ASSESSMENT OF RENAL FUNCTION IN HIV/AIDS PATIENTS ON HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) IN ABUTH, ZARIA

BY

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DEPARTMENT OF CHEMICAL PATHOLOGY, FACULTY OF MEDICINE, AHMADU BELLO UNIVERSITY, ZARIA

OCTOBER, 2009

DECLARATION

I declare that the work in the thesis entitled **Assessment of renal function in HIV/AIDS patients on Highly Active Antiretroviral Therapy (HAART) in ABUTH, Zaria** has been performed by me in the Department of Chemical Pathology under the supervision of **Dr I.S Aliyu, Dr P.O Anaja and Dr H.M Muktar**. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any University.

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DEDICATION

This work is dedicated to my dearest wife Dr (Mrs.) K.H Yusuf and children Ahmed and Asiyah Yusuf for their care, support and prayers.

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ABSTRACT

Acquired immunodeficiency syndrome (AIDS) is a spectrum of disease states characterised by progressive immunosuppression. It results from infection with Human Immunodeficiency Virus (HIV) which is capable of affecting every organ system in the body. Renal complications are important component of advanced HIV disease, and these complications significantly contribute to morbidity and mortality in these patients. Therefore regular assessment of their renal status using sensitive markers such as Creatinine Clearance (CrCl) together with serum electrolytes, creatinine, urea and uric acid is necessary.

The objective of this study was therefore to assess the renal function, determine the appropriate assessment parameters and the prevalence of renal disease among HIV/AIDS patients on highly active antiretroviral therapy (HAART) in ABUTH, Zaria. Renal function of 101 HIV positive patients on HAART and 100 controls was assessed. The data obtained were analysed using statistical programme for the social sciences 11.0 (SPSS 11.0).Two-tailed student's t-test for matched samples and Pearson's linear correlation statistical methods were employed for the analysis. A p-value of equal to or less than 0.05 ($p \leq 0.05$) was considered as statistically significant.

Mean levels of Na^+, K^+, HCO_3^-, Cl^-, Ca^{2+} and PO_4^{2-} obtained in patients were 138.97±0.43, 3.83±0.05, 25.28±0.26, 100.02±0.42, 2.62±0.03 and 1.21±0.03 mmol/L respectively, whereas the corresponding values in controls were 138.84±0.26, 3.96±0.04, 25.78±0.16, 98.90±0.35, 2.31±0.02 and 1.22±0.02 mmol/L respectively. These results showed that K^+ was significantly lower ($p< 0.05$) while Cl^- and Ca^{2+} were significantly higher ($p< 0.05$ and $p< 0.001$ respectively) in patients than in controls. Also the mean levels of estimated creatinine clearance (eCrCl), creatinine, uric acid and urea in patients were 121.52±4.95 ml/min, 65.29±2.61 µmol/L, 258.62±8.32 µmol/L and 4.27±0.20 mmol/L respectively, whereas the corresponding values in controls were

121.04±2.62, 74.46±1.33, 204.00±5.35 and 5.02±0.09 respectively. These results showed that creatinine and urea were significantly lower ($p< 0.01$) while uric acid was significantly higher ($p< 0.001$) in patients than in controls. This study demonstrated that all analytes in both patients and controls were within normal reference ranges for this environment.

The study also showed that 26 (25.7%) patients had decreased eCrCl as against 12 (12.0%) controls. Nineteen (18.8%) of the patients with decreased eCrCl had eCrCl of < 90 ml/min (stage 2 CKD) with the remaining 7 (6.9%) having eCrCl of <60 ml/min (stage 3 CKD). Of all the patients with deranged renal function using eCrCl, only 4 would have been detected using serum creatinine with or without proteinuria.

It was also found that 48(47.5%) of the patients were overweight to mild obesity as against 26 (26.0%) controls.

The findings in this study demonstrates a higher prevalence of renal disease in patients and that eCrCl is a better marker than serum electrolytes, urea, creatinine and uric acid in assessing renal function in these patients. It is therefore recommended that GFR should be assessed regularly using prediction formula (Cockcroft and Gault formula) in HIV/AIDS patients on HAART.

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ABBREVIATIONS/SYMBOLS USED

A	Absorbance
A_{std}	Absorbance of standard
ARF	Acute renal failure
AIDS	Acquired immunodeficiency syndrome
ABUTH	Ahmadu Bello University Teaching Hospital
ADH	Antidiuretic hormone
ARV	Antiretroviral
BMI	Body mass index
CKD	Chronic kidney disease
CRF	Chronic renal failure
C	Concentration
C_{std}	Concentration of standard
Cr	Creatinine
CrCl	Creatinine Clearance
^{o}C	Degree Celsius
DNA	Deoxyribonucleic acid
eCrCl	Estimated creatinine clearance
GFR	Glomerular filtration rate
>	Greater than
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
HIVAN	HIV- associated nephropathy

HCl	Hydrochloric acid
kg	kilogram
kg/m2	kilogram per metre square
<	Less than
≤	Less than or equal
µl	micro litre
µmol	micromole
µmol/L	micromole per litre
mEq/L	milli equivalent per litre
ml	milliliter
mmol	milli mole
mmol/L	milli mole per litre
min	minute
MDRD	Modification of diet in renal disease
nm	nanometer
NNRTIs	Non – nucleoside reverse transcriptase inhibitors
NRTIs	Nucleotide reverse transcriptase inhibitors
%	Per cent
±	plus or minus
PIs	Protease inhibitors
rpm	Revolution per minute
RNA	Ribonucleic acid
STD	Sexually transmitted disease
std	standard

SEM Standard Error of Mean

UNAID Joint United Programme on AIDS

WHO World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Acquired Immunodeficiency Syndrome (AIDS) is a spectrum of disease states characterised by progressive immunosuppression. It results from infection with the Human Immunodeficiency Virus (HIV) (Cotran *et al*, 1999; Olumide, 2003).

