

**UNIVERSITY OF GHANA
SCHOOL OF BIOMEDICAL AND ALLIED HEALTH SCIENCES**

**ASSOCIATION BETWEEN VITAMIN D AND RENAL FUNCTION
AMONG GHANAIAN HIV/AIDS PATIENTS**

BY

BERNICE ACKOM

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN
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DECLARATION

I, Bernice Ackom do hereby; certify that this thesis is my original work of my research which was carried out at the Department of Chemical Pathology under the supervision of Prof. Henry Asare Anane and Dr. Seth Amanquah. In instances where references have been made and cited, full acknowledgement has been made. Neither all nor part of this thesis has been presented for any other degree.

.....
BERNICE ACKOM

(STUDENT)

.....
DATE

.....
PROF. HENRY ASARE ANANE

(SUPERVISOR)

.....
DATE

.....
DR. SETH AMANQUAH

(SUPERVISOR)

.....
DATE

DEDICATION

The Almighty God deserves all the thanks and praise for His love, protection, favour and blessings.

I dedicate this work to my family, especially Dea. Agnes Ackom and Mr. Eric Ghansah for their immerse love, encouragement and support.

This work is again dedicated to my dad, siblings, Jedidiah and Lady Ruth Laryea for their support and Prayers.

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CONTENTS

DECLARATION	ii
DEDICATION	iii
ACKNOWLEDGEMENT	iv
CONTENTS	v
LIST OF FIGURES	vii
LIST OF TABLES	viii
LIST OF ABBREVIATIONS	ix
ABSTRACT	xi
1.0 Background	1
Introduction	1
1.1 Problem Statement	3
1.2 Justification	4
1.3 Hypothesis	5
1.4 General Aim	5
1.5 Specific Objectives	5
2.0 Literature Review	6
3.0 MATERIALS AND METHOD	20
3.1.0 Study Site	20
3.2.0 Study Design and selection of Participants	20
3.2.1 Inclusion Criteria.....	21
3.2.2 Exclusion Criteria.....	21
3.3.0 Sample Size Determination	22
3.4.0 Study Participants	22
3.5.0 Sample Collection, Preparation and Storage	22
3.7.0 Anthropometric measurements and Clinical characteristics	23
3.8.0 Biochemical Analysis	23
4.0 RESULTS	27
4.1.0 Background of Study Population	27
4.1.1 Anthropometric and Baseline Characteristics of the Study Population	28
4.1.2 Biochemical Indices of study participants	29
5.0 DISCUSSION	38

6.0 CONCLUSSION AND RECOMMENDATION	41
6.1 CONCLUSION	41
6.2 RECOMMENDATION	41
Appendix I: Consent Form	49
Appendix II : Participation Information Sheet	50
Appendix III : Questionnaire	53
Appendix IV: Calculation of Sample size	54

LIST OF FIGURES

Figure 1: Vitamin D correlation with serum creatinine, eGFR, albumin and calcium among HAART/Vit. D-naive HIV/AIDS patients

Figure 2: Vitamin D correlation with serum creatinine, eGFR, albumin and calcium among HIV/AIDS patients on HAART and Vitamin D supplement for less than 6 months

Figure 3: Vitamin D correlation with serum creatinine, eGFR, albumin and calcium among HIV/AIDS patients on HAART and Vitamin D supplement for more than 6 months

Figure 4: Distribution of ARTs among HIV/AIDS patients on HAART and Vitamin D supplement for less than 6 months

Figure 5: Distribution of ARTs among HIV/AIDS patients on HAART and Vitamin D supplement for more than 6 months

LIST OF TABLES

Table 1 : Stages of Chronic Renal Disease based on eGFR

Table 4.1: Baseline clinical characteristics of HIV/AIDS patients on HAART/Vitamin D supplements and HAART/Vitamin D supplement-naïve HIV patients

Table 4.2: Biochemical indices of HIV/AIDS patients on HAART/Vitamin D supplements and HAART/Vitamin D supplement-naïve HIV patients

Table 4.3: 25hydroxyvitamin D status and eGFR Staging among HIV/AIDS patients on HAART/Vitamin D supplements and HAART/Vitamin D supplement-naïve HIV patients

LIST OF ABBREVIATIONS

25(OH)D ₃	-	25 hydroxy vitamin D
1,25[OH] ₂ D	-	1,25-dihydroxyvitamin D
AIDS	-	Acquired immunodeficiency syndrome
ART	-	Anti Retroviral Therapy
CKD	-	Chronic kidney disease
DM	-	Diabetes mellitus
DBP	-	Vitamin D Binding Protein
ESRD	-	End stage renal disease
GFR	-	Glomerular filtration rate
FGF23	-	Fibroblast growth factor 23
FSGS	-	Focal segmental glomerulosclerosis
HAART	-	Highly active antiretroviral therapy
HIV	-	Human immunodeficiency virus
HIVAN	-	HIV-associated nephropathy
MDRD	-	Modification of Diet in Renal Disease
NKT	-	Natural Killer T
NNRTIs	-	Non-nucleoside reverse transcriptase inhibitors
NRTIs	-	Nucleoside reverse transcriptase inhibitors
PTH	-	Parathyroid hormone
PIs	-	Protease inhibitors
ROS	-	Reactive oxidative species
SCr	-	Serum Creatinine

UV	-	Ultra Violet
VDR	-	Vitamin D receptor
WHO	-	World Health Organization

ABSTRACT

Background

The prevalence of HIV among Ghanaian youth ranges from 2.4% to 3.5%. Many deaths of HIV patients are caused by complications like renal and cardiovascular diseases. Rapid replication of HIV which is favored by increased oxidative stress and the attack of reactive oxidative species (ROS) on the CD4+ T-cells are the major causes of CD4+ T-cell depletion in HIV/AIDS patients. Effective circulating antioxidant and minerals may play important role in curtailing the deleterious effect of ROS. HIV-infected persons have a high prevalence of vitamin D deficiency and insufficiency. Some antiretroviral drugs are known to interfere with vitamin D metabolism causing deficiency of vitamin D. Thus, the intake of micronutrients including vitamin D is encouraged. Micronutrients like vitamin D when taken in higher doses can lead to hypercalcemia. Hypercalcemia usually contributes to calcium deposits in the kidney causing kidney stones, kidney damage and eventually kidney failure.

General Aim:

This study aimed at determining the serum vitamin D levels and establishing its association with kidney function among Ghanaian HIV/AIDS patients on HAART with or without vitamin supplements

Methodology:

This study was a cross-sectional study carried out at the Fevers Unit of Korle-Bu Teaching Hospital and the Chemical Pathology Department of the College of Health Science, University of Ghana. Two hundred participants confirmed to be positive of HIV-1 and or HIV-2 were selected randomly from the Fevers Unit of Korle-Bu Teaching Hospital. Patients taking HAART and vitamin supplement for more than six months were 100 made up of 30(30%) males and 70 (70%) females.

Fifty patients on HAART and vitamin supplement for less than six months comprised of 17 (34%) males and 33 (66%) females. Patients not on both HAART and vitamin supplement was 50, made up of 14 (28%) males and 36 (72%) females. Blood samples obtained were analysed for 25 hydroxyvitamin D3, creatinine, serum calcium and albumin.

Results

The mean systolic blood pressure (SBP) of patients compared among the three groups was statistically significant ($p < 0.05$). However, the mean diastolic blood pressure (DBP) and body mass index (BMI) was not statistically significant in the patients taking HAART and vitamin supplement for more than six months, patients taking HAART and vitamin supplement for less than six months and those not on both HAART and vitamin supplement ($p > 0.05$).

The mean estimated Glomerular filtration rates, albumin, calcium, creatinine and 25 hydroxyvitamin D levels were not statistically significant in the 3 categories of patients studied ($p > 0.05$).

25 hydroxyvitamin D levels were low ($<15\text{ng/ml}$) in patients on HAART and vitamin supplement for the categories (more than six months, less than six months and not on both HAART and vitamin supplement). The mean eGFR was $> 60 \text{ mL/min/1.73 m}^2$ for about 70% of the patients on HAART and vitamin supplement for more than six months, less than six months and patients not on both HAART and vitamin supplement. Low vitamin D levels were found across the various age groups.

There was no correlation between 25hydroxyvitamin D and creatinine, eGFR, albumin and calcium of the patients on HAART and vitamin supplement for more than six months, less than six months and patients not on both HAART and vitamin supplement.

Conclusion

Long exposure to vitamin D did not cause its increased levels in circulation and did not relay any adverse effect on kidney function in Ghanaian HIV/AIDS patients receiving both antiretroviral and

vitamin supplements.

CHAPTER ONE

1.0 Background

Introduction

Pandemic of HIV/AIDS is one of the deadliest epidemics of modern times. Every year about 2 million people are being diagnosed with the virus with a total of 37 million people in the world living with it (Swanepoel et al. 2018). In 2007, a total of two million men, women, and children died of AIDS worldwide (Bongaarts et al. 2008). HIV infects the immune system and central nervous systems. The virus attacks the T lymphocytes, an important part of the immune system by coordinating the actions of the remaining immunocytes. The virus attaches itself to CD4 and primarily infects the T helper cells. Overtime, HIV infection results in an extreme reduction in the T helper cells present to fight diseases (Husain et al. 2009). Rapid replication of HIV which is favoured by raised oxidative stress and the attack of reactive oxidative species (ROS) on the CD4+ T-cells are the major causes of reducing CD4+ T-cell in HIV/AIDS persons (Stephensen et al. 2005). Effective circulating antioxidant and minerals play significant roles in curtailing harmful effects of ROS. Most HIV-infected persons are vitamin D deficient. A few antiretroviral drugs have been found to interfere with vitamin D metabolism causing deficiency of vitamin D. Thus, addition of micronutrients including vitamin D to antiretrovirals is encouraged. HIV infections and AIDS have various clinical complications such as cardiovascular diseases, bone diseases, renal disease, lipodystrophy with associated dyslipidemia and vitamin deficiencies have been attributed to HIV infection and its treatment (Sharma 2014).

Renal disease is extensively known as one of the common HIV/AIDS complication. HIV Patients experience some form of renal disorders at every stage of the infection. These disorders range from the electrolyte and fluid imbalances to end-stage renal disease (ESRD) (Choi and Rodriguez 2008). There is a high risk for HIV/AIDS patients to develop both chronic and acute kidney disease (CKD)

(Swanepoel et al. 2018). Up to 30% of HIV patients are proteinuric as an indication that the kidneys are not functioning well and an estimate of 10% HIV patients acquire some form of kidney disease (Fabian and Naicker 2009). Renal dysfunction is a state in which the kidneys lose the ability to balance fluids and eliminate wastes. Kidney problems linked to HIV are caused by HIV virus multiplying and damaging the kidney tissues or by some HIV medications given to the patients (Post et al. 2008). Kidney disease associated with HIV is among the major causes of death in Africans (Han et al. 2006); this also has an association with diabetes, hypertension, low CD4+ cell counts, advanced HIV disease, acute bacterial infection of the kidney and sepsis, smoking for over ten years (Kamga et al. 2011) and can present as acute or chronic kidney disease (Roling et al. 2006). Various researches have focused on highly active antiretroviral therapy (HAART) as treatment to repress HIV viral replication and the progression of the disease to AIDS. The World Health Organization (WHO) recommends both macro and micronutrient supplementation for HIV/AIDS patients in addition with HAART treatment. These micronutrient supplementations constitute iron, zinc, B-complex vitamins, vitamins A,C,D and E (World Health Organization 2003).

Many antiretroviral drugs are excreted partly by the kidneys and thus patients with reduced glomerular filtration rate (GFR) need some dosage adjustment of the drugs. There are many side effects of the antiretroviral drugs, and these include hepatotoxicity and nephrotoxicity (Fokunang et al. 2010; Kamga et al. 2011).

