EFFECTS OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY ON THE LIVER AND KIDNEY FUNCTIONS IN HIV PATIENTS AT COAST PROVINCE GENERAL HOSPITAL, KENYA

By

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156/CE/11948/2008

A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science (Medical Biochemistry) in the School of Pure and Applied Sciences of Kenyatta University.

OCTOBER, 2013
DECLARATION

I declare that the work presented in this thesis was carried out at Coast Province General Hospital (CPGH). Any information that was obtained from published work is acknowledged in the text, references and appendices. The contents of this thesis are entirely my original work and have not been presented for a degree or other award in any other University.

Signed…………………………….……    Date…………………………..……

Chris Kipngetich Ngeny

We confirm that the work reported in this thesis was carried out by the candidate under our supervision and has been submitted with our approval.

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    Mombasa, Kenya

Signed ................................. Date........................................
DEDICATION

This thesis is dedicated to my late father, Mr. Joseph Kipngeny Langat who was diagnosed with stomach cancer and passed away during the course of my study. I also dedicate it to my wife, Mrs. Jennifer Ngeny and our three children: Brian Ngetich, Brenda Cherotich and Barbara Chepkemoi for their prayers, understanding, moral support and encouragement while undertaking my studies.
ACKNOWLEDGEMENT

I sincerely register gratitude to my supervisors: Prof. Joseph J.N. Ngeranwa of Kenyatta University, Prof. Charity Gichuki of Presbyterian University of East Africa and Dr. Ng’ali Mbuuko of CPGH-Mombasa for their unreserved advice, guidance, constructive suggestions and reviews that made my entire research study a success. I sincerely thank them for their mentorship and for offering me a shoulder to stand on to see farther into the horizon in quest for new knowledge. I greatly appreciate the financial support awarded to me by Pwani University through the Research Board at the University.

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To God, be the glory.
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<th>Description</th>
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<tbody>
<tr>
<td>ABC</td>
<td>Abacavir</td>
</tr>
<tr>
<td>ACU</td>
<td>AIDS Control Unit</td>
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<tr>
<td>ACTG</td>
<td>AIDS Clinical Trial Group</td>
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<tr>
<td>AIDS</td>
<td>Acquired Immunodeficiency Disease Syndrome</td>
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<tr>
<td>AKI</td>
<td>Acute Kidney Injury</td>
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<tr>
<td>ALB</td>
<td>Albumin</td>
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<tr>
<td>ALP</td>
<td>Alkaline Phosphatase</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine Aminotransferase</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>ARM 1</td>
<td>HAART naïve patients</td>
</tr>
<tr>
<td>ARM 2</td>
<td>HAART treated patients</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate Aminotransferase</td>
</tr>
<tr>
<td>ANC</td>
<td>Antenatal Clinic</td>
</tr>
<tr>
<td>ART</td>
<td>Antiretroviral Therapy</td>
</tr>
<tr>
<td>BIL-D</td>
<td>Direct Bilirubin</td>
</tr>
<tr>
<td>BIL-T</td>
<td>Total Bilirubin</td>
</tr>
<tr>
<td>BUN</td>
<td>Blood Urea Nitrogen</td>
</tr>
<tr>
<td>CCC</td>
<td>Comprehensive Care Centre</td>
</tr>
<tr>
<td>CD4</td>
<td>CD4+ T-cell (T-lymphocyte bearing CD4+ receptor)</td>
</tr>
<tr>
<td>CDC</td>
<td>Centre for Disease Control</td>
</tr>
<tr>
<td>CKD</td>
<td>Chronic Kidney Disease</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CPGH</td>
<td>Coast Province General Hospital</td>
</tr>
<tr>
<td>CREAT</td>
<td>Creatinine</td>
</tr>
<tr>
<td>Acronym</td>
<td>Full Form</td>
</tr>
<tr>
<td>----------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>D4T</td>
<td>Stavudine</td>
</tr>
<tr>
<td>DIH</td>
<td>Drug Induced Hepatotoxicity</td>
</tr>
<tr>
<td>DHS</td>
<td>Demographic and Health Survey</td>
</tr>
<tr>
<td>EFV</td>
<td>Efavirenz</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme Linked Immuno-Sorbent Assay</td>
</tr>
<tr>
<td>ESRD</td>
<td>End-Stage Renal Disease</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration of United States of America</td>
</tr>
<tr>
<td>GFR</td>
<td>Glomerular Filtration Rate</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>HAART</td>
<td>Highly-active Antiretroviral Therapy</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>HIVAN</td>
<td>HIV Acute Nephropathy</td>
</tr>
<tr>
<td>HOPS</td>
<td>Healthier Options for Public Schoolchildren</td>
</tr>
<tr>
<td>HSR</td>
<td>Hypersensitivity Reaction</td>
</tr>
<tr>
<td>IAS</td>
<td>International AIDS Society</td>
</tr>
<tr>
<td>IFCC</td>
<td>International Federation of Clinical Chemistry</td>
</tr>
<tr>
<td>K⁺</td>
<td>Potassium ion</td>
</tr>
<tr>
<td>KAIS</td>
<td>Kenya AIDS Indicator Survey</td>
</tr>
<tr>
<td>KDHS</td>
<td>Kenya Demographic and Health Survey</td>
</tr>
<tr>
<td>KEMRI</td>
<td>Kenya Medical Research Institute</td>
</tr>
<tr>
<td>KNBS</td>
<td>Kenya National Bureau of Statistics</td>
</tr>
<tr>
<td>KNCMAP</td>
<td>Kenya National Clinical Manual for ART Providers</td>
</tr>
<tr>
<td>LFT</td>
<td>Liver Function Test</td>
</tr>
<tr>
<td>MoH</td>
<td>Ministries of Health in Kenya</td>
</tr>
<tr>
<td>NACC</td>
<td>National AIDS Control Council</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>---------</td>
<td>-------------</td>
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<tr>
<td>NASCOP</td>
<td>National AIDS and STI Control Programme</td>
</tr>
<tr>
<td>NCCLS</td>
<td>National Committee for Clinical Laboratory Standards</td>
</tr>
<tr>
<td>NCST</td>
<td>National Council for Science and Technology</td>
</tr>
<tr>
<td>NGO</td>
<td>Non-Governmental Organization</td>
</tr>
<tr>
<td>NVP</td>
<td>Nevirapine</td>
</tr>
<tr>
<td>PI</td>
<td>Protease Inhibitor</td>
</tr>
<tr>
<td>PLWHA</td>
<td>People Living With HIV and AIDS</td>
</tr>
<tr>
<td>PMTCT</td>
<td>Prevention of Mother-To-Child-Transmission</td>
</tr>
<tr>
<td>PROT</td>
<td>Protein</td>
</tr>
<tr>
<td>PSI</td>
<td>Population Services International</td>
</tr>
<tr>
<td>QA</td>
<td>Quality Assurance</td>
</tr>
<tr>
<td>QC</td>
<td>Quality Control</td>
</tr>
<tr>
<td>RFT</td>
<td>Renal Function Test</td>
</tr>
<tr>
<td>RPM</td>
<td>Rational Pharmaceutical Management</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolutions Per Minute</td>
</tr>
<tr>
<td>RSH</td>
<td>Reproductive and Sexual Health</td>
</tr>
<tr>
<td>SAS</td>
<td>Statistical Analysis Software</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SOPs</td>
<td>Standard Operating Procedures</td>
</tr>
<tr>
<td>STI</td>
<td>Sexually Transmitted Infection</td>
</tr>
<tr>
<td>TB</td>
<td>Tuberculosis</td>
</tr>
<tr>
<td>TDF</td>
<td>Tenofovir Disoproxil Fumarate</td>
</tr>
<tr>
<td>ULN</td>
<td>Upper Limit of Normal</td>
</tr>
<tr>
<td>UNAIDS</td>
<td>Joint United Nations Program on AIDS</td>
</tr>
<tr>
<td>UNGASS</td>
<td>United Nations General Assembly Special Session</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>USAID</td>
<td>United States Agency for International Development</td>
</tr>
<tr>
<td>VCT</td>
<td>Voluntary Counseling and Testing</td>
</tr>
<tr>
<td>VL</td>
<td>Viral Load</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
ABSTRACT

The emergence of highly active antiretroviral therapy (HAART) has led to dramatic improvements in prolonging survival of HIV-infected patients on treatment in resource-limited areas. However, the main drawback of HAART that long-term use has the potential to cause liver and kidney derangements that may be life-threatening. These important complications sometimes warrant switch or discontinuation of antiretroviral therapy. Information on the prevalence of the above complications in Kenya is scanty. The current study assessed the prevalence of hepatic and renal toxicity in one hundred and fifty HIV+ patients [50 HAART naïve and 100 HAART treated subjects] based on clinical laboratory assays. Data were matched for HAART status, age, sex and the duration the patients had been on ARV treatment. The data was analyzed using SAS version 9.2. The prevalence of hepatotoxicity based on elevated alanine aminotransferase analyte above upper limit of normal was 18% in HAART treated and 8% in HAART naïve patients. The prevalence of renal derangements based on elevated creatinine analyte above upper limit of normal was 4% in HAART treated and 8% HAART naïve group. However, the prevalence of hepatotoxicity and renal derangements cases did not vary significantly between HAART treated and HAART naïve subjects ($\chi^2$; $P =0.59$ and $P = 0.9$ respectively). Variation in liver and kidney analytes were compared between gender using student’s $t$-test and variation in data for liver and kidney analytes were compared for age and duration the patients were on HAART using ANOVA with statistical significance set at $\alpha=0.05$. The key liver and kidney analytes indicative of hepatotoxicity and renal insufficiency varied significantly between males and females; (ALT; $P=0.001$) and (CREAT; $P=0.001$) respectively. Liver and kidney analytes varied significantly with age; (ALT; $P=0.006$) and (CREAT; $P=0.001$) respectively and the duration the patients had been on HAART; (ALT; $P=0.002$) and (CREAT; $P=0.001$) respectively. In conclusion, prevalence of hepatotoxicity was 17.3% and renal insufficiency was 5.3% in all HIV positive subjects irrespective of HAART status. The prevalence of hepatotoxicity was higher in the HAART treated, female gender, patients aged above 46 years or have been on HAART for more than 4 years. Renal insufficiency was more common in HAART naïve patients, female gender, and patients aged more than 46 years. Results from this study will help healthcare actors and providers to pay greater attention to individualized treatment of HIV and AIDS using HAART so as to reduce toxicities and co-morbidities that reduce the quality of life and increases the risk of death. They can also help in harmonizing HAART regimens and prescription dosage in order to reduce toxicity levels. The study recommends a controlled research study to be carried out to tease out toxic individual drug agents within HAART classes on liver and kidney functions.
CHAPTER ONE

INTRODUCTION

1.1 Background information

About 35.3 million people were living with HIV and AIDS worldwide by 2012 up from 33.4 million in 2008 and more than 25 million have died since the first cases were reported in 1981 (WHO and UNAIDS, 2013). Sub-Saharan Africa is the worst-affected region with an estimated 25.0 million people (70.8%) of the global total. The population of Sub-Saharan Africa accounts for only 11-12% of the world’s population (UNAIDS, 2013). The pandemic killed an estimated 1.4 million people in 2012 of which 1.2 million of the cases were from sub-Saharan Africa. The epidemic is more prevalent in low and middle income-countries where millions of people are infected each year (UNGASS, 2010). About 2.3 million Kenyans live with HIV/AIDS while an estimated 1.5 million have already died of the virus and each year, approximately 200,000 Kenyans develop the AIDS syndrome (Milkowski, 2004). HIV and AIDS pandemic affect all regions and communities and it impacts negatively on households and economic growth of nations. The prevalence and incidence of HIV by region as shown in table 1.

Table 1: Prevalence and incidence of HIV by region (Ages 15-49)

<table>
<thead>
<tr>
<th>World region</th>
<th>Adult prevalence of HIV infection</th>
<th>Adult and child deaths during 2009</th>
<th>Adult prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>World Wide</td>
<td>30.6 - 36.1 million</td>
<td>1.9 to 2.4 million</td>
<td>0.8</td>
</tr>
<tr>
<td>Sub-Saharan Africa</td>
<td>20.9-24.3 million</td>
<td>1.6 million</td>
<td>5.0</td>
</tr>
<tr>
<td>Africa</td>
<td>5.1 - 8.1 million</td>
<td>360,000</td>
<td>1.3</td>
</tr>
<tr>
<td>Asia</td>
<td>1.9 – 2.8 million</td>
<td>79,000</td>
<td>1.1</td>
</tr>
<tr>
<td>America</td>
<td>0.6 - 1.1 million</td>
<td>12,000</td>
<td>0.3</td>
</tr>
<tr>
<td>Europe</td>
<td>1.6-1.7 million</td>
<td></td>
<td>7.1</td>
</tr>
</tbody>
</table>

Source: WHO and UNAIDS, 2013
The introduction of antiretroviral therapy (ART) for use in management of HIV and AIDS, compounded with the routine use of CD4+ T-cell counts as surrogate markers of drug efficacy and disease progression significantly increased the life expectancy among HIV-infected patients. Between 1996 and 1999 the advent of highly active antiretroviral therapy (HAART) dramatically improved the survival of patients with HIV infection with unprecedented changes in disease progression and mortality seen first in the US and European population (Palella, 1998; Pezzotti, 1999). World Health Organization (WHO) and other organizations are providing countries with ongoing guidance, tools and support in delivering and scaling up ART for HIV and AIDS within the public health sector.

The goal of ART is to suppress viral replication and have impaired immunity restored but its major drawback is adverse effects accompanying its use. HAART toxicity has emerged as an important complication and eventually a major reason for ART switch and/or discontinuation (Braitstein et al., 2006). Acute drug toxicities still exist, and although typically not life-threatening, they can affect the quality of life and patients’ willingness to adhere to their treatment regimens (Montessori et al., 2004). Despite substantial benefits of HAART, a variety of short and long-term adverse effects have been associated with their use which reduces adherence and efficacy levels of the medication (d’Arminio et al., 2000). The frequency of drug toxicities is often described in clinical trials but not so thoroughly monitored and evaluated in clinics. Sulkowski et al. (2000) observed that, 18 out of 31 drugs causing hepatotoxicity in humans showed toxicity in animal models and one-third of all drugs associated with hepatotoxicity in animals result in a rise in liver enzymes in humans. Drug-induced toxicity is often detected long after a drug enters the market because animal models cannot always predict human toxicity (Vella and Palmisano, 2000). Detection of
toxicities is done by measuring the levels of organ-specific surrogate markers in blood and/or urine samples then compared with established reference range values of a normalised population when making interpretations.

1.2 Problem statement

Antiretroviral therapy has significantly improved prognosis of HIV and AIDS infections by restoring immune veracity and limiting opportunistic infections. However, HIV treatment results in toxicities that complicate management and increases the cost of health care. Adverse effects have been reported with all antiretroviral drugs and are among the most common reasons for switching or discontinuing therapy as well as for medication non-adherence (O’Brien et al., 2003). According to Ickovics (1997), surveys of people receiving HAART have showed that 30% of patients missed doses within the previous 3 days, and adverse effects account for 10%–15% of those discontinuing treatment. The HAART side effects have become an important public health problem contributing to more than 50% of acute liver failure cases, a fraction of which require immediate transplantation (d’Arminio et al., 2000). Data on HAART toxicities are plentiful, but they are inconsistent and therefore more robust studies are needed (Kramer et al., 2007). This augurs well with the intent of this study. It is anticipated that as the population of HIV-infected patient ages and remains on HAART for longer periods of time HIV and HAART-related metabolic disorders increases. Jevtovic (2008) attested to this when he observed that cumulative long-term toxicities, for instance drug-induced hepatotoxicity and kidney injury, have emerged as significant complications. It is therefore important to elucidate the effects of HAART on the liver and kidney functions in HIV positive patients.
1.3 Justification

HIV patients are more prone to develop adverse effects due to use of a cocktail of antiretroviral drugs. Such cohorts of patients are at high risk of developing short and long-term complications such as hepatotoxicity, cardiovascular disorders and renal insufficiency among others. Hepatotoxicity is associated with many of the antiretroviral agents which make their use a double-edged sword (Sanders et al., 2007). Renal disease and other syndromes encountered in HIV patients are diverse, progressive, and frequently insidious and their presence is subtle until it is far advanced when very little renal function has remained (Ogundahunsi et al., 2008). Despite scaling up of HAART treatment in Kenya, documented data and reports on the prevalence of liver and kidney derangements are still scanty. It is against this backdrop that this study evaluated the prevalence of abnormal liver and renal function analytes in HIV positive patients on HAART. Variation in liver and kidney analytes is compared with age, gender and duration the HIV patients have been on HAART at Coast Province General Hospital (CPGH), Kenya.
1.4 **Research questions**

i. What is the prevalence of liver and renal derangements in HAART treated and HAART naïve HIV positive patients at CPGH?

ii. Do liver and kidney analytes vary with age in male and female patients on HAART?

iii. Do liver and kidney analytes vary with the duration the patients have been on HAART among HIV+ patients at CPGH?

