INFLUENCE OF CYP2B6 POLYMORPHISMS ON CD4+ T CELL COUNT AND HIV RNA VIRAL LOAD CHANGES AMONG INDIVIDUALS ON NEVIRAPINE CONTAINING HIGHLY ACTIVE ANTIRETROVIRAL THERAPY

BY

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DECLARATION

I declare that this thesis is my original work and has not been presented to any other University or Institution for a degree or any other award.

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DEDICATION

To my beloved son Dylan, whose bar has now been significantly raised.

ABSTRACT

Human Immunodeficiency Virus (HIV) remains the leading cause of morbidity and mortality in Kenya with a prevalence of 6.5%. Highly active antiretroviral therapy (HAART) is used to manage the disease by increasing the number of CD4 cells and reducing the viral load concentration. Majority of these HAART based medication contain nevirapine (NVP) that is metabolized through the cytochrome P 450 (CYP450) system. The use of NVP is however, limited by sub-optimal response from patients. This poor response may be caused by genes responsible for its metabolism. Polymorphisms on CYP2B6 gene may interfere with catalytic activity of the enzyme leading to either an increase or reduction in NVP plasma concentrations. The current study aimed at determining the influence of CYP2B6 gene polymorphisms on plasma NVP concentration, CD4+ T-cell number and viral load change among HIV infected individuals on NVP-containing HAART regimen. This was a prospective study among 228 HIV infected adults attending Kenyatta National Hospital in Nairobi, Kenya. Whole blood samples were collected from the study participants at enrolment and six months post-treatment. The samples were genotyped for CYP2B6 516G>T and 983T>C mutations using real-time polymerase chain reaction (RT-PCR) technique. Pharmacokinetic analysis was done six months post treatment using tandem quadruple mass spectrometer to determine NVP plasma concentrations. The CD4 cell count and plasma viral load were analyzed at both enrolment (baseline) and six months post treatment. One way analysis of variance (ANOVA) was used to determine the relationship between the changes in CD4+ cell count, HIV-RNA viral load, and CYP2B6 genotypes The frequency of the T variant allele on the CYP2B6 516G>T polymorphism was 45.2% while the proportion of participants with GG, GT and TT genotypes were 50%, 36%, and 14% respectively. The frequency of the C variant allele on the CYP2B6 983T>C polymorphism was 38.6% while participants with TT genotypes were 61.4%. Nevirapine mean plasma concentrations were higher among homozygous participants with CYP2B6 516TT mutant at 5335.9ng/mL compared to those heterozygous 516GT (4985.5 ng/mL) and 516GG wild type (3725.8 ng/mL). Heterozygous participants with CYP2B6 983T>C genotype had higher mean nevirapine plasma concentration of 4748.9ng/mL compared to the wild-type CYP2B6 TC at 4161.5 ng/mL. There was a lower mean CD4 cell count six months post treatment among individuals with CYP2B6 516TT and CYP2B6 516GT polymorphisms compared to those with the wild-type CYP2B6 516GG. In the general linear model controlling for baseline CD4+ cell count, HAART regimen and NVP plasma level; GG genotype (wild type) and GT genotype (heterozygous mutant) predicted significant change (P = 0.002) in CD4 cell count. HIV-1 RNA plasma viral load concentration was found to be higher among those with 516GT and 516TT compared to those with 516GG. There were no significant effect on CD4+ and viral load concentration among those with 983T>C polymorphisms. This study indicates that CYP2B6 516G>T polymorphism is associated with a significant (p=0.039) reduction in viral load concentration and an increase in CD4 cell count among participants on NVP. The CYP2B6 983T>C polymorphisms were found not to have a significant effect on NVP plasma concentration, increase in CD4+ cell count and reduction in viral load concentration. It can therefore be concluded that polymorphisms on CYP2B6 genotype influence enzymatic activity that affect immunological and virological changes among patients on NVP containing therapy. The findings from this study could be adopted by HIV treatment policy makers in determining those to receive NVP as first line HAART and formulation of treatment strategies.

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LIST OF ABBREVIATIONS

3TC	Lamivudine
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
AZT	Zidovudine
CD4	Subgroup of T lymphocytes carrying CD4 antigens
CNS	Central nervous system
CRDR	Center for respiratory disease research
CYP2B6	Cytochrome P450 2B6
D4T	Stavudine
DNA	Deoxy-ribonucleic acid
EFV	Efavirenz
FDA	Food and drug administration
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
KEMRI	Kenya medical research institute
NNRTIs	Non-nucleoside/Nucleotide reverse transcriptase inhibitors
NRTIs	Nucleoside/Nucleotide reverse transcriptase inhibitors
NVP	Nevirapine
PI	Protease inhibitor
PK	Protein kinase
PMTCT	Preventing mother to child transmission
RNA	Ribonucleic acid
RT	Reverse transcriptase
SID	Subject identification number
SNP	Single nucleotide polymorphism
TB	Tuberculosis

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CHAPTER ONE: INTRODUCTION

1.1 Background Information

The treatment of HIV involves the use of highly active antiretroviral therapy (HAART) which is categorized into four major classes namely: non-nucleoside reverse transcriptase inhibitors (NNRTIs); nucleoside reverse transcriptase inhibitors (NRTIs); protease inhibitors (PIs) and entry inhibitors (EIs) (Michael 2005). Of all the NNRTIs, nevirapine is the most commonly prescribed regardless of the three combination regimen due to its efficacy, availability, low cost and use in prevention of vertical HIV-1 transmission (Penzak 2003; Capparelli 2008). The use of NVP is however limited by sub optimal response from patients. This poor response may be caused by genes responsible for its metabolism.

Nevirapine, a first line antiretroviral therapy regimen component undergoes first pass (oxidative) metabolism primarily by cytochrome P450 system (Seden 2012). The CYP-450 system is a large superfamily of proteins (enzymes) involved in the metabolism of a wide variety of both exogenous (drugs) and endogenous substances (Lamson 1999). The key CYP450 isoenzymes involved are 3A4 (CYP3A4) and 2B6 (CYP2B6) (Penzak 2007). The metabolic activity of CYP2B6 is dependent on the influence of several genetic polymorphisms, inhibitors and inducers that may contribute to variability in plasma drug exposure (Zanger 2008). Polymorphisms on CYP2B6 have been found to be prevalent in African populations (Klein 2005). The major single nucleotide polymorphism (SNP) on CYP2B6 are 516G>T (rs3745274) and CYP2B6 983T>C (rs28399499). The SNPs have been associated with a significant reduction or increase in enzyme catalytic activity in the liver (Jinno 2003; Xie 2003). Specifically for the CYP2B6 516 G>T polymorphisms, the GG, GT and TT variant alleles are considered as extensive, intermediate and slow metabolizers respectively (Penzak 2007). For the CYP2B6 983 T>C polymorphisms, the TT, TC and CC variant alleles are considered extensive, intermediate and slow metabolizers respectively (Penzak 2007; Oluka 2012). The polymorphism at 516 G>T slightly reduces hepatic protein activity and expression and occurs mainly among African populations compared to Caucasian and Asian populations (Klein 2005). Cytochrome P450 2B6 983T>C (rs28399499) which is considered a null allele (Wyen 2008), is not found

among Caucasians, however it has a 4%–11% prevalence in the African populations (Kwara 2009).

This genetic changes has been reported to influence nevirapine pharmacokinetics during chronic treatment (Rotger 2005). Previous studies have shown the importance of CYP2B6 genetic polymorphisms in African populations and people of African origin in western countries (Mehlotra 2007; Klein 2013). This is because CYP2B6 516G>T single nucleotide polymorphisms which occurs more frequently in Africans compared to Caucasian and Asian populations; have been shown to interfere with hepatic CYP2B6 protein expression and activity (Lang 2001). However, there are limited studies on the Kenyan population which is ethnically diverse. A recent Kenyan study by Oluka et al 2015 only focused on CYP2B6 polymorphisms among the female yet Nevirapine is a widely used antiretroviral drug by HIV infected patients in Kenya irrespective of gender. Furthermore, the study was based in Mombasa where the population is not as heterogeneous as Nairobi where the current study was conducted.

Nevirapine plasma concentrations that are within the recommended ranges are used as indicators of treatment efficacy. Plasma concentrations that are sub-therapeutic are associated with treatment failure and the emergence of antiretroviral drug resistance, whereas supra-therapeutic concentrations are associated with toxicity (Kappelhoff 2005). A previous study has evaluated the associations between CYP2B6 516G>T and 983T>C SNPs and Nevirapine plasma concentration (Haas 2009). Studies have confirmed that both SNPs are more frequent in patients of African origin due to ethnic diversity (Klein 2005; Mehlotra 2007). Furthermore, these SNPs have been shown to influence Nevirapine exposure by decreasing Nevirapine clearance hence leading to higher NVP plasma concentrations and drug toxicity (Mahungu 2009). However, there is paucity of data on the influence of 516 G>T and 983 T>C polymorphisms in the CYP2B6 encoding gene on steady state Nevirapine plasma concentration in the Kenyan ethnically diverse population. This study therefore sought to assess the influence of Cytochrome P450-2B6 (CYP2B6) polymorphisms on CD4 T cell and HIV RNA viral load changes among HIV positive individuals on NVP-containing HAART regimen in Kenyatta National Hospital, Nairobi, Kenya

Immunological and virological parameters are often used to assess patient prognosis before initiating them on nevirapine containing antiretroviral regimen (Barlett 2005). The main immunological markers used are cluster of differentiation; (CD4+) T-helper cells and viral load levels for virological response (Autran 1997). At the onset of treatment, patients present with low CD4 levels and elevated viral load concentration. Nevirapine in conjunction with other antiretroviral drugs are expected to elevate the immune levels (CD4+) and reduce on the viral load of the patients. Currently, there are limited studies comparing the influence of CYP2B6 polymorphisms on viral load and CD4+ counts (Oluka 2015). This study therefore aimed to close this gap by comparing CD4+ and viral load concentration changes with CYP2B6 polymorphic genotypic variants.

1.2 Statement of the Problem

Human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS), remains a global health problem of unprecedented dimensions, with an estimated 33.4 million people living with the condition. The incidence of the pandemic is estimated to be 2.7 million annually with a global mortality of 2 million. To reduce the mortality rates of HIV and disease progression to AIDS, the world health organization recommends the use of highly active antiretroviral therapy (HAART). Highly active antiretroviral therapy consists of three combination therapy regimen of an NNRTI and two NRTIs for first-line and an NNRTI, NRTI and Protease inhibitor (PI) for second line treatment. The most commonly used NNRTI in the first line regimen (where most HIV infected participants belong) is NVP. Nevirapine-based antiretroviral combinations are used in resource-limited settings due to their relatively low cost, manageable pill burden and excellent efficacy. The metabolism of NVP is done by the cytochrome P4502B6 enzymes which metabolize it into two major metabolites, namely: 2-hydroxynevirapine and 3-hydroxynevirapine. Single nucleotide polymorphisms (SNPs) in exon 4 (516 G>T) and in exon 7 (983 T>C) are associated with a significant interference in CYP2B6 catalytic activity. Both SNPs have been shown to be more frequent in people of African origin and to be associated with decreased NVP clearance and higher plasma concentration. Despite this fact, limited studies have been done in Kenya to assess the effect of CYP2B6 polymorphisms on plasma NVP concentration. There is also limited data on the effect of CYP2B6 polymorphisms at 516GT and 983TC on immunological and virological response. This

study therefore aims to determine the effect of CYP2B6 polymorphisms on nevirapine plasma concentration among HIV infected individuals.

