INSTITUT D'ENSEIGNEMENT SUPÉRIEUR DE RUHENGERI

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DEPARTMENT OF BIOTECHNOLOGIES

Scientia et Lux

STUDY ON EVOLUTION OF CREATININE AMONG PATIENTS UNDER ANTIRETROVIRAL DRUGS. «CASE STUDY OF MUHIMA HOSPITAL»

A Dissertation submitted in partial fulfillment of requirements for the award of a Bachelor's Degree of science in Biotechnologies

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Musanze, October 2012

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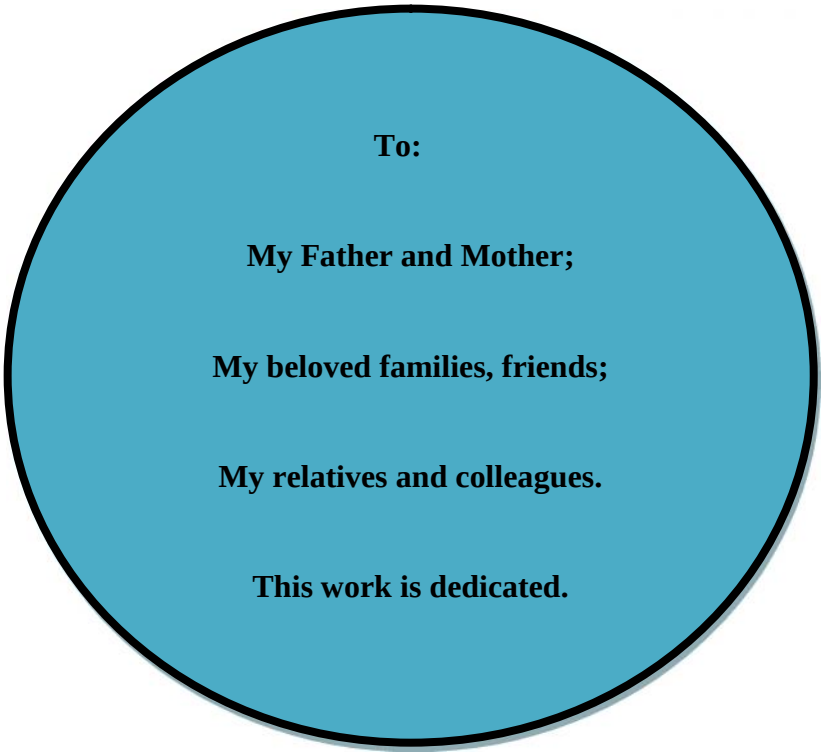
DEDICATION


To:
My Father and Mother;
My beloved families, friends;
My relatives and colleagues.
This work is dedicated.

ACKNOWLEDGEMENTS

First of all, we would like to thank the Almighty God for his protection and strength he gave us to complete this work.

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We are thankful to all who have contributed to my study for their affection and support.

May God bless you all?

Noëlla IRAKOZE

ABSTRACT

HIV/AIDS is a major problem of public health concern in the world especially in developing countries, including Rwanda. The reduction on impact of HIV infections is only done by prevention, monitoring and care of patients living with HIV/AIDS under antiretroviral therapy. There are currently 184 ARTs sites in Rwanda that perform laboratory tests to follow up and care to patients living with HIV/AIDS who are under antiretroviral therapy. It is very important to mention that this crucial activity of following up patients with HIV/AIDS who are under antiretroviral therapy have started in Rwanda since 2003 and there is no research published yet to indicate the side effects of ARVs on kidney functions among HIV/AIDS patients under ARVs treatment in Rwanda. This is the reason why this study has been carried out to improve the care and better follow up of people living with HIV/AIDS infection.

The general objective of this study was to determine whether creatinine is evolved in HIV/AIDS patients who are under ARVs treatments in Kigali rural areas. This study has been carried out at Laboratory of Muhima Hospital where biochemistry tests results from May 2012 up to June 2012 were used to see whether creatinine is evolved in kidney among HIV/AIDS patients under ARVs therapy.

In this regard a total number of 100 HIV/AIDS patients' samples have been analyzed. Our results showed that complications caused by ARVs are found in same drug combination.

The frequency of abnormal concentration of creatinine in HIV patients per 16 peoples between the ages of 0-80 years of the 1st month .The highest frequenty among males is 2 ranging from 51-60 years and in females the highest frequent is 6 ranging from 21-30 years according to ***appendix I.***

The frequency of abnormal concentration of creatinine in HIV patients per 12 peoples between the ages of 0-80 years of the 2nd month. The highest prevalence among males is 1 ranging from 11-20 and 31-40 years and in females the highest prevalence is 5 ranging from 21-30years according to ***appendix II***.

RÉSUMÉ

Sur le plan de la santé publique, le VIH/SIDA est considéré comme un problème majeur mais spécialement dans les pays en voies de développement, dont le Rwanda. La réduction de l'impact de l'infection au VIH est faite seulement par sa prévention ainsi que le suivi des patients vivant avec le VIH/SIDA sous traitement par des ARVs. Actuellement, on compte 184 sites d'ART au Rwanda qui font les tests de laboratoire pour le suivi des patients vivant avec le VIH/SIDA sous ARVs. C'est très important de noter aussi que cette activité cruciale de suivi des patients vivant avec le VIH/SIDA sous ARVs a débuté en 2003, jusqu'ici aucune étude n'a été publiée montrant les effets secondaires sur le fonctionnement rénal causés par ces médicaments au Rwanda. Cette étude a été faite dans le but d'améliorer le suivi des patients vivant avec le VIH/SIDA sous ARVs.

L'objectif général de cette étude est de déterminer s'il y a une évolution au niveau du fonctionnement rénal des patients vivant avec le VIH/SIDA sous ARVs dans le région de Kigali. Cette étude a été effectuée au Laboratoire de L'Hôpital de Muhima, où les résultats des tests biochimiques du Mai 2012 jusqu'à Juin 2012 ont été utilises pour voir le fonctionnement rénal ainsi que le statut sanguin chez les patients vivant avec le VIH/SIDA sous ARVs.

Le nombre total de 100 échantillons des patients vivant avec le VIH/SIDA sous ARVs ont été analysés et nos résultats montrent que ces complications causées par ARV sont trouvées en combinaison dues aux différents types de médicaments.

La fréquence de la concentration d'une créatinine anormale par 16 personnes, entre l'âge de 0-80 ans du 1er mois. La plus grande fréquence parmi les hommes est 2 se trouvant entre 51-60 ans et parmi les femmes la plus grande fréquence est 6 se trouvant entre 21-30 ans ***(annexe I).***

La fréquence de la concentration d'une créatinine anormale par 12 personnes, entre l'âge de 0-80 ans du 2eme mois. La plus grande fréquence parmi les hommes est 1 se trouvant entre 11-20 et 31-40 ans et parmi les femmes la plus grande fréquence est 5 se trouvant entre 21-30 ans ***(annexe II).***

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LIST OF ABBREVIATIONS

%:	Percentage
3TC:	Lamividune
ABC:	Abacavir
AIDS:	Acquired Immune Deficiency Syndrome
ART:	Antiretroviral therapy
ARVs:	Antiretroviral
AZT:	Zidovudine
CA:	Capsid
CCR5:	Chemokine receptor type 5
CD4:	Cluster of Differentiation four (T lymphocyte subpopulation involved)
cDNA:	Complementary Deoxyribonucleic Acid
CXCR4:	Chemokine receptor type 4
D4T:	Stavudine
DC:	Dendric cell
DDI:	Didanosine
DLS:	Dimer Linkage Structure
DNA:	Deoxyribonucleic Acid
E:	Electrolyte
EFV:	Efavirenz
EGFR:	Estimated Glomerular Filtration Rate
Env:	Envelope
ER:	Endoplasmatic reticulum
FDA:	Food and Drug Administration
Gp:	Glycoprotein
HAAR:	High active antiretroviral therapy
HIV:	Human Immune Virus
Http:	Hypertext transfer protocol
IDV:	Indinavir
INES:	Institut d'Enseignement Supérieur

