PATTERNS AND RISK FACTORS FOR ALANINE TRANSAMINASE ELEVATION AMONG HIV POSITIVE PATIENTS ON NEVIRAPINE REGIMENS AT KENYATTA NATIONAL HOSPITAL

A thesis submitted in partial fulfillment of the requirements for Master's degree of the University of Nairobi in Pharmacoepidemiology and Pharmacovigilance

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DECLARATION

I declare that this thesis is my original work, and has not been presented for award of a degree in any other University.

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DEDICATION

I am grateful to the Almighty God for the strength and knowledge to do this work.

I dedicate this thesis to my family, my dear wife Sally, my son Brandon and my daughter Angel Kayla for their love, patience and support during my studies.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACTG	AIDS Clinical Trial Group
ADR	Adverse drug reaction
AIDS	Acquired immunodeficiency syndrome
ALT	Alanine aminotransferase
ART	Antiretroviral therapy
CCC	Comprehensive Care Center
CD4	Subgroup of T-lymphocyte carrying CD4 antigens
CYP	Cytochrome
DILI	Drug induced liver injury
EFV	Efavirenz
eGFR	Estimated glomerular filtration rate
HAART	Highly active antiretroviral therapy
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HT	Hepatotoxicity
KNH	Kenyatta National Hospital
LFTs	Liver function tests
LPV/r	Lopinavir/ritonavir
NNRTIs	Non-nucleoside reverse transcriptase inhibitors
NRTIs	Nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
SNP	Single nucleotide polymorphism.
TB	Tuberculosis
ULN	Upper limit of normal
UoN	University of Nairobi
WHO	World Health Organization

OPERATIONAL DEFINITIONS

Adverse drug reaction: A response to a drug which is harmful and unintended, and which occurs at doses normally used in humans for the prophylaxis, diagnosis or therapy of disease, or for the modification of physiological function

Alanine transaminase elevation: An increase of at least ≥1.25 fold in serum alanine transaminase levels using 40 IU of ALT as the upper limit of normal.

Baseline investigation: Any measurement or investigation done between 60 days prior to or 30 days after antiretroviral drug initiation

Renal disease: an estimated glomerular filtration rate (eGFR) of less than 50ml/min/1.73m^2

Significant hepatotoxicity: An alanine aminotransferase elevation of grade 2–4 in patients who had normal liver enzymes at baseline (using 40 IU of ALT as the upper limit of normal)

ABSTRACT

Background:

Human immunodeficiency infected patients frequently present with elevated levels of serum transaminases. This is often been attributed to hepatic effects of antiretroviral drugs. The introduction of life prolonging anti-retroviral therapy has drastically reduced the morbidity and mortality associated with HIV. Because of improved life expectancy, non-HIV/AIDS defining diseases and drug related toxicities have emerged as key issues in the management and care of people living with HIV/AIDS.

Nevirapine is associated with asymptomatic elevations of alanine transaminase (ALT) levels, and at times life threatening, clinical liver hepatotoxicity. Hepatotoxicity can be fatal when not recognized early and when treatment not interrupted in time.

Objective:

This study aimed to determine the pattern and risk factors for alanine transaminase elevation among HIV positive adult patients on nevirapine containing anti-retroviral regimens at Kenyatta National Hospital.

Methodology:

We obtained ethical approval to carry out this study from the KNH-UoN research and ethics committee. We conducted a retrospective cohort study of HIV positive patients on nevirapine containing regimens who attended the KNH Comprehensive Care Clinic between May and August 2014. We performed generalized linear regression to establish patterns and predictors for ALT elevation. Data obtained from the patient interviews and abstraction of patient files, were analyzed using STATA version 10.

Results:

Two hundred and forty one patients took part in the study. One hundred and sixty two (67.2%) had normal ALT levels throughout the study, seventy-two (29.9%) had mild

elevation and seven (2.9%) developed moderate hepatotoxicity. None of the participants developed severe or very severe hepatotoxicity.

In patients with normal ALT at baseline, the pattern of ALT change was cyclical with peaks and troughs. The peak levels seemed to increase with time. Very sharp peaks were noted from the 5^{th} year of therapy onward. Among patients who had elevated ALT levels at baseline the trend was a gradual decline in ALT levels until about 6 years of therapy, thereafter the ALT levels started rising progressively.

Risk factors for ALT elevation differed across sex. Predictor variables that were significantly associated with ALT elevation in both sexes included; elevated baseline ALT level [β=10.14 (95%CI 7.34- 12.96); P<0.001], [β=13.52(95%CI 9.36 –17.68); P < 0.001] and renal disease [β=5.44 (95%CI 2.62 – 8.25); P <0.001], [β=11.52 (95%CI 3.46 – 19.60); P = 0.005] in females and males respectively. Ethnicity had a protective effect in both sexes; [β-6.61(95%CI-9.28, -3.93); P< 0.001] in males and [β-1.20(95% CI-2.39, -0.01); P= 0.048] in females. Among the different ethnic groups, Nilotes and Cushites had lower ALT levels compared to Bantus.

Other factors that were significant included; smoking (P=0.001), concurrent illnesses (P=0.045), previous adverse drug reactions (P=0.040) in females and a longer duration of anti-retroviral therapy [β 1.81(95%CI 0.89 – 2.73); P < 0.001] in males. Poor adherence had a protective effect [β -1.62(95%CI -3.20, -0.04); P=0.045] among females, whereas initiation on AZT+3TC+NVP had a significant protective effect [β -7.80 (95%CI -13.96, -1.63); P=0.013] in males.

Conclusion

Alanine transaminase elevation might occur in up to one third of HIV/AIDS positive adult patients taking nevirapine based ART. None of the patients developed severe or very severe hepatotoxicity in this cohort.

In setting where transaminase testing is available, monitoring should focus on delayed hepatotoxicity, patients with abnormal baseline ALT and those with impaired renal functioning.

All HIV-infected patients should be screened for liver disease at the time entry into care.

1 CHAPTER ONE: INTRODUCTION

1.1 Background

HIV/AIDS remain one the world's most serious health challenges. Global solidarity in the AIDS response during the past decade continues to generate extraordinary gains especially by making HIV prolonging drugs more accessible (1).

According to the WHO/ UNAIDS 2012 report, 34 million people were living with HIV at the end of 2011. An estimated 0.8% of adults aged 15-49 years worldwide are living with HIV. Sub-Saharan Africa remains most severely affected, with nearly 1 in every 20 adults (4.9%) living with HIV. The region accounts for 69% of the people living with HIV worldwide. In Kenya, the prevalence of HIV/AIDS among adults aged 15-49 years stands at 5.6% which corresponds to approximately 1,600,000 persons living with HIV in 2012 (2).

Nevirapine is one of the recommended first line anti-retroviral drugs and forms the backbone in HIV management. As at June 2013 an estimated 561,774 HIV positive adults Kenyans were on antiretroviral therapy out of which 63% were on nevirapine containing regimen according to the commodity security committee of NASCOP. However, nevirapine can cause potentially life-threatening skin reactions and hepatotoxicity that usually occurs within the first 18 weeks of treatment. Six to seven percent of patients on nevirapine based regimen discontinue the use of antiretroviral drugs (ARVs) because of clinically significant hypersensitivity reactions (3).

Studies have reported that Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI), especially nevirapine, have a class effect in terms of abnormal liver enzyme levels. However, an increased rate of serious clinical (symptomatic) hepatotoxicity has not been demonstrated in this patient population yet. Risk of hepatotoxicity is dependent on several concomitant conditions, such as viral co-infection, plasma drug levels, gender and degree of immune damage (3-5).

Anecdotal reports suggest a high prevalence of adverse drug reactions among HIV patients. Hepatotoxicity forms part of the constellation of adverse drug reactions. In addition, massive increases in ALT levels without accompanying clinical signs of hepatocellular injury have been reported (6). Hepatotoxicity can be fatal when not recognized early and when treatment is not interrupted in time (7).

1.2 Problem statement

HIV associated morbidity and mortality have decreased dramatically since the introduction of life prolonging anti-retroviral therapy. Because of improved life expectancy, non-HIV/AIDs defining diseases and drug related toxicities have emerged key issues in the management and care of people living with HIV/AIDs (8). Liver related conditions are the most frequent cause of non-HIV AIDS related death among HIV infected persons (9).

All classes of antiretroviral drugs are associated with asymptomatic elevations of alanine transaminase (ALT) levels, and much less frequently with serious, and at times, life threatening, clinical liver hepatotoxicity. The relationship between the risk of developing serious clinical liver injury and the rate and severity of elevated asymptomatic ALT levels is poorly understood (4)**.** Severe and potentially life threatening hypersensitivity reactions (skin reactions and hepatotoxicity) have occurred in HIV- infected patients taking nevirapine. These severe events are described in a black box warning in the manufacturer's package insert. Incidences of an asymptomatic increase in ALT levels ranging between 5 to 15% among patients using nevirapine based regimen have been reported in literature while the incidence of clinically symptomatic hepatotoxicity is approximately 4% (9, 10).

Screening for hepatotoxicity during ART is primarily based on serum levels of ALT, a liver enzyme that serves as a "proxy" for liver inflammation and damage. However, laboratory tests, while desirable, are not a prerequisite for initiation or for routine follow up of patients on ART in resource constrained settings as per the guidelines for anti-retroviral therapy in Kenya. Hepatotoxicity from drugs is often difficult to diagnose

because the signs and symptoms vary so much from one drug to the next and symptoms often resemble other commonly diagnosed illnesses. While symptom directed monitoring of liver function is a cost reduction measure, only a very small proportion of subjects with abnormal ALT levels exhibit clinical signs of hepatotoxicity. Early detection of liver injury may be a challenge in settings where there is no routine ALT monitoring and many patients with hepatotoxicity may be underdiagnosed (12).

The bulk of the current knowledge of adverse events associated with anti-retroviral drugs is primary based on data from resource rich countries. Only few studies have been conducted in Sub-Sahara Africa where the burden of HIV/AIDS is highest. This means available information may not be representative as demographics, genetic factors, comorbidities, nutritional status and concomitant use of other drugs may vary substantially (8). It is therefore necessary to determine the frequency of ALT elevation and identify the risk factors for hepatotoxicity among HIV patients using nevirapine-containing regimen in Sub-Sahara Africa.

1.3 Objectives

1.3.1 Main objective

The primary objective of this study was to describe the pattern and risk factors for alanine transaminase elevation during anti-retroviral therapy among HIV positive patients on nevirapine containing regimens.

1.3.2 The specific objectives

The specific objectives were to:

1. To describe the pattern and prevalence of alanine transaminase elevation among HIV positive patients on nevirapine based anti-retroviral therapy.

2. To identify risk factors for alanine transaminase elevation among HIV positive patients on Nevirapine regimens.

2 CHAPTER TWO: LITERATURE REVIEW

2.1 Overview of drug induced hepatotoxicity

The liver is central to metabolism of virtually all foreign substances ingested. Potential toxicants when ingested can damage the liver either directly or as a consequence of the metabolic changes that occur in the liver. The spectrum of drug induced hepatotoxicity is wide, ranging from asymptomatic reversible alteration in liver function tests to fatal acute hepatic necrosis (13).

The liver is involved in approximately 3-10% of all adverse drug reactions (ADRs). Up to 50% of all cases of acute liver failure (which is associated with 90% mortality rate) are allegedly drug related. Furthermore, hepatotoxicity is the main cause of fatal ADRs and the most common reason for withdrawal of drugs from the market (13). Drug hypersensitivity in HIV-infected patients is about 100 times more common than in the general population (1).

