EFAVIRENZ LEVELS AND TREATMENT OUTCOMES IN KENYAN HIV-TB CO-INFECTED PATIENTS AT KENYATTA NATIONAL HOSPITAL

DR. JARRED OKOYO NYAKIBA

DEPARTMENT OF PHARMACEUTICS AND PHARMACY PRACTICE.

SCHOOL OF PHARMACY

UNIVERSITY OF NAIROBI.

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DECLARATION

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

DR. JARRED OKOYO NYAKIBA

Signature.....Nyakiba..... **Date**.....16th Nov 2010.....

SUPERVISORS:

1. DR MARGARET OLUKA

DEPARTMENT OF PHARMACOLOGY AND PHARMACOGNOSY,

SCHOOL OF PHARMACY, UNIVERSITY OF NAIROBI.

This dissertation has been submitted for examination with my approval as University supervisor.

Signature:.....Oluka..... **Date:**.....16/11/2010.....

2. DR FAITH OKALEBO

DEPARTMENT OF PHARMACOLOGY AND PHARMACOGNOSY,

SCHOOL OF PHARMACY, UNIVERSITY OF NAIROBI.

This dissertation has been submitted for examination with my approval as University supervisor.

Signature:.....F-Okalebo..... **Date:**.....16/11/2010.....

COLLABORATING INSTITUTIONS

1. UNIVERSITY OF NAIROBI/ AFRICAN INSTITUTE OF BIOMEDICAL SCIENCE AND TECHNOLOGY (UON/AiBST) LABORATORY.

DEPARTMENT OF PHARMACOLOGY AND PHARMACOGNOSY.
SCHOOL OF PHARMACY, UNIVERSITY OF NAIROBI.

2. NATIONAL AIDS AND STI CONTROL PROGRAMME (NASCOP)

MINISTRY OF HEALTH.

PO BOX 19361 – 00200, NAIROBI, KENYA.

TEL: +254 20 272 9549 or 272 9502 FAX: +254 20 271 0518

Website: www.aidskenya.org

3. NATIONAL QUALITY CONTROL LABORATORY (NQCL).

UNIVERSITY OF NAIROBI, SCHOOL OF PHARMACY BUILDING 2ND FLOOR.

P.O.BOX 29726-00202 KNH, NAIROBI.

TEL: +254 0202726963/3544525/30/+254 020 3544525. FAX: +254 202 2718073.

Email. www.nqcl.go.ke info@nqcl.go.ke

4. COMPREHENSIVE CARE CENTRE

KENYATTA NATIONAL HOSPITAL

P.O.BOX 00202, KNH, NAIROBI.

DEDICATION

I am immensely grateful to the almighty God for giving me the patience, strength, perseverance, knowledge and will to accomplish this work. I dedicate this dissertation to my family; my wife Marion, my son Andrew and my daughter Maria for their love, patience and unwavering support during my studies. I also dedicate this dissertation to my parents, John and Agnes, brother Augustus and sisters Damaris and Phyllis, for their love, patience and unwavering support during my studies.

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LIST OF ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
AiBST	African Institute of Biomedical Science and Technology
ART	Antiretroviral therapy
AUC	Area under the curve
BMI	Body mass index
C	Cytosine
CCC	Comprehensive Care Centre, Kenyatta National Hospital
CD4	Subgroup of T lymphocytes carrying CD4 antigens
CSF	Cerebrospinal fluid
CXR	Chest X ray
CYP450	Cytochrome P450 enzymes
DNA	Deoxy-ribonucleic acid
ESR	Erythocyte sedimentation rate
F	Bioavailability
FHG	Full haemogram
G	Guanine
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
Kgs	Kilograms
KNH	Kenyatta National Hospital

LFTs	Liver function tests
MCV	Mean corpuscular volume
MOH	Ministry of Health, Kenya
NRTIs	Nucleoside reverse transcriptase inhibitors
RNA	Ribonucleic acid
2RHZE/4RH	2 months of rifampicin/isoniazid/pyrazinamide/ethambutol then 4 months of rifampicin/isoniazid in fixed dose combinations.
2RHZE/6EH	2 months of rifampicin/isoniazid/pyrazinamide/ethambutol then 6 months of isoniazid/ethambutol in fixed dose combinations.
SPSS	Statistical Package for Social Sciences
STI	Sexually transmitted infections
T	Thymidine
TB	Tuberculosis
t_{max}	Time to maximum plasma concentrations
WHO	World Health Organisation
Yrs	Years

ABSTRACT

Efavirenz is a non-nucleoside reverse transcriptase inhibitor specific against HIV-1 that is used as a component of HAART in combination with two NRTIs at an adult dose of 600mg daily. Rifampicin is a semi-synthetic derivative of rifamycin that is administered with other anti-TB drugs for the first line management of active TB at an adult dose of 300-600mg per day. Rifampicin induces the cytochrome P450 enzyme system in the liver which also metabolizes efavirenz resulting in decreased levels of efavirenz. When this is coupled with the large inter-patient and intra-patient variability in efavirenz plasma concentrations, it could lead to sub-therapeutic concentrations hence treatment failure or toxic concentrations hence adverse effects. The aim of this study was to determine the plasma levels of efavirenz and the treatment outcomes in Kenyan HIV-TB co-infected patients receiving concomitant Anti-TB therapy and ART at Kenyatta national hospital.

The study was designed as a concurrent descriptive cohort study. HIV-TB co-infected Kenyan patients attending the comprehensive care centre of Kenyatta National Hospital who met the study inclusion criteria were recruited into the study. Following approval by the Kenyatta National Hospital ethics and research committee and a written informed consent, 28 HIV-TB co-infected patients of Kenya origin were recruited into the study from January 2010 to March 2010. Patient demographics, history, baseline data and treatment outcomes were obtained by medical record review. Plasma concentrations of efavirenz were determined by reverse phase high performance liquid chromatography with UV detection (HPLC-UV). Two blood samples were obtained from each subject on two separate occasions separated by a minimum of 2 weeks.

Data analysis was performed by SPSS version 12.0 software. Descriptive and exploratory data analysis was carried out for each variable. Intra and inter-patient variability was determined using the coefficient of variation. Comparison of plasma concentrations at first and second occasions and with different variables was done using various nonparametric tests, Post hoc multiple comparison and Spearman's rho correlation. Multivariate data analysis was done by general linear regression (repeat measures).

The median plasma concentrations (Range) at the first and second sampling occasions were 13.17 (0.04 – 52.48) ug/ml and 10.66 (3.54 – 57.61) ug/ml. On 1st sampling one patient (3.7 %) had sub-therapeutic plasma EFV concentrations (<1 ug/ml) while only one patient (3.7 %) had therapeutic plasma EFV concentrations (1-4 ug/ml). A majority of patients (92.6 %) had toxic

plasma EFV concentrations (>4 ug/ml). Out of these 25 patients with toxic levels, 68 % had levels between 4 and 20 ug/ml while 32 % had levels above 20 ug/ml. On the second sampling occasion, no patient had sub-therapeutic plasma EFV concentrations while only one patient (4.3 %) had therapeutic plasma EFV concentrations. A majority of patients (95.7 %) had toxic plasma EFV concentrations. Out of these 22 patients with toxic levels, 72.7 % had levels between 4 and 20 ug/ml while 27.3 % had levels above 20ug/ml.

A high intra-patient variability of 32.5 % was observed and a high inter-patient variability of 77.8 % on first and 86.4 % on second sampling was observed. Females consistently had higher efavirenz plasma concentrations than male patients though this was not statistically significant. Bodyweight was a statistically significant factor with patients weighing less than 60 kilograms having higher EFV levels than those weighing >=60 kilograms. Ethnicity was also a significant factor with higher efavirenz levels in the Kamba compared to Kikuyu, Kisii, Luhya and Kalenjin being demonstrated. Multivariate analysis of bodyweight, ethnicity and gender showed that they were not statistically significant determinants of efavirenz levels and a large study is required to assess the contribution of these variables to efavirenz levels.

Treatment outcomes were difficult to assess in this study. Although there was a statistically significant difference in the CD4 counts, this was not correlated to the high efavirenz levels. There were 24 episodes of adverse effects in 28 patients implying that nearly every patient would experience an adverse effect during treatment. The prevalence of adverse effects increased on onset of anti-TB therapy and gradually reduced. Skin adverse effects were the most common followed by drug induced hepatitis. Gastrointestinal adverse effects presented early while neurological and metabolic adverse effects presented later.

Contrary to the expectation that efavirenz levels would be lower in patients receiving rifampicin, a large proportion of Kenyan HIV-TB co-infected patients have toxic efavirenz levels and high rates of adverse effects were observed in these patients. Clinicians should therefore be particularly vigilant for signs of toxicity. A priori dose reduction in native African patients, a pharmacogenetic-therapeutic drug monitoring approach to individualised dosing and a prospective clinical dose optimization and treatment outcome study in Kenyans and indeed native Africans are immediately required.

Keywords: efavirenz, rifampicin, HAART, NRTIs, ART, anti-TBs, HPLC, CD4, HIV-TB, SPSS, pharmacogenetic, therapeutic drug monitoring.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Human immunodeficiency virus (HIV) is a major cause of morbidity and mortality especially in Africa and Asia. As per the World Health Organisation (WHO) worldwide estimates of December 2007, there are 33 million people living with HIV/AIDS (PLWHA), 2.7 million people were newly infected with HIV in 2007 and there were 2 million AIDS deaths in the same year.[1] The Kenya AIDS indicator survey of 2007 indicates that the prevalence of HIV in Kenya is 7.4 % among adults aged 15 - 64 years and that more than 1.4 million Kenyans are living with HIV/AIDS.[2] There were an estimated 1.37 million HIV-TB co-infected patients globally in 2007. Around 80 % of these patients live in sub-Saharan Africa which therefore bears the brunt of the HIV fuelled TB epidemic. There were 456,000 people who died of HIV-associated TB in 2007. At least one third of the 33 million PLWHA worldwide are infected with TB and are 20-30 times more likely to develop TB than those without HIV.[1]

In Kenya, there has been a steady increase in the number of TB cases with the case notification rate increasing from 54 per 100,000 in 1991 to 329 per 100,000 in 2006.[3] This increase was fuelled by the growing HIV epidemic. HIV sero-prevalence among the TB patients was 52 % in 2006 and is currently estimated at 60 %. In 2006, all forms of TB cases notified in the public sector were 115,234.[3] HIV is one of the strongest risk factors for developing active TB because immunodeficiency increases the risk of reactivation of latent TB infection and the risk of rapid progression of a recent TB infection. Those with WHO clinical stage 3 or 4 are at most risk of developing active TB infection. The rate of HIV-TB co-infection is increasing in sub-Saharan Africa.[4]

TB is the most common opportunistic infection and the most common cause of death among people living with HIV/AIDS.[4, 5] It is also the most common presenting illness among PLWHA who are taking antiretroviral therapy (ART).[1] Unlike other opportunistic infections (OIs), TB can occur at any point in the course of HIV infection and is not dependent on the CD4 cell count.[4]

WHO guidelines recommend the same TB regimen for HIV-TB co-infected patients as is used in HIV negative TB patients. Both the WHO and the National TB and leprosy guidelines in

Kenya recommend the use of 2RHZE/4RH or 2RHZE/6EH though the latter regimen may be associated with a higher rate of treatment failure and relapse than the former regimen. [6, 7] 2RHZE/4RH is the regimen of choice and is currently in use in all public health facilities in Nairobi. Rifampicin (RFP) induces the cytochrome P450 (CYP450) enzyme system in the liver which also metabolizes the protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) and therefore could cause decreased levels of these antiretrovirals and failure of HIV therapy.[4, 5] Nevirapine levels are reportedly reduced by 37 % and efavirenz (EFV) levels by 25-33 % in patients treated with rifampicin.[4] Studies indicate that early treatment with ART and anti-TB's in HIV-TB co-infected patients improves outcomes.[8] Studies have also shown that EFV based ART regimens are less compromised by concomitant use of RFP and have better virological outcomes in HIV-TB co-infected patients than nevirapine based regimens.[9]

Current WHO guidelines for the management of HIV in the presence of TB recommend a 1st line ART regimen comprising of: Stavudine (D4T)/Zidovudine (AZT) + Lamivudine (3TC) + Efavirenz (EFV). Tenofovir (TDF) or Abacavir (ABC) may be substituted for AZT and D4T.[4] Standard doses of D4T and 3TC are given with Anti-TBs. Guidelines for antiretroviral drug therapy in Kenya (MOH) for the management of HIV in TB recommend switching of patients from nevirapine to efavirenz for patients above 3 years or >10 kilograms or abacavir for patients below 3 years or <10 kilograms.[3]

1.1.1 Problem statement

TB is the most common opportunistic infection and the most common cause of death among people living with HIV/AIDS.[4] HIV positive patients on nevirapine based ART regimens who develop TB, are switched to EFV based ART regimens during anti-TB therapy. TB patients who are HIV positive are started on an EFV based regimens as per the National guidelines for ART in Kenya (2005) and WHO guidelines.[4, 10] EFV plasma concentrations exhibit a large inter-patient and intra-patient variability which coupled with the drug interaction between EFV and RFP could lead to sub-therapeutic or toxic plasma concentrations of EFV.[11] No data exists in Kenyan patients regarding the plasma concentrations of EFV and the treatment outcomes when used concomitantly with rifampicin. Hence there is need to establish if sub-therapeutic, therapeutic or toxic efavirenz levels are attained in Kenyan patients. There is also need to establish whether there is an association between the efavirenz levels and treatment outcomes.

