoral degrees and are each specialists in their areas.

3.12 Case definition

The main outcomes of interest were the steady state plasma concentration of ATV, the prevalence of hyperbilirubinemia and the occurrence of single nucleotide polymorphisms in the CYP3A5 and UGT1A1 genes.

The therapeutic range for atazanavir in plasma is listed as 150 – 800/850 ng/mL in literature (13,20). A single nucleotide polymorphism is a genetic variation with a frequency of greater than 1% in the population. Can also be described as

differences in DNA sequence that give rise to different phenotypic forms. Hyperbilirubinemia as classified by the Division of AIDS (DAIDS) adverse events grading is based on elevations of the total bilirubin levels with reference to the upper limit normal (ULN) of the reference range used within a laboratory. There are grades I -IV (Grade 1 – 1.1 to < 1.6 x ULN; Grade 2 - 1.6 to < 2.6 x ULN; Grade 3 – 2.6 –to < 5.0 ULN and Grade 4 - ≥ 5.0 ULN).

3.13 Data management

Data coding was done to maintain confidentiality. Study records were kept under lock and key accessible only to the principal investigator. Data was entered into an Epi Info (version 7, CDC, Atlanta, GA, USA) database and data cleaning done to ensure completeness of the records. The database was password protected and it was backed up on a daily basis. The data was thereafter exported to STATA version 13 and R i386 version 3.3.1 for statistical analysis.

3.14 Study variables

The main outcomes of interest were the plasma concentration of ATV and the incidence of hyperbilirubinemia. The main predictor variable of interest was the occurrence of mutations in the CYP3A5 genes.

Data such as baseline CD4 count, viral load, sex, age, weight, ethnicity, concurrent drugs, adherence to ARVs and illnesses (including depression) was collected and modelled against the primary outcomes to check for possible associations.

3.15 Data analysis

The specific objectives were to:

1. determine the prevalence of CYP3A5 (*1, *3, *5 & *7) and UGT1A1*28 single nucleotide polymorphisms.
2. examine the influence of genetic polymorphisms of CYP3A5 on the steady state plasma levels of atazanavir.
3. determine the prevalence and risk factors for hyperbilirubinemia
4. examine the influence of UGT1A1*28 on the incidence of hyperbilirubinemia.

Table 3.2: Classification of variables used in the statistical analysis (Part 1)

Variable	Description	classification	Statistical meaning	Reason for inclusion in the model
Sex	Participant's gender, male or female	qualitative, nominal	Confounder	Causally associated with hyperbilirubinemia; non causally associated with ATV trough concentrations
Age	Participant's age in years	quantitative, continuous	confounder	Causally associated with hyperbilirubinemia; non causally associated with ATV trough concentrations
Current OI	1 present; 0 absent	Categorical, binary	Confounder	Causally associated with hyperbilirubinemia; non causally associated with ATV trough concentrations
Anti TB	Treatment for pulmonary tuberculosis	Categorical, binary	Confounder	Causally associated with hyperbilirubinemia; non causally associated with ATV trough concentrations
Conmeds	Concomitant medications	Categorical, binary	Confounder	Causally associated with hyperbilirubinemia; non causally associated with ATV trough concentrations
Herbs	Herb use	Categorical, binary	Confounder	Causally associated with hyperbilirubinemia; non causally associated with ATV trough concentrations
Cigarette use	1 smokes; 0 does not smoke	Categorical, binary	Confounder	Associated with both ATV and bilirubin levels
Duration with HIV	Years since diagnosis of HIV	quantitative, continuous	Instrumental	One of the determinants for the use of ATV
ART duration	Years since initiation of HAART	quantitative, continuous	Instrumental	One of the determinants for the use of ATV
Trough ATV concentrations	Minimum concentrations of ATV	quantitative, continuous	Predictor –model 2	Main independent variable

Table 3.3: Classification of variables used in the statistical analysis (Part 2)

Alcohol use	1 consumes alcohol; 0 does not take alcohol	Categorical, binary	Precision	Associated with bilirubin levels, not with ATV concentrations
		Categorical variable with > 2 levels	Outcome (model 4B)	Main outcome variable for model 4B
Bilirubin concentrations	1 for abnormally high; 0 for normal.	Categorical, binary	Outcome (models 2, 4A & 5)	Main outcome variable
	Continuous in a linear model			
CYP3A5 SNPs	Single nucleotide mutations (*1, *3, *5 &*7)	Categorical variable with > 2 levels	Instrumental (models 2 & 5)	Causes elevated ATV levels that lead to hyperbilirubinemia
			Effect modifier (model 4A)	Association differs by this variable
			Predictor –(model 4B)	Main independent variable
UGT1A1*28 SNPs	Single nucleotide mutations (6/6, 6/7 & 7/7)	Categorical variable with > 2 levels	Instrumental (models 2 & 4A)	Causes elevated ATV levels that lead to hyperbilirubinemia
			Effect modifier (model 5)	Association differs by this variable
WHO stage	Severity of HIV disease classified as I, II & III	Categorical variable with > 2 levels	Instrumental in model 2 only	Related to likelihood of use of ATV

3.15.1 Descriptive analysis

Descriptive and exploratory data analysis

We explored the data to get numerical summaries of measures of central tendency – mean, standard deviation, median – and measures of spread – max, min, IQR, range. Using this strategy, we hoped to identify any errors in the data such as out of range values, identify missing data and patterns of missingness. This worked for variables of a continuous nature such as ATV concentrations, years since diagnosis with HIV and on ART, age and bilirubin concentrations. For other qualitative variables, we computed the counts and proportions.

We also used graphical summaries. To note the manner of distribution of continuous variables – age, ATV concentrations and bilirubin concentrations, histograms were plotted.

For objective 2, to test for a correlation between the plasma ATV concentrations and the indirect bilirubin concentrations, we constructed scatter plots with either maximum or minimum plasma concentrations as the predictor variables or indirect bilirubin concentrations as the response variable. We inspected the plots for direction, strength and form. Stratification by the following variables was done; CYP3A5 SNP, UGT1A1 SNP and gender. The Pearson's and Spearman's correlation coefficients were calculated before and after stratification to check for possible separation and either confounding or effect modification. If there was possible confounding or effect modification, this was factored in the

Chi square test. Continuous variables were transformed to dichotomous variables to allow for use of the chi square test.

To describe the relationship between the SNPs identified and plasma concentrations, we constructed box plots with SNPs as the predictor and the plasma and bilirubin concentrations as the response.

a. For objective 2, CYP3A5 SNPs identified were used as the predictor variable against plasma ATV concentrations. We compared the plots in those patients in whom we have identified the SNPS to those who did not have the SNPs. We expected that the plots would show some heterogeneity.

b. For objective 4, the role of UGT1A1 SNPs were examined in this relationship, using bilirubin concentrations as the response variable. The plots were likely to show heterogeneity in the bilirubin concentrations subset by UGT1A1 SNPs.

3.15.2 Inferential analyses

Specific objective 1

1. The prevalence of each SNP was determined as a proportion of the whole. To determine whether Hardy Weinberg equilibrium was maintained, we used the Pearson's chi- square test if the cell numbers were large. The alpha was set at 0.05, at 1 df and a p value less than the alpha value led to a rejection of the null hypothesis. We reported the chi square statistics, the df and the p value.

2. If the numbers within any of the cells were <5, we used the Fisher's exact test and the significance ascertained as in 1 above.

Specific objective 2

Influence of CYP3A5 SNPs on the steady state concentrations of ATV

Generalized linear modeling was used to test the hypothesis.

$$\textit{Mean } p = \beta_0 + \beta_1\ CYP5A5(*3) + \beta_2\ CYP5A5(*6) + \beta_3\ CYP5A5(*7) + \beta_4 Sex + \beta_5 Age + \beta_6 Alcohol + \beta_7 Conmeds + \beta_8 DuATV + \beta_9 Herb + \beta_{10} CurrOI$$

1. Alpha is still set at 0.05, and associations were considered significant if the p value of the beta coefficients (1-3) is less than 0.05, and the 95% CI does not include zero. We reported the p values, 95% CI and the beta values, taking note of all variables in the model. Robust standard errors were used.

2. The F statistic/wald statistic was examined to determine the overall importance of the CYP3A5 variable in the model. An overall p value of < 0.05 was considered significant which was reported.

3. We examined the model for violations of the regression assumptions using the extracted residuals. To check for outliers, influential or high leverage points, we calculated the dfbetas. We then run the regression model excluding those observations that have large residuals and checked the effect on the value of β_1. If the value of β_1 changed significantly, then we considered the excluded points as influential and vice versa.

4. Model diagnostics were performed following the steps described under objective 2.

Specific objective 3 & 4

Logistic regression analysis was used to test the hypothesis.

$$Logit(p) = \beta_0 + \beta_1[ATV_{trough}] + \beta_2 sex + \beta_4 age + \beta_4 Alcohol + \beta_5 cigarrete + \beta_6 DuATV + \beta_7 * UGT1A1 + \beta_8 WHOstage + \beta_9 CurrOI + \beta_{11} AntiTbs$$

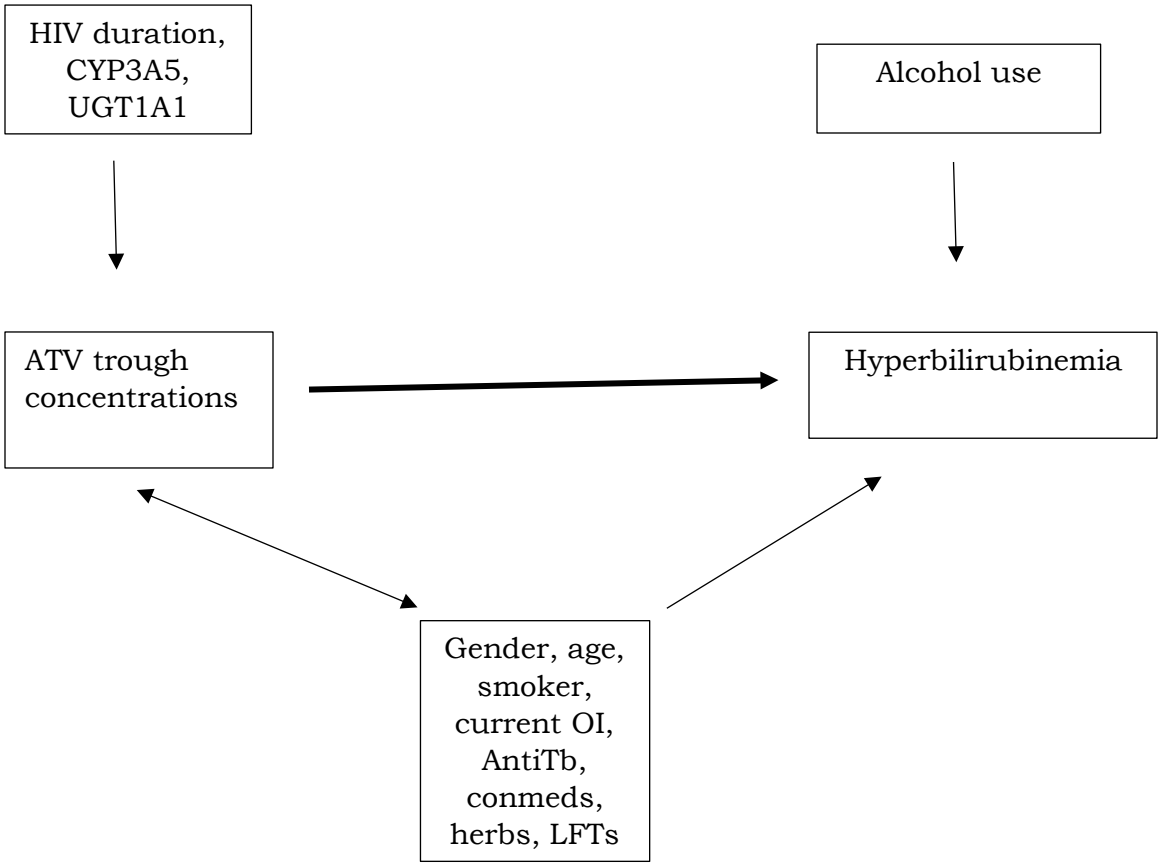


Figure 3.1: Conceptual framework of the variables examined in the study

1. In this analysis, we used an automated technique for model selection –the forward stepwise model building strategy. Starting with an empty model, we set the p value for entry at 0.1, and that of removal at 0.2. The variables that were tested were those displayed in the model and the causal diagram. Once the final model was selected, we run a backward stepwise regression model analysis to confirm that we got the same variables in the two models.

2. Once the model was selected, we ran it with the α value set at 0.05, and therefore, we considered an association statistically significant if the p value of the β_1 coefficient was < 0.05 and the 95% CI did not include 0 (in the raw model and 1 in the transformed model) which led to a rejection of the null hypothesis. We reported the p value associated with the e^β coefficient (including the units), the F statistic and the 95% CI, taking note of all variables within the model. We used robust standard errors.

3.16 Ethical considerations

Ethical approval for study conduct was sought from the KNH/UoN Ethics and Research review committee (KNH-ERC/A/110) (Appendix 4). Any amendments to protocol were to be submitted to the ERC for approval prior to implementation. The investigator provided regular safety and progress reports to the ERC annually and within 3 months of completion of the study.

Written informed consent was required from the patients prior to participation in the study. They were informed that they were free to stop participating in the

study without any prejudicial treatment befalling them at the CCC. There were no direct benefits to the patients who took part in the study. The risks, such as pain and discomfort at the site of blood sampling, were explained to the participants. The informed consent forms are attached in Appendix 5.

This was a minimal risk study because it is non-interventional. However, it entailed provision of blood samples at least 3 times in one day. This caused some discomfort to the patient.

No inducements were offered to the patients to persuade them to join the study. Patients were reimbursed 500 KShs for their transportation cost and for the 4 hours stay in the hospital. They were provided with newspaper and magazines to read during the study period.

Confidentiality of the patients' medical records was maintained and no names were included during data collection or analysis. Patients were assigned study numbers in place of hospital patient identification numbers. Good Clinical Practice (GCP) guidelines were adhered to as outlined by the International Conference on Harmonization (ICH) (46).

The Principal investigator and the Institutional Ethics Committee had the right to terminate the study at any time. Reasons for this termination would be provided to the participants. The Principal Investigator reserved the right to discontinue the study for safety reasons at any time.

3.17 Publication policy/Data dissemination plan

Data generated from this research will be shared via publications and conference presentations. A copy of the thesis report will be shared with the KNH and the UoN library. Publications of the results of this study (listed below) will be governed by KNH/UoN ERC policies.

i. Prevalence and correlates of depression among people living with HIV on atazanavir based second line regimens at a tertiary teaching and referral hospital in Kenya. G. A Mugendi, N. N. Nyamweya, F. A. Okalebo and J. Unadkat, East African Medical Journal Vol. 96 No. 4 April 2019

ii. People living with HIV on atazanavir based second line regimens are not highly adherent to therapy; a report from a tertiary referral hospital in Kenya. Mugendi G, Nyamweya N, Okalebo F, Unadkat J, African Journal of Pharmacology and Therapeutics Vol. 9 No. 2 Pages 44-50, 2020

4.0 RESULTS

OPTIMIZATION AND VERIFICATION OF A HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF ATAZANAVIR IN PLASMA

4.1 Introduction and objectives

There are a number of high-performance liquid chromatographic methods described in literature for the detection of atazanavir in human plasma samples. They differ in their mode of elution, extraction techniques and the detectors that are employed (47–51). In a resource limited clinical setting, optimization and verification of a simple existing HPLC UV method for determination of the plasma levels of atazanavir is desirable.

The original chromatographic conditions of the method described by Cattaneo *et al.* (2008) (45) consisted of an isocratic elution using a mobile phase consisting of a mixture of 45% water, 20% methanol and 35% acetonitrile. The flow rate was 1 mL/min. The stationary phase consisted of reverse phase octyl silane (C_8) packed in a column measuring 150 mm in length, 4.6 mm internal diameter and 4 µm particle size with the oven temperature was set at 30 °C. The UV detection wavelength was set at 260 nm and the injection volume was 30 µL. The internal standard (IS) used was diazepam.

Applying the chromatographic conditions just described resulted in fairly good separation of the components and relatively short run times, but the challenge was finding a short C_8 column that could sustain the high back pressures. The

alternative was to use a 250 mm long by 4.6 mm internal diameter column with a particle size of 5 µm and then optimize the conditions accordingly. Method verification and optimization were therefore undertaken for the determination of atazanavir plasma levels in HIV patients using this larger column.

4.2 Materials and methods

HPLC analysis was performed in the Research and Development unit of the National Quality Control Laboratory (NQCL), Nairobi, Kenya. Sample preparation and extraction was achieved through protein precipitation which was done at the African Institute of Biomedical Sciences and Technology Laboratory of the University of Nairobi (UoN- AiBST) housed within the Department of Pharmacology and Pharmacognosy.

4.2.1 Instrumentation

For analysis, a Merck Hitachi LaChrom HPLC system (Hitachi Ltd, Tokyo, Japan) was utilized. The system was made up of the following components: a L-7100 quaternary low pressure gradient pump; a L-7400 variable wavelength UV/Vis detector; a L-7200 variable injection volume autosampler; and a l-7350 thermostatic column oven. In addition, it comprised of a D-7000 software interface module.

To control the HPLC system, an IBM compatible Windows based desktop personal computer installed with a HPLC System Manager (HSM) application version 4.1 (Merck KGaA, Darmstadt, Germany and Hitachi Instruments Inc., San Jose, USA) was used. This application was coupled to the HPLC system

interface module. A DC 200h MRC ultrasonic water bath (MRC Ltd, UK) sonicator was used to degass the components of the mobile phase for 30 minutes before analysis.

During plasma sample preparation, a vortex mixer (SN 70918146 Vibromix (Thermo Electron Corporation, UK) was used. This resulted in a near homogenous mix of the spiked plasma sample. A D-37520 Osterode Biofuge Pico (Heraeus Instruments, Germany) centrifuge was used to centrifuge the precipitated plasma samples.

During the preparation of stock and working solutions, an AUW220D Shimadzu analytical balance (Shimadzu Corporation, Japan) was used to weigh the chemical reference standards.

4.2.2 Reagents and solvents

Mobile phase was prepared using HPLC grade methanol and acetonitrile (Rankem, Avantor Performance Materials, Ltd, India) and water. The water was ultra-purified and was prepared in house through consecutive reverse osmosis and ultra-filtration on an Arium 613616 Water System (Sartorius AG Gottingen, Germany). Plasma was obtained from the Hematology unit of KNH from patients who had undergone plasmapheresis.

The reference standards of atazanavir and diazepam were obtained by the head of the R & D unit from industry collaborators. Diazepam was kindly donated by Lab and Allied Limited (Nairobi, Kenya) while atazanavir was kindly donated by Mylan Laboratories Limited (India). The other reference standards were provided

by the NQCL from the stocks available which were stored under refrigeration (2-8°C) and protected from light.

4.2.3 Preparation of standard solutions

4.2.3.1 Calibration standard solutions

Stock solutions of atazanavir were prepared by dissolving 20 mg of the analyte in 20 mL of methanol and stored at -20 °C. These stocks were used to prepare several working calibration solutions at different concentrations. Working standards of atazanavir were prepared by diluting the stock solution in methanol tenfold consecutively to obtain 3 final concentrations of 3000 ng/mL (Working standard 1-WS1), 30000 ng/mL (WS 2) to 300000 ng/mL (WS 3).

Blank plasma was spiked using the working standards to achieve concentrations of atazanavir in plasma from 80 to 3000 ng/mL as detailed in Table 4.1. A constant volume of blank plasma was used as shown and methanol was used to top up the difference in the overall volume.

Table 4. 1: Volumes and concentrations of working standards used to prepare calibration standards

Final volume (µL)	Plasma volume (µL)	Diluent volume (µL)	Working standard volume (µL)	Actual concentration ng/mL	Target concentration (ng/mL)
1000	900	20	80 (WS 3)	240	80
1000	900	85	15 (WS 2)	450	150
1000	900	70	30 (WS 2)	900	300
1000	900	40	60 (WS 2)	1800	600
1000	900	0	100 (WS 2)	3000	1000
1000	900	85	15 (WS 1)	4500	1500
1000	900	70	30 (WS 1)	9000	3000

4.2.3.2 Preparation of stock and working solutions of the internal standard.

Diazepam was used as the internal standard because it was associated with a high recovery and good retention time. It is also eluted earlier than atazanavir and was not affected by interfering peaks. A stock solution of 1 mg/mL of diazepam was prepared by dissolving 20 mg of diazepam in 20 mL of methanol and this was stored at -20°C. Working solutions of diazepam were then prepared from the stock by appropriate dilutions using methanol and stored at 4°C. A constant concentration of 4.5 µg/mL was added to the samples under analysis.

4.2.4 Sample work up by protein precipitation

Patient blood samples were centrifuged at 3000 x g for 3 minutes and the plasma thereafter extracted and stored frozen at -40°C. These samples were thawed immediately before use by refrigerating them between 4 - 8 °C. Stock solutions of 1 mg/mL diazepam were added to chilled acetonitrile at a final concentration of 4.5 µg/mL. The solution of chilled acetonitrile containing 4.5 µg/mL of diazepam was added to all plasma samples at a ratio of 1:2 (plasma:acetonitrile with diazepam). This mixture was vortexed for 10 seconds to precipitate the proteins and thereafter centrifuged at 10000 x g for 10 minutes. The clear supernatant was then pipetted out for analysis.

4.2.5 Chromatographic conditions

The mobile phase was reproduced in the exact proportions as described in literature (Cattaneo, 2018) during verification and it consisted of acetonitrile

(35%), methanol (20 %) and water (45%). The elution was isocratic. The flow rate was 1.0 mL/min while the upper limit of the column back pressure was set at 250 bar. The flow rate was varied during the verification process from 0.5, 0.75, 1.0 and 1.5 while continuously checking the column backpressure. The initial column temperature was 30°C and this was increased to 35°C and 40°C during the verification. The initial injection volume was 30 µL and this was varied between 20 to 50 µL to find the optimum volume to work with.

4.2.5.1 Selection of column and detection wavelength

The method was tested using 3 different columns. Catteneo *et al.* had utilized a Ultrasphere octyl, with 4 µm beads, 4.6 mm by 15 cm (Beckman Coulter, Fullerton, CA) column which, we tested, but it could not sustain the high back pressures. A different column, a Zorbax® SB C_8 (4.6 by 250 mm, 5 µm) (Agilent, USA), was tested due to the relative difficulty of acquiring a short C_8 column. The detection wavelength was set at 260 nm, as reported by Cattaneo *et al* (45).

4.2.6 Verification of the HPLC-UV method

Parameters that were evaluated during the verification of the HPLC UV method included: linearity of the calibration curve; accuracy; precision; sensitivity; and recovery.

4.2.6.1 Linearity of the calibration curve

The nominal concentrations of atazanavir were plotted against the ratio of the analyte and internal standard peak areas to obtain the calibration curves. Six

replicates of the calibration standards were analyzed. The relative standard deviations from the nominal concentrations were computed.

Simple linear regression was applied in the back calculations of the concentrations of the calibration standards. The standard curve was acceptable if the 75% of the back calculated concentrations fell within ± 15% of the nominal value except at Lowest Limit of Quantification (LLOQ) where the acceptable limit was 20%. To test the appropriateness of the linear regression, the residuals were checked for normal distribution and homoscedasticity.

4.2.6.2 Accuracy and precision

Accuracy was determined from the percentage recovery of analyte drug (atazanavir) from blank plasma spiked at different concentration levels.

The precision was determined by making replicate injections and the percentage relative standard deviation of the determined concentrations from the nominal levels calculated. Using five replicates of atazanavir QC concentrations, the LLOQ was assessed and the mean value calculated. The deviation from the nominal value was accepted if it was less than 15%.

To determine the repeatability and reproducibility of the method as a measure of precision, intra-day coefficients of variation were calculated. A coefficient of variation less than 20% was termed acceptable for the LLOQ. All solutions were analyzed freshly prepared and therefore the inter day precision was not determined.

4.2.6.3 Sensitivity

The limit of detection (LOD) was defined as the concentration where the analyte peak and that of the blank plasma matrix were distinguishable. A mathematical formula was also applied to determine the LOD as detailed in Equation 4.1.

$$LOD = \frac{3.3 \times \text{Standard deviation of the response}}{\text{Slope of the calibration curve}} \quad \textit{(Equation 4.1)} \ (52)$$

To determine the Limit of Quantitation (LOQ), three-fold dilutions were prepared for concentrations on the lower end of the calibration curve. These concentrations were 40, 50, 70, 100 and 120 ng/ml. The response was determined by calculating the ratio of the peak areas of the analyte and the internal standard. Thereafter the accuracy and the coefficient of variation for each respective concentration was calculated.

The LOQ was defined as the lowest concentration with a precision of 20% and accuracy of 80-120%. This was also determined from the calibration curve using Equation 4.2.

$$LOQ = \frac{10 \times \text{Standard deviation of the response}}{\text{Slope of the calibration curve}} \quad \textit{(Equation 4.2)} \ (52)$$

4.2.6.4 Recovery

To determine the extraction efficiency of the analyte from the plasma matrix, a comparison of the peak area ratios of the analyte and the internal standard for both the extracted spiked plasma samples as well as the unextracted QC samples

to which only methanol was added in place of plasma as done. Recovery was therefore calculated as shown in Equation 4.3.

$$\text{Percentage recovery} = \frac{\text{Peak area ratio of the extracted spiked plasma samples} \times 100}{\text{Peak area ratio of the unextracted QC standards}}$$

(Equation 4.3)

Replicate determinations were conducted and a recovery of ≥ 70% was considered acceptable.

4.2.7 Statistical analysis

Data analysis was done using Microsoft® Office Excel 2016 package. Peak area ratios of atazanavir to diazepam (IS) were plotted against the nominal concentrations of the analyte in order to obtain the calibration curves. Simple linear regression was used to fit the data and a coefficient of determination greater than 0.99 considered acceptable. Residual analysis was done in Microsoft® Office Excel 2016. The mean and standard deviations were computed.

The coefficient of variation was used to determine the precision. To ascertain accuracy, the relative standard deviation of the analyte from the nominal concentration was calculated. The percentage of analyte recovered was also calculated.

4.3 Results

4.3.1 HPLC-UV method optimization

The optimal mobile phase and the mode of elution were the same as described by Cattaneo *et al* (45). Using a longer column (Zorbax® SB C_8 (4.6 by 250 mm, 5

μm) (Agilent, USA)) performed well with longer retention times of up to 26 minutes.

4.3.1.1 Wavelength and injection volume

The detection wavelength was optimal at 260 nm. The injection volume was increased to 50 μL from 30 μL. The smaller volumes described in literature did not provide sufficient levels of drug at the detector which proved to be problematic at atazanavir concentrations less than 100 ng/mL.

4.3.1.2 Flow rate and temperature

A flow rate of 1.0 mL/ min was maintained as detailed by Cattaneo *et al.* (2008). The column temperature was increased to 35 ºC and this was found to be optimum. Temperatures below 35 ºC increased the drift and noise while those above that level did not improve separation and would have shortened the lifespan of the column. Table 4.2 contains a summary of the optimized chromatographic conditions.

Table 4. 2: Chromatographic conditions for the quantification of atazanavir

Parameter	Optimized chromatographic conditions
Column	Zorbax® SB C_8 (4.6 by 250 mm, 5 μm) (Agilent, USA)
UV detector wavelength	260 nm
Temperature	35 ºC
Flow rate	1 mL/min
Injection volume	50 μL
Inter- run wash	Once after every run
Run time	26 minutes
Mobile phase	Acetonitrile 35% Methanol 20% Water 45%

Figure 4.1 represents the separation and resolution of atazanavir and diazepam extracted from spiked plasma samples under the conditions outlined in Table 4.2.