2.2 Types of drug induced reactions

Based on etiology, drug induced liver damage can be classified into two main types, namely predictable (type A) and unpredictable or idiosyncratic reactions (type B). Predictable reactions are dose dependent and are the most common. The administered dose is a stronger determinant of the likelihood of a reaction than the host's metabolic constitution. Examples of dose dependent hepatotoxic agents are acetaminophen, salicylates and tetracyclines. Idiosyncratic reactions are generally less frequent, typically occurring in between 1 in every 10,000 to 1 in every 100,000 patients; however the actual incidence is probably higher due in part to the difficult in diagnosis (14). Idiosyncratic reactions are characterized by a variable delay, ranging from five to 90 days from the initial ingestion of the drug and are usually fatal if the drug is continued once the reaction has begun. Examples of drugs that exhibit such reactions are chlorpromazine, halothane and isoniazid. Both types of reactions can cause similar patterns of liver damage. Several drugs can cause more than one type of damage (15).

2.3 Pathophysiology and biochemical mechanism of drug induced liver injury

The liver is frequently involved in drug-induced toxicities due to its important role in drug metabolism. Liver injury is often a multistep process that involves both direct and indirect drug injury and subsequent activation of inflammatory pathways. The offending drug or the drug metabolites trigger the initial steps of injury. The hepatotoxic metabolites mainly result from phase I drug metabolism but can also arise from conjugative phase II metabolism (14).

Cytochrome 450 (CYP 450) generates toxic metabolites from parent compounds which may cause mitochondrial damage either through intrinsic, extrinsic or direct mitochondrial inhibition. Mitochondrial permeability is affected resulting in membrane disruption. In the presence of adenosine tri-phosphate (ATP), an apoptosome is formed which leads to cell degradation via fragmentation and apoptosis. In the absence of ATP, increased mitochondria permeability leads to increased cytosolic calcium, sodium, cell lysis, necrosis and cytokine release.

Injury can be propagated through either direct cell stress by depleting glutathione stores, binding of metabolites to enzymes, lipids, nucleic acid or other structures. Injury can also occur via direct mitochondrial inhibition by affecting the mitochondrial respiratory chain resulting in ATP depletion and accumulation of reactive oxygen species. Binding of the drug or its metabolite to human leukocyte antigen proteins evokes specific immune responses. These complexes are then presented to T-cells and recognized as antigens. The neo-antigens are then placed on the antigen presenting cells which activate formation of antibodies or activate the immune system to form auto-antibodies against cell structures (16). Table 1 presents some common idiosyncratic drug reactions.

Table 1**: Idiosyncratic drug reactions and the cells that are affected**

Type of reaction	Effect on cells	Examples of drugs
Hepatocellular	Have a direct effect or production by enzyme-drug adduct leads to cell dysfunction, membrane dysfunction, and cytotoxic T-cell response.	Allopurinol, Aspririn, Didanosine, Diclofenac, isoniazid, lovastatin, methyldopa, nefazodone paracetamol.
Cholestasis	Partial or complete obstruction of the common bile duct, resulting in retention of bile acids, this can lead to inflammation, scarring and eventually cirrhosis.	Angiotensin converting enzyme inhibitors, carbimazole, cimetidine, clavulanic acid, ketoconazole, phenytoin, sulfonamides, warfarin.
Immunoallergic	The enzyme-drug adducts on the cell surface induce IgE response	Halothane, phenytoin,sulfamethoxazole
Granulomatoma	Macrophages and lymphocytes infiltrate hepatic lobule	Diltiazem, Sulfa drugs, quinidine
Micro vesicular Steatosis	Altered mitochondrial respiration, β-oxidation leads to lactic acidosis and triglyceride accumulation.	Acetylsalicylic acid, Didanosine, tetracycline, valproic acid
Steatohepatitis	Multifactorial	Amiodarone, tamoxifen
Autoimmune	Cytotoxic lymphocyte response directed at hepatocyte membrane components	Lovastatin, methyldopa, minocycline nitrofurantoin
Fibrosis	Activation of stellate cells	Methotrexate, excess vitamin A
Vascular collapse	Non-thrombotic concentric narrowing of the central hepatic veins by connective tissues	Oral contraceptives, cytotoxic agents
Oncogenesis	Encourages tumor formation	Oral contraceptives, danazol, anabolic steroids
Mixed	Cytoplasmic and canalicular injury, direct injury to bile ducts	Amoxicillin-clavulanate, carbamazepine, cyclosporine, troglitazone

Adopted from Drug induced hepatotoxicity by William M. Lee (2003)

2.4 Clinical presentation

The signs and symptoms of drug induced hepatotoxicity can be as mild as a change in liver function tests presenting no apparent symptoms in the patient, to full blown hepatotoxicity and liver failure. The types and severity of signs and symptoms can vary from one drug to the other and one patient to another. The common signs associated with drug induced liver injury are presented in Table 2.

Table 2: **Some common signs associated with drug induced hepatotoxicity**

Description	**Features**
Non-specific symptoms: may not directly pinpoint to hepatotoxicity	Fatigue Weakness Vague abdominal pain Loss of appetite
Signs and symptoms specific for hepatotoxicity	Jaundice Itching Ease in bruising
Signs of severe liver damage (cirrhosis)	Edema (often at times in the legs) Mental confusion Kidney failure Gastrointestinal bleeding Vulnerability to bacterial infections
Symptoms of hepatitis (inflammation of liver cells)	Loss of appetite Nausea Vomiting Fever Weakness Fatigue Abdominal pain

2.5 Challenges in the diagnosis of drug induced liver injury

Clinically, the differential diagnosis of hepatotoxicity associated with drugs can be problematic due to the vast number of symptoms the patient may experience. Clinical signs of toxic hepatitis are uncommon, most diagnosis being made as a result of raised enzyme levels. However, liver enzyme elevation can also be caused by enzyme induction, immune reconstitution syndrome, exacerbation of hepatitis B or C and can accompany hypersensitivity reactions (1). There are three major problems in understanding drug-induced liver injury (DILI), such problems include; establishing causality, determining the true incidence of and clinical risk factors for drug-induced hepatotoxicity and elaborating the mechanisms by which injury occurs.

Establishing causality requires sufficient and accurate information for medical differential diagnosis. There are no pathognomonic indicators of drug induced liver toxicity; even liver biopsy is not diagnostic. Making the correct attribution of causality requires analyzing the temporal relationship of drug exposure to illness and excluding all other possible causes.

Determination of incidence cannot be done adequately using currently available methods, whether by clinical trials, by spontaneous adverse event reports, or by retrospective epidemiologic studies. There is need for prospective safety studies to establish the true incidence of DILI caused by a drug, to identify risk factors for it, and to collect biologic materials for analytic studies toward better understanding mechanisms of drug induced liver injury (17).

Establishing the mechanism of liver injury is problematic because majority of drug related reactions are idiosyncratic and unpredictable. Metabolic fate of most compounds is a complex process. Several variables other than the toxic potential of the compound itself may play a part in the metabolic outcome (18).

2.6 Prevalence of adverse reactions associated with nevirapine.

Nucleoside reverse transcriptase inhibitors (NNRTI), especially nevirapine, have a class effect in terms of abnormal liver enzyme elevation (19). The post marketing experience

has shown that the most serious adverse reactions are Stevens Johnson syndrome, toxic epidermal necrosis and serious hepatic failure and hypersensitivity reactions. Six to seven percent of patients discontinue the use of nevirapine because of the clinical signs of hypersensitivity reactions (3). The prevalence of adverse reactions associated with nevirapine is presented in Table 3.

Table 3**: Prevalence of ADRs associated with nevirapine**

Adverse event	**Prevalence**
Hepatic Reaction Symptomatic hepatic events regardless of severity	5% (range 0 to 15%)
Asymptomatic transaminase elevations (AST or ALT greater than 5 times ULN)	6% (range 0 to 9%)
Skin rash Grade 1 and 2	13%
Grade 3 and 4	2%

Adopted from a comprehensive hepatic safety analysis of nevirapine in different populations of HIV infected patients by Stern et al. (2003).

2.7 Risk factors for symptomatic hepatic events

The risk of hepatotoxicity has been shown to be dependent on several concomitant conditions. Identified risk factors for developing hepatotoxicity with nevirapine include; higher CD4 cell count prior to starting nevirapine, female gender, chronic hepatitis B or C virus infection, liver cirrhosis, and abnormal baseline hepatic transaminase levels (3,8).

2.7.1 Higher CD4 count prior to starting nevirapine

A number of studies have been conducted to establish the association between a high CD4 count and risk of hepatotoxicity; however these studies have yielded mixed results. Some studies have supported this association while others have not found an association.

In a retrospective analysis of Boehringer-Ingelheim databases, it was found that the risk of symptomatic hepatotoxicity was 12 times greater in women with a CD4 count above 250 cells/μL compared with 0.9 times risk among women with baseline CD4 count <250 cells/μL. In men, there was a 6.3 times risk if the CD4 count was above 400cells/μL compared with 1.2 times when the CD4 count was below 400 cells/ μL. In the case of hepatotoxicity with skin rash, the increased risk was 9.8 (relative risk) for women with a CD4 count above 250 cells/μL and 6.4 (relative risk) for men with a CD4 count above 400 cells/μ (1).

A large collaboration of seven observational cohorts (n= 10,186) found that nevirapine was well tolerated in ART experienced patients with high CD4 count provided there was no detectable viremia (3). Similarly, a multi-center study conducted in Kenya, Zambia and Thailand among women taking Nevirapine-based ART found that severe hepatotoxicity and rash-associated hepatotoxicity were predicted by abnormal baseline ALT levels, but not by a CD4 count ≥ 250 cells/μL (20). These findings are in agreement with other studies conducted in Cote d'Ivoire which found no association between CD4 cell count >250 cells/mm3 and a higher risk of severe hepatotoxicity and/or rash (21).

2.7.2 Chronic hepatitis B or C virus infection

Severe hepatotoxicity occurs throughout the course of NNRTI therapy and is more common among HIV infected patients co-infected with hepatitis B and C virus (8). The risk of severe hepatotoxicity in HIV patients with chronic viral hepatitis is approximated to be about 69% (22). Several studies have demonstrated the association between viral hepatitis and hepatotoxicity during nevirapine therapy including a controlled clinical trials dubbed "Viramune Hepatic Safety Project" conducted by nevirapine innovators(8).

2.7.3 Female gender

For reasons that are unclear, women appear to be more susceptible to drug induced liver toxicity than men (3). Women with a high CD4 counts are at greatest risk of hepatic events, including potentially fatal events. In general, during the first 6 weeks of treatment, women have a 3-fold higher risk than men for symptomatic, often rash-

associated hepatic events (6% versus 2%). Results of a large randomized clinical trial, the 2NN study, demonstrated that women with CD4 count >200cells/μL had a statistically significantly increased risk of developing a rash compared with men (1).

2.7.4 Pre-existing liver disease

Patients with liver disease may be at increased risk of adverse drug reactions (ADRs) because of a reduced drug-metabolizing capacity of the affected liver. Nevirapine is metabolized in the liver by CYP3A4 and CYP2B6 isoenzymes (17). In severe liver disease, the activity of the CYP P450 is greatly decreased. This may affect the rate of drug metabolism. In general, patients with pre-existing liver disease such as alcoholic cirrhosis or chronic viral hepatitis are at greater risk of drug induced adverse events (14, 22).