1.3 Significance of the Study

Human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) pandemic remains a major cause of disease burden in both economically developed and developing countries. To control disease spread and reduce mortality rates, the use of HAART remains the only existing working solution. Nevirapine based HAART are commonly used as the first line regimen for HIV infected individuals. To estimate the concentration of NVP in patients, its plasma concentration assay is used as an indicator of adequate drug bioavailability in the body. The concentrations should be maintained within the optimal levels to avoid drug resistance associated with sub-therapeutic concentrations or toxicity that arise from supra-therapeutic concentrations. This study identified the effect of CYP2B6 gene polymorphisms on Nevirapine plasma concentrations and its relationship to an individual's CD4+ T cell level and HIV RNA viral load concentration.

The findings of the study could be adopted by HIV treatment policy makers in determining which patients could receive NVP as a first line medication and those who should be given alternative HAART combination. The findings may be adopted in personalised medication strategies. With this adoption, there will be a significant reduction in medication wastage often associated with drug reactions, toxicity and resistance.

1.4 Objectives

1.4.1 Broad objective

To assess the influence of CYP2B6 polymorphisms on CD4+ T cell and HIV RNA viral load concentration changes among individuals on nevirapine containing HAART regimen at Kenyatta National Hospital, Nairobi, Kenya.

1.4.2 Specific objectives

1. To determine the CYP2B6 516G>T and 983T>C genotype frequencies among HIV infected individuals at Kenyatta National Hospital, Nairobi, Kenya.

2. To determine the difference in Nevirapine Plasma concentration between CYP2B6 516G>T and 983T>C polymorphisms among HIV infected individuals on nevirapine-containing HAART at Kenyatta National Hospital, Nairobi, Kenya.

3. To determine the influence of CYP2B6 516G>T and 983T>C polymorphisms on initial viral load and CD4 cell count changes among HIV positive individuals on nevirapine containing HAART at Kenyatta National Hospital, Nairobi, Kenya.

1.5 Research Questions

1. What are the genotype frequencies of CYP2B6 516G>T and 983T>C polymorphisms among HIV infected individuals attending care at Kenyatta National Hospital, Nairobi, Kenya.

2. What is the difference in Nevirapine Plasma concentration between CYP2B6 516G>T and 983T>C polymorphisms among HIV infected individuals on nevirapine-containing HAART at Kenyatta National Hospital, Nairobi, Kenya.

3. What is the influence of CYP2B6 516G>T and 983T>C polymorphisms on initial viral load and CD4+ cell count changes among HIV positive individuals on nevirapine containing HAART at Kenyatta National Hospital, Nairobi, Kenya.

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview

Human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) pandemic remains a major cause of mortality globally (WHO 2012). The problem is further compounded in sub-Saharan African countries such as Kenya (UNGASS 2008). In the United States of America, incidence of HIV and AIDS was 49,273 in the year 2011 (CDC 2012). The number of deaths due to HIV and AIDS was 6,966 with a mortality rate of 2.2 per 100,000 deaths (CDC 2012). The prevalence is higher among the black African American population compared to their white counterparts (CDC 2012). The prevalence of HIV and AIDS is higher in Latin America and South Asia compared to the Northern America at 1.4% and 1.2% respectively (WHO 2012). Sub Saharan Africa not only bears the highest morbidity and mortality rates of HIV and AIDS but also the disease burden. Approximately 2.2 million people in Kenya are living with HIV and AIDS and 6.5% of all adults are infected with HIV, with the number of new infections still remaining high (KAIS 2015). The Government of Kenya started offering ART in public sector since 2003. Nevirapine is a key component of first regimen in developing countries being affordable and with manageable pill burden (Penzak 2003).

Nevirapine pharmacokinetics have a large inter-individual variability that contributes to variation in outcomes among HIV-infected individuals (Schipani 2011). Lower than therapeutic plasma concentrations are associated with treatment failure and the development of drug resistance. On the other hand, concentrations above therapeutic levels may lead to toxicity (Penzak 2007). Immunoallergic reactions are believed to be behind hepatic injury for individuals on Nevirapine treatment (Bruck 2008). Hepatic injury is more common among women than men and in patients with higher CD4 T cell counts (Bruck 2008). However, not all cases of nevirapine hepatotoxicity may be immunoallergic in origin, particularly those with a longer latency period to onset (Crommentuyn 2005). Nevirapine has extensive hepatic metabolism and is a substrate for CYP 2B6 and 3A4 and a potent inducer of these enzymes, features that favor potential production of a toxic intermediate that might cause liver injury (Chen 2010).

Cytochrome P450 2B6 is primarily expressed in the liver where its contribution to the total microsomal P450 pool has been estimated to be within a range of about 1–10%, with a large inter-individual variability at protein level of roughly 100-fold (Ward 2003; Gounden 2010). Although some earlier studies reported expression in only a fraction of human livers (Klein 2013) recent studies found CYP2B6 to be present in all investigated human adult liver samples (Hofmann 2008). These studies found up to one-third of paediatric samples contained no detectable CYP2B6 enzyme proteins (Klein 2013).

The cytochrome P450 (CYP) enzyme CYP2B6 is one of about a dozen human CYPs that are primarily involved in the biotransformation of drugs and other xenobiotics (Erickson 1999). The CYP2B6 gene and its closely related pseudogene - CYP2B7 - are located in a tandem head-to-tail arrangement within a large CYP2 gene cluster on the long arm of chromosome 19 (cytochrome P450, family 2, subfamily B, polypeptide 6) (Klein 2013).

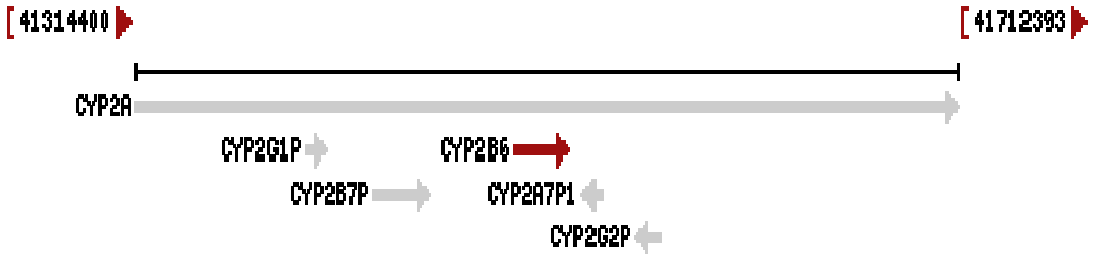

Figure 1.1: CYP2 Super-family (knowledgebase 2014)

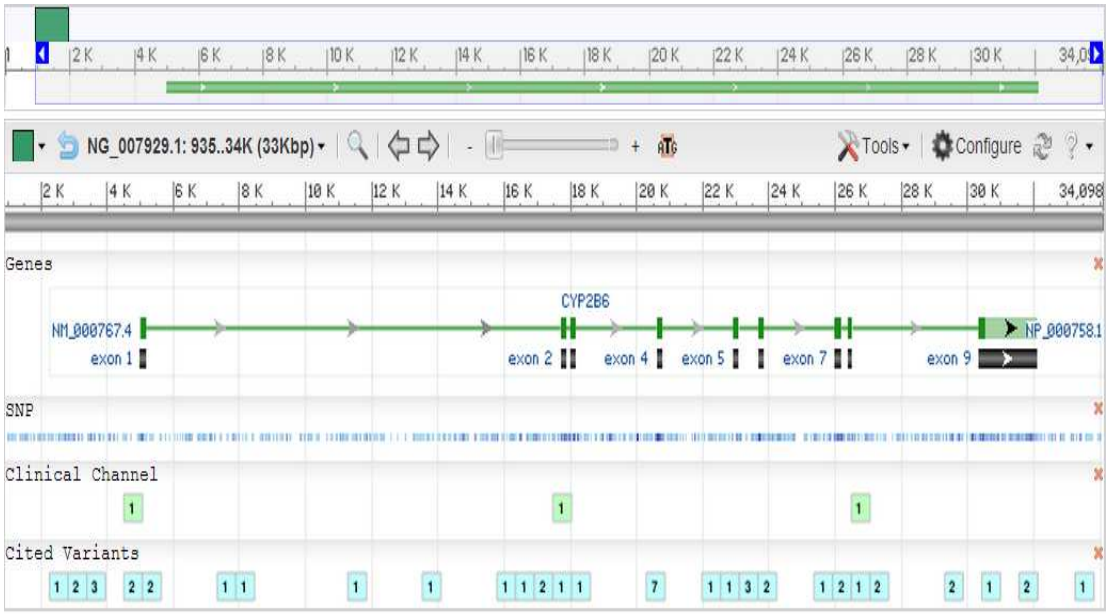

Figure 1.2: CYP2B6 Polymorphisms (knowledgebase 2014)

2.2 Cytochrome P450 2B6 (CYP2B6) Genotype Frequencies

More than 30 amino acid-changing single-nucleotide polymorphisms occur in different combinations and together with additional non-coding variants and many more SNPs not yet assigned to particular haplotypes. There are currently 37 distinct star-alleles; gene haplotypes with a distinct variant amino acid sequence or with demonstrated functional effect (Karolinska Institute 2013). The most common SNPs include, 516G>T (exon 4). A number of studies have been done to examine the allelic frequencies and association of CYP2B6-G516T gene polymorphisms with Nevirapine pharmacokinetics (Rotger 2005). In a Ugandan study the prevalence the CYP2B6 GT genotype at position 516 was 26% (Penzak 2007). A study by Ribaudo (2010), shows CYP2B6 516G>T allelic freguencies of; 24.5%, 34.2% and 32.2% among white, black and Hispanic participants respectively. The worldwide variations in SNP frequencies have been reviewed recently (Klein 2013) as shown in Table 2.1 below.