HIV infection is a worldwide health problem that affects millions of men and women (Jeff, 2002). Since the beginning of the pandemic in the early 1980, more than 60million people have been infected with HIV worldwide, with an estimated 25million deaths, and 5million new infection each year. Sub-saharan Africa carries the highest burden of HIV infection with an estimated prevalence of more than 80% of the world cases (AIDS epidemic update, 2002). In Nigeria, the prevalence of HIV infection continues to rise from 1.8% in 1991 to 5.8% in 2001, and about 3.5million Nigerians were reported to be infected with HIV virus in 2002 (National AIDS/HIV/STD Control programme, 1997, 1999, 2000, 2002, 2003).

The high prevalence in 2002 put Nigeria as the second in the world with the national sero-prevalence of 5.4% of HIV infection (Idoko *et al*, 2002). The prevalence appears to be on the decline as the 2005 sentinel survey by National Action Committee on AIDS (NACA) shows a prevalence rate of 4.4% with

about 2.9 million people presently living with the virus, putting Nigeria presently as the third in the world (Ishaku, 2007).

The virus has the capability to affect every organ system in the body by direct damage or by rendering the host susceptible to opportunistic infections (Jeff, 2002). Since the emergence of Acquired Immunodeficiency Syndrome (AIDS) more than 25years ago, renal disease has been recognised as a common complication associated with Human Immunodeficiency Virus (HIV) infection (Scott and Paul, 2007).

1.1.1 HIV and Renal disease

The association between HIV infection and renal disease was first reported as far back as 1984 by researchers in New York and Miami. They reported series of HIV-1infected patients with renal syndrome characterized by proteinuria and progressive renal failure (Gradenswartz *et al*, 1984; Pardo *et al*, 1984; Michael and Klotman, 2002). Renal disease in HIV can also result from the use of the highly active antiretroviral therapy (HAART) and their associated adverse effects. Also, patients with HIV/AIDS are at a higher risk of developing pre-renal azotemia due to volume depletion resulting from salt wasting, poor nutrition, and nausea or vomiting (Moro *et al*, 2007).

HIV type 1 (HIV-1) infected patients are at a greater risk for the development of a variety of acute and chronic renal diseases (Ross *et al*, 2000).

HIV-1 associated nephropathy is now the 3rd leading cause of end-stage renal disease (ESRD) in blacks' 20-64years of age (Jonathan *et al*, 2001; Lynda *et al*, 2004; Paul and Paul, 2004). These patients typically have proteinuria followed by a reduction in the glomerular filtration rate (GFR) that progresses to end-stage renal disease in few weeks or months (Jonathan *et al*, 2001).

All aspects of medical management become more difficult when a patient with HIV infection develops renal failure (AIDS Clinical care, 2007). Given the risks associated with kidney disease, developing awareness of kidney function and getting the necessary tests are essential to maintaining good health in patients with HIV infection (Lynda, 2006). The kidney has several functions including the excretion of water, soluble wastes such as urea and creatinine, and foreign materials such as drugs. It is responsible for the composition and volume of circulating fluids with respect to water and electrolyte balance and acid-base status. It has an endocrine function playing a part in the production of active vitamin D and erythropoietin, and as a part of the renin-angiotensin-aldosterone axis (David and Christopher, 1986).

Kidney function is assessed using both blood and urine tests to measure two key indicators; serum creatinine and protein in the urine. Abnormality in either of these measures indicates a functional or structural problem in the kidneys (Lynda, 2006). But most doctors use the plasma concentrations of creatinine, urea, and electrolytes to determine renal function (Wikipedia, 2007).

Assessment of renal function may be required for several reasons which include, identify renal impairement, to monitor disease progress, and to assess baseline parameters prior to commencement of treatment with antiretroviral drugs. Additionally, the type of kidney function assessment performed will be determined by the reason for assessing the renal activity (David and Christopher, 1986).

1.2 STATEMENT OF PROBLEMS

Renal complications are important component of advanced HIV disease, and these complications significantly contribute to morbidity and mortality in patients with HIV infection. Therefore, early intervention through clinical and laboratory assessments of the renal status in this group of patients is an important aspect of their management (Agaba *et al*, 2003). The laboratory assessment includes assessing the glomerular and the tubular functions of the kidneys, ie, Renal Function Tests (RFTs). Serum creatinine levels or estimated glomerular filtration rates may be used to monitor changes in renal function in an individual patient (Jamie *et al*, 2006). Unfortunately, serum urea and creatinine will not be outside the normal range until 60% of total kidney function is lost. Hence, Creatinine Clearance (CrCl) is a more accurate measure of glomerular filtration rate (GFR). This is not widely available in many HIV-

infected patients, especially those on Highly Active Anti Retroviral Therapy (HAART).

1.3 JUSTIFICATION

As mentioned earlier, renal diseases significantly contribute to morbidity and mortality in HIV- infected patients. However, appropriate renal assessment is neglected in the management of these patients in most hospitals in Nigeria. Moreover, there is paucity of data on the renal assessment of HIV/AIDS patients on antiretroviral drugs in Nigeria, including Zaria. The reported studies on this aspect were carried out elsewhere in the World (Ross *et al*, 2000; Mathur, 2002; Andia *et* al, 2005; Jamie *et* al, 2006). In view of its clinical importance, there is therefore the need to carry out a study on the renal assessment in HIV/AIDS patients on antiretroviral drugs in ABUTH, Zaria. This could help in the diagnostic, therapeutic and prognostic purposes, and therefore reduce the morbidity and mortality among this important group of patients in Nigeria.