Alcohol and herbal preparations can potentially interact with other medications used concomitantly leading to undesired consequences (15). Chronic alcohol consumption induces the CYP450 system and as a result, can potentiate toxicity induced by certain drugs. Similarly, some herbal preparations enhance drug metabolizing enzymes via induction of CYP450 isoenzymes which may lead to an increased risk of liver damage in individuals exposed to drugs that produce toxic metabolites (24).

2.7.5 Abnormal baseline hepatic aminotransferase levels

A baseline elevation of ALT levels > 2.5 times the upper limit of normal is an independent risk factor for developing liver injury (9). A multicenter study conducted in Kenya, Zambia and Thailand found that the risk of severe hepatotoxicity and rash associated hepatotoxicity was predicted by abnormal (≥ grade 1) baseline transaminases levels (20).

2.7.6 Polymorphism of CYP2B6

Genetic factors are increasingly recognized as potentially important determinants of drug induced liver injury (25). The activity and expression of the CYP450 isoenzyme is genetically determined and this may influence the extent to which an individual may produce toxic metabolites or have reduced protective mechanisms when exposed to a particular drug. Nevirapine and efavirenz are metabolized by hepatic cytochrome P450 CYP2B6 and CYP3A4. Associations have been identified between a frequent CYP2B6 variant (516G→T) and NNRTI pharmacokinetics (25).

The incidence of cutaneous and hepatotoxic reactions to nevirapine differs by race, which reflects differences in the distribution of various alleles for genes coding for human leucocyte antigen (HLA) and CYP2B6. Hepatic adverse events to nevirapine tend to be associated with HLA-DRB*01. CYP2B6 genotype on the other hand tends to be associated with cutaneous reactions. Blacks with CYP2B6 516TT allele are prone to cutaneous reactions (24).

2.8 Timing of hepatotoxicity and clinical manifestations

There are two distinct types of nevirapine-associated hepatotoxicity, each with characteristic time courses. The first type is an immune-mediated hypersensitivity reaction, which develops within 18 weeks of starting nevirapine, with most cases occurring between day 10 and 30 days. Most patients with this type of early nevirapine-associated hepatotoxicity will have concomitant flu-like symptoms (fever, myalgia, fatigue, malaise, nausea, and vomiting) with or without skin rash. The randomized 2NN study demonstrated that most adverse events occur during the first 6 weeks of treatment, as reported in other studies. Only hepatitis co-infection has been significantly associated with developing liver enzyme elevation after 6 weeks of treatment (27).

The second type typically occurs after 18 weeks of nevirapine therapy and most likely represents an intrinsic toxic drug effect and does not appear to correlate with baseline CD4 cell count (22). This delayed hepatotoxicity generally occurs without concomitant

constitutional symptoms. Regardless of whether the hepatotoxicity occurs early or later, an increase in liver aminotransferase levels is most often the first identifiable marker of nevirapine-induced hepatotoxicity (10).

2.9 Monitoring for nevirapine hepatotoxicity

Patients starting nevirapine should have hepatic aminotransferase levels monitored very closely in the first 18 weeks of therapy and continued during NVP therapy. Specifically, the guidelines for antiretroviral therapy in Kenya recommend measuring ALT at baseline, first and third month and in addition, at any point in the course of therapy, if a patient presents with a rash or constitutional symptoms that suggest a possible adverse reaction. Clinicians and other healthcare providers should instruct all patients on NVP treatment to immediately seek medical attention if signs and symptoms hepatitis develop.

2.10 Prevention and management of nevirapine hepatotoxicity

According to the guidelines for antiretroviral therapy in Kenya, nevirapine is not recommended in patients with severe hepatic impairment. The appropriate use of Nevirapine consist of a lead in dose of 200mg once daily for 14 days, followed by an escalation to 200mg twice daily in patients who do not develop complications during initiation phase. Patients who develop clinical hepatitis should immediately stop Nevirapine and seek medical attention. The recommended protocol for management of NVP associated rash by the Kenyan Ministry of Health is presented in figure 1.

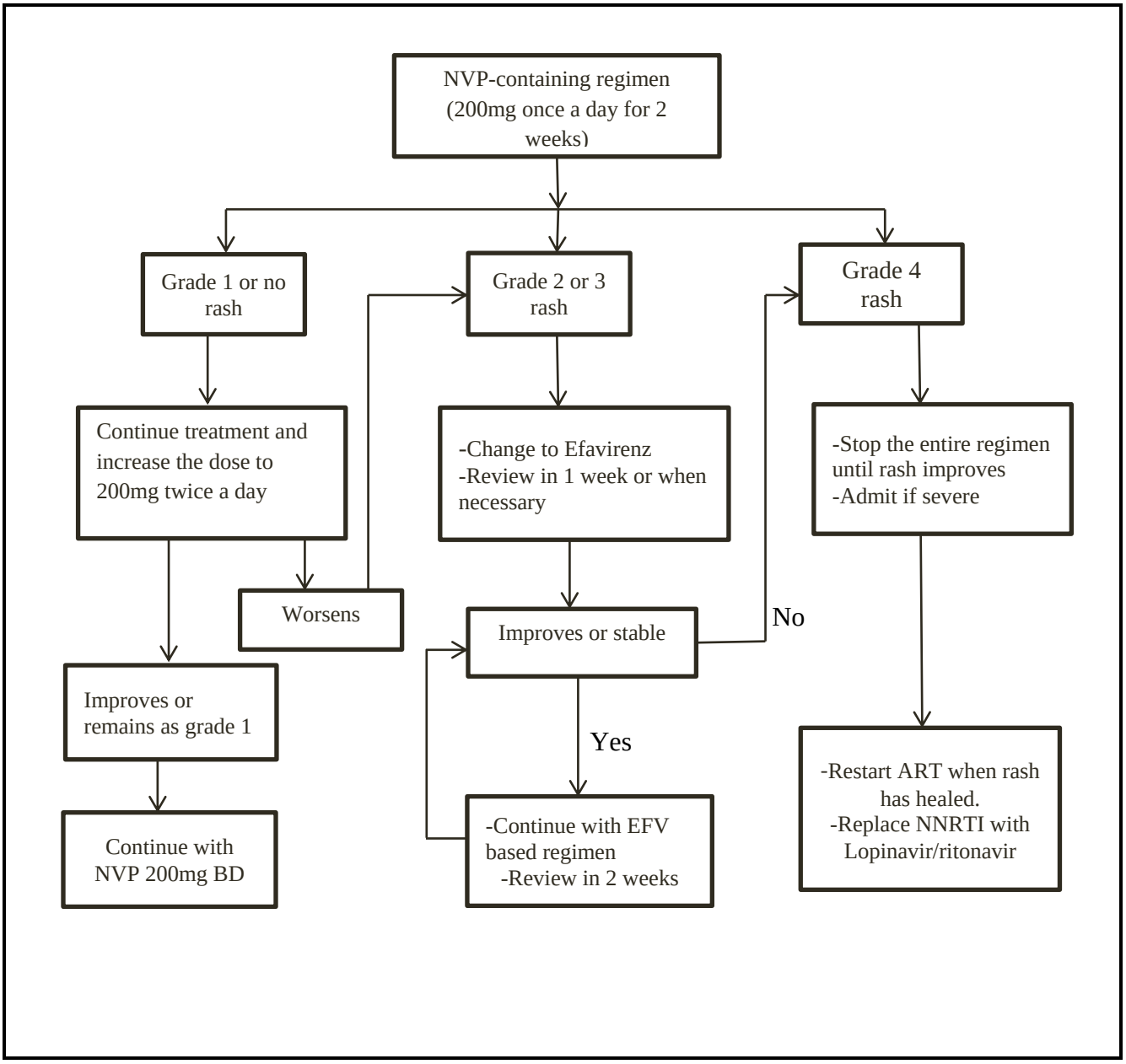

Figure 1**: Management of Nevirapine associated rash**

Adopted from the Ministry of Heath guidelines for antiretroviral therapy in Kenya (4th edition 2011).

In a case series of patients with hepatotoxicity attributed to nevirapine, hepatic aminotransferase levels returned to normal at a median of 45 days after discontinuing Nevirapine (28). Unfortunately, hepatic injury may progress even after discontinuation of nevirapine. Patients with nevirapine–associated hepatotoxicity should receive aggressive supportive management. The use of prednisone during the 14-day lead in period has not proven effective in preventing nevirapine-associated rash or hepatotoxicity and may increase the risk of developing adverse effects (29). There are insufficient data regarding the use of prednisone after an adverse reaction has occurred and is not recommended (26).

3 CHAPTER THREE: METHODOLOGY

3.1 Study design

We conducted a descriptive (right censored arm) hospital based retrospective cohort study to determine the pattern of ALT changes and establish the risk factors for ALT elevation among HIV positive patients during nevirapine-based anti-retroviral therapy.

3.2 Study site and period

This study was conducted from May to August 2014 at Kenyatta National Hospital Comprehensive Care Clinic (CCC). The hospital is located at the Kenya's capital city of Nairobi and is the largest teaching and referral Hospital in the country. The hospital CCC is among the first clinical units established by government to provide specialized HIV/AIDS care and treatment services. The CCC is one of the chronic follow-up clinics, with over 5,000 patients enrolled on care and treatment. Usually patients visit the clinic for routine medical follow up and medication refills. During these visits, patients are routinely monitored for liver and renal function and their CD4 cell count. Viral load testing is conducted selectively to confirm suspected treatment failure.

The site was ideal for this study because of the diversity of patients who are enrolled at the clinic, large number of patients on ART and excellent set up with specialized personnel and facilities.

3.3 Study population

The target population was HIV/AIDS positive adult males and females aged between 18 and 55 years on any nevirapine containing HAART regimen and seen at the Comprehensive Care Clinic between May and August 2014.

3.4 Inclusion and exclusion criteria

Patients were included into the study if they were HIV positive, on nevirapine-containing regimen for at least 6 months, aged between 18 and 55 years and willingly consented to take part in the study.

We excluded participants if they were on nevirapine-containing regimen for less than 6 months, declined to give consent and aged below 18 years or above 55 years. We excluded participants below 18 years because they could not give consent and those above 55 years because of the likelihood of predisposition to hepatotoxicity.

3.5 Sample size

We calculated the sample size using the formula described by Hulley et al (2013) for estimation of sample size of a dichotomous variable in a cohort study. The calculated minimal sample was 138 based on literature review of local studies that reported an expected prevalence of hepatotoxicity of 10% (20). I used the following formula;

$$\mathbf{N=4Z_{\alpha}^{2}p\,(1-P) \div w^{2}}$$

Where

N is the total sample required for the study

Z_{α} is the standard normal deviate for a two sided α (for a 95% confidence level $Z_{\alpha} = 1.96$).

P is the expected proportion (for this study it is 10%)

W is the width of the confidence interval (in this case, set at 10%)

$N= \{4 \times 1.96^{2} \times 0.1(1-0.1)\} \div 0.1^{2}$

$=138$

To accommodate for expected missing files or incomplete data entries of about 20%, the calculated sample size we inflated by 20%. Therefore, we targeted a minimum sample size of 166 participants.

3.6 Sampling method and patient recruitment strategy

We sampled participants by convenient sampling method. Trained Pharmacy personnel were involved in participant recruitment. Participants were recruitment they collected drugs from the CCC pharmacy to minimize service interruption. All adult patients on nevirapine regimens were invited to participate. We used the appended consent form (Appendix B). Recruitment was done until the required sample size was achieved. Out of the 290 patients recruited, 241 met the eligibility criteria. We generated a list of willing participants that was given to the records department for file retrieval.