MOH:	Ministry Of Health
NC:	Nucleocapsid
NNRTIs :	non-nucleoside reverse transcription inhibitors
NRTIs:	nucleoside reverses transcription inhibitors
NVP:	Nevirapin
Psi:	Packaging signal
R:	Receptor
RNA:	Ribonucleic acid
RT:	Reverse transcriptase
SU:	Surface subunit
TDF:	Tenofovir
TM:	Transmembrane subunit
tRNA:	Transfer Ribonucleic acid
U:	Urea
VIF:	Viral infectivity factor
VPU:	Viral protein U
WHO:	World Health Organization
WWW:	World Wide Web

GENERAL INTRODUCTION

The Human Creatinine is a break-down product of creatine phosphate in muscle and is usually produced at a fairly constant rate by the body and chiefly as a function in filtered out of the blood by the kidneys (Sharon L. Lewis et al, 2008)

The research project of the study on evolution of creatinine among patients under ARV activity is aimed to evaluate the risk of glomerular filtration and proximal tubular secretion associated with a particular antiretroviral agent during ARV treatment.

Problems associated with antiretroviral treatment related kidney disorders concern 30% of those patients and can lead to end-stage renal disease (medic.med.Uth.tmc.edu/path/macconk.htm, retrieved on 11th January, 2012)

The human immunodeficiency virus (HIV) is a serious health problem in the world. AIDS is characterized by changes in the population of t- cell lymphocytes. In an infected individual the virus causes depletion of helper t-cells which leave the person susceptible to opportunistic infection and some malignancies (Sharon L. Lewis et al, 2008)

The Human creatinine is produced at a fairly constant rate by the body and is filtered out of the blood by the kidneys.

Problems associated with antiretroviral treatment including chronic kidney disease were produced by the Infectious Diseases itself seems to play a primary aetiological role in what is called HIV-associated nephropathy and immune-mediated glomerulonephritides. (WHO, 2007)

Some specific antiretroviral drugs such as indinavir and tenofovir have been associated with increased risk of acute and chronic renal failure. HIV-infected patients may also be frequently exposed to nephrotoxic drugs, commonly used for treatment or prophylaxis of opportunistic infections (Thomas C et al, 2001)

The human creatinine level is observed only with marked damage to functioning nephrons. Damage of blood vessels in the kidneys (glomerulonephritis) caused by infection or autoimmune diseases (Henry D. Isenberg, 2004)

The level of creatinine is assayed in blood tests as a marker of myocardial infarction (heart attack), rhabdomyolysis acute hemolytic anemia, severe burns, acute renal disease, musculoskeletal diseases, heart, skeletal muscle, kidneys, brain, and red blood cells.(severe

muscle breakdown), muscular dystrophy, the autoimmune myositides and in acute renal failure (Izzedine H , Launay-Vacher et al, 2001)

Creatinine is the best test to measure your level of kidney function and determine your stage of kidney disease they can be measured in blood and urine (Sharon L. Lewis et al, 2008)

1. PROBLEM STATEMENT

Rwanda Biochemistry unit succeeded in enhancement of direct observed therapy for HIV/AIDS; Research done in different countries show that antiretroviral drugs for HIV/AIDS are nephrotoxic (WHO,2007)

WHO recommends monitoring of creatinine function before and after starting treatment? In Muhima hospital, routine monitoring of creatinine function of patients taking antiretroviral drugs is not done. Therefore this research is to evaluate creatinine concentration in patients under antiretroviral drugs after 4 weeks of treatment and 12 weeks of treatment, for detection of kidneys damage caused by antiretroviral drugs and to determine the most susceptible groups according to age and sex (Sharon L. Lewis et al, 2008)

Antiretroviral drugs differ in how commonly they cause particular side effects. For example, indinavir and tenofovir are the drug most associated with psychiatric symptoms. Nevirapine or efavirenz demonstrated acceptable toxicity profiles. Two distinct patterns of drug injury associated with nevirapine use have emerged, hypersensitivity reactions and direct drug-related toxicity.

Hypersensitivity reactions of the patients who are taking nevirapine, the overall incidence of symptomatic events involving the kidney function is approximately 15-20%(Smith DK et al, 2005) However, severe kidneys toxicity, in HIV-infected and HIV-seronegative individuals. The use of nevirapine for postexposure prophylaxis was issued after some individuals developed nephrotoxic failure requiring kidney transplantation. In an HIV treatment trial assessing the efficacy and safety of emtricitabine, a higher incidence of nephrotoxic was observed in patients assigned to the nevirapine arm than in those in the efavirenz arm(Smith DK et al, 2005)

The other antiretroviral drugs which can cause nephropathy and glomerulonephritides, ranging from 7% with zidovudine, 9-13% with stavudine and 16% with didanosine indinavir and tenofovir. In other studies, a different pattern of drug injury with nevirapine use has emerged,

with onset of kidneys elevations occurring beyond 16 weeks of therapy, consistent with direct or idiosyncratic host-mediated kidneys injury (Clinical trial data, 1980)

2. CHOICE AND INTERESTS OF THE STUDY

2.1 Choice of the study

We chose this study because we wanted to realise the whether creatinine is avolved among patients under ARV therapy.

2.2 Interests of the study

2.2.1 Personal interest

This study will help me gain the experience in data analysis basing on the results that will be obtained from participants from Muhima Hospital.

2.2.2 Social interest

This study will facilitate laboratories and service of ARV to know the problem and to do more research on patients under ARV therapy and to take they care.

2.2.3 Academic interest

The outcome of this research will contribute to the increase knowledge; students may utilize the findings, other undergraduate to research more. It also facilitates me to attain my bachelors' degree.

3. HYPOTHEIS OF THE STUDY

The hypothesis of my study among HIV patients on:

- There is no increase in production of creatinine among HIV patients on ARV treatement in Rwanda.

4. OBJECTIVES OF THE STUDY

4.1 Main objective

The main objective of our study was to determine whether the production of increases among patients under antiretroviral drugs for the people of KIGALI rural areas.

4.2 Specific objectives

The specific objectives of our study are to:

- Determine the evolution of creatinine level among the patients under antiretroviral drugs at the beginning of the treatment.
- Follow up appearance of nephrotoxic for the patients under ARV during 2 months of treatment.

5. METHODOLOGY

During my work I used the sampling method of creatinine test by using visual machine during sample collection:

I collected 100 samples from different patients by using the well sterilized tubes and we categorized patients according to sex and age. We analyzed serologically and chemically methods by following an appropriate lab protocol.

6. DELIMITATION OF THE STUDY

Our work is limited in domain, in space and in time.
In domain, our work is limited in immunotechnology and biochemistry domain.
In space, our work is limited in Kigali rural area at health center of Muhima.
In time, our work will be done from May 2012 up to June 2012.

7. SUBDIVISION OF THE STUDY

Besides the general introduction, our study is composed with two parts namely literature review and experimental section with four chapters. The first chapter presents the generalities, second chapter deals with materials and methods, the third chapter is focusing the results presentation, interpretation and discussion while the forth chapter closes our study by a conclusion and recommendations.