Vitamin D is synthesised in the epidermis of the skin by exposure to Ultra Violet (UV) light and sometimes absorbed from foods and supplements. Vitamin D can also be taken in as vitamin D3 or vitamin D2. 1, 25-dihydroxyvitamin D is produced in the kidney and this process can be regulated by calcium metabolism signalling (Dini and Bianchi 2012). The kidneys are involved in the filtering of waste substances from the blood and transforming them to be excreted through the urine. In addition, the kidneys produce calcitriol, the active form of vitamin D that maintains

healthy bones among other roles. People infected with HIV have a high prevalence rate of vitamin D deficiency. A few antiretroviral drugs have been established to cause an interference with the metabolism of vitamin D causing vitamin D deficiency. Thus, intake of micronutrients including vitamin D is encouraged. Hypervitaminosis D occurs as a result of intake of higher doses of supplements containing vitamin D and not by sun exposure or diet. Vitamin D hypervitaminosis results in hypercalcemia and hypercalciuria. Hypercalcemia causes deposition of calcium crystals and a possible kidney damage (Ozkan et al. 2012).

1.1 Problem Statement

Renal dysfunction is relatively common in HIV patients, because of the infection itself or a side effect from HIV medications. Although renal disease can be treated in many cases, the cost of treatment is very high and in HIV patients the disease can be very difficult to manage. This creates much financial burden on the HIV/AIDS individuals, their dependents and the nation as a whole. The prevalence of CKD in HIV infected persons ranges from 2% to 38% and varies widely between geographical regions (Rosenberg et al. 2015). Five new cases of ESRD develop for every 100 cases of CKD in black persons, as compared to non-blacks. Among persons with HIV infection who receive dialysis, 91% are black.

With poor management of HIV/AIDS and its related complications, the mortality rate increases in Ghana each year. As part of management and treatment of HIV/AIDS, patients are administered with highly active antiretroviral drugs alongside with micronutrients containing antioxidants and other deficient vitamins such as vitamin D. However, other researches and studies have reported adverse side effects of these remedies attributing to mostly exposure over long period of time. Consistent and long term exposure to especially fat soluble vitamins such as vitamin D may lead to hypervitaminosis.

Hypervitaminosis and toxicity of vitamin D results in hypercalcemia characterized by increased serum 25-hydroxyvitamin D and calcium (Ozkan et al. 2012). Increased levels of calcium in circulation has been reported as a contributing factor to calcification of kidney tissues leading to damage to kidney tissues (Ozkan et al. 2012). Therefore, vitamin D toxicity leading to hypercalcemia could be a contributing factor to the pathogenesis of kidney disease among HIV/AIDS patients.

Damage to kidney tissues can lead to chronic kidney disease, cardiovascular events including heart failure and death (Whitman et al. 2012).

1.2 Justification

With poor management of HIV/AIDS and its related complications, the mortality rate increases in Ghana each year. The prevalence rate of AIDS as at 2014 was 1.37% (Biney et al. 2016). Various researches and development of remedies to eradicate AIDS are being done in this 21st century. As part of a global call to manage and treat the disease, patients are given highly active antiretroviral drugs together with micronutrients containing antioxidants and other deficient vitamins such as vitamin D (World Health Organization 2003).

This study, therefore seeks to assess vitamin D toxicity among HIV/AIDS patients on Anti-Retroviral Therapy (ART) and Vitamin D supplementation. This study also seeks to establish an association between hypervitaminosis D and renal function among Ghanaian HIV/AIDS patients.

The outcome of the study will provide information on the vitamin D status and its contribution to the kidney dysfunction among Ghanaian HIV/AIDS patients. The outcome will also provide information for further research into hypervitaminosis and renal function among HIV/AIDS patients.

1.3 Hypothesis

Hypervitaminosis D will not cause renal dysfunction in Ghanaian HIV/ AIDS patients

1.4 General Aim

To determine serum vitamin D levels and establish its association with kidney function among Ghanaian HIV/AIDS patients on HAART with or without vitamin supplements

1.5 Specific Objectives

1. To estimate levels of serum 25-hydroxyvitamin D among Ghanaian HIV/AIDS patients on HAART with or without Vitamin supplements as an outcome of renal failure
2. To estimate calcium levels of study participants to determine whether levels can contribute to calcification of kidney tissues
3. To assess the renal function by estimating the glomerular filtration rate (GFR) and to find an association between 25-hydroxyvitamin D, calcium levels and kidney function among study participants.

CHAPTER TWO

2.0 Literature Review

2.1 HIV/AIDS

Acquired immunodeficiency syndrome (AIDS) is a health problem that reduces the effectiveness of the immune system, leading to various life-threatening infections and complications. AIDS is a disease that affects the human immune system and is caused by human immunodeficiency virus (HIV). This virus leads to the onset of a clinical condition which is depicted by gradually minimizing the efficacy of the immune system by depleting CD4 T-cells. Individuals who are infected become susceptible to opportunistic infections by any pathogen (Sharma 2014). Rapid replication of HIV which is favoured by increased oxidative stress and the attack of reactive oxidative species (ROS) on the CD4+ T-cells are the major causes of CD4+ T-cell depletion in HIV/AIDS patients. High levels of reactive oxygen species (ROS) have been proven to be present at the beginning of HIV infection (Sharma 2014). However, effective circulation and administration of exogenous antioxidants and other micronutrients will play important roles in curtailing the deleterious outcome of ROS (Sharma 2014).

The diagnosis in the beginning stages of HIV infection is very significant to ensure that all infected patients are treated. This helps to reduce morbidity and mortality rate as well as decrease the transmission to uninfected people (Biney et al. 2016).

2.1.1 MODE OF TRANSMISSION AND PREVALENCE OF HIV

The virus is spread when one person comes into contact with any bodily fluid of an HIV person like vaginal fluid, breast milk, semen, and blood. Globally, it is estimated that about 37 million individuals are dwelling with HIV and it makes the disease a huge public health concern. About

4.9% of the adult population of Sub-Saharan Africa are living with HIV (Biney et al. 2016). The prevalence of HIV in Sub-Saharan Africa is 68%, with women and young people being the most affected. Roughly 52% of the adult HIV population are made up of women (Lewis 2011). In Africa, the widespread transmission of HIV/AIDS has been linked to some political and socio-economic factors like the poor living and healthcare conditions, less or no education on HIV, infrastructures, transportation and communication problems, and inconsistent availability of highly active antiretroviral therapy (Arendse et al. 2010).

The HIV prevalence among the Ghanaian youth is found to be 0.6% for 15-19 years, 1.1% for 15-24 years and 3.5% for 35-39 years and finally 5.6% for 45-49 years old persons (Biney et al. 2016).

2.2.0 ANTI RETROVIRAL THERAPY

The development of HAART was aimed at managing retroviral infections especially HIV in order to extend the span of life and reduce mortality. The main goal of HAART is the preservation and restoration of immunologic function, maximum suppression of viral load, reduction of HIV-related morbidity and mortality, and the improvement of quality of life (Hawkins 2010). Since 2004 Ghana, like other African countries have been using antiretroviral therapy (ART). With the treatment package, the first-line ART is made up of two nucleotide reverse inhibitors (NRTI) and one non-nucleoside reverse transcriptase inhibitor (NNRTI) in accordance to WHO guidelines (Sarfo et al. 2013). No cure has been found for HIV /AIDS yet, but the antiretrovirals help to slow the progression of the virus to AIDS. Current and future researches are focusing on finding new drugs that are less harmful to internal organs, new regimens to avoid drug resistance, and completely eradicate the virus. In addition, gene therapy, vaccines and neutralizing monoclonal antibodies for HIV is under development (Cihlar and Fordyce 2016).

Antiretroviral medications that are approved by the Food and Drug Administration and administered to patients are the Nucleoside reverse transcriptase inhibitors (NRTIs), Non-nucleoside reverse transcriptase inhibitors (NNRTIs), Protease inhibitors (PIs), Entry inhibitors, Fusion inhibitors and Integrase inhibitors. These medications act in various ways to reduce the progression of the disease.

Nucleoside reverse transcriptase inhibitors (NRTIs) usually attach to the protein that HIV requires for replication and disable reverse transcriptase. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) on the other hand create defective forms of building blocks that HIV requires for replication. Protease inhibitors (PIs) inactivate protease, a protein that HIV requires for replication. Entry and fusion inhibitors act by blocking the entry of the virus into cells. Lastly, integrase inhibitors deform the proteins that HIV uses for inserting its viral genetic material into the genetic material of an infected cell. Aside its good side, antiretroviral therapy has also been proven to cause some side effects, such as osteonecrosis and metabolic abnormalities (Chitra et al. 2014).

2.2.1 ANTI RETROVIRAL THERAPY AND RENAL DAMAGE

Depending on the duration that HAART is administered, it has an effect on the nephrons of the kidney as well as on the hepatocytes of the liver by causing toxicity (Peters et al. 2008). Tenofovir disoproxil fumarate, is the known HAART that has been associated with kidney dysfunction and is actively accumulated in the proximal renal tubule (Cihlar et al. 2001). Most HIV medications are harsh on the kidneys and these include antiretroviral medications and other drugs for the treatment of HIV-related cases.

Various evidence from studies show that Anti-Retroviral Therapy (ART) has helped in the reduction of incidence of HIV-related CKD. ART has also been associated with decreased

proteinuria and slowing of progression to ESRD; however, antiretroviral drugs like tenofovir have been associated with renal damage (Sarfo et al. 2013). Interstitial nephritis and nephrolithiasis have been associated with the intake of some antiretrovirals like atazanavir and indinavir. In a few case reports, some protease inhibitors have been linked to renal damage (Chughlay et al. 2015). Some studies have shown that replacing atazanavir and lopinavir/ritonavir with darunavir increases eGFR and improves kidney function (Swanepoel et al. 2018).

Highly active antiretroviral therapy (HAART) and other HIV treatments have some side effects like lactic acidosis, crystal-induced obstruction, interstitial nephritis and electrolyte abnormalities and these can sometimes be toxic to the kidneys (Atta et al. 2008). There are several drug-drug interactions between ARTs and other administered drugs. Some antiretrovirals either induce or inhibit absorption, hepatic metabolism through the cytochrome P450 system and tubular excretion of the other drugs (Swanepoel et al. 2018).

2.3 HIV/AIDS and Kidney Disease

Currently kidney disease is extensively known as a common complication of HIV infection. Kidney disorders are encountered at every stage of HIV infection, and they range from the electrolyte and fluid imbalances to end-stage renal disease (Choi and Rodriguez 2008). There is a high prevalence of HIV-related kidney damage in the West African region as compared to the eastern and southern regions in some few studies conducted (Sarfo et al. 2013). There is a high risk of chronic kidney disease (CKD) and this can quickly progress to end stage renal disease (ESRD) with an increased rate of death in untreated HIV cases. In 1984, the first case of HIV associated kidney disease was discovered in African-American patients with AIDS. Their histological characteristics were formally described as a unique form of collapsing focal segmental glomerulosclerosis (Arendse et al. 2010). Presently, renal disease among HIV-infected persons is mainly due to comorbidities mainly infections, tumours, diabetes, hypertension and drug toxicities (Tonolini et al. 2013).

Renal damage that ranges from slight increase in serum creatinine levels to end-stage renal failure is seen as an important sign in HIV patients. Kidney dysfunction occurs more in untreated patients and patients with AIDS, and most times encountered even in the early stages of HIV infection. Studies have reported reduced glomerular filtration rate in about 10% of HIV people; however, subclinical renal impairment shown by protein present in urine is highly common (Tonolini et al. 2013).