1.1.2 Objectives

1.1.2.1 Main objective

The main objective of the study was to determine the plasma levels of efavirenz and treatment outcomes in Kenyan HIV-TB co-infected patients receiving concomitant Anti-TB therapy and ART.

1.1.2.2 Specific objective

The specific objectives of the study were to;

1. Determine the plasma concentration of EFV in Kenyan HIV-TB co-infected patients on both ART and Anti-TBs.
2. Measure the inter-patient and intra-patient variability in plasma concentrations of efavirenz in this group of patients.
3. Determine the treatment outcomes in this group of patients using various clinical parameters.
4. Identify factors that affect plasma concentrations of EFV in this group of patients.

1.2. LITERATURE REVIEW

1.2.1 Clinical pharmacology of efavirenz

Efavirenz (EFV), empirical formulae $C_{14}H_9ClF_3NO_2$ and molecular mass 315.68, is a non-nucleoside reverse transcriptase inhibitor (NNRTI) specific against HIV-1 whose activity is mediated by non-competitive inhibition of HIV type 1 reverse transcriptase (RT). EFV has additive antiviral activity without cytotoxicity against HIV-1 when combined with other NNRTIs, nucleoside reverse transcriptase inhibitors (NRTIs), protease inhibitors (PIs) and the fusion inhibitor enfurvitide.[12] It is therefore used as a component of HAART in which it is combined with two NRTIs at an adult dose of 600mg daily[10]. Resistance to EFV emerges rapidly in the presence of the drug with *K103N* being the most common reverse transcriptase mutation responsible for the resistance. Cross-resistance among NNRTIs is common[12].

EFV is moderately well absorbed (F = 45 % & t_{max} = 3-5hrs). Steady state plasma concentrations are reached within 6-10 days. High fat/caloric meals are associated with an increase in the absorption of EFV hence it is taken on an empty stomach to minimise toxicity. It is highly plasma protein bound (99.5 - 99.75 %). It is metabolised by cytochrome P450

system (CYP450) to hydroxylated metabolites which are inactive with subsequent glucuronidation. Studies suggest that CYP3A4 and CYP2B6 are the major isoenzymes involved in its metabolism. It is excreted in urine as the metabolites and in faeces as the parent drug.[12]

EFV is a CYP450 enzyme inducer/inhibitor. It induces CYP3A4, increasing its own metabolism (autoinduction) hence a single dose has a half life of 52-76hrs and multiple doses have a half life of 40-55hrs. It also interferes with other drugs metabolized by CYP3A4 such as PIs, statins, carbamazepine, azole antifungals, lorazepam and rifabutin. It also inhibits CYP2C9 and CYP2C19 at concentrations achieved clinically. Other drugs also have an effect on the plasma concentrations of EFV; PIs, carbamazepine and phenobarbitone will reduce the plasma concentrations of EFV while fluconazole will increase it.[12]

The most significant adverse effects of EFV are nervous system symptoms, psychiatric symptoms and skin rash. Nervous system symptoms include dizziness, insomnia, impaired concentration, somnolence, abnormal thinking and dreaming, hallucinations and depersonalisation. It is therefore taken at bedtime to minimise these CNS side effects[12]. Psychiatric symptoms include severe depression, suicidal ideation, nonfatal suicide attempts, aggression, paranoid reactions and manic reactions. The skin rash is reported as a mild to moderate maculopapular skin eruptions.[12] Congenital anomalies have been reported hence it is avoided in pregnancy.[13]

1.2.2 Clinical pharmacology of rifampicin

Rifampicin (RFP) is a semi-synthetic derivative of rifamycin, an antibiotic produced by *Streptomyces mediterranei.* It is active against gram positive and gram negative cocci, some enteric bacteria, mycobacteria and Chlamydia. There is no cross resistance to other classes of antimicrobial drugs but there is cross resistance to other rifamycin derivatives such as rifabutin and rifapentine.[14] RFP acts by binding to the β-subunit of bacterial DNA –dependent RNA polymerase and thereby inhibits RNA synthesis. Resistance results from mutations in the gene for this β subunit of RNA polymerase.[14]

RFP is well absorbed orally and is excreted through the liver into bile. It then undergoes enterohepatic circulation and is excreted in faeces and urine. It is widely distributed in body fluids and tissues, highly plasma protein bound and adequate CSF concentrations are reached only in the presence of meningeal inflammation. It is administered with other anti-TB drugs for the management of active TB usually at an adult dose of 300-600mg per day depending on the

weight resulted in 10% lower plasma concentrations per additional 10 kilograms and suggested that a low body weight especially <55 kilograms results in higher concentrations and vice versa.[11, 19, 22] EFV levels have been noted to be higher in women than men though the difference was not statistically significant.[11]

Studies have shown that increasing the EFV dose from 600 mg daily to 800 mg daily in patients receiving both EFV and RFP provides plasma concentrations of EFV similar to those observed in patients receiving EFV 600mg daily without RFP. Although increasing the EFV dose to 800mg daily cannot be firmly recommended, it seems that a cautious approach to maintain the same plasma concentrations as in those receiving EFV 600mg daily without RFP would be favourable.[11] This is recommended both in the WHO guidelines[4] and the guidelines for ART therapy in Kenya[10] though not practised within the public sector. Patients weighing <55 kgs, Black, Asian and Hispanic patients in whom higher plasma concentrations and a high rate of adverse effects have been observed due to genetic factors would mostly benefit from a dose of 600 mg daily.[11]

Several studies have also shown that a standard dose of 600 mg of EFV is well tolerated, there is no added benefit from increasing the dose of EFV and that high serum concentrations are associated with increased neurotoxicity. These studies show excellent clinical outcomes and recommend the routine use of 600 mg per day even in the presence of rifampicin and increasing the dose to 800 mg daily for patients >60 kg.[5,23, 24]

CHAPTER TWO

METHODOLOGY

2.1 STUDY POPULATION

The study was conducted at the Comprehensive Care Centre (CCC) of Kenyatta National Hospital (KNH). The study population was made up of HIV-TB co-infected black Kenyan patients aged eighteen years and above who clearly consented to participate in the study. These patients were recruited from the CCC of KNH from January 2010 to March 2010.

2.2 STUDY DESIGN

The study design was concurrent descriptive cohort study.

2.3 SAMPLE SIZE AND SAMPLING METHOD

A minimum of 50 HIV-TB co-infected patients were to be recruited based on the assumption of 20% frequency of sub-therapeutic concentration in this group of patients (Sathia *et al*).[18]

$$n = \frac{Z^2 * p\,(1-p)}{d^2}$$

Where: Z- z value for a 95% confidence interval that is 1.96.

p- prevalence for a certain outcome

d- level of significance (5% or 0.05).

n- sample size.

However due to scarcity of patients and limitation of time only 28 patients were recruited. Sampling was by convenience sampling such that all new HIV-TB patients on ART and anti-TBs who met the study inclusion criteria over a period of 3 months were recruited into the study. The ART dispensing tool in the CCC pharmacy was used to identify these patients.

2.4 INCLUSION/EXCLUSION CRITERIA

HIV infected patients older than 18 years who were newly diagnosed of TB were included into the study. These patients needed to be on an EFV based ART regimen for not less than 1 month and on a RFP based anti-TB regimen for not less than 1 month.

Children less than 18 years of age, pregnant women and patients concomitantly using drugs with potential pharmacokinetic interaction with EFV or RFP that is inducers or inhibitors of CYP450 enzyme system were excluded from this study. Patients involved in any other study that may impact on the results of this study were also excluded.

2.5 STUDY PROCEDURES

2.5.1 Data collection

A data collection form was designed and validated for use on every participant (Appendix A).

Patient demographics and baseline tests such as viral load, CD4, liver function tests, full haemogram, creatinine levels and medical examination at the onset of anti-TB therapy were retrospectively obtained by medical record review. Adherence and reported adverse effects were obtained by medical record review at 1-2 months of anti-TB therapy and repeated after a minimum of 2 weeks from the first evaluation. Clinical outcomes of treatment such as chest X ray, sputum smear, viral load, CD4 and reported adverse effects were obtained from the same medical records at the end of anti-TB therapy.

2.5.2 Blood collection and preparation

Phlebotomists working at the CCC laboratory were familiarised with the study protocols and involved in the blood sample collection. Five millimetre blood samples were collected by mid-arm veno-puncture from patients who met the inclusion criteria and placed into well labelled EDTA tubes. Plasma was then prepared from 3.0 ml of whole blood by centrifugation at 10, 000g for 10 minutes. Two millimetres of whole blood was kept in case of any contingencies. All the samples were stored at -20°C until further use.

A full pharmacokinetic design was used in which two blood samples separated by a minimum of 2 weeks was obtained. Blood sampling was done at 12-16 hours (C12-16) post-dose therefore 9 am to 1pm daily for most patients.

2.5.3 Analytical method for the determination of efavirenz in plasma

The total efavirenz concentrations in plasma at steady state were measured using a reverse-phase high performance liquid chromatography (HPLC) with ultraviolet (UV) detection in accordance with the method of Nyakutira *et al.* (2007) with slight modifications. The analytical method was validated for accuracy, precision, stability as well as the recovery of the analyte sequel to its modification over the concentration range of 0.50 to 16.0µg/ml efavirenz. External standardization method was used to determine the standard curve in the concentration range of 0.2 to 16.0µg/ml. Stock solutions A and B containing 10ug/ml and 100ug/ml respectively of the efavirenz reference standard (obtained from the National Quality Control Laboratory) in 1ml of acetonitrile (HPLC grade) were prepared.

Neat standards over the concentration range of 0.2 to 16 ug/ml were then prepared by diluting the stock solutions with the appropriate amount of distilled water. Spiked plasma standards over the concentration range 0.5 to 16 ug/ml were then prepared by diluting the stock solutions with the appropriate amount of blank human plasma (obtained from the National Blood Transfusion Centre, Nairobi). Efavirenz was then extracted from the plasma by liquid-liquid extraction with ice cold acetonitrile.

1000ul of blank mobile phase was then transferred into a HPLC auto sampling vial at a ratio of 70:30 that is 700mls of mobile phase A and 300mls of B. Blank plasma was also prepared using the same procedure for the samples and then transferred into a HPLC auto sampling vial. Off-column pre-treatment of the samples was carried out by liquid-liquid extraction of efavirenz from the plasma samples.

The HPLC system used was a Merck-Hitachi Interface D-7000 series instrument (Made in Japan) made up of quaternary pumps L-7100 fitted with a gradient mixer (Merck-Hitachi) with a system purge and a variable wavelength (200-800nm) ultraviolet-visible detector model L-7400 (Lachrom, Merck-Hitachi) and an 18µL flow cell. Injection was by a Rheodyne model 7725 valve (Cotati, California, U.S.A.) fitted with a 20µL loop. The system is fitted with on-line auto-sampler L-7200 and column oven L-7350 (Merck-Hitachi). The column used was a Phenomenex (C-18) 5µm particle size and 250 x 4.6 mm I.D, reversed phase stainless steel (Gemini) fitted to a guard column.

The mobile phase was made up of a mixture of solution A and solution B. Both solutions A and B consisted of acetonitrile (HPLC grade), 25 mM ammonium acetate buffer (Analar grade) and glacial acetic acid (Analar grade) in proportions of 90:10:0.1 and 10:90:0.1, respectively. These was pumped through the column at a ratio of 70:30 of solutions A and B respectively with a

flow rate of 1ml/min and the analytical run was performed at a column oven temperature of 40 °C. A 50 ul injection volume was used and repeated at a rate of three injections per vial. UV-detection was done at 254nm. The computer system used was a Hewlett Packard (France) computer connected to a Hp 1000 printer.

2.6 DATA ANALYSIS

Data was stored electronically using excel spreadsheet daily. Data that was incomplete was deleted. Statistical analysis was performed by SPSS version 12.0. All variables were subjected to descriptive data analysis. For each variable the mean and standard error of the mean or median and inter-quartile range were determined depending on the distribution of the variable. Exploratory data analysis was performed to reveal patterns in the population data set. Intra and inter-patient variability was determined using the coefficient of variation of replicate determinations. Comparison of 1st and 2nd sampling occasions plasma concentrations was done using Wilcoxon sign rank test and Spearmann's rho correlation.

Comparison of Efavirenz plasma levels by different categories was done using Mann Whitney U test, Kruskal Wallis test and post hoc multiple comparison (determined using Tukey's honestly significant difference, HSD). Correlation of Efavirenz levels with other variables was done using Spearmann's rho correlation coefficient. Factors affecting Efavirenz levels were determined using univariate and multivariate general linear regression (repeat measures). Only variables that showed significant association with plasma efavirenz levels on univariate were included in the multivariate model.

The dependent variable was plasma concentrations of efavirenz. The independent and confounding variables included patient demographic characteristics, patho-physiological traits such as concurrent illnesses, liver function and renal function; level of adherence, concurrent medications and blood sampling intervals.

2.7 CASE DEFINITIONS

The diagnosis recorded in the patient files was used as evidence of the presence of the outcome of interest. This included the presence and type of TB, HIV, any significant abnormalities on physical examination and concurrent illnesses.

2.8 ETHICAL CONSIDERATIONS

The study protocol was submitted and approved by the Kenyatta National Hospital Ethics and Research Committee (KNH-ERC) before initiation of the study. See Appendix B for a copy of the approval letter.

