THE ANTIRETROVIRAL DRUGS TOXICITY AND IMMUNE STATUS OF HIV PATIENTS ATTENDING RUNYENJES COMPREHENSIVE CARE CENTRE, EMBU, KENYA

NAMU JOHN SAMUEL (BEd SC)
(I56/CE/10957/06)

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTERS OF SCIENCE (IMMUNOLOGY) IN THE SCHOOL OF PURE AND APPLIED SCIENCES OF KENYATTA UNIVERSITY

SEPTEMBER 2011

DECLARATION

I, Namu John Samuel hereby declare that this is my original work and has not been presented for award in any other university

NAMU JOHN SAMUEL (I 56/CE/10957/06)

Signature ---------- **Date** 22/9/11

This thesis has been submitted for examination with our approval as University supervisors

DR. MICHAEL M GICHERU

Department of Zoological Sciences, Kenyatta University

Signature ---------- **Date** 5-10-2011

PROF. ZIPPORAH NG'ANG'A

Jomo Kenyatta University of Agriculture And Technology

Signature ---------- **Date** 23/9/2011

DEDICATION

I dedicate this thesis to my wife, Catherine Njoki and daughters Moreen Mwende and Joylena Muthoni who provided moral and financial support, my mother Mary Gicuku and my father Samuel Kiura for their encouragement and prayers. To my sisters, Joy Wanja and Rebbecca Muthoni, my friends and relatives who have been an encouragement through this work.

ACKNOWLEDGEMENTS

I wish to sincerely thank my supervisors Dr. Michael Gicheru , Department of Zoological sciences, Kenyatta University and Prof. Zipporah Ng'ang'a of Institute of Tropical Medicine and Infectious diseases, Jomo Kenyatta University of Agriculture and Technology for their unlimited time, advice and guidance they provided me throughout this study. I also thank all the staff of Runyenjes hospital for their support especially Dr. James Maina and Dr. Elesban Kihuba who allowed me through the ministry of medical services to carry out this study at Runyenjes comprehensive care centre, Mrs. Anceta Njeru, the incharge of the comprehensive care centre who assisted me to bleed patients for blood samples, the Embu provincial laboratory staff especially Mr. John Munyi who assited me to analyse the blood samples with the laboratory equipments. My gratitude also goes to Kenyatta University for giving me an opportunity to to be their student. My sincere thanks also go to Peter Njeru of Karurumo health centre for his encouragement as I carried out this study. Last but not the least I am grateful to my family especially my father Samuel Kiura for his financial support and friends for their unlimited moral support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS AND ABBREVIATIONS

Abs	Antibodies
Ag	Antigens
AIDS	Acquired Immunedeficiency Syndrome
ARVs	Antiretrovirals
Caf	Cell Antiviral Factors
CCC	Comprehensive Care Centre
CD	Cluster of Differentiation
cDNA	Complementary DNA
CTLS	Cytotoxic T. Lymphocyte
Env	Envelope
FDA	Food and Drug admininstration
Gag	Group Specific Antigens
Gp	Glycoproteins
Hb	Haemoglobin
HIV	Human Immuno Deficiency Virus
Ig	Immunoglobulin
KAIS	Kenya AIDS Survey
LTR	Long Terminal Repeats
MCV	Mean Corpuscular Volume
MHC	Major Histocompatibility Complex
mRNA	Messenger RNA
NASCOP	National AIDS /STI Control Program
Nef.	Negative Regulation Factor
Pol	Polymerase

Rev	Regulator of Viral Proteins
STI	Sexually Transmitted Infections
Tat	Transactivitor
UNAIDS	United Nations AIDS
Vif	Viral Infectivity
Vpr	Viral Protein R
VpU	Viral protein U

ABSTRACT

Human Immune Deficiency Virus (HIV) has become a global problem which has reduced the quality and lifespan of many people all over the world. The World Health Organization (WHO) has established that ARVs reduce the suffering of HIV patients. Currently, there are various ARV regimes in use, and they are taken for a long period of time. This could lead to emergence of ARV associated toxicity. The objective of this study was to determine toxicity and immune status of HIV patients under ARVs (Zidovudine, Stavudine and Nevirapine) which comprises first line ARV regime currently being used in this country. The study design was longitudinal, a case study involving HIV patients attending Runyenjes comprehensive care centre (CCC) during the months of May to November 2009. A total of sixty HIV patients participated in the study after consenting to undergo comprehensive care. A control group of 40 HIV patients on only contrimoxale (septrin), an antibiotic used to control opportunistic infections was also monitored for six months to exclude the compounding effects of accompanying antibiotics. Baseline values of creatinine and haemograms were determined and compared with immunological parameters for different levels of treatment monthly over a period of six months. CD3 and CD4 cell counts were determined in a Fluorescence Activated Cell sorter (FACs) using cross reactive antibodies while mean corpuscular volumes (MCV), platelets and Haemoglobin (Hb) were determined using a blood cell analyser. Using a serum analyser serum creatinine was determined and used as an indicator of toxicity of the ARV drugs. Analysis of Variance (ANOVA), t-test and correlation coeficient were used to analyse the data. The results showed that out of sixty HIV patients sampled twenty (33.3%) had baseline CD4 counts less than 50 cells /µl of blood, 9 (15%) between 50-100 cells/µl of blood, 8 (13.3%) between 101-150 cells/µl of blood and 23 (38.4%) above 150 cells/µl of blood. Correlation analysis showed that creatinine was weakly positively correlated with MCV ($p<0.01$; $r=0.149$), weakly correlated with CD3 ($p<0.01$; $r=0.063$), weakly correlated to haemoglobin level ($p<0.01$; $r=0.059$), weakly correlated with platelet counts ($p<0.01$; $r=0.082$) and positively correlated to CD4 ($p<0.01$; $r=0.178$). Increase in creatinine levels resulted in increased CD4 and CD3 up to the third month and increased levels of haemoglobin, MCV and platelet up to the sixth month. Results also showed significant increase in mean creatinine following 6 months of ARV use ($p<0.01$, $F=22.73$, $df=5$) and that treatment with ARVs may cause toxicity especially to patients with baseline CD4 counts of 50-100 cells/µl of blood by the sixth month of use. Results of the control group of patients showed no significant changes in mean creatinine ($p=0.1$, $F=0.004$, $df=5$) with a mean of 0.99mg/dl of blood which was within normal creatinine range in health. The findings of this study will be useful in understanding the potency of ARV regime and associated toxicity. Based on the data observed in this study it is recommended that the ARV drug combination used may be initiated for patients at baseline CD4 counts of 101-150 cells/µl of blood but change to another combination after three months to minimize toxicity associated with prolonged use.

CHAPTER ONE: INTRODUCTION

1.1 Background

Human immune deficiency virus (HIV) and acquired immune deficiency syndrome (AIDS) is a global pandemic which has reduced quality of life for millions of people (WHO, 2006). HIV is associated with immune suppression which can be measured by the levels of T cell (Roit *et al.*, 2006). Specifically HIV has been shown to reduce levels of CD4 and CD3 in addition to other haematological values (Cheeseborough *et al.,* 2005). Normal levels of CD4 in health ranges are 1000-1200 cells/µl of blood (Cheeseborough *et al.,* 2005). Individuals with or less than 200 CD4 cells/µl of blood have been shown to develop AIDS which is associated with opportunistic infections and malignancies (Gilks,1998).

Currently, 40 million people are living with HIV and AIDS all over the world (WHO, 2009). It is estimated that over 1.2 million people in Kenya are infected with HIV (WHO, 2009, KAIS, 2009). Prevalence of infection in Kenya varies from region to region, with Nyanza having 14% followed by Nairobi with 9% (GOK, 2008). Embu in Eastern Province has reported a prevalence of 4% (GOK, 2008).

World Health Organization has established that ARVs reduce the suffering of HIV patients (WHO, 2006). Antiretroviral drugs have been shown to boost the

immune status of HIV patients and reduce opportunistic infections (Janeway *et al.*, 2005). The Kenya government has made a lot of effort to ensure that ARVs are available in provincial and district hospitals (GOK, 2006). Currently, combination ARV regimes are in use. Nucleoside analogues are most commonly used and HIV patients are initiated with first line combination ARV therapy (GOK, 2008) Antiretroviral combinations are designed to be taken for long periods of time; this could lead to emergence of ARVs associated toxicity (Smith, 2006).

Limited studies on prolonged use of ARV therapy showed swollen feet, kidney failure, liver malfunctions and compromised immune system in some patients (Janeway *et al.*, 2005). Toxicity can be measured by determination of levels of creatinine in serum among others which is more reliable as it indicates kidney filtration rates (Greer, 1999). Normal creatinine values in health is 0.6 to 1.2 mg/dl of blood in male adults and 0.5 to 1.1 mg/dl of blood in females and above 0.2mg/dl of blood in infants. Toxicity of most drugs may be manifested as abnormally elevated creatinine levels in serum (Greer, 1999).

1.2 JUSTIFICATION

Establishment of Comprehensive Care Centres in some hospitals and free ARVs has created high rates of ARV use .Currently the mode of ARV therapy is a combination of three or more drugs. Different ARVs have been associated

with toxicity. There is need to understand toxicity with combinaton ARV therapy. With more than 500 HIV patients enrolled at Runyenjes no comprehensive study has been done to address the effects of combination ARV therapy in Runyenjes, Embu. One way of monitoring toxicity is by monitoring creatinine levels in serum.

1.3 Problem statement

Antiretroviral drugs are freely available in government and private hospitals. Prolonged use of ARVs may contribute to drug associated toxicity. Their complications may include nephrotoxicity, lactic acidiosis, liver damage (hepatotoxicity) and Peripheral neuropathy (nerve damage) (Gerschenson *et al.*, 2000). All ARVs are taken for a long period of time (life long) and they are also highly toxic hence require close monitoring by clinicians. ARV drugs have not been monitored for their associated toxicity or side effects in HIV patients at Runyenjes.

1.4 Research questions

a) What are the effects of first line combination ARV therapy (nevirapine, zidovudine and stavudine) on CD3 and CD4 levels in HIV patients?

b) What are the hematological profiles of HIV patients under first line combination ARV therapy (nevirapine, zidovudine and stavudine)?

c) What are the effects of first line combination ARV therapy (nevirapine, zidovudine and stavudine) on creatinine levels among HIV patients?

1.5 Null hypotheses

a) There is no relationship between first line combination ARV therapy (nevirapine, zidovudine and stavudine) on use and creatinine levels.

b) There is no relationship between first line combination ARV therapy (nevirapine, zidovudine and stavudine) on use and the immune status.

1.6 Objectives

1.6.1 General Objective

To determine ARV drugs toxicity and immune status of HIV patients attending Runyenjes Comprehensive Care Centre, Embu, Kenya.

1.6.2 Specific Objectives

a) To determine the effects of first line combination ARV therapy (nevirapine zidovudine and stavudine) on creatinine levels in HIV patients .

b) To determine the effects of first line combination ARV therapy (nevirapine, zidovudine and stavudine) on CD4 and CD3 counts

c) To determine the effects of first line combination ARV therapy (nevirapine, stavudine and zidovudine) on heamatological values (Hb, MCV, and platelet) in HIV patients.

d) To determine the relationship between creatinine levels and immune status of HIV patients on first line combination ARV therapy (nevirapine, stavudine and zidovudine).

1.7 Significance and expected output

Creatinine level is a measure of kidney function based on glomelular filtration rates. Elevated levels of creatinine indicate kidney function impairment (Gerschenson *et al.*, 2000). Use of ARVs for a long time may change the level of creatinine, which may be a sign of toxicity. Kidney impairment may result to haematological defects which may lead to anaemias related to low production of erythropoietin hence affect haemopoiesis; this may change haemoglobin, platelets and mean corpuscular volumes (Gerschenson *et al.*, 2000). Platelet levels may influence blood clotting. Creatinine may also influence lymphopoiesis which may change CD3 and CD4 levels (Carr *et al.*, 2001). The level of creatinine and immune profiles will be useful in monitoring ARV drug responses and safety in HIV patients.

CHAPTER TWO: LITERATURE REVIEW

2.1 Epidemiological trends

Human immune deficiency virus (HIV) is a retrovirus with unique and strange characteristics (Brostof *et al.,* 2006). It contains two copies of single strand RNA with 9 genes (Roit *et al.,* 2006). Upon entry into the cell the genome is reverse transcribed into complementary DNA and integrated into the human genome to form a provirus (Carmichael *et al.,* 1995). There are two types of HIV: HIV-1 and HIV-2(Costello, 1998). HIV-1 is more prevalent and more virulent than HIV-2 (Costello, 1998). Approximately 40 million people in the world today are living with HIV virus, out of which 24 million hail from Africa (WHO, 2009).

An estimated 2.8 million people die every year and about 5 million new infections of HIV occur every year (WHO, 2009). In Kenya an estimated over (1.2M) 5.9% of the population is infected (MOH, 2008). Nyanza and Nairobi are leading with prevalence rates of 14% and 9% respectively (GOK, 2008). Embu has approximately 6000 infected: a prevalence of 4% (GOK, 2008). Infections with HIV has impacted negatively on Kenya's economy because the infected and affected are less productive (GOK, 2008). This is due to absenteeism from work, time wasting due to over focusing on the HIV-patient and burial costs (WHO, 2009). Globally, there are about 8 million HIV patients on ARVs, of which 3.5million are from Africa (WHO, 2009). In Kenya, an

estimated 0.4M HIV patients are under ARV treatment (NASCOP, 2009). Embu has about 2500 HIV patients on ARVs (GOK, 2009).

2.2 Structure and genome of HIV

The size of HIV is 120nm in diameter and is roughly spherical (Figure 2.1). The virus has two exact copies of single stranded RNA (genome) in the centre of the organism enclosed by a conical capsid comprising the viral proteins *p 24* in tightly packed association with one another, typical of lentiviruses (Figure 2.1). This is sorrounded by an envelope made of lipids and membrane bound proteins (Cullen 1998). It is this membrane – bound protein that binds to a particular protein on the surface of certain immune cells like T cells, macrophages and membrane dendritic cells. The singe stranded RNA is tightly bound to the nucleocapsid protein, *p 7* and enzymes such as reverse transcriptase, intergrase and protease are essential in HIV replication (Chrystie, 1988).

The nucleocapsid proteins, *p7* and *p6* associate with the genomic RNA and protect the RNA from digestion by nucleases. A matrix composed of an association of the viral protein *p17* sorrounds the capsid ensuring the integrity of the virion particle (Grant and Decock, 1998). Enclosed within the particle are accessory proteins viral infectivity factors *(vif), Viral protein R (vpr),* Negative reguratory factor *(Nef)* and *p7.* The envelope is formed when the

capsid buds from the host cell taking some of the host –cell membrane glycoprotein *gp120* and *gp 41* (Kwong *et al.,* 1998).