1.4 GENERAL OBJECTIVE

The study is aimed at assessing the renal function of HIV/AIDS patients on Highly Active Antiretroviral Therapy (HAART) in ABUTH Shika, Zaria and

also to determine the appropriate assessment parameters for renal function in this category of patients.

1.5 SPECIFIC OBJECTIVES

i) To assess the renal function among HIV/AIDS patients on highly active antiretroviral therapy (HAART) by measuring serum electrolytes, urea, creatinine, uric acid, and estimation of creatinine clearance.

ii) To determine the prevalence of renal disease among HIV/AIDS patients on highly active antiretroviral therapy (HAART).

iii) To ascertain the relevant parameters in the assessment of renal function among HIV/AIDS patients on highly active antiretroviral therapy (HAART).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 HUMAN IMMUNODEFICIENCY VIRUS (HIV)

2.1.1 Brief History

HIV is a retrovirus that leads to acquired immunodeficiency syndrome (AIDS), a condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections (Rick, 1999).

In 1981 Clinicians in New York City, San Francisco, and Los Angeles recognised a new immunodeficiency syndrome among male homosexuals (Sepkowitz, 2001; Thomas, 2001). Initially, the illness was called Gay-Related Immunodeficiency Syndrome (GRIDS). However it soon became apparent that this disease was not confined to one segment of the population, but has silently disseminated through other high risk populations. The high risk populations include those who engage in indiscriminate sexual activities (both hetero/homosexuals), intravenous drug abusers, patients who receive multiple unscreened blood and blood products and patients who had organ transplant (Cotran *et al*, 1999). By the end of 1981, several hundred cases had been identified and the disease was renamed AIDS (Thomas, 2001).

Luc Montagnier, Barre Sinoussi with colleagues at the Pasteur institute in Paris and Gallo Robert in the United States of America isolated the retrovirus, responsible for AIDS in 1982 (Weller, 1984; Thomas, 2001). Previous names

for the virus include Human T-lymphotrophic virus-III (HTLV-III), Lymphadenopathy-associated virus (LAV), and AIDS-associated retrovirus (ARV) (Coffin *et al*, 1986; Rick 1999). In 1987 the virus was renamed HIV (Clifford, 1998).

2.1.2 Epidemiology

It is almost thirty years of the initial recognition of the acquired immunodeficiency syndrome among homosexual men in the United States of America (Sepkowitz, 2001; Thomas, 2001). The disease has spread so rapidly that it is currently a pandemic with sub-saharan Africa bearing the greatest brunt of the problem with over 75% of reported cases (Fausi, 1999; Gayle, 2001). HIV/AIDS has touched virtually all aspects of society, and its reach currently extends to every social institution, from families, schools and communities to private businesses, government establishments and various uniformed services (Fausi, 1999; Gayle, 2001).

In 2006, UNAIDS (Joint United Nation programme on AIDS) estimated that 65million people have been infected with the HIV to date. More than 25million people have died from the disease since it was first discovered in 1981, making it one of the most destructive pandemic in recorded history. In 2005 AIDS claimed the lives of 2.8million people and 4.1million people became newly infected while 38.6million people were living with the virus (UNAIDS, 2006).

Almost two third (63%) of all persons infected with HIV are living in sub-saharan Africa retarding economic growth and increasing poverty. An estimated 4.3million (3.6-6.6million) adults and children became infected with HIV in 2006, more than in all other regions of the world combined. The 2.1million (1.8-2.4million) AIDS death in sub-saharan Africa represent 72% of global AIDS deaths (UNAIDS, 2006). According to current estimates, HIV is said to infect 90million people in Africa, resulting in a minimum estimate of 18million orphans (UNAIDS, 2005).

Nigeria, the tenth most populous country in the world and the most populous country in sub-saharan Africa, has the third highest population of people living with HIV, after India and South Africa (Ejembi, 2007). The first reported case of AIDS in Nigeria was in Lagos, in a 13 year old sexually active girl in 1986 (Ejembi, 2007). Since then there has been a steady increase in the prevalence of the disease in the country. Biennial sentinel surveillance surveys conducted in Nigeria showed a steady rise in the HIV sero-prevalence rates from 1.8% in 1992 reaching the highest level of 5.8% in2001. In 2003, there was a decrease to 5%. The rate decreased further to 4.4% in 2005 (Ejembi, 2007).

The National median sero-prevalence rate conceals marked regional and state differences in the prevalence of the disease. From the 2005 survey data, the Southwestern zone of the country had the lowest sero-prevalence rate of

2.6%, while the North Central zone had the highest prevalence rate of 6.1%. The variation in trend in prevalence rates is more marked across states in the country. The 2005 prevalence rates ranged from 1.6% in Ekiti state (South west zone) to 10% in Benue state (North Central zone). Fifteen states had prevalence rates greater than the national average of 4.4% and all states had prevalence rates of between 1% suggesting a generalised epidemic (Ejembi, 2007).

2.1.3 Biology of the virus

HIV is a member of the genus lentivirus, part of the family of *Retroviridae*. Lentiviruses are transmitted as single-stranded, positive sense, enveloped RNA viruses. Upon entry of the target cell, the viral RNA genome is converted to double stranded DNA by a virally encoded reverse transcriptase that is present in the virus particle. This viral DNA is then integrated into the cellular DNA by a virally encoded integrase, along with host cellular co-factor, so that the genome can be transcribed (Smith and Daniel, 2006). Once the virus has infected the cell, two pathways are possible; either the virus becomes latent and the infected cell continues to function, or the virus becomes active and replicates, and a large number of virus particles are liberated that can then infect other cells.