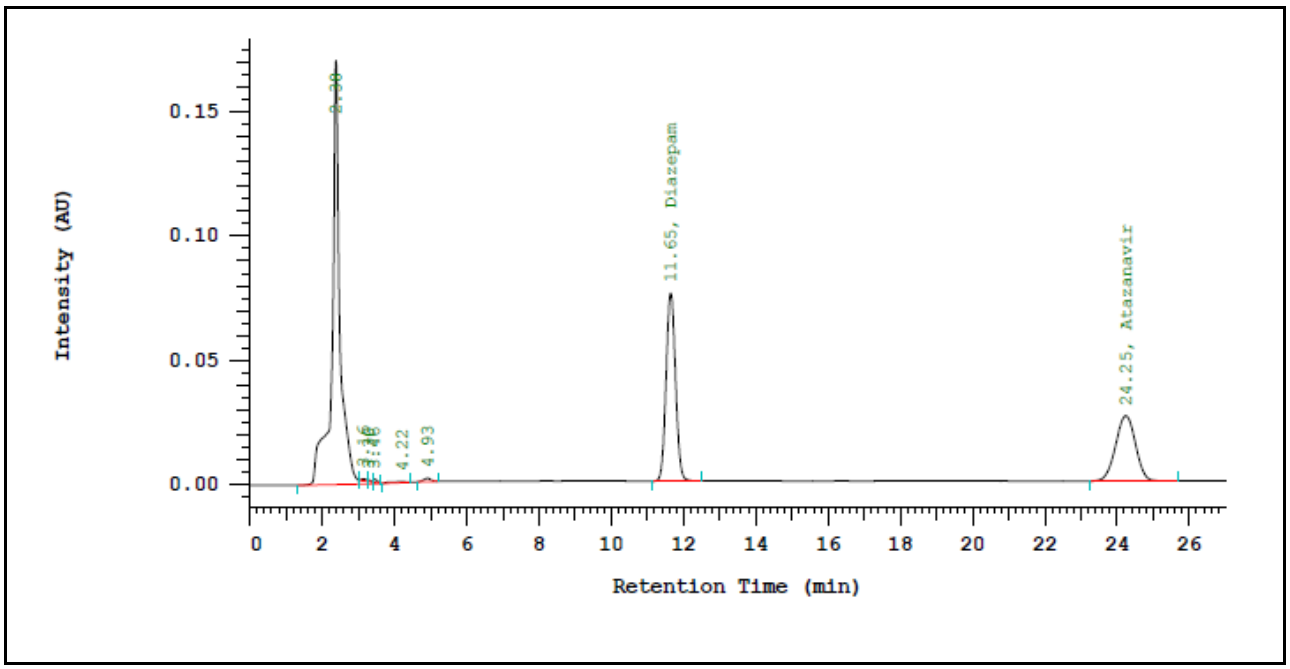


Figure 4. 1: Chromatogram of atazanavir and diazepam extracted from plasma (10µg/mL and 4.5 µg/mL respectively)

4.3.2 HPLC – UV method verification

4.3.2.1 Calibration curve and linearity

The simple regression equation for the calibration curve was y = 0.0001x + 0.0081 with a coefficient of determination of 0.9931 (>0.99). Figure 4.2 represents the calibration plot. The standard deviation of the residuals was 3.9% and thus were within the 15% limit. They were normally distributed and did not display a trend as illustrated in Figure 4.3.

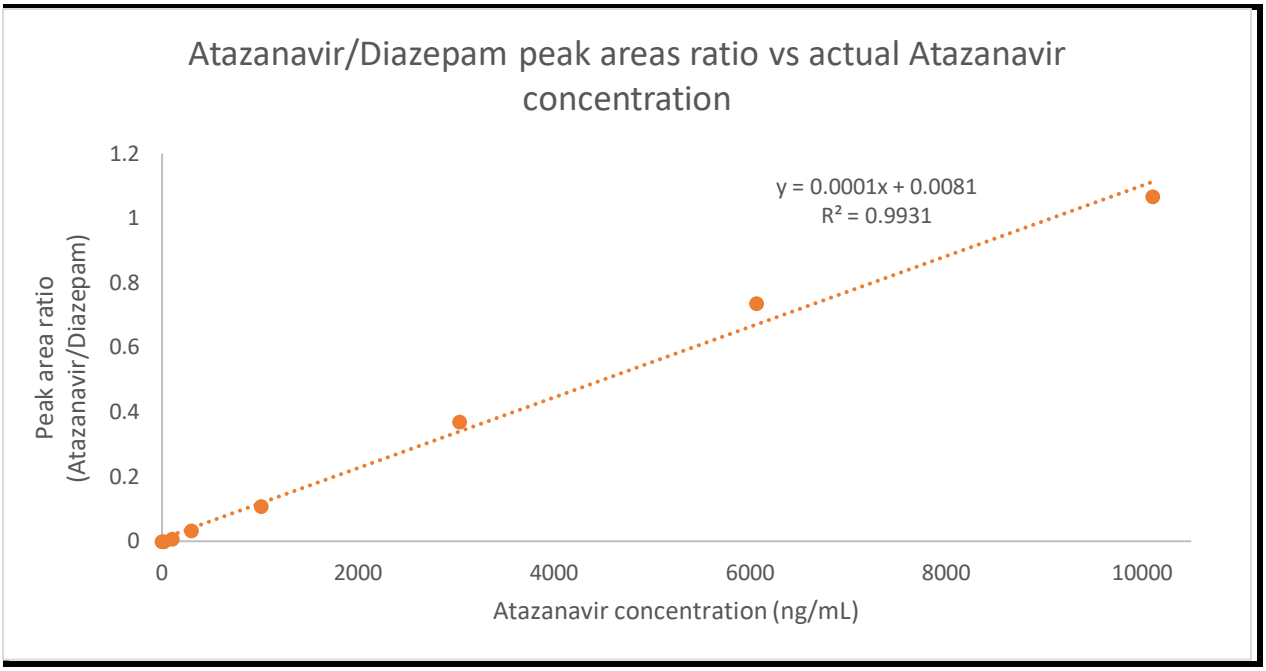


Figure 4. 2: Calibration curve for atazanavir in plasma (20-10000 ng/mL)

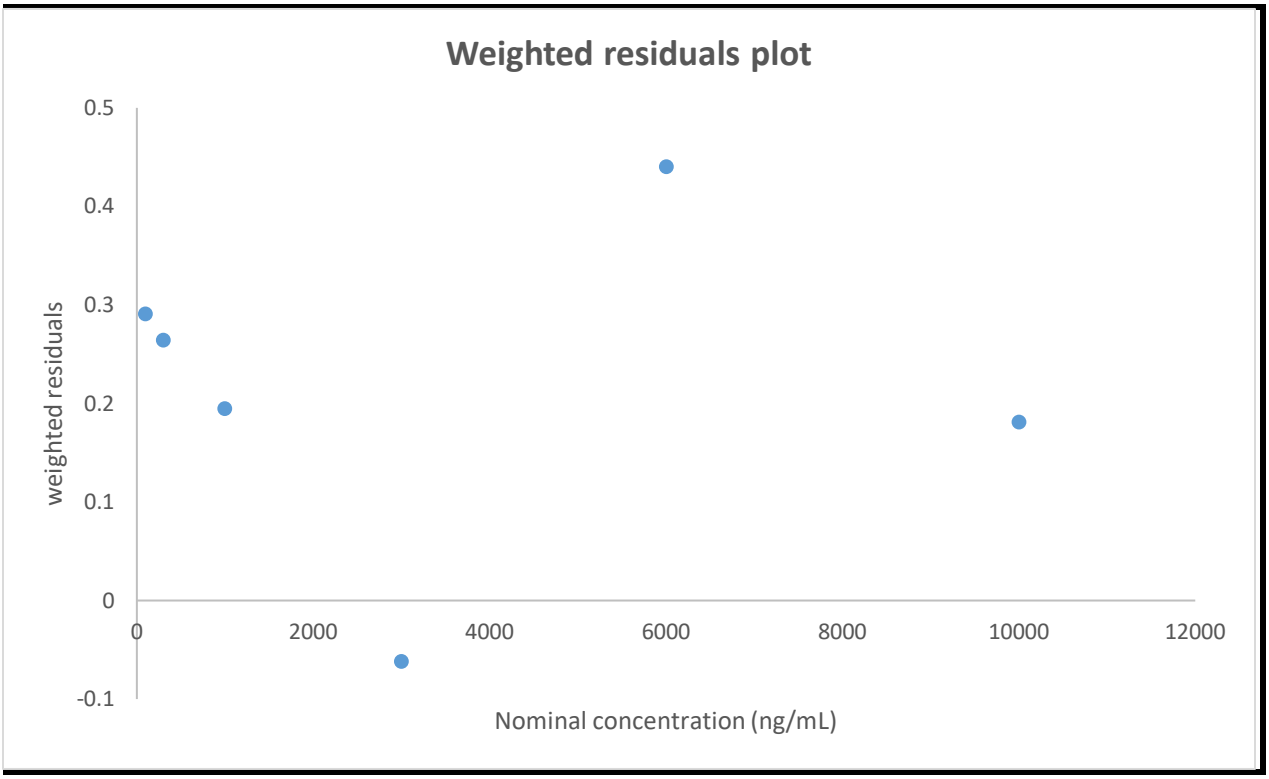


Figure 4. 3: Scatter plot of residuals against nominal concentrations

4.3.2.2 Accuracy, precision, recovery and sensitivity

The precision (presented as the % relative standard deviation, RSD) of the method was a mean of 8.8% (SD = ± 6.2) ranging between 2.18 and 17.02%. Intra-day precision was 5.64% ± 4.17 ranging from 2.69 to 8.59 % (Table 4.3).

Accuracy/recovery of atazanavir from plasma ranged from 91.3 - 120.8%.

Table 4. 3: Accuracy and precision of the method

Accuracy	**40 ng/mL (LOQ)**	**90 ng/mL (Low QC)**	**120 ng/mL (Medium QC)**	**250 ng/mL (High QC)**
Mean ± SD (ng/ml)	44.0 ± 4.3	85.8 ± 3.6	133.0 ± 2.6	253.5 ± 6.8
% recovery	99.3 - 120.8	91.3 - 99.3	108.7 - 113.0	98.7 - 104.1
Precision (% RSD)	17.02	9.38	6.62	2.18

Precision (within day assay)	**80 ng/mL (n=6)**	**250 ng/mL (n=6)**
Precision (% CV)	8.59	2.69

The LOD was 40 ng/mL while the LOQ was 120 ng/mL (Table 4.4).

Table 4. 4: Limit of detection and limit of quantification of atazanavir

Concentration (ng/mL)	Relative Difference (%), n=5	Precision (RSD (%), n=5	Label
40	8.83	17.02	**LOD**
50	3.26.	8.87	
70	0.19	9.52	
100	9.83	7.76	
120	9.64	6.62	**LOQ**

4.4 Discussion

The run time for the optimized HPLC-UV method for the detection and quantitation of atazanavir was 26 minutes. This was 11 minutes longer than what was reported by Cattaneo *et al.*(45) since we used a longer column, but it offered equally good separation of the analyte, the IS and other eluting compounds.

The method proved linear in the range of drug concentrations from 100 -10000 ng/mL with an R^2 of 0.9931, similar to what was reported by Colombo *et al.* (47). The linear regression assumptions were adhered to and the weighted residuals were distributed in a homoscedastic manner.

The method had good sensitivity with the limit of quantification being 120 ng/mL and the limit of detection was 40 ng/mL. This was comparable to other methods reported for quantifying atazanavir in plasma (48,50).

Recovery of atazanavir from plasma ranged between 78.0 - 89.8%, which is comparable to what has been reported in other methods (53,54). The intra-day

precision ranged between 2.7 to 8.6 %, similar to what has been reported in other methods (49,51,55).

4.5 Conclusion

The verified and optimized HPLC-UV method for the detection and quantitation of atazanavir in human plasma accords an accurate and reproducible process. There was good extraction efficiency with a low limit of quantification, given the expected atazanavir plasma concentrations in patients from prior studies.

5.0 THE SOCIODEMOGRAPHIC TRAITS AND GENOTYPIC VARIANTS OF THE STUDY POPULATION

5.1 Introduction

The rapid expansion of information on human genetics has provided an opportunity to understand the variability in response to therapeutic agents among different individuals and populations globally. Despite this rapid expansion, there are groups that have been left behind and their genetic data are yet to be fully characterized (12).

Widely accepted is the fact that a high level of genetic diversity exists within sSA, in addition to the known inter-ethnic variations in genetic makeup (12). Of interest to this study are the uncharacterized variations in CYP3A5 enzyme responsible for the metabolism of atazanavir.

Further impetus for this study is drawn from the fact that most drugs are marketed as safe for use in Africa based on recommendations from the United States Food and Drugs Administration and the European agencies. However, much consideration is not given to the risk of adverse outcomes based on differences in genetic makeup, primarily because that data is not available to the regulators.

Our objectives were to characterize the frequencies of CYP3A5 and UGT1A1 SNPs in a subset of the Kenyan population. We achieved this by conducting PCR on pure DNA extracted from packed cells.

5.2 Baseline characteristics of the study population

One hundred and ten participants were enrolled in the study over a time period of two months. Their baseline sociodemographic characteristics are outlined in Table 5.1. The mean age of the study population was 39.8 (SD ± 11.8) years. More females (n = 64, 58.2%) were enrolled in the study compared to males (n = 46, 41.8%). Over half the participants had attained secondary level of education (n = 56, 50.9%) whereas only one participant had received informal education. Over two thirds of the participants were in gainful employment with the remaining third unemployed. Most neither smoked (98.2%) nor took alcohol (89.1%).

5.3 Baseline medical and antiretroviral therapy (ART) data

A small proportion of the study participants had hypertension (n = 10, 9.1%) or diabetes (n = 5, 4.5%). The median CD4 count was 219 cells/mL [IQR 272] whereas approximately 50% (n = 54) of the participants were virally suppressed. The median duration since diagnosis of HIV infection was 9.4 years [IQR 3.8] while mean duration on antiretroviral therapy was 8.4 years (SD 3.3). Most participants were on regimens consisting of tenofovir and lamivudine (57%, n = 63) followed by zidovudine and lamivudine (37%, n = 41) (Figure 5.1).

Table 5. 1: Baseline characteristics of the study population

Variables	Total (N=110)	%
Sex		
Male	46	41.8
Female	64	58.2
Age in years; mean (SD)	39.8 (11.8)	
Marital status; n (%)		
Single	34	30.9
Married	59	53.6
Separated	10	9.1
Divorced	1	0.4
Widowed	6	5.5
Number of children; median (IQR)	2(0-3)	
Level of education		
Primary	24	21.8
Secondary	56	50.9
Tertiary	29	26.4
Informal education	1	0.9
Occupation; n (%)		
Unemployed	30	27.3
Self employed	43	39.2
In formal employment	37	33.6
Smoke cigarettes; n (%)	2	1.8
Alcohol consumption; n (%)	12	10.9
Hypertension; n (%)	10	9.1
Diabetic; n (%)	5	4.5
CD4 count; median (IQR)	219(272)	
Viral load; median (IQR)	26840(1034000)	
Time since diagnosis of HIV in years; median (IQR)	9.4(3.8)	
Duration on antiretroviral therapy in years; mean (SD)	8.4(3.3)	
Regimen ever modified; n (%)	108	98.2
Reason for regimen modification; n (%)		
Regimen failure	105	95.5
Toxicity	3	2.7
Other	2	1.8
Current opportunistic infections; n (%)	10	9.1
Current regimen		
TDF/3TC/ATV/r	63	57.3
AZT/3TC/ATV/r	41	37.3
ABC/3TC/ATV/r	5	4.5
RAL/ATV/r	1	0.9

None of the participants were on antidepressants, anticoagulants or antibiotics at the time of the study (Table 5.2). Only 6 participants reported that they were currently using or had ever used alternative or complementary medications.

Table 5. 2: Medications used by study participants on second line regimens

Drug name	n	Yes (%)
Cotrimoxazole	109	99.1
Tenofovir	63	57.3
Zidovudine	41	37.3
Antihistamines	8	7.3
Herbs	6	5.5
Abacavir	5	4.5
Anti tuberculous agents	4	3.6
Antidiabetics	3	2.7
Antipsychotics	3	2.7
Pyridoxine	3	2.7
Antifungals	2	1.8
Hematinics	2	1.8
Statins	1	0.9
Multivitamins	1	0.9
Dapsone	1	0.9

Nearly all the participants had switched HAART regimens and the most common reason for this change was regimen failure (Table 5.1). Approximately 95% of the participants were in the WHO stage II or III of HIV infection.

5.4 Prevalence of CYP3A5 (*3, *6 & *7) and UGT1A1*28 single nucleotide polymorphisms

5.4.1 CYP3A5*3 genotypic and allelic frequencies

The prevalence of CYP3A5*3 TT wild type genotype was 26 (24%) whereas that of the heterozygous TC genotype was 77 (70%). The CC variant genotype had a frequency of 7 (6%). The allelic frequencies are summarized in Table 5.3. The T allele occurred at a frequency of 58.6% while that of the C allele was 41.4% (Chi square = 6.56, P = 0.010). Compared to the expected genotypic prevalence in the population, the observed frequencies did not conform to the Hardy Weinberg equilibrium proportions.

Table 5. 3: CYP3A5*3 genotypes and allelic frequencies in the study population

Characteristic	Predicted phenotype (Metabolizer status)	Proportion of patients		Chi square (Test for Hardy Weinberg equilibrium)	P
		n	%		
CYP3A5*3					
Genotypes					
TT	Extensive	26	24		
TC	Intermediate	77	70		
CC	Poor	7	6		
N		110			
Alleles					
T		129	58.6		
C		91	41.4	6.56	**0.010**
N		220			

5.4.2 CYP3A5*6 genotype and allele frequencies

The prevalence of CYP3A5*6 CC wild type genotype was 3 (2.7%) while that of the heterozygous CT genotype was 107 (97.3%). There was no TT variant genotype. The genotype and allelic frequencies are listed in Table 5.4. The frequencies of the C and T alleles respectively were 51.4% and 48.6%, respectively (chi square = 0.164, P = 0.686). This was in conformity with the expected Hardy Weinberg equilibrium proportions.

Table 5. 4: CYP3A5*6 genotypes and allelic frequencies in the study population

Characteristic	Predicted phenotype (metabolizer status)	Proportion of patients		Chi square (Test for Hardy Weinberg equilibrium)	P
		n	%		
CYP3A5*6					
Genotypes					
CC	Extensive	3	2.7		
CT	Intermediate	107	97.3		
TT	Poor	0	0		
N		110			
Alleles					
C		113	51.4		
T		107	48.6	0.164	0.686
N		220			

5.4.3 CYP3A5*7 genotype and allele frequencies

The prevalence of the CYP3A5*AA wild type genotype was 1 (0.9%) while that of the heterozygous A/- genotype and the -/- variant genome was 106 (96.4%) and

3 (2.7%), respectively (Table 5.5). The allelic frequencies were: A = 49.1% and -- = 50.9% respectively. These frequencies were in conformity with the expected Hardy Weinberg equilibrium proportions.

Table 5. 5: CYP3A5*7 genotypes and allelic frequencies in the study population

Characteristic	Predicted phenotype (Metabolizer status)	Proportion of patients		Chi square (Test for Hardy Weinberg equilibrium)	P
		n	%		
CYP3A5*7					
Genotypes					
AA	Poor	2	1.8		
A/-	Intermediate	105	95.5		
--	Extensive	3	2.7		
N		110			
Alleles					
A		109	49.5		
-		111	50.5	0.018	0.893
N		220			

5.4.4 UGT1A1 genotype and allele frequencies

Genotyping for the single nucleotide polymorphism using the rs 887829 (c,-364 C>T; UGT1A1*80) was done. The rs 887829 T allele is in very strong linkage disequilibrium (LD) with the TA_7 and TA_8 alleles, while the rs 887829 C was in very strong LD with the TA_5 and TA_6 alleles.

The prevalence of the CC genotype, otherwise identified as an extensive metabolizer, was 19 (17.3%) while that of the heterozygous CT genotype, also

referred to as the intermediate metabolizers, was 191 (82.7%). The C allele occurred at a frequency of 58.6% while the T allele was at 41.4% (Chi square = 6.56, P = 0.010). Compared to the expected genotype frequencies in the population, the observed proportions did not conform to those of the Hardy Weinberg equilibrium (Table 5.6).

Table 5. 6: UGT1A1 genotypes and allelic frequencies in the study population

Characteristic	Predicted phenotype (Metabolizer status)	Proportion of patients		Chi square (Test for Hardy Weinberg equilibrium)	P
		n	%		
UGT1A1					
Genotypes					
CC	Extensive	18	16.4		
CT	Intermediate	92	83.6		
TT	Poor	0	0		
N		110			
Alleles					
C		128	58.2		
T		92	41.8	5.89	**0.015**
N		220			

5.5 Discussion

We genotyped 110 Kenyan HIV positive patients sampled from among the various ethnic groups that are represented in the country. We found the allelic frequencies for CYP3A5*3, CYP3A5*6 and CYP3A5*7 were 41.4, 48.6, 50.9 %

respectively. Genotypic frequencies for UGT1A1 were 17.3, 82.7 and 0 % for CC, CT and TT respectively.

Cytochrome P450 enzymes are involved in the disposition of most drugs and xenobiotics within the body. There are differences in the distribution of CYP 450 allelic variants between ethnic groups, and these demographic based variations are known to contribute to interindividual variability of drug metabolism and response (56,57). These variations exist between populations and also among persons within the same population. African populations show the greatest genetic heterogeneity (58).

A SNP at nt 22,893 (AC 005020) intron 3 of CYP3A5*3 results in a loss of hepatic expression of CYP3A5. A cryptic splice site is generated and exon 3B producing a stop codon. The protein that is generated upon translation of the abnormal transcript is terminated prematurely at amino acid 109 and has no catalytic activity (59).

The prevalence of the CYP3A5*3 SNP reported in European populations is much higher than that in the Kenyan population studied here. Among Russians, Greeks and Bosnians, the prevalence ranges between 91 – 95 %, which is nearly double of what we observed (57,60,61). Among the American Indians and the Native Alaskans, the prevalence is 92.47%, which is just as high as that in European populations (62).

The prevalence of CYP3A5*3 in Asian populations is far more diverse, particularly among the Chinese; ranging from 35% to as high as 88% across the

different Chinese ethnic groups (Uyghur, Kazakh, Tibetan, Han, Wa and Bai Chinese) (63–66). This SNP is reported to be the most common among Koreans (67), whereas in the Japanese population, the prevalence is approximately 76% (68). In Jordan, a Middle Eastern nation, the prevalence is approximately 93%, similar to that in the European populations (69).

In West Africa, among Ghanaians and Nigerians, the prevalence of CYP3A5*3 SNPs is much lower (15 and 16% respectively) than our finding (70,71). In the East (Tanzania (mainland) and Zanzibar) and Southern Africa, the prevalence ranges between 15 – 18 %, much closer to the Nigerian estimate (70,72), but still much lower than what we report. In contrast to all these findings and ours as well, the prevalence in a Zimbabwean cohort was quite high (76%), representing the diversity of genetic variations within the African continent (73). Within the East African region, the prevalence in Ethiopians was 40.3% which was in concordance to what we observed (12).

The prevalence of CYP3A5*3 SNPs is highest in European populations. It has been postulated that this SNP is at the point of fixation among Europeans (28). From the literature presented, it is clear that the prevalence is lowest in African populations, but at the same time, it is the most diverse region. These differences within African populations could be representative of evolutionary pressures that may have been the result of environmental factors resident within geographically distinct regions (73). One such environmental factor that has been quoted in relation to this genetic diversity is the distance from the equator.

The further away a population is from the equator, the higher the frequency of CYP3A5*3 SNPs (74).

The CYP3A5*6 allele contains a 30597G >A mutation in exon 7 that is associated with the deletion of exon 7 from the CYP3A5 mRNA and consequently has lower catalytic activity. Among East Asian populations, this SNP is rare and was not detected in the aforementioned studies that also sequenced for CYP3A5*3 (63,65–68). Among Jordanians in the Middle East, the prevalence was 2.2% (69).

In African populations drawn from the West, Central and East regions, the prevalence of CYP3A5*6 SNPs ranged from 4 - 33% (12,28,70), all of which were lower than what we found. Our finding is higher than what is reported in literature, but it is in agreement with the fact that this polymorphism is more common in Africans and absent in Caucasians for whom we could not find literature on the prevalence (73). This mutation is believed to be approximately greater than 200,000 years old, and owing to its restricted geographical distribution, it is postulated that it was lost in a population bottleneck in the migration and expansion process of the human race out of Africa (12).

A frameshift mutation is what characterizes the CYP3A5*7 allele and this leads to the synthesis of a truncated protein lacking catalytic activity (29). This polymorphism is rare among Asians and Europeans (67,68,73), but is common among Africans. According to Bains *et al.*, this polymorphism was found in only the Niger-Congo speaking population (0-22%) (12), but in another analysis, it was also found among Ethiopians, though the frequency was much lower (1.4%).

In our study, we report a very high allelic and genotypic frequencies of *7 highlighting the differences between East and West African populations. It is postulated that CYP3A5*7 arose from a recent mutation and spread with the growth of the Niger- Congo speaking group approximately 4000 years ago (75).

The findings support that there is a greater variation in genetic mutations of CYP3A5 amongst Africans. The CYP3A5*6/*7 mutations are more prevalent in this population compared to Asians and Europeans.

Abnormalities in the conjugation of bilirubin to glucuronide causes a benign form of indirect hyperbilirubinemia. Polymorphisms in the gene coding for the enzyme UGT1A1 results in reduced activity (76). The defect is the insertion of a TA repeat in the regulatory TATA box of the UGT1A1 gene promoter. The TA 7 and 8 repeats have reduced activity since the TATA box is the binding site for transcription factor IID that is required for initiating transcription (77). Increased promoter length causes reduced enzyme transcription and consequently decreased bilirubin conjugation. TA 5 repeats cause higher transcription activity than the normal TA6 repeat and these individuals are likely to have lower bilirubin levels (10).

In Asian populations, the prevalence of the CC homozygous genotype (extensive metabolizers) ranges between 0.8 – 82% (78–81). More specifically, the prevalence of the TA 6/6 repeat was between 45 and 82 % (78,80,81) while that of the TA 5/6 repeat, which was reported in only one study, was 0.8% (79). These frequencies were much higher than those described in our population except for

the TA 5/6 repeat which is more common in African populations(65,76), but which we did not identify.

Among Caucasians and Europeans, the CC homozygous genotype frequency was between 7% in Romanians (82) and 43- 46% in Russians and other white populations (80,83). In Saudi Arabians , the 6/6 TA repeat occurred at a frequency of 74.3% (84). However, the 5/6 TA repeat was not reported in either of these populations and this was expected (65,76).

In Africans, the CC homozygous genotype frequency was 49.5 % which consisted of the 6/6 TA repeat (45.5%) and the 5/6 TA repeat (4.0%) (76). This is much higher than we report, but this could be due to a significant contribution by geographical location and possible contribution from a genetic pooling since the higher prevalence reported was from a sample of North and Central Americans with varying degrees of African ancestry.

The CT heterozygous phenotype (consisting of 6/7, 6/8, 5/7 and 5/8 TA repeats) has also been identified in Asian populations in varying frequencies. The 6/7 TA repeat frequency ranges from 11 – 35 % (76,78–80,85,86) whereas the 6/8 TA repeat has a very low frequency of 0.8%, and this is expected (79). These frequencies are much lower than what we report.

The frequency of the 6/7 genotype among Europeans and other Caucasians lies between 39 and 58% (76,80,82,83). In these populations, the 6/8, 5/7 and the 5/8 genotypes are not present and therefore not reported.

The CT genotype is highly prevalent in African populations and three out of the four genotypes have been identified and reported (6/7, 6/8 and 5/7 genotypes) in frequencies of 37, 2.0 and 5% respectively (76), which is nearly half of what we report, but the reason has already been alluded to.

UGT1A1*28 also identified as 7/7 genotype was not identified in our study population. This is otherwise referred to as the TT allele pair. In the Asian continent, including the Far East in Japan, Malays, Indians and the Chinese, the prevalence ranges from 0 - 15% (78–80,85–87). Among Europeans and other Caucasians, the frequency is between 9 and 32% (79,80,82,83,88) whereas among the Saudis, the prevalence is 26% (84). Beutler *et al.* reports that the prevalence among Africans is 19% (76).

The differences in the number of TA repeats between the populations discussed could be adaptational. Bilirubin is known to be both beneficial as an antioxidant and harmful as well, more so in neonates where at high levels, it causes kernicterus and brain damage. Higher TA repeats that confer reduced UGT1A1 activity are more common in Africans compared to Asians and Caucasians. This could be understood as being harmful to the African people, but it has been demonstrated that Caucasians have a higher red cell mass compared to Africans and thus a higher hemoglobin content (76).