1.5 **Null hypotheses**

i. Prevalence of liver and renal derangements is not significantly different between HAART and HAART naïve patients.

ii. Sex and age related differences in liver and renal analytes are not significant among patients on HAART.

iii. Liver and renal function analytes do not vary significantly with duration patient had been on HAART.

1.6 **Objectives of the study**

1.6.1 **Main objective**

To examine the functional integrity of the liver and kidney from the effects of HAART and elucidate whether these episodes are associated with any risks factors.
1.6.2 Specific objectives

i. To determine the prevalence of liver and kidney derangements in HAART-treated and HAART naïve HIV positive subjects at the CPGH.

ii. To compare variation in liver and kidney analytes with age among male and female patients on HAART at CPGH.

iii. To compare variation in liver and kidney analytes with the duration the patients have been on HAART.
CHAPTER TWO

LITERATURE REVIEW

2.1 HIV and AIDS

Human Immunodeficiency Virus (HIV) belongs to the retrovirus family of viruses. HIV affects the immune system of infected persons by destroying T-lymphocytes cells, which the body relies to fight infection (NASCOP, 2002). There are two distinct serotypes of HIV virus: type 1 and type 2. The HIV-1 is the primary cause of acquired immunodeficiency syndrome (AIDS) worldwide while, HIV-2 is found largely in West Africa and its vertical transmission is unusual (Sanders et al., 2007).

Acquired immune deficiency (AIDS), is the late stage of HIV infection, a condition characterized by destruction of CD4+ T cells which help the body fight diseases (NASCOP, 2002). The syndrome was first identified in 1981 among homosexual men and intravenous drug users in New York and California and after its detection evidence of AIDS epidemics grew shortly after among heterosexual men, women, and children in sub-Saharan Africa (CDC, 2009). Although initial infection with HIV can result in flu-like symptoms, infected persons typically can show no symptoms for many years but as HIV replicate in the body, infected persons begin to show signs and symptoms of e.g., shingles, tuberculosis, oral or vaginal thrush, herpes simplex virus, and Kaposi sarcoma (WHO, 2009) which is a reflection of a weakened immune system or loss of the body’s ability to fight infection.
2.2 HIV and AIDS epidemiology

2.2.1 Global HIV and AIDS status

HIV infection since its discovery in the early 1980s has led to quick development of AIDS into a worldwide epidemic, affecting virtually every nation. By end of 2009 an estimated 33.3 million people were living with HIV compared to 26.2 million in 1999 – a 27% increase (UNAIDS, 2010). By 2005, more than 25 million people had died and an estimated 39 million were living with HIV (WHO, 2009). An estimated 4 million people were newly infected with HIV in 2005 and 95 percent of them in sub-Saharan Africa, Eastern Europe, or Asia (Ashford, 2006). Already, more than 30 million people around the world have died of AIDS-related diseases (United Nations, 2011). Globally, 34% of people living with HIV in 2009 resided in the 10 countries in southern Africa; 31% of new HIV infections in the same year occurred in these 10 countries, as did 34% of all AIDS-related deaths. About 40% of all adult women with HIV live in southern Africa (UNAIDS, 2010).

2.2.2 HIV and AIDS status in Africa

The impact of HIV has been most severe in some of the poorest countries in Africa. At the end of 2009, there were 9 countries in Africa where more than one tenth of the adult population aged 15-49 years was infected with HIV (UNAIDS, 2010). In the same year, an estimated 1.8 million new HIV infections occurred in Africa accounting for 69 percent of new infections worldwide and 370,000 children began their lives with HIV, which is a decrease from the previous year when 390,000 African children were infected through mother-to-child transmission (UNAIDS and WHO, 2009; UNAIDS, 2010). Sub-Saharan Africa is more heavily affected by HIV and AIDS than any other region of the world. In 2008, it was home to two thirds (67%) of all people
living with HIV and nearly three quarters (72%) of AIDS-related deaths (UNAIDS and WHO, 2009).

This HIV pandemic has overwhelmed health-care systems, increased the number of orphans and caused life expectancy rates to plummet. In some countries in the southern part of the continent, including Botswana, Lesotho, Swaziland, and Zimbabwe, more than 30 percent of the population has HIV infection or AIDS (CDC, 2009). It has sapped the populations of young men and women in their productive years of between 15-49 years who form the foundation of the labor force (Ashford, 2006). Health care problems have already reached crisis proportions in some parts of the world already burdened by war, political upheaval, or unrelenting poverty.

2.2.3 HIV and AIDS situation in Kenya

In Kenya, the first case of HIV was diagnosed in 1984 and by the end of 1985, 26 cases of AIDS were reported in a study that indicated an HIV prevalence of 59 percent amongst a group of sex workers in Nairobi (AIDS Newsletter, 1986). Towards the end of 1986 there was an average of four new AIDS cases being reported to the World Health Organization each month (AIDS Newsletter, 1987). Since then, the epidemic rates of HIV infection expanded, remained concentrated in marginalized and special-risk groups, including women who were sex workers and their clients, and men in mobile occupations, such as long-distance truck drivers (KAIS, 2009).

HIV prevalence in Kenya has been declining in the last two decades. National HIV prevalence in Kenya among adults aged 15-49 years decreased from a high of around 14% in the mid-1990s to 6.3% in 2008 (KDHS, 2009). The downward trend was especially profound in the urban sites of Busia, Meru, Nakuru and Thika, where
median prevalence declined from 28% in 1999 to 9% in 2003 among 15–49-year-old women attending antenatal clinics, and from 29% in 1998 to 9% in 2002 among those aged 15–24 years (Hallett et al., 2006). The Kenya AIDS Indicators Survey (KAIS) estimated the average HIV prevalence among the general population aged 15-49 years at 7.4 percent while the Kenya Demographic and Health Survey (KDHS) estimated prevalence for the same population at 6.3 percent (KAIS, 2009; KDHS, 2009).

The surveys also confirmed that women still have a higher prevalence compared to men at 8.4 percent against 5.4 percent (KAIS, 2009) and according to KDHS (2009) it stands at 8 percent for women compared to 4.3 percent for men. Sex differential is more pronounced among young women 15-24 age group whose HIV prevalence is four times higher than young men i.e. 5.6 percent against 1.4 percent respectively (KAIS, 2009). HIV prevalence also varies between regions, ranging from as low as 0.9 percent in North Eastern province to as high as 13.9 percent recorded in Nyanza province (NACC and NASCOP, 2006).

2.3 HIV and AIDS transmission

HIV is transmitted when a person is exposed to body fluids infected with the virus, such as blood, semen, vaginal secretions, and breast milk. The primary modes of HIV transmission include having sexual relations with an infected person or sharing hypodermic needles or accidental pricking by a sharp contaminated with infected blood and transfer of the virus from an infected mother to her baby during pregnancy, childbirth, or through breast-feeding (Milkowski, 2004). Sex workers, their clients, men who have sex with men and injecting drug users were together estimated to account for roughly one in three new HIV infections in Kenya in 2006 (Gelmon et al., 2009). In a study in Mombasa, Kenya, 43.0% of men who have sex only with other
men tested HIV-positive, compared with 12.3% of men who reported having sex with both men and women (Sanders et al., 2007).

When HIV enters the body, it infects lymphocytes, the CD4 T cells of the immune system. The virus commandeers the genetic material of the host cell, instructing it to replicate more and more viruses (UNAIDS and WHO, 2010). The newly formed viruses break free from the host, destroying the cell in the process and move on to infect and destroy other new or uninfected lymphocytes.

2.4 Diagnosis of HIV infection
The presence of HIV infection in individuals can be ascertained only through the use of laboratory tests on various body fluids such as blood, plasma, semen or vaginal fluid among others. WHO-UNAIDS has established an algorithm for the use of various tests for screening, surveillance and diagnostic purposes (WHO, 2009). Antibodies to HIV are detectable within four to six weeks of infection by commonly employed tests and in virtually all infected individuals within six months since HIV antibodies persist for lifetime once they appear in blood (Bunnel and Cherutich, 2008).

Diagnosis of HIV infection can be carried out by detecting antibodies to HIV, P24 HIV antigen, HIV nucleic acid (RNA/DNA) in clinical samples (WHO, 2009). The most commonly used test for the diagnosis of HIV infection is by serological tests detecting anti-HIV antibodies. It is economical, rapid and can be performed easily in most laboratories. The standard test to detect HIV antibodies in the blood is the enzyme-linked immune-sorbent assay (ELISA) where a blood sample is mixed with proteins from HIV (WHO, 2009). If the blood contains HIV antibodies, they attach to
the HIV proteins, producing a tell-tale color change in the mixture. This test is highly reliable when performed two to three months after infection with HIV.

Although HIV tests are very sensitive, they can produce false-positive results. So a positive ELISA HIV tests must be confirmed with another HIV test called the Rapid HIV antibody test, which can detect lower levels of HIV antibodies within 10-20 minutes. In the test, a blood sample is applied to a paper strip containing HIV proteins and if HIV antibodies are present, they bind to the HIV proteins, producing a color change on the paper (Bunnel and Cherutich, 2008). The combination of the ELISA and the Western Blot test is more than 99.9 percent accurate in detecting HIV infection within 12 weeks following exposure (WHO, 2005). Once the tests confirm an HIV infection, the health of the infected person’s immune system is monitored periodically by measuring CD4 cell counts and viral load in the blood. Viral load test measures the amount of the virus in the body and is determined using polymerase chain reaction (PCR) test. The viral RNA determines the rate of HIV growth in an infected person (CDC, 2009). The progressive loss of CD4 cells corresponds to an increasing viral load and a worsening state of the disease indicating that the immune system is increasingly becoming impaired or weakened.

2.5 HAART in management of HIV and AIDS

Whereas no medical treatment cures AIDS, antiretroviral therapy (ART) was developed for the management of HIV and AIDS to prolong life. The primary goal of ART is maximal and durable suppression of plasma viral load, preservation and/or restoration of immunologic function, improvement of quality of life and reduction of HIV related morbidity and mortality (NASCOP, 2002). This concurs with the fact that in the early 1980s when HIV/AIDS epidemic began and the corresponding absence of
ART, people with AIDS were not likely to live longer than a few years (Carcelain et al., 1999). UNAIDS estimated that a total of 2.5 million deaths have been averted in low and middle-income countries since 1995 due to the roll out of antiretroviral therapy (UNAIDS, 2010).

The therapy package entails the use of antiretroviral medications that attack the virus itself plus other non-ARV medications to prevent and treat opportunistic infections (OIs) that can occur when the immune system is compromised by HIV (WHO, 2009). Counseling and support mechanisms is also done to help people deal with emotional and traumatizing repercussions as well to enable one to accept to live with a disabling, potentially fatal disease. The extent to which HAART increases longevity suggest that with the most recent advances, individuals diagnosed with AIDS in 2003 and who received treatment would live, on average, 14 more years than if they had not been treated at all (Maria and Soriano, 2006). HAART also lessen the chances of transmitting HIV from one partner to the other through sex though the risk of transmission is not completely eliminated. HAART use among HIV-infected persons has been associated with a 60% reduction in transmission risk behaviour in multiple settings (Bunnel and Cherutich, 2008).

There has been increased expansion and access to ART to those eligible for treatment. Antiretroviral therapy coverage rose from 7% in 2003 to 42% in 2008, with especially high coverage achieved in eastern and southern Africa (48%) (UNAIDS and WHO, 2009). According to UNAIDS and WHO estimates, 47% (6.6 million) of the estimated 14.2 million people eligible for treatment in low and middle-income countries were accessing life-saving antiretroviral therapy in 2010, an increase of 1.35 million since 2009 (UNAIDS and WHO, 2010). In Kenya, AIDS-related deaths have
fallen by 29% since 2002, a decline which is attributed to the use of antiretroviral drugs (NACC and NASCOP, 2006)

Today, there are more than 31 antiretroviral drugs grouped into five classes and approved by the Food and Drug administration (FDA) to treat HIV infections (WHO, 2009). Each of the five classes attack HIV in a different way as depicted in the mechanism of action in column three of table 2 below.

**Table 2: Classes of HAART and their mechanism of action**

<table>
<thead>
<tr>
<th>HAART class</th>
<th>Approval year</th>
<th>Mechanism of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleoside Reverse Transcriptase Inhibitors (NRTI*)</td>
<td>1987</td>
<td>Inhibit reverse transcription by being incorporated into the newly synthesized viral DNA and preventing its further elongation.</td>
</tr>
<tr>
<td>Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI*)</td>
<td>1997</td>
<td>Inhibit reverse transcriptase directly by binding to the enzyme and interfering with its function.</td>
</tr>
<tr>
<td>Protease Inhibitors (PIs*)</td>
<td>1995</td>
<td>Target viral assembly by inhibiting protease enzyme used by HIV to cleave nascent proteins for final assembly of new virons.</td>
</tr>
<tr>
<td>Fusion or Entry Inhibitors</td>
<td>2003</td>
<td>Prevent HIV from binding to or entering human immune cells.</td>
</tr>
<tr>
<td>Integrase Inhibitors</td>
<td>2007</td>
<td>Inhibit integrase enzyme needed by HIV to insert its genetic material into human cells.</td>
</tr>
</tbody>
</table>

*Available in most countries while the rest are available in resource-rich countries. **Source: WHO 2009.**

The standard antiretroviral (ARV) regimen for management of HIV positive patients consists of a combination of 3 active antiretroviral agents: two NRTIs often combined with one medication from either NNRTIs or PIs class (Montessori et al., 2004). The combination of these three or more ARV drugs forms a regimen referred to as highly-active antiretroviral therapy (HAART) which effectively suppresses the viral load to undetectable levels (WHO, 2009). HAART has dramatically decreased the number of
hospital admissions and AIDS patients have achieved an impressive improvement in the quality of life (Palella et al., 1998; Maria and Soriano, 2006).

2.6 HAART-related adverse effects

While the use of HAART medications has had a profound impact on the AIDS epidemic in the world, it should be understood that the drugs carry their own drawbacks. Increasing adverse effects caused by HAART ranging from mild to severe have been well documented in many studies and are a major safety concern (Hawkins, 2010). Each drug in the HAART combination has its own range of side effects and it is not possible to predict how an individual will be affected by the drug therapy although some common adverse effects identified during pre-marketing clinical trials are known. The three common side effects of HIV medications are diarrhea, nausea and fatigue (Emery et al., 2008). Less frequent toxicities like lactic acidosis with hepatic steatosis, progressive ascending neuromuscular weakness and longer term complications for instance dyslipidemia and fat mal-distribution were not recognized until after the drugs had been in use for years (Hawkins, 2010).