Two species of HIV infect humans; HIV-1 and HIV-2. HIV-1 is the virus that was initially discovered and termed LAV. It is more virulent, relatively easily transmitted, and is the cause of the majority of HIV infections globally.

HIV-2 is less transmittable than HIV-1 and is largely confined to West Africa (Reeves and Doms, 2002).See table 2.1.

2.1.4 Viral Structure

HIV virion is spherical and contains an electron dense, cone-shaped core surrounded by a lipid envelope derived from the host cell membrane (Barre-Sinoussi, 1996).

The virus contains:

i) The major capsid protein p24
ii) Nucleocapsid protein p7/p9
iii) Two copies of genomic RNA, and
iv) The three viral enzymes (protease, reverse transcriptase, and integrase). (Melbye, 1986; Cotran *et al*, 1999).

The most readily detected viral antigen is p24. A matrix protein called p17, which lies underneath the viral envelope, surrounds the viral core. Studding the viral envelope are two glycoproteins, gp120 and gp41, which are critical for HIV infection of cells (Cotran *et al*, 1999) (figure 2.1).

The HIV proviral genome contains three groups of structural genes, the *gag* (group specific antigen), *pol*, and *env* genes. The products of the gag are translated initially into large precursors, a protein that is cleaved by the viral protease to yield mature proteins. The *pol* codes for the reverse transcriptase,

Table 2.1: Comparison of HIV species.

Species	**Virulence**	**Transmittability**	**Prevalence**	**Purported origin**
HIV-1	High	High	Global	Common Chimpanzee
HIV-2	Lower	Low	West Africa	Sooty Mangabey

(Reeves and Doms, 2002)

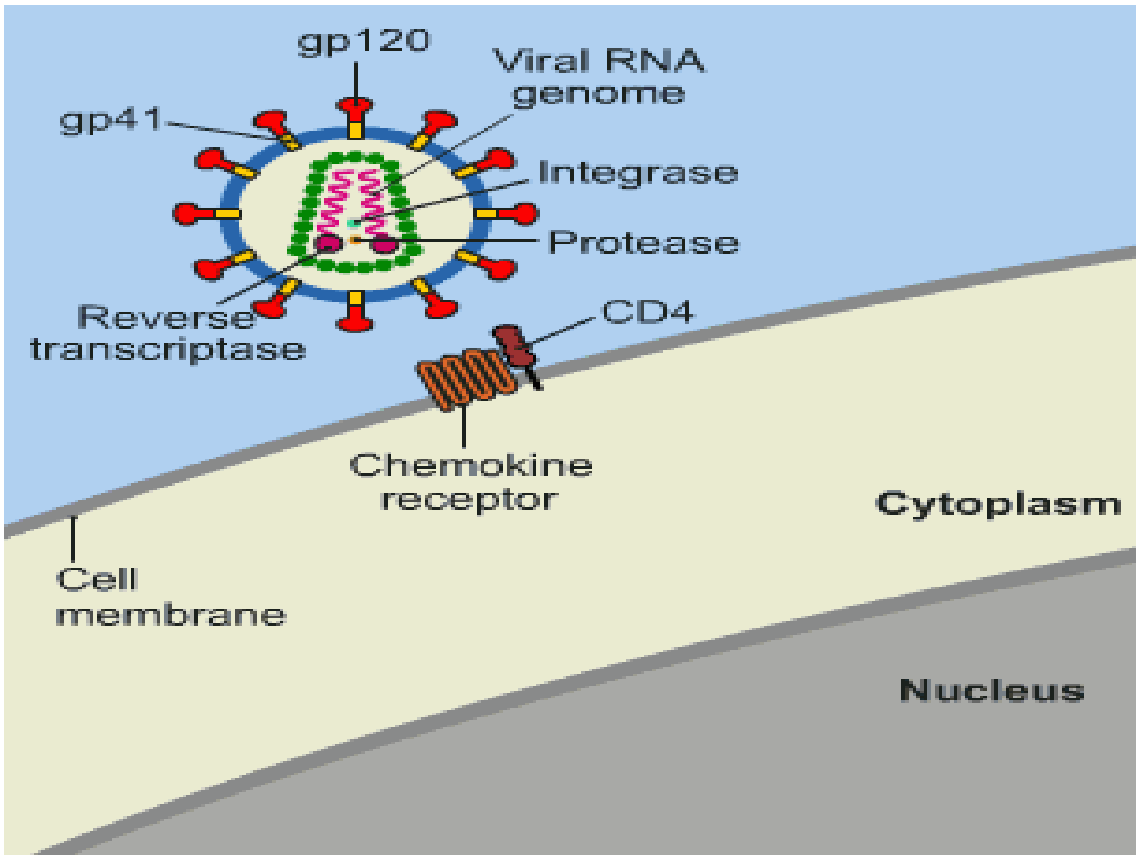


Figure 2.1: Schematic illustration of HIV-1 virion.

which converts viral RNA into DNA within the host cell. The *env* codes for the envelope glycoproteins.

HIV contains other regulatory genes: *tat*, *rev*, *vpv*, *vif*, *vpr* and *nef*, which are responsible for viral replication and infectivity. In addition there are other open reading frames (*Orf*, *Sor*, and 3 *Orf*) whose functions are unknown. The DNA intermediate viral genome has been well studied and is about 10-kilo base in length. It is made up mainly of Long Terminal Repeat (LTR) sequences at the ends and *gag*, *pol* and *env* genes in that sequence. The LTRs are responsible for the regulation of the viral replication (Cotran *et al*, 1999).