Since bilirubin is a degradation product of hemoglobin, Africans produce less of it and therefore reduced UGT1A1 activity does not necessarily confer a disadvantage. In addition, and as alluded to earlier, bilirubin is an antioxidant

and this may have been an adaptation in response to stressful environmental conditions. Moreover, the fact that Africa lies within the tropics means that degradation of any excess bilirubin by way of phototherapy occurs saliently in the background (76).

The allelic frequencies for CYP3A5*3 and UGT1A1 were not in Hardy Weinberg Equilibrium (HWE). There are several ways that this finding can be explained. The Hardy Weinberg equilibrium generally states that allelic frequencies within a population remain stable from one generation to the next. There are exceptions, such as the introduction of new alleles into the population by way of mutations, non-random mating, gene flow, genetic drift and genotyping error (89,90). There is also the possibility of population stratification due to the presence of a systematic difference in allele frequencies between sub populations in a population due to differences in ancestry. The test for HWE however do not distinguish between the causes of disequilibrium. HW disequilibrium is also noted to occur more frequently in unstable regions where there is segmental duplications and single tandem repeats (89). This may be the case for UGT1A1, where variants arise due to a difference in the number of TA repeats as earlier indicated.

Abramovs *et al.* avers that a heterozygote excess (HetExc), which is what was observed, might be one of the reasons for the observed disequilibrium. It is believed that the HetExc could be a sign of natural selection where in the heterozygous state, allele frequencies for autosomal recessive disorders reach a

high frequency compared to the homozygous state, and this offers some survival advantage. Abramovs *et al.* found two known recessive disease-causing variants with evidence of heterozygote advantage in the sickle-cell anemia *(HBB)* and cystic fibrosis *(CFTR)* in an African American population (90). Sickle cell anemia is common in malaria endemic zones of Kenya, some of which also have the highest in country prevalence of HIV.

In Kenya, there are 43 tribes, and within those major tribes, there are sub-tribes. In the modern-day setting, there is a lot of intermarrying between tribes and subtribes, and these individuals may have different ancestry. The lack of equilibrium therefore needs to be viewed in light of this reality. In addition, the Kenyan population has been left out of studies looking at genetic diversity in the past, and as such, our findings provide an opportunity for others to further the pool of knowledge.

This is the first study in Kenya to examine the genetic mutations in drug metabolizing enzymes in relation to atazanavir. The study had several limitations though, one being that the sampled population may not be representative of the general Kenyan population. Secondly, we were not able to get data on ethnicity for each patient which would have greatly enriched the inferences.

5.6 Conclusion

Mutations in CYP3A5 gene were found in Kenyan HIV positive patients. The frequencies were however different from other populations that we compared with, Caucasians, Asians and other African nationalities. We did not find any

individual with the UGT1A1*28 SNP which is contrary to literature regarding the prevalence of this SNP in Africans. However, this study is the first examining these SNPs in our population, and thus adds to the already known fact that the African continent is richly diverse in genetic variations.

6.0 CYP3A5 EXPRESSOR STATUS AND ITS IMPACT ON ATAZANAVIR PEAK AND TROUGH LEVELS AT STEADY STATE

6.1 Introduction and objectives

CYP3A5 is a highly polymorphic enzyme (28) and it displays a bimodal pattern of hepatic expression (91). CYP3A5*1 is the ancestral allele and is the expressor phenotype whereas CYP3A5*3, *6 and *7 are derived alleles that are termed as low/non expresser phenotypes (28). Significant inter-ethnic variability exists in CYP3A5 expression and this can either be as high concentrations (21 -202 pmol/mg) or reduced or undetectable protein levels (<21 pmol/mg) (12).

CYP3A5 is highly expressed in Black Africans (50 -70%), however, among Caucasians, the expression is as low as 10%. CYP3A5*3 is found in all ethnicities (85% in Caucasians, 65 – 85% of Asians, 75% of Hispanics and 55% of African Americans). CYP3A5*6 and CYP3A5*7 have been identified solely in Black African and African American populations) (59).

Data from HIV negative volunteers has demonstrated a faster clearance and lower trough concentrations of atazanavir among expressers compared to non-expressers, though this effect was not demonstrated among African American males (30). To understand these racial differences, atazanavir metabolites were examined in human plasma samples from the previous study. Interestingly, the metabolite ratios in African American and non-African American males were consistent; 2 fold higher in expressors compared to non expressors (25). This finding was in tandem with those of a sub-study of the AIDS Clinical Trials Group

(ACTG) PEARLS study (91). These follow up studies point to the fact that there could be some other factors beyond race that contributed significantly to the differences observed.

Our goal was to explore this field further by examining whether CYP3A5 SNPs had an influence on the recommended trough and peak concentrations of atazanavir at steady state (150 and 850 ng/mL respectively)(20) in Kenyans living with HIV. This was achieved by sampling blood at several time points for the plasma levels as well as for the genetic testing. Plasma levels were determined using HPLC with UV detection while the presence or absence of SNPs was ascertained by use of PCR. The findings of that analysis are presented herein.

6.2 Results

6.2.1 Distribution of steady state plasma concentrations of atazanavir

Three plasma samples were obtained from each patient representing the trough levels (pre-dose), peak levels (two hours post-dose) and post peak sample (four hours post-dose). Median concentrations for Cmin, Cmax and post Cmax concentrations were 631.1 (IQR 1147.1) ng/mL, 4283 (IQR 3970) ng/mL and 3671 (IQR 3671) ng/mL respectively (Figure 6.1).

Females had higher median concentrations at all three time points (Table 6.1). Median trough concentrations were higher in the younger age group (18 -41), but the peak and post peak concentrations were higher in the older age group (>42). Based on the CYP3A5 SNPs identified, the concentrations varied according to the predicted phenotypes (Figures 6.2 & 6.3).

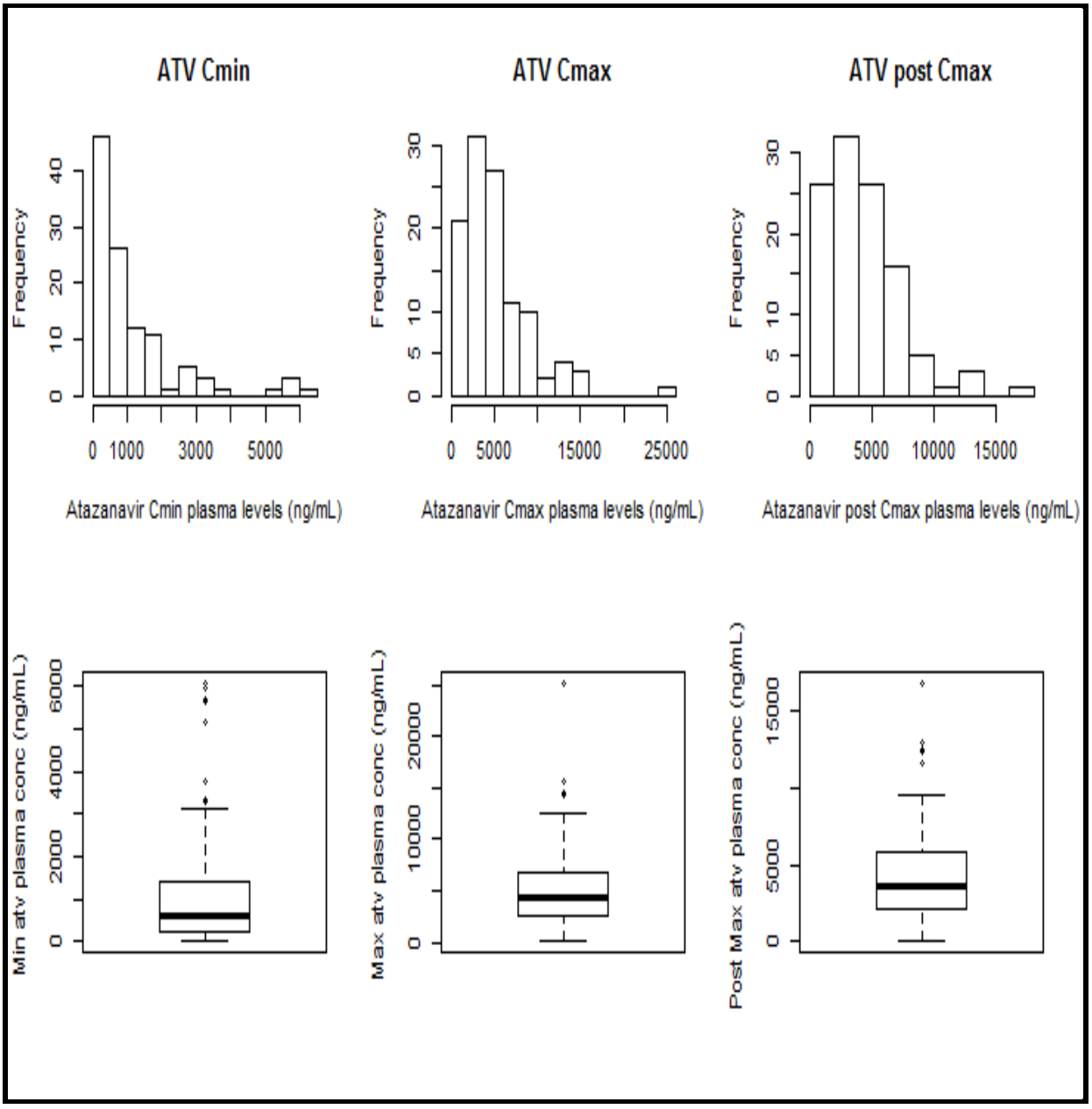


Figure 6. 1: Trough and peak levels of atazanavir at steady state

Table 6. 1: Distribution of atazanavir steady state plasma concentrations by baseline traits

Variable	Atazanavir plasma concentrations (ng/mL)		
	Cmin (Median (IQR))	Cmax (Median (IQR))	Post Cmax (Median (IQR))
Age			
18 - 41	550.6 (965)	4500 (3476)	4107 (3542)
42 - 80	782.6 (1329.1)	3695 (3957)	3415 (3643)
Sex			
Male	493.8 (482.8)	3623 (3644)	3671 (3977)
Female	980.0 (1481)	4500 (4166)	7113 (3552)
CYP3A5*3			
TT	535.0 (826.5)	3695 (3970)	2800 (2785)
TC	650.8 (1115.5)	4859 (3699)	4244 (3936)
CC	1728.0 (2002.4)	2596 (6259)	5168 (3272)
CYP3A5*6			
CC	162.4 (121.6)	1347 (2476)	853.6 (1547.9)
TC	649.5 (1150.1)	4295 (3955)	3962 (3647)
CYP3A5*7			
AA	2922.0 (2760)	6969 (5622)	4822 (3968)
A/-	643.7 (1125.4)	4270 (3536)	3641 (3613)
--	477.7 (1231.5)	6298 (3413)	3701 (1811)

There was evidence of differences in the distribution of atazanavir trough concentrations by sex (Wilcoxon Rank sum test, p = 0.028). Similar differences were not identified in the peak and post peak concentrations by sex.

Regarding the trough levels of ATV, slightly above half (54%) of the participants had concentrations that were within the stipulated therapeutic range (Table 6.2). Approximately 40% had supratherapeutic concentrations while 15% had subtherapeutic concentrations. Above 90% of the participants had

supratherapeutic plasma concentrations at peak time and 2 hours post peak (Table 6.2).

Table 6. 2: Proportions of participants with subtherapeutic, therapeutic and supratherapeutic plasma atazanavir concentrations

Plasma concentrations sample	Subtherapeutic < 150 ng/mL	Within range ≥ 150 - ≤ 850 ng/mL	Supratherapeutic >850ng/mL
C min (n, %, range)	17 (15.5%) (undetectable – 148.9 ng/mL)	60 (54.4%)	43 (39.1%) (900 – 6093.8 ng/mL)
C max (n, %, range)	1 (0.9%) 41.9 ng/mL	7 (6.4%)	102 (92.7%) (993.7 – 25285.8 ng/mL)
2 hrs post C max (n, %, range)	2 (1.8%) Undetectable	3 (2.7%)	105 (95.5%) (853.5 – 16886.3 ng/mL)

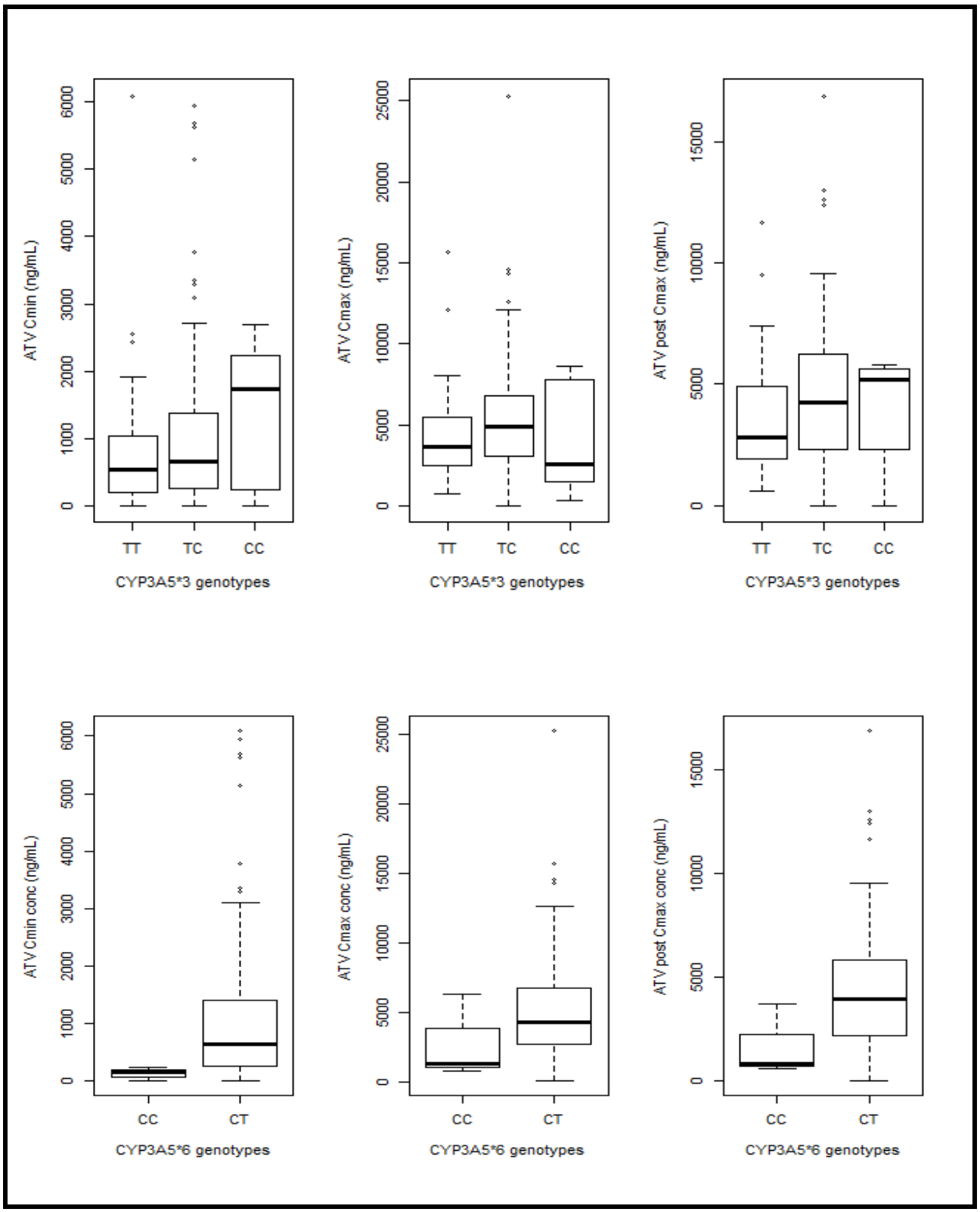


Figure 6. 2: Distribution of concentrations of atazanavir (Cmin, Cmax and post Cmax) by CYP3A5 polymorphisms

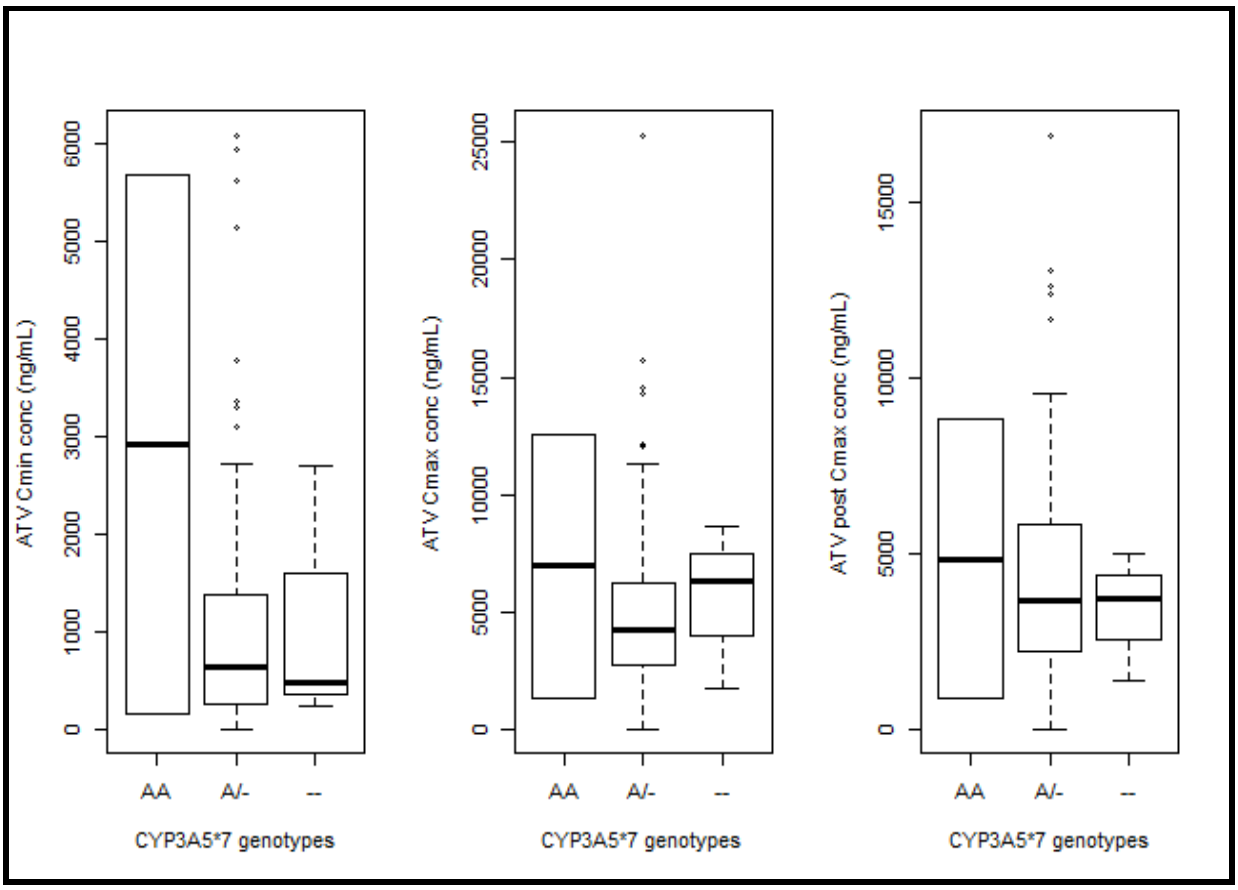


Figure 6. 3: Distribution of atazanavir plasma concentrations (Cmin, Cmax and post Cmax) by CYP3A5*7 polymorphisms

6.3 Influence of CYP3A5 polymorphisms on the steady state plasma concentrations of atazanavir

Generalized linear modeling (GLM) was utilized to examine if an association existed between CYP3A5 polymorphisms and the trough and peak concentrations of atazanavir. Bivariable analysis showed that sex was significantly associated with the trough plasma concentrations of atazanavir (Table 7.3). Women were more likely to have higher mean plasma trough concentrations compared to men (507 ng/mL, 95% CI 15.7, 997.6, p=0.046).

Multivariable GLM showed that CYP3A5*6 SNP influenced the plasma trough concentrations of atazanavir (Table 7.3). Persons carrying the CYP3A5*6 heterozygous allele pair were more likely to have higher mean plasma trough concentrations compared to those with the wild type allele pair, holding all other variables constant (2151.1.9 ng/mL, 95% CI 275.5, 4026.8, p= 0.025).

Similar bivariable and multivariable analysis with peak atazanavir concentrations was performed (Table 7.4) but these did not yield any significant associations.

Table 6. 3: Bivariable and multivariable generalized linear modeling of the association between trough plasma concentrations of atazanavir and several predictors

Variable	Beta coefficient (crude) (ng/mL)	95% CI (L, H)	p value	Beta coefficient (adjusted) (ng/mL)	95% CI (L, H)	p value
CYP3A5*3						
Wild type (**reference**)	1			1		
Heterozygous	-198.8	-1221.6, 821.0	0.704	-20.4	-655.9, 615.0	0.949
Homozygous	-407.3	-1510.5, 695.9	0.471	-259.7	-1478.7, 959.3	0.673
CYP3A5*6						
Wild type **(ref)**	1			1		
Heterozygous	987.1	-2490.3, 516.1	0.201	2151.1	275.5, 4026.8	**0.025**
CYP3A5*7						
Wild type (**reference**)	1			1		
Heterozygous	-83.0	-1577.3 1411.3	0.914	-751.3	-2417.2, 914.5	0.373
Homozygous	1780.0	-549.7, 4109.7	0.137	2048.1	528.3, 4624.5	0.118
Sex	506.7	15.7, 997.6	**0.046**	426.1	-110.5, 962.6	0.118
Age	10.7	-10.3, 31.8	0.320	6.1	-16.7, 28.9	0.597
Alcohol use	-539.9	-1324.7, 244.8	0.180	-480.9	-1295.4, 333.6	0.244
Years on atazanavir	36.8	-140.6, 214.1	0.685	23.5	-159.7, 206.7	0.799
Current opportunistic infection	429.0	-425.4, 1283.3	0.327	-53.3	-1042.3, 935.6	0.694
Use of herbs	115.6	-970.6, 1202.8	0.835	242.4	-997.0, 1461.8	0.915

Table 6. 4: Bivariable and multivariable generalized linear modeling of the association between peak plasma concentrations of atazanavir and several predictors

Variable	Beta estimate (crude) (ng/mL)	95% CI (L, H)	p value	Beta estimate (adjusted) (ng/mL)	95% CI (L, H)	p value
CYP3A5*3						
Wild type (**reference**)	1			1		
Heterozygous	11138.9	-1860.0, 4137.8	0.458	703.5	-1212.8, 2619.7	0.474
Homozygous	64.2	-3170.5, 3298.9	0.969	-1345.4	-5021.5, 2330.7	0.475
CYP3A5*6						
Wild type (**ref**)	1			1		
Heterozygous	-2369.4	-2369.4, -6810.8	0.298	3886.5	-1769.7, 9542.8	0.181
CYP3A5*7						
Wild type (**reference**)	1			1		
Heterozygous	-500.6	-4975.8, 3974.5	0.827	-2207.4	-7231.0, 2816.3	0.391
Homozygous	1403.7	-5573.2, 8380.5	0.694	1912.8	-5856.5, 9682.2	0.603
Sex	1254.4	-200.5, 2709.2	0.094	1602.4	-15.6, 3220.5	0.055
Age	10.1	-52.3, 72.5	0.752	-7.6	-76.4, 61.3	0.852
Alcohol use	-108.2	-2440.2, 2223.9	0.928	323.7	-2132.6, 2779.9	0.797
Years on atazanavir	-8.8	-533.7, 516.1	0.974	-82.3	-634.8, 470.2	0.771
Current opportunistic infection	99.1	-2429.9, 2628.1	0.939	-1037.4	-4019.6, 1944.8	0.497
Use of herbs	484.9	-2715.4, 3685.2	0.767	1288.0	-2389.3, 4965.2	0.494

6.4 Discussion

In our study examining CYP3A5 expression in relation to atazanavir plasma trough concentrations, we demonstrated that persons with one or two functional alleles had lower plasma trough concentrations compared to those without which is consistent with a dominant effect. This is in concurrence with other findings from diverse populations of PLHIV that showed that CYP3A5 non expressers either had higher trough concentrations (10) or slower clearance of atazanavir (92,93). The trough plasma concentrations for approximately half the patients were within the therapeutic range, with a small proportion being sub and supra-therapeutic. The peak and 2 hours post peak plasma concentrations of ATV/r were predominantly supratherapeutic.

CYP3A5*6 was found to influence the trough concentrations of atazanavir. This is in agreement with other reports that CYP3A5 non expression does influence the clearance by slowing it and thus increasing the trough plasma concentrations (92). However, it is contrary to what Castillo-Mancilla *et al.* reported suggesting that CYP3A5 expression did not alter the pharmacokinetics of ritonavir boosted atazanavir and therefore the concentrations in persons of African ancestry (91). Though we did not identify an impact of CYP3A5*3 status on the trough or peak concentrations of atazanavir, Singkham *et al.* was able to demonstrate that Thai patients with the SNP had higher atazanavir concentrations (94).

Female sex was found to be associated with the trough concentrations of atazanavir at univariate and bivariate analysis, but not at the multivariate stage. We acknowledge that this variable may have been a confounder in the analysis, however, it is worth noting that women had higher trough concentrations which is in agreement with findings from other studies (94,95). Women are reported to have 10 -30% lower clearance of the atazanavir, though the mechanism for this has not been clearly elucidated (92,96–98). Some factors vary by sex, such as a slower gastric emptying time in females allowing for more atazanavir absorption (99), and higher ritonavir exposure in females (100).

Prior enquiries about the impact of CYP3A5 expresser status on the concentrations of atazanavir have been inconclusive, particularly in Africans (91,101). Our study has demonstrated an association between CYP3A5 expresser status (for CYP3A5*6 and *7) and trough plasma concentrations, which is a novel finding.

Attainment of desired clinical outcomes such as virologic suppression and rise of CD4 cells count may be slow or retarded based on the differences in plasma trough concentrations that are hinged on CYP3A5 expresser status. Given the potential for genetic variability within the East Africa region (11), periodic surveillance of patients plasma concentrations may be called for to prevent possible erosion of the gains made.

The proportion of patients who had sub-therapeutic trough plasma concentrations of ATV/r in this study was similar to that reported by Gervasoni

et al. at 12% (13). The median trough plasma concentrations were also similar, with Gervasoni *et al.* reporting 650 (IQR 847) ng/mL compared to our median of 631.1 (IQR 1147.1) ng/mL. Our findings however differed with those of Colombo *et al.* and Boffito *et al.* where none of their patients on the boosted regimen were below the lower limit of 150 ng/mL (18,102). Nearly a third of those subtherapeutic plasma concentrations reported in our study were undetectable, and this correlates with adherence data that we collected. Thirteen percent of our participants had low adherence, and this could be the reason for the observation on subtherapeutic plasma concentrations.

Thirty nine percent of our participants had supratherapeutic trough plasma concentrations, which is comparable to 44% reported by Gervasoni *et al.* among patients on ritonavir boosted atazanavir (13). In view of the finding of the impact of CYP3A5*6 heterozygosity on the trough plasma concentrations, this finding is not unexpected.

A major strength of this study was the large sample size for a pharmacokinetic and pharmacogenetic study. In addition, the large sample consisted of a group that was previously underrepresented. There were some limitations. We could not collect data on ethnicity due to the sensitivity surrounding the issue at the time of the study. We are also not certain whether our participants came to the clinic in a fasted state on the morning of the study which could have affected the plasma concentrations of ATV through enhanced absorption.

6.5 Conclusion

In summary, we have demonstrated that among Kenyan PLHIV, CYP3A5 expresser status is significantly associated with atazanavir plasma trough concentrations. The implication of this on clinical outcomes such as viral load and CD4 count warrants further investigation.

7.0 PREVALENCE AND RISK FACTORS FOR HYPERBILIRUBINEMIA.

7.1 Introduction and objectives

Hyperbilirubinemia is a commonly reported side effect in patients treated with boosted or unboosted atazanavir that presents as scleral icterus and jaundice resulting in discontinuation in less than 1% of patients (103). The basis of this side effect is elevated serum indirect bilirubin concentration occasioned by the inhibition by atazanavir of the enzyme UGT1A1 which mediates metabolite conjugation to glucuronide (35).

Atazanavir induced hyperbilirubinemia is perceived to be of little clinical significance as it has been observed to regress completely upon discontinuation of therapy, and in addition, it does not signify hepatic injury (35,103). However, it has also been argued that though seemingly clinically insignificant, it is extremely disturbing to patients and may increase stigma (104). As such, providing clinicians with the ability to predict the likelihood of the occurrence of atazanavir intolerance secondary to hyperbilirubinemia may reduce the rates of treatment discontinuations and modification (35,104).