HAART side-effects may be transient or may persist throughout therapy and are among the most common reasons for switching or discontinuing therapy as well as for medication non-adherence (Hawkins, 2010). Adverse effects play a major role in determining adherence to HAART and it is perhaps the most significant determinant of a regimen’s success (d’Arminio et al., 2000). In a Swiss cohort study, the presence of laboratory adverse events was associated with higher rates of mortality during 6 years of follow-up, highlighting the importance of adverse events in overall patient management (Maria and Soriano, 2006). In rare cases, some drug-related events may result in significant morbidity and even mortality. Antiretroviral drug toxicity
affecting the liver and kidney is monitored after every six months (MoH, 2007) but in most resource-limited settings it is symptom directed. Severity of adverse effects of HAART varies by ethnicity, individual differences, age, region, and interaction with other drugs, including alcohol and type or class of drug (Dieterich et al., 2002).

Antiretroviral therapy can have a wide range of adverse effects which are conveniently identified by class of offending agent used and categorized as short and long-term toxicities (Hawkins, 2010). The most predominant adverse effects for each antiretroviral class used in antiretroviral therapy are summarized in table 3 below.

Table 3: Adverse effects associated with different classes of HAART

<table>
<thead>
<tr>
<th>HAART Class</th>
<th>Examples of drugs</th>
<th>Adverse effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Short term events</td>
<td>Long term events</td>
</tr>
<tr>
<td>NRTIs</td>
<td>Zidovudine, Stavudine, didanosine</td>
<td>Anemia, nausea, lactic acidosis, pancreatitis, rash, myopathy and Nausea.</td>
</tr>
<tr>
<td>NNRTIs</td>
<td>Efavirenz, Nevirapine, Etravirine</td>
<td>Rash and Hypersensitivity reaction.</td>
</tr>
<tr>
<td>PIs</td>
<td>Atazanavir, Indinavir, Lopinavir</td>
<td>Nausea, diarrhea, nephrolithiasis, rash, and Jaundice.</td>
</tr>
</tbody>
</table>

NRTI- Nucleoside reverse transcriptase inhibitors; NNRTI- Non-nucleoside reverse transcriptase inhibitors and PI – Protease inhibitors.

Source: Hawkins, 2010

2.6.1 Short-term HAART adverse effects

Short term HAART side effects occur within weeks to months after initiation of ARVs. Some of the common ones include; gastrointestinal (GI) related toxicities clinically manifested by nausea, vomiting and diarrhea that were the major reasons for discontinuation of ARVs in the acute phase of treatment in a retrospective review
from the HOPS database (O’Brien et al., 2003). Rash is a common short term adverse effect which can be caused by almost any drug. Rash is the main offender in HAART and it has been observed in 10-17% of patients receiving NNRTIs (Carr and Cooper, 2000). Hypersensitivity reaction (HSR) is a common side effect of HAART characterized by fever, rash, myalgia, abdominal pain, elevated liver transaminases, lethargy, respiratory distress, musculoskeletal ache, paresthesia and edema. HSR occur notably with Abacavir (ABC) and Nevirapine (NVP) and can cause renal or hepatic failure (Hawkins, 2010). Central Nervous System (CNS) disorders that are signified by symptoms like vivid dreams, off-balance or unsteady walking, light-headedness or drowsiness, feeling like falling over, spinning or room spinning are commonly associated with Efavirenz (Clifford et al., 2003).

Anemia is primarily associated with Zidovudine (ZDV) drug which results from decreased ability of the bone marrow to produce blood cells, a condition called myelosuppression. Pozniak (2006) reported in a GS 934 study that 6% of patients on ZDV/3TC had been discontinued from the study at 48 weeks because of anemia and jaundice resulting from an increased red blood cell breakdown and in indirect (unconjugated) bilirubin. Other documented short-term HAART side effects includes; fatigue, dizziness, dyspepsia, acute pancreatitis, nephrolithiasis, lactic acidosis, and nail discoloration (Hawkins, 2010).

2.6.2 Long-term HAART adverse effects

Although antiretroviral therapy has been shown to reduce the incidence of both AIDS-defining and non-AIDS conditions, long-term exposure to HAART may also be associated with significant toxicity. Long term side-effects occur within months to years after onset of antiretroviral therapy. The most commonly documented cases
include; Cardiovascular diseases (CVD) for instance myocardial infarction which is associated with ABC as documented in two studies by Lundgren et al., 2008 and Cooper et al., 2009. Association between ABC and decreased flow mediated dilation following brachial artery clamping; a marker for endothelial dysfunction and decreased platelet function has been documented (Hsue et al., 2009). Dyslipidemia, a side effect of many ARVs, especially those including PIs has been associated with an increased risk for CVD. Lipodystrophy an umbrella term for several conditions, including lipoatrophy and/or lipohypertrophy is often associated with dyslipidemia and insulin resistance (Grinspoon and Carr, 2005).

Renal dysfunction is a HAART side effect that has been associated primarily with Tenofovir Disoproxil Fumarate (TDF) since the parent NRTI tenofovir is actively accumulated in the proximal renal tubule (Cihlar et al., 2001). Liver disease occurs from a number of ARV for instance nevirapine (NVP) and efavirenz (EFV) can cause hepatotoxicity via HSR which can result in acute liver necrosis and death. The overall rate of severe hepatotoxicity with NRTI therapy reported by Reisler and colleagues was 12%, which highlights the complexity and difficulty of evaluating and managing hepatotoxicity associated with antiretroviral therapy (Reisler et al, 2001).

2.7 HAART-related liver toxicity and its diagnostic markers

The liver plays a central role in transforming and clearing of chemicals such as drugs and it is susceptible to damage from toxicity of these agents. Due to its unique metabolism and close relationship with the gastrointestinal tract, the liver receives blood coming directly from gastrointestinal organs and then spleen via portal veins which bring drugs and xenobiotics in near-undiluted form (Larry et al., 2004). Certain medicinal agents, when taken in overdoses and sometimes even when
introduced within therapeutic ranges, may injure the liver causing them to be withdrawn from the market due to hepatotoxicity (Sulkowski, 2004). The National Institutes of Health of USA presented findings on liver toxicity in International AIDS Society (IAS) conference and its retrospective analysis showed that hepatotoxicity is associated with all classes of antiretroviral medications in use (Clifford et al., 2003). Liver problems, diarrhea, nausea, and other stomach problems are possible side effects of any HIV medication (Pataki, 2006). Drug induced hepatotoxicity characterized by elevation of AST/ALT levels to at least twice the upper limit of normal (ULN) can occur with drugs from all ARV classes (Sulkowski et al., 2000).

Several mechanisms are responsible for either inducing hepatic injury or worsening the damage process due to HAART. Many chemicals damage mitochondria causing it to release excessive amount of oxidants which, in turn, injure hepatic cells releasing intracellular enzymes into blood circulation (Martinez, 2004). Many HIV patients do often take alternative and complementary medicines in association with HAART and several of those have been associated with clear-cut hepatotoxicity (Mocroft et al., 2005). In patients with HIV, the term hepatotoxicity may then be misleading because some of these elevated liver tests may not be directly caused by the medication in question but acute viral hepatitis, reactivation of chronic hepatitis B or C, alcohol ingestion may all play a role in such events (Sulkowski et al., 2000).

Although most liver diseases cause only mild symptoms initially, it is vital that early diagnosis and detection is done by performing full liver function tests (LFTs). However, diagnosis of drug hepatotoxicity may be complicated by the fact that patients often take several medications so teasing out the actual culprit can present challenges. Patients with HAART-induced hepatotoxicity may be asymptomatic, with
liver injury diagnosed during routine blood testing, while others develop symptoms including nausea, fatigue, itching and jaundice (O’Brien et al., 2003) with the latter symptom being significant. There is a broad variability among studies in the criteria to categorize the severity of hepatotoxicity.

According to Kenya National Clinical Manual for ART providers (KNCMAP), patients with transaminases within normal limits at baseline are considered to develop hepatotoxicity when ALT and/or AST rise above the upper limits of normal (MoH, 2007). It defines severe hepatic injury (the primary study outcome) as defined as grade 3 or 4 change in AST and/or ALT levels during antiretroviral treatment and if AST and ALT grades are discordant; the highest should be used for classification purposes.

Liver function tests (LFTs) are carried out to detect the presence of liver disease, distinguish among different types of liver disorders, and gauge the extent of known liver damage and response to treatment (Prognosis). LFTs are a group of clinical biochemistry laboratory blood assays designed to give information about the state of a patient's liver (Abrescia et al., 2005). Some liver analytes in LTFs are associated with liver functionality e.g. Albumin (ALB) and total proteins (PROT), others are concerned with hepatocellular integrity e.g. aminotransferases (ALT & AST) and some associated with cholestasis - biliary tract blockage- e.g. gamma-glutamyl transferase (ɤ-GT) and alkaline phosphatase (ALP) (MoH, 2007). In most cases, hepatotoxicity due to drug toxicities is not mutually exclusive and mixed types of injuries are often encountered categorized in table 4.
Table 4: Grading of hepatotoxicity

<table>
<thead>
<tr>
<th>Type of injury</th>
<th>ALT</th>
<th>ALP</th>
<th>ALT/ALP RATIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatocellular</td>
<td>≥ 2ULN</td>
<td>&gt;2ULN</td>
<td>High, ≥5</td>
</tr>
<tr>
<td>Cholestatic</td>
<td>Normal</td>
<td>≥ 2ULN</td>
<td>Low, ≤2</td>
</tr>
<tr>
<td>Mixed</td>
<td>≥ 2ULN</td>
<td>≥ 2ULN</td>
<td>2-5</td>
</tr>
</tbody>
</table>

ULN – means upper limit of normal

Source: MoH, 2007

The two liver biomarkers (ALT and ALP) are useful in the monitoring, evaluation and management of patients with hepatic dysfunction due to drug toxicity. Categories of patients at higher risk for drug-induced hepatotoxicity include: females, obese individuals, elderly patients, viral illnesses and pre-existing liver disease (Wit et al., 2002). The most important biochemical analytes of the liver significant in diagnosing drug-induced hepatotoxicity are outlined below.

2.7.1 Total protein (PROT)

Plasma proteins are synthesized predominantly in the liver and are the building blocks of all cells and body tissues. In the course of disease PROT concentration and the percentage represented by individual fraction can significantly deviate from normal values (Koller, 1984). Total protein measurements are used in the diagnosis and treatment of a variety of diseases involving the liver i.e. liver cirrhosis and hepatitis, kidney, bone marrow and other metabolic nutritional disorders (Lindsey, 1986).

2.7.2 Albumin (ALB)

Albumin is a carbohydrate-free protein, representing 55 to 65 percent of the plasma PROT. It maintains the plasma colloidal osmotic pressure, transport and stores a wide variety of ligands and serves as a source of endogenous amino acids. It binds toxic heavy metal ions and many drugs which is why a decrease in Albumin in the blood
can have important pharmacokinetic consequence (Grant et al., 1987). Hyperalbuminemia is of little diagnostic significance except in dehydration but hypoalbuminemia is very common in many diseases. It stems from various factors namely, impaired synthesis as a result of liver disease or due to diminished protein intake and increased catabolism due to tissue damage or inflammation. An albumin measurement also allows for monitoring of the patient’s response to nutritional support and is useful test of liver functionality (Marshal, 1989). In severe hypoalbuminemia, plasma albumin levels are below 25g/L.

\section*{2.7.3 Alanine aminotransferase (ALT)}

Alanine aminotransferase (ALT) is an enzyme present in a variety of tissues and its major source is the liver. Measurement of ALT levels is used in diagnosis of hepatic disease where elevated serum ALT is found in hepatitis, cirrhosis, obstructive jaundice, carcinoma of the liver, and chronic alcohol abuse (Sherwin and Sobenes, 1996). Both serum aspartate aminotransferase (AST) and ALT become elevated whenever disease processes affect liver cell integrity though ALT is liver specific and its activity persist longer than elevations of AST activity (Sherwin and Sobenes, 1996). Elevated ALT/AST above 40U/L is an indicator for hepatotoxicity which can be categorized as mild/grade 1 (40-84 U/L); moderate/grade 2 (85-174 U/L); severe/grade 3 (175-350U/L) and severe/grade 4 (>350U/L) (MoH, 2007).

\section*{2.7.4 Aspartate aminotransferase (AST)}

Aspartate aminotransferase is widely distributed in tissue, principally hepatic, cardiac, muscle, and kidney and elevated serum levels are found in diseases affecting these
tissues (Nagy, 1984). Hepatobiliary diseases such as cirrhosis, metastatic carcinoma, and viral hepatitis also increase serum AST levels.

2.7.5 Alkaline phosphatase (ALP)

Alkaline phosphatase is a group of phosphatases found in almost every tissue in the body. Normal adult males tend to have ALP higher levels than females, but pregnant females have increased levels due to placental secretion of ALP. Normal ALP levels are elevated during periods of active bone growth, like in young children and adolescents (Moss et al., 1987) however, abnormal elevation of ALP levels >160 U/L occurs in diseases such as hepatitis, cirrhosis, malignancy, chemical toxicity, and bone diseases such as metastatic carcinoma, rickets, Paget’s disease, and osteomalacia.

2.7.6 Total bilirubin (BIL-T)

Bilirubin is formed in the reticulo-endothelial system during the degradation of aged erythrocytes. The heme portion from hemoglobin and from heme-containing proteins is removed, metabolized to bilirubin, conjugated with glucuronic acid for solubilization and subsequent transport through the bile duct and elimination via the digestive tract (Fody, 2005). Elevations of circulating unconjugated bilirubin occurs in liver immaturity and several diseases, in which the bilirubin conjugation is impaired causes. Bile tract obstruction or damage to hepatocellular structure causes increase in levels of both direct and indirect bilirubin in the circulation (Balisteri and Shaw, 1987).
2.7.7 Direct bilirubin (BIL-D)

In the liver, bilirubin is conjugated with glucuronic acid for solubilization and subsequent transport through the bile duct and elimination via the digestive tract (Balisteri and Shaw, 1987). Increase in conjugated bilirubin in plasma is highly specific for disease of the liver or bile ducts. Hepatocellular injury or cholestasis is suspected when more than 50% of total bilirubin is conjugated bilirubin (Fody, 2005).

2.7.8 Gamma glutamyl transferase (γ-GT)

Gamma glutamyl transferase is an enzyme involved in the transfer of γ-glutamyl residue from γ-glutamyl peptides to amino acids, water and other small peptides. γ-GT activity is found primarily in brain, prostrate, pancreas and liver (Krefetz and McMillin, 2005). Enzymatic activity of γ-GT is often the only parameter with increased values when testing for diseases affecting the mentioned organs and is one of the most sensitive indicators known. γ-GT activities are found in the serum of patients requiring long term medication with Phenobarbital and phenytoin (Krefetz and McMillin, 2005). Clinical applications of assay however are confined mainly to diagnosis and monitoring of hepatobiliary disease.

2.8 HAART-related kidney injuries and their diagnostic markers

The kidney plays a major role in the metabolism and excretion of waste products of metabolism including drug metabolites. HIV infection hurts the ability of the kidneys to function properly and some HIV medications may also harm the kidneys (Pataki, 2006) making it vulnerable to various types of renal damage including disturbances of fluid and electrolyte metabolism and disturbances in acid-base balance. Clinically, HAART can cause various kidney syndromes including various electrolyte and acid-base disorders, acute kidney injury (AKI), lactic acidosis, and chronic kidney disease.
These injuries occur via multiple mechanisms, including direct tubular toxicity, allergic reactions, and precipitation of insoluble drug crystals within renal tubular lumens (Wyatt et al., 2009).