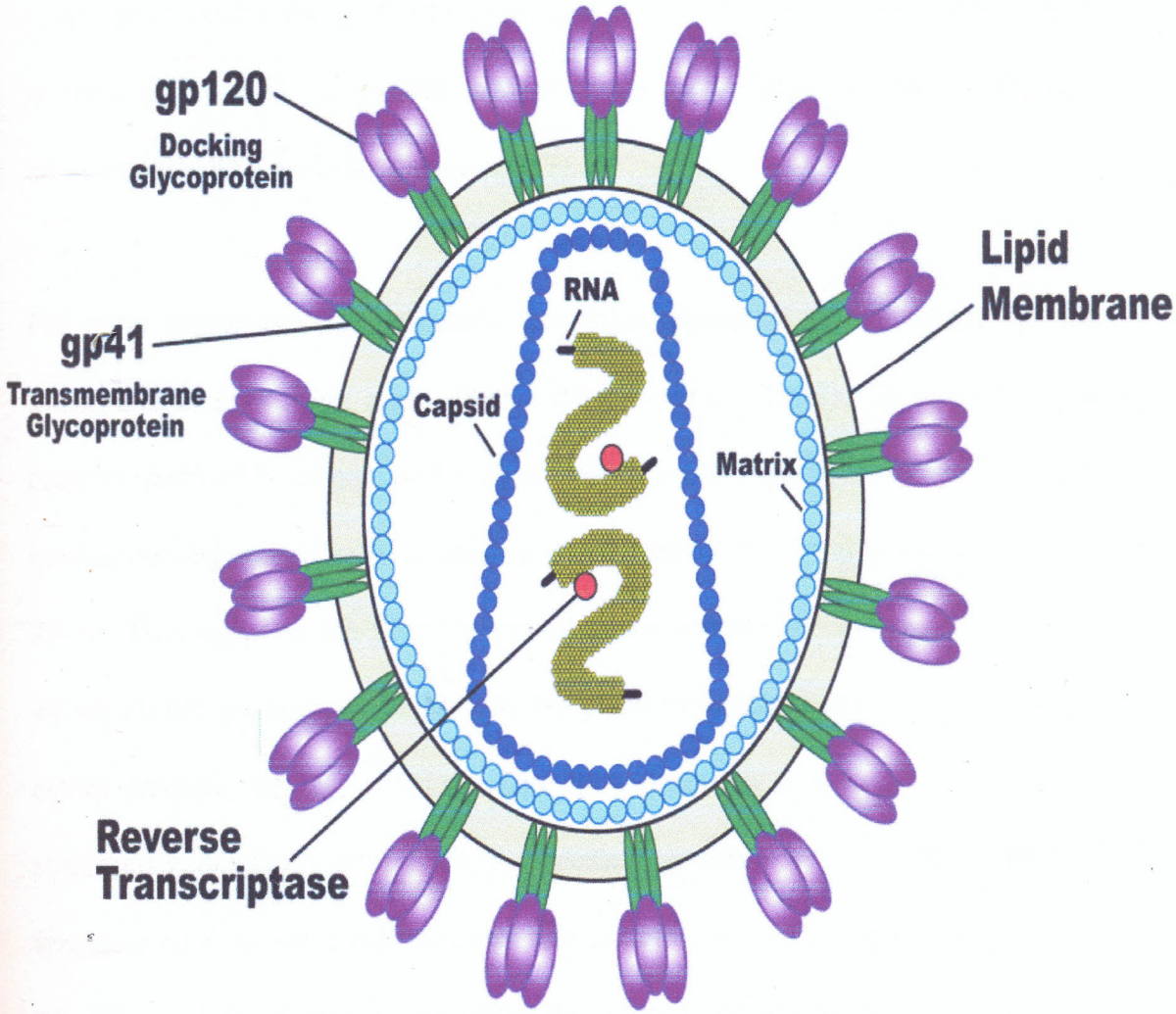


Figure 2.1: Structure And Genome Of HIV (Adapted from Chrystie, 1988).

The major genes of HIV coding for structural proteins are similar to those found in all retroviruses. However several, non structural or accessory genes that are unique are found in HIV (Lori *et al.*, 2005). Each of these genes play an essential role in the structure and function of HIV. Group specific antigen *(Gag)* gene codes for *p24,* the viral capsid , *p6, p7,* the nucleocapsid protein and *p17,* a matrix protein thus providing the basic physical infrastructure of the virus (Taften, 2007).

Pol gene codes for viral enzymes, the most important of which are reverse transcriptase, intergrase, and protease (Kwong *et al.,* 1998). These cleaves the proteins derived from *gag* and pol functional proteins thus providing the basic mechanism by which retroviruses reproduce envelope *(env)* gene (Lori *et al.,* 2005). This supplies the proteins essential for the precursor to *gp 120* and *gp 41* which are proteins embedded in the virus to attach to and fuse with target cells(Carcelain, *et al.,* 2001). *Env, gag* and *pol* are collectively called structural genes (Lori *et al.,* 2005). Accessory proteins *tat, ref, nef, vif, vpr* and *vpu* help HIV to enter the host cell and enhance its replication (Carcelain, *et al.,* 2001). Each of these genes codes for a single protein with same names; *tat* gene for *tat* proteins, *ref* for *Ref, nef* for *Nef, Vif* for *Vif* and *vpu* for *VpU* (Lori *et al.,* 2005; Figure 2.2).

2.3 Cellular invasion by HIV

Bringing the global HIV epidemic under control will require more effective strategies to prevent the spread (Kaplaan,1999). This is due to its high rates of mutation and negative impact on the immune cells (Grouard and Clark, 1997). Once HIV comes into contact with host cell like T cells, attaches and fuses with the cell and injects its genetic material into the cell (Grouard and Clark, 1997). Attachment is specific binding between proteins on the surface of the virus and proteins that serve as co-receptors on the surface of the T cell (Royse *et al.*, 1997). The co-receptors help the cell to communicate with other cells (Doms, 2005). Two receptors, that is, CD4 and CCR5 or CXCR4 depending on the strain of the virus, are used by HIV to attach and enter the cell (Scarlatti *et al.*,1997).

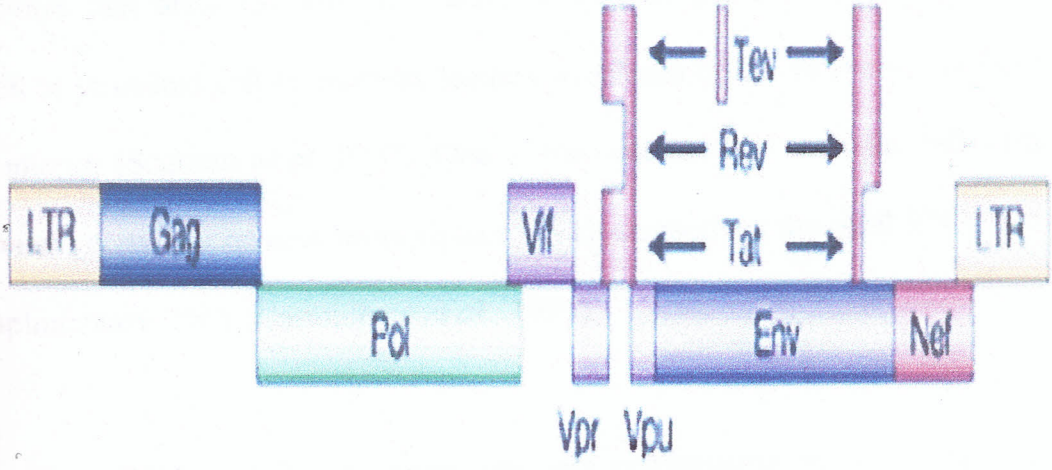


Figure 2.2: Genome organisation of HIV (Adapted from Chrystie, 1988).

HIV is primarily transmitted sexually (Male *et al.*, 2006). CD4 receptor is the main point for viral entry (Carmichael *et al.*, 1995). The binding of T helper cell (CD4) to HIV particle results in conformational changes of glycoprotein of molecular weight of 120 KDa. This exposes gp 41 binding to chemokine receptors allowing viral entry (Moore *et al.*, 1997). CCR5 is the major macrophage and T-cell co receptor used by HIV to establish primary infection *in vivo* to form a provirus (Lehnert., 2002). The combined CD4 binding and co receptor binding results in the formation of a coiled structure that exposes the hydrophobic fusion domain at the N-terminus of *gp 41* which was previously hidden in the viral membrane and loosely bound to *gp120* (Lehnert, 2002). Assembled as a trimmer on the viral membrane, this coiled coil protein splits open, projecting three peptide fusion domains that harpoons the lipid bilayer of the target cell (Udeselberg, 2006). The fusion domains then form hair pin-like structures that draw the viral and cell membranes together promoting the fusion of virus and cell membrane, leading to release of the viral core into the cell interior (Scarlatti *et al.*,1997). One of the proteins that enter the cell with the viral genome is reverse transcriptase which transcribes the viral RNA into complimentary DNA (Carmichael *et al.*, 1995).

The viral cDNA is then integrated into host cell genome by viral integrase which also enters the cell forming a provirus (Arya *et al.*, 1998). The *Gag* and *pol*-proteins are translated from unspliced mRNA. *Vif, Vpr, Vpu* and

Env are translated from singly spliced viral mRNA. *Tat, Rev* and *Nef* are translated from multiple spliced mRNA (Cullen, 2000).

2.4 Immune responses to HIV infection

Upon entry of HIV into host, active replication occurs in lymph nodes accompanied by destruction of CD4 T cell (Bain, 1997). Over the next few weeks viral replication is reduced by specific cytotoxic T lymphocytes (Morgan, 1997). A lowered level of viral replication then continues in lymph nodes and a steady state of viral production is matched by equivalent rates of clearance of virus with T cell death (Carmichael *et al.,* 1995). Many bases of viral sequence may be mutated during reverse transcription, which may affect the delicate balance between the virus and host immune system (Roit *et al.,* 2006).

Infectious virus is present at relatively low level in peripheral blood of infected individual during prolonged asymptomatic time (Johnson, 2002) after which the virus is replicated persistently in the lymphoid tissue (Vergani *et al.,* 2006). During this period CD4 cell count gradually decline but the levels of antibodies and CD8 cells directed against the virus remain high (Lempicki et al., 2000). Eventually the level of antibodies and CD4 cells also decline followed by a progressive increase of infective HIV in peripheral blood (UNAIDS, 1998).

2.5 Chemotherapy in HIV and AIDS patients

Antiretroviral drugs are medications for the management of infection by retroviruses, primarily HIV (Carcelain *et al.*, 2001). When several such drugs, typically three or four, are taken in combination, the approach is known as highly active antiretroviral therapy (HAART) (Dybul *et al.*, 2002). The American National institute of health and other organizations recommend that antiretroviral treatment should be offered to all patients with AIDS (Janeways *et al.*, 2005). Because of the complexity of selecting and following a regime, the severity of the side-effects and the importance of compliance to prevent viral resistance, such organizations emphasize the importance of involving patients in therapy choices, and recommend analyzing the risks and potential benefits to patients without symptoms (Dybul *et al.*, 2002).

2.5.1 Classes of antiretroviral drugs and their mode of action

There are different classes of antiretroviral drugs that act at different stages of the HIV life-cycle (WHO, 2009). Nucleotide reverse trancriptase inhibitors (NRTI) such as zidovudine, nevirapine and stavudine inhibit reverse transcription by being incorporated into the newly synthesized viral DNA and preventing its further elongation (Pierson *et al.*, 2000). Non nucleoside reverse transcriptase inhibitors (NNRTI) inhibit reverse transcriptase directly by binding to the enzyme and interfering with its function (Chrystie, 1988).

Protease inhibitors (PIs) target viral assembly by inhibiting the activity of protease, an enzyme used by HIV to cleave nascent proteins for final assembly of new virions (Chrystie, 1988). Intergrase inhibitors inhibit the enzyme intergrase, which is

responsible for intergration of viral DNA into the DNA of the infected cell (Perelson *et al.*, 1996). There are several integrase inhibitors currently under clinical trial, and raltegrivir in this class was the first to receive FDA approval in October 2007 (WHO, 2008). Other classes of ARVs include entry inhibitors (or fusion inhibitors) which interfere with binding, fusion and entry of HIV-1 to the host cell by blocking one of several targets. Maraviroc and enfovirtite are the two currently available drugs in this class. Maturation inhibitors inhibit the last step in *gag* processing in which the polyprotein is cleaved, thereby blocking the conversion of the polyprotein into mature capsid protein *p24* (Chrystie, 1988). Because these viral particles have a defective core, the virions released consist of mainly non-infectious particles. These maturation inhibitors includes bevirimat and vivecon. AntiViral HyperActivation Limiting Therapeutics or 'virostatics' (AV-HALTs) combine immune-modulating and antiviral properties to inhibit a specific antiviral target while also limiting the hyper-elevated state of immune system activation driving disease progression (Richman and, Wyatt, 1997).

The life cycle of HIV can be as short as about 1.5 days from viral entry into a cell through replication , assembly, and release of additional viruses, to infection of another cell. HIV lacks proofreading enzymes to correct errors made when it converts its RNA into DNA via reverse transcription (Saitoh *et al.*, 2005). Its short life cycle and high error rates cause the virus to mutate very rapidly resulting in high genetic variability (Richman *et al.*, 1987). Most mutations either are inferior or convey no advantage but some of them have superiority to their parents and slip past immune system defences or even ARVs activity (Saitoh *et al.*, 2005). Antiretroviral combination therapy defends against

resistance by suppressing HIV replication as much as possible (Richman and Wyatt, 1997).

2.5.2 Current ARV combinations in use

The current guidelines for adults and adolescents recommend that all patients with HIV and AIDS-defining illness like chronic coughs regardless of CD4+ T cell count, less than 200 CD4+ T cells/µl receive ART (Dainiak *et al.*, 1988). The preferred initial regimes include; nevirapine, zidovudine and stavudine, efavirenz , tenofovir and emtricitabine, boosted with Ritonavir; zidovudine and lamivudine and lopinavir boosted with ritonavir; lopinavir tenofovir and emtricitabine (WHO, 2008).

Combinations of antiretrovirals create multiple obstacles to HIV replication to keep the number of offspring low and reduce the possibility of a superior mutation. If a mutation that conveys resistance to one of the drugs arises, the other drugs continue to suppress reproduction of that mutation (Coovadia *et al.*, 2004). With rare exceptions, no individual antiretroviral drug has been demonstrated to suppress HIV infection for long; these agents must be taken in combinations in order to have a lasting effect. As a result, the standard care is to use combinations of antiretroviral drugs (WHO, 2008). Combinations usually comprise two nucleoside-analogue RTIs and one non-nucleoside-analogue RTI or protease inhibitor. The three drug combination is commonly known as a triple cocktail (WHO, 2006).

Combination of anti HIV chemotherapy has drastically reduced mortality rates and increased life expectancy of infected individuals (WHO, 2006). Prolonged viral chemotherapy may induce side effects which may be fatal

(WHO, 2006). Drugs that block HIV replication lead to rapid decrease in titers of infections virus and an increase in CD4 T-cells (Pierson *et al.*, 2000). These ARV drugs target reverse transcriptase enzyme which is required for synthesis of provirus (Cammack, 2001). Inhibitors of these enzymes prevent the establishment of further infection in the uninfected cell (Cammack, 2001). After inhibiton with such treatment, the virus produced by previously infected cells is no longer infectious and virus production is curtailed as these cells die and no new cells are infected. Reservoirs of lately infected cell remain present for many years (Janeway *et al.*, 2005).

The common combinations in use include fixed-dose combinations which are multiple antiretroviral drugs combined into a single pill and synergistic enhancers which either do not possess antiretroviral properties alone or are inadequate or impractical for monotherapy (Carbonara *et al.*, 2001). When the enhancers are taken concurrently with antiretroviral drugs they enhance the effect of one or more of the ARVs often by altering the metabolism of antiretrovirals (Carbonara *et al.*, 2001). These include ritonavir, which is an antiretroviral drug that belongs to the class of protease inhibitors with the protease inhibitor lopinavir. ritonavir is also used as an enhancer of other protease inhibitors such as saquinavir and atazanavir (Microft *et al.*, 1999).

In countries with a high rate of baseline resistance, resistance testing is recommended prior to starting treatment combination of nevirapine, zidovudine and stavudine

(Moore *et al.*, 1991). In Kenya, most of the HIV patients are under zidovudine, nevirapine and stavudine combination of ARVs which acts as reverse transcriptase inhibitors (GOK, 2006).

2.5.3 Antiretroviral drugs Toxicity and side effects

Several ARVs cause Kidney damage (nephrotoxicity). Drugs such as nevirapine and tenofovir are associated with symptoms like fatigue, frequent urination, swollen feet and increased thirst (Carr *et al.*, 2001). Creatinine has been found to be a fairly reliable indicator of kidney function because as the kidneys become impaired the creatinine levels increase and thus warn of possible malfunction or failure of the kidneys, sometimes even before a patient reports any symptoms (Greer, 1999). It is for this reason that standard blood and urine tests routinely check the amount of creatinine in the blood (Greer, 1999). Other drugs may cause Lactic acidosis which is associated with symptoms like nausea and vomiting, abdominal pain, tiredness, shortness of breath abnormal heart beat and weight loss. These include stavudine and didanosin (De Souz *et al.*, 2000). Liver damage (hepatotoxicity) due to nevirapine and ritonavir may occur in association with symptoms such as fatigue, loss of appetite, abdominal pain and Jaundice (Yellow skin and whites of the eyes, dark urine, pale stools) (Blanche *et al.*, 1999). Drugs like Stavudine, didanosine and lamivudine may cause peripheral neuropathy (nerve damage) which is associated with symptoms like numbness, pins and needles in hands or feet can become very painful and eventually disabling (Gerschenson *et al.*, 2000).