PART ONE: LITERATURE REVIEW

CHAPTER I: GENERALITIES

1.1 Human Immunodeficiency Virus (HIV)

1.1.1 Definition

HIV (human immunodeficiency virus) is a virus that attacks the body's natural defense system. HIV infection is caused by the human immunodeficiency virus. One can get HIV from contact with infected blood, semen, or vaginal fluids (Crorr A et al, 2000)

1.1.2 Natural history of HIV infection

Since the first cases of acquired immunodeficiency syndrome (AIDS) were reported in 1981, infection. With Human immunodeficiency virus (HIV) has grown to pandemic proportions, resulting in more than 65 million infections and 25 million deaths. At the end of 2006, an estimated 39.5 million people were living with HIV, with Sub-Saharan Africa carrying the highest burden (Arpadi S, 2005)

In 2006, an estimated 4.3 million people became newly infected with HIV. More than 95% of these new infections are in low and middle income countries. Each day 11,000 persons become newly infected with the virus; of these, half are women and 40% are young people (15-24 years old). Of the estimated 37 million adults living with HIV worldwide, nearly 18 million are women. At the end of 2006, there were an estimated 2.3 million children living with HIV the increasing number of child deaths due to AIDS threatens to reverse many of the recent gains of child survival programmed. Moreover, the socio-economic impact of HIV/AIDS on children is profound. As their parents fall sick and die of AIDS, children undergo a long trail of painful experiences such as: economic hardship, withdrawal from school; lack of love attention and affection; psychological distress, stigma discrimination and isolation, and malnutrition and illness. Cumulatively, there are a total of 16 million children worldwide who have become orphans because their parents died of AIDS (Brian Gazzard, Jens Lundgren et al, 2010)

1.2 Current transmission of HIV through heterosexual

In sub-Saharan Africa, the predominant mode of transmission of human immunodeficiency virus type 1 (HIV-1) is through heterosexual contact, and the rate of transmission by this means is increasing throughout Asia and in many industrialized countries.

A wide variety of behavioral and biologic risk factors are associated with the risk of transmission, including the frequency and types of sexual contact, the use or nonuse of condoms, immunologic status, and the presence or absence of the acquired immunodeficiency syndrome (AIDS), circumcision (in men), and sexually transmitted diseases.

Other potential factors include plasma HIV-1 RNA levels, the presence or absence of chemokine receptors and the use or nonuse of antiretroviral therapy. Improved understanding of the way in which these factors influence both the infectiousness of and the susceptibility to HIV-1 could facilitate efforts to prevent transmission of the virus (Guyton et al, 2006)

1.3 Current status of aids in africa

Cases of the acquired immune deficiency syndrome (AIDS) have been reported in countries throughout the world. Initial surveillance studies in Central Africa suggest an annual incidence of AIDS of 550 to 1000 cases per million adults. The male to female ratio of cases is 1:1, with age- and sex-specific rates greater in females less than 30 years of age and greater in males over age 40. Clinically, AIDS in Africans is often characterized by a diarrhea-wasting syndrome, opportunistic infections, such as tuberculosis, cryptococcosis, and cryptosporidiosis, or disseminated Kaposi's sarcoma.

From 1 to 18% of healthy blood donors and pregnant women and as many as 27 to 88% of female prostitutes have antibodies to human immunodeficiency virus (HIV). The present annual incidence of infection is approximately 0.75% among the general population of Central and East Africa. The disease is transmitted predominately by heterosexual activity, parenteral exposure to blood transfusions and unsterilized needles, and perinatally from infected mothers to their newborns, and will continue to spread rapidly where economic and cultural factors favor these modes of transmission. Prevention and control of HIV infection through educational programs and blood bank screening should be an immediate public health priority for all African countries (http://www.medterms.com, retrieved on 15th April 2012)

A study conducted in Uganda showed that a total of 415 couples discordant for HIV-1 were enrolled between the first and the fourth survey and followed for a period of up to 30 months. The male partner was infected with HIV-1 at base line in 228 of these 415 couples (55 percent), and the female partner was infected in 187 (45 percent).

Ninety (22%) of the HIV-1–negative partners seroconverted during the course of the study, for an overall incidence of 11.8 per 100 person-years. Fifty (56%) of the partners who seroconvert were female, and 40 (44%) were male. The rate of transmission from male partners to female partners was not significantly different from the rate of transmission from female partners to male partners (12.0 per 100 person-years vs. 11.6 per 100 person-years) (http://www.aids.about.com accessed on the 18thApril 2012). Similar results were obtained in Kigali by Project San Francisco (http://www.aids.about.com; retrieved on 2nd May 2012)

1.4 HIV life cycle

The figure below show that the virus circulates in the bloodstream of the infected person; then after, HIV attaches to a cell and it empties its contents into the cell. The HIV genetic code (RNA) is used by the reverse transcriptase enzyme to build HIV DNA. The HIV DNA is inserted into the cell's DNA by the integrase enzyme. This establishes the HIV infection in the cell. When the infected cell reproduces, it activates the HIV DNA, which makes the raw material for new HIV viruses. Packets of material for a new virus come together. The immature virus pushes out of the infected cell in a process called "budding.

The immature virus breaks free of the infected cell. The new virus matures: raw materials are cut by the protease enzyme and assembled into a functioning virus (Heikens et al, 2008)

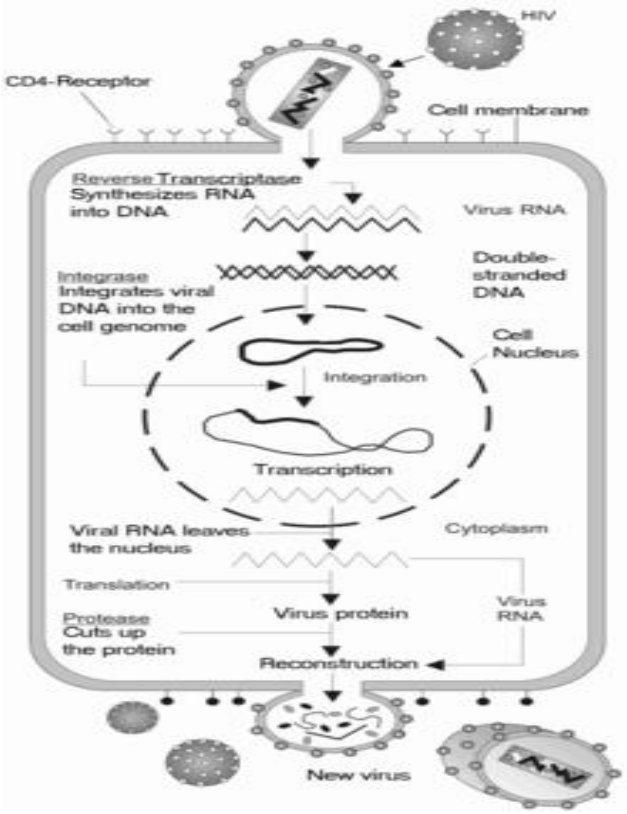

Figure 1: Diagram of HIV life cycle.

Source: (http://www.cnls.gov.rw/hiv sida Rwanda en.php, retrieved on the 8th June 2012)

1.4.1 Entry

HIV can only replicate inside human cells. The process typically begins when a virus particle bumps into a cell that carries CD4 receptor on its surface. HIV-1 infection is initiated by binding of the virion gp120 surface subunit (SU protein) to the CD4 receptor. The SU protein is attached to the virus by a non-covalent binding to the gp41 transmembrane subunit (TM protein). Both SU and TM are proteolytically cleaved from the Envelope (Env) precursor protein by a cellular convertase, furin, within the endoplasmatic reticulum (ER). Both remain noncovalently attached and are targeted to the host plasma membrane by vesicular transport. The SU protein is responsible for receptor recognition on $CD4^+$ T-lymphocytes and the TM protein mediates the fusion between the viral membrane and the host cell membrane.