Literature on the prevalence and risk factors for hyperbilirubinemia in this group of patients is not locally or regionally available. In response to this, we set out to determine the prevalence as well as the risk factors of hyperbilirubinemia in Kenyans living with HIV at a teaching and referral hospital.

To achieve this, serum samples were collected and analyzed for the total and indirect bilirubin levels which were then compared to the normal ranges to

determine those who had hyperbilirubinemia. Risk factors were identified through logistic regression modeling. The results of these analyses are herewith presented.

7.2 Prevalence of hyperbilirubinemia

All participants had their bilirubin levels determined during the study. The median total bilirubin concentration was 24 µmol/L (IQR 24.7) whereas the median indirect bilirubin concentration was 15.1 µmol/L (IQR 17.9) (Figure 7.1).

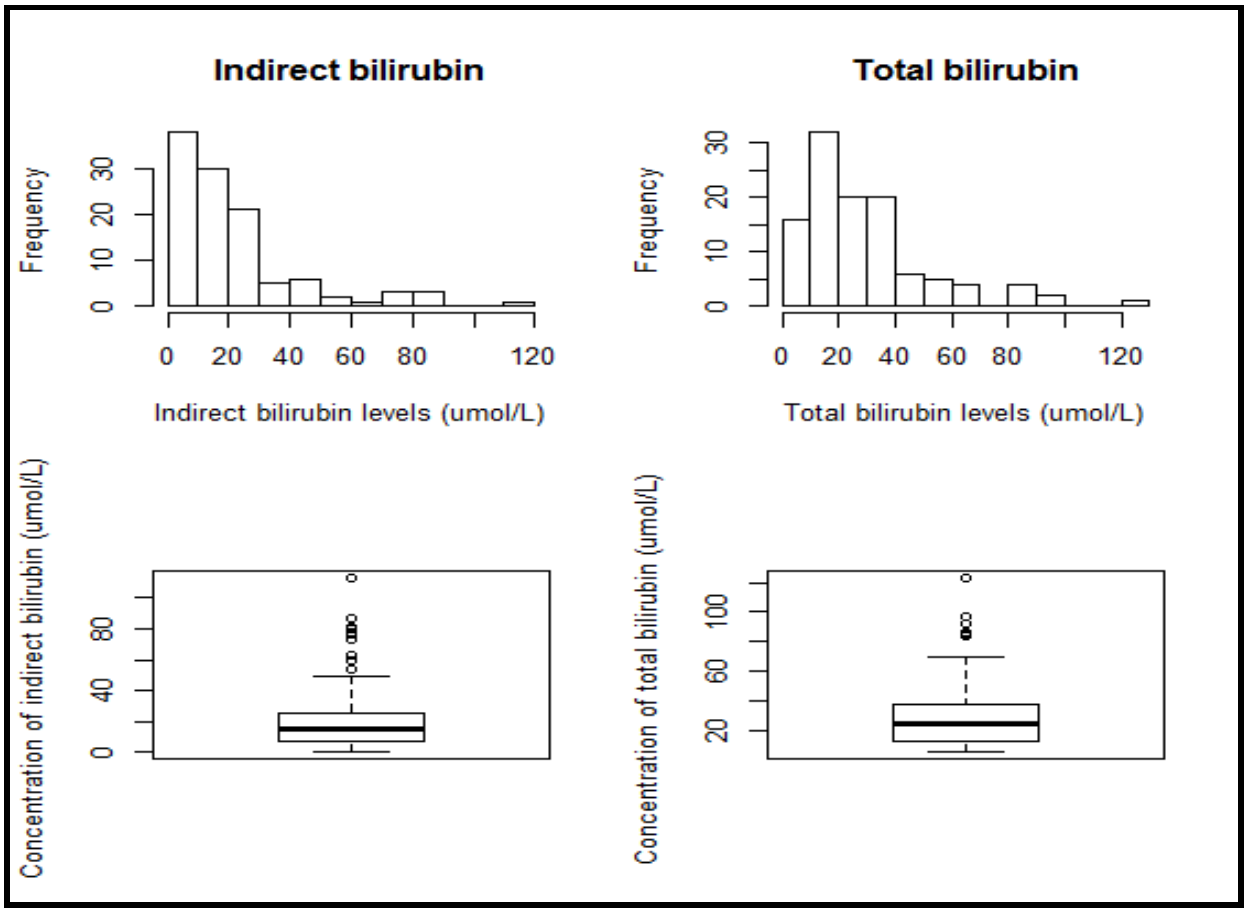


Figure 7. 1: Distribution of indirect and total bilirubin levels

Men had higher mean concentrations of both total (25.2 µmol/L (IQR 16.5, 37.9)) and indirect bilirubin (17.1 µmol/L (IQR 9.9, 27.9)) (Figure 7.2 & Table 7.1).

The median values of both indirect and total bilirubin were notably different when classified by self-reported alcohol consumption and the presence or absence of a current opportunistic infection (Table 7.1). Patients who had an ongoing infection or reported regular alcohol consumption had lower total and indirect bilirubin levels (Figure 7.3 & 7.4).

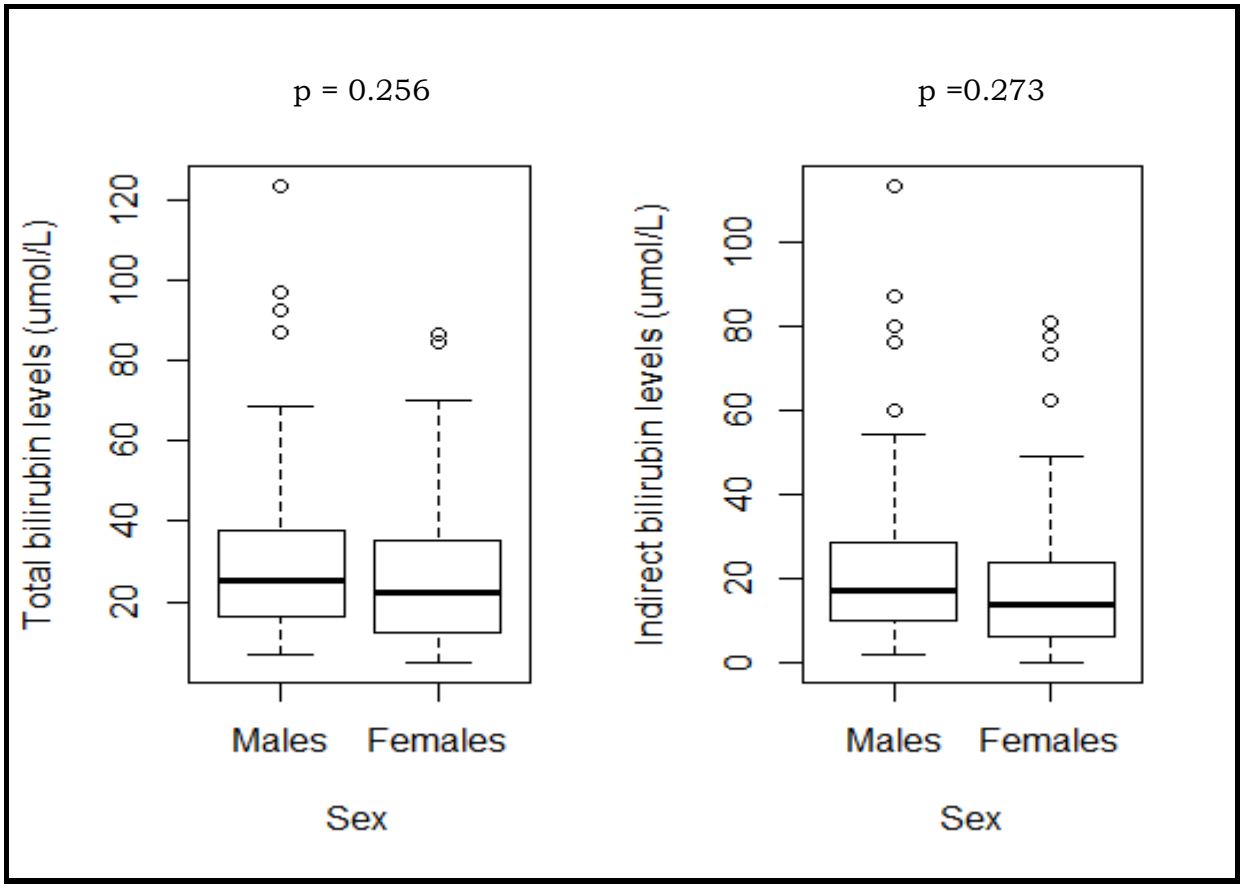


Figure 7. 2: Distribution of indirect and total bilirubin levels by sex

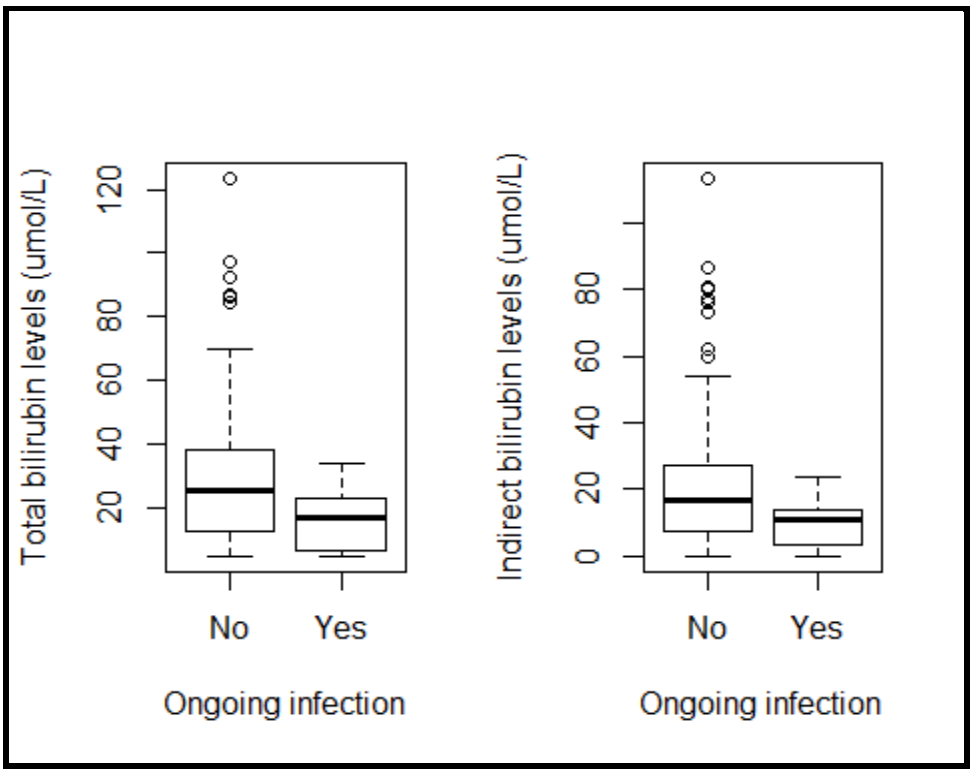


Figure 7. 3: Distribution of total and indirect bilirubin by ongoing opportunistic infection

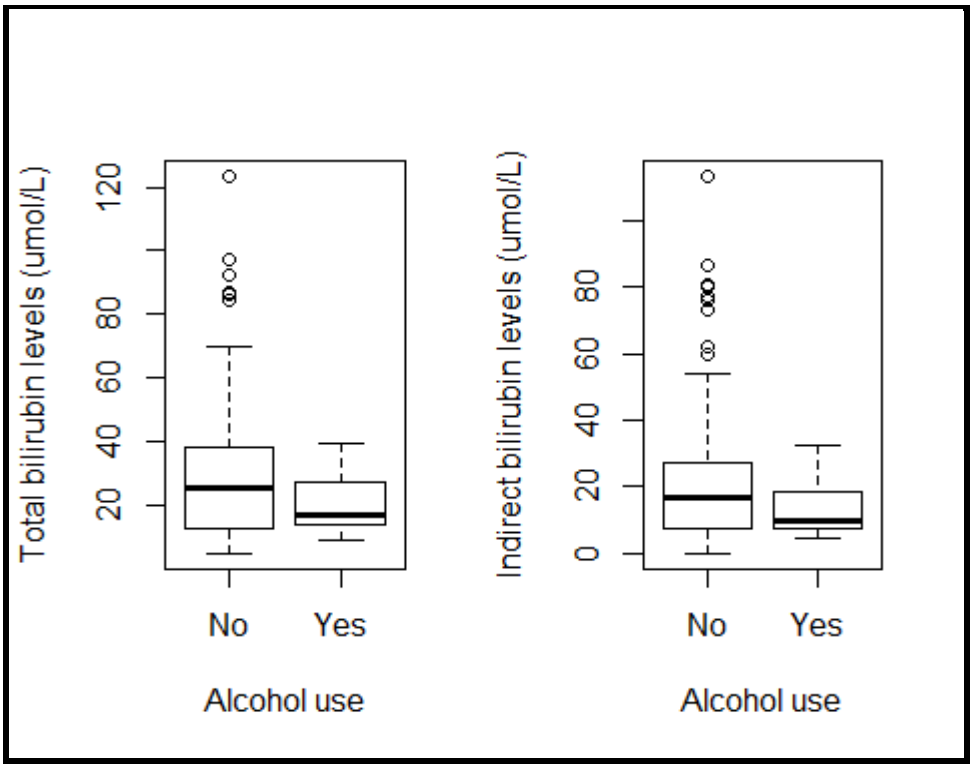


Figure 7. 4: Distribution of total and indirect bilirubin by alcohol use

Table 7. 1: Distribution of indirect and total bilirubin levels by baseline traits

Variable	Bilirubin levels			
	Indirect (median, IQR)	P value	Total (median, IQR)	P value
Age				
18 – 41	12.5 (20.0)	0.987	18.7 (26.2)	0.712
42 - 80	18.5 (13.7)		27.0 (19.2)	
Sex				
Male	17.1 (18.0)	0.273	25.2 (21.4)	0.256
Female	13.7 (17.4)		22.0 (22.7)	
Smoke cigarettes				
No	14.9 (18.4)	0.153	23.5 (25.1)	0.419
Yes	16.9 (1.9)		26.5 (2.4)	
Alcohol consumption				
No	16.8 (19.6)	**0.007**	25.6 (25.2)	**0.007**
Yes	9.8 (8.8)		16.6 (11.3)	
Hypertension				
No	15.1 (18.0)	0.331	24.0 (25.3)	0.374
Yes	14.4 (15.6)		21.2 (16.2)	
Diabetes				
No	15.1 (19.2)	0.093	24.1 (25.2)	0.119
Yes	7.5 (14.7)		15.7 (17.9)	
Regimen modification				
No	13.6 (8.9)	0.525	20.0 (10.0)	0.508
Yes	15.1 (18.2)		24.0 (25.0)	
Current opportunistic infection				
No	16.8 (19.7)	**0.002**	25.6 (25.3)	**0.003**
Yes	10.8 (9.1)		16.9 (14.1)	

7.3 Severity of hyperbilirubinemia

More than half the participants (n= 63, 57%) had elevated total bilirubin concentrations to varying degrees following application of the Division of AIDS (DAIDS) Adverse Events Grading System. The prevalence of grades 1, 2, 3 and 4 hyperbilirubinemia were 20.9%, 21.8%, 12.7% and 1.8% respectively (Figure 7.5). Grades 1, 2 and 3 were cumulatively the most prevalent.

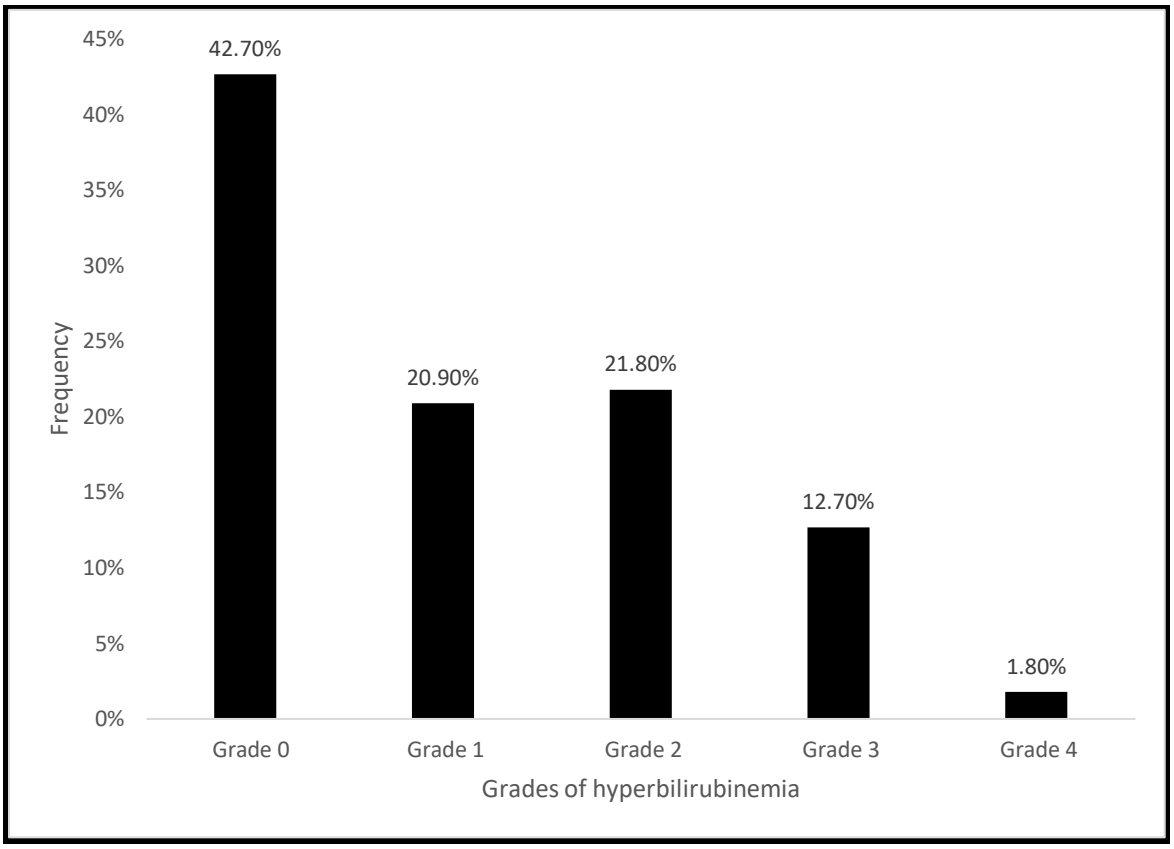


Figure 7. 5: Prevalence of various grades of hyperbilirubinemia among the study participants on atazanavir based second line regimens

7.3.1 Severity of hyperbilirubinemia by UGT1A1 and CYP3A5 genotypes

An examination of the grades of hyperbilirubinemia based on UGT1A1 and CYP3A5 polymorphisms was done and is presented in Figure 7.6. Most of the heterozygous patients had Grade 0 hyperbilirubinemia. Grade 2 hyperbilirubinemia was the second most prevalent class of hyperbilirubinemia among the heterozygotes in all the polymorphisms identified. The lowest frequency was Grade 4 hyperbilirubinemia across all polymorphisms.

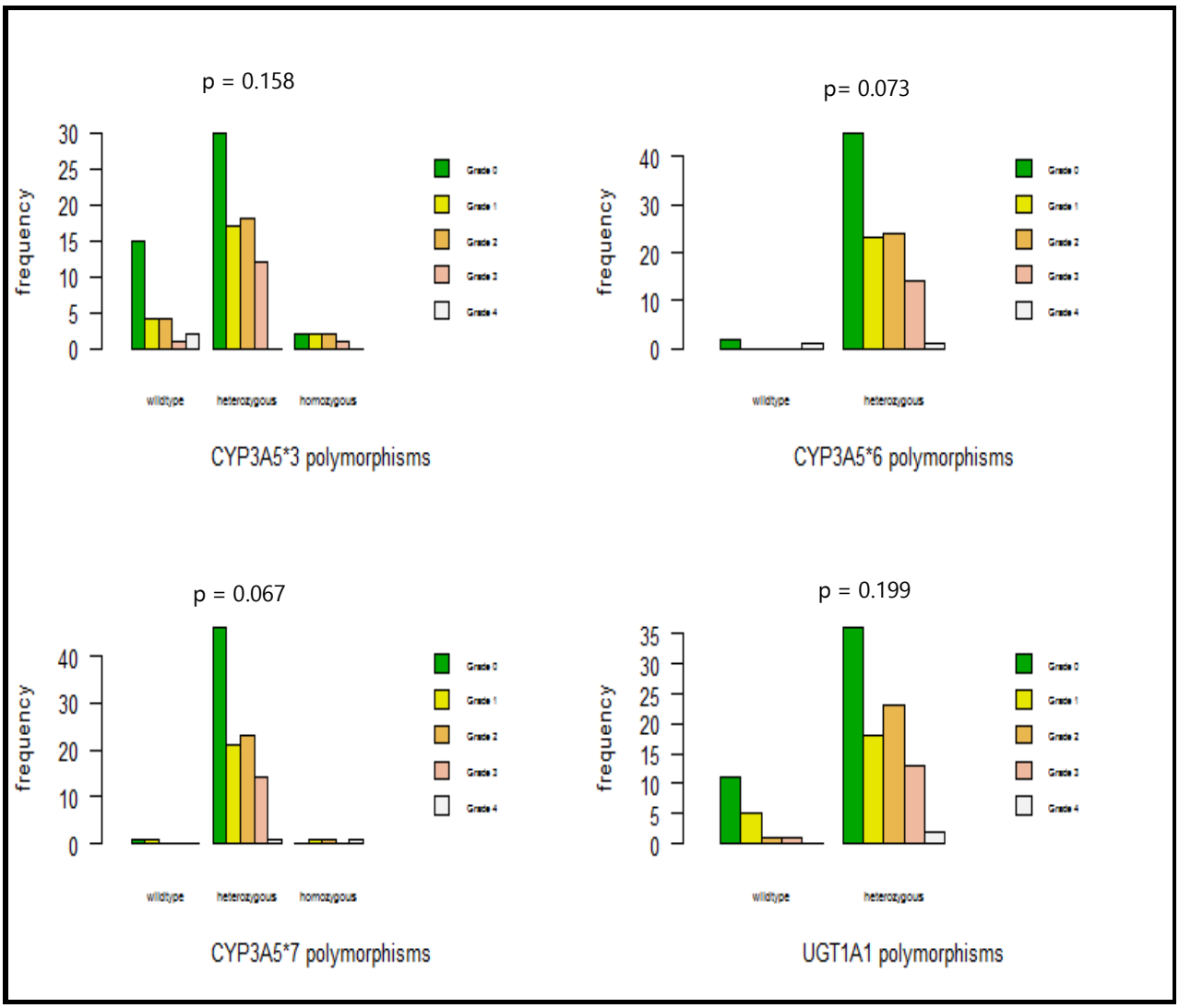


Figure 7. 6: Grades of hyperbilirubinemia by UGT1A1 and CYP3A5 polymorphisms

7.4 Risk factors for hyperbilirubinemia

Multivariable logistic regression was performed to identify patient related risk factors for hyperbilirubinemia. The model had been pre-specified a priori and the analysis was thus confirmatory. Participants with any grade of hyperbilirubinemia were grouped together for the logistic regression analysis. A bivariate analysis was run first before a multivariable model was executed.

Table 7. 2: Bivariable and multivariable logistic regression analysis of hyperbilirubinemia versus participants sociodemographic and genetic traits

Variable	Crude OR	95% CI (L, H)	p value	Adjusted OR	95% CI (L, H)	p value
Trough ATV concentration	1.001	1.000, 1.002	**0.001**	1.001	1.001, 1.002	**0.028**
Sex	0.664	0.303, 1.433	0.301	0.392	0.143, 1.014	0.059
Age						
18-41 (reference)	1			-	-	-
42-80	2.272	1.051, 5.039	**0.039**	-	-	-
Alcohol use	0.331	0.084, 1.124	0.087	0.176	0.030, 0.779	**0.031**
Years on atazanavir	1.12	0.850, 1.589	0.444	-	-	-
Current opportunistic infection	0.463	0.112, 1.723	0.256	0.260	0.037, 1.439	0.139
CYP3A5*3						
Wild type (**reference**)	1					
Heterozygous	2.136	0.872, 5.378	0.099	-	-	-
Mutant	3.409	0.609, 27.037	0.185	-	-	-
CYP3A5*6						
Wild type (**reference**)	1					
Heterozygous	2.756	0.256, 60.367	0.414	-	-	-
CYP3A5*7						
Wild type (**reference**)	1					
Heterozygous	1.283	0.050, 33.018	0.862	-	-	-
Mutant	-	-	0.990	-	-	-
UGTIA1						
Wild type (**reference**)	1			1		
Heterozygous	2.444	0.881, 7.198	0.091	7.008	1.857, 34.014	**0.007**

Only trough concentrations of atazanavir and age were associated with hyperbilirubinemia (Table 8.2). A unit increase in atazanavir concentrations raised the odds of hyperbilirubinemia by 1.001 (cOR = 1.001, 95% CI; 1.000, 1.002, p= 0.001). Older participants (≥ 42 years old) had 2.3 times higher odds of having hyperbilirubinemia compared to younger participants (< 43 years old) (cOR 2.272, 95 % CI 1.051 – 5.039, p = 0.039). The effect of age was lost in the multivariable model, suggesting that this variable may have been a confounder.

Backward stepwise logistic regression was conducted to arrive at the most parsimonius model. Three variables were associated with hyperbilirubinemia, namely the trough concentrations of atazanavir, a positive history of alcohol use and the UGT1A1 polymorphisms (Table 7.2). The finding of UGT1A1 polymorphisms and alcohol use being associated with hyperbilirubinemia in the multivariable model implies that there may have been some confounding in the bivariable analysis that was controlled for in the subsequent analysis.

We found strong evidence that UGT1A1 polymorphisms were associated with having hyperbilirubinemia. The odds of hyperbilirubinemia among participants with a heterozygous allele pair was 7.008 times that of a participant with the wild type allele (aOR =7.008, 95% CI; 1.857, 34.014, p= 0.007) holding all other variables constant. Positive history of alcohol consumption was found to lower the odds of hyperbilirubinemia by 82.4% (aOR 0.176, 95% CI 0.030, 0.779, p = 0.031) holding all other variables constant.

7.5 Discussion

In this analysis, we determined serum bilirubin levels for all the participants and classified them based on the DAIDS Adverse Events grading table. Grades III and IV hyperbilirubinemia were evident in 12.7 and 1.8% of the study population, whereas hyperbilirubinemia of any grade was present in 57.3% of the participants.

Studies among Caucasian populations have varied in their modes of reporting the prevalence of hyperbilirubinemia. For instance, Torti *et al.* while studying an Italian HIV positive cohort reported the incidence of grades III and IV hyperbilirubinemia as 44.6 and 7.2 % respectively (37). Bissio *et al.* on the other hand while carrying out the same inquiry in a Hispanic cohort found a prevalence of 50.5% for grade III and 13.1% for grade IV hyperbilirubinemia (9). These values are much higher than what we report, but this could be due to the fact that our enquiry was cross sectional, whereas Torti *et al.* carried out a prospective study therefore was able to come across more events in a larger group (>2400 patients) compared to the small sample we used. Similarly, Bissio *et al.* did a retrospective analysis among 108 patients but over a period of 12 months on average, and it was not clear if censoring was done (9).

Rodriguez *et al.* reported the risk of grade III and IV hyperbilirubinemia based on the UGT1A1 genotype (105). The incidence of hyperbilirubinemia among individuals with the 7/7, 6/7 and 6/6 genotypes was 80, 29 and 18%

respectively. The study population consisted of 118 patients all Caucasians though follow up was longer.

Laprise *et al.* carried out a longitudinal study to explore the determinants of hyperbilirubinemia in a Canadian cohort (36). The cumulative incidence of grades III and IV elevations in bilirubin at 1, 5 and 8 years of follow up was 30, 73.4 and 83.6 % respectively, which again seems higher than what we report, but the sample size was larger and they were followed up longer. In addition, the incidence was cumulative.

Three risk factors for hyperbilirubinemia were identified in this study, namely high trough concentrations of atazanavir, the heterozygous allele status for UGT1A1 and a positive history of alcohol use. Johnson *et al.* showed that slower clearance of atazanavir was a risk factor for hyperbilirubinemia, and this agrees with our study finding (106).