When ARVs are administered, variants of the virus carry mutations conferring resistance which may result from former levels of plasma virus regained (Roit *et al.*, 2005). Resistance hasnecessited introduction of the current treatment of combination therapy which dramatically reduces mortality and morbidity among patients with advanced HIV infection (Roderick and Herbert, 2005). However, this has been accompanied by significant side effects. These side effects may be as a result of toxicity due to use of these drugs for a long period (WHO, 2006).

There are several concerns about antiretroviral regimes. The drugs can have serious side-effects. Regimes can be complicated, requiring patients to take several pills at various times during the day. However treatment regimes have been greatly simplified in recent years. If patients fail to adhere to treatment regimes, drug resistance can develop (Uberg, 2009). In addition anti-retroviral treatment is costly and resource-intensive, which makes it difficult for majority of the world's infected individuals cannot access treatment services (WHO, 2008). If HIV infection becomes sufficiently resistant to antiretroviral-drugs, treatment becomes more complicated and prognosis may deteriorate (Coovadia, 2004). Treatment options continue to improve as additional new drugs enter clinical trials. However, the limited distribution of many such drugs denies their benefits to patients in the developing world (Carr *et al.*, 2001).

Drug holidays (or "structured treatment interruptions"), are intentional discontinuations of antiretroviral drug treatment. Studies of such interruptions attempt to increase the sensitivity of HIV to antiretroviral drugs (Blanche *et al.*, 1999). The

interruption change the selection pressure from the drug resistance towards resistance to the human immune system, thus breeding a more drug-susceptible virus. HIV spends some of its life-cycle in a state where its DNA is entirely integrated into human DNA (De Souza, 2000). Under certain conditions, drug-resistant strains of the virus can remain dormant in this state, since CD4 T-cells also are dormant when not aroused by invading organisms. The resistant strain can then reemerge when antiretroviral drugs are re-introduced (De Souza, 2000).

On rare occasions, side effects can be life threatening although once started, antiretroviral treatment must be taken every day for life. Every missed dose increases the risk that the drugs will stop working (WHO, 2008). It is therefore vital that people receiving antiretroviral treatment get all the help they need to minimise the impact of side effects. Often there are several ways to lessen the harm, either by treating the side effects or by switching to alternative antiretroviral drugs (Abrescia, 2005). Antiretroviral drugs differ in how commonly they cause particular side effects. For example, efavirenz is the drug most associated with psychiatric symptoms, while protease inhibitors are more likely to raise the levels of cholesterol and triglycerides (Uberg, 2009). This should be considered when deciding which drugs to take (Carr *et al.*, 2001). Side effects vary from person to person and it is impossible to predict exactly how each individual will be affected. Some people take antiretroviral treatment for years with few problems, while others find the same drugs intolerable. Nevertheless some characteristics and pre-existing conditions (such as high blood pressure or hepatitis infection) are known to increase the risk from certain side effects (Uberg, 2009). Some side effects appear shortly after starting an antiretroviral drug and disappear within a few weeks as the body gets used to the new chemicals. This is

often the case with nausea, diarrhoea and headache (Abrescia, 2005). Unfortunately other side effects such as peripheral neuropathy (nerve damage) and lipodystrophy (fat redistribution) tend to worsen over time and may never go away. Also some problems may not emerge until months or even years after treatment is started. Patients should also know how to spot the warning signs of more serious side effects that may require immediate intervention. Other possible causes include opportunistic infections, stress, diet, and non-HIV drugs (Cheesborough, 2005).

Older people living with HIV may experience signs of ageing that could resemble certain side effects. For example, when people get older they might be more susceptible to increased fat in the abdomen, which could look similar to the changes that are caused by lipodystrophy (Blanche *et al.*, 1999). Switching of drugs is often an effective way to reduce or eliminate a side effect when all other approaches have failed. If the viral load is undetectable then it is usually possible to switch only one drug without affecting treatment effectiveness or future treatment options. Otherwise, the entire combination may have to be changed. Switching drugs is not without risks. As already mentioned, it can be difficult to identify the cause of a particular set of symptoms, and it may turn out that the rejected drug or drugs weren't to blame after all. There is also a chance that the new medication may cause even worse side effects, perhaps forcing another switch. Changing drugs repeatedly will narrow future treatment options (Abrescia *et al.*, 2005).

Kidneys may be impaired by use of certain drugs like ARVs which may interfere with ultrafiltration in the glomeruli of the nephron (Greer, 1999). Malfunction of the kidney reduces filtration rates of metabolic wastes, whose retention and circulation

results in serious side effects. Creatinine levels in blood increase with malfunctional kidneys (Greer, 1999).

2.6 Monitoring HIV chemotherapy

Antiretroviral drugs (ARVs) are toxic to the bone marrow and blood-cell system and can cause anaemia (decrease of red blood cells) and leukopenia (decrease of white blood cells (UNAIDS, 2009). Certain ARVs block DNA synthesis in the cells of the body. However, these drugs cannot discriminate between the replicating virus and the rapidly dividing cells, such as those of the bone marrow that are essential to keep the human body alive and in optimum health. The organ system with the highest cell proliferation is the bone marrow, the production site of red and white blood cells. An impaired production/function of red blood cells leads to anaemia whereas an impaired production and function of white blood cells leads to an impairment of the immune system (drug-induced immune deficiency). The toxicity of ARVs on the bone marrow and blood-cell system and the known side effects of anaemia (loss of red blood cells) or leukopenia (loss of white blood cells) is known to the manufacturers of ARV drugs and is even part of the mandatory ARV product information (Richman and Wyatt, 1997). Although the doses of nucleoside analogs have been lowered in the triple drug combination regimes such as Nevirapine, Stavudine and Zidovudine, their toxicity has been reported resulting in thrombocytopenia and anaemia (Carbonara *et al.*, 2001).

Additionally, nucleoside analogs such as Nevirapine, Stavudine and Zidovudine drug regimes are hepatotoxic (Abrescia *et al.*, 2005). Further, nucleoside analogs have been linked to mitochondrial toxicity (Fleischer *et al.*, 2004), lipodystrophy

syndrome, characterised by dyslipidemia, such as a severe impairment of the metabolism and storage of fats in the body (Carr *et al.*, 2001). Many ARVs also have other adverse side-effects. There are a number of metabolic complications associated with the use of some ARVs, including derangement in glucose and lipid metabolism, bone metabolism and lactic acidaemia (sustained accumulation of lactic acid in the blood), which have been documented in industrialised countries (Blanche *et al.*, 1999). Antiretrovirals used in this study were Nevirapine, Stavudine and Zidovudine nucleoside analogs. These are reverse transcriptase inhibitors which target the construction of viral DNA by inhibiting activity of reverse transcriptase enzyme. They are incorporated in the viral DNA leading to chain termination (NASCOP, 2008). Patients had varied responses to ARVs which were attributed to their varied level of baseline CD4 counts and level of ARV chemotherapy. Levels of toxicity were measured by determination of creatinine levels.

2.6.1 Antiretroviral drugs and creatinine

Creatinine levels may vary from one individual to another and from region to region but in health creatinine ranges approximately from 0.6-1.2 mg /dl of blood in adults (UNAIDS, 2009). When ARVs are used for long, drug resistance and toxicity may occur which may change the course of the immune system. Toxicity can be detected by establishing creatinine as a measure of kidney function (Greer, 1999). Creatinine is a metabolic waste that is generated from muscle metabolism. Creatinine is produced from creatine, a molecule of major importance for energy production in muscles. Creatinine is transported through the blood stream to the kidneys. The kidneys filter out most of the creatinine and dispose it off in the

urine (Erich *et al,* 1991). Creatinine has been found to be a fairly reliable indicator of kidney function (Greer, 1999). As the kidneys become impaired the blood creatinine levels increase and thus warn of possible malfunction or failure of the kidneys, sometimes even before a patient reports any symptoms. It is for this reason that standard blood and urine tests routinely check the amount of creatinine in the blood (Greer, 1999).

2.6.2 Antiretroviral drugs and CD3/CD4 counts

Initial ARV administration elevates CD4 cell counts because cell to cell transmission is controlled (Roit *et al.,* 2005). The normal range of CD4 in health is 1000 – 1200 cell/µl of blood (Cheesborough, 2005). CD4 level below 200 cells/ µl of blood indicate an AIDs condition. Where patients are put on ARVs, CD4 cells tend to rise rapidly, but when ARV drugs are used for long CD4 production may altered due to associated toxicity. CD4 counts can be detected in a Fluorescence Activated Cell sorter (FACs) machine by flow cytometry (Roit *et al.,* 2005).

2.6.3 Antiretroviral drugs and mean corpuscular volumes (MCV)

Mean corpuscular volume provides information on red cell size and is measured in femtolitres (fl). The normal range of MCV is 80 – 100 fl, MCV above 100fl indicates macrocytic anaemia particulary of iron deficiency or anaemia of chronic disease (Cheesborough *et al.,* 2005) while newborns have

low MCV of 70-85(fl) (Dacie *et al.*, 2000). Prolonged use of ARVs may lead to altered red cell size (Pierson *et al.*, 2000). The mean corpuscular volume (MCV), is a measure of the average red cell volume volume that is reported as part of a standard complete blood counts in patients with anaemia.

It is the MCV measurement that allows classification as either a microcytic anaemia (MCV below normal range), normocytic anaemia (MCV within normal range) or macrocytic anaemia (MCV above normal range). In presence of haemolytic anaemia_, presence of reticulocytes can increase MCV. In pernicious anaemia (macrocytic), MCV can range up to 150 fl. Vitamin B12 and folic acid deficiency has also been associated with macrocytic anaemia (high MCV numbers). The most common causes of microcytic anaemia are iron deficiency and MCV can be as low as 60 to 70 femtolitres (Tarnesen *et al.*, 1996). Most drugs like ARVs may elevate MCV levels (Dacie and Lewis, 2000).

2.6.4 Antiretroviral drugs and platelets

Patients on treatment with cytotoxic drugs like ARV's or are infected with HIV and AIDs, may suffer thrombocytopenia (Cheeseborough *et al.*, 2005). In health, there are about 150-450 $x10^9$ platelets per liter of blood. Capillary platelets are lower than venous blood platelets (Cheesborough *et al.*, 2005). Associated ARV drugs toxicity may cause platelets destruction (Smith *et al.*, 2006).

2.6.5 Antiretroviral drugs and haemoglobin

Haemoglobin levels are used to detect anaemia and to monitor response to treatment (Dacie *et al.*, 2000). Normal Hb levels vary according to age, gender and altitude at which a person lives (Dacie *et al.*, 2000). Haemoglobin levels in health range is 11-18mg/l of blood Cheesborough, 2005). Anaemia may develop due to reduced red cell production or premature red cell destruction which could be caused by ARV drug associated toxicity (Lipsky *et al.*, 1996).

CHAPTER THREE: METHODOLOGY

3.1 Study Area

The study was carried out at Runyenjes Sub- District Hospital, Embu. The Hospital is along Embu-Meru Road 40 km from Embu Town (Appendix i). Runyenjes Town is the Divisional Headquarters of Embu district mostly populated by Aembu with traces of other tribes. It's a transit town with many travelers from Nairobi to Meru. Embu district has an approximate population of 150,000 people and 6 thousand (4%) of these are infected with HIV (GOK, 2008).

3.2 Study Design

This was a longitudinal study covering HIV patients attending Runyenjes CCC during the month of may to December 2009. A representative sample of those who enrolled was randomly picked from the register forming the experimental group. The control group was a representative sample of HIV patients registered for opportunistic infections management. The baseline values of CD4, CD3, MCV platelets, Hb and creatinine were established. Baseline CD4 counts were used to group the patients into categories. Monitoring of creatinine, CD3, CD4, platelets, MCV and Hb was carried out monthly upto 6 months. Patients were put on first line combination ARV therapy (Nevirapine, Zidovudine and Stavudine) and monitored monthly. A control group of 40 HIV patients on contrimoxale (septrin), an antibiotic

commonly used to control opportunistic infections was also monitored for creatinine, CD3, CD4, platelets, MCV and Hb monthly upto six months. This was to exclude the compounding effects of accompanying antibiotics. Patients on ARVs (experimental group) were HIV patients with less than 200 CD4 cells/µl of blood at baseline while those on septrin (control group) were HIV patients with CD4 counts higher than 200 CD4 cells/µl of blood at baseline as recommended (WHO, 2006).

3.3 Sample size determination

This sample size was estimated using the formula used by Fishers *et al.* (1998) on an estimated population of 100 patients currently on ARVs in the Hospital.

$$n = \frac{Z^2pqD}{d^2}$$

z=1.96 at 95% confidence interval.

P=proportion of target population = 10% (0.1)

q=1-p

d=0.05

D= (design effect)=1

$$n = \frac{1.96^2 \times 0.1 \times 0.9 \times 1}{0.05^2}$$

$$=144$$

Since the population was less than 10, 000, then Fishers formular (1998) was used

to adjust minimum sample size.

$$n_f = \frac{n}{1 + n/N}$$

$$n_f = \frac{144}{1 + 144/100}$$

$$= 59$$

A sample size of 69 patients was used. The extra 10 patients were to cater for losses due to natural attrition and drug defaulters. Usually HIV patients on ARVs are given antibiotics and HIV patients with CD4 counts greater than 200 cells /µl of blood are not given ARVs but antibiotics alone. Therefore a control sample of 40 HIV patients on septrin, to control opportunistic infections was used to exclude the compounding effects of accompanying antibiotics toxicity.

3.4 Inclusion/exclusion criteria

Only HIV patients attending Runyenjes CCC who consented for blood sample analysis were considered for the study. Ethical clearance was sort from the Ministry of Medical services (Appendix ii).

3.5 Sample collection and laboratory analysis

3.5.1 Sample collection

Four milliliters of venous blood was removed from patients at every visit by a qualified clinician. The sample was divided into two aliquots of which one was put into a tube containing Ethylene diamine tetra acetic acid (EDTA) anticoagulant and the other into a tube without anticoagulant. This was used for serum extraction.

3.5.2 CD4 and CD3 enumeration

The sample with EDTA was used for CD4 and CD3 counts in a Fluorescent Activated cell sorter (FACs, Anaspec model) scanner using ant CD3 and ant CD4 cross-reactive monoclonal antibodies coated with magnetic beads from USA. A sample of 20 µl of blood was mixed with coated monoclonal antibodies in a FACs machine. The results were read on a computer connected to the FACs machine as described by Cheesborough (2005)

3.5.3 Measurement of MCV

Mean corpuscular volume was measured by a blood cell analyzer by Beckam Coulter and Symex which counts cells by impendence (Cheesborough, 2005). A volume of 10µl of blood sample mixed with 100µl of buffered electrolyte was passed through an aperture tube between two electrodes. The counts were expressed in femtolitres as described earlier (WHO, 2006).

3.5.4 Determination of haemoglobin levels

Haemoglobin was measured using Haemoglobincyanide (HiCN) technique where whole blood was diluted in 1: 20 in a modified Drawkins solution containing potassium ferricyanide and potassium cyanide (Scott and Lewis, 1995). Absorbance of HiCN was read in a spectrophotometer at wavelength 540 nanometers. The absorbance obtained was compared reference standard solution. Haemoglobin values were directly read out on the haemoglobinometer from the digital display (Scott and Lewis, 1995).

3.5.5 Platelet counts

Platelet count was determined by use of improved Neubauer ruled counting chamber. Blood sample was diluted in 1: 20 in a filtered solution of ammonium oxalate reagent which lyses the red cells. The platelets were then computed and expressed in cells per liter of blood (Pettit, 2000).

3.5.6 Determination of creatinine levels

Serum was used for creatinine determination in a serum analyzer. This assay is based on the reaction of creatinine with sodium picrate as described by Jeff (2008) (Labman Diagnostic UK Ltd). Picric acid (17.5 mol/l) was mixed with sodium hydroxide (0.29mol/l) and 2mls of serum loaded in the serum analyzer. The amount of creatinine was then read from the computer of the

serum analyser and expressed in milligrammes per decilitre of blood (Jeff, 2008).