2.1.5 Molecular Epidemiology of HIV

The geographical origin of HIV is still unclear, but is thought to have originated in non-human primates in sub-Saharan Africa and transferred to humans during the 20th century.

So far, only two major types have been described and these are HIV-1 and HIV-2. HIV-1 is the commonest worldwide. HIV-2 originated in West African region and HIV-1 is the most common type in USA, Europe, and Central Africa (Gurtler, 1996; Cotran *et al*, 1999). There is 40% homology between the two viruses. HIV-2 differs from HIV-1 mostly from its envelope. Other types of retroviruses have been obtained from primate species and exhibit some cross reactivity with human species (Clavel *et al*, 1986; Peter *et al*, 1992).

Analysis of the *env* and *gag* gene sequences for classification of HIV-2 gives five subtypes A, B, C, D and E. Three groups of HIV-1 have also been identified on the basis of differences in *env*; M, N, and O (Thomson *et al*, 2002). Group M is the most prevalent and is subdivided into eight subtypes (based on the whole genome), which are geographically distinct (Carr, 1998). The most prevalent are subtype B (found mainly in North America and Europe), A and D (found mainly in Africa), and C (found mainly in Africa and Asia) (Osmanov *et al*, 2002).

2.1.6 Mode of Transmission

2.1.6.1 Sexual Intercourse (Homosexual/Heterosexual).

This is the predominant mode of transmission in 90% of cases (Akinsete, 1993). The virus is carried in the semen and in cell-free state, and it enters the recipient's body through abrasions in the mucosal linings. The rate of heterosexual transmission has now out paced other means (Bredberg-Raden *et al*, 1995; Royce, 1997). Other sexually transmitted diseases, especially those with associated genital ulcerations such as syphilis, chancroid, genital herpes, gonorrhea and Chlamydia are co-factors in HIV transmission (Kreiss, 1997; Cohen, 1998).

2.1.6.2 Parenteral Transmission.

Parenteral transmission has occurred among intravenous drug abusers, haemophiliacs, recipients of blood and blood components. Organs obtained

from an infected donor can also transmit AIDS (Levy, 1993; Bredberg-Raden *et al*, 1995; Kreiss, 1997). In developing countries, where economic and political problems interfere with screening programme for blood products, 5-10% of HIV infection results from transfusion of sero- negative blood (Piot *et al*, 1988; Cohen, 1998).

2.1.6.3 Vertical Transmission.

This is the cause of paediatrics AIDS. It is either by uteroplacental route, during passage through an infected birth canal, or during lactation (Hira *et al*, 1989).

2.1.6.4 Certain Traditional Practices in Our Environment.

Traditional practices such as circumcision, cutting of umbilical cord with unsterilised instruments, can result in transmission of the virus (Edward, 2001).

HIV cannot be transmitted by close personal contact. No convincing evidence for spread by insect bite has been reported. Although the virus is found in serum, semen, saliva, urine, faeces, and tears, the virus is highly susceptible to dessication. Non sexual transmission by these fluids is improbable. In approximately 6% of cases, the risk cannot be determined (Gershon *et al*, 1990; Bredberg-Raden *et al*, 1995; Edward, 2001).

2.1.7 Pathogenesis

There are two major targets of HIV infection, the immune and the central nervous systems. The CD4 molecule is a high affinity receptor for the HIV and

this explains the selective tropism of the virus for CD4+ T-cells and other CD4+ cells, particularly monocytes, macrophages, langerhans and dendritic cells (Bredberg-Raden *et al*, 1995; Cotran *et al*, 1999). Binding to CD4 antigen is not sufficient to cause infection, but HIV *gp120* must also bind to other cell surface molecule (co-receptor) for entry into the host cell. Two co-receptors are known CCR5 (expressed on macrophages and T cells), and CXCR4 (expressed only on T cells) for β and α chemokines respectively. This binding leads to a conformational change in the *gp41* and results in the insertion of a fusion peptide at its tip into the cell membrane of the target cell (Doms and Peiper, 1997).

The viral core containing the genome now enters the cytoplasm. Once internalised, it undergoes reverse transcription leading to the formation of proviral DNA (cDNA). This circularises in dividing T cells, enters the nucleus and is then integrated into the host genome. In quiescent T cells, the cDNA remains in the cytoplasm in a linear episomal form. After integration, the virus may remain locked in the nucleus for months or years unrecognised by the host immune system (Latency). Alternatively cDNA may be transcribed, with the formation of complete viral particles. This buds off from the cell membrane and cell death follows by direct lysis of the peripheral CD4+ Tcells (Bredberg-Raden *et al*, 1995) (figure 2.2).