Binding to CD4 induces a structural alteration in SU that exposes the binding site for a co-receptor of the chemokine family. The major co-receptors required for entry of HIV-1 are the

chemokine receptor molecules CCR-5 (R5 HIV-1 isolates) and CXCR-4 (X4 HIV-1 isolates), which are used by monocytes/macrophage-tropic and T-cell tropic HIV-1 viruses, respectively.

When the SU protein binds to the co-receptor the result is another structural alteration exposing the N-terminal part of TM. This part, also known as the fusion-peptide, mediates the fusion between the viral and host membranes. The Env protein is also capable of mediating fusion between infected and non-infected cells by a process known as syncytium formation. Current strategies are targeting particularly the CD4-SU interaction, the SU-chemokine co-receptor interaction, and the TM-mediated virus-cell membrane fusion process (Coffin JM et al, 1996)

1.4.2 Reverse Transcription and Integration

After fusion the viral core enters the cytoplasm and the viral RNA is copied into double-stranded cDNA. This process is mediated by the viral reverse transcriptase (RT) enzyme which converts the viral RNA into cDNA, that is compatible with human genetic material. This cDNA is transported to the cell's nucleus, where it is spliced into the human DNA by the HIV enzyme integrase. Once integrated, the HIV cDNA is known as provirus.

RT possesses three essential activities important for replication of the virus: RNA-dependent DNA polymerase (i.e. reverse transcriptase), RNase H activity (i.e. cleaves the genomic RNA in RNA/DNA hybrids during cDNA synthesis), DNA-dependent DNA polymerase activity (i.e. for synthesis of the second strand of the proviral DNA). Because RT is essential for viral replication it has been one of the most popular targets ((Izzedine H, Launay-Vacher et al, 2001)

1.4.3 Viral assembly, release, maturation and budding

The virus particle is assembled at the plasma membrane. In this process the GAG and GAG-POL polyproteins interact with each other by protein-protein interaction, most probably via the capsid (CA) protein domain.

The viral genome is packaged in a process in which the packaging signal, *Psi*, is recognized by the nucleocapsid (NC) protein domain of the GAG protein. Another important function of the NC domain is to mediate the formation of the RNA dimer via a palindromic sequence in the dimer linkage structure (DLS) sequence, which is located in the *Psi* sequence

In addition several cellular tRNAs are packaged. The assembly of the virus particle is partly regulated by the VPU and VIF proteins. The primary function of the VPU protein is to mediate the release of the virus particle from the cell surface, by selectively targeting the CD4 protein to a degradation pathway in the endoplasmic reticulum (ER). This permits the release of ENV protein from the ER, which may otherwise be complexed with the CD4 protein, and further processing of the ENV protein can then proceed (Gelderblom HR et al, 1991)

1.4.3.1 Viral assembly

The packaging signal *Psi* is a highly conserved RNA sequence and contains four stem-loops located near the 5' major splice donor and the start of the *gag* open reading frame and is essential and sufficient for RNA packaging. The structural proteins, GAG and ENV, form multimeric complexes during viral assembly (Gelderblom HR et al, 1991)

1.4.3.2 Viral maturation and Viral release

The HIV-1 protease plays an important role in virus maturation. HIV protease enzyme cleaves the GAG and GAG-POL polyproteins to form the structural and enzymatic proteins. HIV provirus may lie dormant within a cell for a long time. But when the cell becomes activated, it treats HIV genes in much the same way as human genes. First it converts them into messenger RNA (using human enzymes). Then the messenger RNA is transported outside the nucleus, and is used as a blueprint for producing new HIV proteins and enzymes (Gelderblom HR et al, 1991)

1.4.3.3 Budding

The newly assembled virus pushes out ("buds") from the host cell. During budding, the new virus steals part of the cell's outer envelope. This envelope, which acts as a covering, is studded with protein/sugar combinations called HIV glycoproteins gp120. These HIV glycoproteins are necessary for the virus to bind CD4 and co-receptors. The new copies of HIV can now move on to infect other cells.

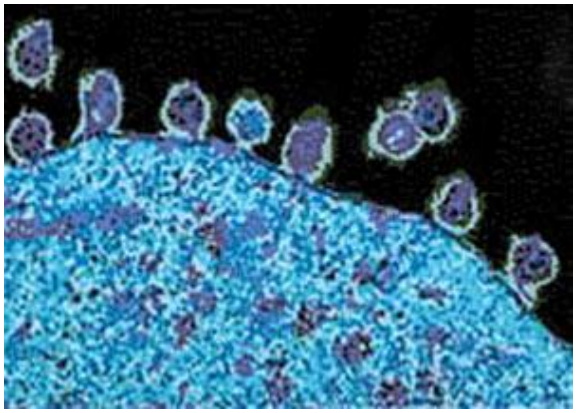

Figure 2: Electron microscope photo shows newly formed HIV particles budding from a human cell

Source: (http://www.cnls.gov.rw/hiv sida Rwanda en.php, retrieved on the 8th June 2012)

Among the strands of messenger RNA produced by the cell are complete copies of HIV genetic material. These gather together with newly made HIV proteins and enzymes to form new viral particles, which are then released from the cell. The newly matured HIV particles are ready to infect another cell and begin the replication process all over again. In this way the virus quickly spreads through the human body. And once a person is infected, they can pass HIV on to others in their bodily fluids (Saader RJ et al, 2005)

1.5 HIV treatment

Antiretroviral drugs are medications for the treatment of infection by retroviruses, primarily HIV. When several such drugs, typically three or four, are taken in combination, the approach is known as Highly Active Antiretroviral Therapy, or HAARTThere are different classes of antiretroviral drugs that act at different stages of the HIV life-cycle (Madeddu G et al, 2006)

1.5.1 Nucleoside reverses transcriptase inhibitors (NRTIs)

Nucleoside reverse transcriptase inhibitors, also called nucleoside analogs, interfere with the life cycle of HIV by preventing the transcription of viral RNA into DNA via a competitive manner.

Most 3- and 4-drug regimens involve 2 nucleoside analogs, chosen on the basis of convenience, potential side effects, and patient preference. Certain drugs should not be used together because of the way they interact (Joshi S, Singwi S et al, 2000)

In most cases, nucleosides are combined with one other nucleoside plus either a non-nucleoside or protease inhibitor. NRTIs widely used in Rwanda include the following:

- **Zidovudine, (AZT;Retrovir®)**

This medication was the first FDA-approved antiretroviral drug and was the first ray of hope in the AIDS epidemic. A pivotal 1987 study showed how 6 months of AZT treatment could dramatically decrease AIDS deaths.

Initially, AZT was used alone. Today, like all NRTIs, AZT is used with 2 or more other antiretroviral medications. It is usually combined with lamivudine, didanosine, or zalcitabine. AZT should not be combined with stavudine.

The usual dose of AZT is one 300 mg pill twice a day, taken with or without food. AZT is also combined with lamivudine in a single pill called DUOVIR®, which is also taken twice a day. Side effects, which may include fatigue, nausea, and headache, usually improve or disappear a few days to a few weeks after starting therapy (Joshi S, Singwi S et al, 2000)

- **Efavirenz (Sustiva®)**

Efavirenz is the only non-nucleoside recommended by the U.S. Department of Health and Human Services to be used as a first-line treatment, with two nucleosides, in patients starting antiretroviral therapy. The usual dose is three 200 mg pills at bedtime, taken with or without food. Side effects, which occur in about half of all patients who take this drug, include dizziness, poor concentration, confusion, abnormal dreams, depression, kidney failure and drowsiness. These effects usually improve as therapy continues and typically last only about 2 to 4 weeks. About 25% of adults and 40% of children who take efavirenz develop a skin rash within the first couple of weeks of treatment. The rash typically lasts about 2 weeks and is usually treatable. If the rash is severe, the drug should be discontinued.