3.7 Data collection procedure

Data collection was divided into 2 parts. The first part involved patient interview using a questionnaire. Second part entailed retrospective assessment of patient records to abstract laboratory and clinical information.

Patient interview

Participants were subjected to a brief interview to obtain information on self-reported medication related problems, alcohol use, use of herbal and non-prescription preparations, marital status, smoking status and educational level. This was used to supplement information obtained from patient medical records.

Abstraction of patient files

The medical files of recruited patients was retrieved and the following information abstracted: demographic characteristics; liver function tests results; history of pre-existing liver disease; CD4 count; history of any skin reaction; renal function tests; any adverse drug event; medication history and documented clinical signs of hepatotoxicity.

3.8 Quality assurance

All personnel received training on Good Clinical Practice at the start of the study. A pilot study was done before initiating data collection and the findings were used to improve the design of the data collection tools and the standard operating procedures. Using the data

collection tools, we collected data from 20 patients. Significant shortcomings in the design of the tools were noted and adjustments made to eliminate ambiguities and improve clarity and the quality of data collected.

Random checks and inspections were done on weekly basis during data collection to ensure that protocols were followed. The research assistants were encouraged to consult and/or report any difficulties with the protocol or any deviations so that these were addressed promptly. All protocol deviations were appropriately reviewed and documented. Those that affected the integrity of the study were reported in the final report.

3.9 Case definitions

Two different criteria's were used to grade the severity of liver toxicity. In the first criterion, I used the AIDs Clinical Trial Group (ACTG) severity grading system. In this system, severity is based on the number of times ALT levels is greater than upper limit of the normal (ULN). This is outlined in table 4. A cut off 40UI/L was used for both men and women. In the second criteria, the ratio of the ALT levels to the baseline value at each sampling point using a cut-off of two was used.

Table 4**: Severity grading in drug induced liver injury**

Severity	**Fold increase in ALT levels**
Grade 0 (Normal)	< 1.25 times ULN
Grade 1 (Mild)	1.25–2.5 times ULN
Grade 2 (Moderate)	2.5–5 times ULN
Grade 3 (Severe)	5-10 times ULN
Grade 4 (Very severe)	> 10 times ULN

ALT, alanine transaminase; ULN, upper limit of normal (40IU/L)

3.10 Variables and outcomes

Any increase by ≥ 1.25 times ULN in ALT (grade 1-4 hepatotoxicity) as by AIDS clinical trial group, ACTG classification during the course of HAART was the primary outcome of interest. Predictor variables that were considered in the identification of the key risk factors for ALT elevation included patient demographics, baseline laboratory tests, co-morbidities ART regimen, renal disease, adherence and treatment duration. Clinical signs of delayed hepatotoxicity were the secondary outcome of interest. This included jaundice, anorexia, abdominal pain, fever, fatigue and vomiting.

3.11 Data management

Participant's confidentiality was maintained by not recording their name or clinic number in the data collection forms. Each study participant was allocated a unique identifier that was used throughout the study. All documents linking the patient's name, file number and data collection number were kept by the principal investigator under lock and key. Reviewing of patient files and data abstraction was carried out within the CCC. The principal investigator kept all raw data under lock and key. Data was stored in re-writable CD and backed up on an external hard drive daily in password protected file limiting the access to the principal investigator and the data analyst only. Abstracted data was copied into an Excel sheet. The data was cleaned and any change made to the original copy of the data was recorded.

All data entries were double checked against the source document by the investigator. The raw data generated during the course of the study and the final report was subjected to inspection and quality audit for conformity to set protocols by the investigator.

3.12 Statistical analysis

I carried out descriptive data analysis on all variables. The Shapiro Wilk test was used to determine those continuous variables that conformed to normal distribution. For those continuous variables that were not normally distributed, the median and interquartile ranges (IQR) were reported. Counts and percentages were used for categorical variables

and the 95% confidence intervals were reported. Pearson Chi square test was done to compare the distribution of various variables with the main outcome of interest.

Generalized linear models were used to establish predictors for development of ALT elevation. All variables with a P-value lower than 0.20 at bivariable analysis were entered into a multivariable model (if clinically meaningful) and model building was conducted using forward stepwise selection method. Since some variables are not significant on bivariable analysis while in reality, they become significant on multivariable analysis a less stringent cut-off of p-value of ≤ 0.2 was used to select variables to include in the multivariable model. All analyses were performed using STATA version 10 (StataCorp 4905 Lakeway Drive College Station, Texas 77845 USA). For multi-variable analysis, P values less 0.05 were considered statistically significant.

3.13 Ethical considerations

To safeguard the rights and safety of study participant, this study protocol along with corresponding informed consent form was reviewed by the Kenyatta National Hospital/University of Nairobi Ethics and Research Committee and permission to carry out the study was granted (Ref: KNH-ERC/A/122). The KNH/UoN ethical approval is appended (Appendix A).

We informed potential participants about the study through an oral presentation regarding the purpose, procedure to be carried out, potential risks and their rights. Participants were required to understand and sign a consent form summarizing the discussion prior to recruitment (Appendix B). A copy of the signed informed consent statement was given to participants while the investigator retained a second copy.

Participants were not compensated on account of their participation in the study.

Participants were informed that they were free to dropout from the study at any time without stating any reason. We made every effort to obtain a complete follow up for any withdrawal.

4 CHAPTER 4: RESULTS

4.1 Baseline characteristics of the study participants

Overall, 185 (76.8%) of the 241 participants who took part in the study were females. The median age was 39 years [interquartile range (IQR) 35, 44]. Thirteen (5.4%) had a body mass index of below 18.5kg/m^2. The median body weight at baseline was 62kg ranging from 56 to 70kg. One hundred and fifty five (64.3%) were married. One hundred and eighty four (76.7%) participants were of the Bantu ethnicity group while Nilotes constituted 18%. Seventy two (29.8%) participants reported taking alcohol occasionally (less than twice a month) while two (0.8%) took alcohol regularly. Five (2.1%) participants were smokers. Most patients had attained either primary or secondary education accounting for 68.5% of the study participants.

At the start of therapy 158 (65.6%) patients had CD4 cell count of less than 250cells/mm^3. The median baseline ALT level was 22 IU/L (range 17 to 32). Most of the participants (91.3%) had a normal ALT level while fourteen (5.8%) had an elevated baseline ALT (above 40UI/L). The main nucleoside reverse transcriptase inhibitor (NRTI) most often used in combination with nevirapine at ART initiation was stavudine (35.3%). The median duration of follow up for the entire cohort was 4.75 years [IQR 3.34 - 6.6].

The prevalence of co-morbidities was less than 10%. The most prevalent conditions were hypertension (14.5%) and chronic pain (2.1%). A summary of the baseline characteristics of the 241 patients included in the study is presented in Table 5.

Table 5**: Demographic and Clinical characteristics of the study cohort**

Variables	Median [IQR] or n (%)
Sex	
Male	56 (23.2)
Female	185 (76.8)
Age at diagnosis (years)	39 [35-44]
Weight at diagnosis(kg)	62 [56-70]
Height (cm)	162 [158-168]
BMI at HAART initiation	
≤18.5	147 (61)
≥18.5	94 (39)
Marital status	
Married	155 (64.3)
Single	57 (23.7)
Divorced	4 (1.7)
Widowed	24 (10.0)
Separated	1 (0.4)
Education	
Primary	48 (19.9)
Secondary	117 (48.6)
Diploma	57 (23.7)
Degree	19 (7.9)
Employment status	
Unemployed	18 (7.5)
Employed	108 (44.8)
Self-employed	115 (47.7)
Alcohol use	
Never	167 (69.3)
Occasionally	72 (29.9)
Regularly	2 (0.8)
Smoking	
Yes	236 (97.9)
No	5 (2.1)
CD4 cell count x10^9/L	206[127-270]
≤250	158 (65.6)
≥251	68 (28.2)
Missing values	15 (6.2)
ALT at initiation of HAART	
Normal	209 (86.7)
Elevated	26 (10.8)
Missing	6 (2.5)
Concurrent illness	
None	182 (75.5)
Hypertension	36 (14.9)
Diabetes	3 (1.2)
PUD	4 (1.7)
Asthma	3 (1.2)
Chronic pain	5 (2.1)
Other conditions	8 (3.6)

Abbreviations: ART, antiretroviral therapy; BMI, body mass index, calculated as the weight in kilograms divided by the square of height in meters; IQR, interquartile range ; PUD-peptic ulcer disease; n= proportion per category.

Antiretroviral Regimens

At ART initiation 234 (97%) patients were started on a nevirapine based regimens, the other 7 (3%) were initiated on efavirenz based regimens. Those on efavirenz-based regimens had been switched to a nevirapine based regimen at the time of the recruitment into the study. The most widely used regimen was a combination of stavudine, lamivudine and nevirapine with 85 (35.3%) patients. This is presented in Table 6.

Table 6**: Regimens at ART initiation**

Regimen type	**Number of Patients (%)**
TDF+3TC+NVP	71 (29.5)
AZT+3TC+NVP	78 (32.4)
D4T+3TC+NVP	85 (35.3)
ABC+3TC+EFV	1 (0.4)
AZT+3TC+EFV	3 (1.2)
TDF+3TC+EFV	3 (1.2)

TDF: Tenofovir; 3TC: Lamivudine; NVP: Nevirapine; AZT: Zidovudine; D4T:Stavudine; ABC: Abacavir; EFV: Efavirenz.

Ninety two patients switched regimens in the course of their therapy. Most of the patients 70 (29.0%) were switched from D4T+3TC+NVP to TDF+3TC+NVP. The most common reason for regimen switch was development of adverse drug reaction especially peripheral neuropathy (38%) and lipodystrophy (15%) associated with stavudine. This is summarized in Table 7 below.

Table 7**: Regimen patients were switched to**

Regimen type	**Patients (%)**
TDF/3TC/NVP	80 (87.0)
AZT/3TC/NVP	11 (11.9)
ABC/3TC/NVP	1 (1.1)
Total	92 (100)

4.2 ALT changes in the study cohort

4.2.1 Patterns of ALT elevation in the course of therapy

A biochemical criterion was used to dichotomize patients to those having normal or elevated ALT levels. Normal maximum value in the laboratory for ALT was 40IU/L and we used the same cut off for both men and women. At baseline, most of the participants 209 (86.7%) had a normal ALT level while 26 (10.8%) had mild elevation. Six patients had no baseline ALT level readings. The median baseline ALT level was 22 IU/L (range 17 to 32).

In order to examine the trend in changes in ALT levels over time for patients with normal values at baseline, a median band plot was generated. The pattern looked cyclical with peaks and troughs. The peak levels seemed to increase with time. The trend is presented in Figure 2.