Renal toxicities including acute kidney injury (AKI), tubulopathies, chronic kidney disease (CKD), and end-stage renal disease are some of the adverse side-effects of HAART requiring renal replacement therapy (Ogundahunsi et al., 2008). Renal damage manifests itself as proximal tubular injury with associated reduction in glomerular filtration and patients often develop increased serum creatinine, glycosuria, tubular proteinuria, and low serum phosphate (Thompson, 2011). Renal disease has been associated primarily with TDF since the parent NRTI tenofovir is actively accumulated in the proximal renal tubule via the action of renal-specific organic anion transporters 1 (Cihlar et al., 2001). Accumulation of the drugs in renal proximal tubules due to a potential imbalance in the uptake and efflux has been implicated in drug-induced Fanconi syndrome (Izzedine et al., 2005). Fanconi syndrome causes acute proximal tubular dysfunction and has been reported in patients receiving TDF and adenovir, most often in patients with poorly controlled HIV disease, causing elevated creatinine (Eaton, 2005).

Diagnosis of HAART induced adverse effects on the kidney involve performing full renal function tests (RFTs or RFs), a group of clinical biochemistry laboratory blood assays designed to give information about the state of a patient's kidney (Daugas et al., 2005). Indirect renal markers namely serum creatinine (CREAT) and blood urea nitrogen (BUN), as well as electrolytes; sodium (SOD), potassium (K⁺) and chloride (CL⁻) are assayed to determine renal function. Serum creatinine levels or creatinine clearance/glomerular filtration rate (GFR) can be measured to determine degree of
renal dysfunction. The scale of renal dysfunction (insufficiency) is graded as mild moderate or severe based on the GFR or serum creatinine level as outlined in table 5.

Table 5: Grading of renal dysfunction

<table>
<thead>
<tr>
<th>Grade of Severity of Renal dysfunction</th>
<th>Glomerular Filtration Rate (ml/minute)</th>
<th>Serum Creatinine (umol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&gt;50</td>
<td>&lt;160</td>
</tr>
<tr>
<td>Mild</td>
<td>20-50</td>
<td>160-300</td>
</tr>
<tr>
<td>Moderate</td>
<td>10-20</td>
<td>300-450</td>
</tr>
<tr>
<td>Severe</td>
<td>&lt;10</td>
<td>&gt;450</td>
</tr>
</tbody>
</table>


Another prognostic marker for kidney disease is an elevated level of protein in the urine, a condition referred to as proteinuria. It is a qualitative test that turns positive when there is presence of albumin in urine which indicates injury to kidney glomeruli due to viral infection or HAART toxicities among other causes. The functional importance of renal biochemical analytes and their levels are significant in diagnosing renal-toxicity. These analytes are summarized below.

2.8.1 Blood Urea Nitrogen (BUN)

Urea is the major end product of protein metabolism. It is synthesized by the urea cycle in the liver from ammonia produced by amino acid deamination. Urea is excreted mostly by the kidneys but minimal amounts are also excreted in sweat and degraded in the intestines by bacterial action. Determination of blood urea nitrogen is the most widely used screening test for renal function (Friedman and Young, 2000). It is used in conjunction with serum creatinine determinations to aid in differential diagnosis of the three types of azotemia: pre-renal, renal and post-renal. Unpredictable levels of BUN occur with liver diseases (Rock et al., 1986). Elevations in BUN concentration occur in inadequate renal perfusion, shock, diminished blood volume (pre-renal causes), chronic nephritis, nephlo-sclerosis, tubular necrosis,
glomerulonephritis (renal causes) and urinary tract obstruction (Friedman and Young, 2000). Transient elevations may also be seen during periods of high protein intake.

2.8.2 Creatinine (CREAT)

Creatinine (CREAT) is produced as a natural by-product from spontaneous muscle activity on creatine phosphate and then removed from the bloodstream by kidneys. It is often measured as a gauge of how well the kidneys are functioning. The rate of creatinine formation is fairly constant with 1 to 2% of the body creatine being converted to creatinine every 24 hours (Rock et al., 1986). Serum creatinine and urea levels are elevated in patients with renal malfunction especially decreased glomerular filtration, however, in the early stages of kidney damage, a rise in the serum urea levels usually precedes an increase in serum creatinine. The advantage is offset by the fact that serum urea levels are affected by factors such as diet, degree of hydration and protein metabolism (Reid et al., 2008).

Urea nitrogen and Creatinine assays are conducted for diagnostic purposes, therapeutic drug monitoring of acute and chronic renal diseases and for monitoring kidney dialysis (Ogundahunsi et al., 2008). They are adequate to determine whether a patient is suffering from kidney disease, unfortunately, they are not raised in most cases above the normal range until 60% of total kidney function is lost (Rao, 2001). It is also important to remember that many patients with HIV may present with muscle wasting while receiving HAART, which can lower serum creatinine concentration and falsely support the presence of normal kidney function. Conversely, with HAART therapy many patients may gain weight, and creatinine may increase without renal
injury (Celesia, 2010) and in such patients, serum creatinine measurement alone is an insensitive marker and should be coupled with a qualitative test for proteins in urine.

2.8.3 Sodium (Na)

Sodium is the major extracellular cation and functions in maintaining fluid distribution and osmotic pressure. Hyponatremia can be caused by prolonged vomiting or diarrhoea, diminished reabsorption in the kidney and excessive fluid retention whereas increased sodium level can be caused by excessive fluid loss, high salt intake, and increased kidney reabsorption (Toffaletti and Jones, 1984).

2.8.4 Potassium (K)

Potassium is the major intracellular cation and is critical to neuromuscular cell activity. Some causes of decreased K⁺ levels include reduced dietary intake or excessive loss from the body due to prolonged vomiting, diarrhea or increased kidney excretion. Increase K⁺ levels may be caused by dehydration or shock, diabetic ketoacidosis, and potassium retention by the kidneys (Toffaletti and Jones, 1984).

2.8.5 Chloride (Cl⁻)

Chloride is the major extracellular anion and serves to regulate the balance of extracellular fluid distribution. Common causes of decreased chloride include reduced dietary intake, prolonged vomiting, reduced renal reabsorption, as well as some forms of acidosis and alkalosis. Increased Cl⁻ values are found in dehydration, kidney failure, some forms of acidosis, high dietary or parental Cl⁻ intake, and salicylate poisoning (Toffaletti and Jones, 1984).
2.9  Risk factors in HAART-related toxicities

The therapeutic goal of HAART is to suppress viral replication and restore the patients’ immunologic function. However, its major drawback is associated organ-specific toxicities which can be aggravated by one or multiple risk factors. Physicians are cautioned to maintain a high level of surveillance especially where there are known or established recent risk factors that aggravating the side effects (NASCOP, 2002). An overarching goal in antiretroviral therapy should be to select a regimen that is not only effective but is also safe. A physician should take this into account in line with assessing individual patient’s underlying medical conditions or history (Vella and Palmisano, 2000).

Risk factors for pharmacological toxicity are numerous and depend mainly on underlying patient characteristics as well as the drug regimen under consideration. The patients’ age, gender, body weight and size, nutrition and overall health status can play a role in how one experience drug’s side effects (Pataki, 2006). Many other exotic factors such as occupation, altitude, race and distance from the ocean have been known to affect results (Waithaka et al., 2009). High risk for development of chronic kidney disease with HIV infection are black race, CD4 count < 200 cells/mm³, family history of CKD and presence of diabetes mellitus, hypertension or hepatitis C co-infection (Naicker and Fabian, 2010). All these considerations underscore the significance of taking blood or urine samples in a standardized and controlled fashion for performing and interpreting laboratory tests with advance knowledge in a wide array of confounding risk factors.
CHAPTER THREE
MATERIALS AND METHODS

3.1 Study area

The study was carried out at the Coast Province General Hospital (CPGH) in the Comprehensive Care Centre (CCC) in liaison with the hospital clinical biochemistry laboratory. CPGH is located within Mombasa county of Coast Province of Kenya as shown in the map (Figure 2). The government of Kenya in partnership with RPM Plus and partners rolled out its extensive ART program and HIV care services to the populace within the region in the year 2003. The technical assistance and funding was provided by United States Agency for International Development (USAID).

The hospital set up a comprehensive care center (CCC) to provide HIV care and treatment services through a comprehensive care-based counselling and testing service for HIV diagnosis; a clinical ability to diagnose, treat and manage opportunistic infections; counselling for treatment adherence and nutrition; and delivery of ART. PLHWA could also access a variety of additional services provided offsite by community health workers that ensured greater coordination in their HIV care and saved them time used while travelling to source to the hospital. These included treatment for tuberculosis, home-based care, inpatient care, services for prevention of mother-to-child HIV transmission (PMTCT) and management of sexually transmitted infections (STI) other than HIV. The site of study is marked out in the African map showing Kenya and where Mombasa is precisely located.
3.2 Study population

The hospital draws patients coming for various health care services from the entire province which covers an area of approximately 83,603 km² with a population of 3,325,307 inhabitants as per the 2009 census (KNBS, 2010). The province had one of the highest adult sero-prevalence of HIV in the country, estimated at 7.9% in 2007 (KAIS, 2009). Based on a baseline survey that was conducted before the start of the study in March 2011, a total of 12,735 HIV positive adults (>18 years) were registered for active HIV care at CCC, CPGH. Out of these, 8,144 (64%) were females and 4,591 (36%) were males. 7,396 (58%) had not been started on antiretroviral therapy (HAART naïve cohort) while 5,339 (42%) had already been started on HAART (HAART treated cohort).
3.3 Design of the study

A longitudinal study was adopted in a design where HAART naïve and HAART treated patients attending HIV care clinics were recruited and placed in two groups; ARM 1 and ARM 2 respectively. In order to compare the occurrence of liver and renal derangements in HAART treated subjects with its occurrence in HAART naïve subjects, the data and health statuses of the two groups were subjected through a protocol with framework design illustrated in figure 3.

Figure 3: Design of the study
3.4 Sample size determination

Surveys of people receiving HAART have shown that its adverse effects account for 10% or more of those discontinuing treatment (Ickovics, 1997). The minimum sample size was determined using Alonzo et al. (2002) formula;

\[ n = \frac{Z^2 \times P \times (1-P)}{\delta^2} \]

Where; \( n \) - minimum sample size, \( P \) – estimated prevalence, \( Z \)- Standard normal deviate that corresponds to 95% confidence limit (1.96) \( \delta \) is the alpha level of significance (5%). Assuming a prevalence rate of 10% as reported by Ickovics (1997), the minimum sample size was;

\[ n = \frac{1.96^2 \times 0.1 \times (1-0.1)}{0.05^2} = 138 \]

Sample size; \( n = 138 \).

Assuming a 10% drop out rate (13.8) in the study, the sample size was adjusted and rounded to 150 participants. Since the major objective was to determine prevalence of HAART toxicities on the liver and kidney, one hundred HAART treated patients who had been on ARVs for not less than one year against fifty HAART naïve patients were recruited into the study.

3.5 Ethical considerations

Ethical approval for the study was obtained by KEMRI/National Ethics Review Committee (Appendix 8) after ethical considerations were addressed adequately in the study protocol. In addition, a research letter of authority (Appendix 7) together with a research permit (Appendix 6) to carry out the study at CPGH was granted by National Council for Science and Technology (NCST).
3.6 Inclusion criteria

The participants recruited into the study included HIV positive males and females aged 18-60 years, with CD4 cell counts not less than 200 cells/μL. Those who were willing to consent and cooperate in attending monthly follow-up clinics till the end of the study were registered. HIV positive participants who met the set inclusion criteria and agreed to participate in the study signed an informed consent form written in English or Kiswahili (Appendix 4 or Appendix 5 respectively). The process of informing sampled patients was witnessed by an either a nurse or clinician-in-charge or a community health worker or any other person or relative accompanying the patient.

3.7 Exclusion criteria

HIV patients with confirmed diabetes, pregnant, hypertensive (blood pressure >145/90 mm/Hg), drugs-users or those with systemic opportunistic infections like TB or hepatitis medications were excluded from the study. Patients recruited into the study who acquire systemic infections or were started on antiretroviral medications during the course of the study were discontinued and recorded appropriately.

3.8 Data collection tools

3.8.1 Questionnaire

A structured questionnaire was administered to participants to obtain information on their age, sex, pregnancy status, drugs use, weight HAART use and duration, clinical history and side-effects occurrences was completed for each patient (Appendix 1).
3.8.2 Patient’s medical record

The patient’s card (Appendix 2) kept at the comprehensive care clinic was used to obtain the patient’s demographic characteristics including sex, age, HIV/AIDS and HAART status and history of various medical conditions and treatment of TB, diabetes, hypertension, hepatitis, among other opportunistic infections if any. The card also has a record CD4 count, adherence satisfactory level, ART side effects, referrals, various laboratory tests results and pregnancy status (female patients). This data from the patient’s card was used to determine eligibility during recruitment in addition to corroborating the data captured in the card from the subjects during the consent taking process.

3.8.3 Laboratory request form

A laboratory request form (Appendix 3) was submitted together with the urine and blood samples collected from the participants and it was used to record results from urinalysis, liver and renal function tests carried out.

3.9 Collection of samples

Five milliliters of whole venous blood were collected aseptically monthly for six consecutive months. The samples were placed into vacutainer tubes containing ethylene-diamine-tetra acetic acid (ETDA) and centrifuged at 3000 revolution per minute for two minutes. The serum samples obtained were used to assay liver and renal function. Urine bottles were given to recruited patients for collection of their urine samples. The samples were used to determine presence of glucose and protein using the urine strip test.
3.10 Laboratory analytical methods

Liver and kidney function tests were analyzed on the blood sera samples based on standard operating procedures (SOPs) written and maintained in the clinical chemistry laboratory at CPGH using Cobas c 111 and Roche 9180 electrode automatic analyzers and their analytical reagents from Roche diagnostics (Germany).

3.10.1 Measurement of liver function analytes

Eight liver function analytes were determined on the sera specimens: total proteins (PROT), albumin (ALB), alanine aminotransferase (ALT) Aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total bilirubin (BIL-T) and direct bilirubin (BIL-D). The method of assay and the relevance of the value of each analyte measured are described below.

3.10.1.1 Alanine aminotransferase (ALT)

The ALT reagent was used to measure ALT in the sample by enzymatic rate method. In the assay, ALT catalyses the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD⁺. Pyridoxal phosphate serves as a coenzyme in the amino transfer reaction to ensures full enzyme activation. The rate of the NADH oxidation is directly proportional to the ALT catalytic activity which is determined by measuring the absorbance at 340 nm. The machine calculated and expressed ALT activity in U/L in the reaction at 37°C within three minutes.

3.9.1.2 Aspartate aminotransferase (AST)

AST reagent was used to measure AST activity by an enzymatic rate method where AST present in the sample catalyzed the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. Oxaloacetate then
reacts with NADH in the presence of malate dehydrogenase (MDH) to form L-malate and NAD+. Pyridoxal phosphate serves as a coenzyme in the reaction to ensure full enzyme activation. The rate of the NADH oxidation is directly proportional to the AST catalytic activity and its absorbance measured at 340 nm. The reaction takes three minutes at 37°C and the machine calculates and expresses AST activity in U/L.

3.9.1.3 Gamma Glutamyl-transferase (GGT)

GGT reagent was used to measure the activity of GGT by enzymatic calorimetric assay. Gamma-glutamyl transferase transfers the γ-glutamyl group of L-γ-glutamyl-3-carboxy-4-nitroanilide to form glutamyl-glycine and 5-amino-2-nitrobenzoate. The amount of 5-amino-2-nitrobenzoate liberated is proportional to the GGT concentration in the sample and it is determined by measuring the increase in absorbance at 409 nm. The machine calculated and expressed the activity of GGT in U/L within three minutes at 37°C.

3.9.1.4 Total proteins (PROT)

Total protein reagent was used to measure the concentration of total proteins by a timed end point biuret method. In the reaction, the peptide bonds in the protein sample bound to divalent cupric ions in an alkaline medium and they formed purple-coloured biuret complex. The colour intensity is directly proportional to the protein concentration and the machine automatically calculated the analyte concentration and expressed it in g/L at 552 nm within seven minutes at 37°C.