The role of genetic mutations in either metabolic enzymes or drug transporters was also explored in some studies. Higher levels of bilirubin were associated with UGT1A1 rs887829 T allele which concurs with our finding (105–107). Mutations in the MDR1 G2677T/A were also associated with an increased risk of hyperbilirubinemia (67).

A metanalyses by Pengqiang *et al.* demonstrated that the increased risk of hyperbilirubinemia differed based on the UGT1A1 allele pair (108). Individuals carrying the heterozygous allele pair were three and a half times as likely to develop hyperibilirubinemia compared to those with the wild type. When

compared to the heterozygous state, the risk of hyperbilirubinemia in the homozygous state was 3.69 times higher. In comparison to the wild type allele pair, the homozygous state had a 10 times increased risk of hyperbilirubinemia (108).

The consequence of bilirubin elevations arising from the use of atazanavir has been termed clinically insignificant by some researchers (109). However, among individuals who possess the UGT1A1*28 polymorphism, this has been associated with discontinuation of atazanavir therapy (34,110). In the protocol A5202 study, the UGT1A1*28 polymorphism was found in blacks (24%), whites (8%) and Hispanics (18%), but was only associated with atazanavir discontinuation in Hispanics (110). Leger *et al.* reported a 4.6% discontinuation rate in 12 months related to atazanavir exposure among homozygous rs887289 TT. Overall, the hazard ratio for discontinuation of atazanavir in the entire cohort was 7.3 (95 C1 1.7 -31.5, p = 0.007). Of greater interest though is that Leger *et al.* demonstrated that there was a racial disparity in the risk of discontinuation when the TT and CC allele pairs were compared (whites-HR =14.1(95 CI 2.6-78.7, p =0.002; blacks -HR =0.8 (95 CI 0.05-12.7, P =0.87)(111).

Other risk factors identified in other studies include higher baseline hemoglobin and bilirubin, older age, ritonavir coadministration, use of atazanavir and zidovudine, all of which increase the risk of hyperbilirubinemia to varying degrees (36,37,106). CD4 count appeared to be either protective or harmful depending on the population under study (37,67). Our findings were in

agreement regarding the role of age as a risk factor, in particular when we examined the influence of CYP3A5 polymorphisms on hyperbilirubinemia. Older patients in our study had a higher risk of hyperbilirubinemia. This is attributed to decreased drug metabolism as age increases.

As alluded to with the role of CD4 count, not all factors had positive harmful associations with hyperbilirubinemia. Female sex, clinical class C and NNRTI therapy were found to be protective against the occurrence of hyperbilirubinemia (106). Regarding sex, our findings were in concurrence in analyses checking for the influence of UGT1A1 polymorphisms on hyperbilirubinemia.

The finding on alcohol as a risk factor for hyperbilirubinemia in this study was a bit confusing since it was found to be protective. This is not biologically plausible since some reports from rat models have postulated that alcohol does the reverse by inhibiting the active transport of bile salts from the hepatocytes to the biliary canaliculi leading to an elevation in unconjugated bilirubin levels (112). This finding and all other subsequent findings from these analyses regarding alcohol use should be treated with caution.

This was the first study in Kenya looking at the prevalence and risk factors of hyperbilirubinemia in this group of patients. Despite that, there were some limitations, the first one arising due to the cross-sectional nature of the study which meant that we could not ascertain causality. Secondly, we were unable to do fragment analysis of UGT1A1 as earlier anticipated, and this limited the inferences that we were able to draw. Finally, our exclusion criteria may have

inadvertently caused a bias with the true prevalence of hyperbilirubinemia being underestimated.

7.6 Conclusion

The prevalence of grades III and IV hyperbilirubinemia in Kenyan HIV positive patients on atazanavir therapy was much lower compared to other populations. Higher plasma trough concentrations of atazanavir and the presence of a UGT1A1*1/28 polymorphism were found to be associated with hyperbilirubinemia.

7.7 Recommendations

7.7.1 Practice oriented

Hyperbilirubinemia following initiation of atazanavir therapy has been termed as clinically insignificant because it resolves within a matter of days after discontinuation of therapy. However, patients should be counselled on the risk of development of this side effect and the options available at that point in regards to their therapy. This counselling should be done at the initiation of atazanavir therapy.

Personalized therapy by way of genetic testing is the ideal, because it would rule out those who possess the Gilbert syndrome from atazanavir use. However, given that we are in a developing state with limited resources and taking into consideration the costs involved, this can be taken as a vision for personalized individualized therapy of the future.

7.8.2 Research oriented

Future research should focus on collecting longitudinal data. Though possibly expensive, this will yield more data on what we consider to be the dynamic variable of bilirubin levels with duration of treatment. Secondly, the influence of UGT1A1 polymorphisms on bilirubin levels should be investigated further with additional more robust genetic analysis.

8.0 DEPRESSION AND ADHERENCE TO ANTIRETROVIRAL THERAPY

8.1 Prevalence of depression and the risk factors

8.1.1 Introduction

Depression is the most common mental health disorder in people living with HIV (PLHIV) (1). The burden of depression is 2 - 4 times higher in PLHIV compared to the general populace (2). This increased risk of depressive disorders is postulated to be related to several factors such as stigma (either self or societal), side effects of antiretroviral therapy (ART) or as a consequence of neuropsychological changes that ensue secondary to HIV infection (3).

Globally, approximately 322 million people are living with depression, making it the leading cause of disability worldwide (4). The prevalence of depression in PLHIV on ART in sub Saharan Africa (sSA) is reported to lie between 9 – 32% (5).

Negative consequences of depression among PLHIV include changes in economic productivity, an increased risk of substance abuse, high risk sexual behaviors, decreased quality of life and increased suicide rates (5–8). Following diagnosis of HIV, depression can set in leading to rapid HIV progression and deterioration possibly due to decreased adherence to ART (9,10).

Kenyan ART guidelines recommend periodic screening for depression using the 9 item Patient Health Questionnaire (PHQ – 9) (11). Challenges such as high clinic volumes within the public health systems make this difficult to implement, meaning therefore that the burden of depression and the associated factors are inadequately documented (12). The consequence of this is that health systems

constantly underestimate the effects of mental health issues on PLHIV, especially in resource limited settings.

In response to the gaps highlighted, we designed and implemented a cross sectional study to determine the prevalence of depression and its correlates in PLHIV on second line therapy. We administered the 9 item Patient Health Questionnaire (PHQ-9).

8.1.2 Prevalence and risk factors of depression

Using the PHQ 9 screening tool, we identified 44 (40%) patients with varying severities of depression (Figure 8.1). Mild and moderate severity of depression were identified in 40 (36.4%) and 4 (3.6%) participants respectively. The median PHQ 9 score was 4 (IQR 6) and the individual symptoms score are listed in Table 8.1. No participant had major depressive disorder (MDD).

Univariate analysis of selected baseline characteristics and severity of depression showed that only occupation and an ongoing opportunistic infection were associated with depression (p values = 0.02 and 0.01 respectively; Table 8.2). These associations remained significant in both the bivariable and multivariable logistic regression models.

In the multivariable model, participants with a current opportunistic infection were approximately 7 times more likely to have depression as opposed to those who did not have an infection (95% 1.24 – 55.48, p = 0.04) holding all other variables constant. Being gainfully employed (formal employment), more years on treatment and being moderately adherent to therapy lowered the risk of

depression by 83 % (aOR = 0.17, 95% CI 0.04 – 0.62, p = 0.01), 23% (aOR = 0.77, 95% CI 0.60 – 0.98, p = 0.03) and 77 % (aOR = 0.23, 95% CI 0.05 – 0.95, p = 0.05) respectively, holding all other variables constant (Table 8.3).

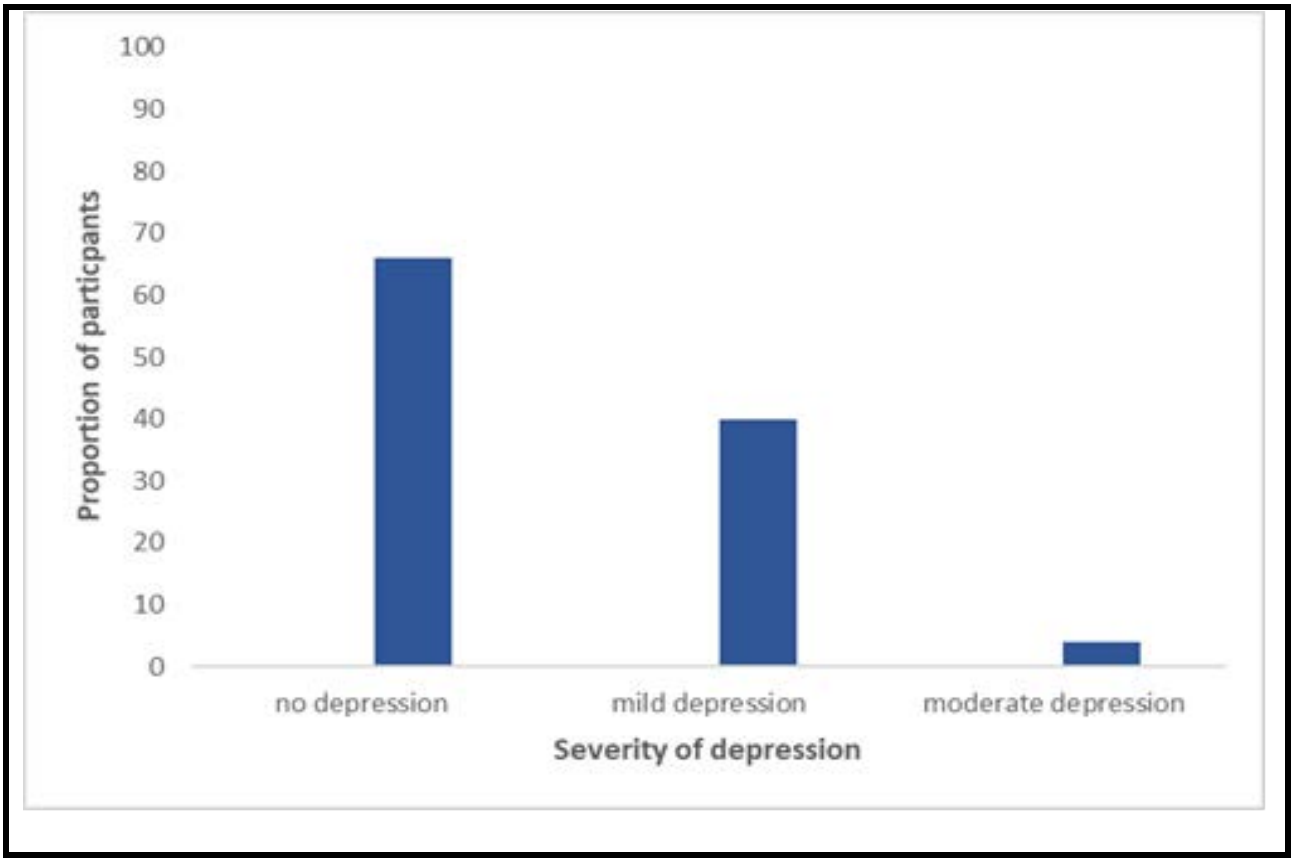


Figure 8.0.1: Prevalence of the severities of depression

Table 8.1 Itemized scores for the 9 items on the patient health questionnaire

	Over the past 2 weeks, how often have you been bothered by any of the following items?	Not at all (n, %)	Several days (n, %)	More than half the days (n, %)	Nearly every day (n, %)
1	Little interest or pleasure in doing things	50 (45.5)	57 (51.8)	3 (2.7)	0 (0)
2	Feeling down, depressed or hopeless	50 (45.5)	56 (50.9)	4 (3.6)	0 (0)
3	Trouble falling asleep, staying asleep, or sleeping too much	49 (44.5)	54 (49.1)	7 (6.4)	0 (0)
4	Feeling tired or having little energy	38 (34.5)	67 (60.9)	5 (4.5)	0 (0)
5	Poor appetite or overeating	79 (71.8)	31 (28.2)	0 (0)	0 (0)
6	Feeling bad about yourself – or that you're a failure or have let yourself or your family down	73 (66.4)	35 (31.8)	2 (1.8)	0 (0)
7	Trouble concentrating on things, such as reading the newspaper of watching television	61 (55.5)	47 (42.7)	2 (1.8)	0 (0)
8	Moving or speaking so slowly that other people could have noticed. Being so fidgety or restless that you have been moving around a lot more than usual	83 (75.5)	25 (22.7)	2 (1.8)	0 (0)
9	Thoughts that you would be better off dead or of hurting yourself in some way	102 (92.7)	7 (6.4)	1 (0.9)	0 (0)

Table 8.2: Comparative analysis of selected baseline sociodemographic traits and depression

Variable	Depression status		p - value
	No depression (n, %)	Possible depression (n, %)	
Age			
18 – 42	37 (33.6)	23 (20.9)	0.70
43 - 80	29 (26.4)	21 (19.1)	
Gender			
Male	30 (27.3)	16 (14.5)	0.45
Female	36 (32.7)	28 (25.5)	
Marital status: n (%)			
Single	21 (19.1)	13 (11.8)	
Married	35 (31.8)	24 (21.8)	0.96
Separated	5 (4.5)	5 (4.5)	
Divorced	1 (0.9)	0	
Widowed	4 (3.6)	2 (1.8)	
Education			
Primary	15 (13.6)	9 (8.2)	
Secondary	32 (29.1)	24 (21.8)	0.94
Tertiary	18 (16.4)	11 (10)	
Informal	1 (0.9)	0	
Occupation; n (%)			
Unemployed	12 (10.9)	18 (16.4)	
Self employed	27 (24.5)	16 (14.5)	**0.02**
In formal employment	24 (24.5)	10 (9.1)	
Smoke cigarettes			
No	65 (59.1)	43 (39.1)	1
Yes	1 (0.9)	1 (0.9)	
Alcohol consumption			
No	57 (51.8)	41 (37.3)	0.36
Yes	9 (8.2)	3 (27.2)	
Hypertension			
No	59 (53.6)	41 (37.3)	0.74
Yes	7 (6.4)	3 (27.3)	
Diabetes			
No	64 (58.2)	41 (37.3)	0.39
Yes	2 (18.2)	3 (27.3)	
Morisky adherence classification			
Low adherence	5 (4.5)	9 (8.2)	0.09
Medium adherence	61 (55.5)	35 (31.8)	
Regimen modification			
No	1 (0.9)	1 (0.9)	1
Yes	65 (59.1)	43 (39.1)	
Reason for regimen modification			
Regiment failure	64 (59.3)	41 (38)	0.56
Toxicity	1 (0.9)	2 (1.8)	
Current opportunistic infection			
No	64 (58.2)	36 (32.7)	**0.01**
Yes	2 (1.8)	8 (7.3)	

Table 8.3: Bivariable and multivariable logistic regression model for the covariates of depression

Variables	Coefficients			
	cOR (95% CI)	**(p-value)**	**aOR (95% CI)**	**(p-value)**
Age	1.00 (0.97- 1.04)	0.862	1.04 (0.99 -1.09)	0.07
Occupation				
Unemployed (**ref**)	1 (-)	1	1 (-)	1
Self employed	0.40 (0.15 – 1.02)	0.057	0.30 (0.08 – 1.05)	0.07
Formally employed	0.25 (0.09 – 0.68)	**0.008**	0.17 (0.04 – 0.62)	**0.01**
Current opportunistic infection	7.11 (1.67 –48.84)	**0.016**	6.81 (1.24 – 55.48)	**0.03**
Years on ART	0.94 (0.83 – 1.05)	0.298	0.77 (0.60 – 0.98)	**0.03**
Morisky adherence scale				
Low adherence (**ref**)	1 (-)		1 (-)	
Medium adherence	0.32 (0.09 – 1.00)	0.055	0.23 (0.05 – 0.95)	**0.05**

8.1.3 Discussion

We identified possible depression in 40% of the participants. This was classified by severity as either mild or moderate depression. No patients had MDD. Being employed, being on treatment for a longer duration, adherence to therapy and having a current opportunistic infection were correlated with screening positive for depression.

Depression is the most prevalent mental health disorder in PLHIV (113). The prevalence among European PLHIV is reported to range from 16% - 63% (114–116). In North America, the burden is higher than our finding at an average of 53% (117,118). Women had a higher burden of depression in these reports which correlates with our findings. In Asia, the prevalence of depression among PLHIV ranges from 9 % in Thailand to 59% in India. It is noteworthy that the tools

employed in all the aforementioned studies are different from what was used in this study, with only one exception.; a study done in a Vietnamese population that used the PHQ-9 (119). In the Vietnamese study, the prevalence of depression was much higher (50.9%) than what we report. In Asia, intravenous drug users who may also have multiple substance abuse challenges constitute a considerable fraction of the PLHIV and this puts them at higher risk of mental health issues.

Sub Saharan Africa (sSA) carries the global burden of HIV, with nearly two thirds of all PLHIV residing in the region. With increased access to antiretroviral drugs, patients are living longer and the surge of non-communicable diseases key among them depression, needs to be addressed urgently (120). The prevalence of depression among PLHIV ranges from 8 to 58%, with the most and least afflicted countries being Nigeria Uganda respectively (121,122). Our findings are in agreement with those of Ndetei *et al.* who reported a prevalence of 42% for depression symptoms among Kenyan PLHIV (123). This was a pooled aggregate from several facilities that was conducted a decade ago and is worrying when compared to our findings, since it implies that not much has changed in the screening, diagnosis or management of the condition in those years.

Patients with opportunistic infections were more likely to screen positive for depression in this study. This is corroborated by findings from other studies in Vietnam, Botswana, Canada and Albania where patients who reported poor health, more HIV/ AIDS symptoms and more frequent health care clinic visits

had a higher likelihood of depression (116,118,124–126). Multiple studies confirmed that being employed is inversely correlated with depression (121,125–129). Etenyi *et al.* while able to demonstrate the existence of a positive correlation between a regular source of income and an improved physical health summary score (PHSS) was not able to show a relationship with the mental health summary score (MHSS) (130). This study was done at the KNH HIV care clinic among in the same cohort of patients. Non - adherence to ART has also been demonstrated to increase the risk of depression in several studies which concurs with our finding (131–133). Being on treatment for a longer duration in years possibly lowered the risk of depression by limiting the neuro inflammation that would ensue in the central nervous system (CNS) due to uncontrolled viral replication (134). This was corroborated by a finding that individuals not on treatment were at a higher risk of depression (116). Factors such as age, CD4 count, female sex, WHO stage, duration of HIV diagnosis, alcohol dependence, education and marital status though not significantly associated with a positive screen on the PHQ-9 in this study, have been found to be predictive of either harm or benefit in other populations (121,125–129,131,132,135–139).

Our study was not without limitations. The sample size was small and some associations may have been missed. Secondly, we did not capture information on aspects such as stigma, disclosure, partner conflict where applicable and living situations at home. All these could have provided greater value in terms of early identification of PLHIV at risk of depression. Moving forward, studies with

larger sample sizes and incorporating all the mentioned aspects may provide greater insight.

In conclusion, a large proportion of PLHIV on second line ART regimens recruited into this study screened positive for depression, though none had MDD. This however needs to be taken a step further through application of the DSM V criteria.

8.2 Adherence to atazanavir based therapy and its determinants

8.2.1 Introduction

Adherence to antiretroviral therapy (ART) is an important and overriding component towards the achievement of optimal treatment outcomes for people living with HIV (PLHIV) on follow up. Adherence levels above 95% have been associated with better outcomes such as viral suppression, slowing of disease progression, decreased morbidity and mortality among others (140). Moreover, high initial adherence is reported to be crucial in determining long term optimal virological and immunological outcomes. High adherence initially and medium adherence in the maintenance phase are demonstrated to result in much favorable values of CD4 count and viral load (141).

Maintaining high adherence levels is challenging. Reports from both high, low and middle countries in North America and sub Saharan Africa (sSA) indicate that the ≥ 95% adherence threshold is not usually attained with pooled estimates of 55% and 77% respectively for the aforementioned continents (142). Though in this meta-analysis, countries in sSA were found to have higher adherence levels

than the North American countries, it is of concern that adherence levels have been declining over time (143–145). This decline and the changes in adherence over time are not unique to these regions and have been reported by Carrieri *et al.* in a French cohort (146).

Failure to adhere to ART has been correlated with changing circumstances over time (147). Some of the factors associated with poor adherence in sSA are patient characteristics such as depression (148), poor service provision (149) and structural factors like distance to the health facility (150). These risk factors for adherence differ between region and are context specific (151). Understanding them will enable healthcare workers to provide tailored care for those at risk of non-adherence.

Patients on second line ART may have be on these regimens for a number of reasons such as virological, immunological or clinical failure. These regimens tend to have a higher pill burden, require an increased dosing frequency and have more severe side effects (152). These reasons, among others yet to be identified in our context, could contribute to low adherence in this group of patients. Poor adherence in this subset of patients could have adverse effects beyond the individual and impacting on the public HIV healthcare system. For instance, the costs of hospitalization are reported to rise from 29% to 51% due to decreased adherence (153). Compounded with risky sexual behavior, transmission of resistant strains to ART naïve patients would worsen the problem (154).

Owing to the paucity of local data on adherence levels among PLHIV on second line ART regimens and the associated factors, we designed a cross sectional study with the aim of filling the gap. In addition, risk factors for poor adherence were evaluated.

8.2.2 Adherence levels and risk factors for poor adherence

Using the MMAS 8, 14 (12.7%) of the participants had low adherence while 96 (87.3%) were moderately adherent to ART (Figure 8.2). None of the participants had high adherence. The median MMAS 8 score was 8 (IQR 7-8) and the scores on each item are listed in Table 8.4.

The most common reason for non-adherence to ART was forgetting to take the medications (20, 18.2%) (Table 8.4). A considerable number of patients also felt that their treatment plans were an inconvenience and this resulted in non-adherence as well (16, 14.5%). Interestingly, all participants reported that they had taken their dose the day prior to their clinic visit.

At univariate analysis, only occupation was found to have a significant association with the adherence status (Table 8.5). On bivariable logistic regression analysis, age, marital status and occupation were found to be significantly associated with adherence level (Table 8.6). In this case, a one-year increase in age was associated with 6% higher adherence levels among the participants (cOR 1.06, 95% CI (1.01, 1.12), p =0.023). Married participants had 3.89 higher odds of being moderately adherent in comparison to those who were single (cOR 3.89, 95% (CI 1.22, 13.79), p=0.026). Self-employed participants had

8.79 times higher odds of being moderately adherent to ART compared to unemployed individuals (cOR 8.79, 95% CI (2.04, 61.17), p=0.09). Similarly, participants who were formally employed had 4.86 times higher odds of being adherent to ART compared to the unemployed (cOR 4.86, 95% (1.29, 23.80), p= 0.029).

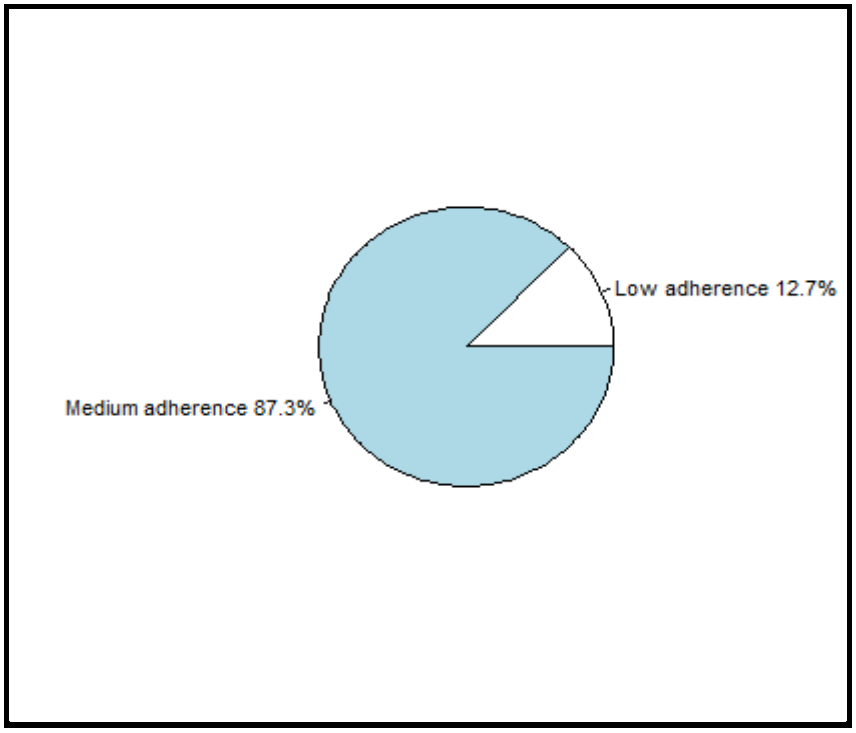


Figure 8.2: Adherence levels among the study participants

Table 8.4: Univariate analysis of sociodemographic and clinical characteristics versus adherence status

Variable	Adherence status		P - value*
	Low adherence (n, %)	Medium adherence (n, %)	
Age			
18 – 42	10 (9.1)	50 (45.5)	
43 - 80	4 (3.6)	46 (41.8)	
Gender			
Male	7 (6.4)	39 (135.5)	0.708
Female	7 (6.4)	57 (51.8)	
Marital status			
Single	9 (8.2)	25 (22.7)	
Married	5 (4.5)	54 (49.1)	**0.013**
Separated, Divorced, Widowed	0	17 (15.5)	
Education			
Primary	1 (0.09)	24 (21.8)	
Secondary	9 (8.2)	47 (42.7)	0.339
Tertiary	4 (3.6)	25 (22.7)	
Occupation;			
Unemployed	9 (8.2)	21 (19.1)	
Employed	5 (4.5)	75 (68.2)	**0.002**
Smoke cigarettes			
No	14 (12.7)	94 (85.5)	1
Yes	0	2 (1.8)	
Alcohol consumption			
No	11 (10.0)	87 (79.1)	0.180
Yes	3 (2.7)	9 (8.2)	
Hypertension			
No	12 (10.9)	88 (80.0)	0.613
Yes	2 (1.8)	8 (7.3)	
Diabetes			
No	13 (11.8)	92 (83.6)	0.501
Yes	1 (0.09)	4 (3.6)	
Depression status			
No depression	5 (4.5)	61 (55.5)	0.090
Possible depression	9 (8.1)	35 (31.8)	
Regimen modification			
No	0	2 (1.8)	1
Yes	14 (12.7)	94 (85.5)	
Reason for regimen modification			
Regiment failure	14 (13.0)	91 (84.3)	1
Toxicity	0	3 (2.8)	
Current opportunistic infection			
No	11 (10.0)	89 (80.9)	0.115
Yes	3 (2.7)	7 (6.4)	

** Chi-square and fisher's exact tests as appropriate*

Table 8.5: Bivariable and multivariable logistic regression analysis for association between demographics, clinical characteristics and adherence level

Variables	cOR (95% CI)	P value	aOR (95% CI)	P value
Age	1.06 (1.01, 1.12)	**0.023**	1.10 (1.04, 1.19)	**0.004**
Gender	1.46 (0.47, 4.59)	0.508	-	-
Marital status			-	-
Single	1			
Married	3.89 (1.22, 13.79)	**0.026**		
Separated, Divorced, Widowed	-	0.992		
Education			-	-
Primary	1			
Secondary	0.22 (0.01, 1.26)	0.159		
Tertiary	0.26 (0.01, 1.92)	0.244		
Occupation			-	-
Unemployed	1			
Employed	6.43 (2.01, 22.91)	**0.002**		
Alcohol use	0.38 (0.10, 1.90)	0.19	0.18 (0.03, 1.21)	0.069
Hypertension	0.55 (0.12, 3.89)	0.475	0.12 (0.01, 1.39)	0.080
Diabetes	0.57 (0.08, 11.51)	0.622	-	-
Depression	0.32 (0.09, 1.00)	0.055	0.17 (0.04, 0.64)	**0.013**
Regimen modification	-	0.993	-	-
Current opportunistic infection	0.29 (0.07, 1.48)	0.102	-	-
Years on ART	0.87 (0.72, 1.03)	0.124	0.84 (0.67, 1.03)	0.106

8.2.3 Discussion

We found that none of the none of the participants were highly adherent to treatment. Most had moderate adherence (96, 87.3%) while a smaller number had low adherence (14, 12.7%). Age and possible depression were identified as being independently associated with adherence status.