HIV related kidney problems can be caused by the virus or HIV medications (Post et al. 2008).

In Sub-Saharan Africa, the commonest HIV-related renal pathology is HIV-associated nephropathy (HIVAN). HIVAN is characterised histologically by focal segmental glomerulosclerosis with glomerular collapse and commonly associated with excessive proteinuria (Sarfo et al. 2013).

HIVAN is also characterized by podocyte hyperplasia, glomerular and tubulointerstitial damage (Burlaka et al. 2016). Up to 30% of HIV patients have protein in urine as a sign of abnormal renal function and roughly 10% of HIV patients develop renal disease (Fabian and Naicker 2009).

HIVAN manifests clinically with an increased creatinine level, proteinuria and a rapid deterioration of the kidney function without hypertension and oedema. Formally HIVAN was one of the major cause of kidney failure in HIV patients, but with introduction of HAART, its occurrence is considerably decreased and is almost limited to HAART-naïve patients (Tonolini et al. 2013).

Currently, there is a concurrent increase in the occurrence of other kidney diseases. Nephrotoxicity, nephrosclerosis and diabetic nephropathy are also other causes of end stage renal disease (Mallipattu et al. 2014).

HIV patients are also exposed to lifelong ART, with the potential to cause or aggravate kidney injury (Swanepoel et al. 2018). Acute renal damage is a common complication in HIV-infected patients treated with HAART and has currently been associated with AIDS, hepatitis C virus infection and liver disease (Wyatt et al. 2006). It has been proven from researches that HIV-1 infects the glomerular podocytes, epithelial cells of the tubules, and the collecting ducts of the

kidney (Arendse et al. 2010).

2.3.1 Renal pathology of HIV infection

There is diversity in the spectrum of renal pathology in HIV patients. This includes lesions associated to comorbidities, abnormalities of the immune system, drug effects, and other infections and lesions that are directly related to intrarenal expression of HIV genes (Rosenberg et al. 2015).

The biopsy of the kidney is normally used to differentiate between these lesions. The classification of the lesions is done according to the type of tissue which is affected; the glomerulus and the tubules.

There are two main Glomerular-dominant diseases which are the immune complex-mediated and podocytopathies. Podocytopathy has four major subtypes seen in the HIV setting which are the focal segmental glomerulosclerosis (FSGS), HIVAN, diffuse mesangial hypercellularity and uncommon cases of minimal change disease (D'Agati and Appel 1997). These subtypes show proteinuria and extensive podocyte foot process effacement, with absent or little immune complex deposition. The causal relationship between HIV infection and HIVAN has been firmly established. This relationship is mediated by the virus directly infecting the nephrons of the kidney, viral gene expression between the renal cells and abnormalities of cell cycle of the host (Ross 2014).

An invariant component of HIVAN is the tubulointerstitial disease. This disease appears out of proportion to the glomerular disease, resulting in enlargement of the kidney and appears hyperechoic using ultrasound. Tubular microcysts are dilated tubules that contains glassy proteinaceous casts which are lined by the epithelium (Swanepoel et al. 2018). Tubular thyroidisation can be differentiated from tubular microcysts depending on their irregular size, larger diameter, and the absence of tubular atrophy casts and these microcysts include every tubular segment (Nadasdy et al. 1994). Tubular degenerative and regenerative changes and interstitial inflammation sometimes occur. Tubular atrophy and fibrosis usually appear after interstitial oedema

in the acute stages of infection (Swanepoel et al. 2018).

Acute tubular necrosis occur in association with toxic insults, volume depletion, and sepsis (D'Agati and Appel 1997). Proximal tubulopathy with a characteristic dysmorphic mitochondria can be caused by the drug tenofovir disoproxil fumarate.

Tubulointerstitial nephritis can occur secondary to proton pump inhibitors, protease inhibitors, antibiotics, nonsteroidal anti-inflammatory drugs, and other medications (Zaidan et al. 2013). Sometimes other pathogens can directly infect the renal parenchyma. There are two uncommon and distinct forms of tubulointerstitial injury and these are related to the immunologic dysfunction in the HIV infection setting. Diffuse infiltrative lymphocytosis syndrome is a hyperimmune reaction against HIV that involves the kidneys in approximately 10% of HIV cases (Swanepoel et al. 2018).

2.4 Glomerular filtration rate and Kidney damage

A raised or elevated serum Creatinine (SCr) level is usually the first sign of renal dysfunction. Creatinine levels differ across various age groups; it could be low in children and older persons and people having low muscle mass. It could also be high in people with high muscle mass, thus creatinine cannot be an accurate measure of renal function. To correct all of these variations in creatinine, a calculated estimate of renal function is a more accurate measure (Choi and Rodriguez 2008).

The simplified Modification of Diet in Renal Disease (MDRD) Study equation is one of the equations for estimation of eGFR and it produces a better estimate of renal function as compared to the Cockcroft-Gault equation, which uses creatinine clearance. Using MDRD, GFR for patients not affected with kidney disease is: $\geq 90 \text{ mL/min/1.73 m}^2$ whilst that for CKD patients is $< 60 \text{ mL/min/1.73 m}^2$. CKD has been grouped into 5 stages, based on estimated renal function (Table 1).

Table 1. Stages of Chronic Renal Disease based on eGFR

Stage	Definition	eGFR (mL/min/1.73 m ²)
1	Renal damage with normal eGFR	≥90
2	Renal damage with mildly decreased eGFR	60-89
3	Renal damage with moderately decreased eGFR	30-59
4	Renal damage with severely decreased eGFR	15-29
5	Kidney failure	<15

2.5 Vitamin D

One of the essential micronutrient is Vitamin D. Vitamin D is a fat-soluble vitamin produced in the skin in the form of cholecalciferol (vitamin D₃) by exposure to sunlight. Cholecalciferol can also be obtained through diet via animal sources (vitamin D₃) and plant sources as ergocalciferol (vitamin D₂) (Yin 2012). Cholecalciferol whether ingested or absorbed from UV is converted to 25 hydroxyvitamin D (25(OH)D₃) or calcidiol in the liver by the enzyme 25 hydroxylase. 25 hydroxyvitamin D is also acted upon by 1- α hydroxylase, predominantly found in the kidney to 1,25-dihydroxyvitamin D (1,25[OH]₂D) or calcitriol, which is the active form of vitamin D (Yin 2012). Through another pathway 25hydroxyvitamin is also converted into 24- hydroxycalcidiol in the kidneys (Ibrahim et al. 2013).

As an essential vitamin, it acts with calcium, in bone mineralization. There are several forms of this vitamin in the human body: however the most important is the active and the circulating form 1,25-dihydroxyvitamin D (Heaney and Weaver 2003).

Even though the active form of vitamin D is $1,25(\text{OH})_2\text{D}$, its serum levels cannot depict the total vitamin D status in the body, thus serum 25 hydroxyvitamin D level is a more clinically important biomarker (Gupta et al. 2014).

The concentration of 25hydroxyvitamin D reflects the total vitamin D synthesized in the body in addition to those obtained from food and supplements. It has a long circulating half-life of about 15 days making it a better indicator of vitamin D status as compared to serum $1,25(\text{OH})_2\text{D}$ which has a half-life of about 15 hours (Jones 2008).

Many supplements contain vitamin D as vitamin D2 (calciferol) and D3 (cholecalciferol). Sufficient intake of vitamin D may be essential in HIV-infected persons with their additional risk factors for neoplasias, osteopenia and cardiovascular disease (Mueller et al. 2010). The activity of vitamin D of 25hydroxyvitamin D is measured in μg ; 1 μg is equivalent to 40 International Units (IU).

2.5.1 Biological Function of Vitamin D

Vitamin D regulates the homeostasis of phosphate and calcium by controlling the action of fibroblast growth factor 23 (FGF23) and parathyroid hormone (PTH) in increasing intestinal absorption of calcium (McComsey et al. 2007). Vitamin D has some immunomodulatory effect both in the innate and adaptive immunity. In innate immunity, it increases cathelicidin gene expression in macrophages which is associated with bactericidal activity and also targets T cells by the activation and recruitment of helper T cells and regulatory T cells in the adaptive immunity (Yin 2012). The synthesis of 1, 25-dihydroxyvitamin D promotes Vitamin D receptor (VDR) mediated transactivation of the antimicrobial peptide cathelicidin and killing of intracellular *Mycobacterium tuberculosis*. 1,25-dihydroxyvitamin D₃ does not just regulate calcium and bone metabolism but it also exerts immunomodulation via the nuclear VDR expressed in antigen-presenting cells and activated tuberculosis cells (Etten and Mathieu 2005).

Cathelicidins have been shown to exhibit an antiviral effect of inhibiting adenoviruses and retroviruses (Dini and Bianchi 2012). Vitamin D also attaches itself to the vitamin D receptors on CD8 cells and natural killer T (NKT) cells. CD8 and NKT cells are involved in regulating cytokine production and in protecting against the generation of autoimmunity (Cantorna 2011). Researchers have shown that 1,25-dihydroxyvitamin D is an effective modulator of the T-cell phenotype as it inhibits the T-helper 1 cells connected with cellular immune response which conversely enhance the response of humoral T-helper 2 cells (Boonstra et al. 2001).

Some prospective studies done on some autoimmune diseases such as Multiple Sclerosis, diabetes mellitus (DM), Rheumatoid Arthritis, and Systemic Lupus Erythematosus have demonstrated the usefulness of vitamin D supplementation in modulating the elements of the immune system responsible for inflammation, such as growth factors, metalloproteinase, nitric oxide and the expression of cytokines (Marques et al. 2010) .

2.5.2 Mechanism of Vitamin D toxicity

Many researchers have suggested three different hypothesis for the mechanism of vitamin D toxicity and these involve the increased concentration of vitamin D metabolites which reach the Vitamin D Receptor (VDR) in the target cells' nuclei and cause exaggerated gene expressions (Gupta et al. 2014). The proposed mechanisms are:

- I.** The elevated levels of serum 1, 25[OH] 2D, results to increased intracellular concentration of 1, 25[OH]2D. This hypothesis is not well supported as only one study revealed elevated levels of 1,25[OH]2D with Vitamin D toxicity (Mawer et al. 1985), and many other studies reported vitamin D toxicity associated with normal or slightly elevated 1,25[OH]D (Gupta et al. 2014).
- II.** The intake of vitamin D increases the concentration of serum 25(OH) D to levels that exceed the binding capacity of vitamin D binding protein (DBP). This leaves free 25(OH)D which enters the cells and directly affect gene expression (Jones 2008). 1,25[OH]2 D has low affinity for the

transport protein DBP and high affinity for VDR making it an important ligand with access to the transcriptional signal transduction machinery. Various Vitamin D metabolites increase in hypervitaminosis D, compromising the capacity of the DBP and allow other metabolites to enter the cell nucleus. Among these inactive metabolites, 25(OH)D has the strongest affinity for the VDR, so at high concentrations it stimulates transcription.

III. The intake of vitamin D increases the concentration of the vitamin D metabolites particularly and 25[OH] D. In hypervitaminosis D, there is the increase of vitamin D metabolites such as 25[OH] D₃; 24, 25[OH]2D₃; 25,26 [OH]2D₃; 25[OH]D₃-26,23-lactone and 1,25[OH]₂ D₃. These concentrations exceed the DBP binding capacity and cause release of free 1-alpha 25[OH]2D₃, which enters target cells (Jones 2008).

The growing evidence from reports and studies of vitamin D toxicity show that 25[OH]D₃ is a good biomarker for toxicity (Gupta et al. 2014).