Table 2.1: CYP2B6 variants in terms of frequency and functional impact and summaries

CYP allele designation[a]	Key mutation(s)[b] rs number	Location, protein effect	Allele frequencies[c]	Functional effect
*CYP2B6*4*	g.18053(c.516) A>G rs2279343	K262R (isolated)	0.00 AA, Af 0.04 Ca 0.05–0.12 As	↑ Expression, moderate substrate-dependent effects
*CYP2B6*5*	g.25505(c.1459) C>T rs3211371	R487C	0.01–0.04AA, Af 0.09–0.12 Ca 0.05–0.12 Hs 0.01–0.04 As	↓ Expression, in part compensated by ↑ specific activity
*CYP2B6*6*	g.15631(c.516) G>T rs3745274 and g.18053(c.785)A>G rs2279343	Q172HK262R	0.33–0.5 AA, Af 0.10–0.21 As 0.14–0.27 Ca 0.62 PNG	↓ Expression; ↓activity with efavirenz *in vivo*; some other substrates show ↑ activity
*CYP2B6*18*	g.21011(c.983)T>C rs28399499	I328T	0.04–0.08 AA 0.05–0.12, Af 0.01 HS 0.00 As, Ca, PNG	↓ Expression and activity
*CYP2B6*22*	g.-82T>C rs34223104	promoter (TATA-box)	0.00–0.025 AA, Af, As 0.024 Ca, Hs	↑ Expression and activity ↑ Inducibility *in vitro*

[a] *According to CYPallele nomenclature homepage http://www.cypalleles.ki.se.*
[b] *Genomic (g.) and cDNA (c.) positions are given in bp.*
[c] *Selected frequencies of individual ethnicities (AA, African American; Af African; As Asian; Ca Caucasian; Hs, Hispanic; PNG, Papua New Guineans) compiled from dbSNP http://www.ncbi.nlm.nih.gov/SNP and from the literature cited in the text.*

A number of studies have been conducted around the world to determine the prevalence of CYP2B6 polymorphisms and their effect on human health (Sanchez 2011). In a West African study, polymerase chain reactions (PCR) were performed on genomic DNA preparations from participants of six different populations to amplify CYP2B6 exons 1, 4, 5, and 9. The most common SNPs of CYP2B6 (C64T [exon 1], G516T [exon 4], C777A, A785G [exon5], and C1459T [exon 9]) were analyzed by a post-PCR ligation detection reaction-fluorescent microsphere assay (LDR-FMA) (Klein 2005). The frequency of CYP2B6*1A was highest (64%) in Asian-Americans and lowest (33%) in Papua New Guineans. In the study, variant alleles *2 (3%–7%),*4 (2%–6%), *7 (1%–3%), and *9 (1%–8%) were present in four or five (out of six) populations (Klein 2005; Rajeev 2007). Variant allele CYP2B6*3 was not observed in any of the study participants. In other previous studies, the frequency of this variant allele was 0.5% in a Caucasian population (Rajeev 2007), therefore CYP2B6*3 is considered a rare allele.

In all populations analysed in the West African study, alleles CYP2B6 *5 and *6 were found. The variant allele which had the highest prevalence in all the West African populations is CYP2B6*6 at 51.6%. The observed frequencies of this allele ranged from 23% in Asian-Americans to 62% in Papua New Guineans. The frequency of CYP2B6*6 in Papua New Guineans was significantly higher than that in any other population. Previously, CYP2B6*6 was reported as the most frequent variant allele in Caucasian (26%) and Japanese (16%) populations (Klein 2005). When West African samples were analysed, the frequency of CYP2B6*1A ranged from 30% (Senegal) to 50% (Ivory Coast). Alleles CYP2B6*3, *4, and *7 were not observed in any of the samples from Africa (Mehlotra 2007). Allele CYP2B6*5 (2%–4%) was present in three countries (Guinea, Ivory Coast, and Sierra Leone) out of five populations. Alleles CYP2B6*6 and *9 (2%–11%) were found in all the populations (Mehlotra 2007). As seen for other populations, CYP2B6*6 was the most prevalent variant allele, with frequencies ranging from 36% (Sierra Leone) to 60% (Senegal) (Klein 2005). From this description, there is no current prevalence data of CYP2B6 polymorphisms in a Kenyan population. This study therefore sought to determine the prevalence of CYP2B6 516 GT and 983 TC polymorphisms among participants at KNH which is a national health referral facility in Kenya.

2.3 CYP2B6 Polymorphisms on Antiretroviral Therapy

The development and refinement of HAART during the last 10 years has dramatically prolonged the survival of HIV-infected individuals (Bhaskaran 2008). In comparison with earlier combination regimens, current options are associated with greater viral suppression and lower discontinuation rates due to improved convenience and tolerability (Ahoua 2009). Nevirapine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) that is potent *in vitro* and *in vivo* against replication of HIV-1 (Saitoh 2007) The drug binds to the viral enzyme and disrupts the enzyme's catalytic site (Saitoh 2007). Resistance to nevirapine develops rapidly when the drug is administered in suboptimal regimens, and the emergence of highly drug-resistant virus has been observed 4 weeks after initiation of therapy (Saitoh 2007). There are strong indications that the virological effect of protease inhibitors is associated with the level of pharmacological exposure (Calcagno 2012).

There is considerable inter-individual variability in the disposition of NVP, at least some of which is caused by polymorphisms in drug-metabolism genes. Nevirapine (NVP) is metabolized primarily by cytochrome (CYP) P450 2B6 (Melissa 2012). Cytochrome P450 2B6 (CYP2B6) variation may predict pharmacokinetic characteristics of its substrate (Gounden 2010). Genetic variation in pathways of drug metabolism contributes to variability in pharmacokinetic parameters of drug levels and clearance in plasma or cells over time such as half life (Zanger 2008; Melissa 2012).

2.2.1 Pharmacokinetics of Nevirapine

2.2.1.1 Absorption of Nevirapine

In clinical studies, NVP is readily (>90%) absorbed after oral administration in healthy volunteers and in participants with HIV infection (Lamson 1995a). After a single 200mg dose, plasma NVP concentrations reach a maximum of 2µg/ml by 4 hour post-dose and decline log linearly thereafter, resulting in a terminal phase half-life of ~45 hour; steady state plasma concentrations of NVP could be higher (Schipani 2011). Nevirapine is an inducer of CYP metabolism, thereby auto inducing its own metabolism and reducing its half-life from 45 hours to 30 hours after two weeks of dosing with 200 mg per day compared with a single oral dose of 400 mg (Lamson 1995b)

2.2.1.2 Biotransformation of Nevirapine

Nevirapine (NVP) is extensively biotransformed via oxidative metabolism by the CYP pathway to form several hydroxylated metabolites *in vivo* and *in vitro*. *In vitro* studies with human liver microsomes suggest that oxidative metabolism of NVP is mediated primarily by CYP3A and CYP2B6 enzymes. Hence, substances that induce or inhibit the CYP enzyme system could have a profound effect on the metabolism of NVP, by decreasing or increasing blood levels of NVP respectively. For example, rifampicin and corticosteroids are inducers and would decrease blood levels while fluconazole is an inhibitor of the CYP enzyme system and could thus increase plasma levels of NVP during concurrent administration (Madan 2003). Nevirapine biotransformation involves extensive hydroxylation and glucuronidation of hydroxylated metabolites. The metabolites are then largely excreted into the urine, where 2-hydroxy, 3-

hydroxy and 12-hydroxy NVP glucuronides account for 68% of the total metabolites. Thus, CYP metabolism, glucuronide conjugation and urinary excretion of glucuronidated metabolites represent the primary route of NVP biotransformation and elimination in humans (Riska 1999). Formation of 2- and 12-hydroxy NVP is mediated by CYP3A4/5, while that of 3- and 8-hydroxy NVP by CYP2B6.

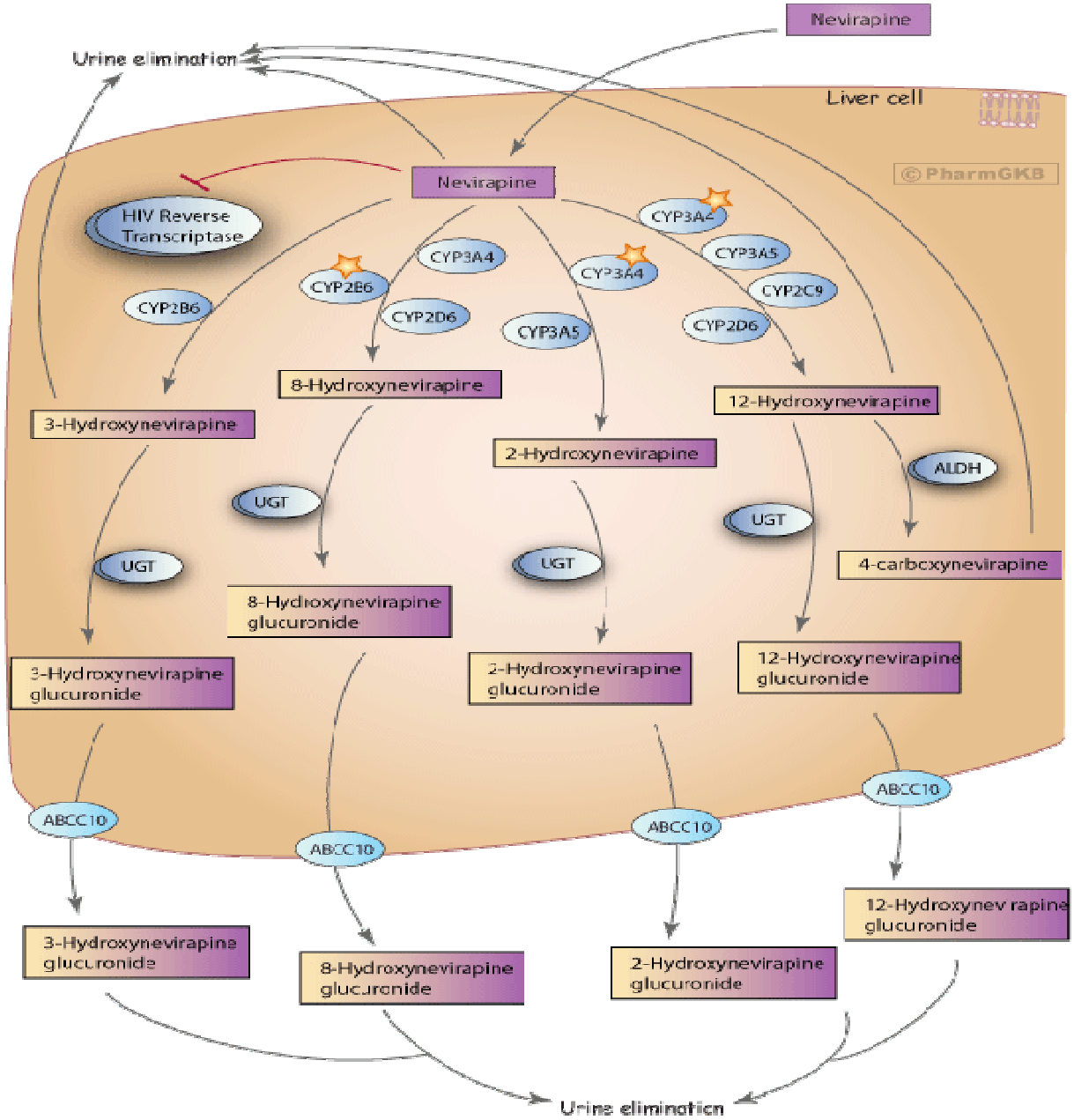

Figure 2.1: CYP2B6 Polymorphisms (knowledgebase 2014)