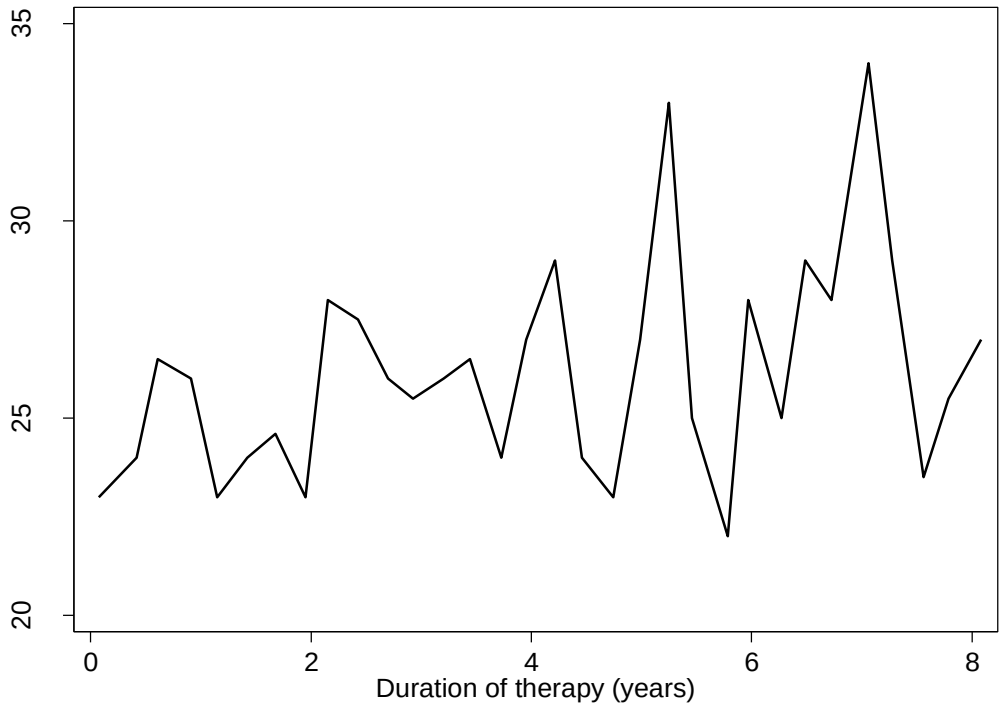

Figure 2**: Median band plot of ALT levels over time for patients with normal values at baseline**

In patients who had abnormal ALT levels, lowess plots were generate to establish the trend. A lowess plot is a summary measure of the weighted median of a series of ALT readings. In this group of patients the trend was a gradual decline in ALT levels till about 6 years of therapy, thereafter the ALT levels started rising steadily (Figure 3). The initial high ALT levels could be attributed to the HIV disease, which is followed by a gradual drop due to positive response to anti-retroviral therapy. The rise thereafter could be due to cumulative toxicity and/or decline in liver function with age.

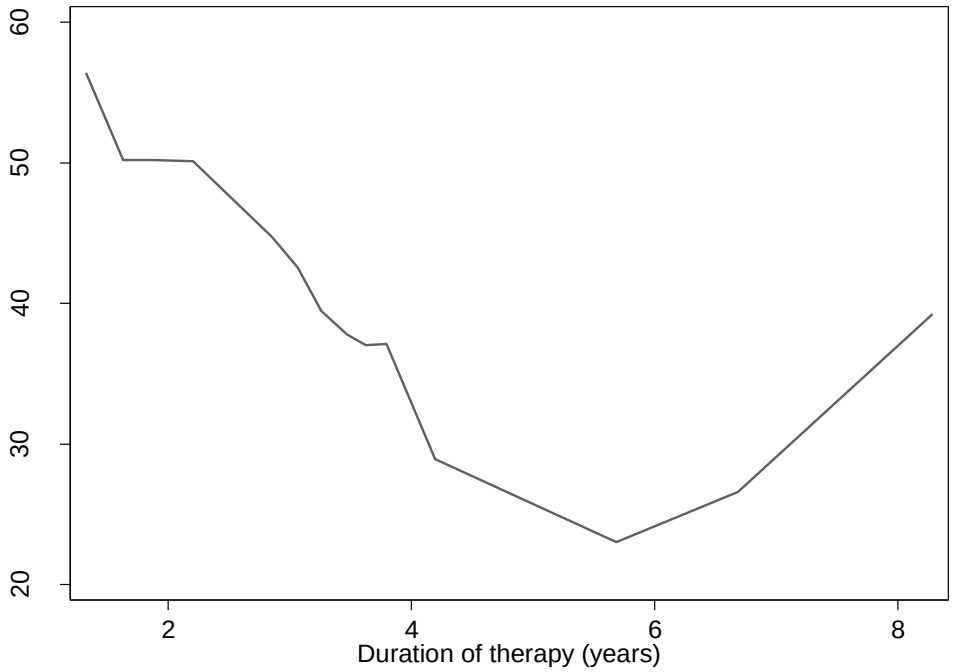

Figure 3**: Lowess plot of ALT levels over time for patients with abnormal values at baseline**

4.2.2 Severity and prevalence of ALT elevation

Based on fold increase in ALT levels criterion, most of the participants (67.2%) had normal ALT levels throughout the study. Seventy two (29.9%) had mild elevation and seven (2.9%) developed moderate hepatotoxicity. None of the participants developed severe or very severe hepatotoxicity. This is presented in Table 8 and Figure 4.

Table 8: **Severity of hepatotoxicity in the cohort according to the AIDS Clinical Trial Group grading system**

Grade	ALT levels	No. of cases n (%)
Normal	< 1.25 times ULN	162 (67.2)
Mild	1.25- 2.5 times ULN	72 (29.9)
Moderate	2.5-5 times ULN	7 (2.9)
Severe	5-10 times ULN	0 (0)
Very severe	>10 times ULN and jaundice and /or lethargy	0 (0)

When the ratio of the ALT levels to the baseline value at each sampling point using a cut-off of two was used, 94% of the patients experienced doubling of ALT from the baseline value. I decided to use the first criterion based on fold increase in ALT levels as it was more conservative.

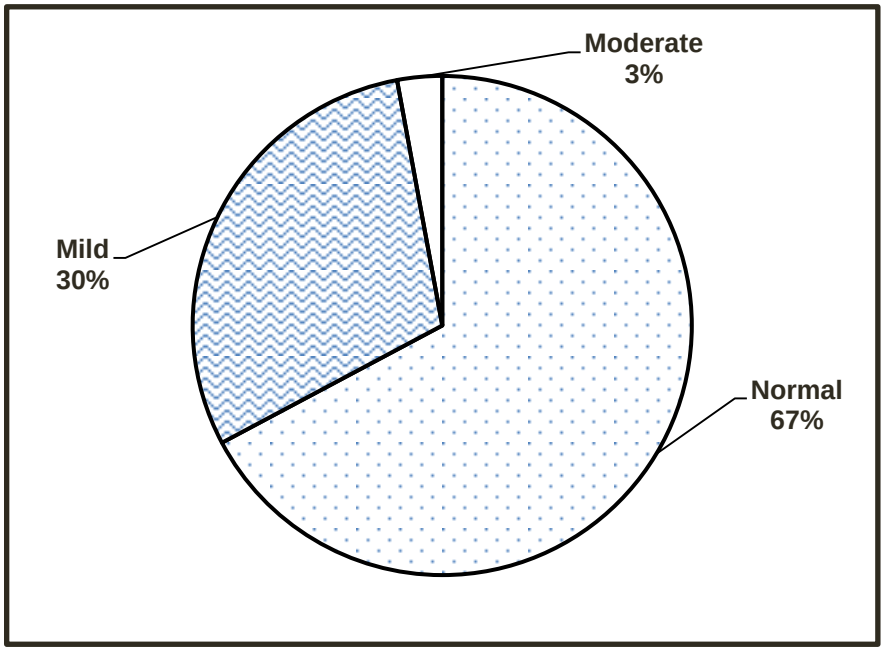

Figure 4: **Severity of hepatotoxicity in the study cohort**

4.2.3 Comparison of baseline traits of patients with normal and elevated ALT levels during therapy.

The baseline characteristics of the study participants according to ALT elevation are summarized in table 9. Severity of hepatotoxicity was based on the fold increase in ALT levels above the upper limit of the normal; 1.25 fold increases was used as a cut off in this study.

Sex was significantly associated with ALT elevation (P <0.001). Thirty out of a total of 56 males (53.6%) in the cohort had elevated ALT compared to 26.5% females. Age at ART initiation was categorized as those below or above 45 years. Eighteen (41%) of the above 45years old had an elevation compared to 31.6% among those below 45 years old.

There was no significant difference in ALT elevation across various marital status with most having a proportion of between 25% and 33%. A larger proportion of Bantus (35.9%) had an elevated ALT as compared to Nilotes (27.9%). None of the six Cushites had elevated ALT levels. Thirty eight percent of those with a BMI of <18.5kg/m^2 had elevated ALT as compared to 32.5% of those with BMI of >18.5kg/m^2. Elevation was common among alcohol users (41.7%) as compared to non-users (29.2%). Most smokers had elevated ALT levels (60%) compared to non-smokers (32.2%).

Abnormal baseline ALT level showed significant association (P <0.001) with ALT elevation. Ninety two percent of patients with abnormal baseline ALT developed subsequent elevation compared to 25.8% with normal ALT at baseline. CD4 cell count was categorized as low (≤ 250 cells/ μL) and high (>250 cells/ μL). There was no significant difference between the CD4 categories with 36.9% among the low CD4 count and 27.9% among high CD4 count developing ALT elevation. Nucleoside/nucleotide backbone used along with lamivudine and nevirapine had a significant impact on the status of liver function (p<0.019), forty out of eighty five (47%) patients initiated on stavudine backbone had an elevation compared to 26.8% and 24.4% patients on Tenofovir and Zidovudine regimens respectively.

Table 9**: Comparison of the traits of patients with normal and elevated ALT levels**

Predictor variable	Normal (<1.25×ULN) n (%)	Elevated (>1.25×ULN) n (%)	P-value
Sex			
Female	136 (73.5)	49 (26.5)	
Males	26 (46.4)	30 (53.6)	**<0.01**
Age at ART initiation (years)			
≤ 45	136(69.1)	61 (30.9)	
> 45	26 (59.1)	18(40.9)	0.204
Marital status			
Married	103 (66.5)	52 (33.5)	
Single	39 (68.4)	18 (31.6)	
Divorced	3 (75)	1 (25)	
Widowed	16 (66.7)	8 (33.3)	0.954
Separated	1 (100)	0 (0)	
Ethnic group			
Bantu	118 (64.1)	66 (35.9)	
Nilotes	31 (20)	12 (15.4)	
Cushites	6 (3.9)	0 (0)	0.129
Body mass index (kg/m^2)			
< 18.5	8 (4.9)	5 (6.3)	
>18.5	154 (95.1)	74 (93.7)	0.654
Alcohol use			
Never	119 (73.5)	49 (62)	
Occasionally	42 (25.9)	30 (38)	
Regularly	1 (0.6)	0 (0)	0.131
Smoking			
No	160 (98.8)	76 (96.2)	
Yes	2 (1.2)	3 (3.8)	0.190
Baseline ALT (U/L)			
≤ 40	155 (98.7)	54 (69.2)	
>40	2 (1.3)	24 (30.8)	**<0.01**
CD4 cell count×10^9/L			
≤ 250	100 (67.1)	58 (75.3)	
>251	49 (32.9)	19 (24.7)	0.202
ART regimen at initiation			
TDF+3TC+NVP	52 (32.1)	19 (24.1)	
AZT+3TC+NVP	59 (36.4)	19 (24.1)	
d4T+3TC+NVP	45 (27.8)	40 (50.6)	
d4T+3TC+EFV	1 (0.6)	0 (0)	
AZT+3TC+EFV	2 (1.2)	1 (1.3)	
TDF+3TC+EFV	3(1.9)	0 (0)	**0.019**
Concurrent illnesses			
None	123 (75.9)	59 (74.7)	
Hypertension	25 (15.4)	11 (13.9)	
Diabetes	2 (1.2)	1 (1.3)	
PUD	1 (0.6)	3 (3.8)	
Asthma	3 (1.9)	0 (0.0)	
Chronic pain	3 (1.9)	2 (2.5)	
cancer	2 (1.2)	0 (0.0)	
Others	3 (1.9)	3 (3.8)	0.197

*Significant P-values are in **bold**

4.3 Identification of risk factors for ALT elevation

We performed generalized linear regression to identify variables predictive of ALT elevation. In bivariable analysis of the whole cohort; gender, ethnic group, smoking, concurrent illness, poor-adherence, baseline ALT level, nucleoside/nucleotide backbone used with nevirapine, duration of therapy and renal disease were significantly associated with ALT elevation ($P < 0.05$); Table 10. The results of all predictor variables that were considered during bivariable analysis are presented in Appendix C.