2.6 HIV and Vitamin D Deficiency

Vitamin D deficiency has a high prevalence in people infected with HIV and also in the general population. Vitamin D deficiency has been reported to be associated with some disease states like diabetes, fractures, falls and cancer (Yin 2012). Yin (2012) defined Vitamin D deficiency as a concentration of 25-hydroxyvitamin D below 20 ng/mL, severe deficiency being levels below 10ng/mL, insufficiency being levels between 20 ng/mL and 30 ng/mL, and finally a normal level of concentration of 25hydroxyvitamin D above 30 ng/mL. Vitamin D deficiency is linked to increased risk for tuberculosis, fractures, cardiovascular disease and diabetes (Yin 2012). There is a high prevalence of vitamin D deficiency and insufficiency among people infected with HIV (Yin 2012). Some antiretroviral drugs interfere with vitamin D metabolism and this causes Vitamin D deficiency in people taking these drugs. Efavirenz, from various studies showed a reduction in the concentration of vitamin D by almost 5 ng/mL after some months of antiretroviral therapy, whiles

there was an increase in the concentration of vitamin D of about 2 ng/mL with antiretroviral therapy excluding efavirenz (Brown and McComsey 2010). The proposed mechanism of efavirenz is the induction of a cytochrome P450 (CYP450) enzyme, 24-hydroxylase which acts by inactivating 1, 25 dihydroxyvitamin D and 25hydroxyvitamin D. Some in vitro studies also indicated that protease inhibitors lower 1,25 dihydroxyvitamin D levels by inhibiting 1- α hydroxylase, the enzyme for converting 25hydroxyvitamin D to 1,25 dihydroxyvitamin D, even though no clinical studies that reported a consistent decreasing vitamin D level (Yin 2012).

Vitamin D deficiency is important in HIV as the epidemiology of the disease corresponds with the individuals at higher risk of vitamin D deficiency and for the negative effect of protease inhibitors on the metabolism of Vitamin D (Cozzolino et al. 2003).

2.7 Hypervitaminosis D and Hypercalcemia

Hypervitaminosis D is defined as the serum concentration of total 25hydroxyvitamin D greater than 30 ng/L (Pérez-Barrios et al. 2016). Hypervitaminosis D is a state where elevated levels of 25-hydroxyvitamin D is associated with hypercalciuria or hypercalcemia or both (Ozkan et al. 2012). Hypervitaminosis D is an uncommon cause of hypercalcemia and is associated with prolonged morbidity (Rizzoli et al. 1994). Hypercalcemia could be the manifestation of a number of primary disorders like hyperthyroidism, Paget's disease, hypervitaminosis A, hypervitaminosis D, milk-alkali syndrome, hyperparathyroidism, and Addison's disease; and some urologic malignancies especially carcinoma of the kidney (Glenn 1995).

Hypercalciuria and hypercalcemia results from increased bone resorption and intestinal absorption. Hypervitaminosis D is associated with a marked morbidity and sometimes permanent damage secondary to extensive metastatic calcification, particularly nephrocalcinosis (Rizzoli et al. 1994). Hypervitaminosis D can lead to irreversible calcification of soft tissues and thus, it is highly necessary to detect early markers of Hypervitaminosis D like hypercalcemia, hypercalciuria and

parathyroid hormone suppression (Pérez-Barrios et al. 2016). Because of the prolonged biological half-life of vitamin D and its metabolite calcidiol (25hydroxyvitamin D₃), the hypercalcemic/hypercalciuric condition can persist for a long time usually weeks or months, with harmful consequences. Hypercalcemia in vitamin D toxicity results from a combination of increased bone resorption, enhanced intestinal calcium absorption, and renal damage (Rizzoli et al. 1994).

Hypervitaminosis D may result from subcutaneous fat necrosis and sarcoidosis, in addition to excess intake of vitamin D. However, depending on the level of hypercalcemia, its effect can be observed on the eyes, skin, kidney, central nervous, gastrointestinal, cardiovascular and musculoskeletal systems. The concentration of calcium and phosphorus levels above 60 mg/dl, leads to the accumulation of calcium phosphate crystals within the soft tissues of the body; vascular calcification, impairment in renal function and the development of renal hypertension (Ozkan et al. 2012).

1, 25-dihydroxyvitamin D is known to cause hypercalcemia but at pharmacological levels, 25-hydroxyvitamin D may directly stimulate transcription by distinct mechanisms (Jones 2008). Evidence gathered suggest that for vitamin D toxicity to occur the concentration of 25hydroxyvitamin D should rise above 375-500 nmol/l, others also reported the occurrence of hypercalcemia at low 25-OHD levels (Jones 2008). In the same way, many case reports show that hypervitaminosis D occurs at serum 25hydroxyvitamin D levels above 250 nmol/L, but there is limited evidence and systematic studies supporting this range are lacking (Pérez-Barrios et al. 2016).

2.8 Hypervitaminosis D and Renal damage

Hypervitaminosis D occurs when there are excessive amounts of vitamin D in the body than the body needs. Vitamin D toxicity mostly occur when higher doses of vitamin D supplements are

taken over a long period of time and not by food or exposure to UV light (Gupta et al. 2014). Vitamin D intoxication from food sources is highly uncommon and over exposure to the sun rays results to metabolic changes in the synthesis of the precursors of vitamin D (Pérez-Barrios et al. 2016).

Vitamin D toxicity causes hypercalcemia and other adverse effects including potentially life-threatening ones (Gupta et al. 2014). Vitamin D toxicity, causing hypercalcemia leads to metastatic calcification, including urolithiasis and perivascular calcifications in various parts of the body. Although Vitamin D has a wide therapeutic index, its toxicity is well known due to off the counter usage of various calcium and Vitamin D supplements, self-medication and malpractice (Nair et al. 2015).

Vitamin D toxicity is characterized by hypercalcemic and hypercalciuric states which is suggestive of excess $1, 25(\text{OH})_2\text{D}$ -like activity. The clearance mechanism for vitamin D metabolites include oxidation and side chain cleavage beyond carbon 23 of the secosteroid molecule. The metabolites are then converted into bile and excreted by the liver (Jones et al. 1987). $25(\text{OH})\text{D}$ and $1, 25(\text{OH})_2\text{D}$ are eliminated by the same clearance mechanisms. Vitamin D toxicity will therefore develop if the intake of vitamin D and its metabolites exceeds the body's adaptive capacity to eliminate them (Jones et al. 1987). Hypervitaminosis D results in hypercalcemia and hypercalciuria. Hypercalcemia causes deposition of calcium crystals on kidney tissues which may lead to kidney damage (Nair et al. 2015). Kidney problems can generate into end-stage renal disease (ESRD) or kidney failure if proper treatment is not given.

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1.0 Study Site

The study was carried out at the Fevers unit of the Department of Medicine, Korle-Bu Teaching Hospital. Korle-Bu Teaching Hospital is a leading tertiary referral hospital in Accra with 2,000 bed capacity and 3,000 members of staff. The hospital serves the Accra Metropolis and the whole of the southern sector of Ghana. The hospital admits patients with HIV/AIDS as well as provides outpatient services to this category of clients. The fevers unit of the Korle Bu teaching hospital, however, holds over 10,000 patient records, and each week, approximately 350 patients attend the clinic from in and around Greater Accra. Calcium levels, creatinine and albumin levels and ELISA was measured at Department of Chemical Pathology and the central laboratory (KBTH) which is the leading laboratory in Korle Bu where samples are collected and analysed. The central laboratories serve over 1000 people in the hospital and beyond which has modern equipment's for laboratory diagnosis and test.

3.2.0 Study Design and selection of Participants

This study was a cross-sectional study which involved random selection of participants from the study site. The study was conducted from July, 2017 to July, 2018.

Ethical and study protocol was reviewed and approved by the Ethical and Protocol Review Committee of College of Health Sciences, University of Ghana (CHS-Et/M.3-P2.4/2018-2019). The subjects for this study constituted confirmed sero-positive HIV patients with HIV-1 and/or HIV-2. All subjects recruited for the study were made to understand the content of the participation

information sheet and signed the consent form. Participants were randomly selected from the out patients' department of the Fevers unit of Korle Bu Teaching Hospital. Recruited participants were put into three groups. The first group represented newly diagnosed HIV cases who were not on HAART and vitamin Supplements, the second group represented participants confirmed to be HIV-1 and/or HIV-2 seropositive and on HAART and vitamins supplement treatment for at least six months. The third group also represented participants also confirmed to be seropositive of HIV-1 and/or HIV-2 on HAART and vitamins supplement treatment for more than six months.

3.2.1 Inclusion Criteria

Participants:

- i. who have been tested and confirmed seropositive of HIV (HIV-1 and/or HIV-2) or seronegative
- ii. older than 18 years of both sexes
- iii. on HAART and vitamins supplement therapy for at least six months
- iv. on HAART and vitamins supplement therapy for more than six months

3.2.2 Exclusion Criteria

- i. Participants with both liver and kidney diseases
- ii. Hypertensive patients
- iii. Diabetic patients

3.3.0 Sample Size Determination

The sample size was determined from the relationship: $n \geq Z^2 p (1 - p) / e^2$ (Cochran 1977) where n is the number of sample size calculated, Z is the Z-score at 95% confidence level, P is the prevalence of case and e is the margin of error taken at 95% confidence level.

The prevalence rate (p) of case in Ghana is taken as 1.37% (Biney et al. 2016). Z-score (z) is taken as 1.96 at 95% confidence level and margin of error (e) taken as 0.10

Therefore,
$$n \geq 1.96^2 \times 1.37 \times (1 - 1.37) / 0.1^2$$

$$n \geq 3.8416 \times 1.37 \times (0.37) / 0.01$$

$$n \geq 194$$

$$n = 200$$

Based on the above estimation, a sample size of 200 participants was recruited for the study.

3.4.0 Study Participants

A total of 200 participants confirmed of HIV1 and/or 2 where recruited from the fevers unit of KBTH, Accra. This number constituted 100 participants on HAART and vitamins supplement treatment for at least six months. 50 participants on HAART and vitamins supplement treatment for more than six months and 50 participants not on HAART and vitamin supplement.

3.5.0 Sample Collection, Preparation and Storage

Five millilitres (mls) of blood samples were collected into serum gel separator tubes. The samples were allowed to clot and centrifuged at 4000rev/min for 5mins to obtain the serum.

The serum was separated into eppendorf tubes and stored in the refrigerator at -20 ° C. The serum was used for analysis of 25 hydroxyvitamin D, Calcium (Ca), albumin, and Creatinine (Cr)

3.6 Reagents and Equipment

The reagents and equipments for biochemical analysis are

Reagents:

Creatinine Jaffe (Elitech clinical systems, France), Calcium Arsena (Elitech clinical systems, France), Albumin (Elitech clinical systems, France), HUMAN 25 hydroxyvitamin D ELISA Kit

Equipments:

Selectra pro S fully automated chemistry analyser (Elitech clinical systems, France), URIT 660 microplate Reader (China)

3.7.0 Anthropometric measurements and Clinical characteristics

The anthropometric measurements taken for each participant included age, sex, height, weight, body mass index (BMI), blood pressure (BP) and type of drug(s) administered. The type of drugs administered to the participants were obtained from the medical folder of the participant.

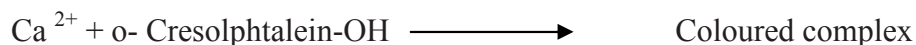
3.8.0 Biochemical Analysis

3.8.1 Serum Calcium (Ca)

Serum calcium was analysed with Selectra pro S fully automated chemistry analyser and an assay kit from Elitech clinical systems, France.

Principle of Assay

The measurement of calcium in the sample is based on formation of colour complex between calcium o- cresolphthalein in alkaline medium



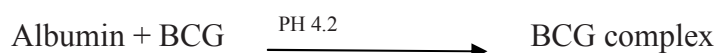
o-Cresolphthalein complex gives violet color in alkaline medium. The intensity of the color formed is proportional to the calcium concentration in the sample.

