AN INVESTIGATION ON THE EFFECTS OF TENOLAM ON KIDNEY FUNCTION IN HIV POSITIVE PATIENTS.

By

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ABSTRACT

The study was carried out to determine the effect of Tenolam drug on kidney function in HIV positive patients at Nkayi District Hospital. The study was done from June 2016 to October 2016. This was done by analysing the change in urine protein concentration after the patients were given the drug. A total of thirty individuals were recruited in the study, fifteen females and fifteen males. Age, gender and CD4 counts of the patients were recorded before tenolam administration. Each patient collected their midstream urine samples and the samples were tested for protein concentration using a protein strip. Sample testing was done three times, at baseline, two months on tenolam treatment and four months on tenolam treatment. At baseline all the patients had no protein in their urine and the urine protein concentration had a significant increase from baseline to two months being on Tenolam drug (t-test p=0.00). The urine protein concentration increased slightly from two months and four months after Tenolam administration (t-test p=0.56). There was a significant increase in urine protein concentration from baseline to four months after tenolam administration (t-test p=0.00). The mean protein concentration at two months after tenolam administration was 70cells/µl and the mean change after four months was 120cells/µl. Gender, sex and Tenolam treatment were the risk factors in the study. Gender did not have a significant effect on the urine protein concentration during the study (anova p=0.632). Age had a significant effect on the urine protein concentration (anova p= 0.012) and Tenolam administration had a significant effect on the urine protein concentration (anova p= 0.00). Therefore Tenolam administration causes the urine protein concentration to increase and this indicates that it alters kidney function.

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DEDICATION

This work is dedicated to my grandmother and son (Mhaka Junior Maundura II)**.**

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CHAPTER 1 : INTRODUCTION

1.1 Background

Human Immunodeficiency Virus (HIV) infection results in the Acquired Immune Deficiency Syndrome (AIDS). The human immunodeficiency virus, once in the body targets the cells of the immune system (Weiss, 1993). If it is not treated at all, the virus can destroy the function of the body fighter cells.

As the immune system is destroyed it becomes difficult for the infected person to fight against any kind of infection that attacks them. Acquired immune deficiency syndrome is a condition that refers to a collection of symptoms and illnesses that occur if an HIV-positive person`s immune system is destroyed such that it cannot fight against the illnesses (Weiss, 1993). Immediately after being infected with the virus there are no signs or evidence of the disease but as the immune system becomes weaker skin problems and upper respiratory tract infections start to develop (Weiss, 1993). In due course, the patient starts to lose weight and experience chronic diarrhoea, persistent fever, fungal or bacterial infections and tuberculosis may follow (Weiss, 1993). The immune system of the patients becomes weak in such a way that it loses its ability to fight against other infections and this later leads to immune deficiency (Weiss, 1993).

HIV infections mostly result from semen, vaginal fluids, anal secretions, and blood or breast milk from an infected person entering an uninfected person’s blood stream (Vernazza, Eron, Fiscus and Cohen, 1999). The virus type that is mainly responsible for the global pandemic is the HIV-1; HIV-2 is not easily transmitted and is confined mainly to West Africa (DeCock, Adjorlo and Ekpini, 1993). The virus can be acquired in a number of ways, from a mother to her baby, parenteral transmission and sexual transmission (Vernazza *et al*., 1999).

Sexual transmission is the most common mode of transmission of the HIV worldwide and the chances of a person to be infected depends on the likelihood of having unprotected sexual intercourse with an infected person (Merson, Dayton and Reilly, 2000). It depends on the state of the infected partner, if the infected person has got high viral loads which are in the later stages of the disease then they have got higher or increased chances of transmitting the HIV to the other partner (Vernazza *et al*., 1999). The presence of a sexually transmitted infection can also enhance the transmission of HIV (Vernazza *et al.*, 1999).

Mother to child transmission is answerable for more than 90% of the infections worldwide (UNIAIDS, 2000). Most of the mother to child transmissions occur in the uterus and only a third of the transmissions occur at birth and during breast feeding (UNIAIDS 2000). A total of 5.1 million children have been infected with HIV worldwide (UNIAIDS, 2000).

Parenteral transmission is when there is direct contact of blood between infected and non-infected people which later leads to infection (Merson *et al.*, 2000). It occurs mainly as a result of sharing non-sterile drug use equipment (Merson *et al*., 2000). It may also occur by the transfusion of infected blood and by using contaminated needles for injections and needle stick injuries among health professionals and infected patients (Merson *et al.*, 2000).

HIV/AIDS is an infectious condition that has spread through human populations worldwide, in 2014, 36.9 million people were living with the virus across the world (DeCock *et al*., 1993). In 2012, 17.2 million people were men, 16.8 million people being women and 3.4 million being people less than 15 years of age (UNIAIDS/WHO, 2000). From the total of 36.9 million people living with HIV, 95% of these people live in developing countries which have a few resources to deal with the pandemic (UNIAIDS, 2000).

Sub-Saharan Africa is the region which is most affected by the virus. It has 68% of all the HIV cases in the world and 66% of all the deaths were a result of the pandemic in 2010 (UNIADS / WHO, 2000). It also had about 5% of its total adult population infected with the virus (UNIAIDS/ WHO 2000). The region has about 10% of the world`s population but more than 60% of the world`s HIV cases (UNIAIDS, 2010).

Women are more infected than men due to the patterns of sexual behaviour. Women tend to have relationships with older men at their tender age (Glynn, Carael and Bure, 2000). The other reason is that women resort to being sexual workers in search of money to look after their families thus giving them an extremely high infection rate in some parts of non-industrialised countries (Glynn *et al*., 2000).

In Zimbabwe 1.4 million people are living with the HIV including 170000 children (NDTPAC, 2010). These values equate to 4% of the global total and the HIV prevalence in the age groups of 15 to 49 years old was 13.71% (NDTPAC, 2010). In 2011 a total of 720000 women are living with the virus of which 70000 are pregnant and this has led HIV to be the most cause of maternal mortality in Zimbabwe (NDTPAC, 2010).

During the first days of the awareness of AIDS patients were not expected to live for more than a year or two with the human immunodeficiency virus (Schwartz and Nair, 1999). Scientists have now developed drugs that can help the infected people to live longer by controlling and minimising the effect of the virus on the patient`s immune system thus they can now live longer and healthy.

There is no cure for the acquired immunodeficiency syndrome but medications have been highly effective in fighting HIV and its complications (Schwartz and Nair, 1999). The HIV drug treatments that have been developed help reduce the virus in one`s body. They keep the immune system at a healthy state as much as possible and also decrease the complications that occur as a result of the infection (Schwartz and Nair, 1999). The availability of antiretroviral drugs has significantly decreased HIV related deaths and increased survival periods of the people living with HIV.

More than 20 antiretroviral drugs have been approved by the Food and Drug Administration Agency (UNIAIDS/ WHO, 2006). The drugs cannot fully eliminate the virus from the body system therefore a lifetime treatment must be administered so as to keep the viral load at a minimum (WHO, 2006). Antiretroviral therapy maintains the suppression of viral replication as a result the virus cannot multiply and attack the body`s fighter cells (WHO, 2006).

Antiretroviral therapy reduces the incidence of HIV/AIDS as well as the number of people dying from the virus but there are challenges that may arise with the therapy (Rudolf and Kirikorlan, 2005). Antiretroviral therapy may fail to supress the viral load of an individual, the virus may develop resistance against the drugs and the medications may cause adverse effects on the patients (Rudolf and Kirikorlan, 2005). Individuals respond differently to the cocktail of drugs given and this results in side effects that later affect the patients. These side effects may cause more harm to the patients if left unattended but can be manageable and controlled if they are noticed and treated early (Coffin, Haase and Levey, 1996).

The diagnosis of HIV infection is no longer a death sentence it used to be in the 1980s. People with the virus now live longer and better lives, thanks to the Antiretroviral Therapy. These medications can be bright spot for someone with HIV as they help stop the virus from attacking the body, boost fighter cell numbers and prolong the life of HIV infected people.

The infection still presents difficulties in that even if the HIV medications are mostly effective and the disease is relatively manageable, there are side effects which come with the use of the medication. People with HIV have a higher risk of developing kidney disease either as a result of infection itself or as a side effect from the cocktail of HIV medications that they are given (Gupta, Eustace, Winston, Boydstun, 2005). Therefore patients end up suffering and dying from the side effects. Kidney disease is treatable but it can be more difficult to manage in someone who already has HIV. HIV raises the risk of having kidney disease. It is estimated that up to 30% of people who have HIV also have some form of kidney disease (UNIAIDS/WHO, 2010).