2.2.2 Influence of CYP2B6 Polymorphisms on Nevirapine Plasma Concentration

Variable responses characterize drug treatment in HIV disease, in terms of both efficacy and toxicity (Owen 2005). Both genetic and environmental factors are considered determinants of this variability; although the relative contributions are unclear and likely to vary with different drugs (Owen 2005). The use of NVP is limited by a potentially fatal, immune-mediated hypersensitivity reaction which can manifest as hepatotoxicity, fever and/or rash (Mahungu 2009). A fragile barrier to the development of drug resistance is also a point of great concern. Among factors that can influence nevirapine exposure is the gene that encodes the cytochrome P450 (CYP) 2B6 enzyme (Penzak 2007). The CYP2B6 isoform, which plays a major role in nevirapine metabolism, is characterized by marked interindividual variability in expression and activity as a result of presence of genetic polymorphisms (Penzak 2007). A few recent studies have investigated the influence of genetic polymorphisms on NVP pharmacokinetics (Kappelhoff 2005). Single nucleotide polymorphisms (SNPs) and haplotype organization of CYP2B6 in Caucasians have been demonstrated to have a reduced hepatic CYP2B6 protein expression and activity in carriers of the G516T (rs3745274) and T1459C (rs3211371) polymorphisms (Lang 2001). However, the association with protein expression and activity was not statistically significant for G516T, although the authors acknowledged the limitation of the sample size (Kappelhoff 2005). Participants with the T516 polymorphism were observed to have a 1.7 fold increase in plasma concentration of NVP compared with G516 participants (Rotger 2005). Similar findings from Uganda (Penzak 2007) and India (Ramachandran 2009) confirmed the prominent role of CYP2B6 in NVP elimination; however the difference in blood levels between participants with the homozygous mutant and wild forms of the allele were less pronounced than in the case of efivarenz (EFV). Moreover, another study indicated that EFV dose reduction according to G516T genotype was a feasible strategy in ensuring optimal drug exposure and prevents toxicity (Saitoh 2007). Recently, the T983C SNP (rs28399499) was described in Black populations. This polymorphism results in an amino acid change in the CYP2B6 protein (Ile328Thr), and heterozygosity has been shown to impact upon EFV pharmacokinetics (Haas 2005). Furthermore, a laboratory-based assessment of this polymorphism indicated that it may represent a null allele (Rajeev 2007). Investigation of the

983 T>C polymorphism in various populations has shown that the allele is absent in Caucasian populations, yet its frequency is as high as 7.5% in African-Americans and Ghanaians (Rajeev 2007) . However, to date, no homozygotes for this polymorphism have been described. The aim of this study was to investigate the frequency of CYP2B6 polymorphisms (516G>T and 983T>C and the influence of heterozygosity and homozygosity for these polymorphisms on plasma concentrations of NVP, CD4+ cell count and initial viral load changes in a cohort of HIV positive participants. In addition, the association with sociodemographic characteristics, glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) was also determined.

Several factors can influence NVP exposure, including gender, concomitant drugs and genetic polymorphisms in the gene encoding for the cytochrome P450 2B6 enzyme (CYP2B6),which is involved in the drug's main metabolic pathway (Calcagno 2012). Single nucleotide polymorphisms (SNPs) in exon 4(516 G> T) and in exon 7 (983 T > C) are associated with a significant reduction in CYP2B6 catalytic activity (Rotger 2007). Both SNPs have been shown to be more frequent in participants of African origin and to be associated with decreased NVP clearance and higher plasma C_{trough}. Other CYP polymorphisms such as CYP2B6 C1459T have not been shown to have much impact on NVP pharmacokinetics (Saitoh 2007; Bakshi 2008). The CYP2B6 785A>G (rs2279343) genetic polymorphism, frequent in Caucasians, has been associated *in vitro* with a lower protein expression (Jinno 2003). The importance of this variant has not yet been studied in patients on nevirapine containing therapy. This association between *CYP2B6* SNPs and NVP plasma concentrations has been confirmed in several middle and low-income countries; these findings suggest a possible reason for the observed higher exposure obtained in non-Caucasian participants (Jinno 2003).

Despite the widespread use of nevirapine in the developing world, the influence of CYP2B6 genotype on NVP exposure has not been investigated in Kenyans. Overall, findings suggest that pharmacogenetics has a potential role as a useful tool in the management of HIV-infected participants and could help design regimen with maximum effectiveness. However, the association between these genetic polymorphisms and pharmacokinetics and pharmacodynamics of NVP have been less frequently studied and in less homogenous populations than that of EFV. The findings from one population may not be generalized to other populations due to the ethnic

differences in drug effect and body weight of the participants. This study therefore sought to determine the difference in NVP plasma concentration between CYP2B6 516 GT and 983 TC polymorphisms of HIV infected individuals on NVP containing HAART in Kenya

2.4 Influence of CYP2B6 Polymorphisms on Viral Load and CD4 Cell Count Changes

The HIV-1 infection is marked by a progressive decline in the number of circulating CD4 T helper cells, which over a period of years leads to death from immune failure and opportunistic infection (Haynes 1996). Although its clinical course is known to vary considerably from patient to patient, with progression to AIDS taking anywhere from 2 to 10 or more years, the reason for this variability remains unclear. In addressing this question, several studies have reported a link between fast progression and high viral load in the blood (Ahoua 2009). Substantially of HAART reduces viral load. Antiretroviral Therapy results in an increase in the number of CD4 cells and the functional reconstitution of the immune system (Haynes 1996; Autran 1997). After initiation of HAART, peripheral CD4 cell count starts rising, continuing for at least 3-5 years (Manuel 2006). The initial increase in CD4 cell count is very rapid and is usually in the first 3-6 months (Renaud-Thery 2007). This initial increase relies on a reduction in T-cell activation and primarily consists of a release of memory CD4 cells trapped in the lymphoid tissue (Autran 1997)

Virological response is defined as a reduction in plasma HIV RNA of at least 2 log10 after 3 months from the beginning of treatment, or an undetectable HIV-RNA load at 6 months in the absence of viral rebound after suppression (Andrea 2001). Participants not achieving an undetectable viral load at the visit of first reported NNRTI regimen use (in the previous six months) or subsequent visit were classified as “non-responders”. This definition conforms to clinical guidelines for treatment with HAART regimen (Barlett 2005). Immunological response was be defined as an increase, at the last measurement, of the CD4 cell count of more than 30% compared with the baseline value (Andrea 2001)

Influence of CYP2B6-G516T genotype on clinical responses has been evaluated in only a few studies (Ribaudo 2006). According to Ribaudo no association was found between 516/983 slow metabolizing genotypes and virological failure in white or Hispanic participants (Ribaudo 2009). This study however recommended replication in other cohorts to validate or dispute their

findings (Ribaudo 2006). According to Saitoh, a better response was seen in children with CYP2B6 516-T/T genotype compared to those with the CYP2B6 516-G/G or G/T genotype (Saitoh 2007). The study was however limited by the fact it was a retrospective study with a relatively small number of participants (Saitoh 2007). An association between metabolizer phenotype and elevated viral load defined as two consecutive HIV viral load measures ≥200 copies/ml, in African-Americans has been suggested (Ribaudo 2009).

The relationship between NVP plasma concentrations and virological response has not been clearly established. Some investigations have found a relationship between nevirapine exposure and virological response, while others have not (Crommentuyn 2005; Duong 2005). Owing to the existence of extensive genetic polymorphism as well as strong inhibitors and inducers, the activity of CYP2B6 enzyme is highly variable in the population. For some clinically used drugs including antiretroviral agents: EFV and NVP, CYP2B6 single nucleotide polymorphisms have been shown to be useful predictors of pharmacokinetics and drug response (Kwara 2009). Current data however, indicate that pharmacogenetic mechanisms are complex and appear on several levels of gene expression from the initial mRNA transcript to splice variants (pre-mRNA splicing and mRNA expression) to altered proteins. They affect function in various ways including substrate-dependent and substrate-independent manner (Sadee 2011). Higher plasma levels of Nevirapine have been reported to be associated with improved virological response and reduced selection of resistant mutations (Penzak 2007).

2.5 Study Limitations

Although the influence of CYP2B6 516 G>T and 983 T>C genotypes were analyzed in this study, the influence of other transporter and binding cassettes such as ATP- Binding Cassette (ABC) were not. This was due to financial constraints.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study Setting

The present study was conducted at Kenyatta National Hospital (KNH) centre for respiratory Disease Research (CRDR) KEMRI in Nairobi, Kenya. Nairobi County lies between latitudes 1.3010° South and 36.8072° East of the equator and has a population of about 3.182 million (KNBS 2009). Kenyatta national hospital (KNH) is the largest referral facility in Kenya offering both clinical and basic research support facilities. It houses Kenya Medical Research Institute (KEMRI) which has the centre for respiratory disease research (CRDR). This centre offers comprehensive HIV care to about 5,000 HIV positive individuals of diverse ethnic background from across the country. It was selected due to its ethnic diversity.

Figure 3.1: Map of Nairobi

3.2 Study Design

This was a prospective study among HIV infected adults on first line NVP containing HAART sampled at enrolment and six months post treatment. A data collection tool (Appendix 1) was used to collect socio-demographic, clinical, CD4+ T cell count and HIV RNA viral load concentration from participants at enrolment. After six months of antiretroviral treatment only CD4+ T cell count and HIV RNA viral load concentration data was collected.

3.3 Study Population

HIV infected adults beginning first line NVP containing HAART attending KNH/ KEMRI/ CRDR program.

3.4 Eligibility Criteria

3.4.1 Inclusion Criteria

1. HIV and AIDS infected adults aged between 18-55 years attending CRDR, HIV treatment program.
2. Participants who were willing to voluntarily give a written informed consent prior to participating in the study and also able to disclose any information that was required during the study period

3.4.2 Exclusion Criteria

1. Pregnant women or those who were intending to become pregnant during the first 24 weeks of the study.

3.5 Sample Size

The sample size was determined using the Fisher's formulae below: (Lessard 2007)

$$n_0 = \frac{z^2 pq}{e^2}$$

Where

n= calculated sample size

z=1.96 i.e. 95% confidence interval.

e=margin of error or precision of the estimate in this case e=0.05.

P= the estimate value of the proportion. In this study, p was the prevalence of individuals who experienced both immunological and virological failure in Uganda (which is almost identical to the Kenyan population) during a 12 month ARV use (P=0.16 referring to a prevalence of 16%) (Ahoua 2009).

q=1-p; where q=

$$n=\frac{1.96^2 \times 0.16 \times 0.84}{0.05^2}$$

=206.52 ~207 participants.

The minimum sample size obtained from the formula was 207: However 21(10%) more participants were added to take care of non respondents and those who would be lost to follow-up. A target sample size of 228 participants on NVP containing regimen were selected as the study participants.

3.6 Sampling Procedure

Systematic random sampling technique was used to sample every second patient enrolled into the KEMRI CRDR HIV program. This sampling interval was arrived at by dividing the minimum sample size with five hundred (the anticipated new enrollment in one year).

500/207 = 2.4.

The first sample was for the initial patient who walked into the clinic and consented to participate into the study. Every second patient (patient 3, 5, 7…..) after the first enrolled participant was recruited into the study.