An informed consent form was designed and the study aims, procedures, benefits and risks were clearly explained to every potential study participant before seeking informed consent (Appendix C). A signed informed consent form was obtained from each study participant and a copy issued to them before they were enlisted for the study.

This study was of no benefit to the patient but the local data will be of great benefit to medical practitioners in designing therapy in this group of patients with respect to optimizing treatment outcomes and minimising occurrence of adverse effects, treatment failure and the development of resistance in this group of patients.

CHAPTER THREE

RESULTS

3.1 HPLC ANALYSIS OF EFAVIRENZ IN PLASMA

The average retention time for efavirenz was 7.015min for the neat standards, 6.929min for the spiked plasma standards and 6.921min for the plasma samples. Figure 3.1 is a representative chromatogram of one patient sample from the first sampling occasion. The HPLC resolution of EFV was very good with no interferences from other concomitantly administered medications as well as endogenous substances in the matrix.

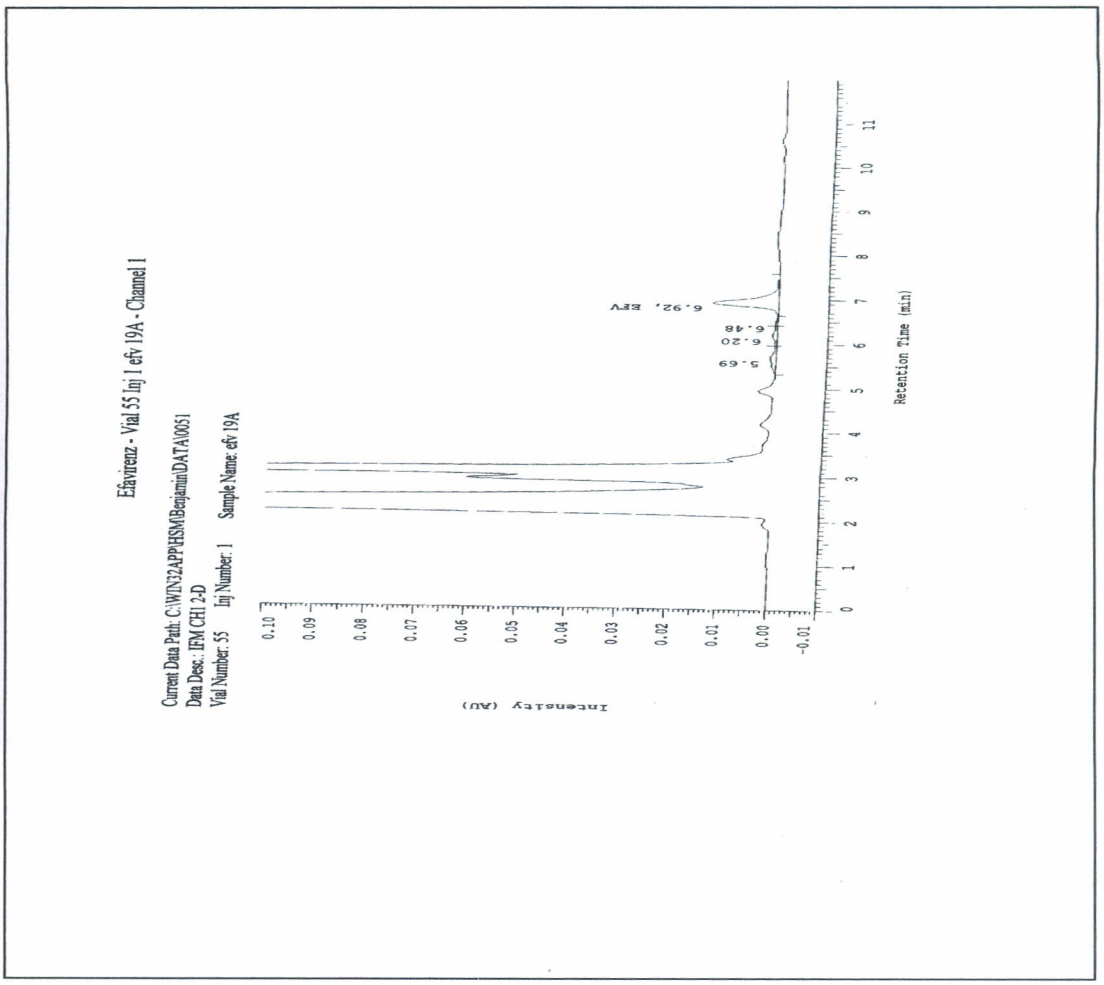

Figure 3.1: HPLC chromatogram of one patient at 1st sampling occasion.

The intra-day precision was measured as the coefficient of variation (CV) of four replicate determinations of the plasma standards analysed on the same day. The inter-day precision was measured as the coefficient of variation of four replicate determinations of the plasma standards analysed on the separate days. The intra-day precision was within acceptable limits as the CV was less than 1 % at every concentration (Table 3.1). The inter-day precision was less than 10 % (Table 3.1). The Lower Limit of Quantitation was 0.2µg/ml.

Table 3.1 Results of Precision studies for Efavirenz in plasma

	Nominal conc.(µg/ml)	**Number of Sample (n)**	**COVAR (%)**
Intra-day	2.0	4	0.27
	4.0	4	0.68
	16.0	4	0.42
Inter-day	2.0	4	3.2
	4.0	4	9.9
	16.0	4	7.9

3.1.1.3 Recovery from plasma

The method also gave a good recovery, which ranged from 93.5% to 109 % for the low (1.0 µg/ml), intermediate (4.0 µg/ml) and high (16.0 µg/ml) concentrations of efavirenz spiked into blank plasma.

3.2 BASELINE CHARACTERISTICS OF THE STUDY POPULATION

Thirty-six patients were identified for this study but 4 patients refused to participate, 2 patients met one or more exclusion criteria and 2 patients died before recruitment. One patient died after the study of cardiovascular arrest. Twenty-eight HIV-TB co-infected patients were successfully recruited into the study.

3.2.1 Demographic characteristics of the study population.

Demographic characteristics are presented in Table 3.2. All patients were Kenyan black patients with different ethnicity. The median age was 37 years with a range of 24 - 62 years, on 1st and 2nd sampling occasions.

Table 3.2: Demographic characteristics at 1st and 2nd sampling occasions.

	1st OCCASION		2nd OCCASION		P value
CHARACTERISTIC	**N=28**	**%**	**N=23**	**%**	
Age in years;					
21 – 30	5	17.9	5	21.7	0.920
31 – 40	12	42.9	10	43.5	
> 40	11	39.3	8	34.8	
Gender;					
Male	12	42.9	10	43.5	0.965
Female	16	57.1	13	56.5	
Bodyweight(kg)					
< 60	18	64.3	14	60.9	
> 60	10	35.7	9	39.1	
Occupation;					
Self employed(Businessman/lady/Farmer)	15	53.6	10	43.5	0.916
Employed(Government/private/Casual)	6	21.4	6	26.1	
Unemployed	4	14.3	4	17.4	
Not indicated	3	10.7	3	13.0	
Place of residence;					
Low socio-economic zones	2	7.1	2	8.7	0.992
CBD / East lands	18	64.3	14	60.9	
Outside Nairobi	6	21.4	5	21.7	
Not indicated	2	7.1	2	8.7	
Educational level;					
None	2	7.1	2	8.7	0.990
Primary	9	32.1	7	30.4	
Secondary	10	35.7	7	30.4	
Tertiary	2	7.1	2	8.7	
Not indicated	5	17.9	5	21.7	
Ethnicity;					
Kamba	9	32.1	8	34.8	0.997
Kisii	1	3.6	7	30.4	
Luo	5	17.9	1	4.3	
Kalenjin	1	3.6	3	13.0	
Luhya	3	10.7	1	4.3	

The median bodyweight was 56.1 kilograms with a range of 43.2 - 74 kilograms, on 1st sampling and 55.7 kilograms with a range of 43.2 - 74 kilograms, on 2nd sampling occasion. There was no statistical difference in age and bodyweight between 1st and 2nd sampling occasion.

Age distribution was similar at 1st and 2nd term. Twelve patients (42.9 %) were males and 16 (57.1 %) were females on 1st occasion while 10 (43.5%) subjects were male and 13 (56.5%) females on 2nd occasion. 64.3 % and 60.9 % of patients had a bodyweight of less than 60 kilograms while 35.7 % and 39.1 % had greater than or equal to 60 kilograms at 1st and 2nd occasions respectively. All demographic characteristics were similar with no statistical difference between 1st and 2nd occasions.

3.2.2 Clinical and laboratory characteristics.

Clinical and laboratory characteristics are summarised in Table 3.3 and 3.4. As expected, a majority of patients, 78.6 % on 1st and 73.9 % on 2nd occasion, were in WHO stage III. Most patients had no significant abnormality detected on physical exam while a significant minority showed varied physical findings with bilateral pedal oedema being the most common while the rest had sensory neural deafness, HIV dementia, abdominal ascites, organomegaly, hepatomegaly, cardiomegaly, abdominal lymphadenopathy, pleural effusion, leg swelling and mild epigastric tenderness. Most patients had no other co-morbidity while approximately 60% had varied co-morbidities on both 1st and 2nd occasion with the most common being oral candidiasis. Others included deep vein thrombosis, esophageal candidiasis, ischaemic heart disease, congestive cardiac failure, heart failure, renal parenchymal disease, epilepsy, convulsive disorder, erythema nodosum, chronic gastroenteritis, chronic kidney disease, HIV associated nephropathy, psychosis, pneumonia, peptic ulcer disease, herpes labialis and glucose intolerance.

A diagnosis of TB and HIV was obtained by medical record review. Diagnostic methods for TB in KNH included microscopy for alcohol fast bacilli (AFBs), chest X ray and clinical diagnosis. At baseline 15 patients had a positive chest X ray while 3 had a negative chest X ray, 4 had a positive and seven a negative sputum smear one had a negative lymph node aspirate and one had a negative lumbar puncture (CSF was clear). On diagnosis therefore, 16 (57.1%) patients had a positive TB test result while 8 had a negative TB test result. Majority of patients had pulmonary TB while five had extra-pulmonary TB.

Table 3.3: Categorised clinical and laboratory characteristics.

VARIABLE	1ST OCCASION N=28	%	2nd OCCASION N=28	%	P VALUE
WHO clinical stage;					
III	22	78.6	17	73.9	0.699
IV	6	21.4	6	26.1	
TB type;					
Pulmonary TB	23	82.1	18	78.3	
TB meningitis	1	3.6	1	4.3	0.998
TB lymphadenitis	1	3.6	1	4.3	
Milliary TB	2	7.1	2	8.7	
TB peritonitis	1	3.6	1	4.3	
TB test;					
Positive	16	57.1	14	60.9	0.964
Negative	8	28.6	6	26.1	
Not indicated	4	14.3	3	13.0	
Albumin;					
Normal (32-50g/L)	5	17.9	5	21.7	
Reduced albumin (<32g/L)	9	32.1	6	26.1	0.875
Not done	14	50.0	12	52.2	
Total Bilirubin;					
Normal bilirubin (<17umol/l)	13	46.4	11	47.8	
Elevated bilirubin	1	3.6	0	0.0	0.658
Not done	14	50.0	12	52.2	
Alanine transaminase (ALT);					
Normal alt (5- 42iu/l)	18	64.3	15	65.2	
Elevated alt (> 42iu/l)	5	17.9	4	17.4	0.998
Not done	5	17.9	4	17.4	
Renal function (ml/min);	24				
Normal (>=90mls/min)	8	28.6	7	30.4	
Mild (60-89mls/min)	8	28.6	8	34.8	
Moderate (30-59mls/min)	7	25.0	4	17.4	0.969
Severe (<=30mls/min)	1	3.6	1	4.3	
Not done	*4*	*14.3*	*3*	*13.0*	
Haemoglobin;					
Normal (>13g/dl for and >	8	28.6	7	30.4	
Anaemic (<13 or <11g/dl)	16	57.1	13	56.5	0.985
Not done	4	14.3	3	13.0	
White Blood cell (*10^{9}cells/l);	*22*				

Normal (4-11)	18	64.3	15	65.2	
Leucopenia (<4)	3	10.7	2	8.7	0.995
Elevated WBC (>11)	1	3.6	1	4.3	
Not done	6	21.4	5	21.7	
Platelets (*10^9cells/l);	23				
Normal (150-400)	19	67.9	15	65.2	
Thrombocytopenic (<150)	4	14.3	4	17.4	0.955
Not done	5	17.9	4	17.4	

Viral load was not done for all the patients. The median CD4 on 1st occasion was 85 and on 2nd occasion 81.5cells/mm^3. 32.1 % patients on 1st and 26.1 % patients on 2nd occasion had hypoalbuminaemia. The median bilirubin on 1st and 2nd occasion was within normal limits. The median Alanine transferase (ALT) levels were 27 iu/l but ranged from 14-229 iu/l on both 1st and 2nd occasion. Renal function test was done by estimating the glomerular filtration rate (GFR) using the Cockroft & Gault equation. The median GFR was 74.5mls/min on 1st and 76.5mls/min on 2nd occasions with 28.6 % of patients on 1st occasion and 30.4 % on 2nd occasion had normal renal function, 28.6 % and 30.4 % had mildly impaired renal function, 25 % and 17.4 % had moderate and only 1 patient (3.6%) on 1st and 2nd occasion had severely impaired renal function.

Table 3.4: Median and range values for clinical and laboratory characteristics.