Medications such as adefovir and tenofovir which can be given singly or in combination affect the kidneys` filtration system. Such medications may crystalize inside the kidneys` drainage system in some patients and this leads to the development of kidney stones or kidney disease (Abby and Cartlin, 2010). Patients given this drug have a tendency of developing kidney malfunction in due course after the administration of the drug.

1.2 Justification of the study

No work has been done to investigate the effect of HIV drugs on the health of the patients in most rural areas. Most of the antiretroviral therapies given to patients are hard on their kidneys (Abby and Cartlin, 2010). Therefore it is necessary for clinical follow up tests to be done on patients after they are given the drugs.

There is no screening for kidney damage in patients taking the drugs in clinics and hospitals in Nkayi. Kidney problems do not really show up as symptoms of diseases therefore it is important to get the composition of urine checked in order to detect any kidney malfunctions. If the urine composition includes proteins then it is an indication that the kidneys are not doing well.

Kidney damage related to antiretroviral therapy is reversible with early recognition and timely discontinuation of the drug. Therefore kidney screening should be done in hospitals so as to note any kidney function changes as a result of Tenolam administration on HIV positive patients.

1.3 Objectives

1.3.1 Main Objective:

- to determine the effects of lamivudine, tenofovir and efavirenz treatment which is marketed as Tenolam on kidney function in HIV positive adults aged between 18-80 years attending the opportunistic infection clinic at Nkayi District Hospital.

1.3.2 Specific Objectives:

- to determine the effect of Tenolam on urine protein concentration of patients after Tenolam administration,
- to determine the effect of age and gender on urine protein concentration of patients taking Tenolam, and
- to evaluate the extent of the safety and continual use of Tenolam drug for HIV treatment in Zimbabwe.

CHAPTER 2 : LITERATURE REVIEW

2.1 Antiretroviral therapy

When AIDS was first recognised, patients with the disease were unlikely to live longer than a year or two (Schwartz and Nair, 1999). Drugs that can help people infected with HIV to live longer and healthier lives have been developed by scientists. HIV is treated using a combination of medicines to fight HIV infection, this is called antiretroviral therapy (ART) (Schwartz and Nair, 1999). ART does not cure HIV but it can control the virus so that one can live a longer, healthier life and reduce the risk of transmitting HIV to others (Schwartz and Nair, 1999). These HIV medicines prevent HIV from multiplying which reduces the amount of HIV in the body. Having less HIV in the body gives the immune system a chance to recover and fight off infections. HIV medicines also reduce the risk of transmitting the virus to others (Schwartz and Nair, 1999).

Eradication of HIV infection has proved to be difficult to find, therefore lifelong treatment must be administered to combat viral replication. HIV medications are in the form of Highly Active Antiretroviral Therapy (HAART) and they are effective at suppressing viral replication (WHO, 2006). HAART reduces the occurrence of HIV and mortality due to HIV and AIDS and also improves the quality of life of people living with HIV and AIDS (Schwartz and Nair, 1999). ART is recommended for all individuals with HIV, regardless of how long they have had the virus or how healthy they are.

Despite significant advances in HAART during the past few years, challenges such as virologic and immunologic failures, adverse effects of antiretroviral medications, drug-drug interactions and viral resistance, still pose significant obstacles to successful treatment (Rudolf and Krikortan, 2005).

There are more than 20 antiretroviral drugs which belong to six classes according to how they fight HIV (WHO, 2006). The six drug classes are: non-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleoside reverse transcriptase inhibitors (NRTIs), protease inhibitors (PIs), fusion inhibitors, CCR5 antagonists (also called entry inhibitors) and intergrase strand transfer inhibitors (INSTIs) (WHO, 2006).

2.1.1. Nucleoside reverse transcriptase inhibitors (NRTIs)

The NRTIs were the first agents available for treatment of HIV infection (Shen *et al.,* 2008). They interrupt the HIV replication cycle using competitive inhibition of HIV reverse transcriptase and termination of the DNA chain (Elion and Witt, 2003). NRTIs are structurally similar to the DNA nucleosides bases and become incorporated into the pro-viral DNA chain and this results in the stopping of the pro-viral DNA formation (Elion and Witt, 2003). A total of nine drugs make up the NRTIs class and these include: Stavudine (d4T), Lamivudine (3TC), Zidovudine (AZT), Tenofovir (TDF), Abacavir (ABC), Didanosine (ddI) and Emitricitabine (FTC) (WHO, 2006). Tenofovir, lamivudine and emtricitabine exhibit activity against hepatisis B virus in addition to HIV and are frequently incorporated into antiretroviral regimens for patients with HIV and HBV coinfection (Guideline panel on Antiretroviral, 2015).

2.1.2 Non-nucleoside reverse transcriptase inhibitors (NNRTIs)

NNRTIs were introduced in 1996 with the approval of neverapine. These exhibit potent activity against HIV-1 and are part of preferred initial regimens (Shen *et al.,* 2008). HIV reverse transcriptase is a hetero dimer composed of two sub units, p66 and p51 (Sluis-Cremer Temiz and Bahar, 2004). NNRTIs cohere to the p66 sub unit at a hydrophobic pocket away from the active site of reverse transcriptase enzyme. This non-competitive binding causes a conformational change in the enzyme that alters the active site and limits its activity (Sluis-Cremer *et al*., 2004).

2.1.3. Protease inhibitors (PIs)

HIV protease inhibitors were introduced in 1995 and they constitute part of the treatment of HIV infection (Guidline panel for Antiretroviral, 2015). HIV protease is a 99 amino-acid protein and is accountable for maturation of virus particles late in the viral life cycle. HIV protease cleaves individual proteins from the polypeptide precursors into functional sub units for viral capsid formation (Flexner, 1998). PIs block the enzyme protease thereby preventing the assembly and release of HIV particles from infected cells (Flexner, 1998). Examples of PIs are: Lopinavir (LPV), Atazanavir (ATV), Indinavir (IDV), Saquinavir (SQV), Ritonavir (RTV), Darunavir, Fosamprenavir and Tipranavir (WHO, 2006).

2.1.4 Fusion inhibitors (FIs)

Fusion inhibitors were the first class of antiretrovirals to target the HIV replication cycle extra cellularly and received FDA approval in 2003 (WHO, 2006). FIs act outside the targeted cell to prevent the fusion of HIV to the CD4 or other target cells. They block the second step in the fusion pathway by binding to the region of glycoprotein 41(Weissenham *et al.,* 1997). Thus, they interfere with the virus' ability to fuse with the cellular membrane proteins thereby blocking entry into the host cell (Weissenham *et al.*, 1997). The only FI approved by FDA is Enfuvirtide (WHO, 2006).

2.1.5 Integrase inhibitors (IIs)

Intergrase inhibitors target HIV's integrase protein thereby blocking its ability to integrate its genetic code into human cells (Craigie, 2001). HIV integrase is responsible for the transport and attachment of pro-viral DNA to host-cell chromosomes, allowing transcription of viral proteins and subsequent assembly of virus particles (Craigie, 2001). The IIs competitively inhibit the strand transfer reaction by binding metallic ions in the active site (Hazuda *et al.,* 2000). The only drug in this class approved by FDA is Raltegravir (Deeks *et al.*, 2008).

2.1.6 Chemokine receptor 5 antagonists (CCR5 Inhibitors)

These drugs block the CCR5 co-receptor that HIV uses to enter and infect the cells. The only drug in this category approved by FDA is Maraviroc (Lieberman-Blum, Fung and Bandres, 2008). Maraviroc is a small molecule that selectively binds the CCR5 coreceptor, blocking the V3 loop interaction and inhibiting fusion of the cellular membranes (Lieberman-Blum, Fung and Bandres, 2008).

These six classes of ARV drugs are used as first-line regimen and second-line regimen. Drugs which are used to start HAART consists of the first line regimen (WHO, 2006). The first-line regimen for must contain two NRTIs plus one NNRTI (WHO, 2006). The durability and efficacy of any first-line regimen is important when a treatment plan is being designed (WHO, 2006).

If the first line regimen has failed, the second line regimen can be used (WHO, 2006). The second line regimens are determined by the composition of the failed first line regimen therefore there are a number of second line regimens (WHO, 2006). Individuals who do not respond well to the first line regimen are treated with another regimen with drugs that were not part of the first regimen (WHO, 2006). The recommended second-line regimen should contain two NRTIs plus one PI.