3.7 Participants enrolment flow chart

A total of two hundred and twenty eight (228) participants attending FACES program at the centre for respiratory Disease Research (CRDR) KEMRI-Nairobi, on Nevirapine-based regimen were enrolled at the beginning of the study. However on the subsequent sixth month visit, eight (8) patient records had incomplete clinical data, six (6) participants were lost to follow-up while seven (7) had developed toxicity and had their HAART regimen changed. Therefore, a total of twenty one (21) participants were discontinued from the study leaving two hundred and seven (207) participants for final statistical analysis as shown in Figure 4.1

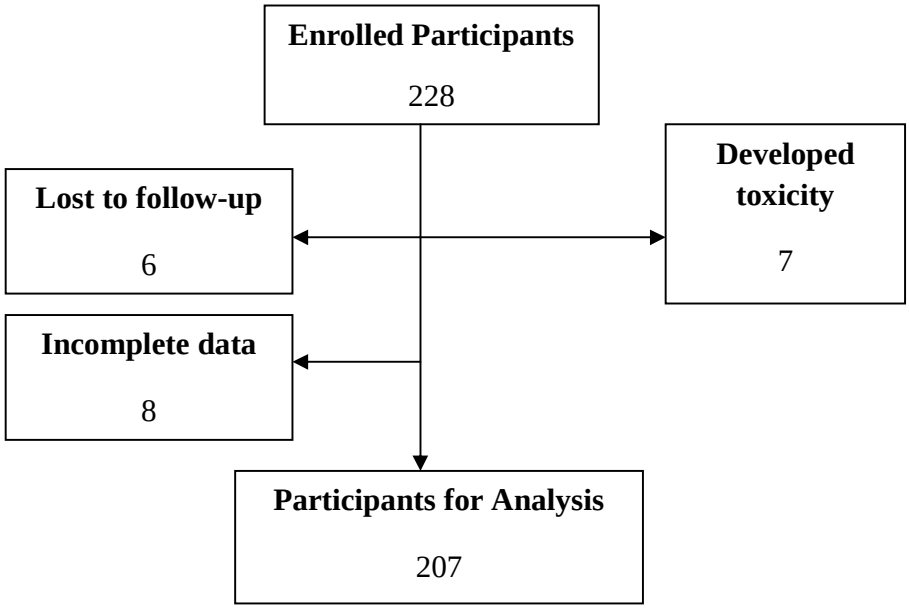

Figure 3.2: Participants enrolment flow chart

3.7 Laboratory Procedures

3.7.1 Sample Collection

About 2.5 ml whole blood was collected from each study participant by a single draw veni-puncture at 8-10 hours after the last dose of nevirapine. About 0.5 ml of the blood was used for CD4 cell count determination and another 1 ml for CYP2B6 polymorphism studies. The

remaining 1 ml of blood was centrifuged at 10,000g for 10 minutes into plasma which was used for virological and biochemical studies.

3.7.2 CD4 T Cell Measurements

The blood cells are stained with fluorescent labelled entibodies that bind specifically to leucocyte surface antigens. During acquisition, the stained cells are introduced to the lesser beam at the flow cell chamber by hydrodynamic pressure created by sheath fluid. The light scattered by individual cells and fluorochrome provide information about the cell size, internal complexity or granularity and relative fluorescence intensity. The scattered fluorescence light signals emitted by cells are picked up by detectors that convertys them proportionally to electron signal. The forward scatter (FSC) correlates with the cell volume while the side scatter (SSC) depends on the inner complexity of the particle (shape of the nucleus, the amount and type of cytoplasmic granules or the membrane roughness). The fluorescence emitted by the cell depends upon the fluorescence tagged specific monoclonal antibodies against the cell surface markers (CD4 PE). The data collected on each cell or event are stored in the computer and is then processed and analyzed to provide information about the cell populations within the sample.

3.7.3 HIV Viral Load Testing

The HIV-1 viral load concentration was determined using the Abbott m2000SP System with an automated sample extraction, amplification and detection system (Abbott Molecular Inc., Illinois, U.S.A). The RNA was extracted from 0.2 mL plasma samples using the 0.2 mL plasma RNA extraction and master mix addition protocol of the Abbott m2000SP sample preparation system. The master mix containing the viral RNA was then transferred to the Abbott m2000rt instrument (Abbott Molecular Inc., Illinois, USA) for viral load detection using the program for 0.2 mL RNA amplification. The RNA was then reverse transcribed into cDNA and amplified using HIV-1 and internal control primers. Real time PCR technology used in the Abbott real time (RT) detection system uses two probes: a fluorescent-tagged longer fragment complementary to the target sequence and a quencher molecule bound onto a shorter fragment. Fluorescence emission of the HIV-1 probe will be proportional to the amount of HIV-1 target sequence in the sample. The fluorescence counts were then converted into viral loads by the analyzer. The lower detection limit of viral load quantification was 40 (2.18 $\log_{10}$) copies/mL of plasma.

3.7.4 Determination of Nevirapine Plasma Levels

The nevirapine (NVP) steady-state plasma levels was determined by a validated bench top, tandem quadruple mass spectrometer designed for ultra-high performance LC/MS Waters ® Xevo™ TQ (Waters Corporation, U.S.A). This involved first inactivation of HIV virus. Briefly into a 1.5ml Eppendorf tube 50ml of plasma and 5ml internal standard (Cnev = 0.005mg/ml, Cboce, tela, rib = 0.02mg/ml, Ctvr = 0.05mg/mL) was added. The mixture was heated at 65^0C for 10min followed by a 10 minutes cooling at room temperature. A 100ml cold methanol (-20^0C) was then added to each samples and kept at -20^0C for 10 minutes. This was followed by 8 minute centrifugation at 20,000g (20^0C) to collect the supernatant in a clean 1.5ml tube. To the collected sample supernatant 850ml ammonium acetate buffer (pH = 3.00) was added and centrifuged for 2 minutes. The sample was then considered safe. The Bond Elut C18 cartridges were prepared and placed onto the Visiprep Vacuum Manifold with Standard Lid. This system has unique flow control valves that allow easy control of flow through each SPE tube. Reusable stainless steel needle directs the sample into the glass basin below. The Bond Elute C18 150 × 4.6 mm, 5-µm column was conditioned by first passing through 1 ml methanol followed by 1 ml ultrapure water. Each column was then charged with 150ml samples containing 850ml ammonium acetate buffer (pH = 3.00) followed by two cleaning cycles using 1 mL ultrapure water. The first cleaning was collected into a clean separate tube while the second water cleaning collected in the waste tubes. The columns were vacuum dried (5-10 kpa in Hg). Nevirapine elution at a flow rate of 1 ml/min was then done twice using methanol, 500ml with vacuum drying between the two elution cycles. Elutes were then completely evaporated using Thermo Scientific™ Reacti-Vap™ Evaporators (Thermo Fisher Scientific Inc, USA) at 37^0C for 30 min). This was then reconstituted using 100ml of equal parts 1:1 acetonitrile and water, vortexed briefly and transferred into 50ml vials and capped. About 1ml of the sample was injected automatically into the LC/MS instrument and quantified within 5 minutes. The analytical results and chromatograph, showing accuracy, precision and linearity data for each standard curve and all quality control samples were provided.

3.7.5 Genomic DNA Preparation and Genotyping of CYP2B6 Polymorphism

Genomic DNA was extracted from EDTA collected blood using the QIA amp DNA blood mini-kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Purity of the DNA was assessed spectophometrically by comparing the A_{260} and A_{280} ratio. The extracted DNA was quantified and normalized to a concentration of 20ng/ml which was used to determine the single nucleotide polymorphisms (SNPs) at CYP2B6. Single nucleotide polymorphisms at CYP2B6 (516G>T and 983T>C) were identified by real-time quantitative polymerase chain reaction (RT-PCR)-based allelic discrimination adopted from the method of Mahungu (2007) Pre-amplification of CYP2B6 gene: To differentiate CYP2B6 from the pseudogene CYP2B7, a pre-amplification step was carried out as follows: 1 µl of DNA was added to a 10-µl reaction mixture consisting of 1µl of x10 PCR Buffer, 0.6µl of $MgCl_2$ (25mM), 0.4ul of forward primers (1mM) and 0.4µl of reverse primers (1mM), 0.2µl of dNTP (10mM), 0.2µl of Taq (5µg / µl) and 6.2µl of RNase-free water (as indicated below). The standard PCR reaction consisted of an initial 5-minute denaturation step, followed by 45 cycles of 95^0C for 30 seconds, 58^0C for 30 seconds and 72^0C for 45 seconds. This was followed by a 5-minute step at 72^0C RT-PCR procedure: For the real-time assay, 2µl (20ng/ml) of amplicons from the exon pre-amplification of DNA were added to a 23-µl reaction mix consisting of 12.5µl of x2qPCRmix, 1.25µl of x20 primer mix, 1.25µl of x20 probe mix and 8µl of RNase-free water. The real-time PCR reaction consisted of an initial 15-minute denaturation step at 95^0C for 15 seconds and 60^0C for 1 minute.

The plate was read by the allelic discrimination settings. The SNP assay was set up using SDS, version 1.3.0 as an absolute quantification assay. Post-assay analysis was done using SDS software. The SNPs were determined by direct sequencing using BigDye method.

CYP2B6 (516G>T) forward primer (5′-GGTCTGCCCATCTATAAAC-3′) and a reverse primer (5′-TCATCCTTTTCTCGTGTGTTCT-3′). CYP2B6 983T>C forward primer (5′-CTTTTCAAATCAATACTCAACTTTAGAGAGTCTACAGGGAGAT-3′) and reverse primer (5′-CAATTAACTACATACAATACATACAGAGAGTCTACAGGGAGAC-3′), and a common 5′-/5Phos/TGAACAGGTGATTGGCCCAC/3Bio/−3′ (Wang 2006).

3.8 Data Analysis

Proportions and percentages were used to present the sociodemographic characteristics. Chi-square test was used for comparing proportions between the groups. Genotype distributions were tested for Hardy-Weinberg equilibrium using chi-square tests. The continuous variables expressed as the mean and median. The t-test and ANOVA were used to compare differences in means for continuous data. Influence of CYP2B6 516G>T and 983T>C polymorphisms on initial viral load and CD4+ cell count determined using General linear model. Statistical analysis was done using Stata version 11 (StataCorp. LP, College Station, USA) at a significance level of $P \leq 0.05$.

3.9 Ethical Consideration

Clearance to collect data was obtained from Maseno University's School of Graduate Studies. This study was approved by the Kenya Medical Research Institutes' (KEMRI) Ethical Review Committee (KEMR-ERC 2539) in May of 2013. Prior to enrolment into the study, a written informed consent was obtained from every participant in a private room. All the objectives and procedures were explained to the participants and freedom to accept or decline study enrolment was emphasised (Appendix 3). Study documents and data were kept in a secure place accessible only to the principal investigator and selected staff to ensure participant privacy. Confidentiality of all study participants was ensured by de-identifying all records that could be attributed to an individual study participant.