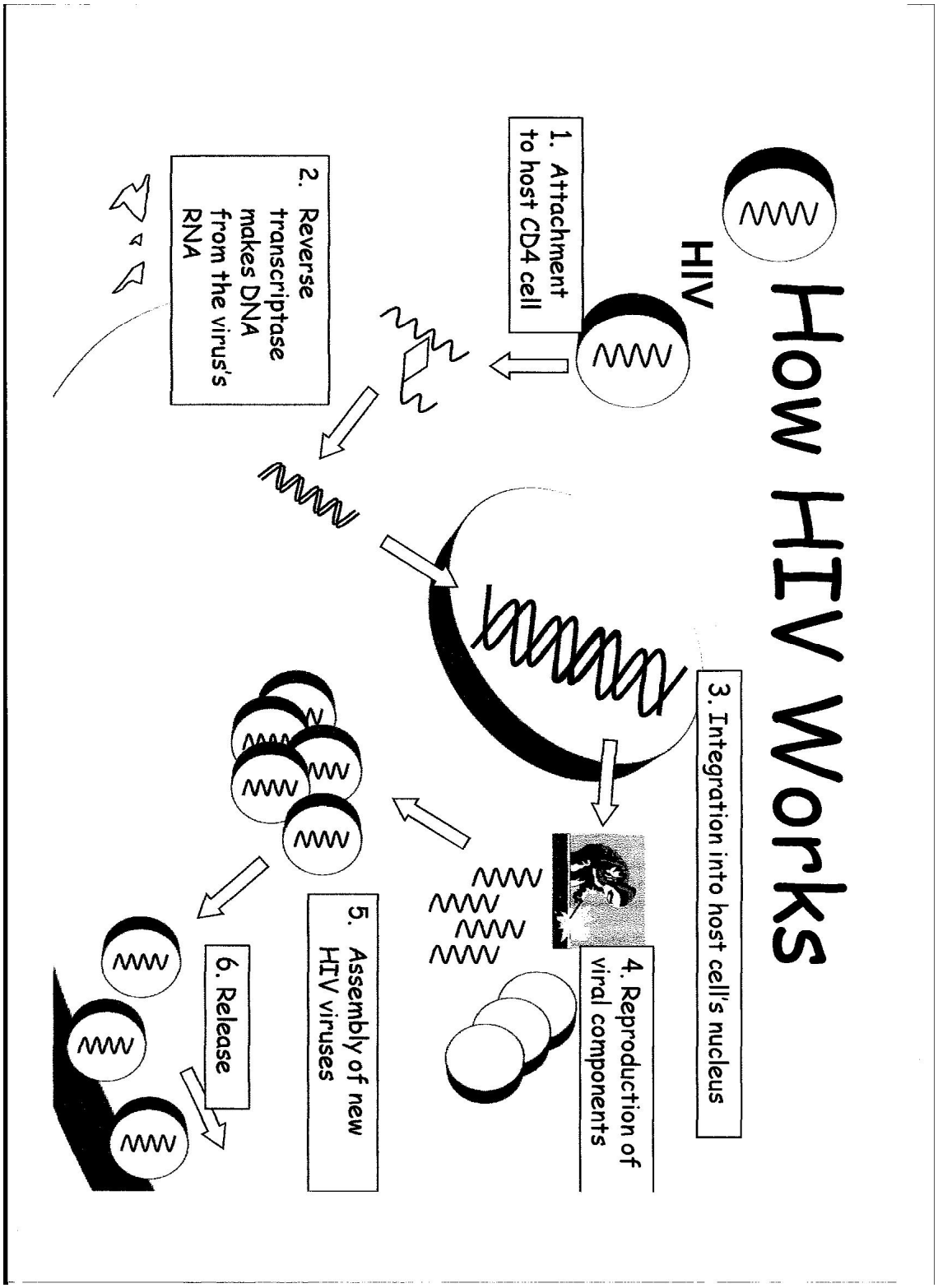


Figure 2.2: Schematic diagram showing how HIV works.

The infected cell is activated by an exposure to antigens presented by an antigen presenting cells, cytokines released from B cells or other viruses and by ultraviolet light. Approximately 10 billion new viral particles are produced everyday, and 1-2billion CD4+ T cells die everyday (Ho, 1997).

Other mechanisms of CD4+ T cell loss include:

i) Apoptosis (programmed self death) of uninfected CD4+ T cells.
ii) Fusion of infected and uninfected cells, syncytia (giant cells) formation, with subsequent ballooning and death.
iii) Direct infection of thymic progenitor cells.
iv) Infection of accessory cells that secrete cytokines essential for CD4+ T cells differentiation (Cotran *et al*, 1999; Edward, 2001; Olumide, 2003).

Fundamental to the pathogenesis of the disease is the interplay of the agent (HIV), the environment and the host. These play a significant role in the mechanism by which HIV infection produces the clinical manifestation of overt disease ie, AIDS (Cotran *et al*, 1999; Edward, 2001). With successful infection, the viral particle enters the CD4+ T cell and destroys it. Reduction of CD4+ T cells is the hallmark of AIDS (Bredberg-Raden *et al*, 1995; Edward, 2001). This accounts for most of the immunodeficiency late in the course of HIV infection (Bredberg-Raden *et al*, 1995; Edward, 2001).CD4+ T cell plays a vital role in regulating the immune response. Therefore loss of this master cell has ripple

effects on virtually every other cell of the immune system (Cotran *et al*, 1999; Edward, 2001).

There is profound B-cell dysfunction (Ho, 1997; Cotran *et al*, 1999). Paradoxically there is hypergammaglobulinaemia and circulating immune complexes owing to polyclonal B cell activation by multiple interacting factors such as viruses (EBV, CMV) (Cotran *et al*, 1999). Cytokine production and function is adversely affected. Factors involved in cell mediated immunity (IL-2, 12, 15) and IFNγ have impaired production, whereas IL-4, 5, 6, 10, 13 which are involved in humoral immunity are spared early in the disease (Ho, 1997). Despite the presence of activated B-cells, they are unable to mount an antibody response to a foreign antigen due to lack of T- cell support. Impaired humoral immunity renders these patients open to disseminated infections caused by capsulated bacteria, such as S. pneumonia, and H. influenza both of which require antibodies for effective opsonisation.

2.1.7.1 Natural History of HIV Infection

Infection with HIV is associated with a progressive decrease of the CD4+ T cell count and an increase in viral load.