3.8.2 Albumin

Serum albumin was analysed with Selectra pro S fully automated chemistry analyser and an assay kit from Elitech clinical systems, France

Principle of Assay

At, pH 4.2 albumin is sufficiently cationic and it binds the anionic dye bromocresol green to form a blue-green coloured complex.



The intensity of the blue-green colour is directly proportional to the concentration of albumin in the sample. The concentration is determined by measuring the increase in absorbance at 620-630nm

3.8.3 Creatinine (Cr)

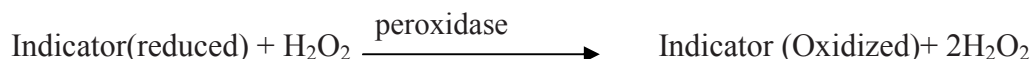
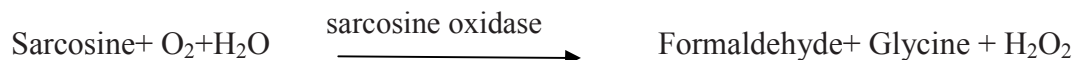
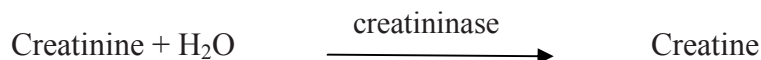
Serum creatinine was analysed with Selectra pro S fully automated chemistry analyser and an assay kit from Elitech clinical systems, France

Principle of Assay

The principle of the assay of creatinine is based on the Jaffe reaction using the Folin and Wu method described in Hawk, Oser, & Summerson, 1947.

Under alkaline conditions creatinine reacts directly with picric ions to form a reddish complex.

The absorbance of the reddish complex is measured at 520 nm.



3.8.4 Determination of 25 hydroxyvitamin D levels

Human 25 hydroxyvitamin D ELISA kit was used for the analysis of 25 hydroxyvitamin D. 50 µl of Calibrator, Control and Samples were pipetted into the appropriate wells. 150 µl of incubation buffer was also pipetted into all the wells. The mixture was incubated for 2 hours at room temperature, on a plate shaker (300 to 700 rpm). The liquid from each well was aspirated and the plate was washed 3 times by: dispensing 0.35 ml of wash solution into each well and aspirating the content of each well. 200 µl of the horse radish peroxidase (HRP) conjugate solution was pipetted into each well and the mixture was incubated for 30 minutes at room temperature, on a plate shaker. The liquid from each well was aspirated and the plate washed 3 times. 100 µl of the chromogenic solution was pipetted into each well within 15 minutes following the washing step and the microtiter plate was incubated for 15 minutes at room temperature, on a plate shaker. 100 µl of stop solution was pipetted into each well and the absorbance was read at 450 nm within one hour on URIT 660 microplate Reader.

3.9.0 Statistical Analysis

Microsoft office Excel and IBM Statistical Package for the Social Sciences (SPSS Statistics 22) were the statistical softwares used for the analysis of data of this study. Continuous data were expressed as Mean \pm SD whilst categorical data were expressed as proportions and percentages. Association between variables was assessed with linear regression analysis. One way analysis of Variance (ANOVA) test was used for comparison between three or more groups having quantitative normally distributed data. Student's (t) test was used for comparison between two groups normally distributed. Statistical significance of proportions was assessed with Chi-square test. All Statistical significance was set at $p < 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1.0 Background of Study Population

A total of two hundred (200) positively confirmed HIV1/2 patients were recruited into the study, consisting of one hundred who were on HAART and vitamin supplement for more than six months, fifty were on HAART and vitamin supplement for less than six months and fifty patients who were not on both HAART and vitamin supplement. Categorical data were expressed as percentage and descriptive data were also presented as mean \pm standard deviation.

Patients taking HAART and vitamin supplement for more than six months were 100 made up of 30(30%) males and 70 (70%) females. Patients on HAART and vitamin supplement for less than six months patients were 50 comprising of 17 (34%) males and 33 (66%) females. Patients not on both HAART and vitamin supplement was 50, made up of 14 (28%) males and 36 (72%) females.

4.1.1 Anthropometric and Baseline Characteristics of the Study Population**Table 4.1:** Baseline clinical characteristics of HIV/AIDS patients on HAART/Vitamin D supplements and HAART/Vitamin D supplement-naïve HIV patients

Parameter	HAART/Vit. naïve n (%)	D- HAART/ Vit. D (<6months) n (%)	HAART/ Vit. D (≥ 6months) n (%)	P-value
Age (years)	44.12 ± 11.34	40.72 ± 7.38	44.71 ± 8.37†	0.035
Gender				0.799
Male	14 (28.0)	17 (34.0)	30 (30.0)	
Female	36 (72.0)	33 (66.0)	70 (70.0)	
Age categories				0.038
20-29	4 (8.0)	3 (6.0)	1 (1.0)	
30-39	15 (30.0)	18 (36.0)	30 (30.0)	
40-49	14 (28.0)	24 (48.0)	42 (42.0)	
50-59	11 (22.0)	4 (8.0)	22 (22.0)	
60-69	6 (12.0)	1 (2.0)	5 (5.0)	
Blood pressure (mmHg)				
SBP	112.92 ± 7.87	113.20 ± 10.79	120.25 ± 23.64*†	0.020
DBP	72.90 ± 8.69	73.74 ± 10.33	74.18 ± 14.96	0.841
BMI (Kg/m²)	25.80 ± 3.46	24.56 ± 3.77	24.97 ± 4.67	0.332
BMI, n (%)				0.168
Underweight	1 (2.0)	2 (4.0)	4 (4.0)	
Normal	16 (32.0)	18 (36.0)	50 (50.0)	
Overweight	26 (52.0)	25 (50.0)	31 (31.0)	
Obese	7 (14.0)	5 (10.0)	15 (15.0)	

Values are presented as mean ± SD or Frequency (percentage) *P<0.05 compared with HAART-naïve group; †P<0.05 compared with HAART (<6months) group.

SD: standard deviation, n represents number of participants, SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: Body mass index, results presented as mean ± SD. Statistically significant level is given at p-value <0.05.

The mean systolic blood pressure (SBP) of patients compared among the three groups were

statistically significant ($p < 0.05$). However, the mean diastolic blood pressure (DBP) and body mass index (BMI) was not statistically significant in the patients taking HAART and vitamin supplement for more than six months, patients taking HAART and vitamin supplement for less than six months and those not on both HAART and vitamin supplement ($p > 0.05$).

4.1.2 Biochemical Indices of study participants

Table 4.2: Biochemical indices of HIV/AIDS patients on HAART/Vitamin D supplements and HAART/Vitamin D supplement-naïve HIV patients

Parameter	HAART/Vit. D- naïve n (%)	HAART/ Vit. D (<6 months) n (%)	HAART/ Vit. D (≥ 6 months) n (%)	P-value
ALB(g/l)	41.06 \pm 5.84	42.12 \pm 5.17	43.32 \pm 6.29*	0.081
Ca(mmol/l)	2.16 \pm 0.35	2.18 \pm 0.22	2.24 \pm 0.36	0.289
25 hydroxyVitamin D (ng/ml)	6.42 \pm 3.17	6.37 \pm 2.80	6.93 \pm 4.33	0.601
Creatinine, umol/l	69.70 \pm 22.56	78.41 \pm 22.38	73.78 \pm 31.92	0.290
eGFR (mL/min/1.73m ²)	97.48 \pm 84.44	84.44 \pm 23.35*	93.65 \pm 33.11	0.099

Values are presented as mean \pm SD or Frequency (percentage) *P<0.05 compared with HAART-naïve group; †P<0.05 compared with HAART (<6months) group.

The mean Glomerular filtration rates,albumin , calcium, creatinine and 25 hydroxyvitamin D levels were not statistically significant in the patients on HAART and vitamin supplement for more than six months,less than six months and not on both HAART and vitamin supplement ($p > 0.05$) (table 4.2).

Table 4.3: 25hydroxyvitamin D status and eGFR Staging among HIV/AIDS patients on HAART/Vitamin D supplements and HAART/Vitamin D supplement-naïve HIV patients

Parameter	HAART/ D –naïve n (%)	Vit. HAART/ Vit. D (<6months) n (%)	HAART/ Vit. D (≥ 6months) n (%)	P-value
25 hydroxyVitamin D (ng/ml)				0.810
< 15 (Low)	48 (96.0)	48 (96.0)	94 (94.0)	
15-60 (Normal)	2 (4.0)	2 (4.0)	6 (6.0)	
eGFR, n (%)				0.472
< 60	7 (14.0)	4 (8.0)	15 (15.0)	
≥ 60	43 (86.0)	46 (92.0)	85 (85.0)	
eGFR Staging n (%)				0.014
≥90	29 (58.0)	16 (32.0)	55 (55.0)	
60-89	14 (28.0)	30 (60.0)	30 (30.0)	
30-59	6 (12.0)	4 (8.0)	14 (14.0)	
15-29	1 (2.0)	0 (0.0)	1 (1.0)	

Values are presented as Frequency (percentage) at P<0.05

Table 4.3 represents the 25hydroxyvitamin D status and the eGFR staging of the patients on HAART and vitamin supplement for more than six months, less than six months and patients not on both HAART and vitamin supplement. 25 hydroxyvitamin D levels were low (<15ng/ml) in patients on HAART and vitamin supplement for the categories (more than six months, less than six months and not on both HAART and vitamin supplement). The mean eGFR was greater than 60 mL/min/1.73 m² for about 70% of the patients on HAART and vitamin supplement for more than six months, less than six months and patients not on both HAART and vitamin supplement.

Table 4.4: Gender and Age association with vitamin D status of HIV/AIDS patients on HAART/Vitamin D supplements and HAART/Vitamin D supplement-naïve HIV/AIDS patients

Characteristics	HAART/ Vit. D –naïve			HAART/ Vit. D (<6months)			HAART/ Vit. D (≥ 6months)		
	Vitamin D status		P-value	Vitamin D status		P-value	Vitamin D status		P-value
	Low n=48(%)	Normal n=2 (%)		Low n=48 (%)	Normal n=2 (%)		Low n=94 (%)	Normal n=6 (%)	
Gender			0.368			0.044			0.043
Male	14 (29.2)	0 (0.0)		15 (31.2)	0 (0.0)		26 (27.7)	4 (66.7)	
Female	34 (70.8)	2 (100)		33 (68.8)	2 (100)		68 (72.3)	2 (33.3)	
Age categories			0.187			0.978			0.67
20-29	3 (6.2)	1 (50.0)		3 (6.2)	0 (0.0)		1 (1.1)	0 (0.0)	
30-39	14 (29.2)	1 (50.0)		17 (35.4)	1 (50.0)		29 (30.9)	1 (16.7)	
40-49	14 (29.2)	0 (0.0)		23 (47.9)	1 (50.0)		39 (41.5)	3 (50.0)	
50-59	11 (22.9)	0 (0.0)		4 (8.3)	0 (0.0)		21 (22.3)	1 (16.7)	
60-69	6 (12.5)	0 (0.0)		1 (2.1)	0 (0.0)		4 (4.3)	1 (16.7)	
Blood pressure									
SBP	112.79 ± 7.99	116.00 ± 2.83	0.577	113.46 ± 10.81	107.00 ± 11.31	0.412	120.32 ± 24.33	119.17 ± 7.47	0.909
DBP	73.06 ± 8.72	69.00 ± 9.90	0.523	73.96 ± 10.49	68.50 ± 2.12	0.470	74.35 ± 14.83	71.50 ± 17.84	0.653
BMI (Kg/m²)	25.77 ± 3.45	26.55 ± 5.02	0.759	24.53 ± 3.84	25.95 ± 0.07	0.608	24.93 ± 4.58	25.63 ± 6.47	0.726
BMI, n (%)			0.353			0.555			0.790
Underweight	1 (2.1)	0 (0.0)		2 (4.2)	0 (0.0)		7 (3.7)	0 (0.0)	
Normal	15 (31.2)	1 (50.0)		18 (37.5)	0 (0.0)		79 (41.6)	5 (50.0)	
Overweight	26 (54.2)	0 (0.0)		23 (47.9)	2 (100)		79 (41.6)	3 (30.0)	
Obese	6 (12.5)	1 (50.0)		5 (10.4)	0 (0.0)		25 (13.2)	2 (20.0)	

Values are presented as Frequency (percentage) at P<0.05

Table 4.4 shows the gender and age association with vitamin D for patients on HAART and vitamin supplement for more than six months, less than six months and not on both HAART and vitamin supplement. Most of the patients had low 25 hydroxyvitamin D levels. Low vitamin D levels were found across the various age groups.