3.6 Data analysis

Creatinine levels and immunological parameters were compared against the base line levels for each patient. These were grouped into four categories based on baseline CD4 levels of 1-50, 51-100, 101-150, and 151-200 (per ul of blood). Baseline CD4 counts were used to group the patients into categories so as to establish the effects of ARVs and associated toxicity when initiated at varying levels of CD4 in HIV patients. The data was plotted to show the relationship of creatinine levels following ARV administration, and relationship of creatinine with CD3, CD4, Hb, platelets and MCV following ARV therapy.

The relationship between ARVs association in toxicity and immune profiles was established by use of correlation coefficient, differences between baseline values of creatinine and immune profiles and values at 6 months of ARV use was analysed using analysis of variance. Data from patients on first line combination ARV therapy (experimental group) and those on septrin (control group) was compared by t- test using paired comparison and differences between months was analysed using post ANOVA (Student Newman-Keul's test). $p<0.05$ was considered significant.

CHAPTER FOUR: RESULTS

4.1 Results overview

The aim of this study was to determine the toxicity of first line ARV drugs (zidovudine, nevirapine and stavudine regime) on HIV patients attending Runyenjes comprehensive care centre. Patient ages ranged between 12-63 years. In this study sampled patients were counseled on how they could improve their immune status by getting medication, how use of ARVs and adherence to the prescribed regime could improve quality of life and prolong their lifespan. Baseline creatinine and immune status was determined and then patients were categorized on basis of baseline CD4 levels. Baseline CD4 counts were used to group the patients into categories so as to establish the effects of ARVs and associated toxicity when iniatiated at varying baseline CD4 levels. The process involved determination of creainine and corresponding immune status based on CD4, CD3, haemoglobin, platelets, and mean corpuscular volumes at baseline and various levels of ARV use for 6 months (Appendix iii). These results were then compared with results of HIV patient under opportunistic infections management with contrimoxale (septrin) antibiotics (Appendix iv).

There was significant difference between creatinine and immune profiles values for patients under ARVs treatment when baseline and values at 6 months was compared, with values at 6 months showing a clear elevation above baseline values (Table 4.1a: $p=0.01$, $F=22.73$, $df= 5$).

Table 4.1a: Mean creatinine and immune profiles of HIV patients at baseline and 6 months of ARV treatment

Profiles	Creatinine	CD4	Hb	Platelets	MCV
Baseline	1.03	101	11	300	88
6 months	1.37	278	13	506	104

Creatinine (mg/dl of blood), CD4 and CD3 (cells/µl of blood), Hb= Haemoglobin (mg/dl of blood), platelets (cellsx10^{9}/l of blood), MCV=Mean corpurscular volume (femtolitres)

In contrast, the control group, showed no significant difference between mean creatinine and immune profiles at baseline compared with mean creatinine and immune profiles at 6 months of contrimoxale use (Table 4.1b: p=0.1, F=0.009, df= 5).

Table 4.1b: Mean creatinine and immune status of HIV patients at baseline and 6 months of contrimoxale use

Profiles	creatinine	CD4	CD3	Hb	Platelets	MCV
Baseline	0.91	250	1600	9.0	150	88
6 months	0.99	209	1237	12	235	84

The results also showed significant differences in creatinine and immune profiles between patients under ARVs treatment and those under septrin, with patients ARVs showing elevated creatinine and immune profiles (Table 4.1c: p= 0.01, t=2.43).

Table 4.1c: Mean creatinine and immune profiles of HIV patients on ARVs and HIV patients on contrimoxale (septrin) at 6 months of treatment

Profiles	Creatinine	CD4	Hb	Platelets	MCV
Patients on ARVs	1.37	278	13	506	104
Patients on septrin	0.99	209	12	235	84

Creatinine (mg/dl of blood), CD4 and CD3 (cells/µl of blood), Hb= Haemoglobin (mg/dl of blood), platelets (cellsx10^{9}/l of blood), MCV=Mean corpurscular volume (femtolitres)

The corresponding creatinine and immune status for each category was determined for 6 months of ARV use (Table 4.2).

Table 4.2: Patients categories based on baseline CD4 levels and their corresponding mean creatinine and immune profiles 6 months of ARV use

CD4 count (category)	No. of patients(%)	creatinine	CD4	CD3	Hb	platelets	MCV
<50	20 (33.3%)	1.18	205	1544	12.1	404.9	100.2
50 -100	9 (15%)	1.25	196	1461	11.5	488	107.7
101-150	8 (13.3%)	1.08	405	2054	12.4	389	98.5
>150	23 (38.4%)	1.21	319	1738	12.3	457	100.9

4.2 Relationship between ARV administration and CD4 counts

In this study, the CD4 levels were determined at baseline and monthly for 6 months following ARV use in various categories of patients. The mean CD4 increased from 101 cells/µl of blood at baseline to a mean of 358 cells/µl of blood in the third month of ARV use then decreased to a mean of 278 cells/µl of blood after 6 months following ARV use.

The CD4 counts increased to a peak of 358 cells/µl of blood in the third month then declined. There were significant differences of CD4 counts between the months of ARV use. CD4 counts at baseline were significantly different with CD4 counts at the third month of ARV use ($p<0.05$, $q=20.9$) , and CD4 counts at third month were significantly different with CD4 counts at sixth month of ARV use (Figure 4.1: $p<0.05$, $q=6.71$). The control group results showed no significant changes in CD4 counts following 6 months of contrimoxale (septrin) use ($p=0.1$, $F=0.003$, $df=5$). After contrimoxale treatment mean CD4 counts decreased from 250 at baseline to 209 cells/µl of blood at 6 months of contrimoxale use.

Among the various categories, patients with baseline CD4 counts of 100-150 cells /µl of blood showed a better and highly significant response with a mean of 405 cells /µl of blood at the 6 months follow up than all other categories. This showed that, use of ARVs resulted in elevated levels of mean CD4 counts especially for patients who started treatment when CD4 counts were 100-150 cells/µl of blood (Figure 4.2; $p=0.01$, $F=15.2$, $df=3$).

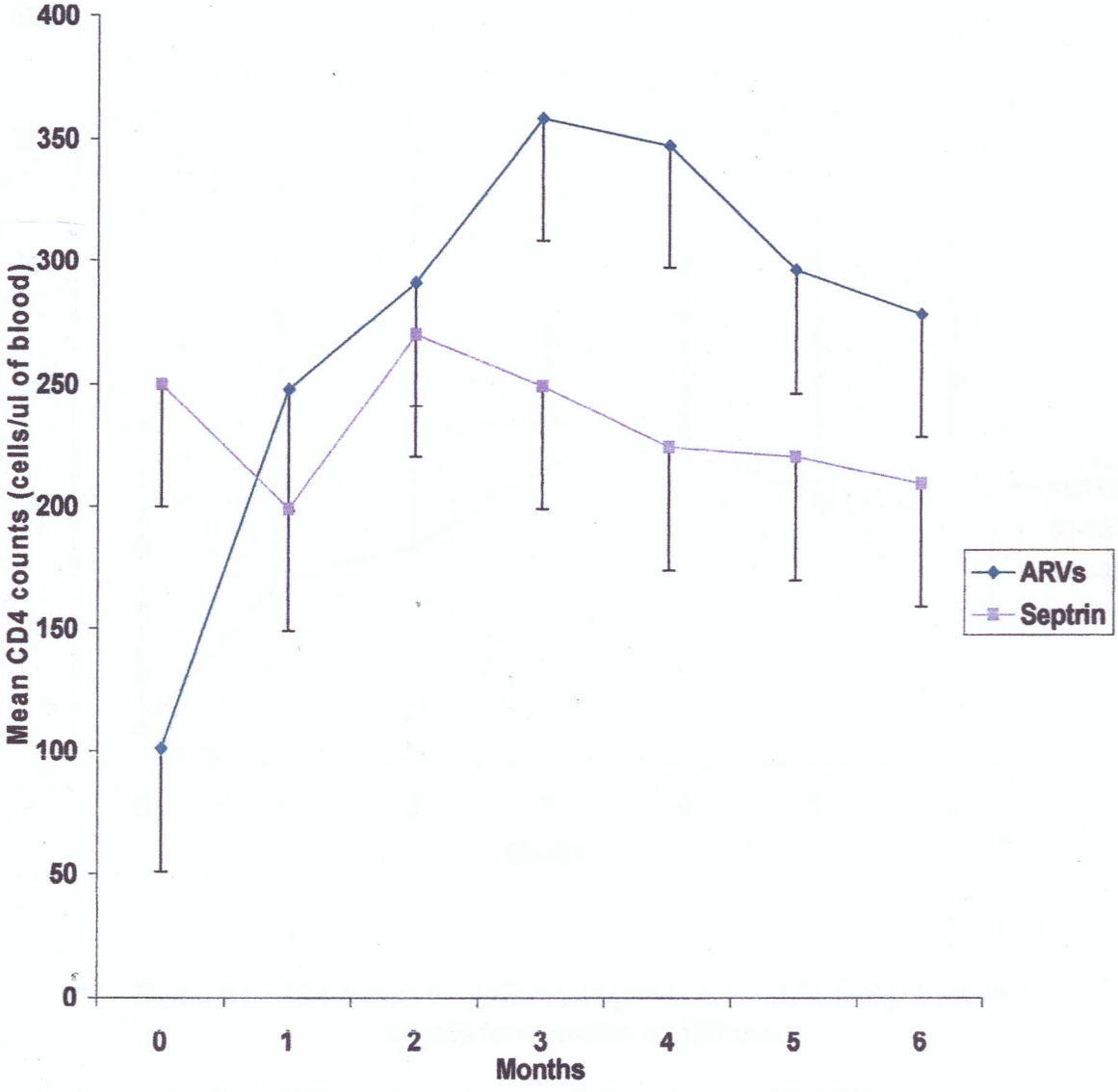


Figure 4.1: Mean CD4 counts and sequential counts 6 months following ARV and septrin use respectively

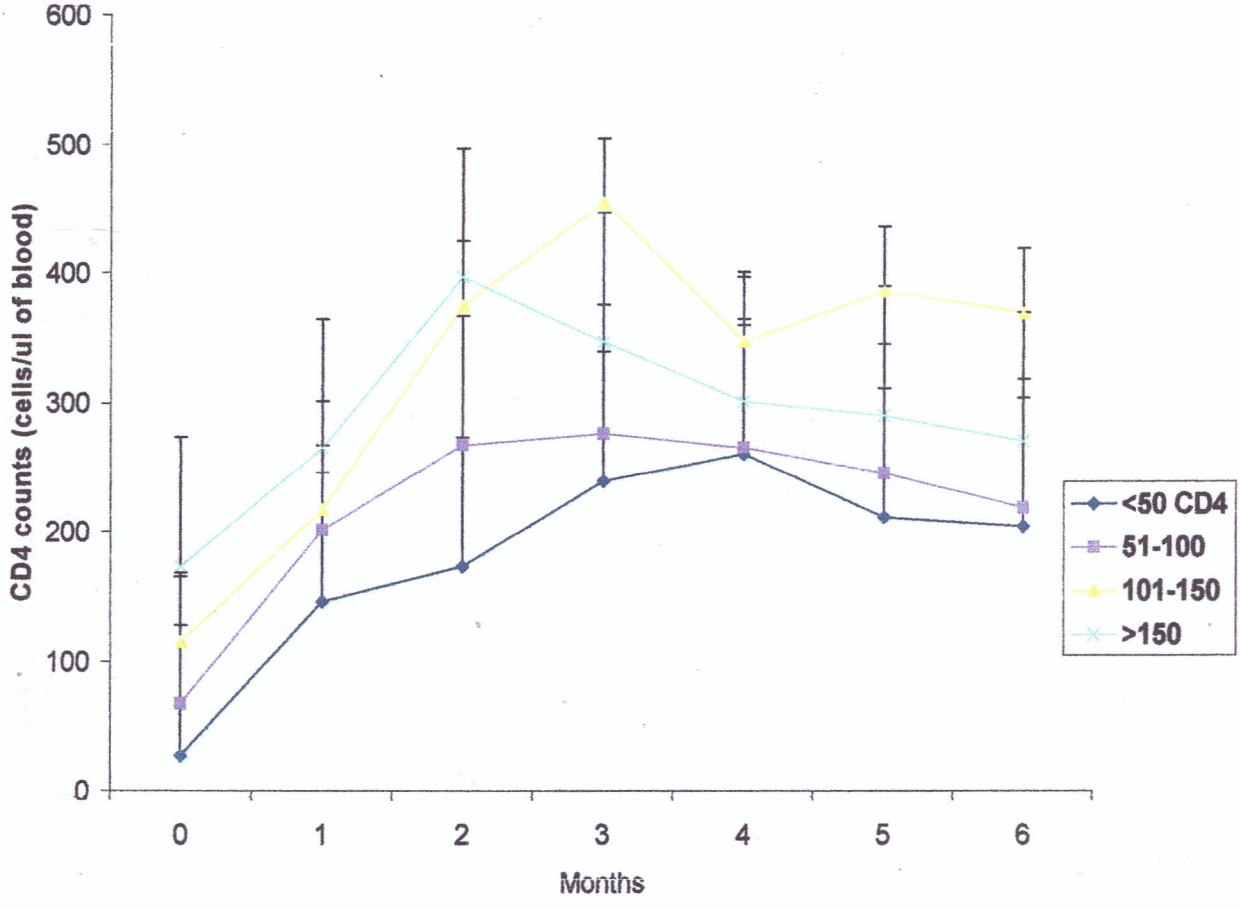


Figure 4.2: CD4 counts for patient categories at baseline and subsequent counts for 6 months of ARV use

4.3 Relationship between ARV administration and CD3 Counts

The CD3 level was determined at baseline and sequentially for 6 months following ARV use in different patient categories based on CD4 baseline counts. The results

revealed that mean CD3 increased from 1306 cell/µl of blood at baseline to 1778 cells/µl of blood following 6 months of ARV use. This showed that use of ARVs for a long time resulted in elevated levels of CD3 counts to a certain level which may then stabilize (Figure 4.3).

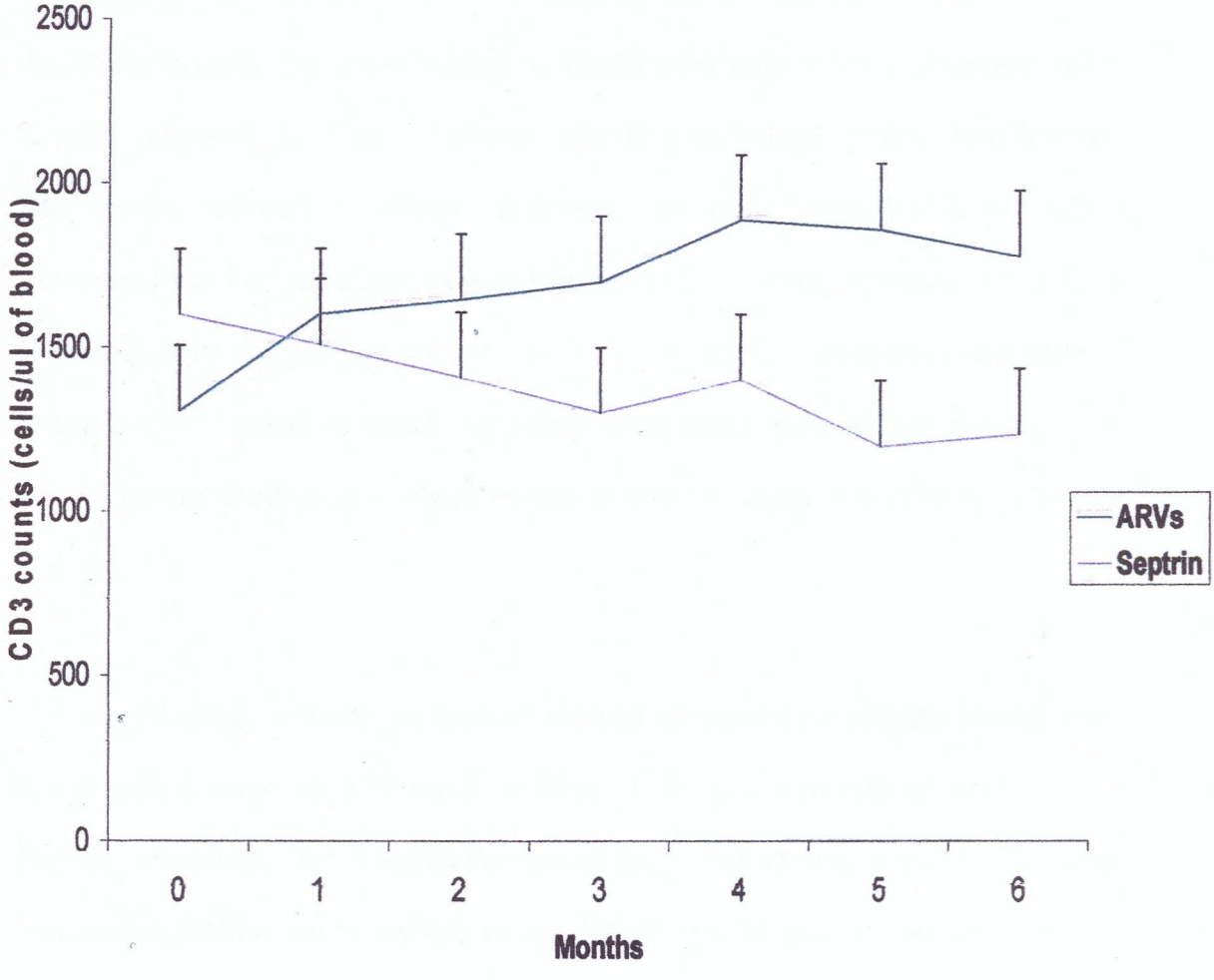


Figure 4.3: Mean baseline CD3 and sequential counts for 6 months during ARVand septrin use respectively

Patient category with baseline CD4 of 101-150 cells/µl of blood showed the best CD3 response (Table 4.3). The control group results showed no significant changes in CD3 counts following 6 months of contrimoxale (septrin) use (p=0.1, F=0.12, df=5). After treatment with contrimoxale mean CD3 counts decreased from 1600 cells/µl of blood at baseline to 1237 cells/µl of blood at 6 months. This showed that contrimoxale use may not improve CD3 counts (Figure 4.3).