2.2 Antiretroviral Therapy in Zimbabwe

AIDS was declared as an emergency by the Government of Zimbabwe in 2002, in-order to mobilise and increase the efforts of making the treatment of AIDS a reality (Guidelines for Antiretroviral Therapy in Zimbabwe, 2010). The development of generic formulation of ARVs globally made it possible for the Ministry of Health and Child Welfare to establish systems to introduce ART in Zimbabwe. Opportunistic Infection (OI) clinics were established in preparation for the introduction of ART in 2003.

The national ART programme in Zimbabwe was initiated in April 2004, to all central and provincial hospitals. The programme has now expanded to mission and district hospitals (Guidelines for Antiretroviral Therapy in Zimbabwe, 2010). The ARV drug classes on offer in Zimbabwe are NNRTs, PIs and NRTIs, therefore the ARV regimens in use in Zimbabwe are a combination of NRTIs, NNRTIs and PIs (Guidelines for Antiretroviral Therapy in Zimbabwe, 2010). The first line regiments are as follows:

- Tenofovir (300mg), Efavirenz (600mg) and Lamivudine (300mg),
- Lamivudine (150mg), Nevirapine (200mg) and Zidovudine (300mg),
- Tenofovir (300mg), Lamivudine (300mg) and Nevirapine (200mg). This is the preferred first-line regimen in Zimbabwe, and
- Stavudine (30mg), Lamivudine (150mg) and Nevirapine (200mg).

The second-line regimens which are currently in use in Zimbabwe for adults and adolescents are as follows:

- if the first-line regimen contained Tenofovir, the second-line regimen should contain Lamivudine (150mg), Zidovudine (300mg) and Lopinavir,
- if the first-line regimen contained Zidovudine or Stavudine, the second-line regimen should contain Tenofovir (300mg), Lopinavir and Lamivudine (150mg), and
- Didanosine (400mg, Abacavir (300mg) and Lopinavir.

2.3 Complications of Antiretroviral Therapy

For many people, the diagnosis of HIV is no longer the seemingly death sentence it used to be in the 1980s. HIV medications are effective and the disease is relatively manageable, but the complications or side effects associated with them have presented a great challenge to the implementation of antiretroviral therapy (Rudolf and Krikorlan, 2005).

The development of side effects in patients on ART can lead to clinically significant diseases and these result from non-adherence to antiretroviral drugs by patients (Rudolf and Krikorlan, 2005). Diagnosis and management of the side effects must be practised by care providers in order to provide optimal long term care to their patients (WHO, 2006).

When a complication has developed it is advisable to assess the extent of the complication and withdraw the drug. The patient would then be given an alternative first-line regimen (WHO, 2006). The second line regimen is used only if all the first line regimens have been tried. ARV side effects are divided into four grades according to the extent of the complication (Rudolf and Krikorlan, 2005).

- Grade 1: Side effects which last for only a short time. In such cases no medical intervention is required so the regimen is not changed,
- Grade 2: Side effects in which there is mild to moderate limitation to activity, minimal medical intervention is required, and
- Grade 3: Side effects have got severe limitation to activity and medical therapy is required.
- Grade 4: Side effects are life-threatening and significant medical intervention is required (Rudolf and Krikorlan, 2005).

2.4 Tenolam

Tenolam E tablet is indicated for the treatment of HIV infection, hepatitis B virus and other conditions. Tenolam is a drug composed of three different tablets which are lamivudine (300mg), tenofovir (300mg) and efavirenz (600mg). It is available in tablet form (Gilead Sciences, 2012). It improves the patient`s condition by, inhibiting the replication of HIV cells, increasing the number of infection fighting cells in the body and blocking the activity of the viral system (Gilead Sciences, 2012).

2.4.1 Tenofovir

It is used in combination with other antiviral drugs in treatment of human immunodeficiency virus infections. It may also be used in very limited cases of long term infections of the liver caused by hepatitis B virus (Viread, 2015). Tenofovir belongs to the class nucleoside reverse transcriptase inhibitors and its structure closely resembles the natural structure of viral DNA (Elion and Witt, 2003). This helps it to incorporate itself into the viral DNA. By so doing it blocks the replication of viral DNA, a process essential for the survival of the virus (Viread, 2015).

2.4.2 Lamivudine

Lamivudine belongs to a class of medication called reverse transcriptase inhibitors. It decreases the amount virus in the blood and also increases the amount of infection fighting cells in the body (Elion and Witt, 2003). It reduces the chances of getting acquired immunodeficiency syndrome and HIV related infections.

2.4.3 Efevirenz

Efevirenz belongs to a class of antiretroviral medications called non-nucleoside reverse transcriptase inhibitors. It inhibits the replication of HIV, thereby reducing the amount of the virus in the blood (Elion and Witt, 2003).

2.5 Kidneys

Kidneys are the body`s filtering system, they remove toxins and excess fluids in the body (Thomas, 2005). The fluid eventually leaves the body through urination. The kidneys contain millions of nephrons which are tiny units made up of blood vessels and fluid-collecting tubes (Thomas, 2005). Kidneys are two bean shaped organs located just below the rib cage one on either side of the spine. They serve several essential regulatory roles in vertebrates.

The main function of the kidneys is to regulate the balance of electrolytes in the body, along with maintaining pH homeostasis (Walter and Boron, 2004). They also remove excess organic molecules from the blood, it is by this action that their best known function which is the removal of waste products of metabolism is performed (Walter and Boron, 2004). Kidneys serve the body as a natural filter of the blood and remove water soluble wastes which are diverted to the bladder. They are also responsible for the reabsorption of water, glucose and amino acids (Thomas, 2005).

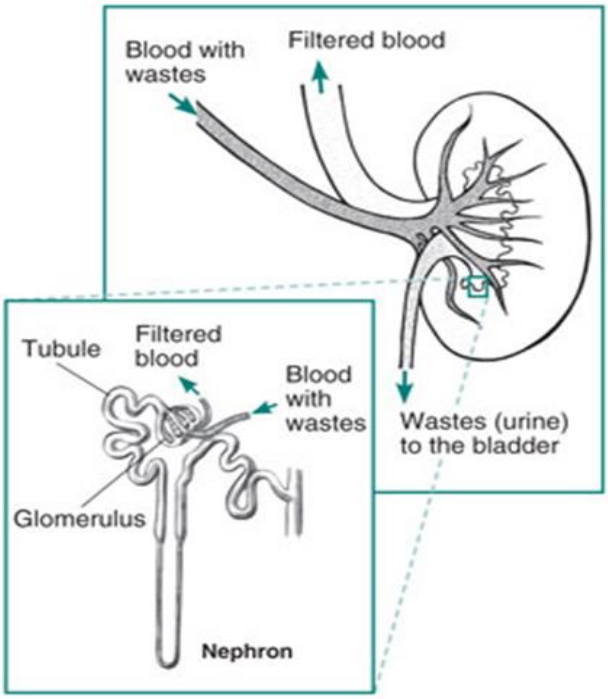

Figure 2.1: Structure of kidneys (Walter and Boron, 2004).

The renal circulation supplies blood to the kidneys via the renal arteries which branch directly from the abdominal aorta into segmental arteries (Walter and Boron, 2004). These penetrate the renal capsule and extend through the renal columns between renal pyramids.

The interlobar arteries then supply blood to the arcuate arteries that run through the boundary of the cortex and the medulla. Each arcuate artery supplies several interlobular arteries that feed into the afferent arterioles that supply glomeruli (Walter and Boron, 2004).

When blood enters the glomeruli it is filtered. Filtration is a process by which cells and large proteins are filtered from the blood to make an ultra-filtrate. The remaining fluid then passes on to the tubule where reabsorption occurs (Figure 2.1). Reabsorption is the transport of molecules from this ultra-filtrate into the blood stream. In the tubule, chemicals and water are either added to or removed from the filtered fluid, according to the body`s needs. The final product is urine which is excreted (Walter and Boron, 2004).

2.5.1 Protein in urine

The kidneys act as filters and keep protein in the body. Very little or no protein normally appears in the urine. Protein in the urine may be an early sign that the kidneys` filters have been damaged thus allowing proteins to leak into the urine (Cotran *et al.,* 2005). An abnormal amount of protein in urine is often a sign of kidney disease (Cotran *et al.,* 2005). Healthy kidneys do not allow a significant amount of protein to pass through their filters but if filters are damaged by kidney disease they may let proteins such as albumin to leak from the blood into the urine. Kidney malfunctions can be divided into chronic kidney disease and glomerulonephritis (Cotran *et al.,* 2005). Chronic kidney disease is defined as a condition where the kidney cannot properly retain wanted substances in the blood. Markers such as proteins are passed out in urine. Glomerulonephritis is a disease that causes inflammation of the glomeruli. Kidney disease often has no early symptoms but one of its signs is proteinuria which is discovered by a urine test (Cotran *et al.,* 2005).