Table 10: **Variables that were significantly associated with ALT on bivariable analysis of the entire cohort**

Variable	**β-coefficient (CI)**	**P-value**
Sex	9.38 (7.65, 11.12)	< **0.001**
Ethnicity	-2.1 (-3.25, -0.95)	< **0.001**
Ethnic group		
Bantus	ref	-
Nilotes	-3.56 (-5.54, -1.59)	< **0.001**
Cushites	-7.88 (-13.04, -2.71)	**0.003**
Smoking	6.37 (1.40, 11.35)	**0.012**
Concurrent illness	0.78 (0.32, 1.24)	**0.001**
Type of concurrent illness	-	-
None	ref	-
Peptic ulcer disease	9.11 (3.03,15.18)	**0.003**
Chronic pain	5.46 (0.45, 10.48)	**0.033**
Non-adherence	-1.77 (-3.38, -0.16)	**0.031**
Baseline ALT level	13.48 (11.20, 15.77)	< **0.001**
Regimen at ART initiation		
TDF+3TC+NVP	ref	-
D4T+3TC+NVP	4.45 (2.55, 6.34)	< **0.001**
Duration of therapy	0.43 (0.05, - 0.81)	**0.027**
Renal disease	9.28 (5.26, 13.30)	<**0.001**

Renal disease- having an estimated glomerular filtration rate (eGFR) < 50ml/min/1.73^2

All variables that were statistically significant at bivariable analysis were included in multivariable analysis models, if clinically meaningful. In all statistical analyses we used a significance level of $P \leq 0.05$. During multivariable analysisis, we found out that sex had a statistical interaction with drug regimen ($P<0.01$). This was interpreted to be a biological interaction since it tended to be additive. Consequently, for subsequent analysis we stratified data across sex. The crude and stratified data is presented in table 11.

In both males and females, elevated baseline ALT level and renal disease were associated with increased risk of ALT elevation. Females who had elevated ALT at baseline had 10 units higher [β 10.14 (95%CI 7.34- 12.96); P<0.001] while males had 13.52 units higher [β 13.52 (95% CI 9.36 – 17.68); P < 0.001] than those with normal levels at baseline.

Renal disease which was defined as having an estimated glomerular filtration rate (eGFR) < 50ml/min/1.73^2 was significantly associated with ALT elevation. Females who developed renal disease had five units higher [(95% CI 2.62 – 8.25); P <0.001] whereas males had 11.52 units higher [(95% CI 3.46 – 19.60); P = 0.005] than those with normal renal function.

Ethnicity had a protective effect in both males and females. Among the different ethnic groups, Nilotes and Cushites had lower ALT levels compared to Bantus. However, ethnicity had a greater effect in males than females. Male Nilotes had 9 units lower [β -9.12 (95% CI -13.17, -5.08); P< 0.001] while Cushites had 13 units lower [β -12.89 (95% CI -25.10, 0.69); P = 0.036] than Bantus. On the other hand, females Nilotes had 4 units [β -3.56 (95% CI -5.54, -1.59); P< 0.001] whereas Cushites had 8 units [β -7.88 (95% CI -13.04, -2.71); P=0.003] lower than Bantus.

Table11: **Factors associated with ALT elevation on stratification**

VARIABLE	CRUDE β (95% CI); P-VALUE	Females	Males
		β (95% C.I); p-value	β (95% C.I); p-value
Ethnicity	-2.10 (-3.25, -0.95); < **0.001**	-1.20 (-2.39, -0.01); **0.048**	-6.61 (-9.28, -3.93); <**0.001**
Ethnic group			
Bantus	-	-	-
Nilotes	-3.56 (-5.54, -1.59); < **0.001**	-3.12 (-5.27, -0.97); **0.005**	-9.12 (-13.17, -5.08); <**0.001**
Cushites	-7.88 (-13.04, -2.71); **0.003**	-6.70 (-11.99, -1.41); **0.013**	-12.89 (-25.10, -0.68); **0.038**
Smoking	6.37 (1.40, 11.35); **0.012**	11.31 (4.46, 18.18); **0.001**	-3.01(-10.82, 4.78);0.448
Concurrent illness	0.78 (0.32, 1.24); **0.001**	0.70 (0.08, 1.32); **0.026**	0.15 (-0.58, 0.90) ;0.677
Type of illness			
None	-	-	-
PUD	9.11 (3.03,15.18); **0.003**	16.72 (9.48, 23.97); <**0.001**	-5.87 (16.76, 5.01);0.290
Chronic pain	5.46 (0.45, 10.48); **0.033**	4.95 (-0.21, 10.12) ;0.06	5.53(-6.35, 17.40) ;0.362
Poor adherence	-1.77 (-3.38, -0.16); **0.031**	-1.62 (-3.20, -0.04); **0.045**	-3.29 (-7.86, 1.28);0.158
Previous ADRs	0.03 (-2.08, 2.15); 0.977	2.02 (0.10, 3.95); **0.040**	-
Baseline ALT level	13.48 (11.20, 15.77); < **0.001**	10.14 (7.34, 12.96); <**0.001**	13.52 (9.36, 17.68); <**0.001**
Regimen at ART initiation	1.23 (0.50, 1.98); **0.001**	0.34 (-0.37, 1.04) ;0.344	4.67 (2.10, 7.24); <**0.001**
Type of regimen			
TDF+3TC+NVP	-	-	-
AZT+3TC+NVP	-1.80 (-3.86, 0.26); 0.086	-1.23 (-3.26, 0.81) ;0.237	-7.80 (-13.96, -1.63); **0.013**
D4T+3TC+NVP	4.45 (2.55, 6.34); < **0.001**	1.53 (-0.40, 3.46) ;0.121	4.26 (-1.27, 9.78) ;0.131
Duration of therapy	0.43 (0.05- 0.81); **0.027**	-0.28 (-0.68, 0.11) ;0.155	1.81 (0.89, 2.73); < **0.001**
Renal disease	9.28 (5.26, 13.30); <**0.001**	5.44 (2.62, 8.25); < **0.001**	11.52 (3.46, 19.60); **0.005**

*Significant P-values are in bold; – reference variable; PUD-Peptic ulcer disease; ADRs-Adverse drug reactions

In females, smoking [β 11.31 (95% CI 4.46, 18.18); P= 0.001], peptic ulcer disease [β 16.72 (95% CI 9.48 -23.97); P <0.001] and previous adverse drug reactions [β 2.02 (95% CI 0.10- 3.95); P=0.040], were associated with increased risk of ALT elevation. However, only two out of the total 185 females (1.08%) were smokers and just three had peptic ulcer disease. Poor adherence had a protective effect [β -1.62(95%CI -3.20, -0.04); P=0.045].

In female a set of variables that best predicted ALT elevation (parsimonious model) were; elevated ALT at baseline, renal disease, ethnic group and previous adverse drug reaction. The results of all variables that were analyzed for association with ALT elevation in females is presented in appendix D.

In males, a longer duration of anti-retroviral therapy was associated with increased risk [β 1.81(95% CI 0.89 – 2.73); P < 0.001], while initiation on AZT+3TC+NVP had a significant protective effect [β -7.80 (95%CI -13.96, -1.63); P=0.013]. Factors that best predicted changes in ALT levels in males include; elevated ALT at baseline, regimen at ART initiation and ethnic group. Other variables that were considered during the analysis are presented in appendix E.

4.4 Prevalence of the clinical signs associated with hepatotoxicity

The clinical signs of hepatotoxicity reported in this cohort were anorexia, abdominal pain and vomiting. None of the patients developed jaundice. Thirteen patients had anorexia, eleven (85%) of whom were females. Eight patients reported vomiting and all of them were females. Abdominal pain was the most widely reported sign with 30 patients.

It is expected that patients who experienced abdominal pain should have showed ALT elevation. Contrary to this expectation, 25 out of 30 patients who experienced abdominal pain had normal ALT levels. There was a negative association between abdominal pain and ALT. Anorexia and vomiting were not statistically significant associated with ALT elevation. The results are presented in table12.

Table 12: **Description of clinical factors associated with ALT elevation**

Predictor variable	**Normal**	**ALT elevated**	**P- Value**
	< 1.25×ULN	**> 1.25×ULN**	
Abdominal pain			
No	136 (84.5%)	73 (93.6%)	
Yes	25 (15.5%)	5 (6.4%)	**0.046**
Vomiting			
No	158 (97.5%)	73 (94.8%)	
Yes	4 (2.5%)	4 (5.2%)	0.274
Anorexia			
No	152 (95.0%)	73 (93.6%)	
Yes	8 (5.0%)	5 (6.4%)	0.653

5 CHAPTER 5: DISCUSSION

This study found that the baseline prevalence of ALT elevation (>40 IU/L) was 10.8%; CI (6.84 - 14.73). This implies that 1 in 10 patients had elevated ALT levels before ART initiation. This elevation could be due to HIV illness. Out of the patients who had normal baseline ALT levels, 30% developed mild elevation while 3% developed moderate elevation throughout the course of therapy. This implies that 3 out of 10 patients will develop some form of liver injury. No case of severe or very severe hepatotoxicity was observed in our study. This was in variance with findings of a multicenter study carried in Kenya, Zambia and Thailand which reported a prevalence of severe hepatotoxicity of 5% (20). Other studies have reported a prevalence of severe and/or very severe hepatotoxicity of between 6-15% (8, 30). This could be explained by the fact that, all our patients were ambulatory and if any patient experienced hepatotoxicity, ARVs were stopped or patient was switched to non-nevirapine based regimens.

In patients with normal ALT at baseline, the pattern of ALT change was cyclical with peaks and troughs. The peak levels seemed to increase with time. Very sharp peaks were noted from the 5^{th} year of therapy onward. Among patients who had elevated ALT levels at baseline the trend was a gradual decline in ALT levels until about 6 years of therapy, thereafter the ALT levels started rising progressively. The initial high ALT levels could be attributed to the HIV disease, which is followed by a gradual drop due to protective effect of ART as it improves clinical status and CD4 count of the patient. The rise thereafter could be due to cumulative toxicity and/or decline in liver function with age. Other studies have reported that nevirapine-associated hepatotoxicity occurs within the first few weeks to months of starting therapy (19, 29). In our study, none of the patients developed hepatotoxicity within this period. This may suggest that these groups of patients are less susceptible to immune-mediated hypersensitivity reaction, which normally develops shortly after starting nevirapine. This may be attributed to the fact that, most of the participants in this study (86.7%) had a normal baseline ALT level at ART initiation. HAART therefore has a beneficial effect on patients who have abnormal baseline ALT levels.

Several unique findings were observed in our study; risk factors for ALT elevation between males and females differed, there were intra-ethnic differences with Bantus being the most susceptible; and initiation on Zidovudine based regimen was protective especially in males.