Table 4.5: Association of vitamin D status with calcium, albumin and renal function parameters of HIV/AIDS patients on HAART/Vitamin D supplements and HAART/Vitamin D supplement-naïve HIV/AIDS patients

Parameters	HAART/ Vit. D –naïve			HAART/ Vit. D (<6months)			HAART/ Vit. D (≥ 6months)		
	Vitamin D status		P-value	Vitamin D status		P-value	Vitamin D status		P-value
	Low (n=48)	Normal (n=2)		Low (n=48)	Normal (n=2)		Low (n=94)	Normal (n=6)	
ALB(g/l)	41.13 ± 5.95	39.35 ± 0.49	0.687	42.06 ± 5.08	43.55 ± 9.69	0.695	43.30 ± 6.37	43.57 ± 5.27	0.921
Ca(mmol/l)	2.16 ± 0.35	2.20 ± 0.14	0.863	2.19 ± 0.22	2.05 ± 0.21	0.400	2.24 ± 0.37	2.25 ± 0.18	0.951
Creatinine, <i>umol/l</i>	70.08 ±			78.49 ±			73.57 ±		
eGFR	22.57	60.75 ± 28.80	0.572	22.61	76.65 ± 22.13	0.911	32.69	76.93 ± 16.66	0.804
(<i>mL/min/1.73m²</i>)	98.67 ±	116.95 ±		83.69 ±	103.00 ±		93.95 ±		
eGFR, n (%)	33.65	66.82	0.422	23.00	33.94	0.256	33.85	89.02 ± 19.03	0.726
< 60			0.560			0.670			0.289
≥ 60	7 (14.6)	0 (0.0)		4 (8.3)	0 (0.0)		15 (16.0)	0 (0.0)	
eGFR Staging n (%)	41 (85.4)	2 (100)		44 (91.7)	2 (100)		79 (84.0)	6 (100)	
≥90			0.879			0.814			0.225
60-89	28 (58.3)	1 (50.0)		15 (31.2)	1 (50.0)		53 (56.4)	2 (33.3)	
30-59	13 (27.1)	1 (50.0)		29 (60.4)	1 (50.0)		26 (27.7)	4 (66.7)	
15-29	6 (12.5)	0 (0.0)		4 (8.3)	0 (0.0)		14 (14.9)	0 (0.0)	
	1 (2.1)	0 (0.0)		0 (0.0)	0 (0.0)		1 (1.1)	0 (0.0)	

Values are presented as mean ± SD or Frequency (percentage), significant at P<0.05

Table 4.5 also shows the association of vitamin D status with calcium, Albumin and eGFR for patients on HAART and vitamin supplement for more than six months, less than six months and not on both HAART and vitamin supplement.

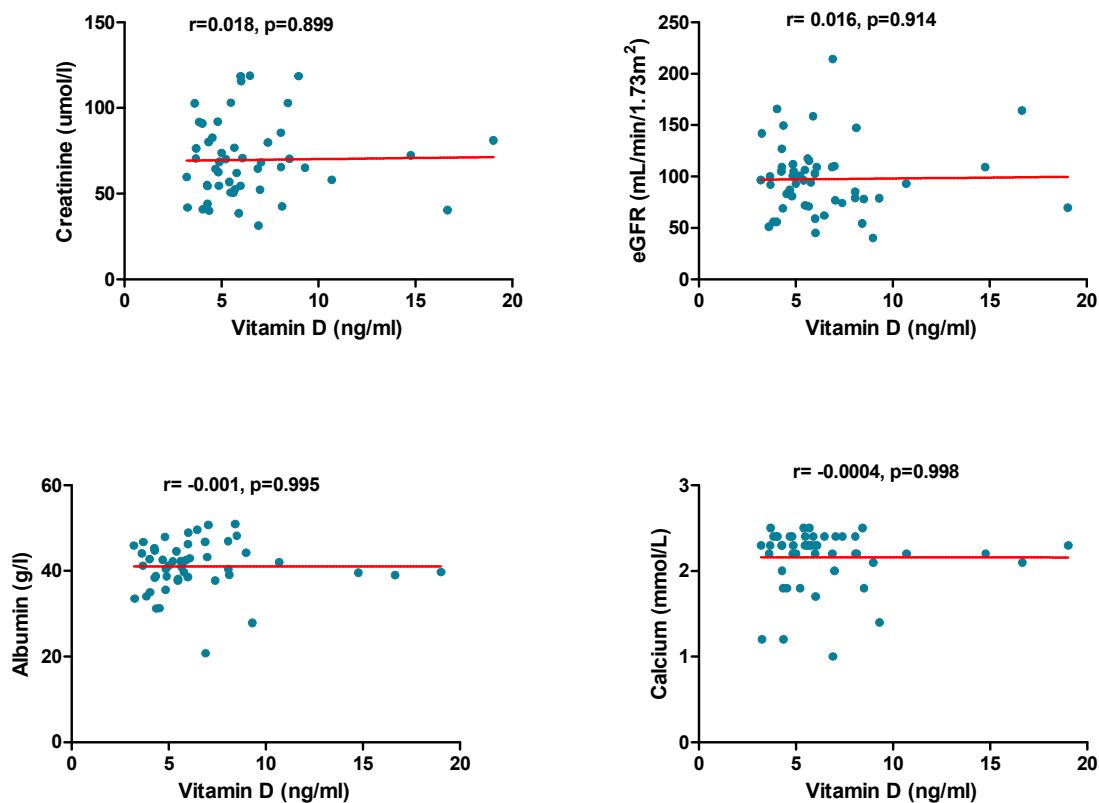


Figure 1: Vitamin D correlation with serum creatinine, eGFR, albumin and calcium among HAART/Vit. D-naïve HIV/AIDS patients

Figure 1 represents the Vitamin D correlation with serum creatinine, eGFR, albumin and calcium among HIV/AIDS patients not on both vitamin supplement. There is no correlation between 25hydroxyvitamin D and creatinine, eGFR, albumin and calcium

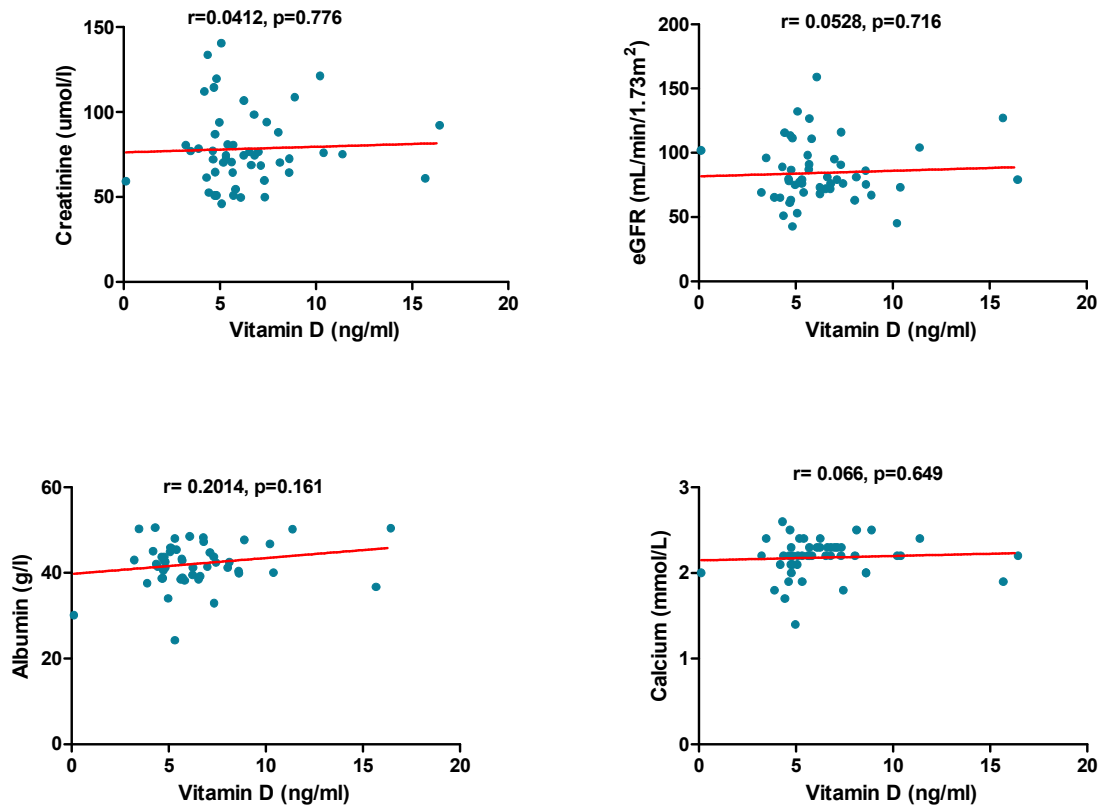


Figure 2: Vitamin D correlation with serum creatinine, eGFR, albumin and calcium among HIV/AIDS patients on HAART and Vitamin D supplement for less than 6 months

Figure 2 represents the Vitamin D correlation with serum creatinine, eGFR, albumin and calcium among HIV/AIDS patients on HAART and Vitamin supplement for less than 6 months. There is no correlation between 25hydroxyvitamin D and creatinine, eGFR, albumin and calcium among HIV/AIDS patients on HAART and Vitamin D supplement for less than 6 months