2.5.2 Protein concentration determination

The urinary protein test is an important part of any physical examination due to the fact that the presence of proteins in urine is often associated with early renal disease. Normal urine contains very little protein usually less than 10 g/l or 100 g/ 24 hours is excreted. The traditional reagent strip testing for protein uses the principle of the protein error of indicators to produce visible colometric reaction.

Certain indicators change colour in the presence of proteins even though the pH of the medium does not change. This is because proteins accept hydrogen ions from the indicator. The protein area of the strip contains different chemicals which contain acid buffers to maintain the pH at a constant level (Gupta *et al.,* 2005).

At pH level 3 the indicators appear yellow in the absence of protein, as the protein concentration increases the colour progresses through various shades of green and finally blue (Gupta *et al.,* 2005). Readings are reported in terms of semi-quantitative values of 30, 100, 300, or 2000 g/l corresponding to each colour change. Trace values are considered to be less than 30 g/l (Gupta *et al.,* 2005).

2.6 Tenolam and kidney disease

Renal disease commonly exists as a co-morbidity in patients with HIV infection. The increase in the life expectancy due to highly active antiretroviral therapy can contribute to the increasing frequency in the recognition of renal impairment in HIV infected patients. Tenofovir, which is one of the Tenolam tablet components has been associated with nephrotoxic drug effects. These include decline in glomerular filtration rate, proximal tubules damage and acute kidney injury (Viread, 2006). Therefore when Tenolam drug is administered it can cause these side effects on kidney function.

A study has shown that a cocktail of drugs with tenofovir resulted in a mean decline in the glomeruli filtration rate after a twelve month follow up period, as compared to patients given lopinavir (Calza *et al.,* 2012). This showed that tenofovir has induced the decline in the kidney function. Another study (Young *et al,* 2012) which showed that tenofovir is associated with reduced renal function was carried out. Tenofovir was combined with boosted protease inhibitor and the patients given the medication followed up for 14, 15 and 19 months (Young *et al.,* 2012). The study showed that the cocktail of medicines led to a greater initial decline in the glomerular filtration rate (Young *et al.*, 2012).

Tenofovir is predominantly excreted by the kidneys and the part of kidneys involved in its excretion is called the proximal tubule (Young *et al.,* 2012). Studies indicate that tenofovir accumulates in the proximal tubule due to interaction with other drugs or prolonged use and is responsible for kidney damage. It results in kidney tubular dysfunction and less frequently in glomerular abnormalities (Young *et al.*, 2012).

Kidney damage may progress over time under long term exposure but it is reversible in most cases on drug discontinuation if noted early before much damage has been done. Studies have shown that after discontinuation of the drug three out of three patients with kidney malfunctions completely recovered (Herlitz *et al.*, 2010).

2.7 Factors that lead to kidney malfunction

There are a lot of factors that might result in kidney damage and these are age, gender, diet, diabetes, high blood pressure and other medications which are taken by the patients. Gender and age are the most studied factors because it is easy to follow up on them as compared to the other factors (Cobo, 2016).

2.7.1 Age and kidney malfunction

Anyone can get chronic kidney disease at any age, however some people are more likely than others to develop (O`Hare *et al.,* 2006). For about one-third of older people kidney function remains steady throughout life, but for the rest of us, kidney function gradually starts to decline around age 35 sometimes worsening quickly in later years with increasing structural and hormonal changes (O`Hare *et al.,* 2006). Older kidneys may not be able to endure as younger ones if they have been stressed. The result may be a higher risk of fluid imbalances, build-up of waste products and other serious consequences in later years (O`Hare *et al*., 2006).

A study was done in which examination of age specific reduction in glomerular filtration rate was monitored among US veterans and it showed that there was great decrease in filtration rates of patients aged 75 years or older (Garg *et al.,* 2004). The studies show that as we age kidney function decreases over time (Garg *et al.,* 2004).

2.7.2 Gender and kidney malfunction

Gender differences are of fundamental importance in most diseases (Cobo, 2006). Men and women, present different symptoms and signs in response to the underlying pathophysiology of the disease and its complications (Cobo, 2016). They respond differently to therapy and tolerate or cope with the disease differently (Cobo, 2016).

Due to the fact that some studies have revealed that there are some factors that contribute to a decline in kidney function despite the effect of the ARV drugs. Therefore gender and age are considered in the current study.

CHAPTER 3 : MATERIALS AND METHODS

3.1: Study area.

The study was done in Nkayi District at Nkayi District Hospital. Nkayi is a district in Matabeleland North in Zimbabwe located about 120km west of Kwekwe and 168km north-east of Bulawayo. It is a second-order administrative division in Zimbabwe. It is located at an elevation of 1.150 meters above the sea level. Its coordinates are 18 49 60 South and 28 49 60 East in DMS (Degrees Minutes Seconds). The patients who attend the Nkayi District Hospital come from around the Nkayi Growth point and the health centres from the Nkayi District

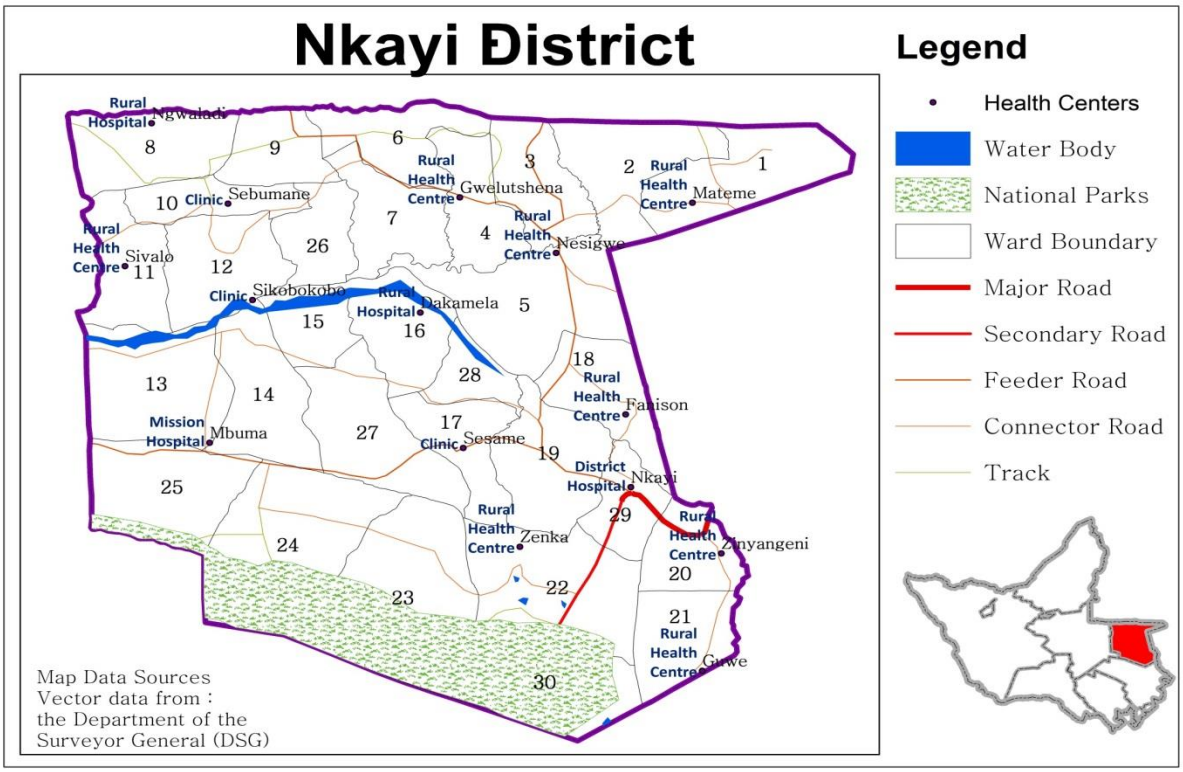

Figure 3.1: Geographical location of Nkayi District Hospital

3.2.1 Sampling

Urine samples were collected in Nkayi from 8-15 June 2016, 3-10 August 2016 and 28 September to 5 October 2016. The samples were from Nkayi growth point and in the surrounding rural areas. The laboratory analyses of samples were conducted at Nkayi District Hospital. A total number of 30 adult participants were recruited into the research study, all coming from Nkayi rural area as well as from the Nkayi growth point.