3.9.1.5 Albumin (ALB)

ALB reagent was used to measure ALB concentration by a timed end point. The reagent combines with bromocresol green (BCG), an anionic dye to form a blue-green complex in a calorimetric assay. At pH value of 4.1, albumin display a sufficiently
cationic character that bind with BCG forming the complex whose colour intensity is
directly proportional to the concentration of albumin in the serum sample. The
absorbance is measured at 583 nm in a reaction that took one and half minutes at
37°C. The machine auto calculated and expressed ALB concentration in g/L.

3.9.1.6 Alkaline phosphatase (ALP)
The ALP reagent was used to measure ALP activity by a standardized kinetic method
in the presence of magnesium and zinc ions. In the standardized method, p-
nitrophenyl phosphate is cleaved by ALP into phosphate and p-nitrophenol. p-
nitrophenol released is directly proportional to the catalytic activity of ALP and is
determined by measuring the increased in absorbance at 409 nm. The machine
calculated and expressed the activity in U/L in a reaction that took place at 37°C
within three minutes.

3.9.1.7 Bilirubin total (BIL-T)
Bilirubin-total reagent was used to measure concentration of bilirubin by Diazo
method. Total bilirubin in the presence of a suitable solvent couples with a diazonium
ion in a strongly acidified medium to form azobilirubin coloured complex. The
intensity of the coloured complex formed is proportional to the total bilirubin
concentration and its absorbance can be measured photometrically at 552 nm. The
machine calculated and expressed the activity of bilirubin in U/L in a reaction that
took place at 37°C within three minutes.

3.9.1.8 Bilirubin direct (BIL-D)
Bilirubin Direct reagent was used to measure concentration of bilirubin by a timed
end point method. Conjugated bilirubin and direct bilirubin react directly with
diazotized sulfanilic acid in an acid buffer to form the red-coloured azobilirubin
complex. The intensity of the coloured complex produced is proportional to the concentration of direct bilirubin in the sample and was determined by monitoring the increase in absorbance at 552 nm. The Cobas c 111 equipment calculated and expressed the activity of direct bilirubin in U/L at 37°C within three minutes.

3.9.2 Measurement of kidney function analytes

Five kidney function analytes were determined on the sera specimens: creatinine (CREAT), blood urea nitrogen (BUN), sodium (Na), potassium (K) and chloride ion (Cl\textsuperscript{-}). The method of assay and the relevance of the value obtained for each analyte measured is described below.

3.9.2.1 Creatinine (CREAT)

Creatinine Jaffe reagent measures creatinine concentration by a modified rate Jaffe method in an alkaline solution. In the reaction, creatinine combines with picrate to form a yellow-orange complex whose rate of formation is directly proportional to the concentration of creatinine in the sample. The change in absorbance of the complex formed was detected at 520 nm. The machine calculated and expressed the concentration of CREAT in μmol/L in a reaction that took place within three minutes at 37°C.

3.9.2.2 Blood urea nitrogen (BUN)

Urea reagent was used to measure the concentration of urea/blood urea nitrogen in the serum sample through a kinetic reaction test. In the reaction, urease enzyme hydrolyzes urea to form ammonium and carbonate ions. The ammonium ion reacts with 2-oxoglutarate in presence of glutamate dehydrogenase and the coenzyme NADH to produce L-glutamate. In this reaction two moles of NADH are oxidized to NAD\textsuperscript{+} for each mole of urea hydrolyzed. The rate of decrease in the NADH
concentration is directly proportional to the concentration of urea in the sample and its absorbance is measured at 340 nm. The machine calculated and expressed the concentration of BUN in mmol/L in a reaction that took three minutes at 37°C.

3.9.2.3 Sodium, Potassium and Chloride electrolytes

The blood sample collected was pipetted from the sample tube into the ion selective electrode (ISE) tower. Each sample (20μl) was diluted with system water (100μl). The other ISE solutions were pipetted from the ISE rack into the ISE tower by the sample probe. The sample was then passed through the ion selective electrodes. ISE reference electrolyte was passed through the reference electrode and into measuring channel of the electrodes. Measurements were made when the ISE reference electrolyte completed the electric circuit for each electrode. The electrolyte concentration of each sample was calculated and the auto-analyzer automatically converted and expressed potassium, sodium and chloride concentrations into mmol/L. The electrolyte module uses flow-through ion selective electrodes and a reference electrode with an open liquid function. Each electrode had a membrane or capillary that was sensitive to a particular type of ion.

3.9.2.4 Urinary Proteins and Glucose

The presence of protein and glucose in urine were estimated qualitatively using urine strip test. All the assays were performed based on SOPs written and maintained at CPGH in the clinical chemistry laboratory.

3.10 Quality control and assurance

Quality control (QC) is a component of quality assurance (QA) that defines all systematic actions necessary to provide adequate confidence that laboratory services will satisfy outlined medical needs for patient care (Elin, 1980). There are two main
QA programmes, internal and external. Internal QA is done for daily monitoring of the precision and accuracy of the analytical methods while external QA serves to maintain long-term accuracy of the analytical methods (Elin, 1980).

External quality assurance evaluations were done by digital labs twice in the course of this study follow-up; one in April 2011 and the other one in July the same year and the results indicated that Cobas c 111 and Roche 9180 electrode auto-analyzers met all calibration and precision standards.

3.11 Data management and statistical analysis

Data for liver and kidney analytes collected were entered into Microsoft excel database, checked and corrected for data entry errors. They were evaluated to determine the prevalence of hepatotoxicity and renal insufficiency based on key liver and kidney surrogate markers respectively. Hepatotoxicity was classified as Hepatocellular (ALT>40U/L) and/or Cholestasis (ALP>160 U/L) whereas renal derangements were classified as renal insufficiency based on CREAT>130 U/L and proteinuria based on a urine strip test (MoH, 2007). The levels of liver and kidney analytes obtained were compared with published reference ranges obtained from a normalized population in Kenya (Waithaka et al., 2009).

Data for liver and kidney function were profiled based on HAART status, sex, age-group and patients’ duration on HAART and imported into SAS 9.2 software. Age was stratified into three categories; 18-32; 33-45; and 46-60 years. The duration patients had been on HAART was grouped into four categories of 0; 1-3; 4-6; and >7 years. Variability in data was tested based on mean± SD (standard deviation) with the alpha level of significance set at 0.05.
The prevalence of hepatotoxicity and renal insufficiency kidney toxicities was tested for significance difference between HAART treated and HAART naïve subjects using non-parametric chi-square ($\chi^2$) test. Independent student’s t-test was used to compare data variability between males and females whereas ANOVA statistical tests were performed to test significant difference in data variability with age and the duration HIV patients have been on HAART.
CHAPTER FOUR

RESULTS

4.1 Characteristics of the study participants

The study was conducted between March and August 2011. Table 6 summarizes the demographic characteristics of study participants.

Table 6: Characteristics of the study participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HAART naïve (N = 50)</th>
<th>HAART treated (N = 100)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>11</td>
<td>26</td>
<td>0.06</td>
</tr>
<tr>
<td>Female</td>
<td>39</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>38.06±8.7</td>
<td>40.2±8.65</td>
<td>0.16</td>
</tr>
<tr>
<td>CD4 count (cells/mm³)</td>
<td>347.0±102.8</td>
<td>397.9±127.5</td>
<td>0.02*</td>
</tr>
<tr>
<td>ARV duration (years)</td>
<td>0</td>
<td>4.77±1.6</td>
<td></td>
</tr>
<tr>
<td>Drop/Fall outs, (n)</td>
<td>6</td>
<td>10</td>
<td>0.87</td>
</tr>
<tr>
<td>Followed, (n)</td>
<td>44</td>
<td>90</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as Mean±SD for age, ARV duration and CD4 of the number of subjects in the HAART treated and HAART naïve columns. *: represent significant difference where P<0.05 by t-Test.

In this study, 89.3% participants were followed to the end of the study while the rest dropped out. 10% of the HAART treated and 12% of the HAART naïve participants dropped out from the study. The number of drop-outs in the study were not significant (P=0.87) hence did not affect the results. Four (4) of the drop outs were discontinued from the study after they were started on ARVs based on the exclusion criteria described. Age distribution differences between the HAART treated and HAART naïve groups did not vary significantly (P = 0.16). However, the mean CD4 count in the HAART treated group was significantly higher than in the HAART naïve group (P = 0.02).
4.2 Liver and kidney function analytes profile in the study groups

Eight liver and five kidney analytes measured during the five-month study period were profiled and their mean values and standard deviations (SD) computed per patient and their values matched for sex, age and the duration the participants on HAART. These values for each analyte were compared with reference ranges obtained from a normal population at Kenyatta National Hospital (Waithaka et al., 2009). The values that that fell outside the mid of reference range were classified as abnormal.

The percentage number of subjects with abnormal liver and kidney analytes in the HAART treated and HAART naïve groups based on their mean±SD are summarized in tables 7 and 8.

<table>
<thead>
<tr>
<th>Analyte (RR, Units)</th>
<th>HAART treated (N=100)</th>
<th>HAART naïve (N=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (0-39U/L)</td>
<td>18 % elevated</td>
<td>8% elevated</td>
</tr>
<tr>
<td>AST (6–40U/L)</td>
<td>16% elevated</td>
<td>10% elevated</td>
</tr>
<tr>
<td>PROT (57–89g/L)</td>
<td>8% lowered</td>
<td>6% lowered</td>
</tr>
<tr>
<td>ALB (29–52g/L)</td>
<td>3% lowered</td>
<td>6% lowered</td>
</tr>
<tr>
<td>γ -GT (7-66U/L)</td>
<td>24% elevated</td>
<td>12% elevated</td>
</tr>
<tr>
<td>ALP (10 – 201U/L)</td>
<td>12% elevated</td>
<td>6% elevated</td>
</tr>
</tbody>
</table>

**RR- Reference range adopted from Waithaka et al. (2009)**

Based on percentage number of subjects with abnormal ALT analyte levels in table 7, 18 (18%) of the HAART treated patients and 4 (8%) HAART naïve patients had elevated ALT which is a key surrogate marker for diagnosing cellular hepatotoxicity whereas, 12 (12%) HAART treated patients and 3 (6%) HAART naïve patients had elevated ALP, a key surrogate marker for diagnosing cholestasis. The percentage number of subjects with abnormal AST, PROT and GGT were higher in the HAART...
treated subjects than the HAART naïve subjects with the exception of abnormal ALB which was higher in the HAART naïve than in the HAART treated group 6% verses 3% respectively).

**Table 8: Percentage of cases with abnormal kidney analytes among HAART treated and HAART naïve participants**

<table>
<thead>
<tr>
<th>Analyte (RR, Units)</th>
<th>% HAART treated (N=100)</th>
<th>% HAART naïve (N=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CREAT (59-127 μmol/L)</td>
<td>4% elevated</td>
<td>8% elevated</td>
</tr>
<tr>
<td>BUN (1.5-5.9 mmol/L)</td>
<td>2% elevated</td>
<td>6% elevated</td>
</tr>
<tr>
<td>SOD (134-153 mmol/l)</td>
<td>2% lowered</td>
<td>6% lowered</td>
</tr>
<tr>
<td>K+ (3-5.3 mmol/l)</td>
<td>8% elevated</td>
<td>4% elevated</td>
</tr>
<tr>
<td>CL (101-110 mmol/l)</td>
<td>3% lowered</td>
<td>6% lowered</td>
</tr>
<tr>
<td>Protein in urine</td>
<td>6% positive</td>
<td>8% positive</td>
</tr>
<tr>
<td>Glucose in urine</td>
<td>0% positive</td>
<td>0% positive</td>
</tr>
</tbody>
</table>

*RR: Reference range. RR values were adopted from Waithaka et al. (2009)*

There were 4 HAART treated patients and 4 HAART naïve patients had abnormal CREAT levels. Overall 5.3% had elevated CREAT, a key surrogate marker for diagnosing renal derangements. 3 HAART treated subjects and 8 of the HAART naïve subjects had proteinuria (overall 9.3%). There were higher percentages of abnormalities in all kidney analytes except K+ in HAART naïve than HAART treated subjects. The prevalence of abnormal liver analytes (ALT and ALP) and renal analytes (CREAT and BUN) which are key surrogate markers for diagnosing drug-induced toxicities affecting the liver and kidney respectively were compared for any variation between HAART naïve and HAART treated groups using chi-square test and results are presented in table 9.
Table 9: Prevalence of abnormal liver and renal analytes in HAART treated and HAART naïve subjects

<table>
<thead>
<tr>
<th>Type of organ injury (Key analytes and their upper limit of normal)</th>
<th>HAART treated (%)</th>
<th>HAART naïve (%)</th>
<th>Sig*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver Toxicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatocellular (ALT&gt;40 U/L)</td>
<td>18</td>
<td>8</td>
<td>0.59</td>
</tr>
<tr>
<td>Cholestasis (ALP&gt;160U/L)</td>
<td>12</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Renal insufficiency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CREAT&gt;127 μmol/L</td>
<td>4</td>
<td>8</td>
<td>0.90</td>
</tr>
<tr>
<td>BUN&gt;5.9 mmol/L</td>
<td>4</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as percentage cases with elevated liver and renal analytes.*- The percentage difference is significant at p<0.05 by chi-square test.

The prevalence of abnormal liver analytes; ALT and ALP did not differ significantly between HAART treated and HAART naïve subjects ($\chi^2$; p=0.59). The prevalence of abnormal kidney analytes; CREAT and BUN did not differ significantly between HAART treated and HAART naïve subjects ($\chi^2$; p=0.90).

4.3 Trends in abnormal liver and kidney analytes in participants

All the data from liver and kidney function test analytes were profiled monthly for the five-month period. The mean±SD values for all analytes were calculated and compared with the reference range established in a normalized Kenyan population (Waithaka et al., 2009). The trends in a number of participants with abnormal liver and kidney function test analytes were presented graphically in figures 4 and 5.
The trends in the number of HAART treated subjects with abnormal liver analyte values in the five-month follow-up period is shown in figure 4.

![Figure 4: Trends in number of HAART treated patients with abnormal liver analytes with time](image)

The number of subjects with abnormal liver function analytes values showed overall increasing trends in three liver analytes namely, AST, ALT, and GGT. However, there was an exception in the other three liver analytes, ALP, PROT and ALB where the abnormal cases showed declining trends in the five-month period (figure 4).
The trends in number of HAART treated subjects with abnormal kidney function analytes is shown in figure 5.

Figure 5: Trends in number of HAART treated patients with abnormal kidney analytes with time

There was fluctuation in most analytes and a general decline in the number of subjects with abnormal creatinine (CREAT), urea (BUN), sodium (Na\(^+\)) and potassium (K\(^+\)) values. There was an exceptional general increase in the number of participants with abnormal chloride (CL) levels during the five-month period (Figure 5).
4.4 Variation in liver and kidney analytes by gender among HAART treated patients

The mean and standard deviation values for liver and kidney analytes from HAART treated patients were determined. The results are summarized in tables 10 and 11.

Table 10: Variation in liver analytes in HIV positive males and females patients on HAART

<table>
<thead>
<tr>
<th>Liver Analytes Mean±SD (Units)</th>
<th>Sex</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females (N=72)</td>
<td>Males (N=28)</td>
<td>P-value</td>
<td></td>
</tr>
<tr>
<td>ALT (18.9±10.5U/L)</td>
<td>29.9±12.6</td>
<td>34.1±14.6</td>
<td>0.001*</td>
<td></td>
</tr>
<tr>
<td>AST (23.5±8.6U/L)</td>
<td>30.8±12</td>
<td>33.9±18</td>
<td>0.04*</td>
<td></td>
</tr>
<tr>
<td>PROT (72.5±8.0g/L)</td>
<td>73.3±5.8</td>
<td>75.5±6.7</td>
<td>0.03*</td>
<td></td>
</tr>
<tr>
<td>ALB (40.7±5.8g/L)</td>
<td>42±3.8</td>
<td>44±3.9</td>
<td>0.001*</td>
<td></td>
</tr>
<tr>
<td>γ-GT (42±3.8U/L)</td>
<td>57.5±70.3</td>
<td>55.5±67</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>ALP (107.3±47U/L)</td>
<td>101.2±35.3</td>
<td>99.5±46.8</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>BIL-D (1.2±0.8U/L)</td>
<td>1.26±0.5</td>
<td>1.42±0.6</td>
<td>0.003*</td>
<td></td>
</tr>
<tr>
<td>BIL-T (3.2±3.8U/L)</td>
<td>3.7±3.1</td>
<td>4.8±4.1</td>
<td>0.01*</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as Mean ±SD of the measured parameter for males and females. *-The sex difference is significant at p<0.05 by t-test.