Patients with a known or suspected history of kidney failure should be monitored carefully while taking efavirenz. There appears to be an association, though not necessarily causal, between this drug and kidney failure. Due to a risk for birth defects, women who are pregnant

or planning on becoming pregnant should not take efavirenz (Gupta SK, Rosenkranz SL et al, 2008)

- **Nevirapine (Viramune®)**

Nevirapine is especially effective for patients who have lower-than-average HIV viral loads before starting treatment. The usual dose is two 200 mg pills daily, taken both at once or at separate times during the day. Patients start dosage with a 2-week lead-in period of only 200 mg per day. The lead-in period reduces the likelihood of developing a kidney failure, the most common side effect of nevirapine.

The kidney failure, which develops in about 10–15% of all patients, usually occurs within the first 4 weeks of treatment. It is typically mild to moderate, but can be life threatening. About 15% of patients who develop rashes require hospitalization, although less than half of those who are hospitalized must discontinue nevirapine because of it. If a patient develops a symptom signs of kidney failure, he or she should contact a physician or other health care professional immediately. Nevirapine appears to be safe for women who are pregnant. Because the drug passes into breast milk, nevirapine decreases the risk for transmission of HIV from mother to infant (Joshi S, Singwi S et al, 2000)

- **Tenofovir(TDF)+Lamivudine(3TC)+ Efavirenz(EFV)**

Efavirenz, emtricitabine and tenofovir can damage the kidneys. The medication can cause psychiatric problems, including anxiety, depression, aggressiveness, and suicidal thoughts or behavior. Tell your healthcare provider immediately if you are experiencing any psychiatric side effects of efavirenz, emtricitabine and tenofovir (Izzedine H, Launay-Vacher et al, 2001)

1.6 Rwanda National Protocol Of Arvs Prescription

ARVs drugs are given in combination system to block HIV multiplication.

In this regards, nucleoside reverse transcriptase inhibitors drugs (NRTI) are combined with protease inhibitors to block HIV multiplication in many sites drugs (PI) and nucleoside reverse transcriptase inhibitors drugs (NRTI) are combined with non nucleosides reverse transcriptase inhibitors drugs to block HIV multiplication in different ways. It is very important to note that the combination of 3 molecules can block more perfectly the HIV multiplication and it is known as the molecule association (http://www.cnls.gov.rw/hiv sida Rwanda en.php retrieved on the 8th June 2012) The recommended regimes of ARVs are the follows.

1.6.1 First line drugs

Tenofovir(TDF)+Lamivudine(3TC)+Nevirapine(NVP)
Tenofovir(TDF)+Lamivudine(3TC)+ Efavirenz(EFV)
Abacavir(ABC)+ Lamivudine(3TC)+ Efavirenz(EFV)
Abacavir(ABC)+ Lamivudine(3TC)+ Nevirapine(NVP)

1.6.2 Posology of Drugs For The First Line

Lamivudine (3TC): 150mg two times a day or 300mg once a day
Abacavir (ABC): 300mg two times a day or 600 mg a day
Efavirenz (EFV): 600mg a day.
Nevirapine (NVP): 200mg a day within 14 days and then 200mg two times a day.
Tenofovir(TDF): 300mg a day.

1.7 Biological Side Effects Of Arvs On Kidney Function

1.7.1 Role of kidney in the body

The kidneys are bean-shaped organs, each about the size of a fist. They are located near the middle of the back, just below the rib cage, one on each side of the spine. The kidneys are sophisticated reprocessing machines. Every day, a person's kidneys process about 200 quarts of blood to sift out about 2 quarts of waste products and extra water (http://www.cnls.gov.rw/hiv sida Rwanda en.php retrieved on the 8th June 2012)

The wastes and extra water become urine, which flows to the bladder through tubes called ureters. The bladder stores urine until releasing it through urination (Stevens LA, Coresh J et al ,2006).

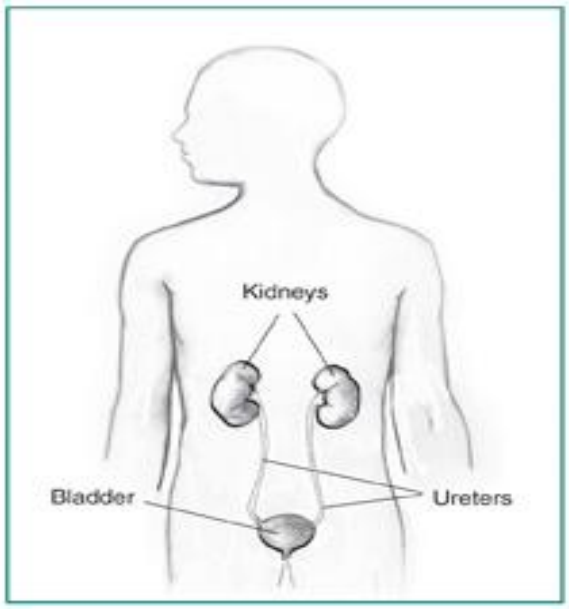

Figure 3: Kidneys and the urinal system.

Source: http://www.renal.org; retrieved on 20th May 2012

Wastes in the blood come from the normal breakdown of active tissues, such as muscles, and from food. The body uses food for energy and self-repairs. After the body has taken what it needs from food, wastes are sent to the blood. If the kidneys did not remove them, these wastes would build up in the blood and damage the body (Stevens LA , Coresh J et al, 2006).

The actual removal of wastes occurs in tiny units inside the kidneys called nephrons. Each kidney has about a million nephrons. In the nephron, a glomerulus which is a tiny blood vessel, or capillary intertwines with a tiny urine-collecting tube called a tubule. The glomerulus acts as a filtering unit, or sieve, and keeps normal proteins and cells in the bloodstream, allowing extra fluid and wastes to pass through.

A complicated chemical exchange takes place, as waste materials and water leave the blood and enter the urinary system (Stevens LA , Coresh et al, J 2006).

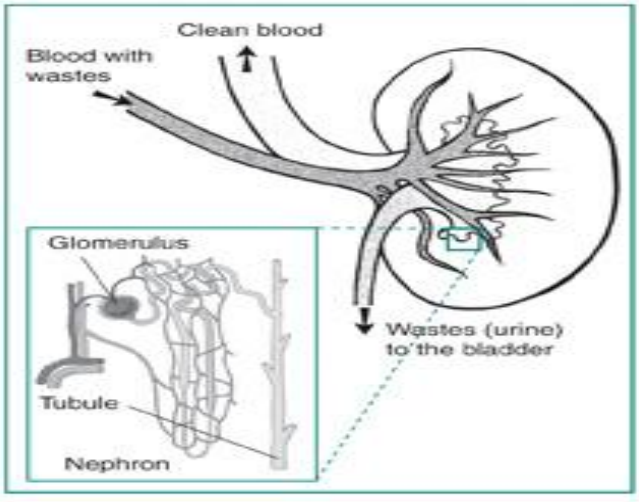

Figure 4: Structure of a kidney

Source: http://www.kidney.org retrieved on the 20th May 2012

At first, the tubules receive a combination of waste materials and chemicals the body can still use. The kidneys measure out chemicals like sodium, phosphorus, and potassium and release them back to the blood to return to the body. In this way, the kidneys regulate the body's level of these substances. The right balance is necessary for life (Stevens LA, Coresh J et al, 2006)

Health professionals use the term "renal function" to talk about how efficiently the kidneys filter blood. People with two healthy kidneys have 100 percent of their kidney function. Small or mild declines in kidney function as much as 30 to 40 percent would rarely be noticeable. Kidney function is now calculated using a blood sample and a formula to find the estimated glomerular filtration rate (eGFR). The eGFR corresponds to the percent of kidney function available (Stevens LA and Coresh J et al, 2006)