Before a patient was commenced on ARVs, intensive counselling was done so that he/she was educated on the importance of drug adherence and was strongly urged to adhere to the review dates given by the nurses. If a patient missed the review date, re-counselling was done before being issued another supply and this was a tedious process which patients did not like to undergo frequently. All these measures were done so as to ensure that patients were followed at regular intervals. After patients had been enrolled at Nkayi District Hospital they were monitored on monthly basis for 6 months and after the medical personnel was satisfied that the patients were stable, they were then transferred to local clinics for drug collections. A total of 30 patients participated in the study. Urine was collected from these patients and their urine-protein levels measured over a period of 4 months at 2 months intervals i.e. just before HAART initiation (baseline), after 2 months and 4 months on HAART.

The inclusion criteria for the study were as follows:

- HIV-positive,
- eligible for initiation on tenolam,
- aged between 18-100 years,
- to be enrolled for HAART at Nkayi district Hospital,
- had consented to participate in the study,
- a permanent resident of Nkayi so as to be easily followed up,

- had a life expectancy of at least 4 months based on clinical assessment-so as to last the 4- months follow-up period for the study.

A patient was excluded from the study if he/she had the following characteristics:

- outside the 18-100 years age group,
- was on anti-TB treatment, hypertensive drugs, hypoglycemics or any long-term drug treatment,
- kidney problems before drug administration, and
- if proteins were present in urine at baseline.

3.2.2 Urine test

A twenty four hour urine protein test was used to test for the presence of protein in the urine of patients before they were given the tenolam drug. Successive urine tests were done after the patients had been given tenolam on two months interval for four months. The urine sample collections were done after the patients were tested, their CD4 cell count done using cell cytometry in the laboratory so as to determine the degree of infection and they had consented to take part in the research study.

Patients were given sterile containers in which they collected and stored their urine over 24 hours. Mid-stream urine was collected after the urine bottles were labelled with the name, age, sex and date. The patients then took the samples to the hospital where they were stored in a cool environment. Prior to sample diagnosis the urine samples were all shaken to get an even distribution of the urine components. The urine test strip was immersed completely in the well mixed sample of urine. The strip was extracted from the container after 10 minutes, the edge of the strip was supported over the mouth of the container so as to drain the excess urine from the test strip.

The strip was left to stand for some 5 minutes allowing for all the necessary reactions to take place. The colours that appeared on the strips were then compared against the chromatic scale provided in the laboratory and the protein concentration recorded. The samples were tested thrice, at baseline, after 2 months and after four months.

3.3 Data analysis

Data analysis focused on comparing the effects of age, sex and tenolam administration. Two way ANOVA and t-tests were used to analyse the data. The dependent variable was the urine protein concentration and the independent variables were the gender, tenolam treatment and age of the participants. The significance level used was 0.05 and all statistical analysis were done using IBM SPSS version 21.

CHAPTER 4 : RESULTS

4.1 Baseline characteristics of the study population

From the total of 30 individuals who participated 15 were females and 15 were males (Table 4.1). The age of the participants ranged from 20 to 72 years. The participants had CD4 cell counts ranging from 35 to 320 cells/µl and their urine protein samples at baseline were all zero (Figure 4.5).

Table 4.1: Baseline characteristics of the study population

Characteristics	N=30
Number of participants	30
Gender: male	15
Female	15
Age, median years	39
CD4 cell count (cells/µl)	
<100	8
100-200	12
200-300	4

4.1.2 Age distribution of the participants

The study individuals were mainly composed of young adults, most of the participants were in the age range of 20-49 years (Figure 4.1). There were seven participants in the age range of 50-74 years. The age group of 35-39 had seven participants and the age group from 60-74 years had a single individual (Figure 4.1). The mean age of the participants was 39 years (Table 4.1).

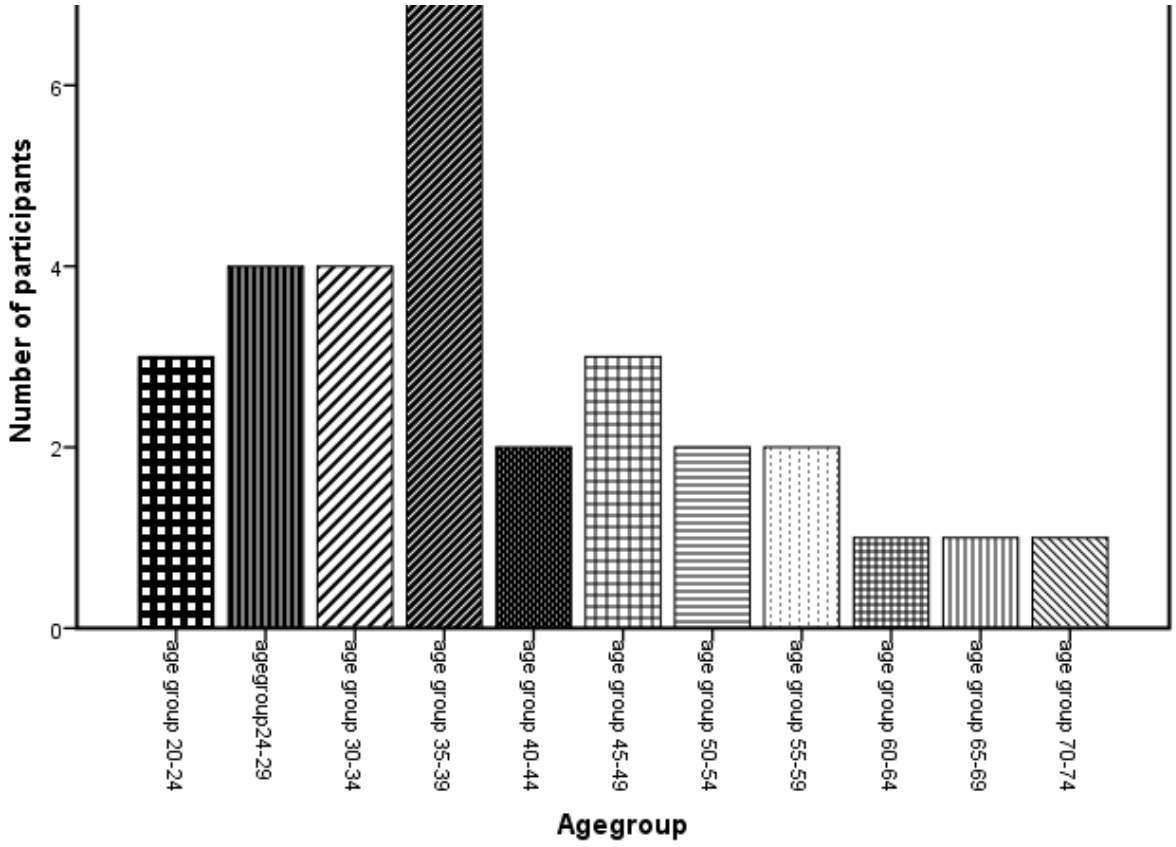

Figure 4.1: Age distribution of the participants

4.1.3 Age and sex distribution of the study participants

There were 15 males and 15 females which took part in the study. The female participants were more than the male participants in the age groups 25-29 and 30-34 (Figure 4.2). In the age groups 55-69 only the females participated. There were equal numbers of males and females in age groups 50-54 and 70-74 (Figure 4.2). Male participants were more than females in age groups 20-24 and 35-39 (Figure 4.2) and age group 40-44 was composed of males only.