CHAPTER FOUR: RESULTS

4.1 Socio-Demographic and Clinical Characteristics

A total of two hundred and twenty eight (228) participants attending centre care at the Center for Respiratory Disease Research (CRDR) KEMRI-Nairobi, on Nevirapine-based regimen were enrolled at the beginning of the study. However on the subsequent sixth month visit, eight (8) patient records had incomplete clinical data, six (6) participants were lost to follow-up while seven (7) had developed toxicity and had their HAART regimen changed. Therefore, a total of twenty one (21) participants were discontinued from the study leaving two hundred and seven (207) participants for final statistical analysis as shown in Figure 4.1

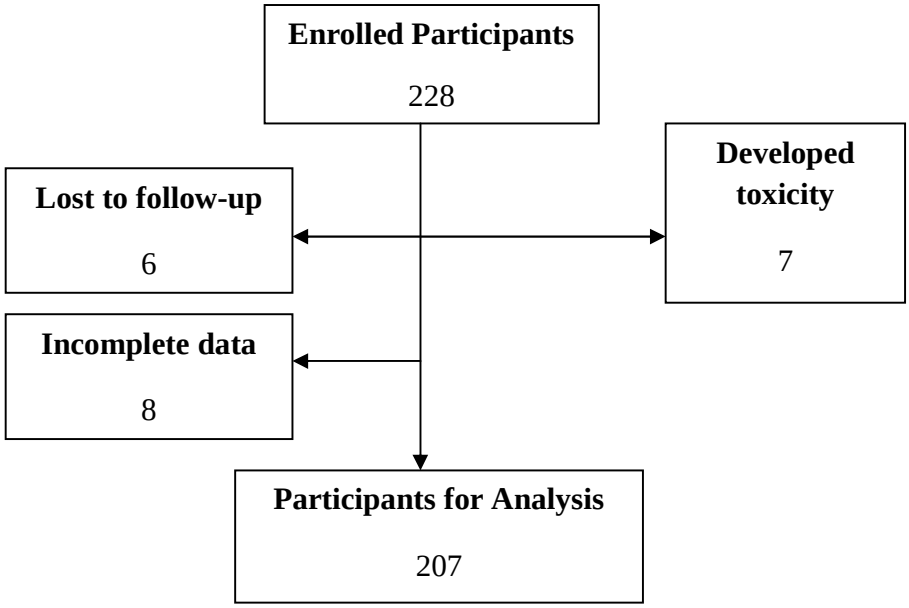

Figure 4.1: Participants enrolment flow chart

Of the 207 participants 56.6% (n=117) were female while 43.4% (n=90) were male. The mean age of all the participants was 40.4 (22-66) years. Majority were aged 31 to 40 years (42.5%) and did not engage in social habits of taking alcohol and smoking (95.2%). There was no significant difference among the males and females that possessed CYP2B6 polymorphisms: gender did not affect the acquisition of mutations. Similarly, alcohol intake was equally high both in males (75.6%) and female (85.5%): sampling was equally distributed within gender, as such, if we find

that alcohol intake affected polymorphisms, it would be due to other patient factors and not gender. The t-test analysis reported that males presented with significantly lower CD4+ T cell count (mean=245) compared to females (mean=316) *P*=0.006. There was no significant differences in the HIV RNA viral load levels between the genders. The participants' socio-demographic and clinical characteristics are presented in Table 4.1

Table 4.1: Participants socio-demographic and clinical characteristics

CHARACTERISTIC	Study participants		*p-value*
	Male (n=90)	**Female (n=117)**	
983 T>C Polymorphisms, n (%)			
TT-Homozygous Wild type	67 (74.4)	88 (75.2)	0.513[a]
TC-Heterozygous Mutant	23 (25.6)	29 (24.8)	
516 G>T Polymorphisms, n (%)			
GG-Homozygous Wild type	47 (52.2)	65 (55.6)	0.737[a]
GT-Heterozygous Mutant	33 (36.7)	37 (31.6)	
TT-Homozygous mutant	10 (11.1)	15 (12.8)	
Alcohol intake, n (%)	68 (75.6)	100 (85.5)	0.076[a]
Immunological factors, Mean (std. dev.)			
Age	41.0 (8.6)	40.0 (8.3)	0.404[b]
CD4 Baseline	245 (172)	316 (200)	**0.006[b]**
HIV Viral load Copies/ml Baseline	83,901 (179,732)	83,842 (182,951)	0.998[b]

Data presented are as indicated as number (n) and proportions of subjects (%, percentage) or means (standard deviation). Statistical analysis of data was conducted using the Chi-square test for comparing proportions between the groups ([a]), and t-test for comparing differences in means for continuous data ([b]). Significant *P*-value is shown in bold.

4.2 Frequency of CYP2B6 Genotypes

For the CYP2B6 516G>T SNP, the frequency of the T variant allele was 45.2% (N=103); 95% CI, 42.7% to 56.4%). The number of study participants with GG, GT and TT genotypes were 104(50.2%), 74(35.7%), and 29(14.1%), respectively (Table 4.2).

For the CYP2B6 983T>C SNP, the frequency of the C variant allele was 38.6% (N=80) (95% CI, 31.9% to 45.2%). Most individuals (61.4%) had the homozygous wild type TT genotype, whereas 80 participants (38.6%) had the heterozygous mutant TC genotype and none had the homozygous mutant CC genotype as shown in Table 4.2. CYP2B6 516G>T polymorphism met Hardy-Weinberg (HW) equilibrium expectations (P=0.26). Hardy-Weinberg equilibrium could not be tested for CYP2B6 983T>C, because the homozygous mutant CC was not detected in this population.

Table 4.2: CYP2B6 Genotype Frequency

SNP	Genotype	N(%)	Frequency of T Variant allele (516 G>T SNPs)
516G>T	**GG**	104 (50.2%)	45.2% (95% CI, 42.7% - 56.4%)
	GT	74 (35.7%)	
	TT	29 (14.1%)	
	Total	207(100%)	
			C variant allele (983T>C SNPs)
983T>C	TC	127 (61.4%)	38.6% (95% CI, 31.9% - 45.2%)
	TT	80 (38.6%)	
	CC	**0**	
	Total	**207 (100%)**	

Data are presented as number (N) and proportions (%) of study participants. Descriptive statistics like percentage was used for the presentation of genotypes. 516G>T; GG (Homozygous Wild type), GT (Heterozygous mutant), TT (Homozygous mutant), 983TC; TC (Heterozygous mutant) and TT (Homozygous mutant) and CC (Homozygous wild type).

4.3 Difference in Nevirapine Plasma concentration between CYP2B6 516G>T and 983T>C polymorphisms of HIV Infected Individuals on Nevirapine Containing HAART

The difference in NVP plasma concentration between CYP2B6 polymorphisms of HIV infected individuals on NVP containing HAART is shown in table 4.3. Majority of the participants (64.7%) had NVP plasma concentration within the durable viral suppression range of >4300ng/ml compared to 17.9% with concentration in viral mutant selection window (3100-4300 ng/ml and 17.4% with poor viral suppression (<3100ng/ml) P = 0.001). The mean Nevirapine plasma concentration was 5155.2 ng/ml (SD 20492) and a median of 4720 ng/ml ranging from 640 to 11800 ng/ml. Among the 207 participants with detectable Nevirapine plasma levels, the mean plasma Nevirapine levels were higher among individuals who were homozygous for the mutation (CYP2B6 516TT, 5335.9ng/mL) and in those who were heterozygous for the mutation (CYP2B6 516GT, 4948.5 ng/mL) compared to those with the wild-type (CYP2B6 516GG, 3725.8 ng/mL, P = 0.0001 across groups. For CYP2B6 983T>C

genotypes, the mean plasma Nevirapine levels were higher among individuals who were heterozygous for the mutation (CYP2B6 983TC, 4748.9ng/mL) compared to those with the wild-type (CYP2B6 983TT, 4161.5 ng/mL, (P = 0.046 across groups).

Table 4.3: Mean difference in Nevirapine Plasma concentration between the genotypes

Polymorphism	Study participants Mean Nevirapine plasma concentration (ng/ml)	*p-value*
983 Polymorphisms, mean (std.dev)		
TT-Homozygous Wild type	4161.5(1925)	0.046[a]
TC-Heterozygous Mutant	4748.9(2384)	
516 Polymorphisms, mean (std. dev)		
GG-Homozygous Wild type	3725.8 (1786)	**0.0001[b]**
GT-Heterozygous Mutant	4948.5 (2170)	
TT-Homozygous mutant	5335.9 (2718)	

Data presented are indicated as mean (standard deviation) and also numbers (n) and percentages (%). Nevirapine plasma concentration between study participants. Statistical data analysis was conducted using t-test ([a]) and ANOVA ([b]) for comparing differences in means for continuous data. Significant *P*-values are shown in bold.
{*<3100 (poor viral Suppression) 3100-4300 (viral mutant selection window) 4300-5000 (Durable viral suppression)}*

4.4 Influence of CYP2B6 516G>T and CYP2B6 983 T>C Genotypes on CD4 T Cell Count and HIV RNA Viral Load Changes

4.4.1 The CD4 T Cell Count Change

For the CYP2B6 516 G>T polymorphisms, controlling for baseline CD4+ cell count, ART regimen and NVP plasma level, compared to TT genotype (homozygous mutant), only GG genotype (wild type) predicted greater change in CD4 cell count (P = 0.007) six months post ART initiation (Table 4.4). on the contrary, for the 983 T>C polymorphisms, controlling for baseline CD4+ cell count, ART regimen and NVP plasma level, compared to TC genotype

(heterozygous mutant), the TT genotype (homozygous wild type) was not associated with a significant change in CD4+ cell count (P = 0.194) six months post ART initiation (Table 4.4).

4.4.2 The Viral Load Change

For CYP2B6 516 G>T polymorphisms, controlling for ART regimen, baseline CD4 cell count, NVP plasma level and baseline viral load, compared to TT genotype (homozygous mutant) only GG genotype (wild type) predicted significant decrease in viral load count (P = 0.05) six months post ART initiation. On the contrary, for CYP2B6 983 T>C genotypes, controlling for baseline CD4 cell count, ART regimen and NVP plasma level, the TC genotype did not predict a significant viral load change six months post ART initiation (Table 4.4).

A similar trend was observed for CYP2B6516G>T genotypes, with mean plasma HIV-1 RNA which was higher among individuals who were homozygous for the mutation (CYP2B6 516TT, 56,239.8 copies/mL) and in those who were heterozygous for the mutation (CYP2B6 516GT, 28,325.8 copies/ml) compared to those with the wild-type (CYP2B6 516GG, 11,167.5 copies/ml (P = 0.039 across groups). On the contrary, for CYP2B6 983T>C genotypes, the mean plasma HIV-1 RNA levels were lower among individuals who were heterozygous for the mutation (CYP2B6 983TC, 16,612.6ng/mL) compared to those with the wild-type (CYP2B6 983TC, 28,027.3 ng/ml. This difference was however not significant (P = 0.359 across groups).