Intra-race differences have not been extensively investigated. However, a number of studies have reported inter-race variability. Kesselring et al, found out that Asians were more susceptible to nevirapine induced hepatotoxicity [HR (95% CI) = 2.24 (1.43 – 3.52); P < 0.001] compared to Caucasians. In our cohort, ethnic grouping had a significant effect in ALT levels in both males and females. However, ethnicity had a greater effect in males compared to females. Male Nilotes [β -9.12 (95% CI -13.17, -5.08)] and Cushites [β -12.89 (95% CI -25.10, 0.69)] had significantly lower ALT levels compared to Bantus. The intra-ethnic differences could be due to genetic, environmental or dietary factors. A study has been conducted that compares the distribution of polymorphisms of CYP 450 across the two major ethnic groups (Nilotes and Bantus) of Kenya. The study found a significant variability in the distribution of CYP2D6*4 and CYP2D6*17 between Bantu and Nilotes (32). Our finding that Nilotes and Cushites had lower ALT levels compared to the Bantus should be approached with caution. We encourage further studies in Africans to investigate the intra-ethnic genetic variations for better understanding of the observed association.

Abnormal baseline transaminase levels has been reported to be an independent risk factor for antiretroviral associated hepatotoxicity (30). Our study has also demonstrated that abnormal baseline ALT levels > 1.25 times the upper limit of normal is a risk factor for subsequent ALT elevation in both males and females. A multicenter study conducted in Kenya, Zambia and Thailand reported that the risk of severe hepatotoxicity and rash associated hepatotoxicity was predicted by abnormal (≥grade 1) baseline transaminase levels (20).

Although the association between renal disease and hepatotoxicity has not been extensively investigated, this study found a correlation between renal disease and ALT elevation. Females who developed renal disease had five units higher [(95% CI 2.62 – 8.25); P <0.001] whereas males had eleven units higher [(95% CI 3.46 – 19.60); P =

0.005] than those with normal renal function. This was a very significant finding although it was not possible to assess whether renal disease precedes liver disease. We speculate that, patients with compromised liver function may accumulate nevirapine that may be toxic to the kidney. Conversion of nevirapine to metabolites may ameliorate it is nephrotoxicity given that there is a paucity of literature of the possible link between nephrotoxicity and hepatotoxicity in patients on nevirapine based regimens.

For the first time we found that poor adherence was associated with a low risk for ALT elevation. We used a very crude measure for poor adherence, patients who reported that they had missed at least one dose the previous week were considered to have poor adherence. Missing doses of ARVs had a protective effect as compared to patients with perfect adherence. This was only significant among females. It is likely that individuals who miss their regular doses of ARVs could be having suboptimal levels of the drugs in plasma. This finding should be approached cautiously as the plasma drug levels were not determined to establish whether there were significant differences among those who reported perfect and poor adherence.

Type of anti-retroviral drug regimen was one of the predictors of ALT elevation but the findings differed across sex. On bivariable analysis, initiating patients on stavudine based NRTI was associated with increased risk of ALT elevation. However, on stratification across sex, the association was not significant in females. In males, treatment initiation on AZT+3TC+NVP had a significant protective effect [β -7.80 (95%CI -13.96, -1.63)]. The finding of our study, contradicts a review of cohort studies investigating the incidence of hepatotoxicity among patients on ARV therapy. This review suggested that the overall rate of ALT elevation is similar among all ARV drugs (5,11).

A longer duration of anti-retroviral therapy was associated with increased risk of ALT elevation among males ([β 1.81(95% CI 0.89 – 2.73); P < 0.001]) but not in females ([β -0.28 (95% CI -0.68, 0.11); P < 0.155]). A study carried out in Barcelona-Spain by Kovari et al support this assertion. They reported that, patients who tolerate ARV drugs for the first few months of therapy were likely tolerate it in long-term therapy (8). The

rise in ALT later in the course of therapy could be due to cumulative toxicity and/or decline in liver function with age.

Smoking and peptic ulcer disease was also associated with increased risk of ALT elevation in females but not in males. However, only two out of the total 185 females (1.08%) were smokers and about 1.6% had PUD, hence the study was not sufficiently powered to enable us draw conclusions about the observed association between this two variables and ALT elevation.

From literature, the most important clinical signs and symptoms of drug induced liver injury are; anorexia, vomiting, abdominal pain and jaundice. In this study, anorexia and vomiting were not statistically associated with ALT elevation. One of the likely reasons for this finding is that only a small proportion of the study participants developed and/or reported these signs during the clinical visits. Therefore, the study did was not sufficiently powered to find an association between the clinical signs and ALT elevation. Abdominal pain was negatively associated with ALT elevation.

Study strengths limitation

A major strength for this study was the study was carried out in a facility with a capacity to investigate laboratory parameters on regular basis. Also, the sample size of the studied populations was representative of the major ethnic communities in Kenya.

The study had a number of limitations that are inherent to most retrospective observational cohort studies in general. The prevalence of nevirapine-induced hepatotoxicity could not be ascertained precisely. There is a possibility that some patients who developed severe or very severe hepatotoxicity in the course of therapy were either discontinued or switched to other regimens. Secondly, the study relied heavily on pre-recorded information that may have been incomplete, missing, and inaccurate or could not be verified, this may have negatively affected the veracity of the study. Lastly, some variables could not be reliably evaluated in the study. Although ethnicity was found to be a predictor variable for ALT elevation, this study could not establish whether there was any genetic variation in the drug metabolizing enzymes among the different ethnic groups.

6 CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

We conclude that ALT elevation might occur in up to one third of HIV infected adult patients taking nevirapine based ARV drugs. While larger studies are needed, our data suggest that nevirapine based regimens are well tolerated. We found that having renal disease, abnormal baseline ALT levels and long duration of therapy were each independently associated with increased risk for ALT elevation.

In a resource limited setting where transaminase testing is available, we recommend that testing and monitoring should focus on delayed hepatotoxicity, patients with renal disease and those with abnormal baseline ALT for improved patient care.

We also recommend further studies to establish interethnic variability in clinically relevant single nucleotide polymorphism of CYP2B6 and CYP3A4. This will provide much need pharmacogenetic data specific to African populations. It will also enable tailoring of therapy based on genetic information that offers the potential to reduce the cases of adverse drug reactions and optimizing treatment.

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APPENDICES

APPENDIX A: KNH/UoN ETHICAL APPROVAL

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Ref: KNH-ERC/A/122 **Link:www.uonbi.ac.ke/activities/KNHUoN** 7th May 2014

Dr. Makori Jones Obonyo
Dept.of Pharmacology and Pharmacognosy
School of Pharmacy
University of Nairobi

Dear Dr. Obonyo

RESEARCH PROPOSAL: PREVALENCE AND RISK FACTORS FOR SYMPTOMS OF HEPATOTOXICITY IN PATIENTS ON NEVIRAPINE AT KENYATTA NATIONALHOSPITAL (P10/01/2014)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and approved your above proposal. The approval periods are 7th May 2014 to 6th May 2015.

This approval is subject to compliance with the following requirements:

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

For more details consult the KNH/UoN ERC website **www.uonbi.ac.ke/activities/KNHUoN.**

Protect to Discover

Yours sincerely

PROF. M. L. CHINDIA
SECRETARY, KNH/UON-ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director CS, KNH
The Chairperson, KNH/UoN-ERC
The Assistant Director, Health Information, KNH
The Dean, School of Pharmacy, UoN
The Chairman, Dept.of Pharmacology and Pharmacognosy, UoN
Supervisors: Dr. Margaret A. Oluka, Dr. Kipruto A. Sinei, Dr. Faith Apolot Okalebo

APPENDIX B: VOLUNTEER INFORMATION AND CONSENT FORM.

Consenting process

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. Once you understand and agree to take part, I will request you to sign your name on this form. You should understand the following general principles which apply to all participants in a medical research.

i. Your agreement to participate in this study is voluntary
ii. You may withdraw from the study at any time without necessarily giving a reason for your withdrawal
iii. Refusal to participate in the research will not affect the services that you are entitled to receive in this Clinic.

Introduction to the study

Nevirapine is a (NNRTI) anti-retroviral agent and a key component in HIV management. It is used in combination with other anti-retroviral drugs. However, Nevirapine has been associated with Liver injury and hypersensitivity reactions. The degree of these adverse events varies from one individual to another and can range from mild reaction to potentially life-threatening hypersensitivity reactions.

Patients on ART should be monitored closely for drug intolerance and side effects. Screening for potential Liver injury during ART is primarily based on serum levels of alanine transaminase, a liver enzyme which serves as a "proxy" for liver inflammation and damage.

In this study I am assessing the risk factors for developing Hepatotoxicity and the changes in liver enzymes in patients using Nevirapine containing ART. Permission is requested from you to enroll in this medical research study.

Purpose of the study

The primary objective of this study is to determine the changes in liver enzymes during ARV therapy among HIV patients using Nevirapine containing regimen. The second objective is to determine the risk factors for developing liver injury

Procedures to be followed

With your permission we will go through your medical records to obtain information on Laboratory investigations which have been conducted since you were initiated on ART. We will also check whether you have suffered any bad reactions to drugs suggestive of liver disease.

You will be asked a few questions about your ethnicity, if you are using any other drugs (prescription or over the counter) or herbal products, whether you drink or smoke, how regularly you take medication and whether you have experienced any bad reactions to drugs that you are taking. Herbal products, over the counter products and alcohol have a significant effect on the functioning of the Liver.

Selection criteria

You will be selected to take part in this study if you meet the following criteria:

a) You are an adult above 18 years.

b) You must have been on a Nevirapine based ART for at least 6 months.

c) You must have attending the Comprehensive Care Clinic at KHN for at least one year.

d) You must have agreed to take part in the study.

Risks or/and discomfort.

There will be no risks involved in this study to you.

Rights and safety

To safeguard your rights and safety as a participant taking part in this study, the Kenyatta National Hospital/University of Nairobi Research and Ethics Committee will review the study protocol and the informed consent process before commencing the research.

Benefits

The study may be of benefit to you and other HIV patients in that it will be used to enhance detection of early warning signs of hepatotoxicity. It may also inform policy makers on the need to review guideline on laboratory monitoring of toxicity.

Assurance on confidentiality

Utmost care will be taken to keep your participation in this study confidential. All information obtained from your file and laboratory investigation will be kept confidential and used for the purpose of this study only. Your name will not be used during data handling or in any resulting publications, codes will be used instead. Your medical records will be kept under lock and key and information will be accessible to authorized persons only.

Contacts

For any further information about this study you may contact me, my academic department or the Kenyatta National Hospital/University of Nairobi Ethics and research Committee using the contacts provided below:

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Dr. Margret Oluka,
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The chairperson,
The Kenyatta National Hospital/University of Nairobi Research and Ethics Committee,
P.O Box, 19676- Nairobi. Tel: 020-2726300 Ext 44102

STATEMENT OF CONSENT

I have understood the information on the consent form. I have had a chance of discussing the research study with the investigator and I have had my concerns addressed. 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 the consent form, or have had it read to me YES/NO

I agree to participate in this research study YES/NO

I agree to have my medical records used in this study YES/NO

Participant signature _____ Date _____

I confirm that I have explained the nature and effect of the study to the participant named above and believe that the participant has understood and has willingly given his/her consent.