In a French cohort of PLHIV who were also intravenous drug abusers (IVDU), 22.9% of the participants had low adherence which is much higher compared to our findings (147). Adherence was determined by self-report which is subjective, but nonetheless, the high non-adherence rate was also understandable since substance abuse has been identified as a determinant in a meta-analysis (155).

Across Asia, the levels of adherence vary greatly, not unexpectedly, as it has been noted that the facilitators and barriers of adherence are context specific. The rates of adherence in China and Indonesia are 72% and 54% respectively, both of which are lower than what we report (156,157). However, in Myanmar, the documented adherence level is congruent to ours at 84% (158). Differences in the tools used to assess adherence may also explain the variances in part, since Aye *et al.* and Kipsang *et al.* both used the 30-day Visual Analogue Scale (VAS) (157,158).

Regionally, adherence levels to ART are comparable to our findings. In Botswana and South Africa, the documented adherence levels are approximately 87% (159,160), though this was ascertained through pill counts. In West Africa, adherence levels to ART range between 78 – 90%; these were determined by pill

counts and self-report (161,162). In East Africa, specifically Tanzania, Uganda and Ethiopia, adherence levels between 52 and 95% have been reported, measured by a number of methods including the VAS, pharmacy refills, appointment keeping and pills consumed in a week (149,163,164). A prospective study conducted in Kenya at the African Medical Research Foundation (AMREF) clinic in Kibera reported that close to 38% of the patients were non adherent (165). This is about three times of what we report as poor adherence, yet these two clinics and patients are drawn from the Nairobi metropolitan area. The differences could be attributed to the fact that Kibera is an area mostly inhabited by individuals of low social economic status and as such issues such as stigma and food scarcity compete for attention with adherence to ART.

Being married and either self-employed or formally employed were associated with better adherence levels at bivariable analysis. This is comparable to findings from other studies in diverse populations (164,166). Married persons offer additional support to each other to adhere to treatment, whereas having a steady source of income may also motivate one to adhere to ART and keep providing for their family. Being older was identified as being significantly associated with better adherence at multivariable analysis, and this has been reported in multiple studies (149,163,164,166–168). Older individuals are more likely to be stable – socially and economically, mature and with more responsibilities and may therefore take their treatment more seriously than young individuals (149).

We also identified depression as being significantly associated with adherence, which has also been reported across multiple studies in diverse settings (147,155,164,167). Depression is a known risk factor for non-adherence based on the premise that persons with mental illness are less likely to find motivation in keeping with the prescribed treatment regimens.

Alcohol use is a known determinant of non-adherence (161,163,167,169,170), though this was not identified in this study. Persons who use alcohol excessively are less likely to remember to take their medication on time or even to take them at all. Though not a risk factor in this population, duration on ART has been identified as a determinant for adherence in South African and Chinese cohorts (152,157). We hypothesize that patients who have been on ART longer are more likely aware of its benefits and become more adherent.

Nine percent of the study population had hypertension as a comorbidity, which was not unexpected as protease inhibitors are associated with an increased risk of metabolic syndrome (171). PLHIVs on ART can expect a near normal population life expectancy, but due to the metabolic syndrome, conditions such as hypertension and diabetes are highly prevalent. In an urban population of PLHIV on therapy, 31.7% were found to have hypertension, which was much higher than what we found (172). Many comorbidities are known to impede adherence, primarily due to the pill burden (173), but also due to competing interests and a lack of understanding of the conditions (172).

Forgetfulness is documented to be the most common cause of non-adherence, a finding that we replicated (157,164,166,174). To decrease the contribution of this factor, use of innovative methods such as setting alarms to remind patients to take their medications might work. Other documented reasons for non-adherence include side effects associated with ART and travelling.

Our study provides insights to healthcare providers on how best to improve adherence among PLHIV. The cross-sectional nature of the study design did not allow us to investigate causality or to determine the dynamic nature of adherence with time. There are also variables such as disclosure and adherence self-efficacy that were not investigated upon and could potentially influence adherence to ART. Data on these variables and others could be collected in future studies that may be longitudinal in nature.

Optimum adherence to ART is key in ensuring treatment success and reducing adverse outcomes such as increased mortality (156). Treatment programs should ensure that measures such as frequent counselling (159), having family support (164), encouraging patients to have adherence partners (159) as well as diagnosis and treatment of mental illness are prioritized to enhance adherence.

References

1. UNAIDS. Global AIDS update 2016. WHO Press, Geneva; 2016.

2. National AIDS and STI Control Programme (NASCOP), Kenya. Kenya HIV County Profiles. 2014.

3. National AIDS and STI Control Programme, Kenya. Kenya HIV Prevention Revolution Road Map. 2014.

4. The Joint United Nations Programme on HIV/AIDS (UNAIDS). UNAIDS 2016-2021 Strategy. WHO Press, Geneva; 2016.

5. National AIDS and STI Control Programme (NASCOP), Kenya. Guidelines for antiretroviral therapy in Kenya, 4th Edition. NASCOP; 2011.

6. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection; Recommendations for a public health approach. WHO Press, Geneva; 2016.

7. Havlir DV, O'Marro SD. Atazanavir: New Option for Treatment of HIV Infection. Clin Infect Dis. 2004;38(11):1599–604.

8. Fuster D, Clotet B. Review of atazanavir: a novel HIV protease inhibitor. Expert Opin Pharmacother. 2005;6(9):1565–72.

9. Bissio E, Lopardo GD. Incidence of Hyperbilirubinemia and Jaundice Due to Atazanavir in a Cohort of Hispanic Patients. AIDS Res Hum Retroviruses. 2013 Mar;29(3):415–7.

10. Gammal RS, Court MH, Haidar CE, Iwuchukwu OF, Gaur AH, Alvarellos M, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for UGT1A1 and Atazanavir Prescribing. Clin Pharmacol Ther. 2016 Apr;99(4):363–9.

11. Bains RK. African variation at Cytochrome P450 genes Evolutionary aspects and the implications for the treatment of infectious diseases. Evol Med Public Health. 2013 Jan 1;2013(1):118–34.

12. Bains RK, Kovacevic M, Plaster CA, Tarekegn A, Bekele E, Bradman NN, et al. Molecular diversity and population structure at the Cytochrome P450 3A5 gene in Africa. BMC Genet. 2013;14(1):1–18.

13. Gervasoni C, Meraviglia P, Minisci D, Ferraris L, Riva A, Landonio S. Metabolic and kidney disorders correlate with high atazanavir concentrations in HIV infected patients: is it time to revise atazanavir dosages? PLoS One. 2015;10(4):1–12.

14. Bunupuradah T, Kiertiburanakul S, Avihingsanon A, Chetchotisakd P, Techapornroong M, Leerattanapetch N, et al. Low-dose versus standard-dose ritonavir-boosted atazanavir in

virologically suppressed Thai adults with HIV (LASA): a randomised, open-label, non-inferiority trial. Lancet HIV. 2016;3(8):e343–50.

15. Brunton L, Chabner B, Knollman B. Goodman and Gilman's the pharmacological basis of therapeutics. 12th ed. New York: Mc Graw Hill; 2011.

16. Croom K, Dhillon S, Keam S. Atazanavir: a review of its use in the management of HIV-1 infection. Drugs. 2009;69(8):1107–40.

17. Piliero P. Atazanavir: a novel HIV-1 protease inhibitor. Expert Opin Investig Drugs. 2005;11(9):1295–301.

18. Colombo S, Buclin T, Cavassini M, Décosterd LA, Telenti A, Biollaz J, et al. Population Pharmacokinetics of Atazanavir in Patients with Human Immunodeficiency Virus Infection. Antimicrob Agents Chemother. 2006 Nov 1;50(11):3801.

19. Goutelle S, Baudry T, Gagnieu M-C, Boibieux A, Livrozet J-M, Peyramond D, et al. Pharmacokinetic-Pharmacodynamic Modeling of Unboosted Atazanavir in a Cohort of Stable HIV-Infected Patients. Antimicrob Agents Chemother. 2013 Jan 1;57(1):517.

20. Colombo S, Buclin T, Cavassini M, Decosterd L, Telenti A, Biollaz J. Population pharmacokinetics of atazanavir in patients with human immunodeficiency virus infection. Antimicrob Agents Ch. 2006;50(11):3801–8.

21. Venuto C, Mollan K, Ma Q, Daar E, Sax P, Fischi M. Sex differences in atazanavir pharmacokinetics and associations with time to clinical events: AIDS Clinical Trials Group Study A5202. J Antimicrob Chemother. 2014;69(12):3300–10.

22. Kiser J, Anderson P, Kakuda T. 6th International Workshop on Clinical Pharmacology of HIV Therapy. Expert Opin Pharmacother. 2006 Mar 1;7:229–41.

23. Solas C, Gagnieu M-C, Ravaux I, Drogoul M-P, Lafeuillade A, Mokhtari S, et al. Population Pharmacokinetics of Atazanavir in Human Immunodeficiency Virus-Infected Patients. Ther Drug Monit [Internet]. 2008;30(6). Available from: https://journals.lww.com/drug-monitoring/Fulltext/2008/12000/Population_Pharmacokinetics_of_Atazanavir_in_Human.5.aspx

24. Li F, Lu J, Wang L, Ma X. CYP3A- mediated generation of aldehyde and hydrazine in atazanavir metabolism. Drug Metab Dispos. 2011;39(3):394–401.

25. Wempe M, Anderson P. Atazanavir metabolism according to CYP3A5 status: An in vitro- in vivo assessment. Drug Metab Dispos. 2011;39(3):522–7.

26. Johnson D, Venuto C, Ritchie M, Morse G, Daar E, McLaren P, et al. Genome-wide association study of atazanavir pharmacokinetics and hyperbilirubinemia in AIDS Clinical Trials Group Protocol A5202. Pharmacogenet Genomics. 2014;24(4):195–203.

27. Perloff E, Duan S, Skolnik P, Greenblatt D, von Moltke L. Atazanavir: effects on P-glycoprotein transport and CYP3A metabolism in vitro. Drug Metab Dispos. 2005;33(6):764–70.

28. Bains RK. African variation at Cytochrome P450 genes: Evolutionary aspects and the implications for the treatment of infectious diseases. Evol Med Public Health. 2013;2013(1):118–34.

29. Quaranta S, Chevalier D, Allorge D, Lo-Guidice JM, Migot-Nabias F, Kenani A, et al. Ethnic differences in the distribution of CYP3A5 gene polymorphisms. Xenobiotica [Internet]. 2006;36. Available from: http://dx.doi.org/10.1080/00498250600944300

30. Anderson P, Aquilante C, Gardner E, Predhomme J, McDaneld P, Bushman L. Atazanavir pharmacokinetics in genetically determined CYP3A5 expressors versus non expressors. J Antimicrob Chemother. 2009;64(5):1071–9.

31. Zhang D, Chando T, Everett D, Pattern C, Dehal S, Humphreys G. In vitro inhibition of UDP glucuronosyltransferases by atazanavir and other HIV protease inhibitors and the relationship of this property to in vivo bilirubin glucuronidation. Drug Metab Dispos. 2005;33(11):1729–39.

32. Rotger M, Taffe P, Bleiber G, Gonthard H, Furrer H, Vernazza P. Gilbert syndrome and the development of antiretroviral therapy associated hyperbilirubinemia. J Infect Dis. 2005;192(8):1381–6.

33. Anderson PL, Aquilante CL, Gardner EM, Predhomme J, McDaneld P, Bushman LR, et al. Atazanavir pharmacokinetics in genetically determined CYP3A5 expressors versus non-expressors. J Antimicrob Chemother. 2009 Nov 1;64(5):1071–9.

34. Lubomirov R, Colombo S, di Iulio J, Ledergerber B, Martinez R, Cavassini M, et al. Association of pharmacogenetic markers with premature discontinuation of first-line anti-HIV therapy: an observational cohort study. J Infect Dis. 2011 Jan 15;203(2):246–57.

35. Vardhanabhuti S, Ribaudo HJ, Landovitz RJ, Ofotokun I, Lennox JL, Currier JS, et al. Screening for UGT1A1 Genotype in Study A5257 Would Have Markedly Reduced Premature Discontinuation of Atazanavir for Hyperbilirubinemia. Open Forum Infect Dis [Internet]. 2015 Jul 1 [cited 2019 Sep 7];2(3). Available from: https://doi.org/10.1093/ofid/ofv085

36. Laprise C, Baril J, Dufresne S, Trottier H. Atazanavir and other determinants of hyperbilirubinemia in a cohort of 1150 HIV-positive patients: results from 9 years of follow up. AIDS Patient Care STDs. 2013;27(7):378–86.

37. Torti C, Lapadula G, Antinori A, Quirino T, Maserati R, Castelnuovo F, et al. Hyperbilirubinemia during Atazanavir Treatment in 2,404 Patients in the Italian Atazanavir Expanded Access Program and MASTER Cohorts. Infection. 2009 May 26;37(3):244.

38. Rodriguez Novoa S, Barreiro P, Rendón A, Barrios A, Corral A, Jiménez-Nacher I, et al. Plasma Levels of Atazanavir and the Risk of Hyperbilirubinemia Are Predicted by the 3435C->T Polymorphism at the Multidrug Resistance Gene 1. Clin Infect Dis Off Publ Infect Dis Soc Am. 2006 Jan 15;42:291–5.

39. Ribaudo HJ, Daar ES, Tierney C, Morse GD, Mollan K, Sax PE, et al. Impact of UGT1A1 Gilbert Variant on Discontinuation of Ritonavir-Boosted Atazanavir in AIDS Clinical Trials Group Study A5202. J Infect Dis. 2013 Feb 1;207(3):420–5.

40. Ministry of Health; National AIDS and STI Control Programme (NASCOP). Guidelines for use of antiretroviral drugs for treating and preventing HIV infection in Kenya 2016. 2016.

41. Kelsey J. Methods in observational epidemiology. 2nd ed. New York: Oxford University Press; 1996.

42. Morisky DE, Ang A, Krousel-Wood M, Ward HJ. Predictive validity of a medication adherence measure in an outpatient setting. J Clin Hypertens Greenwich Conn. 2008 May;10(5):348–54.

43. Kroenke K, Spitzer RL, Williams JBW. The PHQ-9. J Gen Intern Med. 2001 Sep 1;16(9):606–13.

44. Ette E, Roy A. Designing population pharmacokinetic studies for efficient parameter estimation. In: Pharmacometrics; The science of quantitative pharmacology. 1st ed. United States of America: John Wiley & Sons, Inc, Hoboken, New Jersey; 2007. p. 303–44.

45. Cattaneo D, Maggiolo F, Ripamonti D, Perico N. Determination of atazanavir in human plasma by high-performance liquid chromatography with UV detection. J Chromatogr Sci. 2008 Jul;46(6):485–9.

46. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) adopts Consolidated Guideline on Good Clinical Practice in the Conduct of Clinical Trials on Medicinal Products for Human Use. Int Dig Health Legis. 1997;48(2):231–4.

47. Colombo S, Guignard N, Marzolini C, Telenti A, Biollaz J, Decosterd LA. Determination of the new HIV-protease inhibitor atazanavir by liquid chromatography after solid-phase extraction. J Chromatogr B Analyt Technol Biomed Life Sci. 2004 Oct 15;810(1):25–34.

48. Dailly E, Raffi F, Jolliet P. Determination of atazanavir and other antiretroviral drugs (indinavir, amprenavir, nelfinavir and its active metabolite M8, saquinavir, ritonavir, lopinavir, nevirapine and efavirenz) plasma levels by high performance liquid chromatography with UV detection. J Chromatogr B Analyt Technol Biomed Life Sci. 2004 Dec 25;813(1–2):353–8.

49. Sparidans RW, Dost F, Crommentuyn KML, Huitema ADR, Schellens JHM, Beijnen JH. Liquid chromatographic assay for the protease inhibitor atazanavir in plasma. Biomed Chromatogr BMC. 2006 Jan;20(1):72–6.

50. Verbesselt R, Van Wijngaerden E, de Hoon J. Simultaneous determination of 8 HIV protease inhibitors in human plasma by isocratic high-performance liquid chromatography with combined use of UV and fluorescence detection: amprenavir, indinavir, atazanavir, ritonavir, lopinavir, saquinavir, nelfinavir and M8-nelfinavir metabolite. J Chromatogr B Analyt Technol Biomed Life Sci. 2007 Jan 1;845(1):51–60.

51. Schuster A, Burzawa S, Jemal M, Loizillon E, Couerbe P, Whigan D. Quantitative determination of the HIV protease inhibitor atazanavir (BMS-232632) in human plasma by liquid chromatography-tandem mass spectrometry following automated solid-phase extraction. J Chromatogr B Analyt Technol Biomed Life Sci. 2003 May 25;788(2):377–86.

52. Shrivastava A, Gupta VB. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. Chron Young Sci. 2011 Jan 1;2(1):21.

53. Charbe N, Baldelli S, Cozzi V, Castoldi S, Cattaneo D, Clementi E. Development of an HPLC-UV assay method for the simultaneous quantification of nine antiretroviral agents in the plasma of HIV-infected patients. J Pharm Anal. 2016 Dec;6(6):396–403.

54. Notari S, Bocedi A, Ippolito G, Narciso P, Pucillo LP, Tossini G, et al. Simultaneous determination of 16 anti-HIV drugs in human plasma by high-performance liquid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci. 2006 Feb 2;831(1–2):258–66.

55. Loregian A, Pagni S, Ballarin E, Sinigalia E, Parisi SG, Palu G. Simple determination of the HIV protease inhibitor atazanavir in human plasma by high-performance liquid chromatography with UV detection. J Pharm Biomed Anal. 2006 Oct 11;42(4):500–5.

56. Dandara C, Lombard Z, Du Plooy I, McLellan T, Norris SA, Ramsay M. Genetic variants in CYP (-1A2, -2C9, -2C19, -3A4 and -3A5), VKORC1 and ABCB1 genes in a black South African population: a window into diversity. Pharmacogenomics. 2011 Dec;12(12):1663–70.

57. Semiz S, Dujic T, Ostanek B, Prnjavorac B, Bego T, Malenica M, et al. Analysis of CYP3A4*1B and CYP3A5*3 polymorphisms in population of Bosnia and Herzegovina. Med Glas Off Publ Med Assoc Zenica-Doboj Cant Bosnia Herzeg. 2011 Feb;8(1):84–9.

58. Krishna DR, Shekar MS. Cytochrome P450 3A: genetic polymorphisms and inter-ethnic differences. Methods Find Exp Clin Pharmacol. 2005 Oct;27(8):559–67.

59. Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, et al. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat Genet. 2001 Apr;27(4):383–91.

60. Mustafina OE, Tuktarova IA, Karimov DD, Somova R sh, Nasibullin TR. [CYP2D6, CYP3A5, and CYP3A4 gene polymorphism in Russian, Tatar, and Bashkir populations]. Genetika. 2015 Jan;51(1):109–19.

61. Arvanitidis K, Ragia G, Iordanidou M, Kyriaki S, Xanthi A, Tavridou A, et al. Genetic polymorphisms of drug-metabolizing enzymes CYP2D6, CYP2C9, CYP2C19 and CYP3A5 in the Greek population. Fundam Clin Pharmacol. 2007 Aug;21(4):419–26.

62. Fohner A, Muzquiz LI, Austin MA, Gaedigk A, Gordon A, Thornton T, et al. Pharmacogenetics in American Indian populations: analysis of CYP2D6, CYP3A4, CYP3A5, and CYP2C9 in the Confederated Salish and Kootenai Tribes. Pharmacogenet Genomics. 2013 Aug;23(8):403–14.

63. Lai Y, Zhang J, Wang Y-X, Wang X-D, Li J-L, Wang Y-H, et al. CYP3A5*3 and MDR-1 C3435T single nucleotide polymorphisms in six Chinese ethnic groups. Pharm. 2011 Feb;66(2):136–40.

64. Li D, Zhang G-L, Lou Y-Q, Li Q, Wang X, Bu X-Y. Genetic polymorphisms in MDR1 and CYP3A5 and MDR1 haplotype in mainland Chinese Han, Uygur and Kazakh ethnic groups. J Clin Pharm Ther. 2007 Feb;32(1):89–95.

65. Chen Z, Li J, Chen P, Wang F, Zhang N, Yang M, et al. Genetic polymorphisms of the drug-metabolizing enzyme cytochrome P450 3A5 in a Uyghur Chinese population. Xenobiotica Fate Foreign Compd Biol Syst. 2016 Sep;46(9):850–6.

66. Balram C, Zhou Q, Cheung YB, Lee EJD. CYP3A5*3 and *6 single nucleotide polymorphisms in three distinct Asian populations. Eur J Clin Pharmacol. 2003 Jun;59(2):123–6.

67. Park SY, Kang YS, Jeong MS, Yoon HK, Han KO. Frequencies of CYP3A5 genotypes and haplotypes in a Korean population. J Clin Pharm Ther. 2008 Feb;33(1):61–5.

68. Saeki M, Saito Y, Nakamura T, Murayama N, Kim S-R, Ozawa S, et al. Single nucleotide polymorphisms and haplotype frequencies of CYP3A5 in a Japanese population. Hum Mutat. 2003 Jun;21(6):653.

69. Yousef A-M, Bulatova NR, Newman W, Hakooz N, Ismail S, Qusa H, et al. Allele and genotype frequencies of the polymorphic cytochrome P450 genes (CYP1A1, CYP3A4, CYP3A5, CYP2C9 and CYP2C19) in the Jordanian population. Mol Biol Rep. 2012 Oct;39(10):9423–33.

70. Adehin A, Bolaji OO, Kennedy MA. Polymorphisms in CYP2C8 and CYP3A5 genes in the Nigerian population. Drug Metab Pharmacokinet. 2017 Jun;32(3):189–91.

71. Kudzi W, Dodoo ANO, Mills JJ. Genetic polymorphisms in MDR1, CYP3A4 and CYP3A5 genes in a Ghanaian population: a plausible explanation for altered metabolism of ivermectin in humans? BMC Med Genet. 2010 Jul 14;11:111.

72. Marwa KJ, Schmidt T, Sjogren M, Minzi OMS, Kamugisha E, Swedberg G. Cytochrome P450 single nucleotide polymorphisms in an indigenous Tanzanian population: a concern about the metabolism of artemisinin-based combinations. Malar J. 2014 Nov 3;13:420.

73. Roy J-N, Lajoie J, Zijenah LS, Barama A, Poirier C, Ward BJ, et al. CYP3A5 genetic polymorphisms in different ethnic populations. Drug Metab Dispos Biol Fate Chem. 2005 Jul;33(7):884–7.

74. Thompson EE, Kuttab-Boulos H, Witonsky D, Yang L, Roe BA, Di Rienzo A. CYP3A variation and the evolution of salt-sensitivity variants. Am J Hum Genet. 2004 Dec;75(6):1059–69.

75. Beleza S, Gusmao L, Amorim A, Carracedo A, Salas A. The genetic legacy of western Bantu migrations. Hum Genet. 2005 Aug;117(4):366–75.

76. Beutler E, Gelbart T, Demina A. Racial variability in the UDP-glucuronosyltransferase 1 (UGT1A1) promoter: a balanced polymorphism for regulation of bilirubin metabolism? Proc Natl Acad Sci U S A. 1998 Jul 7;95(14):8170–4.

77. Ostanek B, Furlan D, Mavec T, Lukac-Bajalo J. UGT1A1(TA)n promoter polymorphism--a new case of a (TA)8 allele in Caucasians. Blood Cells Mol Dis. 2007 Apr;38(2):78–82.

78. Kobayashi M, Hazama S, Takahashi K, Oba K, Okayama N, Nishioka M, et al. Is there diversity among UGT1A1 polymorphism in Japan? World J Gastrointest Oncol. 2012 Jul 15;4(7):170–5.

79. Lampe J, Bigler J, Horner N, Potter J. UDP-glucuronosyltransferase (UGT1A1*28 and UGT1A6*2) polymorphisms in Caucasians and Asians: relationships to serum bilirubin concentrations. Pharmacogenetics. 1999 Jun;9(3):341–9.

80. Liu JY, Qu K, Sferruzza AD, Bender RA. Distribution of the UGT1A1*28 polymorphism in Caucasian and Asian populations in the US: a genomic analysis of 138 healthy individuals. Anticancer Drugs. 2007 Jul;18(6):693–6.

81. Akiyama Y, Fujita K, Nagashima F, Yamamoto W, Endo H, Sunakawa Y, et al. Genetic testing for UGT1A1*28 and *6 in Japanese patients who receive irinotecan chemotherapy. Ann Oncol Off J Eur Soc Med Oncol. 2008 Dec;19(12):2089–90.

82. E Radoi V, I Ursu R, Poenaru E, Arsene C, L Bohiltea C, Bohiltea R. Frequency of the UGT1A1*28 Polymorphism in a Romanian Cohort of Gilbert Syndrome Individuals. J Gastrointestin Liver Dis. 2017 Mar 1;26:25–8.

83. Kanestri V, Mironov K, Kravchenko A, Pokrovskaya A, Dribnohodova O, Dunayeva E, et al. Clinical significance of the UGT1A1*28 allele detection in HIV-infected patients. J Int AIDS Soc. 2014 Nov 2;17(4 Suppl 3):19579–19579.

84. Alkharfy KM, Alghamdi AM, Bagulb KM, Al-Jenoobi FI, Al-Mohizea AM, Al-Muhsen S, et al. Distribution of selected gene polymorphisms of UGT1A1 in a Saudi population. Arch Med Sci AMS. 2013 Aug 30;9(4):731–8.

85. Ando Y, Ueoka H, Sugiyama T, Ichiki M, Shimokata K, Hasegawa Y. Polymorphisms of UDP-Glucuronosyltransferase and Pharmacokinetics of Irinotecan. Ther Drug Monit

[Internet]. 2002;24(1). Available from: https://journals.lww.com/drug-monitoring/Fulltext/2002/02000/Polymorphisms_of_UDP_Glucuronosyltransferase_and.18.aspx

86. Ando Y, Saka H, Ando M, Sawa T, Muro K, Ueoka H, et al. Polymorphisms of UDP-Glucuronosyltransferase Gene and Irinotecan Toxicity: A Pharmacogenetic Analysis. Cancer Res. 2000 Dec 15;60(24):6921.

87. Teh LK, Hashim H, Zakaria ZA, Salleh MZ. Polymorphisms of UGT1A1*6, UGT1A1*27 & UGT1A1*28 in three major ethnic groups from Malaysia. Indian J Med Res. 2012 Aug;136(2):249–59.

88. von Ahsen N, Oellerich M, Schütz E. DNA Base Bulge vs Unmatched End Formation in Probe-based Diagnostic Insertion/Deletion Genotyping: Genotyping the UGT1A1 $(TA)_n$ Polymorphism by Real-Time Fluorescence PCR. Clin Chem. 2000 Dec 1;46(12):1939.

89. Graffelman J, Jain D, Weir B. A genome-wide study of Hardy-Weinberg equilibrium with next generation sequence data. Hum Genet. 2017/04/03 ed. 2017 Jun;136(6):727–41.

90. Abramovs N, Brass A, Tassabehji M. Hardy-Weinberg Equilibrium in the Large Scale Genomic Sequencing Era. Front Genet. 2020 Mar 13;11:210–210.

91. Castillo-Mancilla JR, Aquilante CL, Wempe MF, Smeaton LM, Firnhaber C, LaRosa AM, et al. Pharmacogenetics of unboosted atazanavir in HIV-infected individuals in resource-limited settings: a sub-study of the AIDS Clinical Trials Group (ACTG) PEARLS study (NWCS 342). J Antimicrob Chemother. 2016 Feb 17;71(6):1609–18.

92. Kile DA, MaWhinney S, Aquilante CL, Rower JE, Castillo-Mancilla JR, Anderson PL. A Population Pharmacokinetic-Pharmacogenetic Analysis of Atazanavir. AIDS Res Hum Retroviruses. 2012 Mar 6;28(10):1227–34.

93. Savic RM, Barrail-Tran A, Duval X, Nembot G, Panhard X, Descamps D, et al. Effect of adherence as measured by MEMS, ritonavir boosting, and CYP3A5 genotype on atazanavir pharmacokinetics in treatment-naive HIV-infected patients. Clin Pharmacol Ther. 2012 Nov;92(5):575–83.

94. Singkham N, Avihingsanon A, Thammajaruk N, Ruxrungtham K, Bunupuradah T, Kiertiburanakul S, et al. Influence of CYP3A5 and SLCO1B1 polymorphisms on atazanavir/r concentrations in Thai HIV-infected patients. Pharmacogenomics. 2019 May 1;20(7):517–27.