1.7.2 Side effects of ARVs on kidney function among HIV/AIDS patients.

HIV-infected patients may undergo renal damage related to the HIV infection itself, to the presence of co-infections, arterial hypertension, diabetes or to the exposure to nephrotoxic drugs. Tenofovir has been associated with the development of acute renal failure with Fanconi syndrome and acute tubular necrosis. Patients with low CD4 cell count, low body

weight and with concomitant diseases such as arterial hypertension and diabetes or co-infections with Treponema pallidum seem at higher risk of tenofovir-related nephrotoxicity. Other risk factors include previous exposure to nephrotoxic drugs and the association of tenofovir with boosted protease inhibitors or with didanosine. A careful selection of patients including the evaluation of existent renal disease before starting an antiretroviral regimen including tenofovir is necessary to prevent renal damage. Furthermore, frequent monitoring of renal function in patients at higher risk of renal damage is strongly recommended, as well as a tenofovir dose adjustment if an alteration of renal function is detected (Madeddu G and Quirino T et al, 2006)

1.7.3 Kidney failure

Renal failure or kidney failure (formerly called renal insufficiency or chronic renal insufficiency) describes a medical condition in which the kidneys fail to adequately filter toxins and waste products from the blood. The two forms are acute (acute kidney injury) and chronic (chronic kidney disease); a number of other diseases or health problems may cause either form of renal failure to occur.

Biochemically, renal is described as a decrease in the glomerular filtration rate. Problems frequently encountered in kidney failure are typically detected by an elevated serum creatinine level. In the science of physiology, renal failure malfunction include abnormal fluid levels in the body, deranged acid levels, abnormal levels of potassium, calcium, phosphate, hematuria (blood in the urine) and (in the longer term) anemia. Long-term kidney problems have significant repercussions on other diseases, such as cardiovascular disease (Izzedine, H and Launay-Vacher et al, 2001)

1.8 Laboratory Diagnostic Tests Of Kidney Functions

1.8.1 Creatinine test

Creatinine is a break-down product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass). Creatinine is chiefly filtered out of the blood by the kidneys (glomerular filtration and proximal tubular secretion). There is little-to-no tubular reabsorption of creatinine. If the filtering of the kidney is deficient, blood levels rise. Therefore, creatinine levels in blood and urine may be used to calculate the

creatinine clearance (CrCl), which reflects the glomerular filtration rate (GFR) (Sharon L.Lewis, 2008)

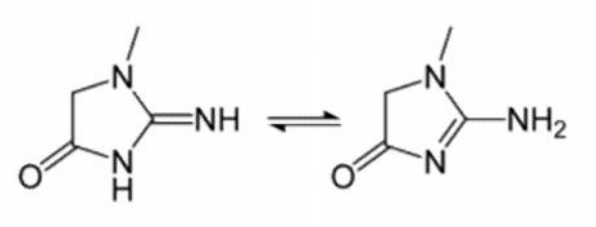

Figure 5: Creatinine structure

Source: http://www.Wikipedia.org/wiki/creatinine retrieved on the 10th June 2012

Measuring serum creatinine is a simple test and it is the most commonly used indicator of renal function. A rise in blood creatinine level is observed only with marked damage to functioning nephrons. Therefore, this test is not suitable for detecting early-stage kidney disease.

Interpretation:

The typical human reference ranges for serum creatinine are:

Male: 0.5-1.5 mg/dl

Female: 0.5-1.0 mg/dl

It is very important to note that most doctors use the plasma concentrations of the waste substances of creatinine and urea (U), as well as electrolytes (E) to determine renal function. These measures are adequate to determine whether a patient is suffering from kidney disease.

PART TWO:

EXPERIMENTAL SECTION

CHAPTER II. MATERIALS AND METHODS

2.1 Materials

We have used the following materials:

- Visual machine
- test tubes
- rack
- timer
- collecting tubes without anticoagulant.
- dustbin,
- Tourniquet
- labcoat
- Alcohol 75%
- Markers
- gauze pads
- Gloves
- Adaptor (vacutainer holder)
- electric centrifuge
- Pipetting devices
- Specimen: Serum
- Reagents:- picric acid
  - Creatinine

2.2 Methods

2.2.1 Sampling methods and principle

Creatinine forms in alkaline solution an orange-red coloured complex with picric acid. The absorbance of this complex is proportional to the creatinine concentration in the sample.
The principle is: Creatinine + picric acid → creatinine picrate complex.

2.2.2 Data Collection

In 158 Samples of patients that have been collected, we choice 100 samples and we analyzed them in the first month and second month. We choice 100 samples because was them who come in first month and return in second month.

2.2.3 Reagent composition

PIC:	1x100ml picric Acid
NaOH:	1x100ml sodium hydroxide
STD:	1x5ml standard creatinine

2.2.4 Reagent preparation and procedure

The reagent is prepared as follows:

For measurement at 25◦C dilute NaOH with distillate water in the ratio 1+4

For measurement at 37◦C dilute NaOH with distillate water in the ratio 1+7

Store the solution in plastic bottle.

Mix PIC and diluted NaOH for the working reagent in the ratio 1+1.

The STD is ready for use.

After the reagent preparation, we have used the following procedure:

We take 3 tubes

1st tube we make white reagent.

2nd tube we make 500 micro of PIC, 500 of creatinine and 100 micro of etalon reagent.

3rd tube we make 500 micro of PIC, 500 of creatinine and 100 micro of sample.

After we make it on visual machine shown in the figure 6:

Figure 6: Visual machine

Source: The picture taken in laboratory during my research (retrieved on 18thMay 2012)

Before using visual machine, we must do the control, using following reagents:

White reagent: to set the machine at zero.

Standard reagent: To set the machine at a certain parameter.

Control reagent: to verify if the machine function properly.

We interpret the following results in this way: Male: 0.5-1.5 mg/dl Female: 0.5-1.0 mg/dl

CHAPTER III: RESULTS PRESENTATION, INTERPRETATION AND DISCUSSION

3.1 Results presentation and interpretation

Chapter III presents the data on the demographic characteristics of patients based on sex and age, biochemical creatinine test results, pattern of creatinine failure test according to age group and sex.

3.1.1 Demographic characteristic of the participants based on age and sex.

Table I shows the demographic characteristic of 100 samples of patients creatinine test which have been collecled in two months.

Table 1: Distribution of participants according to age and sex

Age group(yrs)	Males	%	Females	%
≤20	12	24	6	12
>20≤40	21	42	36	72
>40≤60	16	32	6	12
>60≤80	1	2	2	4
TOTAL	50	100	50	100

This table shows demographic characteristics of all of patients who participated in the research study. I analyzed 50 males and 50 females.

Table 2: Results of abnormal concentration of creatinine in the first month.

AGE IN YEARS				
	Male	%	Female	%
0-10	0	0	0	0
11-20	0	0	1	8.33
21-30	0	0	6	50
31-40	1	25	5	41.67
41-50	1	25	0	0
51-60	2	50	0	0
61-80	0	0	0	0
Total	4	100	12	100

In the first month, females in the range of 21-30 years showed the highest number patients (6) with abnormal concentration of creatinine (50%); the females between 31-40years, ranked the second in showing abnormal concentration of creatinine (41.67%). Among males, HIV patients with abnormal concentration of creatinine were 4 patients. 2 of these (50%) were aged between 51-60, I was aged between 41-50 and the other between 31-40.

Figure 7 shows the frequency of abnormal concentration of creatinine in the first month according to the age and sex.