4.4 Relationship between ARV administration and creatinine levels

To assess toxicity, the mean creatinine levels were determined at baseline and 6 months following ARV use in various categories of patients to determine toxicity. The results revealed significant differences in mean creatinine levels which increased from 1.03 mg/dl of blood at baseline to 1.37 cells/ul of blood at 6 months following ARV use. (Figure 4.4; p=0.01, F=22.73, df=5). The normal creatinine in health is 0.6-1.2 mg/dl of blood and therefore the results showed that prolonged use of ARVs leads to elevated levels of creatinine which is indicative of development of toxicity.

The control group (patients on septrin) showed no significant changes in creatinine levels with a mean of 0.99 mg/dl of blood following 6 months of contrimoxale (septrin) treatment. This is within the normal range of creatinine in health. Creatinine levels changed from mean baseline values of 0.91 to 0.99mg/dl of blood at 6 months of contrimoxale treatment. However these changes were not significant (p=0.1, F=0.004, df=5).

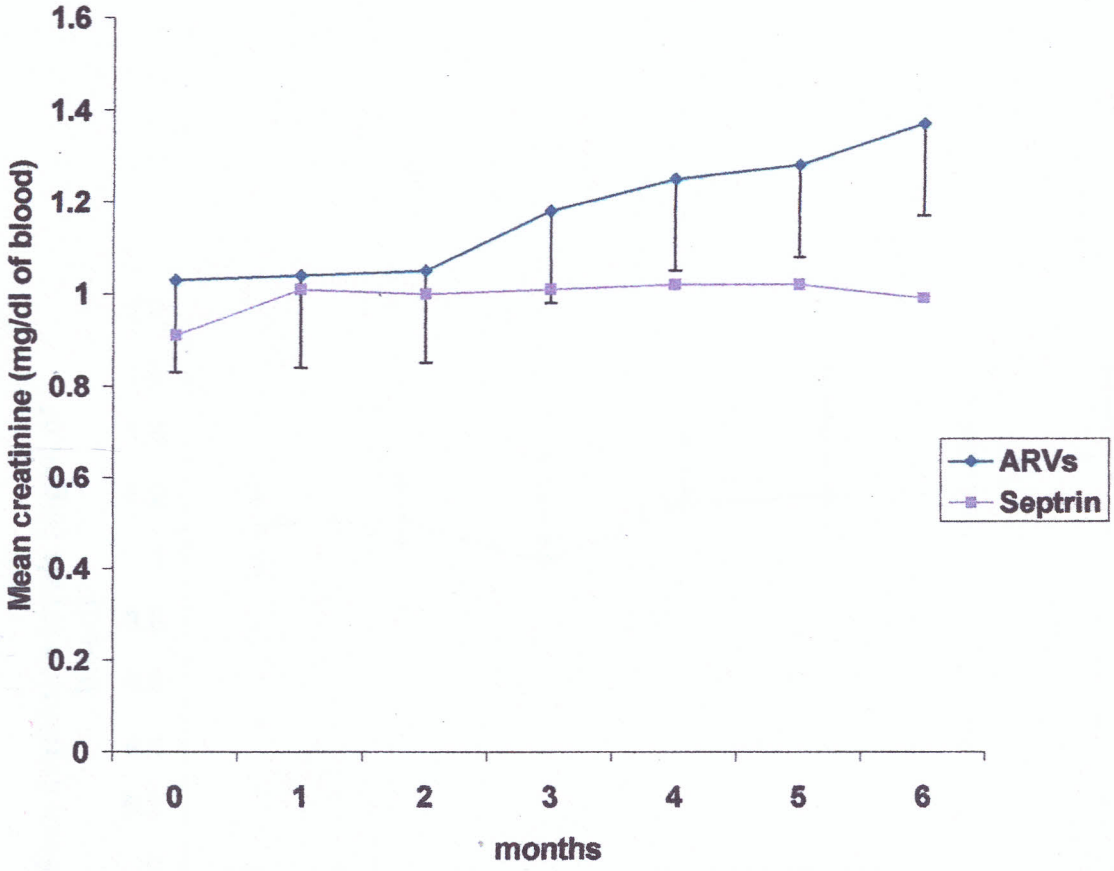


Figure 4.4: Mean creatinine values and sequential values 6 months of ARV and septrin use respectively

Among the patient categories there were significant differences in creatinine levels. Patients with a baseline CD4 counts of 101-150 cells/µl of blood had a better response to ARVs treatment with a mean creatinine of 1.08 mg/dl of blood compared to other categories. Patients with baseline CD4 counts of 50-100 cells /ul of blood had mean creatinine of 1.25 mg/dl of blood which was significant when compared to baseline levels (Figure 4.5; p=0.01, F=5.66, df=3). This category of patients had least CD4 response.

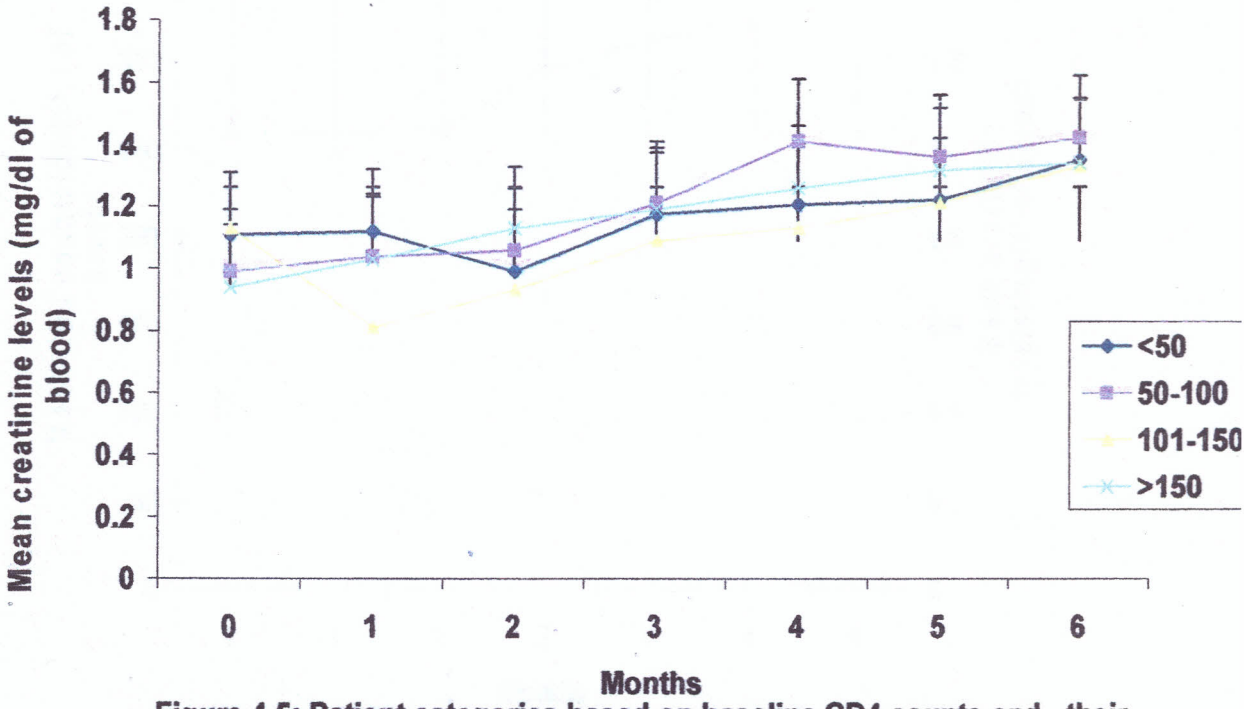


Figure 4.5: Patient categories based on baseline CD4 counts and their creatinine levels at baseline and subsequent levels 6 months during ARV use

4.4.1 Relationship between creatinine and CD4 counts

The mean CD4 counts were compared with mean creatinine for all the patients from baseline to 6 months of follow up during ARV administration. Creatinine was found to be positively correlated with CD4 counts upto three months of ARV treatment (Figure 4.6; $p<0.01$; $r=0.178$).

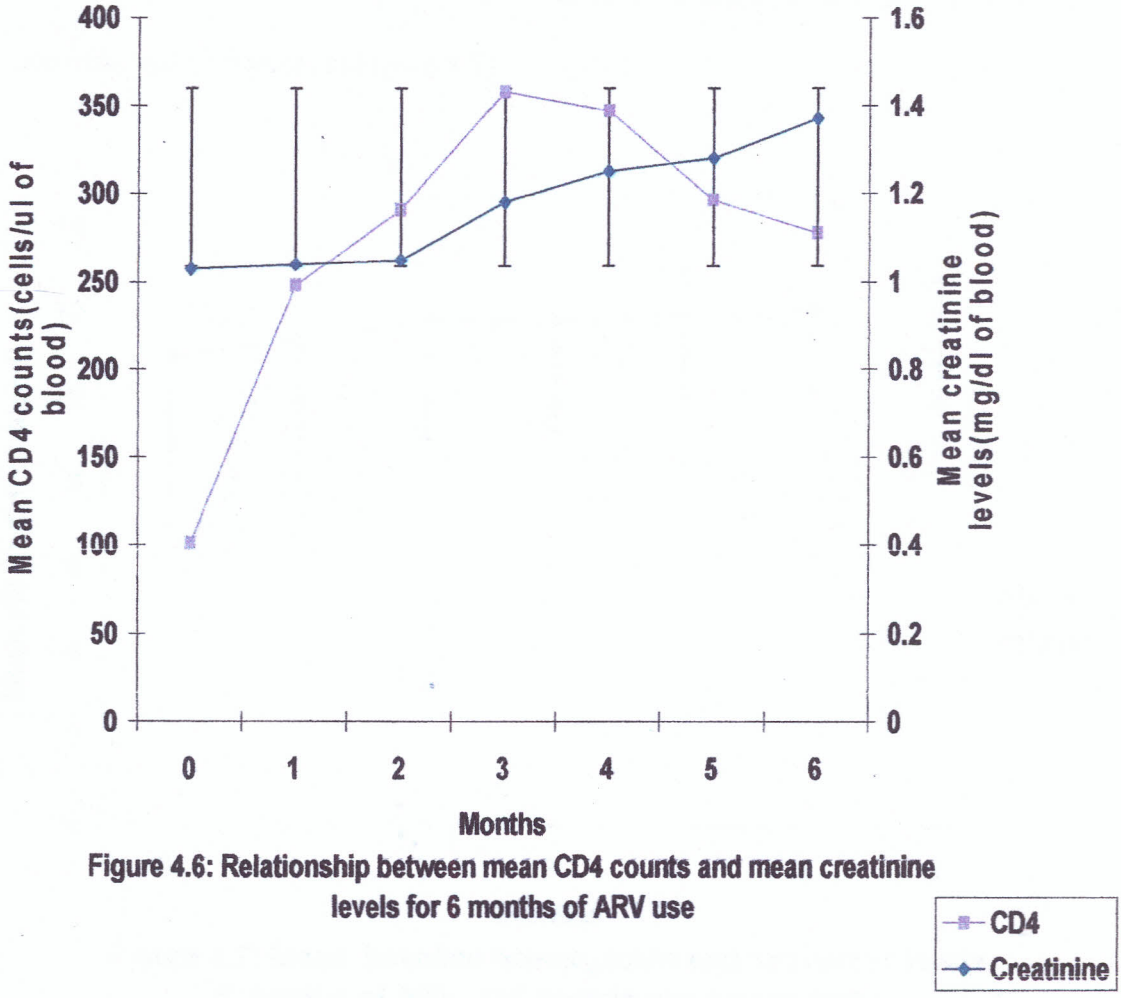


Figure 4.6: Relationship between mean CD4 counts and mean creatinine levels for 6 months of ARV use

4.5 Relationship between ARV administration and haemoglobin levels

The haemoglobin (Hb) levels were determined at baseline and sequentially for 6 months following ARV use and in various categories of patients to determine the effect of ARVs on Hb. The results revealed mean Hb increased from 11mg/dl of blood at baseline to 13 mg/dl of blood following 6 months of ARV use. Using ARVs increased Hb to higher levels but within normal range. Hence Hb had no significant differences during ARV use ($p=0.1$, $F=0.09$, $df=5$). The control

group results showed no significant changes (p=0.5, F=0.007, df=5) in Hb levels with a mean of 12mg/dl of blood at 6 months of (septrin) treatment. The mean Hb level increased from 9.0 at baseline to 12 mg/dl of blood at six months of contrimoxale treatment (Figure 4.7).

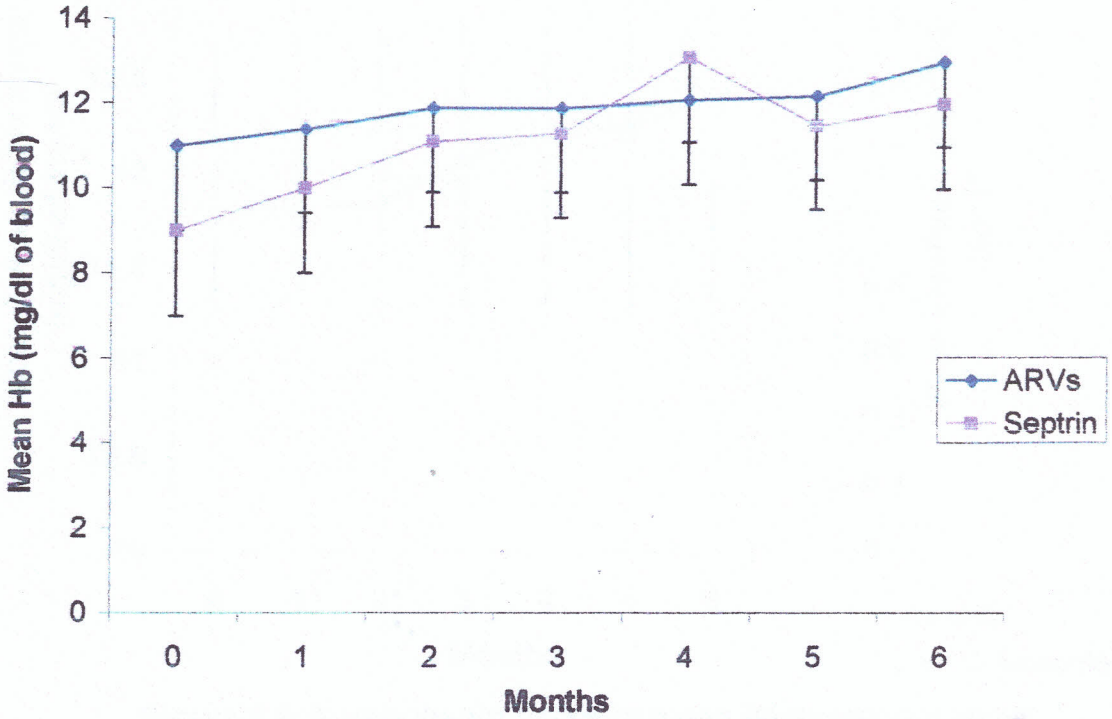


Figure 4.7: Mean baseline haemoglobin and sequential levels 6 months of ARV and septrin use respectively

4.5.1 Relationship between creatinine and haemoglobin levels

The mean Hb levels were compared with mean creatinine of all the patients from baseline to all the levels of ARV administration. Creatinine was found to be weakly correlated with haemoglobin (Figure 4.8; $p<0.01$; $r=0.0061$).