95. Punyawudho B, Thammajaruk N, Ruxrungtham K, Avihingsanon A. Population pharmacokinetics and dose optimisation of ritonavir-boosted atazanavir in Thai HIV-infected patients. Int J Antimicrob Agents. 2017 Mar 1;49(3):327–32.

96. von Hentig N, Babacan E, Lennemann T, Knecht G, Carlebach A, Harder S, et al. The steady-state pharmacokinetics of atazanavir/ritonavir in HIV-1-infected adult outpatients is not affected by gender-related co-factors. J Antimicrob Chemother. 2008 Sep;62(3):579–82.

97. Dickinson L, Boffito M, Back D, Waters L, Else L, Davies G, et al. Population pharmacokinetics of ritonavir-boosted atazanavir in HIV-infected patients and healthy volunteers. J Antimicrob Chemother. 2009 Mar 28;63(6):1233–43.

98. Venuto CS, Mollan K, Ma Q, Daar ES, Sax PE, Fischl M, et al. Sex differences in atazanavir pharmacokinetics and associations with time to clinical events: AIDS Clinical Trials Group Study A5202. J Antimicrob Chemother. 2014 Dec;69(12):3300–10.

99. Gandhi M, Aweeka F, Greenblatt RM, Blaschke TF. Sex differences in pharmacokinetics and pharmacodynamics. Annu Rev Pharmacol Toxicol. 2004;44:499–523.

100. Umeh O, Currier J, Park J, Cramer Y, Hermes A, Fletcher C. Sex differences in lopinavir (LPV) and ritonavir (RTV) pharmacokinetics (PKs) among HIV-infected females and males. J Clin Pharmacol. 2011 Dec;51(12):1665–73.

101. anderson. Atazanavir pharmacokinetics in genetically determined CYP3A5 expressors versus non-expressors | Journal of Antimicrobial Chemotherapy | Oxford Academic. [cited 2020 Jan 31]; Available from: https://academic.oup.com/jac/article/64/5/1071/716814

102. Boffito M, Jackson A, Amara A, Back D, Khoo S, Higgs C, et al. Pharmacokinetics of Once-Daily Darunavir-Ritonavir and Atazanavir-Ritonavir over 72 Hours following Drug Cessation. Antimicrob Agents Chemother. 2011 Sep 1;55(9):4218.

103. Uglietti A, Novati S, Gulminetti R, Maserati R. Correlations between atazanavir Ctrough and hyperbilirubinemia: a case report. J Med Case Reports. 2009 Dec 1;3(1):9307.

104. Avihingsanon A, Tongkobpetch S, Kerr SJ, Punyawudho B, Suphapeetiporn K, Gorowara M, et al. Pharmacogenetic Testing Can Identify Patients Taking Atazanavir at Risk for Hyperbilirubinemia. JAIDS J Acquir Immune Defic Syndr [Internet]. 2015;69(1). Available from: https://journals.lww.com/jaids/Fulltext/2015/05010/Pharmacogenetic_Testing_Can_Identify_Patients.21.aspx

105. Rodríguez-Nóvoa S, Martín-Carbonero L, Barreiro P, González-Pardo G, Jiménez-Nácher I, González-Lahoz J, et al. Genetic factors influencing atazanavir plasma concentrations and the risk of severe hyperbilirubinemia. AIDS [Internet]. 2007;21(1). Available from: https://journals.lww.com/aidsonline/Fulltext/2007/01020/Genetic_factors_influencing_atazanavir_plasma.5.aspx

106. Johnson DH, Venuto C, Ritchie MD, Morse GD, Daar ES, McLaren PJ, et al. Genomewide association study of atazanavir pharmacokinetics and hyperbilirubinemia in AIDS Clinical Trials Group protocol A5202. Pharmacogenet Genomics. 2014 Apr;24(4):195–203.

107. Park WB, Choe PG, Song K-H, Jeon JH, Park SW, Kim HB, et al. Genetic Factors Influencing Severe Atazanavir-Associated Hyperbilirubinemia in a Population with Low UDP-Glucuronosyltransferase 1A1*28 Allele Frequency. Clin Infect Dis. 2010 Jul 1;51(1):101–6.

108. Du P, Wang A, Ma Y, Li X. Association between the UGT1A1*28 allele and hyperbilirubinemia in HIV-positive patients receiving atazanavir: a meta-analysis. Biosci Rep. 2019 May 2;39(5):BSR20182105.

109. Nettles RE, Child MJ, Bertz RJ, Schnittman S. Gilbert Syndrome and the Development of Antiretroviral Therapy–Associated Hyperbilirubinemia: Genetic Screening Is Unnecessary. J Infect Dis. 2006 Jun 1;193(11):1611–2.

110. Ribaudo HJ, Daar ES, Tierney C, Morse GD, Mollan K, Sax PE, et al. Impact of UGT1A1 Gilbert variant on discontinuation of ritonavir-boosted atazanavir in AIDS Clinical Trials Group Study A5202. J Infect Dis. 2013 Feb 1;207(3):420–5.

111. Leger P, Chirwa S, Nwogu JN, Turner M, Richardson DM, Baker P, et al. Race/ethnicity difference in the pharmacogenetics of bilirubin-related atazanavir discontinuation. Pharmacogenet Genomics. 2018 Jan;28(1):1–6.

112. Hossain MR, Pandey RK, Islam MF, Datar P, Gayam V, Puri P, et al. Acute Isolated Hyperbilirubinemia as a Presentation of Alcoholic Liver Disease: A Case Report and Literature Review. Case Rep Hepatol. 2018;2018:3.

113. Tate D, Paul RH, Flanigan TP, Tashima K, Nash J, Adair C, et al. The Impact of Apathy and Depression on Quality of Life in Patients Infected with HIV. AIDS Patient Care STDs. 2003 Mar 1;17(3):115–20.

114. Robertson K, Bayon C, Molina J-M, McNamara P, Resch C, Muñoz-Moreno JA, et al. Screening for neurocognitive impairment, depression, and anxiety in HIV-infected patients in Western Europe and Canada. AIDS Care. 2014 Dec 2;26(12):1555–61.

115. Kittner JM, Brokamp F, Thomaidis T, Schmidt RE, Wiltink J, Galle PR, et al. Disclosure and Experienced Social Support are not Related to Anxiety or Depression in a German HIV Patient Cohort. Infect Chemother. 2014 Jun;46(2):77–83.

116. D Morrison S, H Banushi V, Sarnquist C, Gashi V, Osterberg L, Maldonado Y, et al. Levels of self-reported depression and anxiety among HIV-positive patients in Albania: a cross-sectional study. Croat Med J. 2011 Oct 15;52:622–8.

117. Vyavaharkar M, Moneyham L, Corwin S, Tavakoli A, Saunders R, Annang L. HIV-Disclosure, Social Support, and Depression Among HIV-Infected African American Women Living in the Rural Southeastern United States. AIDS Educ Prev. 2011 Feb 1;23(1):78–90.

118. Williams P, Narciso L, Browne G, Roberts J, Weir R, Gafni A. The Prevalence, Correlates, and Costs of Depression in People Living With HIV/AIDS in Ontario: Implications for Service Directions. AIDS Educ Prev. 2005 Apr 1;17(2):119–30.

119. Tran XB, Dang KA, Truong TN, Ha HG, Nguyen LH, Do NH, et al. Depression and Quality of Life among Patients Living with HIV/AIDS in the Era of Universal Treatment Access in Vietnam. Int J Environ Res Public Health. 2018;15(12).

120. Abas M, Ali G-C, Nakimuli-Mpungu E, Chibanda D. Depression in people living with HIV in sub-Saharan Africa: time to act. Trop Med Int Health. 2014 Dec 1;19(12):1392–6.

121. Ro S, Issa B, GT O, AO M, Odeigah L, Salami K, et al. Prevalence and Correlates of Depressive Disorders among People Living with HIV/AIDS, in North Central Nigeria. J AIDS Clin Res. 2013 Oct 12;4:1–7.

122. Kinyanda E, Hoskins S, Nakku J, Nawaz S, Patel V. Prevalence and risk factors of major depressive disorder in HIV/AIDS as seen in semi-urban Entebbe district, Uganda. BMC Psychiatry. 2011 Dec 30;11(1):205.

123. Ndetei DM, Khasakhala LI, Kuria MW, Mutiso VN, Ongecha-Owuor FA, Kokonya DA. The prevalence of mental disorders in adults in different level general medical facilities in Kenya: a cross-sectional study. Ann Gen Psychiatry. 2009 Jan 14;8(1):1.

124. Thai TT, Jones MK, Harris LM, Heard RC, Hills NK, Lindan CP. Symptoms of Depression in People Living with HIV in Ho Chi Minh City, Vietnam: Prevalence and Associated Factors. AIDS Behav. 2018 Jul 1;22(1):76–84.

125. Matsumoto S, Yamaoka K, Takahashi K, Tanuma J, Mizushima D, Do CD, et al. Social Support as a Key Protective Factor against Depression in HIV-Infected Patients: Report from large HIV clinics in Hanoi, Vietnam. Sci Rep. 2017 Nov 14;7(1):15489.

126. Gupta R, Dandu M, Packel L, Rutherford G, Leiter K, Phaladze N, et al. Depression and HIV in Botswana: A Population-Based Study on Gender-Specific Socioeconomic and Behavioral Correlates. PLOS ONE. 2010 Dec 8;5(12):e14252.

127. Aljassem K, Raboud JM, Hart TA, Benoit A, Su D, Margolese SL, et al. Gender Differences in Severity and Correlates of Depression Symptoms in People Living with HIV in Ontario, Canada. J Int Assoc Provid AIDS Care JIAPAC. 2014 Jun 4;15(1):23–35.

128. Bhatia MS, Munjal S. Prevalence of Depression in People Living with HIV/AIDS Undergoing ART and Factors Associated with it. J Clin Diagn Res JCDR. 2014 Oct;8(10):WC01-WC4.

129. Ngum PA, Fon PN, Ngu RC, Verla VS, Luma HN. Depression Among HIV/AIDS Patients on Highly Active Antiretroviral Therapy in the Southwest Regional Hospitals of Cameroon: A Cross-Sectional Study. Neurol Ther. 2017 Jun 1;6(1):103–14.

130. Etenyi JO, Okalebo FA, Oluka M, Sinei KA, Osanjo GO, Kurdi A, et al. Comparison of Zidovudine and Tenofovir Based Regimens With Regard to Health-Related Quality of Life and Prevalence of Symptoms in HIV Patients in a Kenyan Referral Hospital. Front Pharmacol. 2018;9:984.

131. Seth P, Kidder D, Pals S, Parent J, Mbatia R, Chesang K, et al. Psychosocial Functioning and Depressive Symptoms Among HIV-Positive Persons Receiving Care and Treatment in Kenya, Namibia, and Tanzania. Prev Sci. 2014 Jun 1;15(3):318–28.

132. Beyene Gebrezgiabher B, Huluf Abraha T, Hailu E, Siyum H, Mebrahtu G, Gidey B, et al. Depression among Adult HIV/AIDS Patients Attending ART Clinics at Aksum Town, Aksum, Ethiopia: A Cross-Sectional Study. Depress Res Treat. 2019;2019:8.

133. Tesfaw G, Ayano G, Awoke T, Assefa D, Birhanu Z, Miheretie G, et al. Prevalence and correlates of depression and anxiety among patients with HIV on-follow up at Alert Hospital, Addis Ababa, Ethiopia. BMC Psychiatry. 2016 Nov 2;16(1):368.

134. Berk M, Williams LJ, Jacka FN, O'Neil A, Pasco JA, Moylan S, et al. So depression is an inflammatory disease, but where does the inflammation come from? BMC Med. 2013 Sep 12;11(1):200.

135. L'akoa RM, Noubiap JJN, Fang Y, Ntone FE, Kuaban C. Prevalence and correlates of depressive symptoms in HIV-positive patients: a cross-sectional study among newly diagnosed patients in Yaoundé, Cameroon. BMC Psychiatry. 2013 Sep 22;13:228–228.

136. Aguocha C, Uwakwe R, Duru C, Diwe K, K. Aguocha J, Enwere O, et al. Prevalence and Socio-demographic Determinants of Depression among Patients Attending HIV/AIDS Clinic in a Teaching Hospital in Imo State, Nigeria. Am J Med Sci Med. 2015 Jan 1;3:106–12.

137. Obadeji A, Ogunlesi A, Adebowale T. Prevalence and Predictors of Depression in People living with HIV/AIDS Attending an Outpatient Clinic in Nigeria. Iran J Psychiatry Behav Sci. 2014 Jan 16;8:26–31.

138. Pappin M, Wouters E, Booysen FL. Anxiety and depression amongst patients enrolled in a public sector antiretroviral treatment programme in South Africa: a cross-sectional study. BMC Public Health. 2012 Mar 27;12(1):244.

139. Oppong Asante K. Social Support and the Psychological Wellbeing of People Living with HIV/AIDS in Ghana. Afr J Psychiatry. 2012 Sep 1;15:340–5.

140. Conway B. The role of adherence to antiretroviral therapy in the management of HIV infection. J Acquir Immune Defic Syndr 1999. 2007 Jun 1;45 Suppl 1:S14-18.

141. Carrieri MP, Raffi F, Lewden C, Sobel A, Michelet C, Cailleton V, et al. Impact of early versus late adherence to highly active antiretroviral therapy on immuno-virological response: a 3-year follow-up study. Antivir Ther. 2003 Dec;8(6):585–94.

142. Mills EJ, Nachega JB, Buchan I, Orbinski J, Attaran A, Singh S, et al. Adherence to Antiretroviral Therapy in Sub-Saharan Africa and North AmericaA Meta-analysis. JAMA. 2006 Aug 9;296(6):679–90.

143. Liu H, Miller LG, Hays RD, Golin CE, Wu T, Wenger NS, et al. Repeated measures longitudinal analyses of HIV virologic response as a function of percent adherence, dose

timing, genotypic sensitivity, and other factors. J Acquir Immune Defic Syndr 1999. 2006 Mar;41(3):315–22.

144. Parruti G, Manzoli L, Toro PM, D'Amico G, Rotolo S, Graziani V, et al. Long-term adherence to first-line highly active antiretroviral therapy in a hospital-based cohort: predictors and impact on virologic response and relapse. AIDS Patient Care STDs. 2006 Jan;20(1):48–56.

145. Byakika-Tusiime J, Crane J, Oyugi JH, Ragland K, Kawuma A, Musoke P, et al. Longitudinal antiretroviral adherence in HIV+ Ugandan parents and their children initiating HAART in the MTCT-Plus family treatment model: role of depression in declining adherence over time. AIDS Behav. 2009 Jun;13 Suppl 1:82–91.

146. Carrieri P, Cailleton V, Le Moing V, Spire B, Dellamonica P, Bouvet E, et al. The dynamic of adherence to highly active antiretroviral therapy: results from the French National APROCO cohort. J Acquir Immune Defic Syndr 1999. 2001 Nov 1;28(3):232–9.

147. Carrieri MP, Chesney MA, Spire B, Loundou A, Sobel A, Lepeu G, et al. Failure to maintain adherence to HAART in a cohort of French HIV-positive injecting drug users. Int J Behav Med. 2003;10(1):1–14.

148. Mayston R, Kinyanda E, Chishinga N, Prince M, Patel V. Mental disorder and the outcome of HIV/AIDS in low-income and middle-income countries: a systematic review. AIDS Lond Engl. 2012 Dec;26 Suppl 2:S117-135.

149. Watt MH, Maman S, Golin CE, Earp JA, Eng E, Bangdiwala SI, et al. Factors associated with self-reported adherence to antiretroviral therapy in a Tanzanian setting. AIDS Care. 2010 Mar;22(3):381–9.

150. Lankowski AJ, Siedner MJ, Bangsberg DR, Tsai AC. Impact of geographic and transportation-related barriers on HIV outcomes in sub-Saharan Africa: a systematic review. AIDS Behav. 2014 Jul;18(7):1199–223.

151. Ware NC, Idoko J, Kaaya S, Biraro IA, Wyatt MA, Agbaji O, et al. Explaining adherence success in sub-Saharan Africa: an ethnographic study. PLoS Med. 2009 Jan 27;6(1):e11.

152. Moosa A, Gengiah TN, Lewis L, Naidoo K. Long-term adherence to antiretroviral therapy in a South African adult patient cohort: a retrospective study. BMC Infect Dis. 2019 Sep 5;19(1):775–775.

153. Nachega JB, Leisegang R, Bishai D, Nguyen H, Hislop M, Cleary S, et al. Association of antiretroviral therapy adherence and health care costs. Ann Intern Med. 2010 Jan 5;152(1):18–25.

154. Cambiano V, Bertagnolio S, Jordan MR, Lundgren JD, Phillips A. Transmission of drug resistant HIV and its potential impact on mortality and treatment outcomes in resource-limited settings. J Infect Dis. 2013 Jun 15;207 Suppl 2:S57-62.

155. Langebeek N, Gisolf EH, Reiss P, Vervoort SC, Hafsteinsdóttir TB, Richter C, et al. Predictors and correlates of adherence to combination antiretroviral therapy (ART) for chronic HIV infection: a meta-analysis. BMC Med [Internet]. 2014 Aug 21 [cited 2019 Dec 1];12. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4148019/

156. Rai S, Mahapatra B, Sircar S, Raj PY, Venkatesh S, Shaukat M, et al. Adherence to Antiretroviral Therapy and Its Effect on Survival of HIV-Infected Individuals in Jharkhand, India. PloS One. 2013 Jun 18;8(6):e66860–e66860.

157. Kipsang J, Chen J, Tang C, Li X, Wang H. Self reported adherence to antiretroviral treatment and correlates in Hunan province, the Peoples Republic of China. Int J Nurs Sci. 2018 Apr 14;5(2):162–7.

158. Aye WL, Puckpinyo A, Peltzer K. Non-adherence to anti-retroviral therapy among HIV infected adults in Mon State of Myanmar. BMC Public Health. 2017 May 5;17(1):391–391.

159. Ehlers VJ, Tshisuyi ET. Adherence to antiretroviral treatment by adults in a rural area of Botswana. Curationis. 2015 May 29;38(1):1255.

160. Mabunda K, Ngamasana EL, Babalola JO, Zunza M, Nyasulu P. Determinants of poor adherence to antiretroviral treatment using a combined effect of age and education among human immunodeficiency virus infected young adults attending care at Letaba Hospital HIV Clinic, Limpopo Province, South Africa. Pan Afr Med J. 2019 Jan 18;32:37–37.

161. Yaya I, Landoh DE, Saka B, Patchali PM, Wasswa P, Aboubakari A-S, et al. Predictors of adherence to antiretroviral therapy among people living with HIV and AIDS at the regional hospital of Sokodé, Togo. BMC Public Health. 2014 Dec 19;14:1308–1308.

162. Potchoo Y, Tchamdja K, Balogou A, Pitche VP, Guissou IP, Kassang EK. Knowledge and adherence to antiretroviral therapy among adult people living with HIV/AIDS treated in the health care centers of the association “Espoir Vie Togo” in Togo, West Africa. BMC Clin Pharmacol. 2010 Sep 17;10:11–11.

163. Sangeda RZ, Mosha F, Aboud S, Kamuhabwa A, Chalamilla G, Vercauteren J, et al. Predictors of non adherence to antiretroviral therapy at an urban HIV care and treatment center in Tanzania. Drug Healthc Patient Saf. 2018 Aug 21;10:79–88.

164. Letta S, Demissie A, Oljira L, Dessie Y. Factors associated with adherence to Antiretroviral Therapy (ART) among adult people living with HIV and attending their clinical care, Eastern Ethiopia. BMC Int Health Hum Rights. 2015 Dec 28;15:33–33.

165. Unge C, Södergård B, Marrone G, Thorson A, Lukhwaro A, Carter J, et al. Long-term adherence to antiretroviral treatment and program drop-out in a high-risk urban setting in sub-Saharan Africa: a prospective cohort study. PloS One. 2010 Oct 25;5(10):e13613–e13613.

166. Semvua SK, Orrell C, Mmbaga BT, Semvua HH, Bartlett JA, Boulle AA. Predictors of non-adherence to antiretroviral therapy among HIV infected patients in northern Tanzania. PloS One. 2017 Dec 18;12(12):e0189460–e0189460.

167. Protopopescu C, Raffi F, Roux P, Reynes J, Dellamonica P, Spire B, et al. Factors associated with non-adherence to long-term highly active antiretroviral therapy: a 10 year follow-up analysis with correction for the bias induced by missing data. J Antimicrob Chemother. 2009 Jul 14;64(3):599–606.

168. Charurat M, Oyegunle M, Benjamin R, Habib A, Eze E, Ele P, et al. Patient retention and adherence to antiretrovirals in a large antiretroviral therapy program in Nigeria: a longitudinal analysis for risk factors. PloS One. 2010 May 11;5(5):e10584–e10584.

169. Bukenya D, Mayanja BN, Nakamanya S, Muhumuza R, Seeley J. What causes non-adherence among some individuals on long term antiretroviral therapy? Experiences of individuals with poor viral suppression in Uganda. AIDS Res Ther. 2019 Jan 21;16(1):2–2.

170. Denison JA, Koole O, Tsui S, Menten J, Torpey K, van Praag E, et al. Incomplete adherence among treatment-experienced adults on antiretroviral therapy in Tanzania, Uganda and Zambia. AIDS Lond Engl. 2015 Jan 28;29(3):361–71.

171. Barbaro G. Highly Active Antiretroviral Therapy–Associated Metabolic Syndrome: Pathogenesis and Cardiovascular Risk*. Am J Ther [Internet]. 2006;13(3). Available from: https://journals.lww.com/americantherapeutics/Fulltext/2006/05000/Highly_Active_Antiretroviral_Therapy_Associated.12.aspx

172. Monroe AK, Rowe TL, Moore RD, Chander G. Medication adherence in HIV-positive patients with diabetes or hypertension: a focus group study. BMC Health Serv Res. 2013 Nov 25;13(1):488.

173. Krentz HB, Cosman I, Lee K, Ming JM, Gill MJ. Pill burden in HIV infection: 20 years of experience. Antivir Ther. 2012;17(5):833–40.

174. Croome N, Ahluwalia M, Hughes LD, Abas M. Patient-reported barriers and facilitators to antiretroviral adherence in sub-Saharan Africa. AIDS Lond Engl. 2017 Apr 24;31(7):995–1007.

Appendices

Appendix 1: Case report forms (CRF)

Date ☐☐ ☐☐ ☐☐☐☐

dd *mm* *yyyy*

ATV study eligibility form

Screening ID ☐☐☐☐☐

Inclusion criteria	
1	Is the participant 18 years of age or older? Yes ☐ No ☐
2	Is the participant HIV positive? Yes ☐ No ☐
3	Was the participant willing and able to provide independent, written, informed consent for screening? Yes ☐ No ☐ 3a. When was the informed consent for screening marked or signed? ☐☐ ☐☐ ☐☐☐☐ *Dd* *mm* *yyyy*
4	Is the participant enrolled for follow up and care at the KNH or Mbagathi DH CCC? Yes ☐ No ☐
5	Is the participant on an ATV containing regimen? Yes ☐ No ☐
6	If affirmative in question 4, has the participant been on the ATV containing regimen continuosly for at least 4 weeks? Yes ☐ No ☐
Exclusion criteria *questions 7-11 need to be answered in the negative for eligibility*	
7	Is the participant pregnant? Yes ☐ No ☐
8	Does the participant have an acute HIV infection? Yes ☐ No ☐
9	Does the participant have an active opportunistic infection or stage IV illness? Yes ☐ No ☐
10	Does the participant have any condition that, in the opinion of the investigators, would preclude provision of informed consent, make participant in the study unsafe, complicate interpretation of the study outcome data, or otherwise interfere with achieving the study objectives? Yes ☐ No ☐
11	Is the participant currently on using any of the following classes of drugs – antacids, proton pump inhibitors or H1 receptor antagonists? Yes ☐ No ☐
11	Based on the information from item 1-11, is the participant eligible or not? Eligible ☐ Not eligible ☐
	Completed by:_____ **(***initials/date***)**

Baseline demographics 1- social (BDEM1-S)

Date ☐☐ ☐☐ ☐☐☐☐

dd *mm* *yyyy*

Participant ID ☐☐☐☐☐

Screening Demographics *This is an interviewer administered form. Read each item aloud to the participant*	
1.	Sex of the participant Male ☐ Female ☐
2.	When were you born? ☐☐ ☐☐ ☐☐☐☐ (*dd mm yyyy*) OR What is your age? *Years* ☐☐
3.	What is your marital status? a. Single ☐ b. Married ☐ c. Separated ☐ d. Divorced ☐
4.	How many living children do you have? ☐☐ Number of children
5.	What is your; a. Father's ethnic group or tribe? b. Mother's ethnic group or tribe?
6.	What is the highest level of education completed? ☐☐ *a. Primary* ☐ *b. Secondary* ☐ *c. Tertiary* ☐ *d. Informal education* ☐
7.	What is your occupation? a. Unemployed ☐ b. Self employed ☐ c. In formal employment ☐
8.	Do you smoke cigarettes? Yes ☐ No ☐
9.	Do you consume alcohol? Yes ☐ No ☐
	Completed by: _____*initials/date)*

Baseline demographics 2-comorbidities, labs and ART history (BDEM1-CLA)

Date ☐☐ ☐☐ ☐☐☐☐

dd *mm* *yyyy*

Participant ID ☐☐☐☐☐

Part 1: Medical history	
1.	Is the participant hypertensive? Yes ☐ No ☐
2.	Is the participant diabetic? Yes ☐ No ☐
Part 2: Laboratory parameters	
3.	Latest CD4 count ☐☐☐☐ cells/mm^3 a. Date test was done ☐☐ ☐☐ ☐☐ (*dd* *mm* *yy*)
4	Latest viral load ☐☐☐☐☐☐ copies/mL a. Date test was done ☐☐ ☐☐ ☐☐☐☐ (*dd* *mm* *yyyy*)
5.	What is the participant's latest serum creatinine concentration? ☐☐☐☐ Micromoles/L Date test was done ☐☐ ☐☐ ☐☐☐☐ (*dd* *mm* *yyyy*)
6.	Using the MDRD 4 formula, calculate the estimated glomerular function (eGFR). $eGFR(ml/min/1.73m^2) = 175 \times (serumcreatinine)^{-1.154} \times Age^{-0.203} \times (0.742\ if female) \times (1.212\ if black)$ ☐☐☐☐ mL/min/1.73m^2
Part 3: Antiretroviral Therapy (ART) history	
7.	When was the participant diagnosed with HIV? ☐☐ ☐☐ ☐☐☐☐ (*Dd* *mm* *yyyy*)
8.	When was the participant started on antiretroviral therapy (ART)? ☐☐ ☐☐ ☐☐☐☐ (*Dd* *yy* *yyyy*)

9.	What is the current ART regimen that the participant is using? *(Please key in the abbreviations of the ARVs in the spaces provided)* ☐☐☐ ☐☐☐ ☐☐☐
10.	Please list all other concomitant medications that the patient is on. a. N/A ☐ ***(proceed to question 12)*** b. _____ _____(strength) _____(frequency) c. _____ _____(strength) _____(frequency) d. _____ _____(strength) _____(frequency) e. _____ _____(strength) _____(frequency) f. _____ _____(strength) _____(frequency)
11.	Is the participant on herbal medications? Yes ☐ No ☐ If yes, please list the medications below. a. _____ b. _____ c. _____ d. _____
12.	Has the participant switched ART regimens before? Yes ☐ No ☐ a. If yes, please indicate the previous regimen. *(Please key in the abbreviations of the ARVs in the spaces provided)* ***Most recent regimen*** ☐☐☐ ☐☐☐ ☐☐☐ ***Initial regimen*** ☐☐☐ ☐☐☐ ☐☐☐ ***Date of switch*** ☐☐ ☐☐ ☐☐☐☐ ***mm dd yyyy*** b. Please indicate the reason/s for switching ART regimens. a. Regimen failure ☐ b. Toxicity ☐ c. Other reason (*specify)*_____
13.	What is the participant's WHO clinical staging of HIV/AIDS? a. I ☐ b. II ☐ c. III ☐ d. IV ☐
14.	Is the participant currently suffering from any opportunistic infection? Yes ☐ No ☐ If yes, please indicate the infection and the date of diagnosis._____ Date diagnosed ☐☐ ☐☐ ☐☐☐☐ *dd mm yyyy*
	Completed by: _____(*initials/date)*