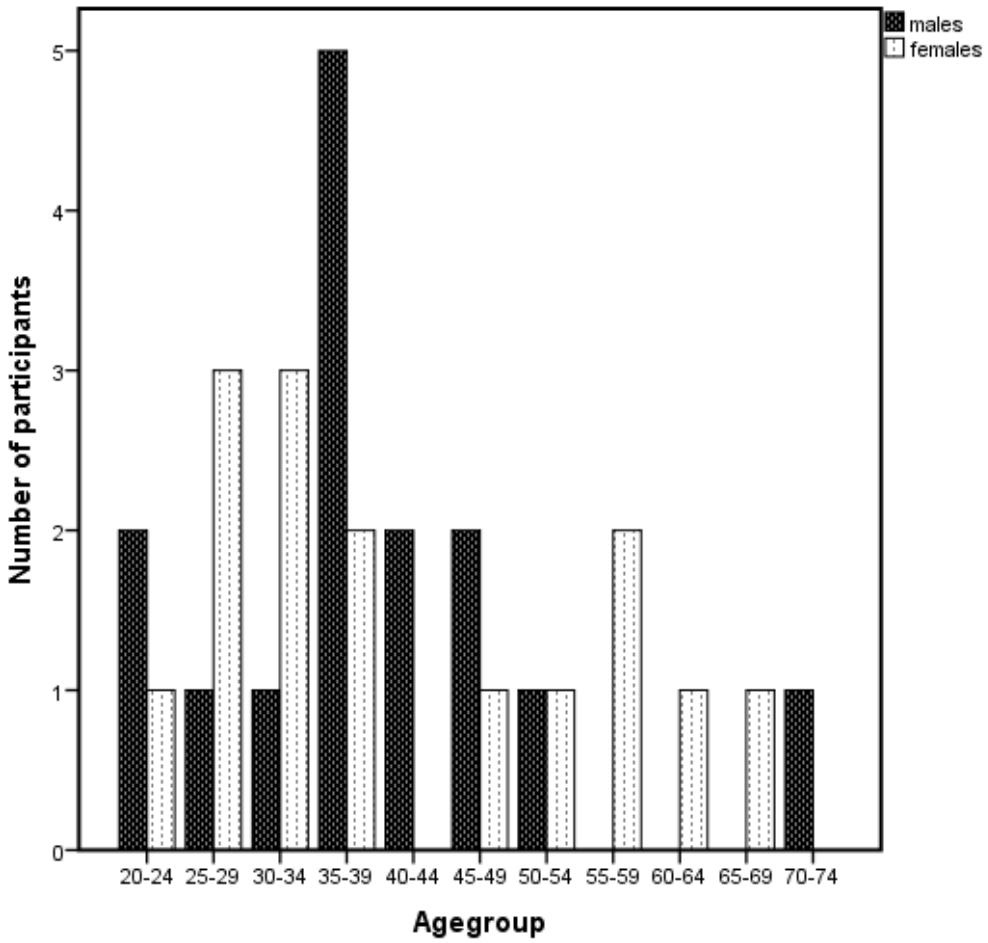

Figure 4.2: Age and sex distribution of the study population

4.1.4 CD4 counts of the participants

CD4 counts of the participants ranged from 50 to 320 cells/µl. Most of the participants had their CD4 counts within the range 100-200 cells/µl. There were 8 individuals with their CD4 counts below 100 cells/µl, 18 participants being within the range 100-200 cells/µl and 4 individuals having the highest CD4 counts ranging between 200-300 cells/µl (Figure 4.3).

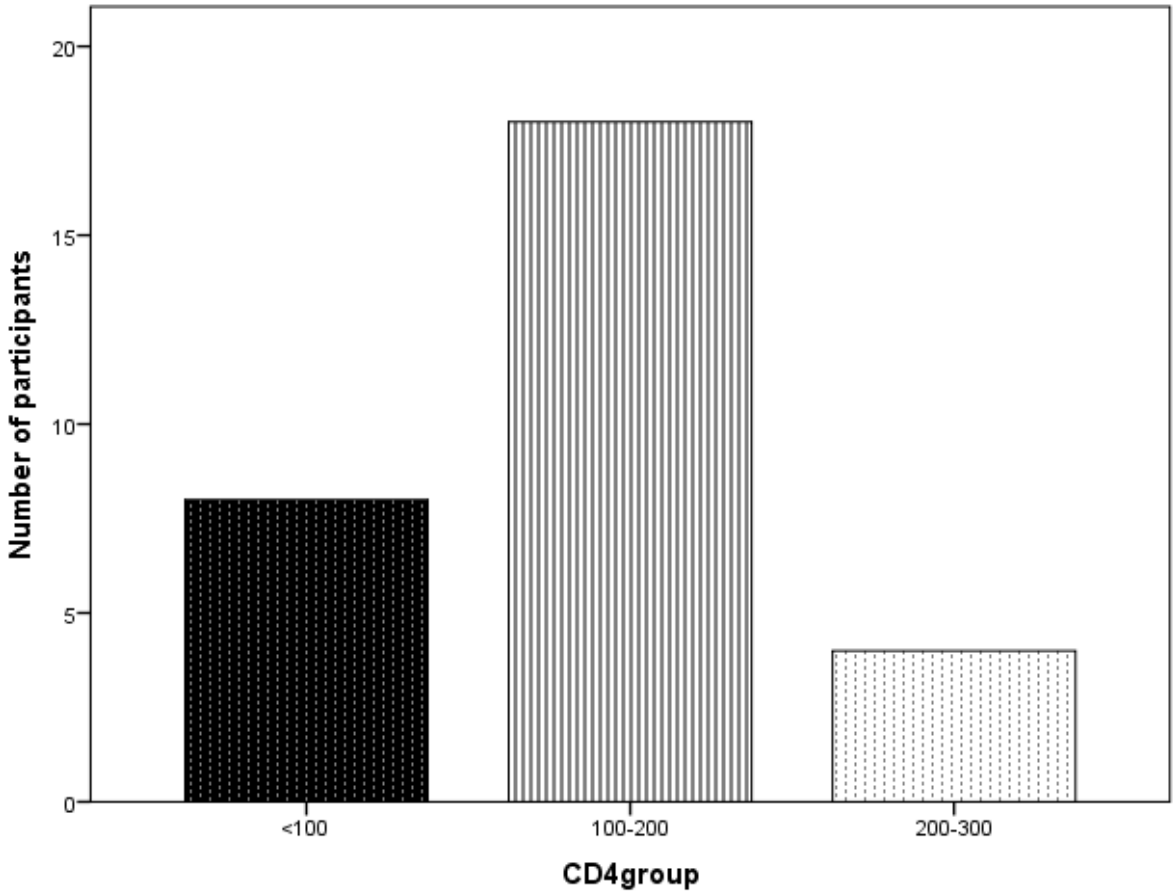

Figure 4.3: Baseline CD4 counts of the study participants

4.2 Individual protein concentrations over the study period

The individual graphs of each patient`s urine protein levels over the four months follow up period show that in most patients the protein concentrations were increased (Figure 4.4). Only three participants had their protein concentrations at a constant and two individuals did not have any protein increase at all. After the 4 month period the highest protein concentration was at 300 g/l and most individuals had concentration of 100 g/l (Figure 4.4).