VARIABLE	1ST OCCASION		2nd OCCASION		P value
	Median	RANGE	Median	RANGE	
CD4 Count (cells/mm^3)	85	3 - 424	81.5	3 – 263	0.786
Albumin (g/L)	29.5	19 – 43	31	19 – 43	0.851
Bilirubin(Umol/l)	8.9	3 - 22.5	7.9	3 - 16.6	0.805
ALT (IU/l)	27	14 – 229	27	14 – 229	1.000
Renal failure (ml/min)	74.5	22 - 152	76.5	22 – 152	0.654
Haemoglobin(g/dl)	10.75	4.14 - 15.3	10.75	4.14 - 15.3	1.000
WBC (*10^{12}/L)	6.07	2.35 - 12.9	6.23	2.35 - 12.9	0.946
Platelets(*10^9/L)	224	97 – 536	219	97 – 420	0.800
ESR (mm/hr)	61	7 – 72	61	7 – 72	1.000
MCV (fl)	84	64.4 – 109	83.8	64.4 – 109	0.980

(P significant at <0.05)

On full haemogram, the median haemoglobin was 10.75 g/dl with 57.1 % of patients on 1st occasion and 56.5% on 2nd occasion being anaemic. Most patients had normal white blood counts (WBC) and platelets. All clinical and laboratory characteristics were similar with no statistical differences between 1st and 2nd occasions.

3.2.3 Anti-retroviral, anti-TB and concurrent medication.

Anti-retroviral, anti-TB and concurrent medication are summarised in Table 3.5. All patients were on a rifampicin based anti-tubercular regimen. Fifty percent of patients on 1st occasion and 43.5 % patients on 2nd occasion were on TDF+3TC+EFV (Tenofovir 300mg OD+ Lamivudine 300mg OD + Efavirenz 600mg OD). Another 28.6 % and 34.5 % of patients on 1st and 2nd occasion were on AZT+3TC+EFV (Zidovudine 300mg BD + Lamivudine 150mg BD+ Efavirenz 600mg OD). About 17.9% and 17.4% of patients on 1st and 2nd occasion were on D4T+3TC+EFV (Stavudine 30mg BD + Lamivudine 150mg BD + Efavirenz 600mg OD) while only 1 patient on 1st and 2nd occasion was on Stavudine 15mg OD + Lamivudine 150mg OD + Efavirenz 600mg OD due severely impaired renal function (Table 3.4).

About 90 % of patients were on co-trimoxazole 960mg once daily, 68 % were on pyridoxine daily, 89 % were on multivitamins and 1 patient was on dapsone daily. Use of over the counter medication and herbal medications were not reported for all patients. Three patients had drug allergies, one a quinolone allergy, one a Sulfadoxine/ Pyrimethamine allergy and the other a non specific drug allergy.

There were 3 cigarette smokers on both 1st occasion and 2nd occasion while there were 7 alcohol users on 1st occasion and 6 on 2nd occasion. Adherence was good in all the patients at 1st and 2nd occasion. Use of medication was similar at 1st and 2nd occasion.

Table 3.5: Drugs used on 1st and 2nd occasion.

CHARACTERISTIC	1st OCCASION N=28	%	2nd OCCASION N=23	%	P value
TB regimen;					
2RHZE/4RH	27	96.4	22	95.7	1.000
2SRHZE/1RHZE/5RHZ	1	3.6	1	4.3	
ART regimen;					
TDF/3TC/EFV	14	50.0	10	43.5	0.962
AZT /3TC /EFV	8	28.6	8	34.8	
D4T/3TC/EFV	5	17.9	4	17.4	
D4T15mgOD/3TC150mgOD/EFV	1	3.6	1	4.3	
Smoking;					
Smoker	3	10.7	3	13.0	0.893
Non-smoker	20	71.4	15	65.2	
Not indicated	5	17.9	5	21.7	
Alcohol consumption;					
Takes alcohol	7	25.0	6	26.1	0.923
Does not take alcohol	16	57.1	12	52.2	
Not indicated	5	17.9	5	21.7	

(P significant at <0.05)

3.3 STEADY STATE EFAVIRENZ PLASMA CONCENTRATIONS

3.3.1 Efavirenz plasma concentrations.

Efavirenz plasma levels on the first and second sampling occasions are presented in Table 3.6.

Twenty-seven concentrations from twenty-eight patients were available on the first sampling occasion. Four patients were not available or refused the second sampling while one was available for the second and not first evaluation. Therefore 23 concentrations from 28 patients were available for second sampling occasion. The median plasma concentrations (Range) at the first and second sampling occasions were 13.17 (0.04 – 52.48) ug/ml and 10.66 (3.54 – 57.61) ug/ml. On 1st sampling one patient (3.7 %) had sub-therapeutic plasma EFV concentrations (<1 ug/ml) while one patient (3.7 %) had therapeutic plasma EFV concentrations (1-4 ug/ml). A majority of patients (92.6 %) had plasma EFV concentrations above the therapeutic range (>4 ug/ml). Out of these 25 patients with elevated levels, 68 % had levels within >4-20 ug/ml while 32% had levels >20 ug/ml.

Table 3.6: Efavirenz plasma concentrations on the first and second sampling occasions.

VARIABLE	N=28	(%)	Median	RANGE
FIRST SAMPLING OCCASION				
Efavirenz levels	**27**		**13.17**	**0.04 - 52.48**
Therapeutic categorization;				
Subtherapeutic (<1ug/ml)	1	3.7		
Therapeutic (1 - 4	1	3.7		
Above therapeutic >4ug/ml	25	92.6		
Interval sampling time	**27**		**15**	**11 - 17**
SECOND SAMPLING OCCASION				
Efavirenz levels	**23**		**10.66**	**3.54- 57.61**
Therapeutic categorization;				
Sub-therapeutic (<1ug/ml)	0	0.0		
Therapeutic (1 - 4 ug/ml)	1	4.3		
Above therapeutic >4ug/ml	22	95.7		
Interval sampling			**14**	**12 – 17**

On the second sampling occasion, no patient had sub-therapeutic plasma EFV concentrations (<1 ug/ml) while only one patient (4.3 %) had therapeutic plasma EFV concentrations (1 - 4 ug/ml). A majority of patients (95.7 %) had toxic plasma EFV concentrations (>4 ug/ml). Out of these 22 patients with elevated levels, 72.7 % had levels between 4 and 20 ug/ml while 27.3 % had levels above 20ug/ml.

The median interval sampling time, (interval between the time of intake of the last dose and time of blood sample collection), was 15 hours with a range of 11 – 17 hours on first sampling and 14 hours and a range of 12 – 17 hours on second sampling. Missing interval sampling

times were replaced with the respective median. Adherence was 100 % for all the patients both on 1st and 2nd sampling occasions.

Figure 3.3 is a scatter plot of the efavirenz levels at first and second sampling occasions. This figure demonstrates the distribution of efavirenz levels below, within and above the therapeutic range. The two horizontal lines in the chart area correspond to the therapeutic range of 1-4 ug/ml.

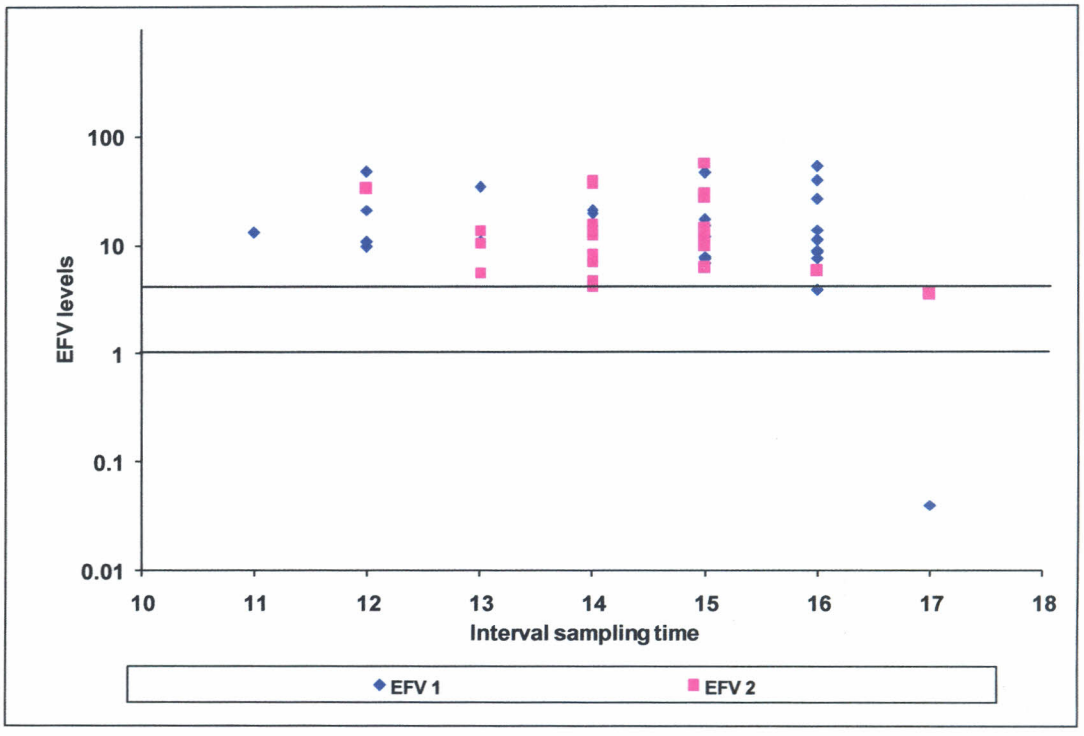

Figure 3.3: A scatter plot of efavirenz levels at 1st and 2nd sampling occasion against the interval sampling time.

3.3.2 Intra and inter-patient variability

Intra-patient variability was calculated as a mean of the coefficients of variation between the two plasma concentrations for each of the patients. A mean of the coefficients of variation of 32.5% was obtained (Table 3.7). Inter-patient variability was calculated as the coefficient of variation between the plasma concentrations of the various patients on the same sampling occasion. This resulted in 77.8% variability between patients in the 1st sampling occasion and 86.4% variability between patients in the 2nd sampling occasion.

Table 3.7: Intra-patient variability of Efavirenz plasma concentrations.

Patients	Mean	SD	CV
P1	11.6	4.9	42.5
P2	10.5	4.8	45.5
P3	7.2	5.1	71.5
P4	37.0	13.2	35.6
P5	9.0	2.0	22.6
P6	42.2	8.1	19.1
P7	48.5	12.8	26.4
P8	23.3	8.8	37.7
P9	34.0	0.6	1.8
P10	6.4	3.1	48.6
P11	12.4	3.6	28.9
P12	9.2	2.1	22.9
P13	13.7	0.7	5.2
P14	12.0	4.1	34.4
P15	7.9	0.1	1.1
P16	17.5	5.1	29.1
P17	11.0	1.8	16.0
P18	32.1	8.2	25.5
P19	4.3	0.5	11.7
P20	6.3	0.8	12.1
P21	8.4	3.2	38.1
P22	2.9	4.0	139.4
	Mean CV		32.5%

(SD-standard deviation)

Patient 22 in Table 3.7 above had a very high intra-patient variability (see 3.3.3 below).

The median concentration ratio of the 1st sampling occasion to the 2nd sampling occasion was 1.24. A median plasma EFV concentration comparison between 1st and 2nd sampling occasions in patients who were available for both gave a median concentration ratio of 1.14 and coefficients of variation of 79.9% and 87.2% for 1st and 2nd sampling occasions. The difference between 1st and 2nd sampling median concentrations was not significant using the Wilcoxon sign rank test (P = 0.6).

A linear correlation of the 1st sampling versus the 2nd sampling EFV plasma concentrations for the 22 patients available at both 1st sampling and 2nd sampling showed a Spearmans rho

correlation coefficient of 0.729 with a strength (correlation of determination) of 0.53 (Figure 3.4). This plot shows that variability of 2nd occasion efavirenz levels is explained to an extent of 53% by variability in 1st occasion efavirenz levels.

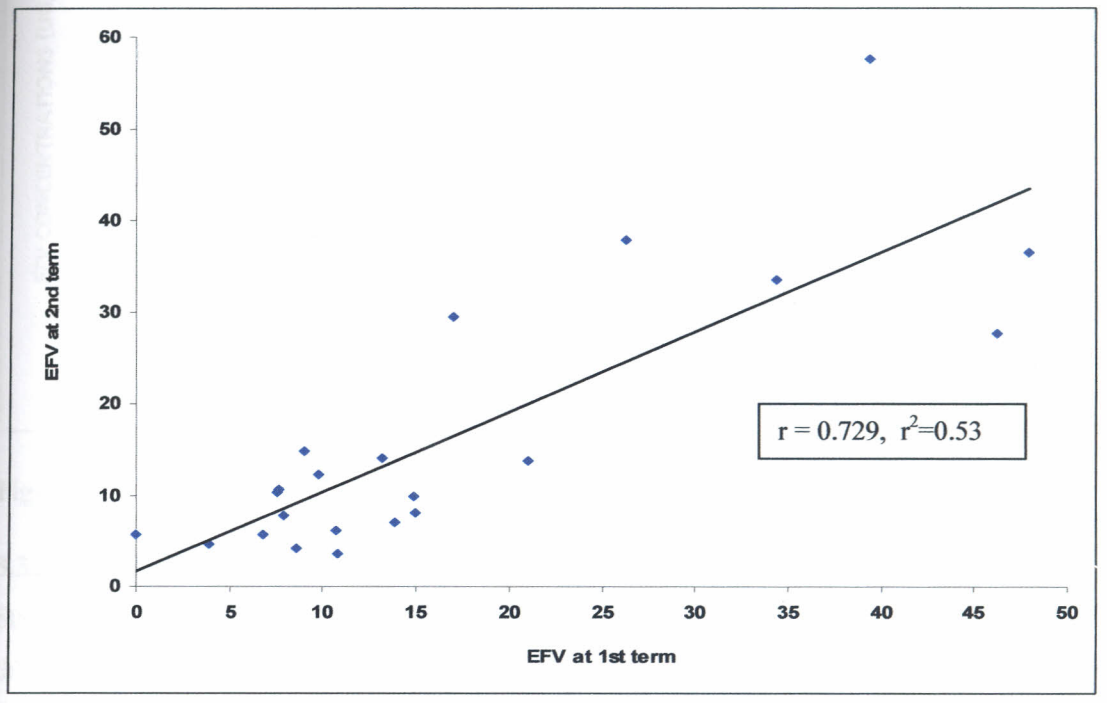

Figure 3.4: Linear correlation (r=Spearman's rho correlation coefficient) of the intra-observed measurements of the EFV at 1st and 2nd sampling occasions (n=22).