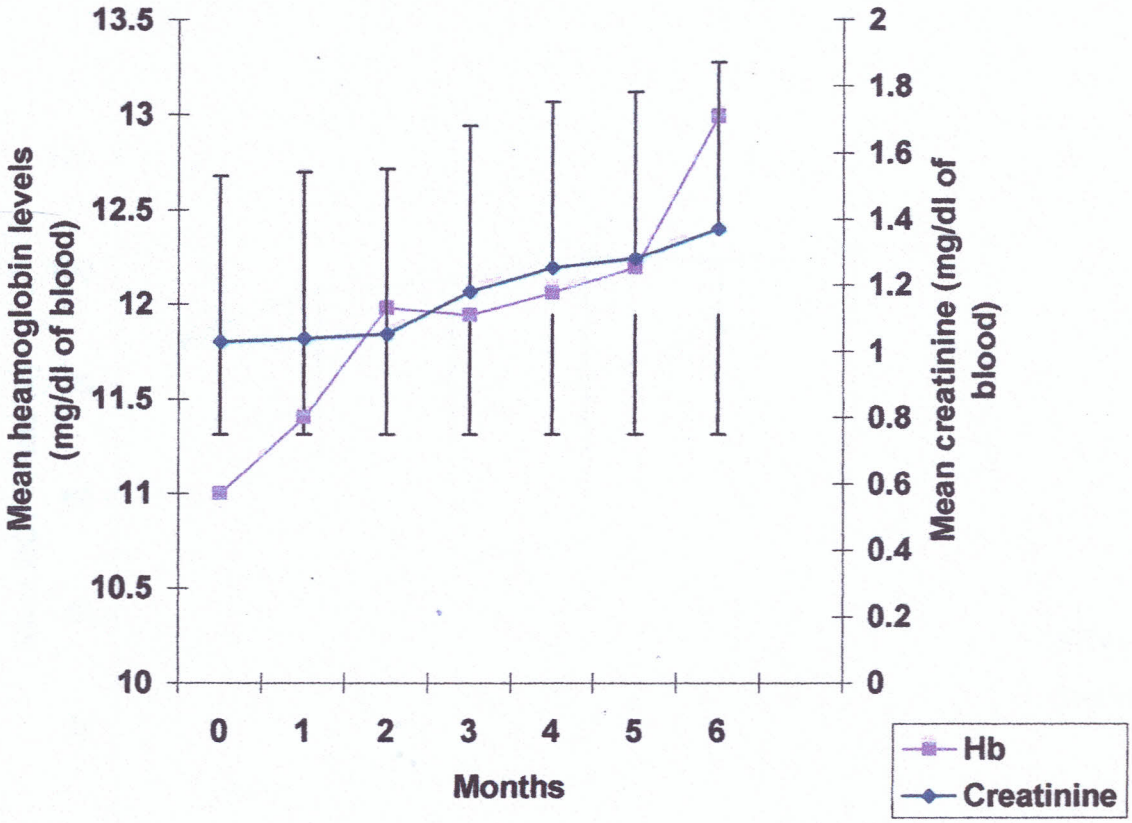


Figure 4.8: Relationship between mean heamoglobin levels and mean creatinine values 6 months of ARV use

4.6 **Relationship between ARV administration and MCV Levels**

The MCV levels were determined at baseline and 6 months following ARV use in various categories of patients. The mean MCV level increased signinficantly from 88 to 104fl at six months of treatment (p=0.01, F=5.10, df=5). This showed that use of ARVs for a long time may result in elevated levels of MCV levels. The control

group showed no significant changes (p=0.01, F=0.002, df=5) in MCV levels following contrimoxale (septrin) use for 6 months. The mean MCV levels changed from baseline mean of 88fl to 84 fl at 6 months (Figure 4.9).

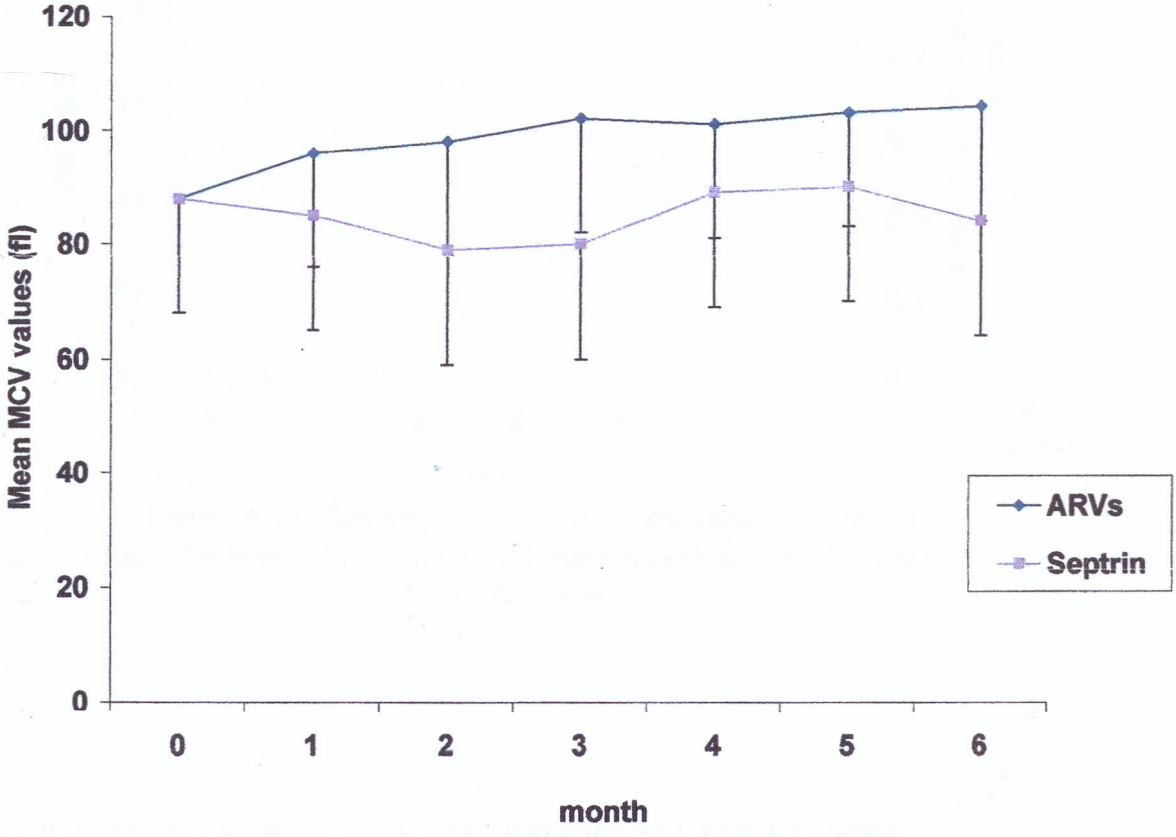


Figure 4.9: Mean MCV baseline values and sequential values 6 months of ARV and septrin use respectively

4.6.1 Relationship between creatinine and MCV Levels

The mean MCV levels were compared with mean creatinine for all the patients from baseline to 6 months of ARV administration. Creatinine was found to be positively correlated with MCV levels (Figure 4.10; $p<0.01$; $r=0.149$).

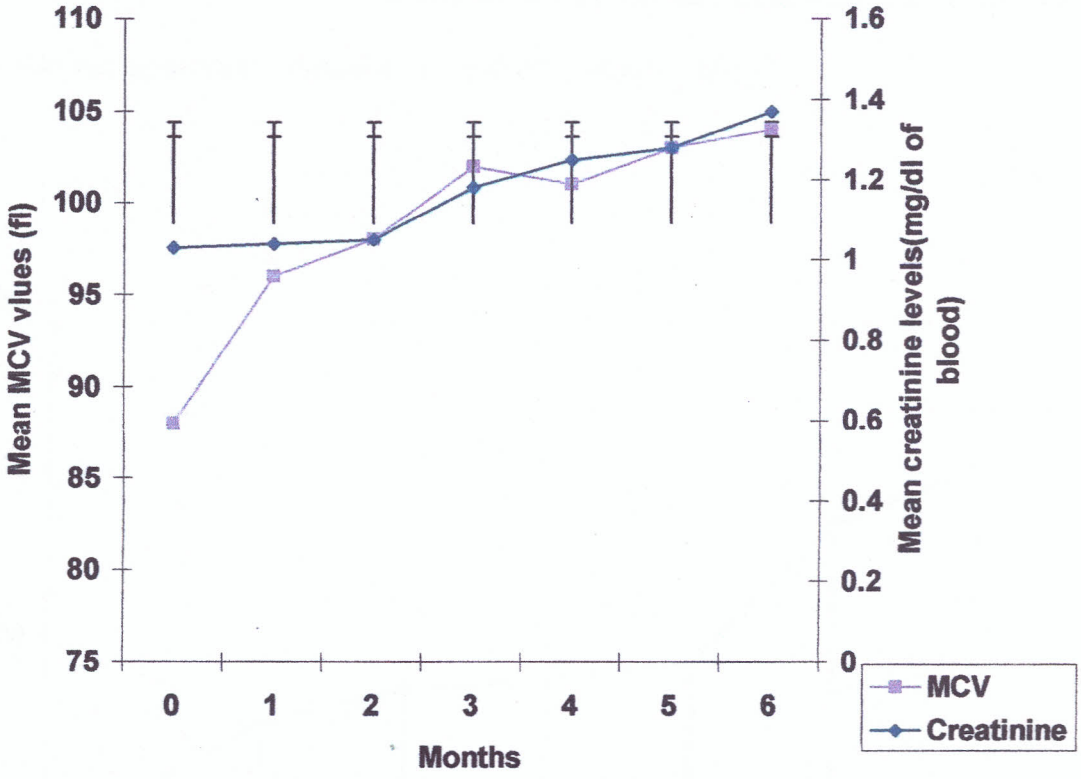


Figure 4.10: Relationship between mean MCV and mean creatinine levels at baseline and subsequent levels for 6 months of ARV use

4.7 Relationship between ARV administration and platelet counts

The platelet levels were determined form baseline to 6 months following ARV use in the various HIV categories. The results revealed significant differences ($p=0.01$, $F=9.34$, $df=5$) with mean platelets increasing from 300 x 10^9 cells/l of blood at baseline to a mean of 506 x10^9 cells/l of blood 6 months following ARV use (Figure 4.12). This showed that use of ARVs may result to elevated levels of platelets counts. The control group results showed no significant rise in platelets counts levels

with a mean of 150 x10^9 cells /l of blood at baseline that changed to 235 x10^9 cells /l of blood following 6 months of contrimoxale treatment. However, this change was not significant (Figure 4.11 : p=0.07, F=0.006, df=5).

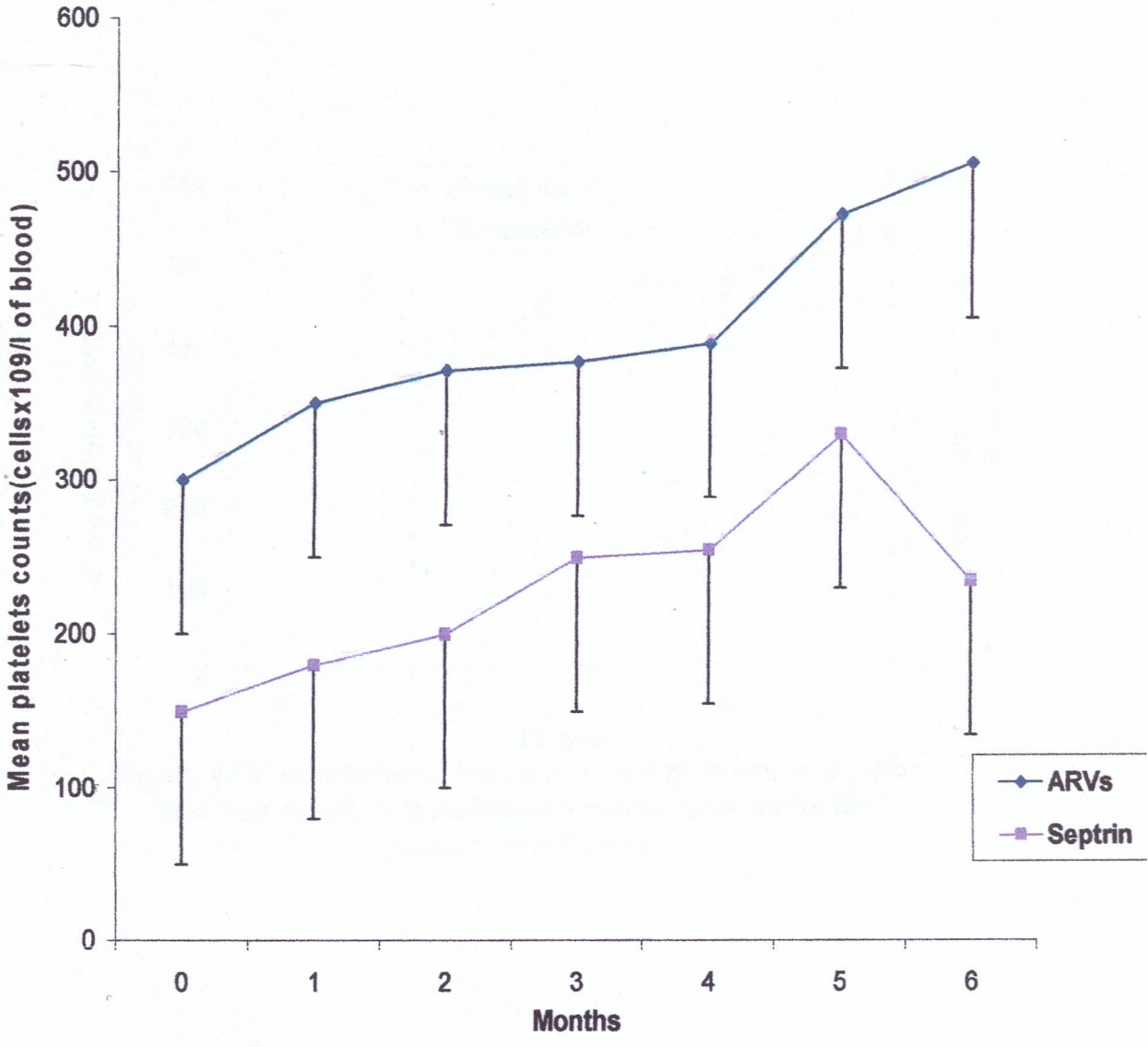


Figure 4.11: Mean platelets counts and sequential counts 6 months of ARV and septrin use

4.7.1 Relationship between creatinine and platelet levels

The mean platelet counts were compared with mean creatinine of all the patients from baseline to 6 months of ARV administration. Creatinine was found to be positively correlated with platelets (Figure 4.12; $p<0.01$; $r=0.082$).