Printed name _____ Date _____

Signature _____ Role in the study _____

APPENDIX C: **Factors associated with ALT elevation on bivariable analysis of the whole cohort**

VARIABLE	**BETA COEFFICIENT(CI)**	**P-VALUE**
Sex	9.38 (7.65, 11.12)	< **0.001**
Age at ART initiation	0.30 (-1.71, 2.32)	0.770
BMI at ART initiation	2.64 (-0.28, 5.57)	0.077
Marital status		
Married	ref	-
Single	-0.77 (-2.63, 1.07)	0.412
Divorced	-2.78 (-8.12, 2.56)	0.307
Windowed	-2.29 (-4.87, 0.29)	0.082
Separated	-0.20 (-18.43, 18.03)	0.983
Occupation		
Un-employed	ref	-
Employed	2.72 (-0.45, 5.88)	0.093
Self-employed	-0.03 (-3.18, 3.12)	0.983
Education	-0.47 (-1.38, 0.43)	0.302
Primary	-	-
Secondary	-0.08 (-2.11, 1.95)	0.937
Diploma	0.86 (-1.46, 3.19)	0.467
Degree	-3.50 (-6.69, -0.33)	0.031
Ethnic group	-2.10 (-3.25, -0.95)	< **0.001**
Bantus	ref	-
Nilotes	-3.56 (-5.54, -1.59)	< **0.001**
Cushites	-7.88 (-13.04, -2.71)	**0.003**
Others	-2.19 (-6.71, 2.33)	0.342
Alcohol use	1.45 (-0.11, 3.01)	0.068
Smoking	6.37 (1.40, 11.35)	**0.012**
Concurrent illness	0.78 (0.32, 1.24)	**0.001**
None	ref	-
Hypertension	1.08 (-0.98, 3.14)	0.305
Diabetes	-0.93 (-7.01, 5.15)	0.764
Peptic ulcer disease	9.11 (3.03,15.18)	**0.003**
Chronic pain	5.46 (0.45, 10.48)	**0.033**
Adherence		
Days missed dose	-1.77 (-3.38, -0.16)	**0.031**
Delayed taking dose	-1.05 (-2.57, 0.46)	0.173
Grade	0.03 (-2.08, 2.15)	0.977
Baseline CD4 count	-0.09 (-1.83, 1.65)	0.919
Baseline ALT level	13.48 (11.20, 15.77)	< **0.001**
Regimen at ART initiation	1.23 (0.50, 1.98)	**0.001**
TDF+3TC+NVP	ref	-
AZT+3TC+NVP	-1.80 (-3.86, 0.26)	0.086
D4T+3TC+NVP	4.45 (2.55, 6.34)	< **0.001**
ABC+3TC+EFV	-7.0 (-16.07, 2.12)	0.133
AZT+3TC+EFV	-2.53 (-8.93, 3.86)	0.438
TDF+3TC+EFV	2.36 (-6.73, 11.45)	0.611
Duration of therapy	0.43 (0.05- 0.81)	**0.027**
Renal disease	9.28 (5.26, 13.30)	<**0.001**

APPENDIX D: Factors analyzed for association with ALT elevation in females

VARIABLE	CRUDE β-Coefficient (95% CI)	P-VALUE	ADJUSTED β-Coefficient (95% C.I)	P-VALUE
Age at ART initiation	0.30 (-1.71, 2.32)	0.770	-0.10 (-2.31, 2.09)	0.924
BMI at initiation	2.64 (-0.28, 5.57)	0.077	3.06 (-0.13, 6.25)	0.060
Marital status				
Married	ref	-	ref	-
Single	-0.77 (-2.63, 1.07)	0.412	2.43 (0.58, 4.28)	**0.010**
Divorced	-2.78 (-8.12, 2.56)	0.307	0.73 (-4.07, 5.54)	0.765
Windowed	-2.29 (-4.87, 0.29)	0.082	1.23 (-1.15, 3.60)	0.311
Separated	-0.20 (-18.43, 18.03)	0.983	3.32 (-12.98,19.62)	0.690
Occupation				
Un-employed	ref	-	ref	-
Employed	2.72 (-0.45, 5.88)	0.093	-1.34 (-4.49, 1.79)	0.401
Self-employed	-0.03 (-3.18, 3.12)	0.983	-1.75 (-4.82, 1.31)	0.262
Education	-0.47 (-1.38, 0.43)	0.302		
Primary	-	-	-	-
Secondary	-0.08 (-2.11, 1.95)	0.937	-0.40 (-2.51, 1.70)	0.710
Diploma	0.86 (-1.46, 3.19)	0.467	-1.57 (-3.95, 0.81)	0.196
Degree	-3.50 (-6.69, -0.33)	0.031	-3.14 (-6.36, 0.08)	0.056
Ethnic group	-2.10 (-3.25, -0.95)	**< 0.001**	-1.20 (-2.39, -0.01)	**0.048**
Bantus	ref	-	ref	
Nilotes	-3.56 (-5.54, -1.59)	**< 0.001**	-3.12 (-5.27, -0.97)	**0.005**
Cushites	-7.88 (-13.04, -2.71)	**0.003**	-6.70 (-11.99, -1.41)	**0.013**
Others	-2.19 (-6.71, 2.33)	0.342	1.05 (-3.53, 5.63)	0.653
Alcohol use	1.45 (-0.11, 3.01)	0.068	0.41 (-1.18, 2.01)	0.610
Smoking	6.37 (1.40, 11.35)	**0.012**	11.31 (4.46, 18.18)	**0.001**
Concurrent illness	0.78 (0.32, 1.24)	**0.001**	0.70 (0.08, 1.32)	0.026
None	-	-	-	-
Hypertension	1.08 (-0.98, 3.14)	0.305	1.56 (-0.54, 3.65)	0.146
Diabetes	-0.93 (-7.01, 5.15)	0.764	3.33 (-4.75, 11.43)	0.419
Peptic ulcer disease	9.11 (3.03,15.18)	**0.003**	16.72 (9.48, 23.97)	**<0.001**
Chronic pain	5.46 (0.45, 10.48)	**0.033**	4.95 (-0.21, 10.12)	0.060
Adherence	ref		ref	
Non-adherence	-1.77 (-3.38, -0.16)	**0.031**	-1.62 (-3.20, -0.04)	**0.045**
Baseline CD4 count	-0.09 (-1.83, 1.65)	0.919	-0.11 (-1.89, 1.66)	0.899
Baseline ALT level	13.48 (11.20, 15.77)	**< 0.001**	10.14 (7.34, 12.96)	**<0.001**
Regimen at ART initiation	1.23 (0.50, 1.98)	**0.001**	0.34 (-0.37, 1.04)	0.344
TDF+3TC+NVP	-	-	-	-
AZT+3TC+NVP	-1.80 (-3.86, 0.26)	0.086	-1.23 (-3.26, 0.81)	0.237
D4T+3TC+NVP	4.45 (2.55, 6.34)	**< 0.001**	1.53 (-0.40, 3.46)	0.121
ABC+3TC+EFV	-7.0 (-16.07, 2.12)	0.133	-5.92 (-14.18, 2.33)	0.159
AZT+3TC+EFV	-2.53 (-8.93, 3.86)	0.438	-1.48 (-7.30, 4.33)	0.616
Duration of therapy	0.43 (0.05- 0.81)	**0.027**	-0.28 (-0.68, 0.11)	0.155
Renal disease	9.28 (5.26, 13.30)	**<0.001**	5.44 (2.62, 8.25)	**< 0.001**

APPENDIX E: Factors analyzed for association with ALT elevation in Males

VARIABLE	CRUDE β-COEFFICIENT (95% CI)	P-VALUE	ADJUSTED β-COEFFICIENT (95% C.I)	P-VALUE
Age at ART initiation	0.30 (-1.71, 2.32)	0.770	-2.91 (-7.06, 1.24)	0.170
BMI at initiation	2.64 (-0.28, 5.57)	0.077	4.85 (-1.17, 10.88)	0.114
Marital status				
Married	-	-	-	-
Single	-0.77 (-2.63, 1.07)	0.412	-6.64 (-11.86, -1.4)	0.073
Divorced	-2.78 (-8.12, 2.56)	0.307	-	-
Windowed	-2.29 (-4.87, 0.29)	0.082	-	-
Separated	-0.20 (-18.43, 18.03)	0.983	-	-
Occupation				
Un-employed	-	-		
Employed	2.72 (-0.45, 5.88)	0.093	12.40 (3.19, 21.61)	0.080
Self-employed	-0.03 (-3.18, 3.12)	0.983	8.65 (-0.76, 18.08)	0.072
Education	-0.47 (-1.38, 0.43)	0.302		
Primary	-	-	-	-
Secondary	-0.08 (-2.11, 1.95)	0.937	0.98 (-3.64, 5.61)	0.675
Diploma	0.86 (-1.46, 3.19)	0.467	11.17 (5.66, 16.69)	<0.001
Degree	-3.50 (-6.69, -0.33)	0.031	-3.43 (-11.29, 4.42)	0.392
Ethnic group	-2.10 (-3.25, -0.95)	< **0.001**	-6.61 (-9.28, -3.93)	<0.001
Bantus	-	-	-	-
Nilotes	-3.56 (-5.54, -1.59)	< **0.001**	-9.12 (-13.17, -5.08)	<**0.001**
Cushites	-7.88 (-13.04, -2.71)	**0.003**	-12.89 (-25.10, -0.68)	**0.038**
Others	-2.19 (-6.71, 2.33)	0.342	-14.10 (-25.18, -3.02)	**0.013**
Alcohol use	1.45 (-0.11, 3.01)	0.068	3.58 (-0.26, 7.42)	0.067
Smoking	6.37 (1.40, 11.35)	**0.012**	-3.01 (-10.82, 4.78)	0.448
Concurrent illness	0.78 (0.32, 1.24)	**0.001**	0.15 (-0.58, 0.90)	0.677
None	-	-	-	-
Hypertension	1.08 (-0.98, 3.14)	0.305	-1.48 (-6.51, 3.55)	0.563
Diabetes	-0.93 (-7.01, 5.15)	0.764	-10.81 (-20.60, -1.03)	0.130
Peptic ulcer disease	9.11 (3.03,15.18)	**0.003**	-5.87 (16.76, 5.01)	0.290
Chronic pain	5.46 (0.45, 10.48)	**0.033**	5.53 (-6.35, 17.40)	0.362
Non-adherence	-1.77 (-3.38, -0.16)	**0.031**	-3.29 (-7.86, 1.28)	0.158
Patient reported ADRs	0.03 (-2.08, 2.15)	0.977	-	-
Baseline CD4 count	-0.09 (-1.83, 1.65)	0.919	1.48 (-2.91, 5.86)	0.510
Baseline ALT level	13.48 (11.20, 15.77)	< **0.001**	13.52 (9.36, 17.68)	<**0.001**
Regimen at ART initiation	1.23 (0.50, 1.98)	**0.001**	4.67 (2.10, 7.24)	<0.001
TDF+3TC+NVP	-	-	-	-
AZT+3TC+NVP	-1.80 (-3.86, 0.26)	0.086	-7.80 (-13.96, -1.63)	**0.013**
D4T+3TC+NVP	4.45 (2.55, 6.34)	< **0.001**	4.26 (-1.27, 9.78)	0.131
ABC+3TC+EFV	-7.0 (-16.07, 2.12)	0.133	-	-
AZT+3TC+EFV	-2.53 (-8.93, 3.86)	0.438	-	-
TDF+3TC+EFV	2.36 (-6.73, 11.45)	0.611	-	-
Duration of therapy	0.43 (0.05- 0.81)	**0.027**	1.81 (0.89, 2.73)	< **0.001**
Renal disease	9.28 (5.26, 13.30)	<**0.001**	11.52 (3.46, 19.60)	**0.005**