Males on HAART showed significantly higher mean values than females in the following six liver function test analytes namely, AST, PROT, ALB, BIL-D, BIL-T, and ALT. However, mean values for GGT and ALP analytes did not vary significantly between males and females in the HAART treated group (Table 10). In the HAART treated group, 13 (18%) females and 5 (17.8%) males had elevated ALT while 2 (3%) females and 2 (7%) males had elevated ALT analyte in the HAART naïve group.
Table 11: Variation in kidney analytes in HIV positive males and females on HAART

<table>
<thead>
<tr>
<th>Kidney Analytes</th>
<th>Sex</th>
<th></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females (N=72)</td>
<td>Males (N=28)</td>
<td></td>
</tr>
<tr>
<td>CREAT (88.0±17.0μmol/l)</td>
<td>77.68±36</td>
<td>87±26</td>
<td>0.001*</td>
</tr>
<tr>
<td>BUN (3.7±1.1mmol/l)</td>
<td>3.26±0.77</td>
<td>3.55±1.0</td>
<td>0.001*</td>
</tr>
<tr>
<td>Na⁺ (132.3±10.8mmol/l)</td>
<td>139.7±2.6</td>
<td>140.1±2.9</td>
<td>0.08</td>
</tr>
<tr>
<td>K⁺ (4.1±0.58mmol/l)</td>
<td>4.3±0.38</td>
<td>4.4±0.41</td>
<td>0.06</td>
</tr>
<tr>
<td>Cl⁻ (105±3.8mmol/l)</td>
<td>106.8±3.6</td>
<td>106.2±3.4</td>
<td>0.048*</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ± SD of the measured parameter for males and females. *The sex difference is significant at p<0.05 by t-test.

The mean values for two kidney analytes; BUN and CREAT were significantly higher in males compared to females. However, females had significantly higher mean values in Cl⁻ analyte compared to males. Two kidney analytes; K⁺ and Na⁺ were not significantly different in males and females (Table 10). In the HAART treated group, 4 (5.6%) females and 0 (0%) males had elevated CREAT whereas only 1 (3.6%) female and 3 (10.7%) males had elevated CREAT analyte in the HAART naïve group.
4.5 Variation in liver and renal analytes by age in males and females on HAART

The mean and standard deviation values for liver and kidney analytes from HAART-treated males and females in the three age categories were determined. The results are summarized in the tables 12 and 13.

Table 12: Variation in liver analytes by sex and age groups in HIV positive patients on HAART

<table>
<thead>
<tr>
<th>Liver Analytes</th>
<th>Sex</th>
<th>Age group (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N 1 (18-31 yrs)</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>F</td>
<td>12 30±12</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>5   32±11*</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>F</td>
<td>10 32.1±11</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>4   31.8±12*</td>
</tr>
<tr>
<td>PROT (g/L)</td>
<td>F</td>
<td>10 77.5±6.5</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>5   78±5.5*</td>
</tr>
<tr>
<td>ALB (g/L)</td>
<td>F</td>
<td>11 42.7±4.1</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>3   41.9±4.4</td>
</tr>
<tr>
<td>γ-GT (U/L)</td>
<td>F</td>
<td>9   51.5±32</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>4   50.6±28*</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>F</td>
<td>12 96.3±26</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>5   98.3±24*</td>
</tr>
<tr>
<td>BIL-D (U/L)</td>
<td>F</td>
<td>10 1.39±0.5</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>3   1.49±0.7*</td>
</tr>
<tr>
<td>BIL-T (U/L)</td>
<td>F</td>
<td>11 3.7±0.32</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>3   3.8±0.47*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD of the number of subjects (N).  
*represents significant sex difference in each age group where p<0.05;  
arepresents significant specific sex difference in age group 1 and 2 where p<0.05;  
brepresents significant specific sex difference in age group 1 and 3 where p<0.05;  
crepresents significant specific sex difference in age group 2 and 3 where p<0.05;

Significant sex-based differences were observed in ALT, AST, PROT, ALP BIL-D, BIL-T and GGT in all age groups except for ALB analyte. In males, AST analytes were significantly higher in age group 3 compared to age group 2 whereas in females, GGT analyte were significantly higher in age category 3 compared to age category 1.  
In both sexes, GGT analyte was significantly higher in age category I compared to age group 2.
Table 13: Variation in kidney analytes by sex and age groups in HIV positive patients on HAART

<table>
<thead>
<tr>
<th>Kidney Analytes</th>
<th>Sex</th>
<th>Age categories (years)</th>
<th>N</th>
<th>1 (18-31 yrs)</th>
<th>N</th>
<th>2 (32-45 yrs)</th>
<th>N</th>
<th>3 (46-59 yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CREAT (μmol/l)</td>
<td>F</td>
<td>12</td>
<td>43</td>
<td>76.80±26</td>
<td>14</td>
<td>75.4±23</td>
<td>11</td>
<td>81.9±32a</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>4</td>
<td>10</td>
<td>83.60±36*</td>
<td>12</td>
<td>87.4±42</td>
<td>9</td>
<td>90.9±48b</td>
</tr>
<tr>
<td>BUN (mmol/l)</td>
<td>F</td>
<td>11</td>
<td>42</td>
<td>3.50±0.74</td>
<td>13</td>
<td>3.43±0.72</td>
<td>10</td>
<td>3.57±1.00</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>5</td>
<td>8</td>
<td>3.62±0.57*</td>
<td>12</td>
<td>3.46±0.68</td>
<td>11</td>
<td>3.74±0.76c</td>
</tr>
<tr>
<td>SOD (mmol/l)</td>
<td>F</td>
<td>10</td>
<td>44</td>
<td>139.7±2.60</td>
<td>15</td>
<td>139.3±3.10</td>
<td>12</td>
<td>139.8±2.40</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>4</td>
<td>9</td>
<td>139.4±2.85</td>
<td>10</td>
<td>139.7±2.80</td>
<td>11</td>
<td>139.6±2.45</td>
</tr>
<tr>
<td>K+ (mmol/l)</td>
<td>F</td>
<td>9</td>
<td>42</td>
<td>4.29±0.36</td>
<td>13</td>
<td>4.31±0.42</td>
<td>10</td>
<td>4.35±0.38</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>4</td>
<td>9</td>
<td>4.23±0.57</td>
<td>9</td>
<td>4.28±0.47</td>
<td>9</td>
<td>4.32±0.41</td>
</tr>
<tr>
<td>CL (mmol/l)</td>
<td>F</td>
<td>10</td>
<td>44</td>
<td>109.3±2.90</td>
<td>12</td>
<td>109.9±2.60</td>
<td>8</td>
<td>108.5±3.65</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>3</td>
<td>8</td>
<td>109.7±2.75</td>
<td>11</td>
<td>109.4±3.10</td>
<td>11</td>
<td>109.5±3.30</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD of the number of subjects (N).
*represents significant sex difference in each age group where p<0.05;
a represents significant specific sex difference in age group 1 and 2 where p<0.05;
b represents significant specific sex difference in age group 1 and 3 where p<0.05;
c represents significant specific sex difference in age group 2 and 3 where p<0.05;

Significant sex-based differences were observed in CREAT and BUN analytes with analyte values in males greater than those in females. No significant sex differences were observed in SOD, K+ and CL analytes in all age groups. In females, CREAT analyte was significantly higher in age group 3 than age group 2. In males, CREAT analyte was significantly higher in age group 3 than age group 1. In males, BUN analyte was significantly higher in age group 3 than age group 2.
4.6 Variation in liver and kidney analytes by the patient’s duration on HAART

The mean and standard deviation values for liver and kidney analytes from HAART-treated patients in four HAART-duration categories were determined. The results are summarized in the tables 14 and 15.

Table 14: Variation in liver analytes with the duration HIV positive patients have been on HAART

<table>
<thead>
<tr>
<th>Liver Analytes (units)</th>
<th>HAART-duration (years)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-HAART (n=50)</td>
<td>1-3 yrs (n=21)</td>
</tr>
<tr>
<td>ALT (18.9±10.5U/L)</td>
<td>29±12*</td>
<td>27±10*</td>
</tr>
<tr>
<td>AST (23.5±8.6U/L)</td>
<td>30±12*</td>
<td>28±10*</td>
</tr>
<tr>
<td>PROT (72.5±8.0g/L)</td>
<td>76±7.3*</td>
<td>75.6±5.8</td>
</tr>
<tr>
<td>ALB (40.7±5.8g/L)</td>
<td>41.2±4.4</td>
<td>43.1±2.9</td>
</tr>
<tr>
<td>γ-GT (42±3.8U/L)</td>
<td>45.5±22.8*</td>
<td>45.3±28.1*</td>
</tr>
<tr>
<td>ALP (107.3±47U/L)</td>
<td>92±26*</td>
<td>99±50</td>
</tr>
<tr>
<td>BIL-D (1.2±0.8U/L)</td>
<td>1.28±0.56</td>
<td>1.28±0.52</td>
</tr>
<tr>
<td>BIL-T (3.2±3.8U/L)</td>
<td>4.1±3.7</td>
<td>3.5±2.7</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ± SD. *: Significant difference in analyte values in the respective HAART duration categories at p<0.05. Sig* = significance

The mean values for three liver analytes; ALT, AST and GGT were significantly higher in the patients who had been on HAART medications for at least 4-6 years. The mean values for PROT parameter were significantly lower in patients who had been on HAART for more than 7 years (category 4) compared to HAART naïve patients (category 1). There was no significant difference in the mean values of four liver analytes: ALB, ALP, BIL-D and BIL-T with respect to HAART duration categories (Table 14).
Table 15: Variation in kidney analytes with the duration the patient have been on HAART

<table>
<thead>
<tr>
<th>Kidney Analytes (Units)</th>
<th>HAART-Duration (Years)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-HAART (n=50)</td>
<td>1-3 yrs (n=21)</td>
</tr>
<tr>
<td>CREAT (88.0±17.0μmol/l)</td>
<td>94.9±85</td>
<td>81.8 ±24</td>
</tr>
<tr>
<td>BUN (3.7±1.1mmol/l)</td>
<td>3.5±0.7</td>
<td>3.3 ±1</td>
</tr>
<tr>
<td>Na⁺ (132.3±10.8mmol/l)</td>
<td>139.6 ±0.2</td>
<td>140 ±0.3</td>
</tr>
<tr>
<td>K⁺ (4.1±0.58mmol/l)</td>
<td>4.4 ±0.43</td>
<td>4.2 ±0.36</td>
</tr>
<tr>
<td>Cl⁻ (105±3.8mmol/l)</td>
<td>106.3 ±3.1</td>
<td>106.9 ±3.5</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ± SD. *: Significant difference in analyte values in the respective HAART duration categories at p<0.05. Sig* = significance

The mean values for CREAT analyte were significantly higher in the non-HAART group compared to the three HAART groups however there was no significant difference in CREAT in the three HAART experienced groups. The mean values for BUN, SOD, K⁺ and Cl⁻ analytes were not significantly different in all HAART duration categories (Table 15).
CHAPTER FIVE
DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

In this study, the mean ages of the HAART treated and HAART naïve groups were not significantly different (P=0.164). Similarly, sex distribution of the HAART naïve and HAART treated subjects in the study did not vary significantly (0.06). However, there were more females than males recruited into the study in the two groups which could be attributed to increasing feminization to HIV epidemic in the developing world (Kumarasamy et al., 2008). The HAART treated group had a significantly higher CD4 mean compared to HAART naive group (P=0.02). This could be attributed to positive effects of ARV treatment which suppressed HIV replication in the body allowing CD4 cells to increase dramatically as corroborated by Vella and Palmisano (2000).

The trends in liver and kidney function analytes during the five month study period gave a fluctuating pattern in HAART treated subjects. Whereas AST, ALT, CL and GGT analytes showed increasing trends, ALP, PROT and ALB showed a declining one in the five-month study period. Elevated ALT levels associated with increased levels of ALP and GGT may be associated with hepatic degeneration (Larry et al., 2004). Similarly, kidney function analytes in the same group gave fluctuating trends during the five-month study period. The trends in assayed biochemical analytes observed in this study pointed to hepatotoxicity and renal insufficiency in HAART treated subjects that may increase morbidity and mortality among HIV-infected patients (Núñez and Soriano, 2005).
Drug induced hepatotoxicity characterized by elevation of AST/ALT levels to at least twice the upper limit of normal (ULN) can occur with drugs from all ARV classes (Sułkowski et al., 2000). The 18% prevalence in hepatotoxicity among the HAART treated patients in this study was lower compared to one reported in Madrid, Spain, which was at 31% (Evans and Scadden, 2000) but higher 10.8% of AIDS clinical trial study group (Reisler et al., 2001). Thompson (2011) noted that hepatotoxicity with a prevalence interval of 7% to 13% with grade 3 or 4 elevation in ALT/AST is more prone to occur in persons with more advanced HIV disease, a reason which can be attributed to the 8% prevalence noted among the HAART naïve patients in this study.

In this study, prevalence of hepatotoxicity did not differ significantly between the HAART treated and HAART naïve subjects (p=0.59) implying that HAART may not be the only cause of hepatotoxicity in HIV-positive patients. Hepatotoxicity in HIV era may be attributed to co-morbid factors like acute and chronic viral hepatitis, opportunistic infections, and non-steatotic antiretroviral toxicity (Larry et al., 2004). HIV infection could be singled out in this study as the main cause of elevated liver analytes observed in HAART naïve subjects, given that hepatitis B or C, systemic opportunistic infections e.g. TB, diabetes, hypertension, or pregnancy were controlled. Jevtovic (2008) observed that, HIV infection is associated with more rapid progression of viral hepatitis-related liver disease, including cirrhosis, end-stage liver disease, hepatocellular carcinoma, and fatal hepatic failure. The mechanisms of accelerated liver disease in HIV-infected patients have not been fully elucidated, but Pozniak et al.(2006) attributed HIV-related immunodeficiency and direct interaction of HIV with hepatic stellate and Kupffer cells as the main cause of hepatotoxicity in HAART naïve patients.
In this study, elevated CREAT/BUN levels and positive presence of proteins in urine were translated to be indicative of renal insufficiency. This study found a prevalence of 8% in renal derangements in HAART naïve and 4% in HAART treated group based on CREAT analysis. Thompson (2011) reported a prevalence of 6% in kidney derangements as a cause of complications amongst HIV patients. This study noted a higher prevalence of kidney derangements in HAART naïve group than the HAART experienced group however, it was not significant (P=0.9). This could be due to HIV replication effects of the virus on the kidney, a reservoir for HIV replication as documented by Rao (2001) who stated that HIV causes injury to the kidney resulting in loss of function and elevated creatinine. Presence of protein in urine was found in the 10 (6.7%) patients in this study which was low compared to a study by Sterling et al. (2001) in which 5 out of 30 patients (16.7%) patients had evidence of proteinuria with no other identifiable cause. HIV replication taking place in the kidneys could perforate the glomeruli and tubules causing protein to leak out due to renal inflammation and loss of its function in HIV patients.