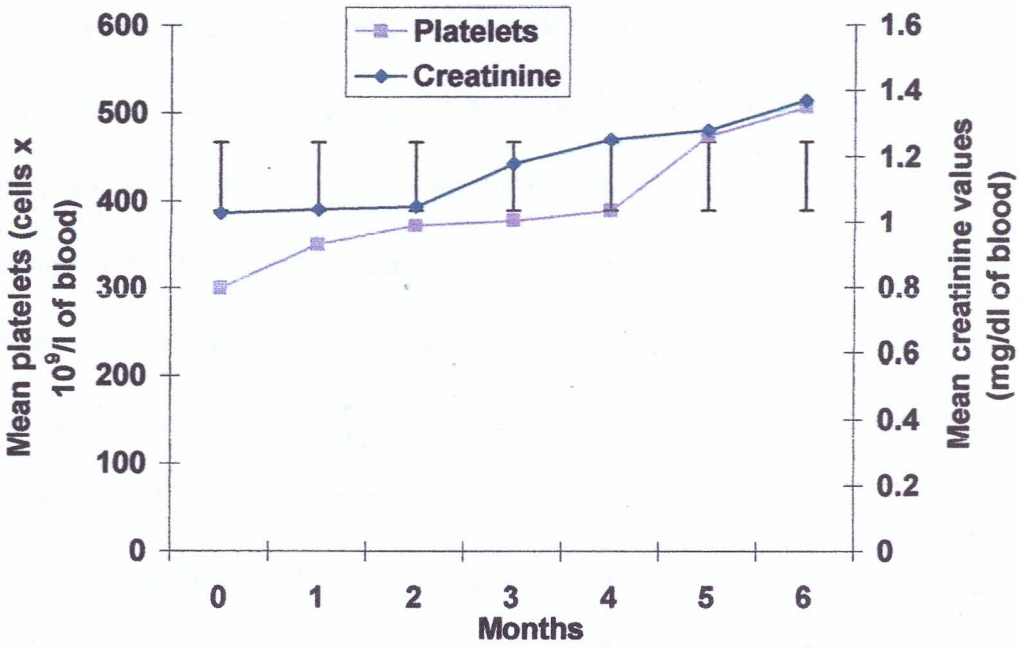


Figure 4.12: Relationship between mean platelets and mean creatinine levels at baseline and subsequent levels for 6 months of ARV use

CHAPTER FIVE : DISCUSSION

5.1 Study overview

Antiretroviral drugs are freely available in all hospitals. Prolonged use of ARVs can contribute to drug resistance and drug associated toxicity. Limited studies on prolonged use of ARVs demonstrated swollen feet, kidney failure, liver malfunction and compromised immune system in some of the patients (Janeway *et al.*, 2005). Toxicity can be measured by determination of levels of creatinine in serum (Greer, 1999). Although ARVs reduce the suffering of HIV patients, it is not clearly known whether and at what stage their prolonged use may result in associated complications. Six months may not be long enough for clinical manifestations but is long enough for immunological manifestations. The study was investigating immune manifestations. Therefore there is need to monitor the immune status and ARV associated toxicity in patients on first line combination ARV regime. This study was aimed at establishing toxicity of ARVs as indicated by creatinine levels and how they relate to the immune status of the HIV patients. Results showed that creatinine beyond a certain level could be used to indicate the development of ARVs associated toxicity among patients living with HIV and AIDS.

5.2 Effects of ARVs (zidovudine, stavudine and nevirapine) on creatinine levels in HIV patients

In health normal creatinine ranges from 0.6-1.2 mg/dl of blood. Creatinine higher than 1.2 mg/dl of blood indicates poor kidney glomelural filtration rates which

would be a sign of kidney impairement. In this study, all the patients were given fixed doses of ARVs. Creatinine levels increased with continued chemotherapy from mean of 1.03 mg/dl at baseline to 1.37mg/dl of blood at the sixth month of ARV treatment.

The results showed that prolonged use of ARVs may lead to elevated levels of creatinine indicating potential of toxicity in HIV patients. The increase in creatinine was an indication that ARVs at some level impair kidney function hence reduce glomelura filtration rates resulting in elevation of creatinine in serum. This observation is in agreement with earlier work done by Hirsch and Gunthad, (2005) which reported that impaired kidneys lead to increased serum creatinine. The control group which received contrimoxale for the management of opportunistic infection showed no significant changes in creatinine with a baseline mean of 0.91 mg/dl of blood and changed to of 0.99 mg/dl of blood following 6 months of treatment. This showed that contrimoxale had no significant effect on creatinine levels since this was within normal creatinine range of 0.6-1.2 mg/dl of blood.

5.3 Effects of ARVs (Zidovudine, Stavudine and Nevirapine) on CD4 and CD3 Counts

The normal range of CD4 is 1000-1200 cells/µl of blood in a healthy person (Janeway *et al.*, 2005). In this study CD4 counts increased from mean values of 101cells/µl of blood at baseline to 358 cells/µl of blood in the third month of ARV chemotherapy, followed by a decline to 278 cells/µl of blood in the sixth month of chemotherapy. This is in agreement with results of studies by Uberg,

(2009) which reported that, CD4 counts increase rapidly in the initial levels of ARV therapy then decline, may be due toxic pressure of ARVs. Studies by Antoni *et al.* (2002) showed that the toxicity of ARVs on the bone marrow and blood-cell system could result in re-infection with HIV. The control group showed no significant changes in CD4 counts with a mean of 231 cells/µl of blood following 6 months treatment with contrimoxale for opportunistic infection management. This showed that septrin had no significant effect on CD4 levels.

CD3 counts increased from baseline mean values of 1306 cells/µl of blood to a mean of 1887 cells/µl of blood in the fourth month of ARV chemotherapy followed by a decline to 1778 cells/µl of blood in the sixth month of chemotherapy. The peak mean CD3 count of 1887 cells/µl of blood was observed in the fourth month of treatment then declined to 1778 cells/µl of blood in the sixth month corresponding with decreasing CD4 counts. T cells response is crucial in all intracellular infections like HIV. CD3 is a marker of all T cells and its changes indicate levels of cellular immune responses(Roit *et al.*, 2006). CD4T cell response induces both humoral and cellular response but also crucial as a major receptor of HIV entry. Hence changes in CD4counts could result changes in CD3 counts. This is in agreement with results of studies by Carbonara *et al.* (2001) which reported that, CD3 levels increase with increase in CD4 counts and vise versa.

5.4 Effects of ARVs (zidovudine, stvudine and nevirapine) on Haematological values

The normal range of haemoglobin in health is usually 11-18mg/l of blood (Janeway *et al.,* 2005). In this study, haemoglobin levels increased from baseline mean values of 11.0mg/l of blood to 13 mg/l of blood in the sixth month of ARV chemotherapy. The mean Hb observed was within the normal range in health. The results showed that treatment with ARVs resulted in increased Hb levels but within normal range. May be the level of toxicity generated was not high enough to influence Hb negatively to change out of normal range. The control group results showed no significant changes in Hb levels with a mean of 12 mg/dl of blood following 6 months of contrimoxale use. This showed that contrimoxale had no significant effect on Hb levels.

Platelet counts increased from baseline mean values of 300 x 10^9 cells/l of blood and increased to a mean of 506 x 10^9 cells/l of blood in the sixth month of ARV chemotherapy. The mean platelets counts observed after 6 months of ARV use were slightly beyond the normal range in health which is usually 150-450 x 10^9 cells /l of blood. This is in agreement with results of studies by Grohskopf and Black (2005) which reported that, ARVs such as Nevirapine, is associated with thombocytosis but disagrees with results of studies done by Smith *(2006) which reported that most drugs are associated with platelet destruction. May* be platelet elevation was responding to ARV drugs in circulation and the level of drug toxicity observed in this study was not high enough to cause platelet

destruction. The control group showed no significant changes in platelets counts levels with a mean of 235 $x10^9$ cells /l of blood following 6 months of contrimoxale use. This showed that septrin may not have negative effects on platelet levels.

Mean corpuscular volumes is a measure of the average red blood cell volume that is reported as part of a standard complete blood counts. Patients may indicate microcytic anaemia (MCV below normal range), normocytic anaemia (MCV within normal range) or macrocytic anaemia (MCV above normal range) (Fleischer, 2004). MCV levels increased from baseline mean values 88 fl to a mean of 104 fl in the sixth month of ARV chemotherapy. The mean MCV observed were beyond the normal range in health which is usually 80-100 (fl). May be toxicity experienced induced elevation of MCV. This is in agreement with results of studies by Grohskopf and Black (2005) which reported that, ARVs such as Nevirapine, is associated with macrocytic anaemia due to increase in red cell volume in response to the drug . The control group showed no significant changes in MCV levels with a mean of 84 fl following 6 months of contrimoxale use. This showed that contrimoxale had no significant effect on MCV levels as it was less toxic.

5.5 Relationship between creatinine and immune status of HIV patients on zidovudine, stavudine and nevirapine

5.5.1 Creatinine and CD4 Counts

The mean CD4 counts and creatinine levels of patients under ARVs were compared from baseline to 6 months of follow up during. Creatinine was found to be positively

correlated with CD4 counts for up to the third month of follow up. The mean creatinine observed in the third month of treatment was 1.18mg/dl of blood and indicated the peak mean CD4 count of 358 cells/µl of blood. Creatinine above 1.18mg/dl/of/blood, was corresponded with mean CD4 counts decreasing rapidly from mean of 358 cells/ul of blood in the third month to a mean of 278 cells/µl of blood in the sixth month of treatment. This could have been due to toxic effects of ARVs increasing beyond tolerance and affecting the blood cell system. This is supported by results of Antoni (2002)) which showed that the toxicity of ARVs on the bone marrow and blood-cell system decreases CD4 levels. In this study, decrease in CD4 may be explained by increased toxicity as a result of ARVs prolonged administration. This showed that only creatinine beyond a certain level can indicate ARV toxicity.

Patients with baseline CD4 counts of 50-100 cells /µl of blood had highest creatinine profiles of 1.25 mg/dl of blood. This was associated with lowest CD4 response of 205 cells /µl of blood to ARVs treatment while patients with baseline CD4 counts of 101-150 cells /µl of blood had the lowest creatinine profiles of 1.08 mg/dl of blood with the highest CD4 response of 405 cells /µl of blood to ARVs treatment following 6 months of ARV use. This may suggest that when toxic pressure is experienced, the CD4 response is poor.

The control group showed no significant changes in CD4 counts and creatinine levels with CD4 mean of 209 cells/µl of blood and mean creatinine of 0.99

mg/dl of blood following 6 months of treatment with contrimoxale. The creatinine observed in the third month of treatment was 1.01mg/dl of blood while mean CD4 count was 249 cells/µl of blood. Creatinine was within the normal range with no significant changes while CD4 counts were still low. This may suggest that viral load was still high and hence CD4 response was poor even after administration of contrimoxale (WHO, 2006).

5.5.2 Creatinine and haemoglobin levels

The mean Hb levels were compared with mean creatinine of all the patients from baseline up to 6 months of ARV administration. Creatinine was found to be weakly positively correlated with haemoglobin. The highest mean creatinine observed after 6 months of treatment was 1.37mg/dl of blood and a peak Hb level of 13mg/dl of blood. This is in agreement with results of studies by Kuhn (2001) which showed that haemoglobin levels may not be dependent on drug toxicity but may depend on nutritional factors.

Among the patient categories those with baseline CD4 counts of 101-150 cells /µl of blood had the lowest creatinine profiles after ARVs treatment. They had a creatinine mean of 1.08 mg/dl of blood corresponding with highest Hb with a mean of 12.4 mg/dl of blood. Patients with baseline CD4 counts of 50-100 cells /µl of blood had highest creatinine profiles after 6 months of ARVs treatment with a mean of 1.25 mg/dl of blood and the lowest Hb with a mean of 11.5 mg/dl of blood following 6 months of ARV treatment. These results showed that in all categories Hb was within the normal range probably because toxicity generated was not high enough to have a

negative effect on Hb. The control group results showed no significant changes in Hb levels and creatinine levels with. Hb changed from 11 mg/dl of blood at baseline to 13 mg/dl of blood after 6 months while creatinine changed from 0.91 at baseline to 0.99 mg/dl of blood following 6 months treatment with contrimoxale.

5.5.3 Creatinine and MCV levels

The mean MCV levels were compared with mean creatinine for all the patients from baseline up to 6 months of ARV administration. Creatinine was found to be highly positively correlated with MCV levels. The highest mean creatinine observed in 6 months of treatment was 1.37mg/dl of blood and indicated the peak mean MCV level of 104 fl. This is in agreement with results of studies by Hirsch and Gunthad (2005) which reported that, increased drug toxicity may increase MCV above normal range resulting from increased red cell volumes. Among the patient categories those with baseline CD4 counts of 101-150 cells /µl of blood had their creatinine and MCVprofiles least affected by ARVs treatment. With a creatinineof 1.08 mg/dl of blood and MCV of 98.5 fl. Patients with CD4 counts of 50-100 cells /µl of blood had their creatinine profiles greatly influenced by ARVs treatment with creatinine of 1.25 mg/dl of blood and MCV of 107.7 fl after 6 months of ARV treatment. The control group showed no significant changes in MCV levels and creatinine levels. MCV changed from 88fl at baseline to 84fl while creatinine changed from 0.91fl to 0.99 mg/dl of blood after 6 months of contrimoxale use.

5.5.4 Creatinine and platelet levels

The mean platelet counts were compared with mean creatinine of all the patients from baseline up to 6 months of ARV administration. Creatinine was found to be positively correlated with platelets. The highest mean creatinine observed in 6 months of treatment was 1.37mg/dl of blood and indicated the peak mean platelets level of 506 x 10^9 cells /l of blood. This is in agreement with results of studies done by Carbonara and David (2001) which reported that, ARVs toxicity is associated with thrombocytosis. Thrombocytosis is associated with elevated platelets levels in serum.

Among the patient categories, patients with baseline CD4 counts of 50-100 cells /µl of blood highest creatinine profiles with a mean of 1.25 mg/dl of blood corresponding with highest platelets of 488 x 10^9 cells /l of blood. Patients with CD4 counts of 101-150 cells /µl of blood had the least creatinine profiles of 1.08mg/dl of blood and lowest platelets of 389 x 10^9 cells /l of blood. The control group showed no significant changes in platelets levels and Creatinine levels changed from 0.91 at baseline to 0.99 mg/dl of blood following 6 months of contrimoxale use.

5.6 Relationship of Creatinine and Immune Responses

In this study, it was observed that the mean creatinine and mean immune profiles (MCV, platelets and Hb attained in the third month were within the normal ranges in health except CD4 counts which were far below normal. This could be due to the fact that at this level of ARV therapy, it is possible that there was gradual restoration of the immune system as indicated by the rising CD4 counts. It may also be suggested

that normal CD4 levels in health may not be easily attained in HIV patients under ARV therapy. This may be due to the fact that drugs suppress body cells proliferation and destroy cells too (Smith, 2006). Beyond three months of ARV treatment of HIV patients, ARV drug may have impaired the immune system leading to impaired immune responses.

The results of the control group showed no significant changes in creatinine levels and immune responses with mean creatinine of 0.994 mg/dl of blood following 6 months treatment with septrin. The mean creatinine observed in the third month of treatment was 1.01mg/dl of blood and indicated mean CD4 count of 249 cells/ul of blood. Creatinine was within the normal range with insignificant changes while CD4 counts were still low. This showed that contrimoxale toxicity had no significant effect on the immune responses.

This study showed that serum creatinine levels beyond 1.18mg/dl of blood may be a good indicator of ARV drug toxicity. This level may be used by clinicians to change the ARV drug regime to boost the immune system in management of HIV patients. The study also showed that ARV chemotherapy could be started at baseline CD4 counts of 101-150 cell/ul of blood because this is when there is least ARV associated negative impact on the immune responses based on observed creatinine levels. It is important to note that Zidovudine and Stavudine have been withdrawn from use as antiretrovirals for HIV patient from September 2010 as recommended by Ministry of Medical Services due to their associated side effects (NASCOP, 2010). However,

creatinine levels in serum may be used as an indicator of ARV toxicity in HIV patients.

CHAPTER SIX : CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

i) The results showed prolonged use of first line combination ARV therapy in HIV patients resulted in elevated creatinine which is an indicator of toxicity

ii) CD4 response improved rapidly in the first three months of follow up then started declining which corresponded with elevated levels of creatinine in HIV patients on first line combination ARV therapy

iii) Elevated MCV and platelets above normal levels in health may have indicated macrocytic anaemia and thrombocytosis respectively in HIV patients on first line combination ARV therapy

iv Based on this study chemotherapy with first line combination ARV therapy could be started at baseline CD4 counts of 101-150 cells /µl of blood when there is less chance of HIV patient body experiencing toxicity but experience maximum benefit from ARVs

6.2 Recommendations

i) There is need for more studies to be done on toxicity of individual drug type rather than a combination regime to find out the impact on the immune profiles.

ii) There is need to determine further effects of accompanying antibiotics in ARV regime on a larger sample of patients

iii) There is need for carrying out a similar study for more than six months and in other parts of the world.