On the other hand, an inverse trend was observed for CYP2B6 516G>T genotypes, with mean CD4 count which was lower among individuals who were homozygous for the mutation (CYP2B6 516TT, 272 cells/mL) and in those who were heterozygous for the mutation (CYP2B6 516GT, 328.2 cells/ml) compared to those with the wild-type (CYP2B6 516GG, 479 cells/ml, P = 0.004 across groups). For CYP2B6 983T>C genotypes, the mean plasma HIV-1 RNA levels were lower among individuals who were heterozygous for the mutation (CYP2B6 983TC, 352.3 cells/ml) compared to those with the wild-type (CYP2B6 983TT, 423.8 cells/ml. This correlation was however not significant (P = 0.359 across groups).

Table 4.4: CYP2B6 516G>T and CYP2B6 983 T>C genotype and change in immunological and viralogical levels six months post ART

Parameter	OR		95% CI	t	P value
CD4 CELL COUNT					
GG (Homozygous wildtype)	206.850	55.890	357.810	2.702	**0.007**
GT(Heterozygous mutant)	57.360	-99.960	214.690	0.719	0.473
TT (Homozygous mutant)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
ART regimen	5.572	-60.890	72.010	0.165	0.869
CD4 count	.232	-.029	.494	1.753	0.081
CYP2B6 983T>C genotype					
TT (Homozygous wildtype)	69.126	-35.340	173.630	1.304	0.194
TC (Heterozygous mutant)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
ART regimen	-12.489	-79.889	54.911	-0.365	0.715
CD4 count	.236	-.033	.505	1.731	0.085
VIRAL LOAD					
CYP2B6 516G>T genotype					
GG (Homozygous wildtype)	-0.536	-1.071	-.001	-1.976	0.05
GT(Heterozygous mutant)	-.378	-0.923	.168	-1.365	0.174
TT (Homozygous mutant)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
ART regimen	.137	-.349	.622	.555	0.579
CD4 count	.001	-.001	.001	.343	0.732
Log10 transformed basline viral load count	.286	.161	.411	4.528	**0.001**
CYP2B6 983T>C genotype					
TT (Homozygous wildtype)	-0.091	-0.446	0.264	-0.505	0.614
TC (Heterozygous mutant)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
ART regimen	.062	-.420	.545	.254	0.8
CD4 count	.001	-.001	.002	.289	0.773
Log10 transformed viral load count	.287	.163	.411	4.554	**0.001**

Data presented are indicated as mean. Statistical data analysis was general linear model. Significant *P*-values are shown in bold.

CHAPTER FIVE: DISCUSSION

5.1 Summary of Results

The study found out the following;

The most frequent polymorphism on CYP2B6 516G>T genotype was the heterozygous G>T variant allele at 50.2%. Among participants with CYP2B6 983T>C genotypes the most frequent variant allele was the homozygous TT at 61.4%. For the 516 G>T genotype nevirapine plasma concentrations were highest among individuals with the TT variant allele and lowest among those with GG variant at 5335.9 ng/ml and 3725.8 ng/ml respectively. For those with CYP2B6983T>C genotype the TC variant allele individuals had the highest CD4+ levels at 4748.9 compared to their TT counterparts at 4161.5 ng/ml. for the 516 G>T genotype individuals those with the GG variant allele recorded the highest CD4+ T cell levels at 479 Cell/ml compared to the TT variant allele individuals at 272 cells/ml. For the CYP2B6 983 T>C genotype, those with the TC and TT variants recorded lower CD4+ levels of 352.3 cells/ml and 423 cells/ml respectively. The viral load was least among those with the GG variant and highest among the TT variant at 11167.5 copies/ml and 56239.8 copies/ml respectively among the CYP2B6 516 G>T genotype individuals.

5.2 Frequencies of CYP2B6 516G>T Genotypes

In this study, for the CYP2B6 516G>T SNP, the frequency of the T variant allele was (45.2%) while the number of subjects with GG, GT and TT genotypes were 50%, 36%, and 14% respectively. This prevalence of 516G>T, T variant and genotypes was similar to that reported for other African populations and Kenyan ethnic groups (Penzak 2007). In a study among HIV-infected women taking nevirapine-based ART in Mombasa, Kenya; Oluka et al., (2015) identified the prevalence of 31.2% T variant allele and a 50.7% GG, 36.2% GT and 13 TT genotypes. In South Africa, Gounden et al., (2010) reported the prevalence of the allelic variant CYP2B6 TT (poor metabolisers) to be 23% amongst study population. Haas et al., (2005) reported a 20% prevalence of the TT genotype amongst the African-American cohort in the Adult AIDS Clinical Trials Group. An Indian study by Ramachandran et al., (2013) reported a genotype frequency for GG, GT and TT genotypes of 32%, 45% and 18% respectively which

except for the GG variant are higher than the current study. A multi-site clinical study done in Haiti, India, Malawi, South Africa, Tanzania and Uganda Vardhanabhuti et al., (2013) reported a prevalence of 40.6% (GG), 46.5% (GT) and 12.9% (TT) almost similar to this study.

For the CYP2B6 983T>C SNP, the frequency of the C variant allele was (38.6%) while the number of subjects with TT genotypes were 61.4%. CYP2B6 983T>C occurs predominantly in African subjects, with allele frequencies of between 4% to 11% (Dahri 2007). The prevalence of the C allele has been shown to be present at a frequency of 4.4% in African–Americans and 6.6% in a Ghanaian cohort, but is absent in Caucasians (Klein 2005). A study by Oluka et al., (2015) identified a lower frequency of C variant allele at 10.1% compared to our study. The study identified 79.9% individuals with homozygous wild type TT genotype and 20.3% had the heterozygous mutant TC genotype. In our study we did not detect Homozygosity for the mutant allele CYP2B6 983 CC, in concurrence with previous studies (Mehlotra 2007; Oluka 2015). CYP2B6 983 CC has been associated with none nucleoside reverse transcriptase inhibitors (NNRTI) central nervous system (CNS) toxicity (Rotger 2007). This lack of 983 CC homozygosity among the study participants could be attributed to the excluded participants who developed toxicity and had their regimen changed. Furthermore, the elevated C variant allele frequency found in this study, could be attributed to the difference in the study settings, sampling frame and the increased genetic diversity in East African population.

5.3 CYP2B6 516G>T and 983T>C Genotypes and Nevirapine Plasma Level

In this study, the mean plasma Nevirapine levels were higher (5335.9ng/ml) among individuals who were homozygous for the mutation (CYP2B6 516TT) and in those who were heterozygous for the mutation (CYP2B6 516GT) at 4948.5ng/ml compared to those with the wild-type (CYP2B6 516GG) at 3725.8 ng/ml. Similar observation were seen with CYP2B6 983T>C genotypes at 4748.9ng/ml while the homozygous CYP2B6 983 TT was at 4161.5 ng/ml. Heterozygosity for the mutation CYP2B6 983TC was associated with higher plasma nevirapine levels in our study. The durable viral suppression range should be greater than 4300ng/ml. A concentration of 3100-4300 ng/ml was considered to be within the viral mutant selection window while concentrations below 3100ng/ml were considered to lead to poor viral suppression. These

findings were similar to a Ugandan study (Penzak 2007) which found a higher Nevirapine plasma concentration among those with 516TT (7607 ng/ml), followed by 516GT (5559 ng/ml) and 516GG at 4181ng/ml. The difference in Nevirapine plasma concentration could be attributed to the follow-up period. In this study, the follow-up period was 6 months while in the Penzak study, the follow-up period was 14 days. Nevirapine plasma concentrations tend to be elevated during the first few weeks of treatment onset. The study findings also had a similar trend with a Burundian study by (Calcagano) which found a mean Nevirapine plasma concentration of 3000ng/ml among those with both 516GT and 983 TC genotypes (Calcagno 2012).

This study found that despite the form of polymorphism a participant presented with, they were able to suppress their viral load levels; however those with CYP2B6 516 TT had viral load levels above the durable viral suppression range (4300-5000 ng/ml). This levels predispose the Study participants to Nevirapine toxicity and selection for NVP resistance mutations and unfavourable outcomes. For those homozygous for CYP2B6 983 TT genotype, their mean plasma NVP concentration (4161.5ng/ml) was within mutant selection window. Despite this, individuals in this category were characterised by better immunological outcomes compared to their TC variant allele counterparts. This observation could be due to the use of mean which may be distorted by outliers (very large or small values) hence giving a false impression. Therefore, a homozygous polymorphism on CYP2B6 516 TT confers a better therapeutic effect compared to those with CYP2B6 983 TT. The findings on viral suppression are similar to those of a French study which found that those individuals with CYP2B6 516TT had a better viral suppression compared to those with the other CYP2B6 genotypes (GG and GT) (Gozalo 2011). This difference in findings could be attributed to study population genetic differences and a different study setting. In this study, although 516TT presented a higher nevirapine plasma concentration, it did not necessarily lead to an elevated CD4 cell count and a reduction in viral load.

A higher mean plasma Nevirapine levels in this study was associated with both the homozygosity for the mutation (CYP2B6 516TT) and heterozygosity for the mutation (CYP2B6 516GT) genotypes. This is similar to the findings of Oluka *et al*., (2015) which reported a similar trend of a significant association between both CYP2B6 516G>T and CYP2B6 516 T>T genotypes and higher plasma nevirapine concentrations. In South Africa, Gounden *et al* (2010),

showed a significant relationship between 516 G>T SNP and plasma NVP concentrations and increased reporting of CNS side effects. It is likely that those with the 516 G>T allele still had high plasma NVP concentrations despite improvement of symptoms.

5.4 Association of CYP2B6 516G>T and 983T>C Genotypes and Changes in CD4+ Cell Count and Viral Load

Plasma NVP levels often predict a change in CD4+ T cell count (O'Brien 1997). Elevated plasma nevirapine levels within or above viral suppression range led to a greater change in CD4+ cell count 6 months post treatment. In this study, there was a lower mean CD4+ level six months post ART among individuals who were homozygous for the mutation (CYP2B6 516TT) and in those who were heterozygous for the mutation (CYP2B6 516GT) compared to those with the wild-type (CYP2B6 516GG). In the general linear model controlling for baseline CD4 cell count, ART regimen and NVP plasma level, GG genotype (wild type) predicted greater change in CD4 cell count (P=0.007). This is contrary to studies by Haas (2005) and Oluka (2015) which reported no association between CYP2B6 516G>T genotypes and change in CD4 cell count. This is also contrary to studies by Saitoh (2007), who observed that the CYP2B6 516TT genotype predicted a greater increase in CD4+ cell count percentage compared to those with the GT and GG genotypes (9.0% vs. 5.0% vs. 3.2% increases respectively. This might be due to difference in study population (children vs. adults) and follow up period (3 months vs. 6 months) between the two studies. High NVP plasma concentration (5335.9 ng/ml) for individuals with the TT variant allele may have predisposed study participants to toxicity and suboptimal drug utilisation {4300-5000 (durable viral suppression range)}.

Similarly, higher mean plasma HIV-1 RNA was seen among individuals who were homozygous for the mutation (CYP2B6 516TT) and in those who were heterozygous for the mutation (CYP2B6 516GT) compared to those with the wild-type (CYP2B6 516GG). In GLM controlling for ART regimen, baseline CD4 cell count, NVP plasma level and baseline viral load, compared to TT genotype (homozygous mutant) only GG genotype (wild type) predicted greater decrease in viral load count six months post ART initiation (P=0.05). Concurrent results were shown by Ramachandran *et al* (2013) where CYP2B6 516 G>T genotypes predicted better virologic outcomes compared to CYP2B6 516 T>T genotypes among participants receiving NVP.