On the other hand, Figure 3.5 depicts the dispersion of efavirenz levels both at first and second sampling occasions. There seems to be a clustering effect with majority of patients having plasma concentrations within 0 – 20 ug/ml and a significant minority inclusive of the outliers being above 20 ug/ml. As a result an arbitrary antinode of 21 ug/ml was seen.

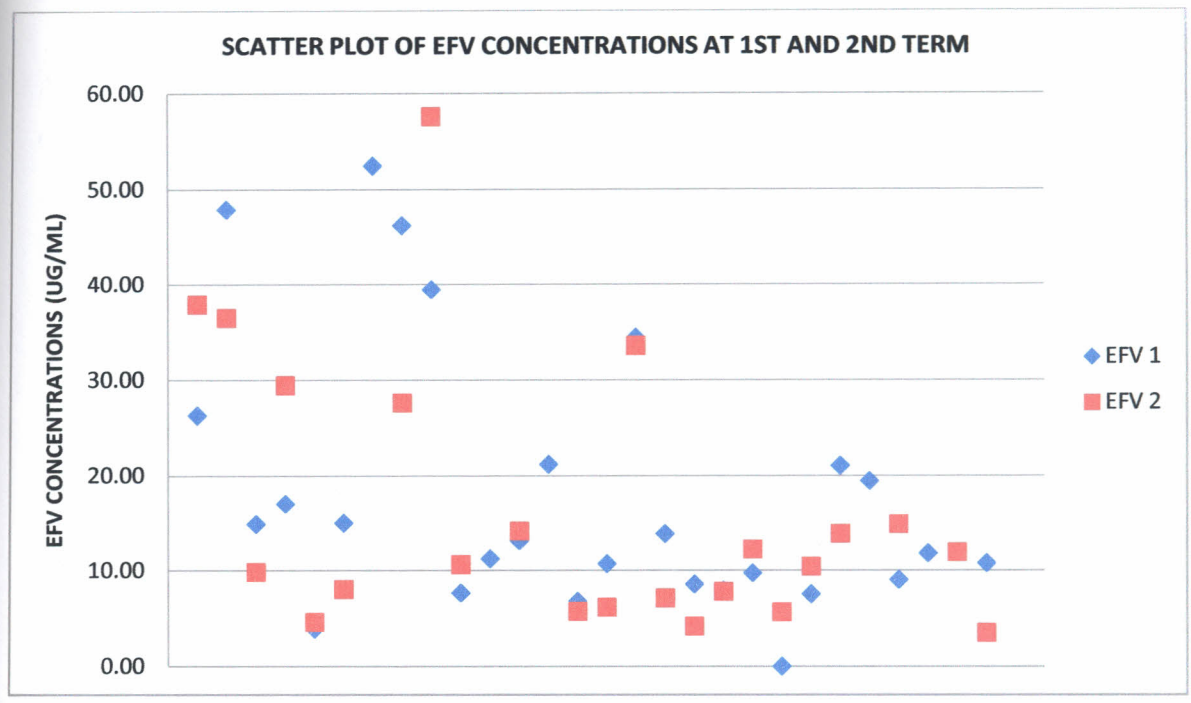

Figure 3.5: Scatter plot of Efavirenz concentrations on 1st and 2nd term evaluation.

3.3.3 Distribution characteristics of efavirenz levels

Steady state efavirenz plasma concentrations were not normally distributed and were skewed to the right (high EFV levels). A log transformation and a square transformation were attempted but could not convert the data to normality. Figures 3.6 (a) and (b) present this distribution. A bimodal distribution is suggested at both first and second sampling though this was not statistically significant due to a small sample size.

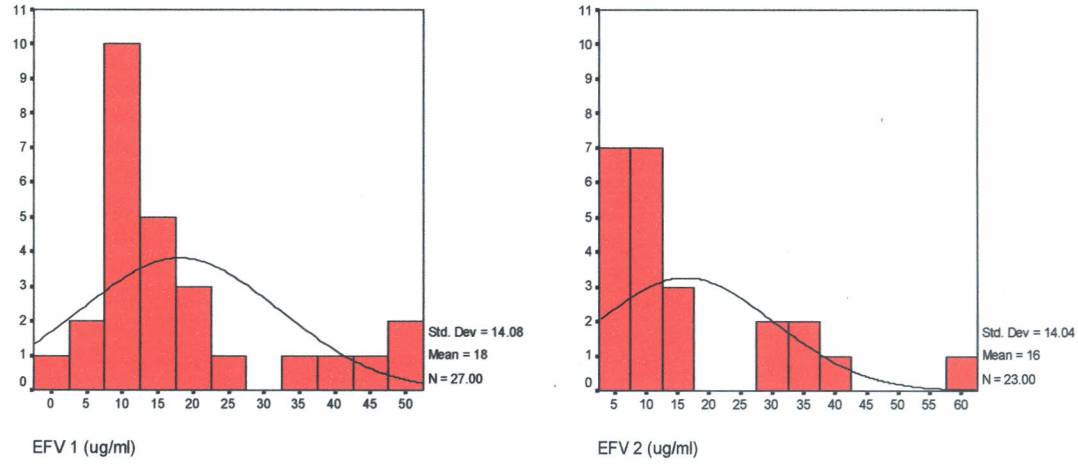

Figure 3.6 (a) and (b): Distribution polygons of efavirenz levels at 1st sampling (a) and 2nd sampling (b) occasions.

An outlier test using a box plot was performed. Four patients were identified as outliers, three on 1st sampling and one on second sampling occasions. A search for common traits was conducted. Interestingly, three of these patients were female and one was male. All had a bodyweight of less than 60 kilograms and in fact had an average bodyweight of 47.6 kilograms. Three were of Kamba ethnicity while one was Luo. All were on co-trimoxazole prophylaxis, pyridoxine and multivitamins. The one patient picked on 2nd sampling had severe renal failure.

One patient had very low levels of 0.04 ug/ml on 1st sampling but had 5.67 ug/ml on 2nd sampling clearly raising issues on adherence. This same patient had a very high intra-patient variability of 139.4 % (Table 3.7). Though there was no indication of poor adherence in the medical records, this patient was a former street boy with who skipped clinic visits frequently.

3.4 FACTORS THAT INFLUENCED EFAVIRENZ LEVELS.

Exploratory data analysis was carried out to identify key variables that were determinants of efavirenz plasma levels.

3.4.1 Correlation of EFV levels with continuous variables.

A Spearman rho correlation was performed between EFV levels at 1st and 2nd sampling and various continuous variables. Only bodyweight and bilirubin levels correlated with efavirenz levels at both first and second sampling occasions (Table 3.8). Bodyweight depicted a negative correlation such that increasing bodyweight resulted in lower EFV levels. Bilirubin depicted a positive correlation such that high EFV levels resulted in higher bilirubin levels. All the other continuous variables were found not to be correlated with efavirenz levels.

Table 3.8: Correlation between EFV at 1st and 2nd sampling occasions and other continous variables.

Variable	1ST OCCASION			2ND OCCASION		
	N	R	P value	N	r	P value
Age (yrs)	27	-0.034	0.866	23	0.103	0.640
Bodyweight(kg)	27	-0.524	**0.005***	23	-0.407	**0.054***
CD4 Count	26	-0.047	0.819	22	0.058	0.799
Albumin	13	-0.143	0.641	11	-0.200	0.555
Bilirubin (umol/l)	13	0.589	**0.034***	11	0.610	**0.046***
Alt (IU/l)	22	0.001	0.996	19	-0.031	0.901
Renal function (ml/min)	23	-0.290	0.180	20	-0.365	0.113
Haemoglobin	24	-0.354	0.090	20	-0.167	0.482
White Blood Cells	22	-0.290	0.191	18	-0.408	0.093
Platelets	23	0.205	0.349	19	0.100	0.684
ESR (mm/hr)	13	0.452	0.121	12	0.014	0.965
Mean Corpuscular	23	-0.224	0.304	19	-0.274	0.256
Interval (sample conc.	27	-0.262	0.187	21	-0.082	0.724
CD4 change (outcome)	16	0.026	0.922	23	0.103	0.640

*** significant at P<0.05.** (**r**-Spearman rho correlation coefficient)

3.4.2 Efavirenz levels across categorical variables.

Efavirenz levels were compared across various subgroups. Only three categories showed statistically significant differences across the groups namely ethnicity, bodyweight and gender. Table 3.9 presents the nonparametric comparison and post hoc multiple comparison (determined using Tukey's honestly significant difference, HSD) of EFV levels by different categories of variables.

Table 3.9: Efavirenz comparisons by different categories.

					P value (Man Whitney U test)	
	BODYWEIGHT	**N**	**Median**	**Range**	**Variable (I : J)**	**P value**
1ST OCCASION	< 60 kg	17	17.06	6.81 - 52.48	<60 : >=60	**0.023***
	>= 60 kg	10	9.90	0.04-19.46		
2ND OCCASION	< 60 kg	14	13.07	3.54 - 57.61		
	>= 60 kg	9	8.08	4.63 - 14.90		0.224
	GENDER					
1ST OCCASION	Male	12	10.8	0.04 - 46.3	Female : Male	**0.064**
	Female	15	17.1	3.9 - 52.5		
2ND OCCASION	Male	10	7.5	3.5 - 27.7		
	Female	13	12.3	4.2 - 57.6		0.107
	ETHNICITY					
1ST OCCASION	Kikuyu	8	10.2	3.9-34.4	Kikuyu : Others	1.000[a]
	Kamba	9	21.1	8.6-47.9		
	Others	10	12.5	0.04-52.5		
	P value (Kruskal wallis test)			0.112	Kamba : Kikuyu	**0.076**[a]
2ND OCCASION	Kikuyu	8	10.1	4.6-33.6		
	Kamba	7	27.7	3.5-57.6	Kamba : Others	**0.048**[a] *
	Others	8	9.4	5.7-29.5		
	P value (Kruskal wallis test)			0.453		
	REGIMENS					
1ST OCCASION	TDF	14	12.86	7.69 - 47.93	TDF : AZT	1.000[a]
	AZT	7	9.07	6.81 - 34.44		
	D4T	6	20.68	0.04 - 52.88		
	P value (Kruskal wallis test)			0.564	D4T : TDF	1.000[a]
2ND OCCASION	TDF	10	10.26	3.54 - 36.51		
	AZT	8	12.92	4.20 - 33.58	D4T : AZT	1.000[a]
	D4T	5	8.08	4.63 - 57.61		
	P value (Kruskal wallis test)			0.969		

(*significant at P value<0.05, [a]- P value calculated by post hoc multiple comparison)

3.4.2.1 Gender

The median (Range) plasma EFV concentration on 1st and 2nd sampling respectively was 17.1(3.9 – 52.5) ug/ml and 12.3 (4.2 – 27.7) ug/ml for females and 10.8 (0.04 – 46.3) ug/ml and 7.5 (3.5 – 27.7) ug/ml for males. This difference was not statistically significant (Figure 3.7 and Table 3.9). Though females had higher plasma EFV concentration than men though the difference was not statistically significant (P = 0.064).

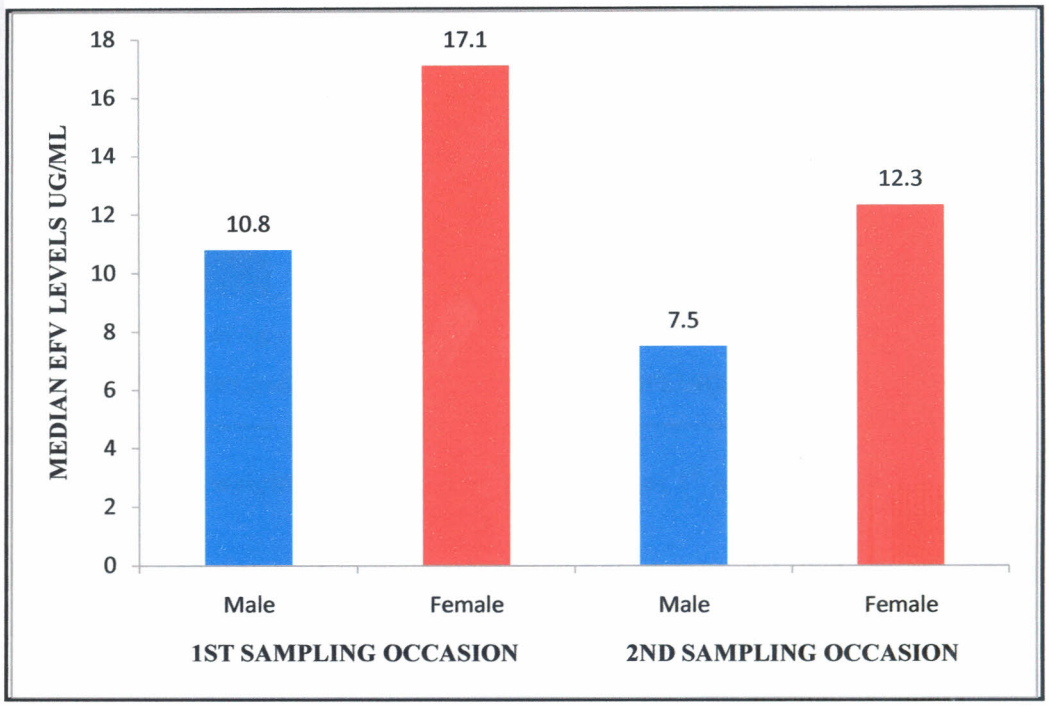

Figure 3.7: Gender comparison of EFV levels on 1st and 2nd sampling occasions.