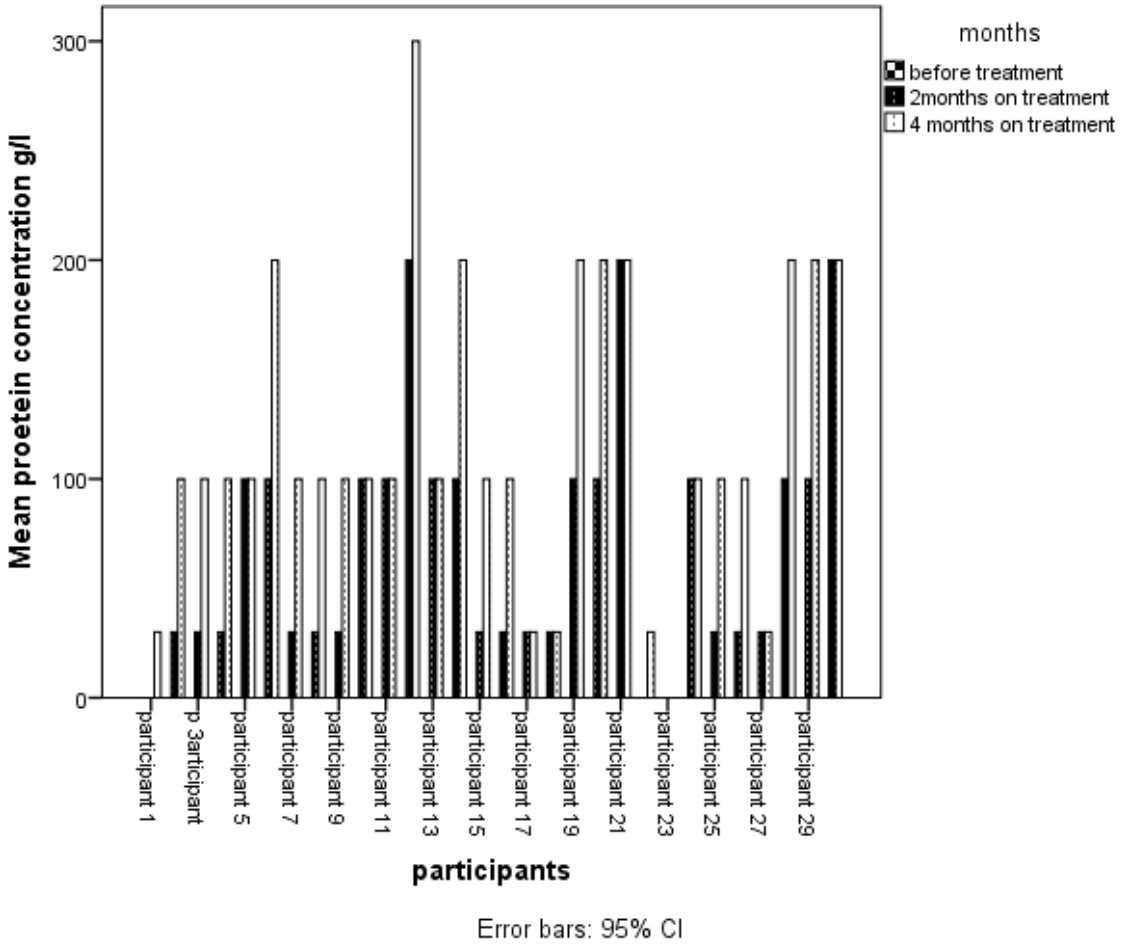

Figure 4.4: Individual protein concentrations over the study period

At baseline all the individuals had protein levels at zero (Figure 4.5). After two months on treatment the protein concentrations increased to an average of 70 cells/µl (Figure 4.5). After four months the mean protein concentration of protein in urine showed an increase to 120 cells/µl (Figure 4.5).

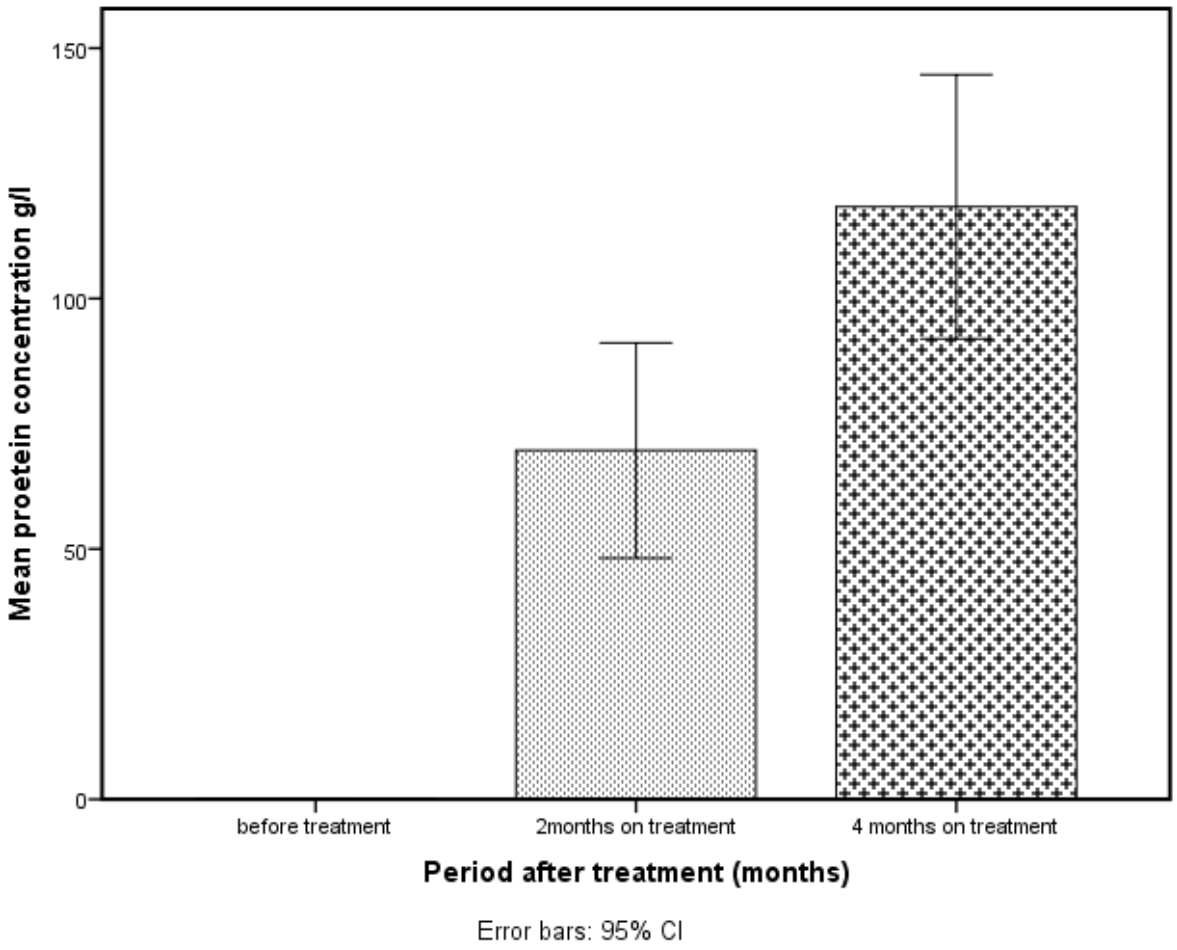

Figure 4.5: Urine protein concentration over the study period

The urine protein concentrations increased over the study period. There was a significant increase in urine protein concentration between 0 and 2 months (t-test p = 0.00, Appendix 3). There was a minimum increase in the urine protein concentration between 2 and 4 months though the increase was not significant (t-test p= 0.56, Appendix 3). From baseline and 4 months there was also significant increase in the urine protein concentration (t-test p= 0.00, Appendix 3).

4.3 Factors influencing change in urine protein concentration

Tenolam treatment had a significant effect on the increase in urine protein concentration of the patients (anova p= 0.00, Appendix 9). Gender of the participants did not have a significant effect on the increase in urine protein concentration (anova p=0.632, Appendix 9). Age of the participants also had a significant effect on the increase in urine protein concentration of patients (anova p=0.012, Appendix 13).

CHAPTER 5 : DISCUSSION

5.1 Changes in urine protein concentrations over the study period

The major objective of the study was to determine the changes in urine protein concentrations of the patients after being given tenolam drug. The increase in the urine protein concentration was a result of the drug activity once it was administered by the patients. From baseline to two months there was a huge increase in urine protein concentration. This might be due to the fact that during the first months of initial drug administration a lot of changes take place in the patients. Metabolic systems and the adjustment of the body to the presence of the drug might have increased the concentration of proteins in urine. The presence of protein in urine is a result of the kidney malfunction. The kidneys would have failed to filter the protein back into the blood system due to damage on the proximal tubules caused by the drugs and other risk factors.

Between 2 and 4 months nine individuals had an increase in their urine protein concentrations. The changes were less because the kidneys had adjusted to the changes brought by the administration of the drug and they had regained their normal functioning. Most of the levels of protein concentrations from the patients under the study fell within low levels of proteins which is below 200 g/l, though there are exceptions in which the levels were above 200 g/l. Low levels in proteins in urine are normal especially after drug administrations. Temporal high levels of protein are also not unusual either particularly in younger people after exercises or during an illness. If urinalysis show prolonged increase in urine protein concentrations to high levels a follow up test must be done in order to determine the amount of protein present and whether it is a cause for concern or not.

5.2 Effect of age on kidney function

Age is one of the risk factors that was observed during the study. Age had a significant effect on the increase in urine protein concentration over the study period. Proteins present in urine indicate some form of kidney`s failure to filter in the wanted substances and keep them in the blood system (O`Hare *et al.,* 2006). In most individuals, kidney function declines with age and this is a result of structural and hormonal changes (O`Hare *et al.,* 2006). Age associated loss of kidney function has been recognised for decades in many kidney malfunction studies done (Garg *et al.,* 2004). With aging many subjects exhibit progressive decreases in glomerular filtration and renal blood flow and this widely varies with individuals (Garg *et al.,* 2004).

From the study, only the participants with no urine protein concentration at baseline were recruited into the study. The concentrations started to rise after tenolam medication was administered. Therefore even if age has an impact on kidney function in general it did not ultimately cause the increase over the study period.

5.3 Effect of gender on urine protein concentration

Gender did not have any effect on the increase in urine protein concentration of the patients over the study period. This means that the increase in urine protein concentration was not a result of gender. Therefore gender did not have any effects on the functioning of the kidneys. However, there are studies that indicate that sex hormones are likely to contribute to the deterioration of the kidney`s function (Silbiger and Neugarten, 1995). Conclusions have been made that chronic kidney disease tends to be slower in females both experimentally and clinically (Silbiger and Neugarten, 1995). Gender may also have an effect due to the fact that males and females respond differently to therapy and tolerate diseases differently (Cobo, 2016).