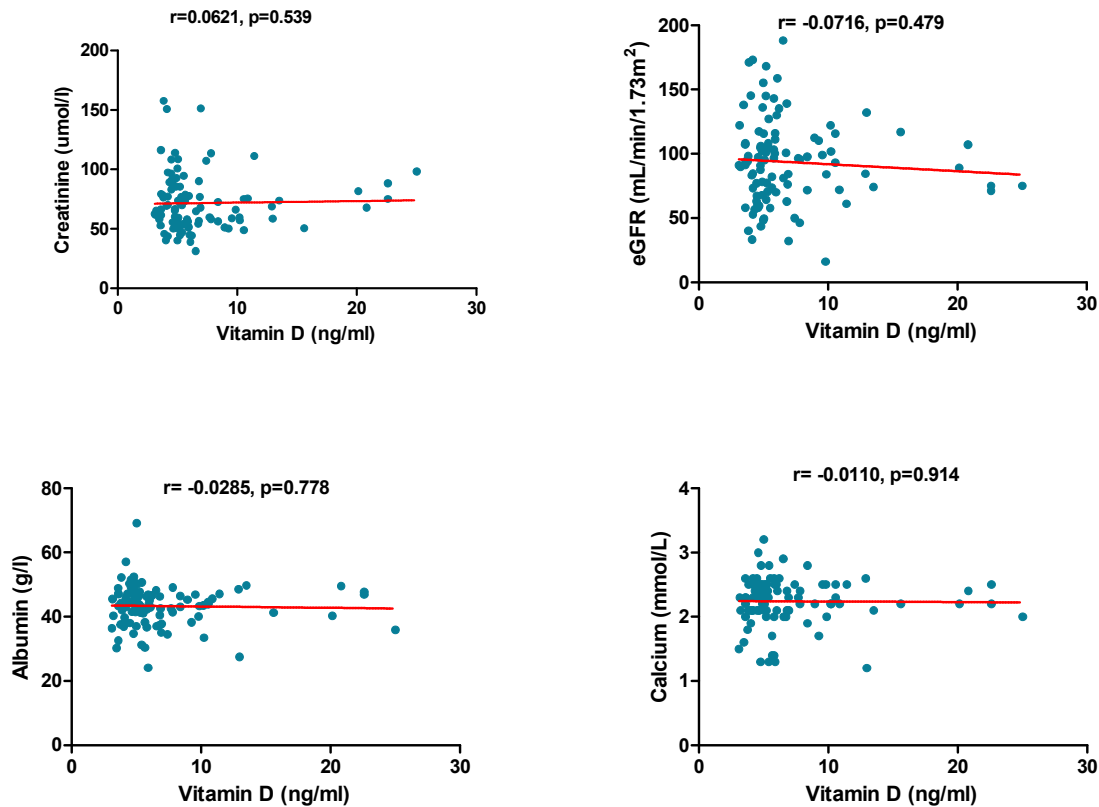
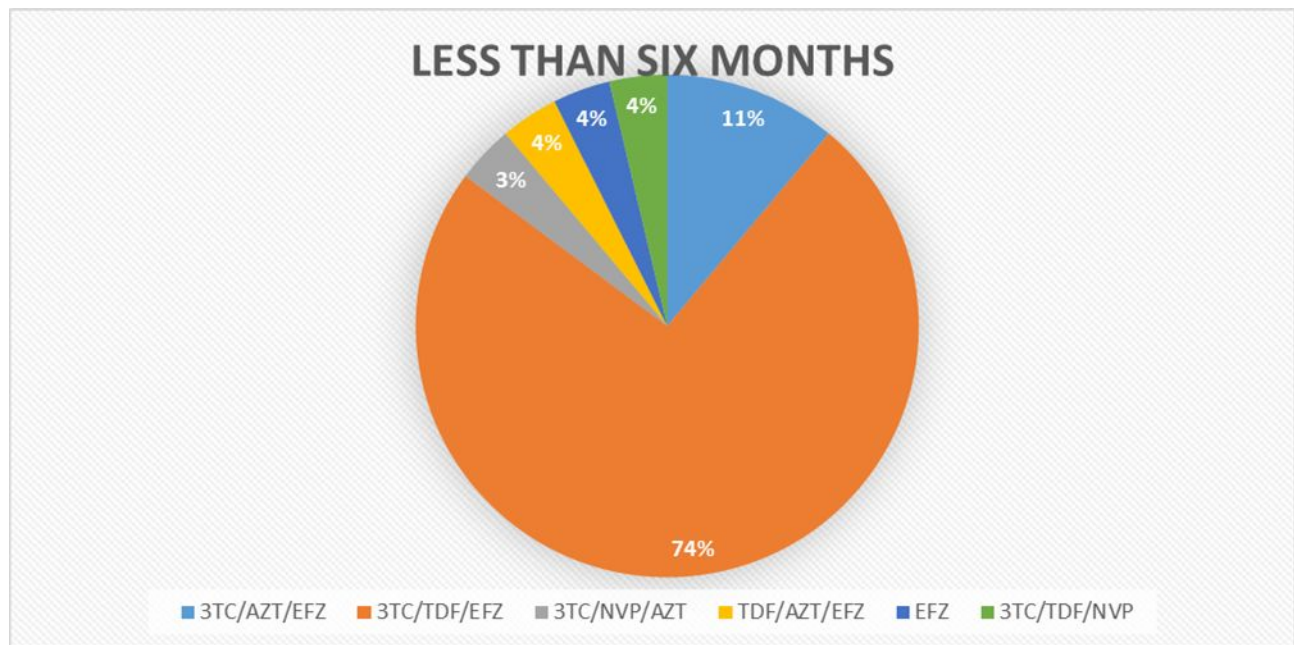


Figure 3: Vitamin D correlation with serum creatinine, eGFR, albumin and calcium among HIV/AIDS patients on HAART and Vitamin D supplement for more than 6 months

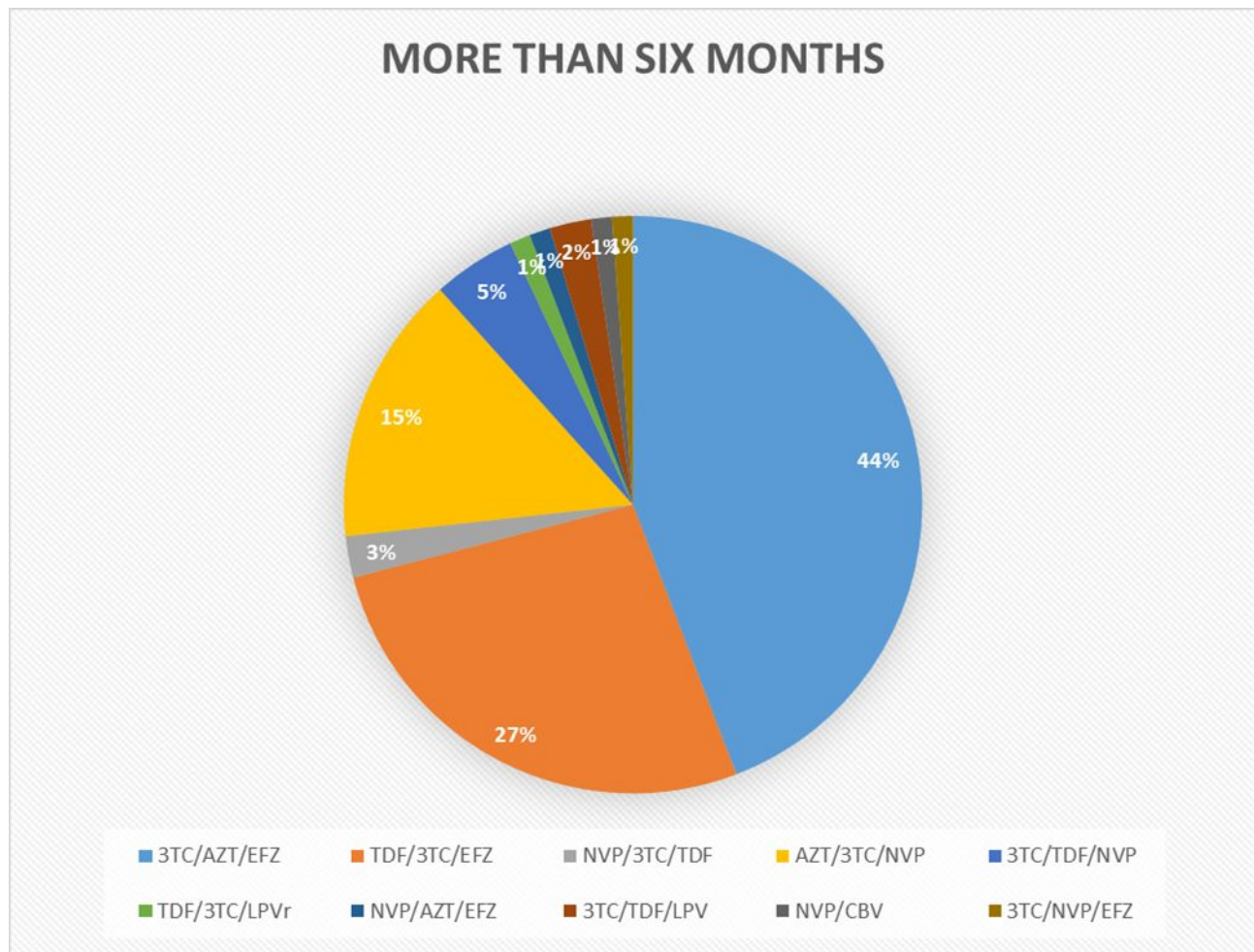
Figure 3 represents the Vitamin D correlation with serum creatinine, eGFR, albumin and calcium among HIV/AIDS patients on HAART and Vitamin supplement for more than 6 months. There is no correlation between 25hydroxyvitamin D and creatinine, eGFR, albumin and calcium among HIV/AIDS patients on HAART and Vitamin D supplement for more than 6 months



Key: Lamivudine (3TC), Zidovudine (AZT), Tenofovir (TDF), Efavirenz (EFZ), Nevirapine (NVP), Lopinavir (LPV)

Figure 4: Distribution of ARTs among HIV/AIDS patients on HAART and Vitamin D supplement for less than 6 months

Figure 4 represents the distribution of ARTs among patients on HAART and vitamin supplement for less than six months. More than 70% of the patients on vitamin supplement were also taking Efavirenz in the HAART combination. 74% of the patients were taking 3TC/TDF/EFZ, 11% of the patients were taking 3TC/AZT/EFZ, 4 % of the patients were taking TDF/AZT/EFZ; EFZ; 3TC/TDF/NVP and finally 3% of the patients were taking 3TC/NVP/AZT



Key: Lamivudine (3TC), Zidovudine(AZT), Tenofovir(TDF), Efavirenz(EFZ), Nevirapine(NVP), Lopinavir(LPV), Combivir (CBV)

Figure 5: Distribution of ARTs among HIV/AIDS patients on HAART and Vitamin D supplement for more than 6 months

Figure 5 represents the distribution of ARTs among patients on HAART and vitamin supplement for more than six months. More than 70% of the patients on vitamin supplement are also taking Efavirenz in the HAART combination. 44% of the HIV patients were taking 3TC/AZT/EFZ, 27 % of them were taking TDF/3TC/EFZ, 15% of the patients were taking AZT/3TC/NVP, 5% of them were also taking 3TC/TDF/NVP, 3% of the patients were taking NVP/3TC/TDF, 2% of them were taking 3TC/TDF/LPV, with 1% of them taking TDF/3TC/LPVr, NVP/AZT/EFZ, NVP/CBV, 3TC/NVP/EFZ

CHAPTER FIVE

5.0 DISCUSSION

The study was a randomized case study which consisted of a total of two hundred (200) HIV/AIDS infected participants. Patients who had been on HAART in less than six (6) months and those naïve of this treatment represented 50 (25%) each of the total study population. Those on treatment for more than six months represented 100 (50%) of the study population. Males and females naïve of treatment were 14 (28%) and 36 (72%) respectively. Those on HAART treatment in less than six months were 33 (66%) for females and 17 (34%) for males. Females were more than males in patients who had received HAART for over six months represented as 30 (30%) males and 70 (70%) females. Considering the age distribution across the study population, there was no significant difference in age between HAART-naïve and HAART patients for less than six months of treatment. However, patients receiving HAART for more than six months were significantly older than their counter parts with treatment less than six months. This difference may be as a result of early treatment with HAART for HIV infection.

This study was aimed at finding an association between vitamin D and kidney function in Ghanaian HIV/AIDS patients by determining serum levels of albumin, calcium, Vitamin D, creatinine and the estimation of glomerulus filtration rate (eGFR).