The cases of renal derangements found in the HAART naïve patients in this study indicates that HAART may not be the exclusive cause for kidney dysfunctions in HIV positive persons. Kidney derangements prevalent in HAART naïve patients may be ameliorated upon initiation of ARV to suppress the viral load and relieve the kidney’s loss of function due to high viral load. Causes of renal disease in HIV-infected patients are multi-factorial and may include HIV infection itself, co-infections, co-morbidities, and HAART medications as attested by Roling et al. (2006) in his findings. Kidney damage related to antiretroviral therapy is typically reversible with early recognition and timely discontinuation of the offending agent (Jao and Wyatt, 2010). There may be a need to distinguish renal injury from progression of HIV-
associated nephropathy or other HIV-related kidney diseases caused by other infections unrelated to HIV infection and its treatment. Antiretroviral agents are relatively free of renal toxicity although drug-related renal injury can occur (Daugas et al., 2005). Nephrologists should be familiar with the potential toxicity of ARV agents that cause kidney damage to avoid delays in diagnosis.

It has been documented that HIV acute nephropathy (HIVAN) is the most common cause of chronic kidney disease in HIV-infected individuals that may lead to end-stage kidney disease (Wyatt et al., 2009). The HAART administration seems to have had a positive impact in resolving kidney derangements among the HAART treated group in this study which supports findings advocating for ART to be started in HIV patients with HIVAN. Antiretroviral therapy in patients with HIVAN has been associated with both preserved renal function and prolonged survival (Ogundahunsi et al., 2008). Steel-Duncan and others (2005) found in a prospective study that renal syndromes in HAART naive patients resolve after eight months of HAART initiation. Thompson (2011) further observed that with increasing availability and use of ARV, the risk of end-stage-renal-disease (ESRD) decreases by more than 50% in some populations which prolongs the survival of HIV-infected persons with ESRD. Renal toxicity is more likely to occur in HIV patients with pre-existing kidney disease or poorly controlled HIV infection with elevated baseline creatinine concentration, female gender, CD4 nadir <200cells/mm3, and concomitant administration of other nephrotoxic drugs (Nelson et al., 2008; Crum-Cianflone et al., 2010).

Gender based differences were observed in six liver analytes; AST, PROT, ALB, BIL-D, BIL-T, and ALT and three kidney analytes; CREAT, BUN and CL. The mean values of these analytes were significantly higher in males compared to female
patients on HAART. It is documented that women can have more severe adverse event from nucleoside therapy than men, including hepatotoxicity-driven regimen alterations (Currier et al., 2000). The gender-based differences observed in AST, ALT, BIL-T, BIL-D and CREAT analytes in this study were consistent with those reported in an established normalized population in studies carried out at KNH, MTRH and Kericho (Kibaya et al., 2008; Waithaka et al., 2009; Juma et al., 2011) and in Uganda (Eller et al., 2008). Notably, ALP analyte from this study showed no sex differences contrary to studies where males have significantly higher ALP values than females due to differences in large muscle mass and bone formation in males (Waithaka et al., 2009; Juma et al., 2011).

The contrast observed in this study could be attributed to substantial weight loss from accelerated muscle wasting and bone loss due to HIV infection in males than females which lowers ALP levels considerably in males (Waithaka et al., 2009). In addition, liver injury caused by ARV releases additional ALP to blood circulation which may rise beyond the reference range. Alkaline phosphatase analyte is a surrogate marker used in diagnosis of cholestasis, thus when levels are higher in females than males it means women experience more hepatotoxic events from ARVs than men. Sex related differences were observed in CL and could be due to variation in dietary salts intake, sex-related hormonal effects and health physiological factors (Ogundahunsi et al., 2008).

In this study, the mean values for the three liver and two kidney analytes were significantly higher in the age category of 46-60 years than 18-32 years and 33-45 years. This is an indication reduced hepatic and kidney function frequently afflict the elderly people more commonly than the younger ones. In a study of 445 patients
initiated on TDF, 51 (11%) developed a decline in kidney function with a significant association between decline in kidney function and age over 50 years (Atta et al, 2008). Likewise, advanced age was associated with an increased risk of renal tubular (Celesia, 2010). Renal toxicity is more likely to occur in HIV patients with pre-existing kidney disease, older age, elevated baseline creatinine concentration, CD4 nadir <200 cells/mm³, and concomitant administration of other nephrotoxic drugs (Nelson et al., 2008; Crum-Cianflone et al., 2010). This could be attributed to medications having more adverse effects on older people because as they age, their bodies are not able to repair and rebuild damaged cells, organs or tissues as rapidly as those of younger people. HIV infection and antiretroviral drugs have been associated with many complications including, kidney and liver dysfunction with increasing age (Cordery and Cooper, 2011). Similarly, advanced age has been independently associated with renal function decline among HIV-infected subjects (Mocroft et al., 2005). To sum it all, most randomized clinical trials with new drugs tend to avoid the enrolment of subjects over 50 years with proper co-morbidities of old age (Celesia, 2010) indicating that the efficacy and toxicity results from the trial would not reflect an optimized test process due to age factor.

Although other risk factors are not well established, observational studies have associated co-morbid renal dysfunction with advanced age (Thompson, 2011). The presence of multiple opportunistic infections is generally common in HIV-infected patients, however, age-associated co-morbidities can compound this problem in older HIV-infected people. HIV infection that attack and destroy the body's defenses can exacerbate with old age and increase the risk of developing additional medical problems like diabetes and high blood pressure, than in younger adults with HIV.
Several studies found that older adults had lower CD4 counts at diagnosis, faster progression to an AIDS diagnosis, more opportunistic infections, and a shorter survival rate than younger adults, regardless of when they were first diagnosed with HIV. As people age, their bodies are not able to repair and rebuild damaged cells, organs or tissues as rapidly as those of younger people. In the early years of HIV epidemic (before HAART), health in older adults deteriorated more rapidly than that of younger individuals regardless of CD4 count (Pataki, 2006). As individuals age and continue receiving HAART they are faced with an increased risk of adverse reactions to drugs toxicities and drug interactions with the disease (Population Reference Bureau, 2009).

Most randomized clinical trials with new drugs tend to avoid the enrolment of >50 years patients with proper co-morbidities of old age. A study showed that even while receiving highly active antiretroviral treatment (HAART), middle-aged men with HIV had a reduced ability to exercise and lower functional performance (Population Reference Bureau, 2009). It is also important to note that, a person's age should not interfere with the ability of HAART to reduce viral load, but there may be age determined differences between younger and older people which influence how well the immune system responds to treatment.

There were significantly higher values for liver analytes ALT, AST, and GGT for HAART duration of 4-6 years than 0 & 1-3 years. However, PROT analyte for HAART duration of 0 years was significantly higher than 4-6 and above 7 years. This implies that the duration in which HIV patients have been on HAART medication determine how toxicity related complications evolve. This study noted that with an aging HIV-infected population and increased survival, drug-induced liver injury will
probably continue to present a relevant entity in HIV therapeutic management. Creatinine was the only kidney parameter that had significantly higher mean in the HAART naïve patients (0 years category) than any of the other three HAART treated (>1 years) categories. This implies that renal damage may be caused by others factors other than ARV itself and that initiation on HAART ameliorates the state of renal disorder caused by other multi-factorial factors other than ARV medications. Jao and Wyatt (2010) observed in a more similar study that early initiation of antiretroviral therapy probably improves outcomes among persons with HIV acute nephropathy.

The data for kidney function tests from this study demonstrated that untreated HIV infection may have detrimental effects on the kidney whereas HAART-treated HIV infection improves function of the kidney as reflected by creatinine levels. Many findings from reviewed publications indicate that renal function typically improves with ARV treatment especially in those with pre-existing kidney disease (Thompson, 2011). Application of HAART is beneficial as it may alleviate the kidney-damage associated with HIV replication during early stages of infection and even when initiated later in infection (Ogundahunsi et al., 2008). It is then probable from this study that renal dysfunction caused by HIV alone may resolve over time after patients are started on HAART. Kidney injuries regardless of the above cited information may occur due toxicity effects of antiretroviral as the body attempts to clear or excrete the drug by-products via the urinary system. The prevalence of renal dysfunction defined by proteinuria was 7.4% in patients on HAART for 4.24±1 years (Ogundahunsi et al., 2008). It is possible that the HAART treated subjects may have had elevated creatinine levels before the start of ARV medications but may have resolved on initiation of ARV or due to increased kidney tolerance to constant exposure to the drug. Kidney damage associated to antiretroviral therapy is typically reversible with
early recognition, tolerability of the organ or timely discontinuation of the offending agent (Jao and Wyatt, 2010).

5.2 Conclusion

- HAART has become increasingly effective but also increasingly complex owing to many adverse effects of therapy causing a variety of liver and renal derangements with the consequences of patient non-adherence, low efficacy, treatment failure and emergence of drug resistance.

- It emerged from the study that hepatotoxicity and renal derangements based on their key surrogate biomarkers are prevalent in HIV positive patients irrespective of being on antiretroviral medications or not, an indication that the complications is independent of antiretroviral therapy.

- Liver and kidney derangements caused by HAART and/or HIV inflammation are more prevalent in an HIV aging population on antiretroviral agents and mitigating them through supportive treatment and other clinically tested remedies like change of medication or discontinuation of the offending drug remains critical.

- As the population of HIV-infected patients’ age and remains on HAART for longer periods of time, HIV and HAART-related liver derangements tends to become increasingly common. HAART-derived liver derangements are aggravated by the patients’ age and increasing duration on HAART unlike renal derangements which resolve in HAART naïve patients once started on HAART.

- Females-gender patients bear the greatest propensity to developing HAART adverse events affecting liver and kidney functions. This notwithstanding, HIV
patients with such risk factors should not be denied appropriate HAART regimens but regular clinical reviews and monitoring of adverse effects be adopted to prevent their emergence.

- As efforts continue in the development of antiretroviral medications with less adverse effect profiles, treating physicians must remain aware of new and developing syndromes associated with their use.

- Although randomized and controlled clinical trials is the gold standard for evaluating efficacy and toxicity of antiretroviral drugs before FDA approves for human consumption, it became apparent from the study that long-term toxicities which affect the liver and kidney in the general population are underestimated, compounded by the desire of subjects to stay for a short period in a clinical trial.

- In addition, severity of HAART adverse effects may vary as a result of patients’ genetics, nutrition, and non-specific laboratory diagnostic tests precipitated by clinical approach of a symptom-directed diagnosis especially in resource limited health settings.

- Finally, the use of adequate and specific clinical laboratory tests to monitor routinely early outcomes of HAART toxicities that can cause life threatening liver and kidney derangements remains an essential prognostic tool useful in surveillance and managing adverse effects of HAART.
5.3 Recommendations

- Although occurrence of hepatic and renal derangements due to use of HAART and/or HIV inflammation are prevalent, HIV patients who are clinically and virologically stable should continue with HAART unless severe or complex complications emerge. If they occur, adequate treatment options for the adverse events are prescribed like regimen change, temporarily withdrawal or complete stoppage after a thorough clinical evaluation.

- Surveillance of liver and renal derangements in HIV/HAART patients with presence of co-morbid factors like advanced age, female-gender and prolonged HAART duration >4years should be stepped up through liver and kidney function tests. This should be done by adopting a periodic 3-month monitoring approach which can help prevent their exacerbation and avoid clinical morbidity, a shift from the current clinical symptoms-directed approach.

- Lastly, following the ubiquitous and essential use of HAART in the management of HIV and AIDS, the study recommends a controlled research study to be carried out to evaluate the potential toxicity effects of individual agents within HAART on liver and kidney by utilizing in vitro assays, animal models and pharmaco-genetics in clinical trial populations and cohort studies whose outcome can probe further redefining or fine tuning of the medication package (HAART).
REFERENCES


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**Friedman and Young.** (2000). Effects of Disease on Clinical Laboratory Tests, 5th ed. IFCC.


Roche Cobas c 111. (2005). \textit{Manufacturer’s Method Manual}, ver. 3.0, Roche diagnostics, Germany.


APPENDIX 1: QUESTIONNAIRE FOR CLIENTS IN THE STUDY

Section 1: Introduction
This questionnaire is set to assess the prevalence of hepatotoxicity and renal insufficiency accompanying the use of antiretroviral medication. Kindly answer all the questions as accurately as possible. All the information you give in this questionnaire is private and confidential. Do not indicate your name on the form. Your contribution is important and will be used to serve you and others.

Section 2: Client information
1: Gender: Male □ Female □
2. Age (Years) __________
3. Religion __________________
4. Marital status (Tick as appropriate): Single □ Married □ Separated □ Divorced □ Widowed □ Widower □
5. Highest level of education attained ___________________________ 
6. Your occupation __________________________________________ 
7. How far is your residence from the hospital? (Tick in the appropriate box)
   0-5 kilometers □ 5 -10 kilometers or less □ over 10 kilometers □
8. How much does it cost you to and from the hospital? Kshs ……..
9. Do you know how HIV/AIDS is transmitted and/or prevented? Yes -- No --
10. Do you know how HIV/AIDS is treated? Yes ------- No -------
11. Are you on ARVs /ART – HAART? Yes ------ No -------
12. If yes, for how long? (years): 1 –3……. 4-6 ..... 7 and above ……
13. Do you take alcohol and/or any other drugs? Yes ----- No --------
14. If yes, specify and state how regularly?
   Drug ……………………….. Daily ---- Weekly ------ Monthly ----- Occasionally --
15. Are you on any treatment for? (i) TB: Yes ----No ---
   (ii) Diabetes: Yes ----No --- (iii) Hypertension: Yes ----No --- (iv) Other (specify): Yes ----No ---
16. a) If yes, specify condition and duration? Condition…….. Duration ………
   b) Are you on any medication? Yes … No ……..
17. Are ARVs always available when you come for refill? Yes ---- No ---
18. How long do you wait to get ARV refill at the hospital? 30mins….. 1hr….. 2hrs…… 3hrs …… 4hrs …. 5hrs …..
19. Do you take the medications as always prescribed?  Yes … No …
20. If not, why? Forget …… Side effects …… Shortage of medication …..
21. Which ARV side effects have you experienced?
   1. Skin rash  Yes ---- No --  2. Headaches  Yes ---- No ---
   3. Diarrhoea  Yes ---- No ---  4. Vomiting  Yes ---- No ---
   5. Pancreatitis  Yes ---- No ---  6. Lipodystrophy  Yes ---- No ---
   7. Liver injury  Yes ---- No ---  8. Kidney disease  Yes ---- No ---
22. How did you cope with the ARV side effects problem?
   1. Given medication by doctor ….  2. Stopped taking ARV medications ….
   3. They subsided on their own ….  4. They are still affecting me…………
23. Did the side effect problem compel you to come to the hospital
   1. Yes ………………  2. No ……………

THANK YOU FOR AVAILING YOUR TIME AND INPUT TO THIS STUDY. GOD BLESSES YOU.
APPENDIX 2: A SAMPLE OF PATIENT CARD

The image shows a patient card form with various sections and fields to be filled out. The form includes details such as patient profile, ART therapy, ART history, ART treatment interruptions, and other medical conditions. The form is divided into sections for different types of information, such as patient demographics, medical history, treatment details, and adherence. The form contains fields for patient identification, contact information, medical history, and treatment regimen. The form also includes space for additional notes and comments.
APPENDIX 3: A SAMPLE OF LABORATORY REQUEST FORM

<table>
<thead>
<tr>
<th>MINISTRY OF HEALTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab No ............</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LABORATORY REQUEST AND REPORT FORM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Note: Incompletely filled forms will not be processed</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>I. Patient details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name .................................................</td>
</tr>
<tr>
<td>Age (yrs/months) ..................</td>
</tr>
<tr>
<td>Sex  M .................. F ..................</td>
</tr>
<tr>
<td>Residence/Village ..................................</td>
</tr>
<tr>
<td>IP/OP No ...........................................</td>
</tr>
<tr>
<td>Report to (Specify clinic / ward/dinician) ..................................</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>II. Specimen destination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tick appropriate box</td>
</tr>
<tr>
<td>Histology/Cytology ......</td>
</tr>
<tr>
<td>Bacteriology .........</td>
</tr>
<tr>
<td>Serology ..........</td>
</tr>
<tr>
<td>Parasitology ....</td>
</tr>
<tr>
<td>Hematology .........</td>
</tr>
<tr>
<td>Biochemistry ......</td>
</tr>
<tr>
<td>Sputum New .... Follow-up ......</td>
</tr>
<tr>
<td>1st .... 2nd .... 3rd ....</td>
</tr>
<tr>
<td>Others (specify) ..................</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>III. Previous Report:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous Lab No ..................................</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IV. Specimen:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collection date ___ / ___ / ___</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>V. Investigation requested:</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>VI. History (including drugs used)</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>VII. Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Requesting Clinician’s Name ........ Signature ............ Date ___ / ___ /20___</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>VII. Report (including macroscopic examination):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test done by (name) .... Sign .........</td>
</tr>
<tr>
<td>Designation .................................. Date ___ / ___ /20___</td>
</tr>
<tr>
<td>Approved by (name) ................................ Sign .............</td>
</tr>
<tr>
<td>Designation .................................. Date ___ / ___ /20___</td>
</tr>
</tbody>
</table>
APPENDIX 4: CONSENT FORM 1 (ENGLISH VERSION)

PATIENTS’ INFORMATION SHEET

Effects of Highly-active antiretroviral therapy on the Liver and Kidney functions in HIV Patients at Coast Province General Hospital, Kenya

Description of the research study
Good morning/afternoon. My name is Chris Ngeny, a Postgraduate student doing Master of Science degree (Medical Biochemistry) at Kenyatta University and carrying out this research in collaboration with Coast Province General Hospital (CPGH). The common treatment of HIV/AIDS involves the use of antiretroviral (ARV) medications that reduces viral numbers slowing down rate of progression into full-blown AIDS. Some of the ARV medications are associated with side effects that may affect the liver and kidney. In this study we want to find out effects of ARV on the liver and kidney among HIV+ patients at CPGH.