Polymorphisms in CYP2B6 516 G>T gene are also probably responsible for the wide inter-patient variability observed in NVP blood levels (Ramachandran 2013). A higher proportion of participants belonging to GG/GT genotype are among the favorable responders in terms of viralogical outcomes (Chen 2010). These findings are also similar to a study conducted in Thai HIV/TB co-infected participants receiving NVP; the authors observed that a higher proportion of TT genotypes achieved virologic failure compared to GG and GT genotypes, though not statistically significant (Uttayamakul 2010).

For those with the CYP2B6 983 T>C polymorphisms, the TT variant allele predicted a greater increase in CD4+ T cell count (423.8 cells/ml) compared to the TC variant allele (352.3 cells/ml). This association was however not significant. This is contrary to the Kenyan study by Oluka (2015) where participants with the heterozygous genotype 983 TC had the highest CD4+ levels 12 months post treatment. This difference could be attributed to the follow up periods and study population and sample size between the two studies. The study by Oluka (2015) was conducted in among 66 women in Mombasa, Kenya. The study population is considered more homogeneous compared to the Nairobi population. Ethnicity though not included in the current study may actually influence variability of CYP2B6 genotypes and their outcomes (Klein 2005) and NVP plasma concentration (Klein 2005).

CHAPTER SIX: SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

In this study, the most frequent genotype for CYP2B6 516G>T was the GG genotype while for CYP2B6 983T>C was the TT genotype. The mean plasma Nevirapine levels were higher among HIV-1 infected individuals who were heterozygous for the mutation CYP2B6 516G>T and CYP2B6 983T>C genotypes. CYP2B6 516G>T genotypes were associated with significant change in viral load count whereas CYP2B6 983T>C genotypes were not. Lastly, CYP2B6 516G>T genotypes were associated with significant change in mean CD4+ cell count while CYP2B6 983T>C genotypes were not. Generally the CYP2B6 516G>T genotype was associated with better immunologic and virological response compared to CYP2B6 983T>C.

6.2 Conclusion

1 Majority of the study participants had the GG and TT polymorphisms among those with CYP2B6 516 G>T and CYP2B6 983T>C genotypes respectively.

2 The TT genotype of CYP2B6 516 G>T polymorphisms and TC genotypes for 983 T>C polymorphisms were associated with higher NVP plasma concentration among HIV infected participants. Higher plasma NVP concentration among those with 516 TT genotype did not confer immunological and viralogical benefits to the participants as they were above the viral suppression range and may have predisposed the participants to nevirapine toxicity.

3 Participants with CYP2B6 516GG and 983TC SNPs were found to have better immunological and virological outcomes on NVP due to plasma concentrations within optimum viral suppression levels, lower viral load levels and elevated CD4 cell count.

6.3 Study Recommendations

1. More studies should be conducted to determine the role of other enzymes and binding cassettes on NVP metabolism.

2. Nevirapine treatment should be tailored to different patients based on their genetic make up to reduce on toxicity and other unwanted effects.

3. Larger prospective studies should be conducted to validate the value of the findings of this study.

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APPENDICES

APPENDIX 1: DATA COLLECTION FORM.

Study participant code _____

Interview date_____/_____/_____

PART I: PARTICIPANTS' SOCIODEMOGRAPHIC CHARACTERISTICS

1. Age _____(Years) Birth date_____/_____/_____

2. Gender:(Tick Appropriately)

☐ Male ☐ Female

7. Social Habit

☐ Alcohol/Smoking ☐ Non alcoholic/Non smoker

PART II; LABORATORY INVESTIGATIONS.

1. Biochemical Parameters
    a. Nevirapine plasma levels _____ ng/ml
    b.
2. Immunological/ Virological Parameters
    a. CD4 Levels _____ Cells/ml
    b. Viral Load_____ Copies/ml
    c.
3. CYP2B6 Genotypes(Tick appropriately)
    a. 516
        ☐ GT
        ☐ GG
        ☐ TT
    b. 983
        ☐ TC
        ☐ TT
        ☐ CC

APPENDIX 2: ETHICAL APPROVAL LETTER.

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **May 21, 2013**

TO: **MUSA OTIENO NG'AYO,**
PRINCIPAL INVESTIGATOR

THRO': **DR. WILLY SANG**
THE Ag. DIRECTOR, CMR,
NAIROBI

Forwarded
30/05/2013

Dear Sir,

RE: **SSC PROTOCOL NO. 2539 (*INITIAL SUBMISSION*): ETIOLOGY OF SUB-OPTIMAL RESPONSES TO NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR (NNRTI) AMONG HIV PATIENTS ON ANTIRETROVIRAL TREATMENT IN NAIROBI KENYA**

This is to inform you that during the 215th meeting of the KEMRI/ERC meeting held on 21st May 2013, the above study was reviewed.

The Committee notes that the above referenced study aims to determine the levels, consequences and etiology of Efavirenz (EFV) and Nevirapine (NVP) sub-optimal response among patients on first line ART attending the HIV treatment program in the Family AIDS Care and Education Services (FACES), KEMRI Nairobi.

Due consideration has been given to ethical issues and the study is hereby **granted approval** for implementation effective this **21st day of May 2013,** for a period of twelve (12) months.

Please note that authorization to conduct this study will automatically expire on **20th May 2014.** If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by **9th April 2014**.

You are required to submit any amendments to this protocol and other information pertinent to human participation in this study to the ERC prior to initiation. You may embark on the study.

Yours faithfully,

EAB

Dr. ELIZABETH BUKUSI,
ACTING: SECRETARY,
KEMRI/ETHICS REVIEW COMMITTEE

APPENDIX 3: INFORMED CONSENT FORM

English Version.

Title: **Influence of CYP2B6 polymorphisms on CD4 t cell count and HIV RNA viral load changes among individuals on nevirapine containing highly active antiretroviral therapy**

Dear participant:

My name is Stephen B. Tanui currently pursuing a masters degree in Medical Immunology at Maseno University Department of Biomedical Sciences. I am currently conducting a study on the Influence of CYP2B6 Polymorphisms on immunological changes among HIV positive individuals on nevirapine containing highly active antiretroviral therapy.

You have been selected to participate in this study since you are on Nevirapine containing therapy. The study will involve drawing 2.5 ml of whole blood which will be used to conduct biochemical tests and genetic analysis. There will be no direct benefit to you as a participant. However the findings will be used to inform future management of participants on HAART. The samples will not contain your name and the results obtained will be kept privately and used only for the research purpose and the tests indicated for the study.

You are free to accept or decline to participate in this study and your lack of participation will not interfere with the current services you are receiving in this program. Participation in this study is important as the findings of the study have the potential of being used to improve care and inform physicians decisions on the best combination of drugs based on individuals genetic makeup.

The risks in this study include possible discomfort due to questions on health and personal behaviour/history. In addition minimal pain may be experienced while a blood sample is being obtained. Every effort will be made to keep your study records confidential.

By signing my name below, I confirm the following:

I have read (or been read to) this entire consent document. All of my questions have been answered to my satisfaction. The study's purpose, procedures, risks and possible benefits have been explained to me. I agree to let the study team use and share the health information gathered

for this study. I voluntarily agree to participate in this research study. I agree to follow the study procedures as directed. I have been told that I can withdraw from the study at any time.

Participant Sign---------- Date----------

principle Investigator---------- Date----------

Note: Below are some of the key contacts

Principle investigator – Stephen Biwott Tanui - **0722154131** ; KEMRI-ERC - **0733400003**

Kiswahili Version

Fomu ya Idhini Kwa Mhusika;

Mada Ya Utafiti: Kutathmini mabadiliko ya chembechembe katika miili na jinsi yanavyoathiri matokeo ya matibabu kwa wagonjwa wanaotumia dawa za kupunguza makali ya ugonjwa wa ukimwi yenye mchanganyiko wa Nevirapine.

Umechaguliwa kushiriki kwenye utafiti huu kwa sababu unatumia dawa za kupunguza makali ya ukimwi zenye mchanganyiko wa Nevirapine. Madhumuni hasa ya utafiti huu ni kujaribu kutathmini mabadiliko ya chembechembe katika miili na jinsi yanavyoathiri matokeo ya matibabu kwa wagonjwa wanaotumia dawa za kupunguza makali ya ugonjwa wa ukimwi yenye mchanganyiko wa Nevirapine. Ukichagua kushiriki kwenye utafiti huu utahitajika kutolewa kiasi cha mililita mbili na nusu ya damu kwa ajili ya upimaji wa hiyari wa viwango vya virusi vya ukimwi, mabadiliko katika chembechembe na pia viwango vya dawa ya Nevirapine kwenye damu yako.

Unaweza kutoa uamuzi wa kushiriki kwenye utafiti huu au pia kukataa. Ukisema la matibabu yako ya kawaida hayataathirika. Si lazima kubaki kama mshiriki unaweza ukakatiza kushiriki wakati wowote. Ni muhimu kufahamu kwamba hakuna faida za kifedha kwa kushiriki kwenye utafiti huu. Zaidi ya yote hautagharamika kifedha kwa njia yoyte. Kushiriki katika utafiti huu ni muhimu kwa sababu, uvumbuzi ama majibu ya utafiti huu yatasaidia katika matibabu na afya ya msingi kwa wagonjwa wote wanaotumia dawa za kupunguza makali siku zijazo.

Hatari zinazoambatana na kushiriki katika utafiti huu ni kama usumbufu kutokana na maswali ya kiafya na ya kibinafsi hasa tabia na historia yako. Kadhalika utahisi uchungu kiasi wakati wa kutolewa damu. Juhudi zote zitafanywa kwa ajili ya kuhifadhi historia yako ya kiafya kwa njia ya siri.

Kwa kuweka sahihi jina langu nathibitisha yafuatayo:

1) nimesoma (ama nimesomewa) karatasi hii ya kutoa idhini ya kukubali, na maswali yangu yote yamejibiwa na nimeridhika; 2) Nimeelezewa nia, hatari na faida zinatokana na utafiti huu kwa njia mwafaka; 3) nakubali na kuruhusu timu ya utafiti kutumia na kugawa habari za kiafya ama

aina yoyote ya habari zitakazokusanywa kutokana na utafiti huu; 4) nimekubali kwa hiyari kushiriki kwenye utafiti huu. Nakubali kushirikiana na watafiti hawa wakati wowote ninapohitajika; 5) Nimeelezwa kwamba ninaweza kukataa kushiriki wakati wowote.

Jina la mshiriki..... Sahihi..... Tarehe.....

Mtafiti mkuu/Msaidizi..... Sahihi..... Tarehe.....

Kwa lolote la ziada wasiliana na wafuatao

Mtafiti mkuu - Stephen Biwott Tanui – **0722154131** ; Ama KEMRI-ERC - **0733400003**