3.4.2.2 Bodyweight

As depicted in Figure 3.8, patients weighing less than 60 kg had higher median EFV plasma concentrations compared to those weighing above 60 kg on both first and second occasions. The median plasma concentrations across the body weight categories are presented in Table 3.9. This difference in plasma concentrations was statistically significant on 1st occasion (P = 0.023) but not on second sampling (P = 0.224).

Most females (86.7 %) weighed less than 60 kilograms compared to 33.3% of male patients. In patients weighing less than 60 kilograms, female patients (median (IQR); 21.08 (0.04 – 19.46) ug/ml) still had higher EFV levels than male patients (median (IQR); 12.34 (6.81 – 46.26)

ug/ml) in this bodyweight category though the difference in median plasma concentrations was not statistically significant (P = 0.412).

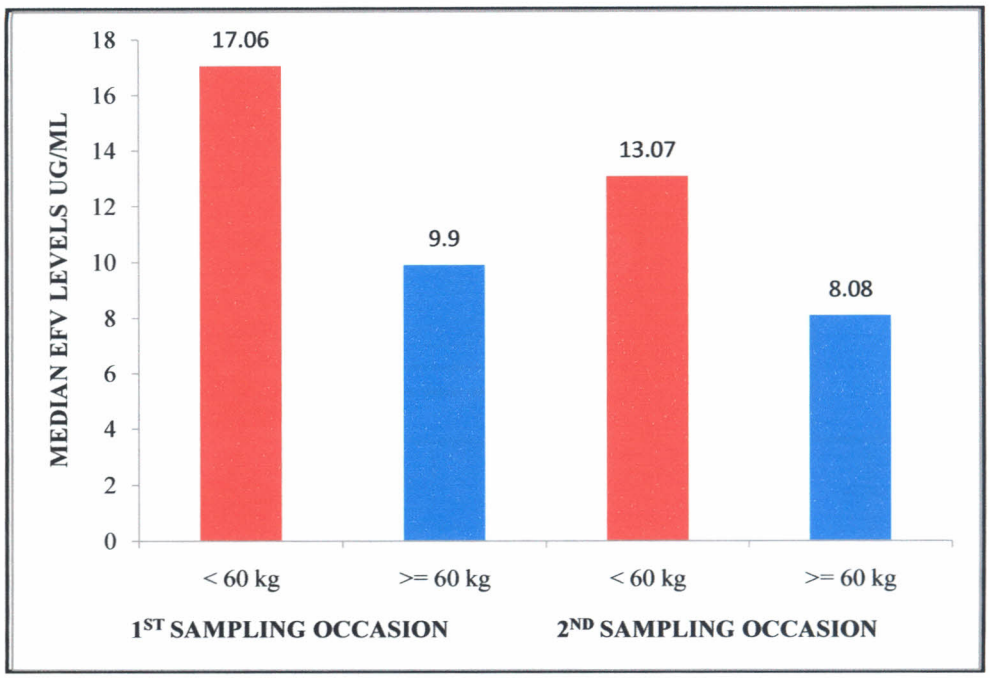

Figure 3.8: Bodyweight comparison of EFV levels at 1st and 2nd sampling occasions.

Only 13.3 % of female patients compared to 66.7 % of male patients had bodyweights above or equal to 60 kilograms. In these patients weighing above or equal to 60 kilograms, male (median (IQR); 9.90 (0.04 – 19.46) ug/ml) and female (median (IQR); 9.90 (0.04 – 19.46) ug/ml) patients had similar EFV levels in this bodyweight category (probably because only two females were in this category) and the difference in median plasma concentration was not statistically significant (P = 1.00).

3.4.2.3 Ethnicity

As presented in Figure 3.9, the Kamba had higher median plasma efavirenz concentrations than the Kikuyu, Kisii, Luhya and Kalenjin. The median plasma efavirenz concentrations across the various ethnic groups are shown in Table 3.9. Though highest for the Kamba, this difference was not statistically significant by Kruskal Wallis test. Post hoc multiple comparison (Table 3.9) showed no significant difference between kikuyu and the others. However between Kamba and Kikuyu, a difference emerges though not statistically significant (P = 0.076). Between the Kamba and Kikuyu, a significant difference is present (P = 0.048).

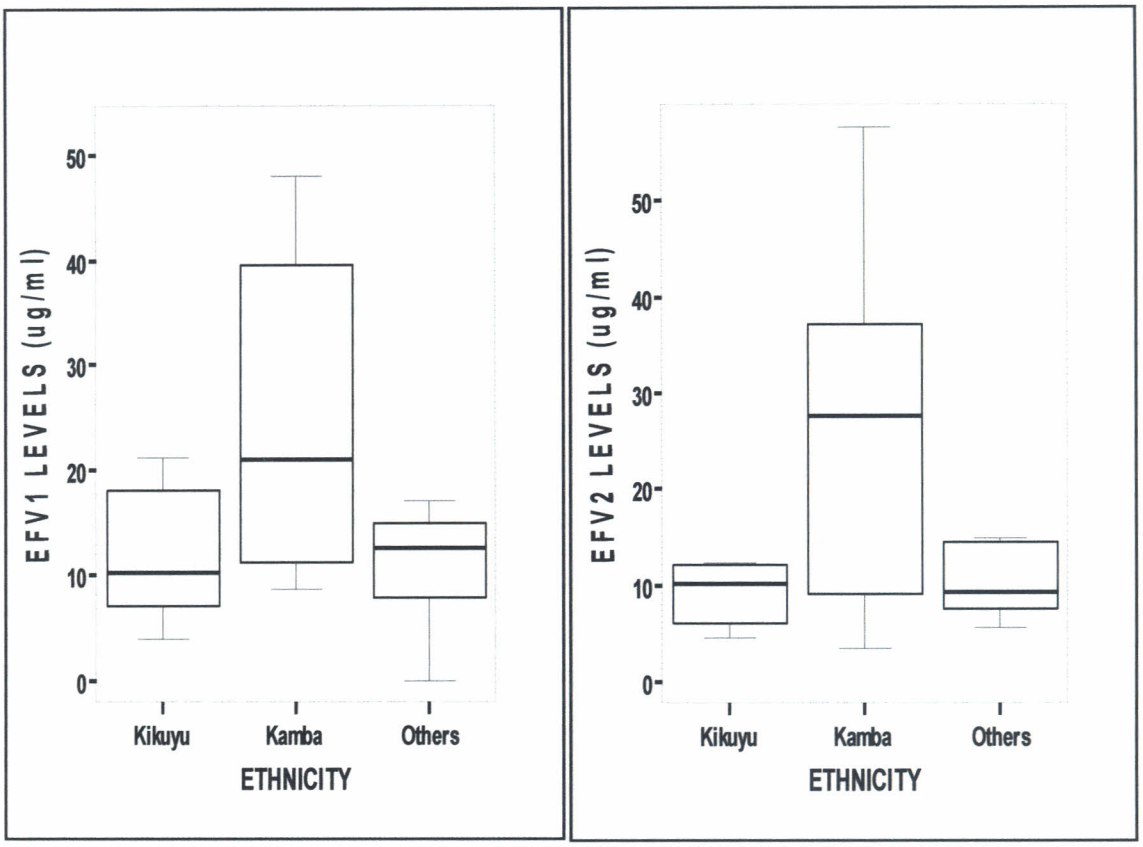

Figure 3.9 (a) and (b): Ethnic comparison of efavirenz levels at 1st (a) and 2nd (b) sampling occasions.

3.4.2.4 Other factors

Although the median efavirenz concentrations varied across the ART regimens (Figure 3.10), these differences were not statistically significant (P = 0.564). D4T presented with the highest variation, 89.7% on 1st sampling and 104.8% on 2nd sampling. Post hoc multiple comparison showed no significant difference between this regimens (Table 3.9).

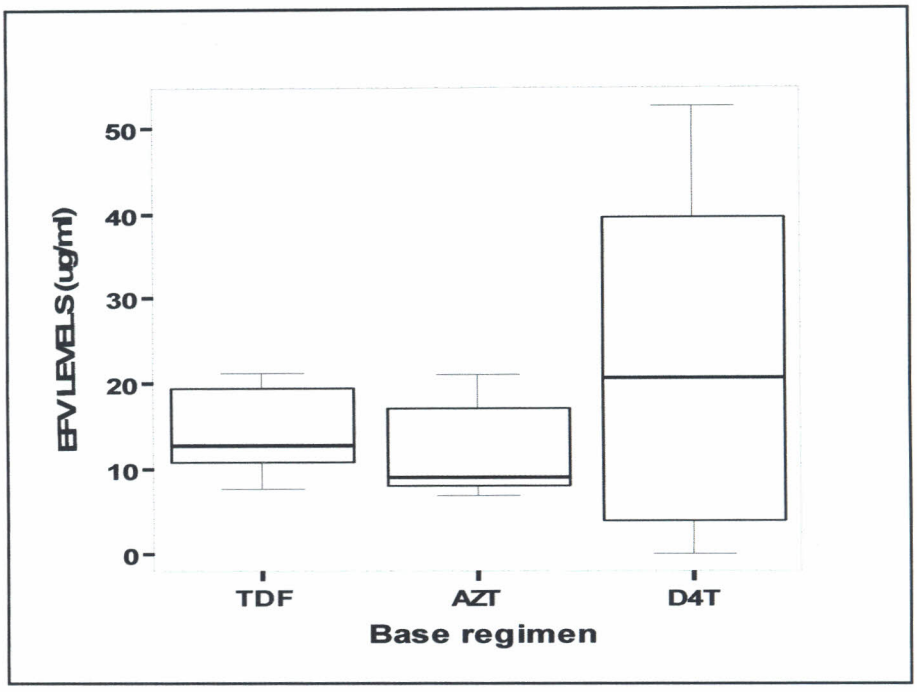

Figure 3.10: Box-plot of EFV levels by different ART regimens.

Though not statistically significant, hypo-albuminaemic patients did show higher efavirenz levels compared to patients with normal albumin levels (Table 3.9). Patients with moderate or severe renal impairment had higher efavirenz levels compared to those with mild renal impairment and normal renal function. These differences were also not statistically significant (Table 3.9).

Interestingly, non-smokers and non-alcohol consumers had a higher EFV levels than smokers and alcohol consumers respectively though not statistically significant. The other categorical variables such as age, presence of co-morbidities, WHO clinical stage, anaemia, occupation, residence, education, white blood cells, platelets, mean corpuscular volume and erythrocyte sedimentation rate were found not to be key determinants of efavirenz levels.

3.4.3 Multivariate analysis using General Linear Regression.

Key variables identified by exploratory data analysis were subjected to univariate and multivariate analysis using a general linear regression (repeat measures). This was done to ascertain the magnitude of the contributions of each factor (independent variable) to the total variation in EFV levels (continuous dependent variable) both at 1st and 2nd sampling occasions (Table 3.10). A factor was considered significant if the P value was < 0.05.

Table 3.10: Univariate and multivariate analysis of the plasma efavirenz concentration using a General Linear Regression.

Source of variability	Univariate analysis			Multivariate analysis		
	R^2	F	P value	R^2	F	P value
Body weight	0.214	5.453	**0.030**	0.040	0.701	**0.414**
Ethnicity	0.310	4.263	**0.030**	0.203	2.166	**0.145**
Gender	0.133	3.074	**0.095**	0.064	1.168	**0.295**
Residence	0.165	1.685	0.215			
Smoking	0.092	1.519	0.237			
Albumin	0.117	1.061	0.333			
Use of pyridoxine	0.045	0.857	0.367			
CYP40	0.040	0.836	0.372			
Age	0.036	0.750	0.397			
MCV	0.040	0.710	0.411			
WHO clinical staging	0.032	0.668	0.423			
Occupation	0.069	0.593	0.564			
Level of education	0.035	0.539	0.474			
Renal failure	0.058	0.493	0.620			
ART Regimen	0.026	0.256	0.777			
CD4 change	0.027	0.247	0.631			
Alcohol consumption	0.015	0.224	0.643			
Haemoglobin	0.012	0.222	0.643			
Co-morbidity	0.010	0.193	0.665			

***significant at P<0.05.**

Bodyweight (R^2=.214, F=5.453, P value=0.030) and ethnicity (R^2=.310, F=4.263, P value=0.030) were statistically significant in contributing to the total variation in plasma EFV concentration (P value= 0.03 and 0.03 respectively). All other factors entered into the univariate model were not significant contributors to the variation in plasma EFV concentrations.