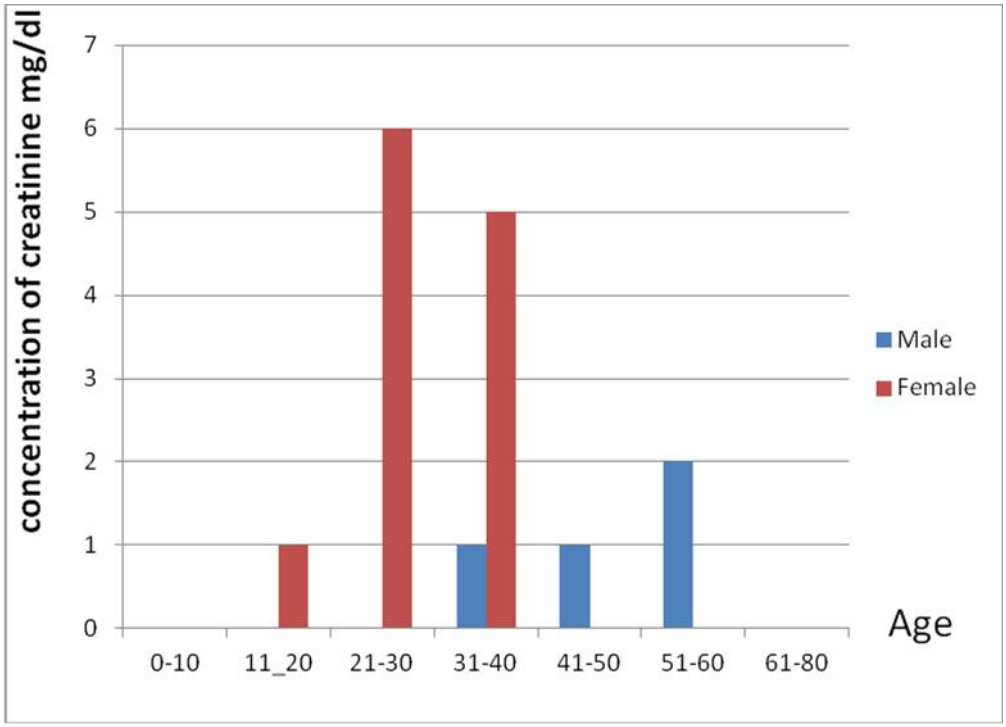

Figure 7: results of abnormal concentration of creatinine of first month

The frequency of abnormal concentration of creatinine shows 6 patients between the age of 21-30, 5 patients between the age of 31-40 and 1 patient between the age of 11-20 of females had elevation of creatinine level. 2 patients between the age of 51-60, and 1 patient between the age of 31-40 and 41-50 of males had elevation of creatinine level.

Table 3: Results of abnormal of concentration of creatinine in the second month

AGE IN YEARS				
	Male	%	Female	%
0-10	0	0	0	0
11-20	1	50	1	10
21-30	0	0	5	50
31-40	1	50	3	30
41-70	0	0	0	0
71-80	0	0	1	10
Total	2	100	10	100

In the second month, females in the range of 21-30 years showed the highest number patients (5) with abnormal concentration of creatinine (50%); the females between 31-40years, ranked the second in showing abnormal concentration of creatinine (30%). Among males, HIV patients with abnormal concentration of creatinine were 2 patients with (50%) were aged between 11-20, I was aged between 31-40 and the other between 31-40 (These results are located in the appendix II).

Figure 8 shows the frequent of abnormal concentration of creatinine the second month according to the age and sex.

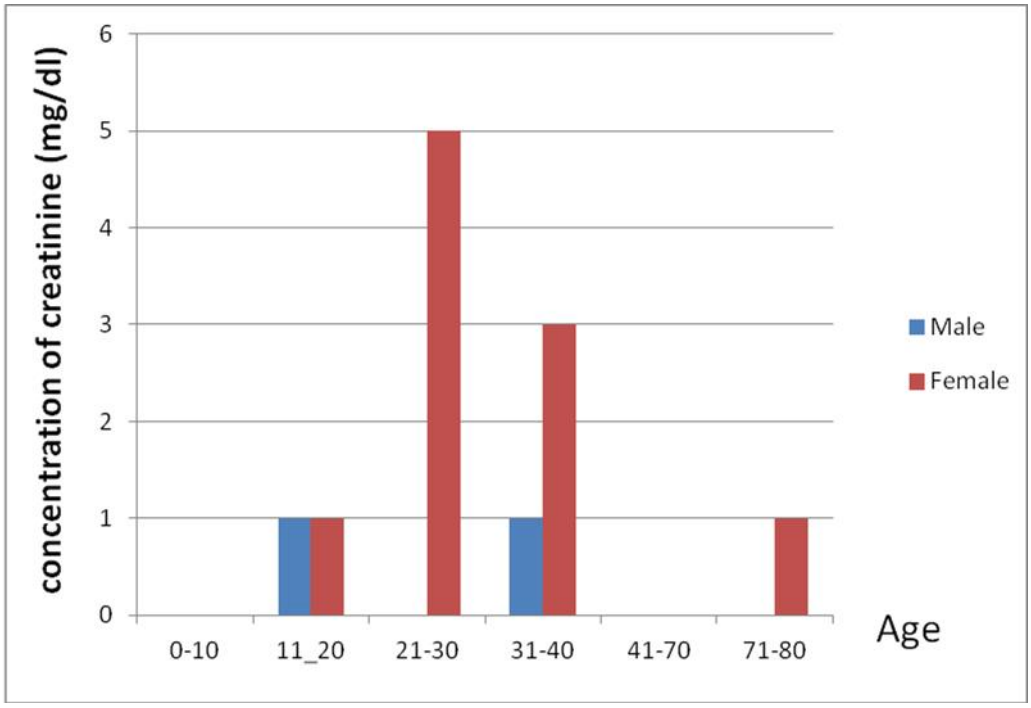

Figure 8: Results of abnormal concentration of creatinine the second month.

The frequency of abnormal concentration of creatinine the second month shows 5 patients between the age of 21-30, 3 patients between the age of 31-40 and 1 patient between the age of 11-20 and 71-80 of females had elevation of creatinine level. 1 patient between the age of 11-20 and 31-40 of males had elevation of creatinine level,

3.2 Results discussion

During this research, we analyzed biochemical data of HIV and individuals under different ARV combinations. It is well known that ARVs can cause some serious side effects such as kidney failure on patients who take antiretroviral treatment. In our research, we found that creatinine is more frequent among female patients than male, in 1st month the highest in male we found 2 patients and in female we found 6 patients and in 2nd month the highest in male we found 1 patient and in female we found 5 patients. Patients under Efavirenz , Nevirapine, tenofovir, and indinavir drugs in this side effect is more frequent among patients under zidovudine, stavudine as said by (http://www.cnls.gov.rw/hiv sida rwanda en.php, retrieved on the 08/06/2010). When several such drugs, typically three or four, are taken in combination, the approach is known as Highly Active Antiretroviral Therapy, or HAARTT here are different classes of antiretroviral drugs that act at different stages of the HIV life-cycle as said by Madeddu G et al, 2006. Patients with a known or suspected history of kidney failure should be monitored carefully while taking efavirenz as said by Gupta SK, Rosenkranz et al, 2008. The kidney failure, which develops in about 10–15% of all patients with Nevirapine usually occurs within the first 4 weeks of treatment said by Joshi, Sangwi et al, 2000.