Vitamin D deficiency is a common condition in HIV/AIDS patients (Rodríguez et al. 2009). In order to prevent this deficiency, recommendations and guidelines from WHO requires the inclusion of Vitamin D contained supplements alongside antiretroviral in HIV patients. Although hypervitaminosis of vitamin D is a rare occurrence as reported by some other studies, Pettifor et al., 1995 reported of a possible occurrence of hypervitaminosis of vitamin D with an associated hypercalcemia. This was attributed to large dose or long term exposure with a safe dose. This

report, on the contrary, was different in this study. The study showed rather low levels of vitamin D in patients not on both HAART and vitamin supplement, patients who had been on treatment for less than six months and those on treatment for more than six months of vitamin D contained supplements. However healthy people taking over a million IU (>25,000 g) of vitamin D supplement each day for several months would develop hypervitaminosis (Vieth 2007)

The mean 25hydroxyvitamin D of patients not on both HAART and vitamin supplement, patients on HAART and vitamin supplement for less than six months and more than six months were less than 15ng/ml indicating vitamin D deficiency (Yin 2012). Interestingly, large numbers of patients with more than 6 months of vitamin D exposure were deficient of the vitamin D. A similar finding was reported by Allavena et al., (2012) of which it was stated that Vitamin D deficiency is frequent in this HIV-infected population.

Patients on antiretroviral therapy have a high risk of being vitamin D deficient than other patients not on the therapy, with a higher risk in patients taking efavirenz (Brown and McComsey 2010). This study is similar to the report by Brown and McComsey, as most of the patients on vitamin supplement were also taking efavirenz in the HAART combination and this could lead to the low vitamin D status. The recognised mechanism of efavirenz is the induction of 24-hydroxylase, a cytochrome P450 (CYP450) enzyme which inactivates 1,25(OH)₂D and 25(OH)D (Yin 2012). The low 25hydroxyvitamin D status findings were also supported with the absence of hypercalcemia among all the study groups. Other antiretrovirals like ritonavir and saquinavir also reduces the vitamin D levels through the activation of pregnane x receptor which causes the expression of 24 hydroxylase (Gröber and Kisters 2012). In addition some protease inhibitors inhibit 1 alpha hydroxylase which converts 25hydroxyvitamin D to 1,25 dihydroxyvitamin D (Yin 2012).

Some studies and researches have reported the incidence of renal dysfunction among HIV/AIDS patients (Puinta and Achidi 2011; Roling et al. 2006). Related renal disease which can manifest as an acute or chronic condition, is among the high causes of death in HIV/AIDS persons particularly

among African descents (Puinta and Achidi 2011). The cause of this as reported by Roling et al., (2006) can be caused by the activity of the virus and/or by drug related effects that are directly nephrotoxic or may change the entire integrity of the renal function. Côté et al., 2002 indicated in their studies that renal dysfunction in HIV/AIDS patient population can be mediated through mitochondrial nephrotoxicity by some antiretrovirals.

The measurement of serum creatinine levels and its use in estimating the glomerular filtration rate (eGFR) of the kidney has been a gold standard method of assessing renal function (Stevens et al. 2008). In this study, creatinine levels and eGFR were determined in all the study groups. Creatinine levels were within normal pathological reference range (61 – 120 $\mu\text{mol/L}$). On the contrary, increased serum creatinine and renal lesion have been observed in HIV patients with long term exposure of tenofovir (Créput et al. 2003). Some of the antiretroviral drugs are known to cause nephrotoxicity in HIV/AIDS patients (Izzedine et al. 2005). This study noticed no significant difference in creatinine level between HAART-naïve and patients treated with HAART for both less and more than six month period. The findings from this study suggest safety of the antiretroviral drugs. Estimated glomerular filtration (eGFR) was greater than 60 mL/min/1.73m^2 in over 80% of both HAART and HAART-naïves. The eGFR has been used widely to assess performance of the kidneys. This study suggests no form of renal dysfunction in all the study groups. There was no association of Vitamin D status with creatinine levels and eGFR in this study. Vitamin D therefore, may not contribute to adjustment in creatinine and eGFR.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

This study therefore concluded that long exposure to vitamin D did not significantly increase its levels in circulation and did not relay any adverse effect on kidney function in Ghanaian HIV/AIDS patients receiving both antiretroviral and vitamin supplements. Doses of vitamin D contained in the supplement received by HIV/AIDS were safe but inadequate.

6.2 RECOMMENDATION

An upward adjustment to the dosage of vitamin D supplement may be beneficial to patients on antiretroviral therapy; as those in this study were still deficient inspite of intake of the Vitamin supplements. Further studies should also be done in this area as the major findings are largely in contrast to what is known in literature.

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APPENDICES

Appendix I: Consent Form

Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information, about risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

DATE: _____ SIGNATURE: _____

NAME: _____

Statement of person giving consent:

I have read the information on this research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction. I understand that my participation is voluntary. I know enough about the purpose, methods, risks and benefits of the research to judge that I want to partake in it. I understand that I may freely stop being part of this research at any time. I have received a copy of this information leaflet and consent form to keep for myself.

NAME OF PARTICIPANT: _____

DATE: _____ SIGNATURE/THUMB PRINT: _____

WITNESS' SIGNATURE (if participant is non-literate): _____

WITNESS' NAME: _____

Appendix II : Participation Information Sheet

TITLE OF RESEARCH: Association between Hypervitaminosis D and Renal Function Among Ghanaian HIV/AIDS Patients

Dear Participant,

I kindly request your permission to participate in this research which I have detailed below. Please read (or have read to you) and further sign a consent form in order to be part of this study. You will be given copies of this form and a signed consent form. I will be readily available to explain to you any unfamiliar words for clarification and better understanding.

PURPOSE OF RESEARCH

Kidney disease is a relatively common complication in patients with HIV disease, because of the infection itself or a side effect from HIV medications. Kidney disease can be treated in many cases but it can be very difficult to manage in an HIV patient.

HIV patients are usually administered with highly active antiretroviral drugs alongside with micronutrients and other deficient vitamins such as vitamin D. However consistent and long term exposure to especially fat soluble vitamins such as vitamin D may lead to high levels of vitamin D in the body. High levels of vitamin D in the body results in increased levels of calcium characterized by increased serum 25-hydroxyvitamin D. Increased levels of calcium in circulation has been reported as a contributing factor to calcification of kidney tissues leading to the damage of the kidney. Therefore, vitamin D toxicity leading to increased levels of calcium could be a contributing factor to the pathogenesis of kidney disease among HIV/AIDS patients.

The purpose of this study is to measure the vitamin D levels and find its association with kidney disease in HIV/AIDS patients.

The findings from this study will help provide further information for improving the management of HIV/AIDS disease and its related complications. This study will also provide information for further studies and research.

STUDY PROCEDURE

Participants shall be HIV 1 and (or) 2 sero-positive patients who have been on HAART and vitamin supplements for at least six months and HIV sero-positive patients on HAART with no vitamin supplements. Participants will initially be served with questionnaires which will provide relevant information needed for the study.

VENIPUNCTURE

Five (5) mls of blood shall be taken from the median cubital vein on the anterior forearm (the side within the fold of the elbow) by qualified phlebotomist for analysis.

MEASURE OF BLOOD PRESURE:

Mercury sphygmomanometer or digital blood pressure meter shall be used to estimate blood pressure (mmHg) by qualified personnel. You (Participant) shall be stabilised for 10minutes before blood pressure is taken at sitting position.

WEIGHT AND HEIGHT MEASUREMENT:

Weight (Kg) and height (cm) measurement shall be done with you (participant) barefoot but with cloth on.

In all a total of 200 recruits are expected to participate in the study.

Risk(s): All the procedures are routine, researched into and proven to be safe. Besides, you (participant) shall be handled by qualified personnel only.

POSSIBLE BENEFITS

Your participation will help determine your vitamin D and calcium levels. This will also serve to assess your kidney function and status and further help in improving management of HIV/AIDS patients.

POSSIBLE RISKS

The amount of blood collected is harmless, although there may be a slight pain at the bleeding site. Sterile techniques and disposable, single-use equipment will be used always.

WITHDRAWAL FROM STUDY

This study is strictly voluntary. Should you, at any point during the study, decide that you do not wish to participate any further, you are free to terminate the participation. Your decision will not affect the health care you would normally receive at the hospital.

CONFIDENTIALITY

All information gathered would be treated in strict confidentiality. When results of this study are reported in medical journals or at medical meetings, identities will be kept anonymous. All medical records will be stored by the researchers in safe cabinets.

CONTACTS

If you have any questions about this study or study-related problems, you may contact Prof. Henry Asare-Anane (Tel: 024 6024002), Dr. Seth Amanquah (024 4293987) and Ms. Bernice Ackom (Student – 0269101131) all of Department of Chemical Pathology, Korle-Bu Teaching Hospital (KBTH). You are free to ask any questions for further clarification. Thank you.

Participant: I understand all the above information and hereby agree to be a participant of this study.

.....
Name of Participant

.....
Signature or Thumb print of Participant

.....
Date

.....
Name of witness

.....
Signature or Thumb print of witness

.....
Date

.....
Name of Investigator

.....
Signature of Investigator

.....
Date

Appendix III : Questionnaire

TOPIC: Association between Hypervitaminosis D and Renal Function Among Ghanaian HIV/AIDS Patients

1. ID..... 2. AGE.....
3. SEX.....
4. Weight (kg)..... 5. Height(cm).....
6. Body Mass Index
7. Systolic Blood Pressure(SBP)..... 8. Diastolic Blood Pressure(DBP)
9. Have you been diagnosed of HIV/AIDS disease? a. Yes b. No c. Don't know
10. If yes when? a. Less than 3 months b. Between 3 months – 1year c. Between 1 -2 years d. More than 2 years
11. Are you on Antiretroviral drug(ART) if diagnosed of HIV/AIDS disease? a. Yes b. No c. Don't know
12. If yes to question 11, for how long have you been on antiretroviral therapy? a. less than 3 months b. between 3 months – 1year c. between 1 -2 years
13. What type of antiretroviral therapy are you receiving?

Please select, by ticking from the list of drugs, the drugs you are taking currently.

Underline the appropriate therapy:

Class	Generic name
Nucleoside Analogue Reverse Transcriptase Inhibitors (NRTIs)	Abacavir(ABC), Didanosine(ddl), Stavudine(d4T), lamivudine(3TC), Zidovudine(AZT), Tenofovir(TDF)
Non nucleoside Reverse Transcriptase Inhibitors (NNRTIs)	Efavirenz(EFZ), Nevirapine(NVP), Delaviradine
Protease Inhibitors(PI)	Fosamprenavir(FAPV), Atazanavir(ATV), Darunavir(DRV), Indinavir(ID V), Lopinavir(LPV), Nelfinavir(NFV), Ritonavir(RTV), Saquinavir(SQV)

11. Are you on any other medication aside the antiretroviral therapy? a. Yes b. No c. Don't know

12. Are you on micronutrient supplement and other vitamins drugs ? a. Yes b. No c. Don't know
13. If yes, how long have you been taking the micronutrient supplement and other vitamins drugs?
a. less than 3 months b. between 3 months – 1year c. between 1 -2 years
14. Have you been diagnosed of any liver disease? a. Yes b. No c. Don't know
15. If yes when? a. Before antiretroviral therapy b. After antiretroviral therapy c. Don't know
16. Have you been diagnosed of been hypertensive? a. Yes b. No c. Don't know
17. If yes when? a. Before antiretroviral therapy b. After antiretroviral therapy c. Don't know
18. Have you been diagnosed of any kidney disease? a. Yes b. No c. Don't know
19. If yes when? a. Before antiretroviral therapy b. After antiretroviral therapy c. Don't know

Appendix IV: Calculation of Sample size

The sample size was determined from the relationship: $n \geq Z^2 p (1-p) / e^2$ (Cochran,1977) where n is the number of sample size calculated, Z is the Z-score at 95% confidence level, P is the prevalence of case and e is the margin of error taken at 95% confidence level.