HIV infection has basically four stages; incubation period, acute infection, latency stage and AIDS.

i) The initial incubation period upon infection is asymptomatic and usually lasts between two and four weeks.

ii) An early acute infection phase representing the initial response of an immunocompetent adult to HIV infection and is characterized by an initial high level of viraemia. Seeding of the lymphoid tissues follows this, which is readily cleared by the development of an initial antiviral response. The symptoms are nonspecific, an infectious mononucleosis like syndrome develop 3 to 6 weeks after an infection. It resolves spontaneously in 2 to 4 weeks (Shacker, 1996; John and Joel, 2003).

iii) The third chronic phase is a period of clinical latency. The immune system is intact but there is viral replication in lymphoid tissues. The individual remains asymptomatic. This may last for several years (7-10 years) or they develop persistent generalised lymphadenopathy (Shacker, 1996; Cotran *et al*, 1999, John and Joel, 2003).

iv) Final crisis phase is characterised by breakdown of host defense, increase in viraemia and clinical disease. Typically the patient presents with long lasting fever (>1 month), fatigue, weight loss, and diarrhoea. The CD4 count is reduced below 200cells/mm^3 (Shacker, 1996; Cotran *et al*, 1999, John and Joel, 2003) (figure 2.3).

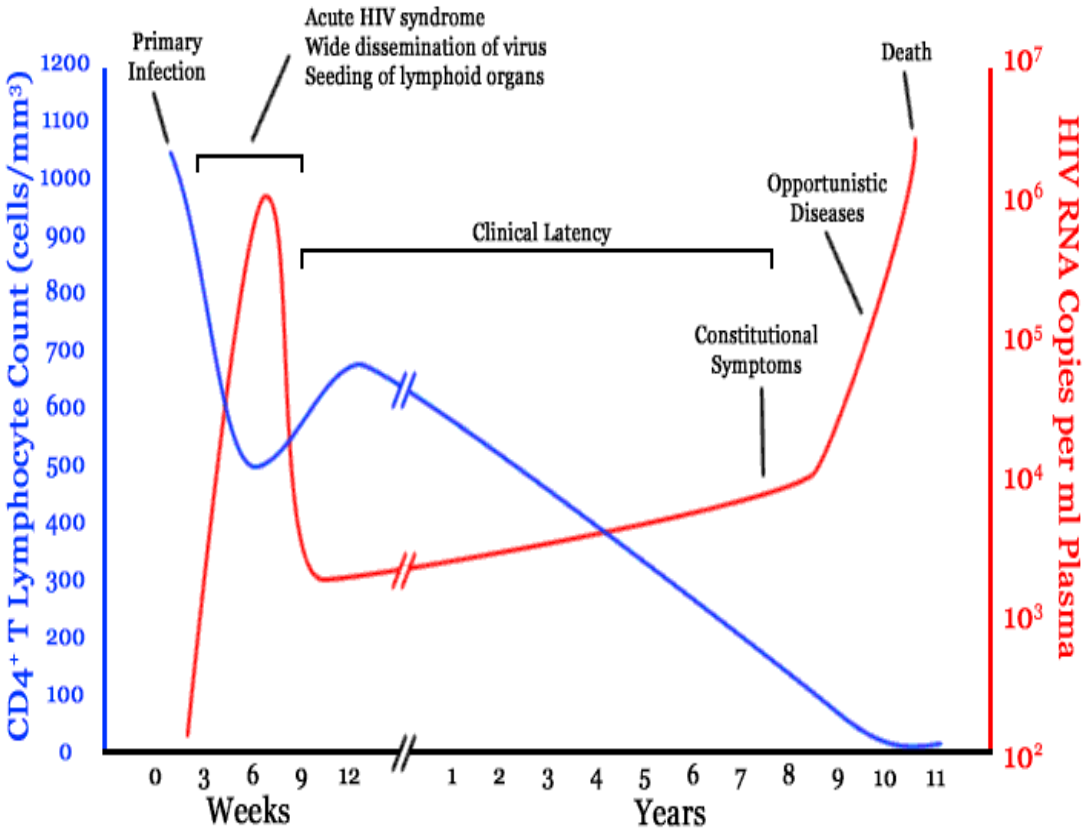


Figure 2.3: Graph showing typical course of HIV infection.

In the absence of treatment, most patients will progress to AIDS after the chronic phase except the long-term nonprogressors and rapid progressors. Nonprgressors are HIV- infected individuals who remain asymptomatic for 10 years or more, stable CD4 counts and low level of viraemia. In rapid progressors the third chronic phase is shortened to 2 to 3 years after primary infection (Cotran *et al*, 1999).

2.1.8 Diagnosis

There are three phases of diagnosis:

i) Presumptive diagnosis (1981-1987)

ii) Definitive diagnosis (1987-1993)

iii) Definitive diagnosis with assessment of;

- Severity of disease at presentation

- Response of the disease to antiretroviral agents to date (Akanmu, 2001).

2.1.8.1 Laboratory diagnosis:

Table 2.2: Laboratory diagnosis of HIV.

Test	Methods
1. Detecting presence of antibody to the virus	Serologic screening tests - Direct (Competitive) and Indirect (Non competitive) Enzyme Linked Immunoabsorbent Assay. Serologic confirmatory test - Western blot test.
2. Detecting presence of the virus	- Viral antigen detection (P24 antigen detection test) - Viral culture - Viral genome detection tests.

(Akanmu, 2001).

2.1.9 Management

There is currently no vaccine or cure for HIV or AIDS. The only known method of prevention is avoiding exposure to the virus.

A. Counseling: This is a multistage process of information to patient about the existence of the virus and implications of infection or otherwise as indicated by the serological tests (Bredberg-Raden *et al*, 1995; John and Joel, 2003).