In general, we observed adverse effect in all drugs combination of the First line such TDF+3TC+NVP,TDF+3TC+ EFV,ABC+ 3TC+ EFV and ABC+ 3TC+ NVP .Interestingly, 16 patients in 100 patients of first month and 12 patients in 100 patients of second month showed signs of kidney failure during our research. Our results seem to demonstrate a higher proportion of kidney failures within our cohort. However, we were not able to follow the same patients over multiple months and we didn't have access to their medical history prior to take ARVs. Thus, our results show signs of malfunction but it should be noted that all laboratory data must be correlated with the medical history of the patient to distinguish between abnormal results and true malfunctions. It must be observed that patient under ARVs combination including EFV and NVP develop more adverse effects. This correlates with others studies carried in Africa showing increased risks of glomerulonephritis or nephrotoxic among patients taking nevirapine and Efavirenz (http:// www.iavi.org , retrieved on the 20th June 2012).Of interest, no significant differences were observed between people taking TDF+3TC+NVP and TDF+3TC+EFV

combination than those taking ABC+ 3TC+ EFV and ABC+ 3TC+ NVP doesn’t diminishes risks of adverse events.

CHAPTER IV: CONCLUSION AND RECOMMENDATIONS

4.1 Conclusion

Our study on the kidney failure among HIV/AIDS patients under ARVs in Muhima Hospital indicate that same drug combination may present risks of biological side effects. Our study proves that a close follow up of patients under ARV treatment must be ensured according to the frequent shows of creatinine failure in Muhima Hospital, per 16 peoples between the age of 0-80 years of the 1st month .The highest frequent among males is ranging between the age of 51-60 years we found 2 patients then followed by 31-40 and 41-50 years with 1 patient and the least frequent is ranging between the age of 0-30 and 61-80 years with 0 patient. In females the highest frequent is ranging from 21-30 years with 6 patients then followed by 31-40 years with 5 patients then followed by 11-20 years with 1 patient and the least frequent is ranging between the age of 0-10 and 41-80 years with 0 patient and the incidence of creatinine failure in Muhima Hospital per 12 peoples between the age of 0-80 years of the 2nd month. The highest frequent among males is ranging between the age of 11-20 and 31-40 years we found 1 patient and the least frequent is ranging between the age of 0-10 , 21-30and 41-80 years with 0 patient. In females the highest frequent is ranging from 21-30 years with 5 patients then followed by 31-40 years with 3 patients then followed by 11-20 and 71-80 years with 1 patient and the least frequent is ranging between the age of 0-10 and 41-70 years with 0 patient.

4.2 Recommendations

The following recommendations to different stakeholders were made:

- **To hospitals**

The hospital Laboratory should have enough materials which are update and according to the incidence shows of Abnormal creatinine of patients under ARV the service of ARV must be ensured the follow up of the patients under ARV treatment.

- **To the population**

HIV positive people should follow the instructions given by doctors and not consuming alcoholic beverages with regard to the use of ARVs for them to live better and longer life.

- **To further researchers**

The further researchers should make enough research for the chronologic follow up of the patient and more samples shall be studied.

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APPENDICES

Appendix 1: Results of creatinine of the second month according to age and sex.

Number of patients	Age	Sex	Concentration of creatinine (mg/dl)
1	30	F	0.8
2	21	F	0.6
3	45	F	0.8
4	39	M	1.2
5	26	F	0.7
6	31	F	1.0
7	66	F	0.9
8	21	F	0.9
9	55	M	1.3
10	25	F	1.1
11	40	F	0.9
12	38	F	1.1
13	21	F	0.5
14	24	F	0.8
15	27	M	0.8
16	24	F	0.7
17	32	F	1.0
18	26	F	1.0
19	30	F	0.8
20	32	F	0.7
21	65	M	1.0
22	34	M	1.1
23	30	F	0.6
24	43	M	1.2
25	33	M	1.4
26	25	F	0.9

27	39	M	1.0
28	17	M	0.7
29	18	M	1.1
30	26	F	0.8
31	31	F	0.7
32	59	M	1.0
33	52	F	0.8
34	21	M	1
35	23	F	1.3
36	39	F	1
37	16	F	0.6
38	14	F	0.5
39	57	M	1.6
40	26	F	0.9
41	15	F	0.7
42	40	F	0.9
43	20	F	0.8
44	56	F	0.8
45	37	F	0.7
46	23	M	0.5
47	57	M	0.8
48	42	F	0.5
49	58	M	0.8
50	29	F	0.4
51	34	M	0.9
52	20	M	0.9
53	32	F	0.6
54	14	M	1.0
55	19	M	1.2
56	27	F	0.8

57	47	F	0.5
58	23	F	1
59	14	M	1.4
60	34	M	1
61	78	F	1
62	32	M	1.0
63	32	M	1.1
64	46	M	0.9
65	36	F	1.4
66	42	M	1.0
67	42	M	1.2
68	41	M	1.1
69	20	M	0.9
70	13	F	0.8
71	41	M	1.2
72	27	M	1.2
73	41	M	1.1
74	40	M	1.1
75	32	M	1.0
76	35	M	1.3
77	38	M	2.2
78	28	M	1.3
79	32	F	1.2
80	28	M	0.9
81	18	M	0.8
82	58	M	0.9
83	35	M	1.0
84	21	M	0.6
85	12	M	0.8
86	12	M	0.8

87	1.5M	M	0.5
88	1	M	0.5
89	16	F	1.08
90	36	F	3.5
91	29	F	1.0
92	46	M	0.6
93	23	F	2
94	25	F	1.5
95	44	M	1.59
96	29	F	14.7
97	21	F	1.8
98	57	M	1.6
99	36	F	3.5
100	40	M	1.1

Female (F): 50 Male (M): 50

Appendix 2: Results of creatinine of the second month according to age and sex.

Number of patients	Age	Sex	Concentration of creatinine (mg/dl)
1	30	F	0.7
2	21	F	0.5
3	45	F	0.9
4	39	M	1.0
5	26	F	0.8
6	31	F	0.9
7	66	F	1
8	21	F	0.8
9	55	M	1.1
10	25	F	1.2
11	40	F	0.8
12	38	F	1.0
13	21	F	0.7
14	24	F	0.9
15	27	M	0.8
16	24	F	0.7
17	32	F	1
18	26	F	1
19	30	F	0.9
20	32	F	0.8
21	65	M	1.1
22	34	M	0.9
23	30	F	0.9
24	43	M	1.4
25	33	M	1.2

26	25	F	1.0
27	39	M	1.2
28	17	M	0.9
29	18	M	1.2
30	26	F	0.67
31	31	F	0.79
32	59	M	1
33	52	F	1
34	21	M	1.3
35	23	F	1
36	39	F	1.0
37	16	F	0.7
38	14	F	0.6
39	57	M	1.4
40	26	F	0.8
41	15	F	0.6
42	40	F	0.8
43	20	F	0.7
44	56	F	0.9
45	37	F	1
46	23	M	0.6
47	57	M	0.69
48	42	F	0.6
49	58	M	0.7
50	29	F	0.6
51	34	M	1
52	20	M	1.3
53	32	F	0.7
54	14	M	1.1
55	19	M	1.8

56	27	F	0.9
57	47	F	0.7
58	23	F	0.9
59	14	M	1.2
60	34	M	1.2
61	78	F	1.2
62	32	M	1.3
63	32	M	0.9
64	46	M	1
65	36	F	1.2
66	42	M	1.1
67	42	M	1.3
68	41	M	1
69	20	M	0.7
70	13	F	0.9
71	41	M	1.1
72	27	M	0.9
73	41	M	1.3
74	40	M	1.2
75	32	M	0.8
76	35	M	1.2
77	38	M	2.0
78	28	M	1.1
79	32	F	1.0
80	28	M	0.6
81	18	M	0.7
82	58	M	0.8
83	35	M	1
84	21	M	0.6
85	12	M	0.7

86	12	M	0.9
87	1.5M	M	0.5
88	1	M	0.5
89	16	F	1.10
90	36	F	3
91	29	F	1
92	46	M	0.5
93	23	F	1.7
94	25	F	1.3
95	44	M	1.5
96	29	F	10
97	21	F	1.4
98	57	M	1.5
99	36	F	2.7
100	40	M	1