REFERENCES

Abrescia, J. P. (2005). Viral subversion of the immune system. *Curr. Pharm. Des.* **11**: 3697-3710).

Antoni, F. G. (2002). Drugs and toxicity of bone marrow. *J. haem.*, **105**: 12-15.

Arya, O. P and Hart, C. A. (1998). Sexually transmitted diseases and AIDs in the tropics. CABL publishing, London, UK. *Lancet;* **154**: 10- 18.

Bain, B. J. (1997). The haematological features of HIV Infection. *J. haem.*, **99**: 1-8.

Brostof, M., Roit, N. and Male, B. (2006). Immunology. Oxford University press. London, UK. **24**: 359-390.

Blanche, D. N. (1999). Persistent mitochondrial dysfunction and perinatal exposure to antiretroviral nucleoside analogues. *Lancet;* **354**: 1084- 1089.

Cammack, N. (2001). The potential for HIV fusion inhibition. *Curr. Opin. Infect. Dis.*, **14**:13-16.

Carmichael, A. (1995). Molecular biology of HIV. Oxford University press Edinburgh, UK. **14**: 215-235.

Carcelain, G., Debre, P., and Autran, B. (2001). Reconstitution of CD4 T. cell in HIV infected individuals following ARTs. *Curr.Opin. Immunol.*, **13**:183-188.

Cheesborough, M. (2005). District Laboratory practice in tropical countries, (Part 2). Cambridge University press, Edinburgh, UK. **6**: 435-471.

Cullen, B. R. (2000). Connections between the processing and nuclear export of mRNA : evidence of export licence? *Pro. Natl, Acad. sci. USA.*, **97**:4-6.

Carr, A., Workman, C. and Smith, D. E. (2001). Abacavir substitution for nucleoside analogues in patients with HIV lipoatrphy. *Lancet;* **357**: 1412-1414.

Chrystie, K. L. (1988). Replication and structure of HIV. Lancet; **60**: 14-25.

Costello, M. B. (1988). The West African Aids Virus. *J. Clin. Pathol.*, **41**: 711-715.

Carbonara, S., David , M. (2001). The AIDS pandemic. *J. Infect.Dis.*, (2001) **42** : 251-256.

Coovadia, H. (2004). "Antiretroviral agents--how best to protect infants from HIV and save their mothers from AIDS". *N. Engl. J. Med.*, **351 :** 289–92.

Dacie, J.V. and Lewis, S. M. (2000). Practical haematology. Churchill living-stone, London, UK. **9**:.56-79.

De Souza R. S. (2000). Effect of prenatal zidovudine on disease progression in perinatally HIV-1-infected infants. *J. AIDS* . **24 (2)**: 154-161.

Dainiak , S. M. (1988). HIV and the CC-R5. *J. Haem.* Britain. **69**: 299-304.

Doms, E. N. (2005). Adverse effects of ARVs . University of California San Francisco. **6**;35.

Dybul. M. (2002). Practices for Treatment of HIV. *Ann. Intern. Med.*, **137** (5 Pt 2): 381–433.

Erich, E. (1991). Handbook of renal physiology. Oxford publishers, London , UK. **12**: 166-175.

Fishers, H. (1998). Sample size determination. **2:** 29.

Fleischer, R. (2004). Chemokine – receptor polymophisms. *J. Clin. Infect. Dis.*, **38:** 4 10.

Gerschenson, M. (2000). Fetal mitochondrial heart and skeletal muscle damage in Erythrocebus patas monkeys exposed in utero to 3'-azido-3'-deoxythymidine. AIDS *Res Hum. Retroviruses.* **16 :** 635-644.

Government of Kenya (GOK), (2006). HIV and AIDS in Kenya report. Pp.35.

Government of Kenya (GOK), (2008). HIV Prevalence in Kenya. National AIDs control Council, Nairobi Kenya. Pp. 16.

Gilks, C. F. (1998). Acute bacteria infection and HIV Disease in tropical medicine achievements and prospect. *British Med. Bulletin,* 55: 1-11.

Grant, A. D., Decock, K. M. (1998). The growing challenge of HIV/AIDs in developing countries in tropical medicine achievement and prospects. *British med. bulletin,* **54**:2 -369.

Greer, J. P. (1999). Wintrobes clinical Haematology. Appincott Williams and Wilkin, Britain, UK. **5**: 133-148.

Grohskopf, L. A., Black R .J, (2005). "Antiretroviral postexposure prophylaxis after sexual, injection-drug use, or other nonoccupational exposure to HIV in the United States: recommendations from the U.S. Department of Health and Human Services. *Recomm. Rep.*, **54.**: 1–20.

Grouard, G. and Clark, E. A. (1997). Role of dendritic and follicular dendritic cells in HIV infection and Pathogenesis. *Curr. Opin. Immunol.*, **9**:563-567.

Hirsch, M. S., Gunthad H. F, (2005). UK Group of Transmitted HIV Drug Resistance Time trends in primary resistance to HIV drugs in the United Kingdom: multicentre observational study. *J. Brit. Med.*, **331** 1368–71.

Janeway, Jr. (2005). Immunobiology. Garland Science Publishing New York, USA. **23**: 356-399.

Jeff, B. (2008). Labman diagnostic kit for creatinine. London, UK. Pp. 23.

Johnson, W. E. (2002). Viral persistence: HIV strategies of immune system. *Annu. Rev. Med.*, **253**: 199-518.

KAIS, (2009). Know your HIV status. Pp. 21.

Kaplaan, J. E. (1999). Preventing opportunistic diseases in human immune deficiency virus infected persons. Implication for the developing world. *J. Med. Hyg.*, 77:731-739.

Kuhn, L. (2001). Disease progression and early viral dynamics in human immunodeficiency virus-infected children exposed to zidovudine during prenatal and perinatal periods. *J. Infect Dis.*, **183 :** 1540-1542.

Kwong, P. Wyatt, R., Robinson, J., Sweet, R. (1998). Structure of an HIV gp 120 envelope in complex with CD4 receptors and a neutralizing human antibody. *NaT.*, **393**: 630-631.

Lehnert, T. (2002). The role of CCR5 chemokine ligand and antibodies to CCR5 co receptors in preventing HIV infection. *Trends Immunol.*, **23:** 347-351.

Lempicki, R. A., Kovas, J. A., Baseler, M.W., Adelsberger, J. W., Dewar, R. L., Natarajan, V., Bosche, M. C., Meticalf, J. A., Stevens, R. A., Lambert R.L., Alvord, W. G., Polis, M. A., Davey, R. T., Dimitor, D. S. and Lane, H. C. (2000). Impacts of HIV infections and highly active ARTs on the kinetics of CD4 and CD8 T. cell turnover in HIV patients. *Proc. Natl. Acad. Sci., USA*, **97**:13778-13783.

Lipsky, J. (1996). Antiretroviral drugs for AIDS. *Lancet*,**348**: 800-803.

Lori, F. and Trocio., J. (2005). *Derma. Vir* , a novel HIV immunization technology. **56**:123-125.

Male, B. (2006). Immunology. Oxford university press. London, UK. **13**:356-359.

Moore, J. P., Trkola, P., and Dragic T. (1997). Coreceptors of HIV -1 entry. *Curr.Opin. Immunol.*, **9**:551-562.

Moore, J. P. (1991). HIV and AIDS. *Arch. Intern. Med.*, **151**: 981-986.

Microft, E. M. (1999). AIDS. *Lancet,* **13**: 943-950.

Morgan, D. (1997). HIV -1 disease progression and AIDs defining disorders in rural Uganda. *Lancet,* **350**: 245-255.

NASCOp, (2008). HIV and AIDS in Kenya. Pp. 22.

NASCOP, (2010). HIV and AIDS news. Pp. 16.

Perelson, A. S., Neumann, A. U., Markowitz, M., Leonard, J. M., Ho D. D. (1996). HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. *Sc.*, **271** (5255): 1582–1586.

Pettit, M. T. (2000). Whole blood counts. *Annu. Rev. Immunol.*, **56**: 59-70.

Pierson, T., MCArther, M. C. and Siliciano, R. F. (2000). Reservours of HIV-1 Mechanisms for viral persistence in the presence of antiviral immune responses and ARTs. *Annu. Rev. Immunol.*, **18**: 665-708.

Roderick, N. and Herbret, M. (2005). Immunology for medical students. Elserviar Mosby. London, UK. **24**:25-65.

Richman F. C. and Wyatt X. (1997). Viral dynamics in HIV-1 infection. Nat. 1997, **373**:117-122.

Roit, N. (2006). Immunology. Oxford university press. London, UK. **12**: 356-361.

Royse, R. A., Sen, A., Cate, W. and Cohen, M. S. (1997). Sexual transmission of HIV. *N. Engl. J. Med.*, **336**: 870-1072.

Saitoh, A., Hull, A. D., Franklin, P., Spector, S. A., (2005). Myelomeningocele in an infant with intrauterine exposure to efavirenz. *J. Perinatol.*, **25** : 555– 556.

Scarlatti, G. (1997). In vivo evolution of HIV-1 coreceptor usage and sensitivity to chemokine mediated suppression . *Nat. Med.*, **3**: 1259-1265.

Scott, G. and Lewis, M. (1995). A simple and reliable method of estimating haemoglobin. Bulletin of WHO pp 369-373.

Smith, K.A. (2006). To cure HIV infection: a new therapeutic strategy needed. *Curr. Opin. Immunol.*, **13**: 617-624.

Tarnesen, H., Hejberg L., Prberrins S.and Anderson J. (1996). Erythroycte mean cell volume. *Actn . Med. Scand.*, **219**: 515-518.

Taften, P. R. (2007). Antiretrovirals and immune response. *Actn . Med. Scand.*, **29**:115-118.

Uberg, M. D. (2009). Renal impairement in HIV infected population updates. New York University Medical Center. *Curr. Opin. Immunol.*, **16**: 619-621.

UNAIDS, (1998). Global summary of HIV/AIDs epidermic. AIDs epidermic updates report. Pp 45.

UNAIDS, (2007). AIDs treatment new internet directory. Pp.79.

Udesselberg, M. (2006). Medical Virology. Oxford University press. Britain , UK. **6**: 251-268.

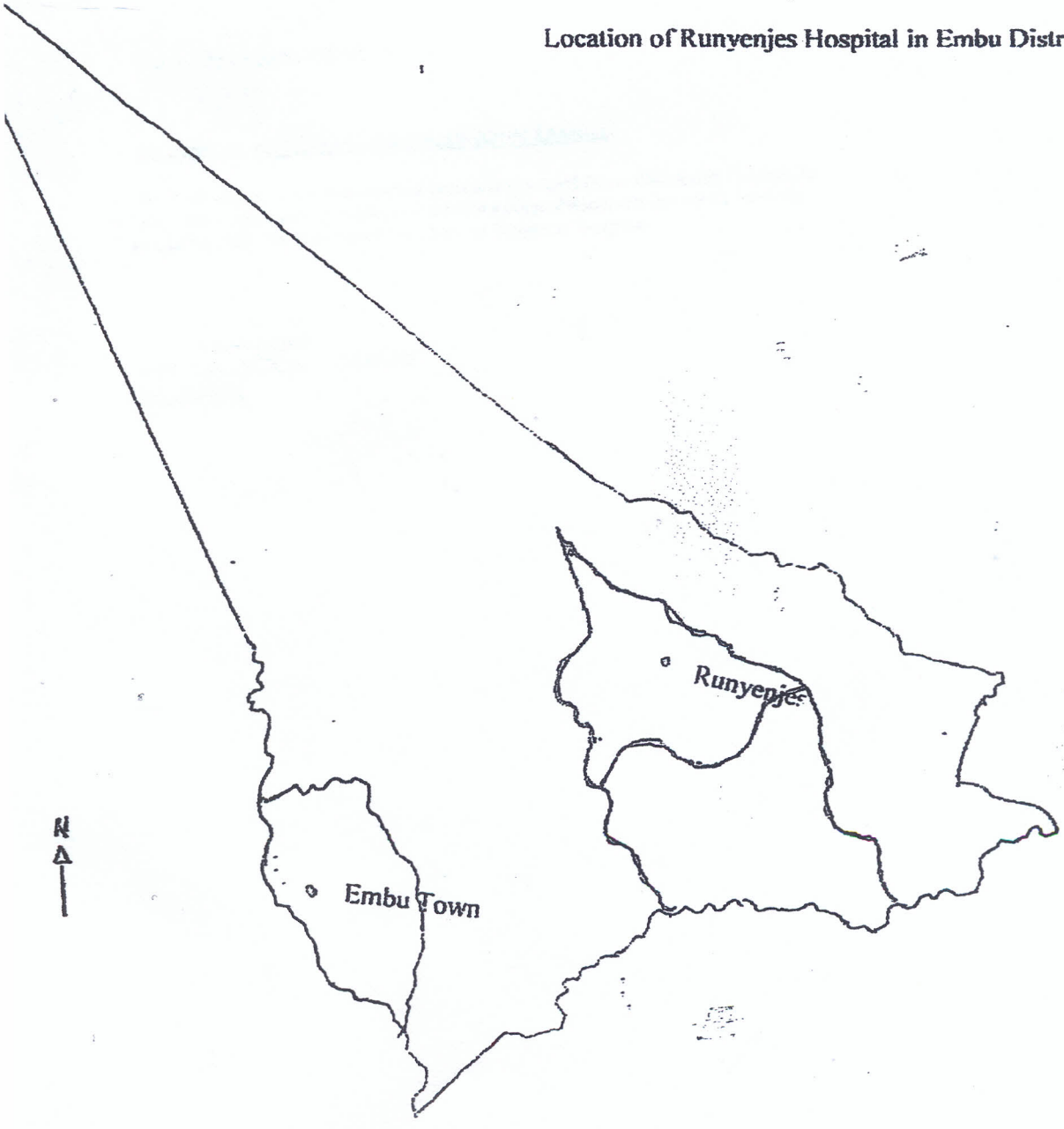
World Health Organistion (WHO), (2006). Global effects of HIV. Pp. 66.

World Health Organistion (WHO), (2008). HIV and antiretrovirals. Pp. 99.

World Health Organistion (WHO) (2009). HIV epidemiology. Pp. 12.

Appendix i.

Location of Runyenjes Hospital in Embu District



Appendix ii.

MINISTRY OF MEDICAL SERVICES

Telephone: 068-62049/068-62128/068-62018
Mobile No: 0721316728.
When replying please quote:

RUNYENJES DISTRICT HOSPITAL
P.O. BOX 193
RUNYENJES

Ref.No. RNJ/T.I/VOL.1/(85)

Date 13th Nov.2008

TO WHOM IT MAY CONCERN

RE: ETHICAL APPROVAL - MR NAMU JOHN SAMUEL

This is to certify that the above named student from Kenyatta University has been allowed to carry out Immunology Research on ARVs Toxicity in our facility for award of masters of Science Degree.

DR ELESBAN KIHUB
MEDICAL OFFICER I/CHARGE
RUNYENJES

Appendix iii

Mean creatinine and immune profiles of HIV patients at baselineand for 6 month under ARVs

Month	creatinine	CD4	CD3	Hb	Platelets	MCV
0	1.03	101	1306	11	300	88
1	1.04	248	1603	11.4	350	96
2	1.05	291	1647	11.9	371	98
3	1.18	358	1722	11.9	377	102
4	1.25	347	1887	12.1	389	101
5	1.28	296	1860	12.2	473	103
6	1.37	278	1778	13	506	104

Appendix iv

Mean creatinine and immune profiles of HIV patients at baseline and 6 months of . (septrin) contrimoxale antibiotic for opportunistic infection treatment

months of follow up	0	1	2	3	4	5	6
creatinine (mg/dl of blood)	0.91	1.01	1.00	1.01	1.02	1.02	0.99
CD4 (cells/ul of blood)	250	199	270	249	224	220	209
CD3 (cells/ul of blood)	1600	1509	1408	1301	1402	1201	1603
Hb (mg/dl of blood	9.0	10	11.1	11.3	13.1	11.5	12
Platelets (cells/l of blood	150	180	200	250	255	330	235
MCV (fl)	88	85	79	80	89	90	84