5.4 Effect of tenolam on kidney function

Tenolam had a significant effect on the increase in urine protein concentration of the patients during the four month study period. When tenolam drug is administered in the body one of its constituents (tenofovir) is excreted from the body by the proximal tubules of the kidneys (Young *et al.,* 2012). Therefore Tenolam drug may have an effect in the functioning of the kidneys.

The results indicated that in some patients the urine protein concentration continued to increase between 2 and 4 months. A prolonged increase in the protein concentration can be a result of the kidneys failing to keep proteins in the blood and this may be early signs of kidney malfunction (Young *et al.,* 2012). The presence of proteins in the urine is an indication of kidney malfunction.

Though the protein concentrations of the participants did not reach high life threatening levels, the increase can actually indicate that tenolam can have profound effects if the study is to be carried out over a long period of time. A true picture was likely going to be obtained if the study was done for a period of between a year and two years just like the other research studies on tenofovir (Young *et al.,* 2012). This was not possible due to the short period of time available for collecting data.

5.5 Conclusions

The results from the study showed that Tenolam has a great impact on the increase in urine protein concentration of the patients that are given the drug as their antiretroviral therapy. Although there were no patients who had their urine protein concentration at high life threatening levels the increase in the levels, indicated that with time the concentrations can reach high levels. This increase in urine protein suggests that a change in the kidney function was initiated by the administration of the tenolam drug.

5.6 Recommendations

The findings of the study indicate that tenolam administration has profound effects on the increase in urine protein concentration with time. This also implies that kidney damage may progress over time. It is therefore recommended that patients given the Tenolam drug should be followed up doing the urinalysis tests in order to observe changes that may result from drug administration. If the urine protein concentration levels continue to rise, the treatment regimen should be stopped and an alternative one be instituted.

The follow ups on patients are also recommended due to the fact that the symptoms of kidney malfunction are not shown unless the kidney has been drastically damaged. Therefore follow ups will help with the diagnosis of any damages and control measures being taken early. If drug administration leads to the continuous increase in protein concentration discontinuous use of the drug is recommended so as to stop the damage being caused by the drug. The patient would then be given another regimen as the new antiretroviral therapy.

It is also recommended that the age of the participants should also be included in the criteria used when deciding the type of regimen to be given to patients. Old individuals should be given drugs that are less toxic and do not need much metabolism because most of their vital organs like the kidneys would have been worn out.

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APPENDICES

Appendix 1. Paired sample statistics
Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Baseline	.00	30	.000	.000
	twomnths	69.67	30	57.565	10.510
Pair 2	twomnths	69.67	30	57.565	10.510
	fourmnths	78.00	30	62.885	11.481
Pair 3	Baseline	.00	30	.000	.000
	fourmnths	78.00	30	62.885	11.481

Appendix 2 Paired sample test

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	baseline - twomnths	-69.667	57.565	10.510	-91.162	-48.172	-6.629	29	.000
Pair 2	twomnths - fourmnths	-8.333	77.419	14.135	-37.242	20.575	-.590	29	.560
Pair 3	baseline - fourmnths	-78.000	62.885	11.481	-101.482	-54.518	-6.794	29	.000

Appendix 3. Paired sample correlations

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	baseline & twomnths	30	.	.
Pair 2	twomnths & fourmnths	30	.176	.352
Pair 3	baseline & fourmnths	30	.	.

Appendix 4. Tests of normality

Tests of Normality[a]

	gender	Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	Df	Sig.	Statistic	df	Sig.
Sqrttrans	Male	.208	15	.080	.893	15	.075

a. treatment = 3, gender = male

b. Lilliefors Significance Correction

Appendix 5. Tests of normality

Tests of Normality[a]

	gender	Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	Df	Sig.	Statistic	Df	Sig.
sqrttrans	female	.363	15	.000	.788	15	.003

a. treatment = 3, gender = female

b. Lilliefors Significance Correction

Appendix 6. Tests of normality

Tests of Normality[a]

	gender	Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	Df	Sig.	Statistic	Df	Sig.
sqrttrans	Male	.254	15	.051	.860	15	.024

a. treatment = 2, gender = male

b. Lilliefors Significance Correction

Appendix 7. Tests of normality

Tests of Normality[a]

	gender	Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	Df	Sig.	Statistic	df	Sig.
Sqrttrans	female	.208	15	.081	.895	15	.079

a. treatment = 2, gender = female

b. Lilliefors Significance Correction

Appendix 8. Multiple comparisons

Multiple Comparisons

Dependent Variable: trans

Tukey HSD

(I) TENOLAMTREATMEN	(J) TENOLAMTREATMEN	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
before treatment	two months on treatment	-7.4543*	.80106	.000	-9.3656	-5.5430
	four months on treatment	-10.2615*	.80106	.000	-12.1728	-8.3502
two months on treatment	before treatment	7.4543*	.80106	.000	5.5430	9.3656
	four months on treatment	-2.8071*	.80106	.002	-4.7184	-.8958
four months on treatment	before treatment	10.2615*	.80106	.000	8.3502	12.1728
	two months on treatment	2.8071*	.80106	.002	.8958	4.7184

Based on observed means.

The error term is Mean Square(Error) = 9.625.

*. The mean difference is significant at the .05 level.

Appendix 9. Tests of between subject effects

Tests of Between-Subjects Effects

Dependent Variable: trans

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1692.965[a]	5	338.593	35.177	.000
Intercept	3138.496	1	3138.496	326.062	.000
GENDER	2.219	1	2.219	.231	.632
TENOLAMTREATMEN	1687.446	2	843.723	87.655	.000
GENDER * TENOLAMTREATMEN	3.300	2	1.650	.171	.843
Error	808.538	84	9.625		
Total	5640.000	90			
Corrected Total	2501.504	89			

a. R Squared = .677 (Adjusted R Squared = .658)

Appendix 10 Multiple comparisons

Multiple Comparisons

Dependent Variable: trans

Tukey HSD

(I) TENOLAMTREATMEN	(J) TENOLAMTREATMEN	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
before treatment	two months on treatment	-7.4543*	.80106	.000	-9.3656	-5.5430
	four months on treatment	-10.2615*	.80106	.000	-12.1728	-8.3502
two months on treatment	before treatment	7.4543*	.80106	.000	5.5430	9.3656
	four months on treatment	-2.8071*	.80106	.002	-4.7184	-.8958
four months on treatment	before treatment	10.2615*	.80106	.000	8.3502	12.1728
	two months on treatment	2.8071*	.80106	.002	.8958	4.7184

Based on observed means.

The error term is Mean Square(Error) = 9.625.

*. The mean difference is significant at the .05 level.

Appendix 11 Levene`s test of equality of variance

Levene's Test of Equality of Error Variances[a]

Dependent Variable: trans

F	df1	df2	Sig.
10.132	5	84	.000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

Appendix 12 levene`s test of error variances

Levene's Test of Equality of Error Variances[a]

Dependent Variable: proteinconcentration

F	df1	df2	Sig.
.	32	0	.

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Agegroup + ttreatment

Appendix 13 Tests of between subject effects

Tests of Between-Subjects Effects

Dependent Variable: proteinconcentration

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	110151.058[a]	13	8473.158	8.496	.000
Intercept	99177.650	1	99177.650	99.445	.000
Agegroup	35773.572	11	3252.143	3.261	.012
Ttreatment	77009.342	2	38504.671	38.608	.000
Error	18948.945	19	997.313		
Total	261264.080	33			
Corrected Total	129100.002	32			

a. R Squared = .853 (Adjusted R Squared = .753)

Appendix 14 Multiple comparisons

Multiple Comparisons

Dependent Variable: proteinconcentration

Tukey HSD

(I) ttreatment	(J) ttreatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Baseline	after 2 months	-75.509*	13.4659	.000	-109.718	-41.300
	after 4 months	-114.345*	13.4659	.000	-148.555	-80.136
after 2 months	baseline	75.509*	13.4659	.000	41.300	109.718
	after 4 months	-38.836*	13.4659	.025	-73.046	-4.627
after 4 months	baseline	114.345*	13.4659	.000	80.136	148.555
	after 2 months	38.836*	13.4659	.025	4.627	73.046

Based on observed means.

The error term is Mean Square(Error) = 997.313.

*. The mean difference is significant at the .05 level.