Gender was the third most significant factor in the univariate analysis though not statistically significant (P = 0.095). Since majority of females (86.7 %) had their bodyweight less than 60kgs while a majority of males (66.7 %) had their bodyweight greater than or equal to 60kgs, gender was included into a multivariate model together with bodyweight and ethnicity. The contribution of bodyweight, gender and ethnicity to the total variation in plasma efavirenz concentrations was reduced and in fact, bodyweight and ethnicity were no longer statistically significant (P = 0.414 and 0.145 respectively). This reduction was greatest for bodyweight

followed by gender and ethnicity. Therefore multivariate analysis showed that none of the variables was a significant contributor to the variation in the levels of EFV.

3.5 TREATMENT OUTCOMES

The outcomes of interest were viral load, CD4 counts and cure from TB. Unfortunately vira load was not available for all patients since it's not considered a vital baseline test. The mediar CD4 at the end of treatment was 188cells/mm^3 with an IQR of 103-316.75cells/mm^3. A comparison of median CD4 counts at baseline with another CD4 count at a minimum of 3months from the baseline CD4 gave a median difference of 86 cells/mm^3 and this difference was statistically significant (P = 0.013). Correlation of this CD4 change with efavirenz levels at first and second sampling was not significant (Table 3.11). On general linear regression (Table 3.10), CD4 change was not a significant factor (R^2 = 0.27, F = 0.247, P = 0.631)

Chest X ray and sputum smear results were not available for many patients. Three patients had a negative sputum smear and another three patients a negative chest X ray. Only one patient had a positive lymph node aspirate. Therefore, one patient still had a positive TB test result while four had a negative TB test result.

Table 3.11: Treatment outcomes of interest.

VARIABLE	N=28	%	Median(IQR)	RANGE
CD4 count	16		188 (103 - 316.75)	21 – 453
Baseline CD4	16		102 (71.25 - 194.5)	(4 - 424)
Median Difference			86	
P value			**0.013**	
TB;				
Positive	1	3.6		
Negative	4	14.3		

***significant at P<0.05**

3.6 ADVERSE EFFECTS EXPERIENCED DURING THE STUDY

As presented in Table 3.12, 82.1% of patients reported no adverse effects at baseline. That is 17.9 % reported an adverse effect including one (3.6 %) patient who reported abdominal pain,

nausea and vomiting; one (3.6 %) patient reported a skin reaction; one (3.6 %) patient drug induced hepatitis and two (7.1 %) patients skin rash with pruritus.

Nineteen (67.9 %) patients reported no adverse effects at first sampling. Therefore 32.1 % had experienced an adverse effect including; two patients (7.1 %) who reported headaches, one patient (3.6 %) reported dizziness, four patients (14.3 %) reported drug induced hepatitis, two patients (7.1 %) reported a skin rash, one (3.6 %) reported peripheral neuropathy and three (10.7 %) reported pruritus.

Twenty-five (89.3%) patients reported no adverse effects at second sampling. Therefore 10.7 % experienced an adverse effect which included e patient (3.6 %) who reported lactic acidosis, one patient (3.6 %) reported peripheral neuropathy, one (3.6 %) reported darkening of skin and two (7.1 %) reported pruritus. Interestingly, **no** adverse effects were reported in all patients at the end of TB treatment. These adverse effects were categorised into minor adverse effects (occurred early in treatment) and major adverse effects (occurred late in treatment) as shown in Table 3.12.

Table 3.12: Prevalence and timing of adverse effects.

ADVERSE EFFECT	NUMBER OF EPISODES(%)	AT BASELINE	1ST SAMPLING	2ND SAMPLING	END OF ANTI-TBS
Prevalence (%)		17.9 %	32.1 %	10.7 %	0 %
MINOR (EARLY) ADVERSE EFFECTS;					
Abdominal pain, nausea, vomiting	1 (4.2%)	√			
Skin reaction	1 (4.2%)	√			
Skin rash	4 (16.7%)	√√	√√		
Pruritus	7 (29.2%)	√√	√√√	√√	
MAJOR (LATE) ADVERSE EFFECTS;					
Drug induced hepatitis	5 (20.3%)	√	√√√√		
Headaches	2 (8.3%)		√√		
Dizziness	1 (4.2%)		√		
Peripheral neuropathy	1 (4.2%)			√	
Lactic acidosis	1 (4.2%)			√	
Darkening of skin	1 (4.2%)			√	
Total	24				NONE

CHAPTER FOUR

DISCUSSION

In this study, the plasma concentrations of efavirenz, the intra-patient and inter-patient variability, treatment outcomes and factors affecting plasma EFV concentrations in Kenyan HIV-TB co-infected patients were investigated. This is the first study of EFV levels in HIV-TB co-infected patients in Kenya. This study was also significant in that it identifies the key variables that may determine efavirenz levels in the Kenyan population.

Only 3.6 % of patients on first and none on second occasion had sub-therapeutic levels (<1ug/ml) while only 3.6 % of patients on first and 4.5 % on second occasion had therapeutic levels (1-4ug/ml). Over 90 % of patients had toxic levels (>4ug/ml) on the first and second sampling occasions. About 70 % of patients with toxic levels had levels between 4 and 20 ug/ml while about 30 % had levels above 20ug/ml. As a result an arbitrary antinode of 20 ug/ml was observed which could be used to separate the slow metabolizers from the rest of the subjects.

This antinode could correspond to two groups, those with less than 20 ug/ml for extensive and intermediate metabolizers and those with above 20 ug/ml for poor metabolizers. Sub-therapeutic plasma concentrations have been associated with treatment failure and may select for viral resistance while toxic plasma concentrations increase the risk of adverse reactions especially central nervous system effects[15, 17, 25]. In a study by Gounden *et al*, patients with no side effects had significantly lower efavirenz plasma concentrations versus those that experienced the most side effects[27].

This high EFV levels are in agreement with other studies in African populations. In one study by Nyakutira *et al*, EFV plasma concentrations were above 4mg/l in 50 % of HIV/AIDS outpatients in Zimbabwe[25]. Race has been identified as a predictive factor with consistently higher EFV plasma concentrations in non-caucasian patients. Lower clearance values have been observed in Asian and Black subjects relative to Caucasian subjects[25]. This could be explained by a high prevalence of the CYP2B6 516G→T (*6) allele variant of 49 % in people of African origin that is associated with reduced enzyme activity and high EFV plasma concentrations. The prevalence's of extensive metabolizers (GG) of 30 %, intermediate

metabolizers (GT) of 44 % and poor metabolizers (TT) of 27 % has been reported in Zimbabwe[25]. In agreement, a frequency of GG genotype of 36 %, GT genotype of 41 % and TT genotype of 23% has also been reported in South Africa[27].

An intra-patient variability of 32.5 % was observed and this could be explained by variation in the interval sampling times and slight physiological changes that may affect drug absorption and elimination. Inter-patient variability was 77.8 % at first sampling and 86.4 % variability at second sampling. This is in agreement with several studies that have reported a wide inter-individual variability of EFV plasma concentrations as high as 118 % [25] and110 % [26]. A high inter-individual variability in efavirenz concentrations has also been reported in South Africa[27]. This high inter-patient variability is attributed to gender, race and genotype[17, 26]. Adherence could have also contributed to this since it was based on the opinion of the attending doctor.

Females consistently had higher efavirenz plasma concentrations than male patients though not statistically significant in this study. Lopez-Cortez *et al* also found higher EFV levels in women than men though the difference was also not statistically significant[11] while Burger *et al* and Nyakutira *et al* found that females had significantly higher plasma EFV levels than males[17]. Female patients have been shown to be 1.5-1.7 times more susceptible to efavirenz adverse drug reactions and also have a 2.2 times higher discontinuation rate of efavirenz therapy[17, 25]. African females have been estimated to have approximately 70 % metabolic capacity compared to African males[25]. The reason for this metabolic difference between men and women is unknown[25]. Female patients had a lower mean bodyweight than male patients with 86.7 % of female patients compared to 33.3 % of male patients weighing less than 60 kilograms. A lower bodyweight in females could therefore also explain the higher plasma levels in females. This is in agreement with the findings of Burger *et al*[17]. Single nucleotide polymorphisms (SNP's) in the regulatory regions of CYP2B6 might also explain the gender difference in CYP2B6 expression and activity hence the need to genotype for both SNP's associated with reduced activity and SNP's in the regulatory region[25]. In contrast, product literature for Sustiva® states that the pharmacokinetics of efavirenz in patients appears to be similar between men and women[12].

Bodyweight was a significant factor in contributing to the total variation of efavirenz levels with patients weighing less than 60 kilograms had significantly higher EFV levels than those weighing >=60 kilograms. Bodyweight depicted a negative correlation such that increasing bodyweight resulted in lower EFV levels. This is in agreement with several studies that showed

that increasing bodyweight resulted in lower plasma EFV levels and vice versa[11, 17, 19, 22]. However, in a multivariate analysis of gender, ethnicity and bodyweight, bodyweight was no longer associated with higher plasma concentrations (P = 0.41). This is in agreement with the findings of Burger *et al.*[17].

Ethnicity was also a significant factor in contributing to the total variation of efavirenz levels. This is the first study to demonstrate ethnicity based differences in the plasma efavirenz levels in Kenyan HIV-TB co-infected patients. Higher efavirenz levels in the Kamba compared to Kikuyu, Kisii, Luhya, Luo and Kalenjin are demonstrated. This could probably be due to pharmacogenetic differences between these ethnic groups and it could be hypothesised that the Kamba are by majority poor metabolizers. However on multivariate analysis, ethnicity also became not significant. So although bodyweight and ethnicity were significant determinants of efavirenz levels on univariate analysis, multivariate analysis of bodyweight, ethnicity and gender showed that they were not statistically significant determinants of efavirenz levels. This was attributed to a small sample size. A larger study is therefore required to determine significance of these variables as determinants of efavirenz levels.

Median EFV levels, inter-quartile range, range and inter-patient variability were highest on the stavudine based regimen compared to tenofovir or zidovudine based regimens. This observation was not clearly understood since drug interaction studies provided in product literature for Sustiva® indicates minimal interaction between efavirenz and AZT, 3TC and TDF. Tenofovir is reported to have no change or an increase or decrease of < 10 % of Cmax, Cmin and AUC of EFV and vice versa. EFV is reported to have no change or an increase or decrease of < 10 % of Cmax and AUC of AZT and 3TC but increases the Cmin for lamivudine by 265 % and increases the Cmin for zidovudine by 225 %. No drug-drug interaction between efavirenz and stavudine is described.

While not statistically significant, bilirubin depicted a positive correlation with efavirenz such that high EFV levels resulted in higher bilirubin levels depicting increasing hepatotoxicity with increasing efavirenz levels. However, this was not matched by elevated alanine transaminase levels. In fact, nearly all hepatic events were associated with onset of anti-TB therapy.

While also not statistically significant, hypoalbuminaemic patients had higher EFV levels than patients with normal albumin levels. This negative correlation is expected since efavirenz is highly plasma protein bound (99.5-99.75 %), especially to albumin[12, 17].

The correlation between renal function and efavirenz levels was not statistically significant. However, patients with moderate or severe renal impairment had higher efavirenz levels compared to those with mild renal impairment and normal renal function. This is in agreement with the product literature for Sustiva® which states that although the pharmacokinetics of efavirenz have not been studied in patients with renal insufficiency, less than 1% of efavirenz is excreted unchanged in urine and so the impact of renal impairment on efavirenz elimination should be minimal[12].

Treatment outcomes were difficult to assess in this study. Viral load was not determined at baseline while CD4 counts were irregularly done. TB patients are referred to peripheral health facilities with no follow up on TB treatment or outcome. As a result a good record of TB diagnosis, monitoring and treatment was not available. Although there was a statistically significant difference in the CD4 counts a baseline and endpoint (median difference of 86 cells/mm^3, $P = 0.013$), this was not attributed to the high efavirenz levels since the correlation between efavirenz levels and CD4 change was not significant.

There were 24 adverse effect episodes in 28 patients resulting in a 85.7 % prevalence within six months of treatment. This implies that nine out of ten patients experienced an adverse effect at one point during the six month treatment period. Pruritus was the most commonly reported adverse effect (29 % of all adverse effects) followed by drug induced hepatitis (20 % of all adverse effects) and skin rash being the third most common (20 % of all adverse effects). However, put together the skin adverse effects were by far the most common accounting for over 50 % of all adverse effects. Minor adverse effects occurred early in treatment while major adverse effects occurred late in treatment. Gastrointestinal adverse effects occurred early after onset of treatment and were not present by the 1st sampling occasion implying tolerance. Skin adverse effects occurred early but also throughout the treatment period. One patient did present with liver toxicity early but a large majority presented late. Neurological toxicity presented late after one to two months of treatment. Headaches and dizziness presented first then peripheral neuropathy. Metabolic toxicity (lactic acidosis) also presented late. Patients reported an increase in the incidence of adverse effects on onset of anti-TB therapy and a gradual reduction with time.