Date ☐☐ ☐☐ ☐☐☐☐ ***Participant specimen collection form***

dd mm yyyy **Participant ID** ☐☐☐☐☐

	Section 1: Pharmacokinetic sampling
1	Have you missed any doses of your medications the past week? Yes ☐ No ☐ If yes, how many doses have you missed? ☐ Doses
2	When did you last take your dose of atazanavir/ritonavir 300mg/100mg tablet? _____ (date *dd/mm/yr)*
3	In the last two days, at what time did you take your dose of atazanavir? Day 1 _____ Time ☐☐☐☐ (24 hour clock) (*dd/mm/yyyy)* Day 2 _____ Time ☐☐☐☐ (24 hour clock) (*dd/mm/yyyy)*
4	**Sample 1: Pre- morning dose sample (-0.5 hour)** Date of sampling ☐☐ ☐☐ ☐☐☐☐ *dd mm yyyy* Sampling time ☐☐☐☐ (24 hour clock) Volume sampled ☐☐☐ mL Sampled by _____(initials/date)
5	**Time of morning dose of atazanvir/ritonavir** ☐☐☐☐ (24 hour clock) Dose administered/observed by _____*(initials/date)*
6	**Sample 2: 2.0 hour post morning dose sample** Date of sampling ☐☐ ☐☐ ☐☐☐☐ *dd mm yyyy* Sampling time ☐☐☐☐ (24 hour clock) Volume sampled ☐☐☐ mL Sampled by _____(initials/date)

7	**Sample 3: 4.0 hour post morning dose sample** Date of sampling ☐☐ ☐☐ ☐☐☐☐ *dd mm yyyy* Sampling time ☐☐☐☐ (24 hour clock) Volume sampled ☐☐☐ mL Sampled by _____(initials/date)
	The samples in Sections 2 and 3 are to be collected at the same time as the trough sample.
	Section 2: Genotyping sample collection
7	**Sample 1:** Date of sampling ☐☐ ☐☐ ☐☐☐☐ *dd mm yyyy* Sampling time ☐☐☐☐ (24 hour clock) Volume sampled ☐☐☐ mL Sampled by _____(initials/date)
	Section 3: Liver function tests sample collection
8	**Sample 2:** Date of sampling ☐☐ ☐☐ ☐☐☐☐ *dd mm yyyy* Sampling time ☐☐☐☐ (24 hour clock) Volume sampled ☐☐☐ mL Sampled by _____(initials/date)
	Completed by_____*(initials/date)*

Date ☐☐ ☐☐ ☐☐☐☐
dd *mm* *yyyy*

Laboratory results form

Participant ID ☐☐☐☐☐

	Section 1: Pharmacokinetics
1	**Sample 1: Pre- morning dose sample (-0.5 hour)** Date of analysis ☐☐ ☐☐ ☐☐☐☐ (*dd* *mm* *yyyy*) Concentration ☐☐☐☐☐ ng/ml Analysed by _____(initials/date)
2	**Sample 2: 2.0 hr post morning dose sample** Date of analysis ☐☐ ☐☐ ☐☐☐☐ (*dd* *mm* *yyyy*) Concentration ☐☐☐☐☐ ng/ml Analysed by _____(initials/date)
3	**Sample 3: 4.0 hr post morning dose sample** Date of analysis ☐☐ ☐☐ ☐☐☐☐ (*dd* *mm* *yyyy*) Concentration ☐☐☐☐☐ ng/ml Analysed by _____(initials/date)
	Section 2: Genotyping (tick as appropriate for SNP identified)
4	**CYP3A5** Date of analysis ☐☐ ☐☐ ☐☐☐☐ (*dd* *mm* *yyyy*) ***1** ☐ ***3** ☐ ***5** ☐ ***7** ☐ Analysed by_____ *(initials/date)*

5	**UGT1A1*28** Date of analysis ☐☐ ☐☐ ☐☐☐☐ *dd* *mm* *yyyy* Wild type- 6/6 ☐ 6/7 ☐ 7/7 ☐ Analysed by _____(initials/date)
	Section 3: Liver function tests
6	*Concentration* *Units* Direct bilirubin ☐☐☐☐ Indirect bilirubin ☐☐☐☐ Total bilirubin ☐☐☐☐ ALT ☐☐☐☐ AST ☐☐☐☐ GGT ☐☐☐☐ Date of analysis ☐☐ ☐☐ ☐☐☐☐ *dd* *mm* *yyyy* Analysed by _____(initials/date)

Date ☐☐ ☐☐ ☐☐☐☐ ***Repeat PK samples collection form***

dd mm yyyy **Participant ID** ☐☐☐☐☐

	Section 1: Pharmacokinetic sampling
1	Have you missed any doses of your medications the past week? Yes ☐ No ☐ If yes, how many doses have you missed? ☐ Doses
2	When did you last take your dose of atazanavir/ritonavir 300mg/100mg tablet? _____ (date *dd/mm/yr)*
3	In the last two days, at what time did you take your dose of atazanavir? Day 1 _____ Time ☐☐☐☐ (24 hour clock) (*dd/mm/yyyy)* Day 2 _____ Time ☐☐☐☐ (24 hour clock) (*dd/mm/yyyy)*
4	**Sample 1: Pre- morning dose sample (-0.5 hour)** Date of sampling ☐☐ ☐☐ ☐☐☐☐ *dd mm yyyy* Sampling time ☐☐☐☐ (24 hour clock) Volume sampled ☐☐☐ mL Sampled by _____(initials/date)
5	**Time of morning dose of atazanvir/ritonavir** ☐☐☐☐ (24 hour clock) Dose administered/observed by _____*(initials/date)*
6	**Sample 2: 2.0 hour post morning dose sample** Date of sampling ☐☐ ☐☐ ☐☐☐☐ *dd mm yyyy* Sampling time ☐☐☐☐ (24 hour clock) Volume sampled ☐☐☐ mL Sampled by _____(initials/date)

Date ☐☐ ☐☐ ☐☐☐☐ ***Participant specimen collection form***

dd mm yyyy **Participant ID** ☐☐☐☐☐

7	**Sample 3: 4.0 hour post morning dose sample** Date of sampling ☐☐ ☐☐ ☐☐☐☐ *dd mm yyyy* Sampling time ☐☐☐☐ (24 hour clock) Volume sampled ☐☐☐ mL Sampled by _____(initials/date)
	Completed by_____*(initials/date)*

Date ☐☐ ☐☐ ☐☐☐☐ ***Laboratory results form (repeat PK sampling)***

dd *mm* *yyyy* **Participant ID** ☐☐☐☐☐

	Section 1: Pharmacokinetics
1	**Sample 1: Pre- morning dose sample (-0.5 hour)** Date of analysis ☐☐ ☐☐ ☐☐☐☐ *dd* *mm* *yyyy* Concentration ☐☐☐☐☐ ng/ml Analysed by _____(initials/date)
2	**Sample 2: 2.0 hr post morning dose sample** Date of analysis ☐☐ ☐☐ ☐☐☐☐ *dd* *mm* *yyyy* Concentration ☐☐☐☐☐ ng/ml Analysed by _____(initials/date)
3	**Sample 3: 4.0 hr post morning dose sample** Date of analysis ☐☐ ☐☐ ☐☐☐☐ *dd* *mm* *yyyy* Concentration ☐☐☐☐☐ ng/ml Analysed by _____(initials/date)

Appendix 2: Morisky Medication Adherence Scale

Date ☐☐ ☐☐ ☐☐☐☐

dd *mm* *yyyy*

Participant ID ☐☐☐☐☐

This is an interviewer administered form. Read each item aloud to the participant	
1.	Do you sometimes forget to take your medicines? No =1 ☐ Yes = 0 ☐
2.	People sometimes miss taking their medicines for reasons other than forgetting. Over the past two weeks, were there any days when you did not take your medicines? No =1 ☐ Yes = 0 ☐
3.	Have you ever cut back or stopped taking your medicine without telling your doctor because you felt worse when you took it? No =1 ☐ Yes = 0 ☐
4.	When you travel or leave home, do you sometimes forget to bring your medicine? No =1 ☐ Yes = 0 ☐
5.	Did you take all your medicine yesterday? Yes =1 ☐ No = 0 ☐
6.	When you feel like your symptoms are under control, do you sometimes stop taking your medicine? No =1 ☐ Yes = 0 ☐
7.	Taking medicine every day is a real inconvenience for some people. Do you ever feel hassled about sticking to your treatment plan? No =1 ☐ Yes = 0 ☐
8.	How often do you have difficulty remembering to take all your medicine? (A) Never/rarely (B) Once in a while (C) Sometimes (D) Usually I All the time A=4 ☐ B=3 ☐ C=2 ☐ D=1 ☐ E=0 ☐
	Divide the score by 4 =
	Score <6=low adherence; 6-<8=medium adherence; 8= High adherence
	Completed by: _____*initials/date)*

Appendix 3: Patient Health Questionnaire 9

Over the last 2 weeks, how often have you been bothered by any of the following problems? *(Use "✔" to indicate your answer)*	**Not at all**	**Several days**	**More than half the days**	**Nearly every day**
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed, or hopeless	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself — or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed? Or the opposite — being so fidgety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead or of hurting yourself in some way	0	1	2	3

FOR OFFICE CODING *0* **+** _____ **+** _____ **+** _____

=Total Score: _____

If you checked off any problems, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

Not difficult at all	**Somewhat difficult**	**Very difficult**	**Extremely difficult**

Developed by Drs. Robert L. Spitzer, Janet B.W. Williams, Kurt Kroenke and colleagues, with an educational grant from Pfizer Inc. No permission required to reproduce, translate, display or distribute.

Appendix 4: Copy of KNH/UoN ERC approval letter -page 1

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

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

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

KENYATTA NATIONAL HOSPITAL APPROVED 27 MAR 2017 KNH/UoN-ERC P.O. Box 20723-00202 Nairobi

Ref: KNH-ERC/A/110

27th March 2017

Dr. George A. Mugendi
Principal Investigator
Dept.of Pharmaceutics and Pharmacy Practice
School of Pharmacy
College of Health Sciences
University of Nairobi

Dear Dr. Mugendi

REVISED RESEARCH PROPOSAL – CHARACTERIZATION OF THE STEADY STATE CONCENTRATIONS AND PHARMACOGENETICS OF ATAZANAVIR IN KENYAN HIV POSITIVE PATIENTS (P939/12/2016)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above revised proposal. The approval period is from 27th March 2017 – 26th March 2018.

This approval is subject to compliance with the following requirements:

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

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

"Protect to Discover"

Appendix 4: Copy of KNH/UoN ERC approval letter -page 2

Yours sincerely,

PROF M. L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Director, CS, KNH
The Assistant Director, Health Information, KNH
The Chair, KNH-UoN ERC
The Dean, School of Pharmacy, UoN
The Chair, Dept.of Pharmaceutics and Pharmacy Practice, UoN
Supervisors: Dr.Faith Okalebo(UoN), Prof. Jashvant Unadkhat(UoW)

"Protect to Discover"

Appendix 5: Enrolment consent forms

Enrolment Consent Form

Characterization of the steady state concentrations and pharmacogenetics of atazanavir in Kenyan HIV positive patients

Version 1.2

30 October 2017

INVESTIGATORS

George Mugendi	Bpharm.,Mpharm,	University of Nairobi	020 4915029
Faith Okalebo	PhD	University of Nairobi	020 4915027
Jashvant Unadkat	PhD	University of Washington	+1(206)6852869
Nasser Nyamweya	PhD	University of Nairobi	020 4915029

INFORMED CONSENT

We are asking you to volunteer to participate in this study. The study is for clients enrolled at the Kenyatta National Hospital comprehensive care centre (KNH-CCC).

If you decide to take part in this study you will be asked to sign this consent form or make your mark in front of a witness. We will give you a copy of this form. This consent form might contain some words that are unfamiliar to you. Please ask us to explain anything you may not understand.

PURPOSE OF THE STUDY

Infection with HIV is primarily managed using anti-retroviral agents in combination, what is commonly referred to as antiretroviral therapy (ART). There are different groups of antiretrovirals that can be used in therapy, one of them being atazanavir.

There are aspects of this drug that we do not fully understand especially regarding its use in Africans with HIV infection. Our aim is to try and understand how this drug is acted upon by the body in an effort to make therapy safer and more effective

We intend to enroll approximately 109 patients on atazanavir who are on follow up at the KNH CCC.

YOUR PARTICIPATION IS VOLUNTARY

Before you learn about the enrolment and study tests, it is important that you know the following:

- You do not have to be in this study if you do not want to.
- You may decide not to have the enrolment and study tests, or to stop the enrolment and study tests at any time, without losing your regular medical care.
- You may be asked if you are taking part in other studies.
- Even if you are eligible, you do not have to join this study.

STUDY VISIT AND PROCEDURES

Enrolment procedures will begin today, after you read, discuss, and sign or make your mark on this form. There is only one study visit which will take about 6 hours and will be on an agreed date within a two week window. As a study participant, it is expected that you will continue taking your medication as directed. This is extremely important not only for this study, but for your own well-being as well.

During this study visit, you will be asked questions regarding your health by a study staff. The study staff will ask you the following:

- Where you live, your age, your occupation and your lifestyle habits.
- Your health status now and in the past.
- Your HIV status and confirm this from your patient file.

If you are found to be eligible for the research study, the study staff will fully explain the study to you and answer any questions you have. If you decide to take part in the research study, you will be asked to sign this consent form.

SAMPLES FOR DRUG LEVELS IN BLOOD

We will collect five blood samples from you over a period of 6 hours. The exact sampling times are as follows; half an hour to your morning dose and the subsequent samples at the following intervals after the morning dose; 2 and 4 hours. These three samples are meant to depict the lowest to the highest concentration of the drug in your body. The samples will be drawn from your arms. Each blood samples will be approximately 4 mLs. You may be selected to return to the clinic within a week to two weeks for additional blood sampling, and this will be communicated to you during the first visit.

SAMPLES FOR GENETIC TESTING AND CLINICAL MONITORING

We will perform a genetic test on a 4 mL blood sample to be drawn separately. This will help us to understand the factors that may contribute to success or failure of therapy in different individuals based on the varying levels of activity of the enzymes that metabolize atazanavir. Once the sample is drawn, it will be labelled using a code that will not directly identify you, and then once analysis is done, a small volume will be stored for 3 years in a secure facility to answer other related questions that may arise in the near future. We shall apply for ethical approval for future studies based on the stored samples.

The results of this test will only be provided to you if you want them, and counselling by the clinician will be provided prior to provision of the results. Depending on the nature of the results, a modification of your regimen may be suggested or not.

In order to monitor your therapy, we will also perform liver function tests, viral load and CD4 count from an additional 2mL sample.

RISKS AND/OR DISCOMFORTS

You may become anxious when talking about your HIV status. The study staff will help you understand this situation. Trained study staff will be available through the study to help you deal with any feelings or questions you may have.

During the blood draws, you may experience some mild pain form the prick of the needle, but our study staff are skilled and they will do their best to minimize this discomfort.

BENEFITS

You may get no direct benefit from the study tests. You will get information on HIV and how to protect others against HIV. If you require further medical assistance, the study staff will refer you to the CCC clinician for treatment.

Your participation may contribute to the improvement of therapy among HIV positive patients using atazanavir.

REASONS WHY YOU MAY BE WITHDRAWN FROM THE STUDY PROCESS

You may be removed from the study process without your consent for the following reasons:

- The research study is stopped or cancelled.
- You are not able to complete the study tests.

COSTS TO YOU

There is no cost to you for study participation.

REIMBURSEMENT

You will be offered 500 KShs as reimbursement for travel costs to the clinic.

CONFIDENTIALITY

Efforts will be made to keep your personal information confidential. However absolute confidentiality cannot be guaranteed. Your personal information may be disclosed if required by law. Any information about you will be identified only by code and not by name. The link between your name and code will be kept in a secure location by the principal investigator. Any publication of this study will not use your name or identify you personally.

The records of your study tests may be reviewed by study staff and representatives of:

- The Kenyatta National Hospital/University of Nairobi Ethics and Research Review Committee (KNH/UoN ERC)

PROBLEMS OR QUESTIONS

If you ever have any questions about the study tests, you should contact George Mugendi on 020 4915029.

If you have questions about your rights as a research participant, you should contact the Secretary of the KNH/UoN ERC, P. O. Box 19676-00202, Nairobi: Telephone number: 0202-272-6300 Ext 44355. Email address: uonknherc@uonbi.ac.ke

NEW FINDINGS

Any new information that we find out during the course of this research that may affect your health, welfare and/or might change your decision to be in the study will be provided to you in a timely manner.

STATEMENT OF CONSENT AND SIGNATURES

I have read this form or had it read to me. I have discussed the information with study staff. My questions have been answered. I understand that my decision whether or not to take part in the study is voluntary. I understand that if I decide to join the study, I may withdraw at any time. By signing this form I do not give up any rights that I have as a research participant.

I agree:

i.	to take part in the genetic study	Yes ☐	no ☐
ii.	to have my blood samples stored for later study	Yes ☐	no ☐
iii.	to have my blood samples analyzed in a lab outside Kenya	Yes ☐	no ☐
iv.	to provide contact information for follow up.	Yes ☐	no ☐

_____	_____	_____
Participant Name (print)	Participant Signature/Thumbprint	Date

I, the undersigned, have fully explained the pertinent aspects of this study to the above named participant who has understood and has willingly and freely given his/her consent.

_____	_____	_____
Study Staff Conducting Consent Discussion (print)	Study Staff Signature	Date

_____	_____	_____
Witness Name If required (print)	Witness Signature	Date

Enrolment Consent Form

Characterization of the steady state concentrations and pharmacogenetics of atazanavir in Kenyan HIV positive patients

Toleo 1.2
30 October 2017

WAPELELEZI

George Mugendi	BPharm., MPharm,	University of Nairobi	020 4915029
Faith Okalebo	PhD	University of Nairobi	020 4915027
Jashvant Unadkat	PhD	University of Washington	+1(206)6852869
Nasser Nyamweya	PhD	University of Nairobi	020 4915029

NAKALA YA ITIKIO

Tunakuuliza ujitolee kwa hiari ili ushiriki katika utafiti huu. Utafiti huu utajumuisha wale wanaotafuta matibabu katika hospitali ya kitaifa ya Kenyatta kituo cha CCC.

Ikiwa utaamua kuhusishwa katika utafiti huu, utaulizwa kuweka sahihi katika nakala hii au kuweka alama ya kidole mbele ya shahidi. Tutakupa nakala ya fomu hii. Nakala hii ya itikio huenda ikawa na maneno mengine ambayo huelewi. Tafadhali uliza tukuelezee chochote ambacho huenda ukakosa kuelewa.

LENGO LA UTAFITI

Ugonjwa wa HIV hutibiwa kwa kutumia dawa zinazopunguza makali ya virusi. Kuna aina tofauti za haya madawa, moja yao ikiwa atazanavir.

Kuna maswala kadha wa kadha kuhusu atzanavir ambayo hatuelewi haswa tukianganzia matumizi yake na wanaAfrika walioadhiriwa na HIV. Lengo letu ni kujaribu kuelewa vile ambavyo mwili na hii dawa huhusiana ili tufanye matibabu yawe ya hali ya juu.

Tunatarajia kuwa tatasajili wanautafiti mia moja na tisa ambao wanatumia atazanavir na wanaopata matibabu katika Hospitali Kuu ya rufaa ya Kenyatta.

KUSHIRIKI KWAKO NI KWA HIARI

Kabla ya kujua kuhusu vipimo vya kuandikishwa na vya kufuatiliwa, ni muhimu ujue yafuatayo;

- Sio lazima kuwa katika utafiti huu ikiwa hutaki.
- Unaweza kuamua usifanyiwe vipimo vya kuandikishwa na vya kufuatiliwa, au kusimamisha vipimo vya kuandikishwa na vya kufuatiliwa wakati wowote, bila kupoteza huduma zako za matibabu za kawaida.
- Unaweza kuulizwa ikiwa unashiriki kwa tafiti zingine.
- Hata ikiwa umehitimu kujiunga na utafiti, sio lazima kujiunga na huu utafiti.

MATEMBEZI NA TARATIBU ZA UTAFITI

Taratibu za kuandikishwa zitaanza leo, baada ya kusoma, kujadili, na kuweka sahihi au alama ya kidole kwa nakala hii. Katika utafiti huu, kuna tembezi moja pekee ambalo litachukua takriban masaa sita. Tembezi hilo litakuwa siku ya makubaliano, katika mwanya wa majuma mawili kutoka leo. Kama mwana utafiti, tunatarajia kwamba utaendelea kutumia dawa kulingana na mawaidha uliyopewa. Swala hili ni muhimu sio tu kwa lengo la utafiti, bali pia kwa afya yako kijumla.

Katika utafiti huu, utaulizwa maswali kuhusu afya yako na mfanyikazi wa utafiti. Maswali ambayo utalulizwa na mfanyikzai wa utafiti ni kuhusu:

- Mahali unapoishi, umri wako, kazi unayojikimu nayo na hali yako ya maisha kwa jumla
- Hali yako ya afya kwa wakati huu na uliopita
- Hali yako ya HIV na kuhakikisha kutoka kwa nakala yako ya hospitali

SAMPULI ZA KUDHAMINI KIWANGO CHA DAWA KWENYE DAMU

Sampuli tano za damu zitachukuliwa kutoka kwako kati ya masaa sita. Sampuli hizi za damu zitatolewa kulingana na taratibu ifuatayo ya masaa; nusu saa kabla ya kunywa dawa za asubuhu, kisha masaa mawili na manne baada ya kumeza dawa. Tunatarajia kwamba hizi samuli za damu zitalingana na wingi wa dawa mwilini. Sampuli hizi za damu zitatolewa kutoka kwa mishipa ya mikono. Kila sampuli itakuwa takriban mili lita nne. Huenda ukachaguliwa kurudi hospitali baada ya juma moja au mbili ili utolewe damu zaidi na utajulishwa kabla ya tembezi la kwanza kuisha.

KIPIMO CHA KUDHAMINI MAUMBILE

Tunalenga kufanya kipimo cha maumbile kwa sampuli nyingine ya damu ya mililita nne ili tuweze kuelewa kwanini watu wengine wananawiri kwa dawa hii na wengine wanadhofia ki afya. Sampuli hiyo ya damu itapewa nambari ambayo itahifadhi usiri wako. Baada ya kipimo kufanywa, sampuli itakayo baki itahifadhiwa katika hali ya usalama kwa minajili ya kufanya vipimo vingine katiak muda wa miaka tatu ijayo. Kabla ya kutumia sampuli hizo kwa utafiti mpya, tutaomba ruhusa kutoka kwa ERC ya Kenyatta.

Utafahamishwa kuhusu matokeo ya kipimo hichi kama unataka kujua, lakini kama hauna haja kujua, pia ni sawa. Utapokea mawaidha kutoka kwa mhudumu wa afya iwapo ungependa kujua haya matokeo. Dawa unazokutumia kudhibiti makali ya HIV yanaweza badilishwa kulingana na matokeo ya kipimo hiki.

Sampuli ya mwisho ya mililita mbili itatusaidia kudhamini vile ini lako linafanya kazi, kiwango cha virusi mwilini pamoja na ile ya CD4.

TATIZO NA/AU KUKOSA STAREHE

Huenda ukawa na hofu au wasiwasi unapoongea kuhusu hali yako ya HIV. Mashauri utakayo pokea kutoka kwa mfanyikazi wa utafiti yatakusaidia kuelewa hali hii zaidi. Washauri waliohitimu watakuweko wakati wote wa

utafiti na watakusaidia kukabiliana na hisia au maswali ambayo unaweza kuwa nayo.

Wakati unapotolewa damu huenda ukahisi uchungu kidogo wa sindano inapotoboa ngozi. Wafanyikazi wa utafiti wako na ujuzi mwingi na watajaribu sana ili kiwango cha uchungu kiwe cha chini kabisa.

FAIDA

Huenda ukakosa kupata faida ya moja kwa moja kwa kushiriki katika utafiti huu. Utapata maelezo ya jinsi kukinga wengine dhidi ya HIV. Ikiwa utahitaji matibabu zaidi, mtafiti, mfanyikazi wa utafiti atakuelekeza kwa mhudumu wa afya katika kliniki ya CCC. Kushiriki kwako huenda kukachangia kuimarika kwa matibabu kwa wale wanaopokea atazanavir.

SABABU AMBAZO ZINAWEZA KUFANYA UTOLEWE KWA UTARATIBU WA UTAFITI HUU

Unaweza kutolewa kutoka kwa taratibu za utafiti huu bila idhini yako kwa sababu zifuatazo:

- Utafiti ukisimamishwa au kufutiliwa mbali.
- Ukishindwa kukamilisha vipimo vya utafiti.

GHARAMA KWAKO

Hakuna gharama kwako kwa kushiriki katika utafiti huu.

NAULI

Wanaoshiriki katika utafiti huu watarejeshewa shilingi mia tano za Kenya kama nauli yao ya kusafiri hadi hospitali.

USIRI

Juhudi zitafanywa kuweka maelezo yako ya kibinafsi kwa usiri. Hata hivyo, usiri kabisa hauwezi kuhakikishiwa. Maelezo yako ya kibinafsi yanaweza kufichuliwa ikiwa yatahitajika kwa sheria. Sampuli yoyote kutoka kwako au maelezo yoyote

yanayokuhusu yatatambuliwa kwa nambari spesheli wala sio kwa majina. Linganisho kati ya jina lako na hiyo nambari spesheli itawekwa mahali salama kwa kliniki pekee. Uchapishaji wowote wa utafiti huu hautatumia jina lako au kukutambua wewe mwenyewe.

Rekodi zako za utafiti huenda zikapitiwa na wafanyikazi wa utafiti na wawakilishi wa:

- Kamati ya Maadili ya Utafiti ya Hospitali ya kitaifa ya Kenyatta na Chuo Kikuu cha Nairobi.

SHIDA AU MASWALI

Ikiwa utawahi kuwa na maswali kuhusu utafiti huu, yafaa uwasiliane na George Mugendi kwa simu 020 4915029.

Ikiwa una maswali kuhusu haki zako kama mshiriki wa utafiti, yafaa uwasiliane na karani wa kamati ya maadili ya utafiti ya Hospitali ya Kitaifa ya Kenyatta na Chuo Kikuu cha Nairobi Sanduku la Posta 19676-00202, Nairobi, Nambari ya simu: 0202-272-6300 Ext 44355; barua pepe: uonknh_erc@uonbi.ac.ke

UVUMBUZI MPYA

Maelezo yoyote mapya yatakayopatikana wakati wa muda wa utafiti ambayo yanaweza athiri afya yako, hali yako na /au huenda yakabadilisha uamuzi wako wa kuwa kwa utafiti yatapeanwa kwako kwa wakati ufaao.

KAULI YA ITIKIO NA SAHIHI

Nimesoma nakala hii ya itikio au imesomwa kwangu. Nimejadili maelezo na mfanyikazi wa utafiti. Maswali yangu yamejibiwa. Nimeelewa uamuzi wangu ikiwa au sitaki kushiriki kwa utafiti huu ni kwa hiari. Nimeelewa ikiwa nitaamua kujiunga kwa utafiti, naweza kutoka wakati wowote. Kwa kuweka sahihi fomu hii, sipatiani haki zangu zozote nilizo nazo kama mshiriki wa utafiti.

Nimekubali:

i. Kushiriki utafiti wa kiumbile Ndio ☐ la ☐

ii. sampuli za damu kuwekwa kwa ajili ya utafiti wa siku zijazo.
Ndio ☐ la ☐

iii. Sampuli za damu kupelekwa katika mahabara nje ya Kenya
Ndio ☐ la ☐

iv. kuwapa wnautaafiti nambari zangu za simu ili waweze kuwasiliana nami katika muda wa utafiti. Ndio ☐ la ☐

_____ _____ _____

Jina la mshiriki (chapa) Sahihi ya mshiriki/kidole gumba Tarehe

Mimi kama mfanyikazi wa utafiti aliyetia sahihi hapa chini, nadhibitisha ya kwamba nimeeleza mwanautafiti aliyetia sahihi hapa juu mambo yote muhimu kuhusu huu utafiti. Naamini kwamba aemeelewa na amekubali kujihusisha na utafiti huu kwa hiari yake.

_____ _____ _____

Mfanyikazi wa utafiti anaye Endeleza itikio (chapa) Sahihi ya mfanyikazi wa utafiti Tarehe

_____ _____ _____

Jina la shahidi Sahihi ya shahidi Tarehe