Purpose of the research study
The research study intents to find out if there are any cases of liver and kidney diseases caused by use of ARV in both female and male HIV+ patients.

Procedures of the study
When you come to hospital for review clinics, blood samples will be taken for determination of the CD4+ cells. The blood sample is usually not for research purposes but to help in the management of HIV and AIDS. In addition, we are requesting to collect a small extra amount of blood of 5mls (1 teaspoonful) from the vein to measure markers that show normalness of the kidney and liver. Two groups will be recruited with one group comprising of HIV+ patients not started on ARV and the second group of HIV+ who have been on ARV for more than one year. Once recruited, all participants’ will attend HIV clinics monthly for five consecutive months where their blood and urine samples will be measured to know the health state of the two organs.

Benefits of the study
There is no immediate individual benefit at now for those participating in the research other than additional laboratory investigation of their liver and kidneys to know if they are in good health as they continue on ARV. However, if any secondary ailment is found one will receive adequate treatment. No incentives are offered and we hope that you will support the aims of this research and contribute to the development of better ways of individualizing ART among HIV+ patients at CPGH and elsewhere in Kenya. Therefore, the benefits will be to the community as a whole.
Risks of being in the study
Participation in this study does not carry any major risk except that participants will experience some pain and discomfort during collection of venous blood. All in all, expert clinicians and nurses will do collect blood with ease and care. One may only get some inconvenience since prolonged time and attention is required of you as we carry out the investigation than in the standard treatment.

Confidentiality
To ensure privacy and confidentiality of all information, the records of the laboratory results will be kept under a code number and not your name. Similarly, all the samples taken will be labeled with a laboratory reference number that link your name. Your name will not appear when reporting the outcome of my findings. Results of individual laboratory tests will not be given to the public.

Voluntary Participation
There is no compulsion at all for you to participate in this study and there will be no reward or penalty of any kind if you decide to or not to participate. If you agree to participate in the study but later change your mind, you may either stop participation or contact us and ask for all records to be destroyed. However, you will continue getting standard medical services in the same way as everyone else.

Storage of Records
Your records will be kept in a file that links you to a code number for future reference but nobody will access it without written permission of the hospital. This will ensure that your privacy is not threatened by the results.

Approval of the Study
The investigator was granted ethical approval by KEMRI/ERC, (Non-SSC Protocol No. 253) and a research permit from National Council for Science and Technology (NCST/RR1/12/1MED011/106) to carry out the study. The study was done at CPGH under supervision of Dr Ng’ali M., (Medical consultant) and Biochemistry laboratory technologists in collaboration with Prof. Ngeranwa JN., of Kenyatta University and Prof. Gichuki C., of Presbyterian University of East Africa as my research supervisors.

Do you have any Questions?
If you have any queries or concerns about this research, please contact Chris Ngeny, Principal Investigator at P.O. Box 195, Kilifi or Tel 0722405003, Dr. Mbuuko Ngali, Medical Pathologist at CPGH on Tel. 0713959766: You can also contact KEMRI/ERC Secretariat at Box 54840, Nairobi or Tel 0722205901/0733400003. At Kenyatta University you can contact Prof. Joseph Ngeranwa on 0722268068 or Prof. Charity Gichuki on 0722614027.
CONSENT AGREEMENT

I ________________________________ (name of patient or guardian) have been given all information concerning the study entitled: Effects of HAART on the Liver and Kidney functions in HIV patients, under the direction of Mr. Chris Ngeny and have been read the document. The nature, implications, duration, purpose, voluntary nature and inconveniences or risks expected have been explained to me by __________________________ (name of investigator) this ________ day of ______, 20_________. I have been given the opportunity to ask questions concerning the study and my questions have been answered to my satisfaction. For further questions, I have been asked to contact Mr. Chris Ngeny, Principal Investigator of P.O. Box 195, Kilifi or Tel 0722405003 or Dr. Mbuuko Ngali, a Medical Pathologist at CPGH on Telephone 0713959766. I understand that I may at any time during the study revoke my consent and/or withdraw from the study without any loss or penalty. My refusal to participate in this study will not accompany any penalty or loss of benefits to which I am otherwise entitled to.

Witness
I observed the process of consent. The participant was given the chance to ask questions, accepted the answers and signed to be enrolled in the research study.

Name ...................................... Designation ..............................

Signature ............................... Date ..............................
APPENDIX 5: CONSENT FORM 2 - (SWAHILI VERSION)

FOMU YA IDHINI KWA WAHUSIKA

Madhara ya dawa za kupunguza makali ya virusi vya HIV kwenye viungo vya ini na figo kwa wanaotumia dawa za ARV katika hospitali kuu ya Mkoa wa Pwani nchini Kenya.

Maelezo ya uchunguzi huu kwa wahusika

Malengo ya uchunguzi
Uchunguzi wangu una lengo la kutaka kujua matatizo ya maini na figo ambayo hutokana na matumizi ya madawa ya kupunguza makali (ARV) ya virusi (HIV) vinavyosababisha ukimwi (AIDS). Uchunguzi huu unalenga wagonjwa wakilivyobadilisha na virusi, wanaume kwa wawawake. Matumizi ya dawa za kupunguza makali ya virusi vinavyosababisha ukimwi yana madhara aina mbalimbali. Katika uchunguzi huu tuna lengo la kujua madhara yake na virusi vya HIV vya viungo hivi. Tunatarajia kutimiza lengo letu ili tupate picha kamili itakayowezesha madaktari na watumizi wa dawa hizi kutambua madhara haya mapema kabla kusababisha kifo.

Taratibu za utafiti
Waathiriwa wa virusi vya HIV watakapofika hospitalini kwa uchunguzi wao wa mara kwa mara au kliniki, damu zao hupimwa au kujua idadi ya chembe za CD4+. Damu hizi hazitumika kwa uchunguzi wowote. Tutahitaji damu zaidi ya kawaida, kiasi cha vijiko viwili (mililita kumi) na mkojo kiasi hicho vilevile ili ituwezeshe tufanye uchunguzi zaidi. Damu hizi zitatumika kwa upimaji wa chembe za maini na figo itakayotuonyeshwa picha ya afya ya viungo hivi. Ilivuweze kufanikika, tunahitajika kufanyia uchunguzi wanaume na wawawake wako na virusi na wawino na umri kati ya miaka 18-60. Tunahitajika wakati viwili ambayo kundi la kwanza ni wana virusi vya HIV hawajanza kutumia dawa za ARV na kundi la pili wana virusi vya HIV na wametumia dawa za ARV zaidi ya mwaka mmoja. Tutahitaji kupima damu na mkojo ili kuthibitisha afya ya maini na figo kila baada ya mwezi moja kwa miezi tano mfululizo.
Manufaa ya uchunguzi
Kwa sasa, uchunguzi huu hauna manufaa yatakayofaidi mtu binafsi bali wahusika wataweza kuja hali yao ya viungo (maini na figo) kadri wanavyoendelea na ARV. Hakuna zawadi zozote tunazopeana unapokubali kushiriki bali omba ni watu wajitolee ili matokeo ya uchunguzi huu utaifaidi nchi yetu hasa tunapotazamia kuimarisha afya za watu ambao wameathirika na janga hili la ukimwi. Kwa ujumla manufaa ya uchunguzi huu utaifaidi jamii yetu yote.

Tahadhari za kushiriki
Watu ambao watahitishwa kwa uchunguzi huu watahitajika kutolewa damu na kupe ana mkojo wao. Hii ni hatua isiyoni na madhara isipokuwa hali ya kawaida ya kuhisi uchungu kidogo wakati wanapotelea damu. Hata hivyo maafisa watahakikisha hali ya kawaida ya kuhisi uchungu kidogo wakati wanapotazamia kwa uchunguzi huu kutoaji wa damu kutoka kwa wagenjwa bila shida.

Kuweka siri
Nitahakikisha kwamba matukeo ya majibu ya watu ambao watahitishwa na uchunguzi huu hayatatolewa hadharani kwa vile nambari zitatu mika kwa vipimo na majibu kati katika mahabara wala jina la mtu binafsi. Pia jina lako halitatokezea watu ambao watahitajika kutolewa damu na mkojo wao. Hii ni hatua isiyo na madhara isipokuwa hali ya kawaida ya kuhiuka uchungu kidogo wakati wanapotazamia kwa uchunguzi huu.

Kujitolea kwa uchunguzi
Uko huru kujiwa uchunguzi na ukia kati na uchunguzi huu wa utafiti wa kawaida na binafsi. Vile vile ukiwakubali kushiriki na utafiti una uchungu kidogo wakati wanapotelea damu. Hata huhiwa kuhiuka uchungu kidogo wakati wanapotelea damu na utafiti wa binafsi.

Kuhifadhi matukeo kwa siku zijazo
Tungependa kuhifadhi matukeo ya utafiti huu kwa watu ambao watahitishwa na uchunguzi huu haya uchungu kidogo wakati wanapotelea damu. Hata huhiwa kuhiuka uchungu kidogo wakati wanapotelea damu na utafiti wa binafsi.

Kupitishwa kwa utafiti
Mwenye kufanya utafiti ama kiongozi ana kibali wa KEMRI/ERC (Non-SSC Protocol No. 253) na kutoka NCST (NCST/RRI/12/1/MED011/106). Utafiti huu utafanywa katika hospitali kuu ya Mkova wa Mwamburi na Chuo kikuu cha Kenyatta.

Je uko na maswali?
Ukiwa na maswali yote utawasiliana na Bw. Chris Ngeny, kiongozi wa utafiti huu wa SLP 195. Kilifi au nambari ya simu 0722405003; Daktari Mbuuko Ngali kwa nambari ya simu 0713959766. Waweza kuwasiliana pia na KEMRI/ERC, SLP 54840,
Nairobi, au nambari ya simu 0722205901/0733400003. Vile vile Professa Joseph Ngeranwa, wa nambari ya simu 0722268068 na Professa Charity Gichuki, nambari ya simu 0722614027 wa Chuo kikuu cha Kenyatta.

**MAKUBALIANO YA KUSHIRIKI**


Jina--------------------------------- Sahihi-----------------------------------Tarehe---------------------------------

**Shahidi**

Nilishuhudia utaratibu wa idhini hii. Muhusika alipewa nafasi ya kuuliza maswali, na akakubali majibu na akatia sahihi kujandikisha kwenye utafiti huu.

Jina--------------------------------- Sahihi-----------------------------------Tarehe---------------------------------

**Mawasiliano**

APPENDIX 6: RESEARCH PERMIT: NCST/RRI/12/1/MED011/106

THIS IS TO CERTIFY THAT:

Prof./Dr./Mr./Mrs./Miss CHRIST KIPNGETICH NGENY

of (Address) KENYATTA UNIVERSITY
P.O BOX 43844, NAIROBI

has been permitted to conduct research in:

Location, MOMBASA District, COAST Province

on the topic:

THE EFFECTS OF ANTIRETROVIRAL DRUGS ON INTEGRITY OF THE LIVER AND KIDNEY, A CASE OF HIV PATIENTS ON HAART AT COAST PROVINCE GENERAL HOSPITAL IN KENYA

for a period ending 30th JULY 2012

CONDITIONS

1. You must report to the District Commissioner and the District Education Officer of the area before embarking on your research. Failure to do so may lead to the cancellation of your permit.
2. Government Officers will not be interviewed without prior appointment.
3. No questionnaire will be used unless it has been approved.
4. Excavation, filming and collection of biological specimens are subject to further permission from the relevant Government Ministries.
5. You are required to submit at least two (2)/four (4) bound copies of your final report for Kenyans and non-Kenyans respectively.
6. The Government of Kenya reserves the right to modify the conditions of this permit including its cancellation without notice.

REPUBLIC OF KENYA

RESEARCH CLEARANCE PERMIT
APPENDIX 7: NCST AUTHORIZATION LETTER

REPUBLIC OF KENYA

NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

Telephone: 254-020-2213471, 2241349
254-020-310571, 2213123, 2219420
Fax: 254-020-318245, 318249
When replying please quote
secretary@ncst.go.ke

Our Ref: NCST/RRI/12/1/MED011/106

Date: 8th August, 2011

NCST/RRI/12/1/MED011/106

Chris Kipngetich Ngeny
Kenyatta University
P.O.Box 43844-00100
Nairobi.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on “Effects of Anti-Retroviral drugs on integrity of the liver and kidney, a case of HIV patients on HAART at Coast Province General Hospital in Kenya,” I am pleased to inform you that you have been authorized to undertake research in Mombasa District for a period ending 30th July, 2012.

You are advised to report to the Chief Administrator, Coast Provincial General Hospital before embarking on the research project.

On completion of the research, you are expected to submit two hard copies and one soft copy in pdf of the research report/thesis to our office.

DR. M. K. RUGUTT, PhD, HSc.
DEPUTY COMMISSION SECRETARY
NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Copy to:

The Chief Administrator
Coast Provincial General Hospital.
APPENDIX 8: KEMRI/NERC APPROVAL - (NON-SSC PROTOCOL NO.253)

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840 - 00200 NAIROBI, Kenya
Tel: (254) (020) 2722541, 2713749, 0722-005901, 0722-400003; Fax: (254) (020) 2720000
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1

2nd December, 2010,

TO: CHRIS K NGENY (PRINCIPAL INVESTIGATOR)
156/CE/11948/2008
KENYATTA UNIVERSITY

THRO: DR. JOSEPH J.N. NGERANWA,
CHAIRMAN, DEPT. OF BIOCHEMISTRY & BIOTECHNOLOGY


Make reference to your letter dated 10th November, 2010 received on 1st December, 2010. Thank you for your response to the issues raised by the Committee. This is to inform you that the issues raised during the 18th meeting of KEMRI/National Ethical Review Committee held on 9th November, 2010, have been adequately addressed.

Due consideration has been given to ethical issues and the study is hereby granted approval for implementation effective this 2nd day of December 2010, for a period of twelve (12) months.

Please note that authorization to conduct this study will automatically expire on 1st December 2011. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by 17th October 2011.

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

Yours sincerely,

R. C. KITHINJI,
FOR: SECRETARY,
KEMRI/NATIONAL ETHICS REVIEW COMMITTEE