Some studies have supported increasing the EFV dose from 600mg per day to 800 mg per day in patients receiving both efavirenz and rifampicin[11], while other studies have shown excellent clinical outcomes with 600mg per day and recommended the routine use of 600mg per day

even in the presence of rifampicin. These studies recommend that a dose increase to 800 mg should be restricted to patients weighing more than 60 kilograms[55, 11, 23, 24]. As a result, this dose increase is recommended by both WHO guidelines[4] and the Guidelines for ART therapy in Kenya[10]. However, these studies were mainly done on Caucasian patients and not African patients. This study clearly demonstrates an immediate need to re-evaluate EFV dosing in Kenyan HIV-TB co-infected patients and indeed native African patients. A priori dose reduction in native African patients to minimise toxicity and cost is very important in this group of patients[25, 26]. Simulation of clinical trials by non-linear mixed effect modelling has shown that dose reductions to 400mg per day for poor metabolizers (TT genotype), 500mg per day for intermediate metabolizing females and even 300 mg per day in poor metabolizing females are feasible without compromising therapeutic efficacy[25].

Pharmacogenetic testing for example using genotype screens together with therapeutic drug monitoring (TDM) is recommended in these patients because the polymorphism is a determinant of the high plasma levels while therapeutic drug monitoring would allow dose reductions hence TDM individualised dosing[23, 25, 28].

4.1 STUDY LIMITATIONS

This study was limited by several factors including variables that could not be accurately measured such as potential drug interactions with EFV, patho-physiological conditions and dietary intake habits of the patient, self reporting of adherence, cost implications that limited the sample size and extent of investigations, time constraints, incomplete medical records and lack of genotype data.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSION

This study found out that a large proportion (over 90 %) of Kenyan HIV-TB co-infected patients have efavirenz levels that are above the therapeutic level (>4 ug/ml) and a high prevalence of adverse effects. A large intra-patient and inter-patient variability was also demonstrated in these patients. Bodyweight, bilirubin and ethnicity are important covariates of plasma efavirenz levels in this population. However their association with efavirenz levels was not statistically significant on multivariate analysis. Increasing bodyweight resulted in lower EFV levels. Higher efavirenz levels were observed in the Kamba community compared to the others. Though not statistically significant, females consistently had higher Efavirenz plasma concentrations than male patients. The inter-ethnic differences in efavirenz levels suggested that genotypic differences could have influenced efavirenz levels whose distribution seemed to follow a bimodal pattern.

5.2 RECOMMENDATIONS

Considering that over 90 % of the subjects had elevated efavirenz levels clinicians should therefore be particularly vigilant for signs of toxicity. This finding also highlights the need to reduce doses of efavirenz in Kenyan and possibly other African patients. A clinical study is therefore required to determine the efficacy and safety of low doses of efavirenz in African patients. The feasibility of genotype and therapeutic drug monitoring driven dose adjustments should also be evaluated.

The finding that efavirenz levels vary between ethnic groups suggested that there were significant inter ethnic genotypic difference. A large study is therefore required to investigate genotypic and phenotypic differences in efavirenz drug metabolism.

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APPENDIX A

EFAVIRENZ LEVELS AND TREATMENT OUTCOMES IN KENYAN HIV-TB CO-INFECTED PATIENTS AT KENYATTA NATIONAL HOSPITAL.

DATA COLLECTION FORM

DATE:.....

PATIENT DEMOGRAPHICS					
CODE		BODY WEIGHT		SES: -OCCUPATION -PLACE OF RESIDENCE -EDUCATIONAL LEVEL	
AGE		HEIGHT			
GENDER		BMI		ETHNICITY	
TB REGIMEN		ART REGIMEN			

PATIENT HISTORY				
PHYSICAL EXAM: ANY SIGNIFICANT ABNOMALITIES	CONCURENT ILLNESSES	MEDICATION HISTORY: PRESCRIPTION, NON-PRESCRIPTION AND HERBAL DRUGS	ADHERENCE	ADVERSE EFFECTS

BASELINE TESTS					
VIRAL LOAD	CD4	SPUTUM SMEAR	CHEST X RAY	LFT	CrCL
FHG					

EFAVIRENZ LEVELS AT 1ST SAMPLE EVALUATION					
EFV LEVELS (mg/ml)	ADHERENCE	TIME OF THE LAST DOSE (A)	TIME OF BLOOD SAMPLE COLLECTION (B)	INTERVAL SAMPLING TIME (=A-B) IN HRS	ADVERSE EFFECTS
CHEST XRAY			SPUTUM SMEAR		

EFV LEVELS AT 2ND SAMPLE EVALUATION					
EFV LEVELS (mg/ml)	ADHERENCE	TIME OF THE LAST DOSE	TIME OF BLOOD SAMPLE COLLECTION	INTERVAL SAMPLING TIME (=A-B) IN HRS	ADVERSE EFFECTS

TREATMENT OUTCOMES AT 6 MONTHS			
VIRAL LOAD NADIR	CD4 NADIR	SPUTUM SMEAR NADIR	CHEST XRAY NADIR
ADVERSE EFFECTS			
DATA COLLECTED BY:			

APPENDIX B (KNH-ERC APPROVAL LETTER)

KENYATTA NATIONAL HOSPITAL
Hospital Rd. along, Ngong Rd.
P.O. Box 20723, Nairobi.
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi.
Email: KNHplan@Ken.Healthnet.org

Ref: KNH-ERC/ A/365

December 8, 2009

MANAGER
KNH-UON ETHICS & RESEARCH COMMITTEE
Kenyatta National Hospital
P.O. Box 20723-00202, Nairobi

Approved
Please provide necessary
assistance -
Gut 21/01/2010

Dr. Jared Okoyo Nyakiba
School of Pharmacy
University of Nairobi

Dear Dr. Nyakiba,

RESEARCH PROPOSAL: "EFAVIRENZ LEVELS AND TREATMENT OUTCOMES IN KENYAN HIV-TB CO-INFECTED PATIENTS AT KENYATTA NATIONAL HOSPITAL" P299/10/2009

This is to inform you that the Kenyatta National Hospital/UON Ethics and Research Committee has reviewed and **approved** your above revised research proposal for the period 8th December 2009 - 7th December 2010.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimen must also be obtained from KNH-ERC for each batch.

On behalf of the Committee, I wish you fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of database that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely

DR. L. W. MUCHIRI
AG SECRETARY, KNH/UON-ERC

KENYATTA NATIONAL HOSPITAL
APPROVED
10 DEC 2009
RESEARCH & DEVELOPMENT

c.c. Prof. K.M. Bhatt, Chairperson, KNH/UON-ERC
The Deputy Director CS, KNH
The Dean, School of Pharmacy, UON
Supervisors: Dr. Margaret Oluka, Department of Pharmacology & Pharmacognosy
Dr. Faith Okalebo, Department of Pharmacology & Pharmacognosy

APPENDIX C

VOLUNTEER INFORMATION AND CONSENT FORM

TITLE OF THE STUDY: EFAVIRENZ LEVELS AND TREATMENT OUTCOMES IN KENYAN HIV-TB CO-INFECTED PATIENTS AT KENYATTA NATIONAL HOSPITAL

INVESTIGATOR: JARRED OKOYO NYAKIBA

MPHARM IN CLINICAL PHARMACY: YEAR 2

SCHOOL OF PHARMACY, UNIVERSITY OF NAIROBI.

SUPERVISORS: 1. DR MARGARET OLUKA

DEPARTMENT OF PHARMACOLOGY AND PHARMACOGNOSY,

SCHOOL OF PHARMACY, UNIVERSITY OF NAIROBI.

2. DR FAITH OKALEBO

DEPARTMENT OF PHARMACOLOGY AND PHARMACOGNOSY,

SCHOOL OF PHARMACY, UNIVERSITY OF NAIROBI.

COLLABORATING INSTITUTIONS:

1. UON/AiBST LABORATORY
DEPARTMENT OF PHARMACOLOGY AND PHARMACOGNOSY
SCHOOL OF PHARMACY, UNIVERSITY OF NAIROBI.

2. COMPREHENSIVE CARE CENTRE

KENYATTA NATIONAL HOSPITAL

P.O.BOX 00202, KNH, NAIROBI.

STUDY SITE: COMPREHENSIVE CARE CENTRE, KENYATTA NATIONAL HOSPITAL, NAIROBI.

COORDINATORS: 1. JARRED OKOYO NYAKIBA

MPHARM IN CLINICAL PHARMACY: YEAR 2

SCHOOL OF PHARMACY, UNIVERSITY OF NAIROBI.

2. DR MARGARET OLUKA

DEPARTMENT OF PHARMACOLOGY AND PHARMACOGNOSY,

SCHOOL OF PHARMACY, UNIVERSITY OF NAIROBI.

PREAMBLE

We are requesting you to volunteer freely in this study. Before you decide to join, we would like to provide you with information about the study. This document is a consent form; it has information about the study and will be discussed with you by the investigators. Please, study it carefully and feel free to seek any clarification especially concerning terminologies or procedures that may not be clear to you. If you agree to join this study, you will be asked to sign this consent form and a copy will be given to you for safekeeping.

PURPOSE OF THE STUDY

The main objective of this study is to determine the plasma levels of efavirenz and treatment outcomes in Kenyan HIV-TB co-infected patients receiving concomitant anti-TB therapy and antiretroviral therapy. Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) antiretroviral agent used as a component of HAART (highly active antiretroviral therapy) in which it is combined with two NRTIs (nucleoside reverse transcriptase inhibitors) at an adult dose of 600mg daily. Rifampicin is a semi-synthetic antibiotic of the rifamycin class. It is used in combination with other anti-tuberculous drugs in the treatment of TB. Efavirenz plasma concentrations exhibit a large interpatient and intrapatient variability which coupled with the interaction between rifampicin and efavirenz could lead to sub-therapeutic or toxic concentrations of efavirenz. No data exists in Kenyan patients regarding the plasma

concentrations of efavirenz and the treatment outcomes when used concomitantly with rifampicin hence the purpose of this study.

STUDY PROCEDURES

Plasma efavirenz concentration and clinical outcome studies

With your consent, 5.0 ml of blood will be withdrawn from your arm by veno-puncture and transferred into a clean EDTA tube. Strict observation of aseptic conditions will be ensured. This blood sample will be used to determine the concentrations of efavirenz in your plasma. The patient demographic data, baseline tests and the outcomes of treatment at the end of TB treatment will be obtained from your medical records.

Inclusion/exclusion criteria

The following criteria determine whether you are eligible to participate in this study or not.

a) Inclusion criteria;

- You must be a HIV infected patients older than 18 years
- You must be a newly diagnosed TB patient
- You must be on an efavirenz based ART regimen
- You must be on a rifampicin based anti-TB regimen.

b) Exclusion criteria

- If you are a child less than 18 years of age.
- If you are a pregnant woman.
- If you are concomitantly using drugs with potential pharmacokinetic interaction with efavirenz or rifampicin that is inducers or inhibitors of CYP450 enzyme system.
- If you are involved in any other study that may impact on the results of this study.

Your sample

Only blood samples will be collected from you, which will be used solely to determine the plasma concentration of efavirenz.

Risks and Discomforts

Participating in this study may be associated with minimum risk and discomfort during blood collection which may include:

- Pain
- Bleeding
- Swelling

Benefits

This study may be of benefit to you in that you will be monitored during the 6 months of TB treatment and your plasma concentrations of efavirenz could be used to adjust your treatment to avoid treatment failure of toxicity. The findings of this study may be of benefit to other HIV-TB co-infected patients in Kenya.

Some possible benefits are:

- Increased knowledge about the plasma concentrations of efavirenz in HIV-TB co-infected Kenyan patients.
- Adjustment of drug dosage depending on individual patient's plasma concetrations.
- Reduced adverse reactions and treatment failures.
- Reduced development of resistance.
- Improved clinical outcomes in HIV-TB treatment.
- Improved rational use of drugs.

Confidentiality

The study staff will take utmost care to keep your participation in this study confidential. Your samples will be identified only by a coded number. Information from this study will be used in reports, published papers or presented in public but your name will never be used. Your name will only be known to the principal investigators for purpose of follow-up.

Voluntary participation/withdrawal from study

The decision to take part in this research study is your choice. You may choose not to take part or to stop participating at any time.

Questions

You are free to ask any questions at any time about the study and regarding your right as a research volunteer. You will not be giving up any of your legal rights by signing this consent form.

Further Information

For further information about this study you may contact Jarred Nyakiba tel: 0724708497, Mpharm in clinical Pharmacy (2nd year), Department of Pharmaceutics and Pharmacy practice, School of Pharmacy, University of Nairobi , P O Box 19676, Nairobi. Tel: +254 02 2725099, 0727499537.

Alternatively you may contact the supervisor of this study, Dr Margaret Oluka tel: 0722604216, Department of pharmacology and Pharmacognosy, School of Pharmacy, University of Nairobi, P O Box 19676, Nairobi. Tel: +254 02 2725099, 0727499537.

For questions related to your rights as a volunteer in this research study; you may contact Prof A. N. Guantai, Secretary of the Kenyatta National Hospital Ethics and Research Committee (KNH – ERC), School of Pharmacy, P. O. Box 19676, Nairobi. Tel: +254 020 2726300 Ext 44102.

STATEMENT OF CONSENT

I have read, or have had this consent form read to me. I have had the chance of discussing this research study with the investigators. I have had my questions answered in a language I understand. The risks and benefits have been explained to me. I understand that my participation in this study is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this research study.

By signing this consent form, I have not given up any of the legal rights that I have as a participant in a research study.

- I have read, or have had it read to me YES/NO
- I agree to participate in this research study YES/NO
- I agree to have my blood collected and analyzed for plasma concentrations of efavirenz YES/NO

Participant's signature: _____ Date: _____

I, the undersigned have fully explained the relevant details of this research study to the participant named above and believed that the participant has understood and has knowingly given his consent.

Printed Name: _____ Date: _____

Signature: _____

Role in this study: _____