B. Therapy:

i. Supportive: These are measures to treat infective, immunological, neoplastic and other complications of HIV infection. Prevention of opportunistic infections (OIs) accordingly and advice to avoid high risk behaviours and the use of latex condoms to avoid further exposure.

ii. Definitive: Current treatment for HIV infection consists of the use of highly active antiretroviral therapy (HAART). This has been seen to be highly beneficial to many HIV-infected individuals since its introduction in 1996, when the protease inhibitor-based HAART initially became available (Pallela *et al*, 1998). Current HAART options are combinations (or cocktails) consisting of at least three drugs belonging to at least two types, or classes, of antiretroviral agents. Typically, these classes are two nucleoside analogue reverse transcriptase inhibitors (NARTIs or NRTIs) plus either a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor (NNRTI).

HAART allows the stabilisation of the patient′s symptoms and viraemia, it does not cure the patient, but alleviates the symptoms (Martinez-Picado *et al*, 2000; Dybul *et al*, 2002). Despite this, many HIV-infected patients have experienced remarkable improvements in their general health and quality of life, which has led to a large reduction in HIV-associated morbidity and mortality in the developed world (Pallela *et al*, 1998; Chene *et al*, 2003; Wood *et al*, 2003).

The timing for starting HIV treatment is still debated. There is no question that treatment should be started before the patient′s CD4 count falls below 200, and most national guidelines advocated commencement of treatment once the CD4 count falls below 350, but there is some evidence from cohort studies that treatment should be started before the CD4 count falls below 350 (Wood *et al*, 2003; Wang *et al* 2004).

2.1.9.1 Classes of Drugs

Antiretroviral drugs are broadly classified based on the HIV life-cycle that the drug inhibits.

i) Nucleoside and Nucleotide reverse transcriptase inhibitors (NRTIs) inhibit reverse transcription by being incorporated into the newly synthesised viral DNA and preventing its further elongation. eg; of nucleoside reverse transcriptase inhibitors (NRTIs) include Zidovudine,

Lamivudine, Stavudine, Zalcitabine, and Didanosine. Nucleotides reverse transcriptase inhibitors include; Tenofovir, Fumarate, Disoproxil.

ii) Non-nucleoside reverse transcriptase inhibitors (NNRTIs) inhibit reverse transcriptase directly by binding to the enzyme and interfering with its function. eg; Nevirapine, Efavirenz, Delavirdine, Atevirdine, Tetradrodiazepine.

iii) Protease inhibitors (PIs) target viral assembly by inhibiting the activity of protease, an enzyme used by HIV to cleave nascent proteins for final assembly of new virions. eg; Ritonavir, Indinavir, Saquinavir, Lopinavir, Amprenavir, Nelfinavir.

iv) Integrase inhibitors inhibit the enzyme integrase, which is responsible for integration of viral DNA into the DNA of the infected cell. eg; Ralteoravir.

v) Entry inhibitors (or fusion inhibitors) interfere with binding, fusion and entry of HIV to the host cell by blocking one of several targets. eg; Maraviroc, Enfuvirtide.

vi) Maturation inhibitors inhibit the last step in gag processing in which the viral capsid polyprotein is cleaved, thereby blocking the conversion of the polyprotein into the mature capsid protein (p24). Because these viral particles have a defective core, the virions released consist mainly of non-infectious particles. There are no drugs in this class currently available, though two are under investigation, Bevirimat and Vivecon.

2.1.9.2 Limitations of Antiretroviral Drug Therapy

i) If an HIV infection becomes resistant to standard HAART, there are limited options. One option is to take larger combinations of antiretroviral drugs, an approach known as **mega-HAART** or salvage therapy. Salvage therapy often increases the drugs side-effects and treatment cost.

ii) If an HIV infection becomes sufficiently resistant to antiretroviral drugs, treatment becomes more complicated and prognosis may deteriorate.

iii) It is still unclear whether suppressing or even eliminating HIV will be adequate to restore normal immune function in the long term, since HIV can damage the ability of the thymus to produce normal diverse T-cells.

iv) Also, rapid suppression of HIV and partial restoration of the immune system sometimes produces a dangerous hypersensitivity reaction, immune reconstitution inflammatory syndrome (Wikipedia, 2007).

2.1.9.3 Side Effects

Adverse effects of antiretroviral drugs vary by drug, by ethnicity, and by individual, and by interaction with other drugs, including alcohol. Hypersensitivity to some drugs may also occur in some individuals (Ian, 2005). The adverse effect of antiretroviral drugs include the following;-

- Abdominal pain
- Anaemia
- Alopecia
- Diarrhoea
- Dizziness (Vertigo)
- Fanconi syndrome
- Flatulence
- Headache
- Hepatitis
- Hyperbilirubinaemia
- Hypercholesterolaemia (Dyslipidaemia, hyperlipidaemia, high cholesterol)
- Hyperpigmentation (of nails, palms, or soles)
- Insomnia
- Jaundice
- Lipodystrophy (Fat accumulation/Fat atrophy)
- Liver failure
- Malaise
- Mitochondria toxicity
- Myalgia
- Nausea

- Neutropenia
- Oral ulcers
- Pancreatitis
- Peripheral neuropathy
- Rash
- **Renal failure or insufficiency**
- Somnolence (drowsiness)
- Steven-Johnson syndrome
- Vomitting
- Xeroderma (dry skin)
- Xerostomia (dry mouth)

2.2 THE KIDNEY

The kidneys are a pair of fist-sized organs located outside the peritoneal cavity on each side of the spine. They range from 9 to 13 cm in diameter; the left slightly larger than right (Wikipedia, 2007). Kidney of the adult human weighs about 150 grams (Arthur and John, 2006). The kidney is a highly specialized organ that maintains the internal environment of the body by selectively excreting or retaining various substances according to specific body needs. The importance of urine formation and excretion as a life-sustaining

function is highlighted in situations in which kidney function is suddenly lost. Without at least one functioning kidney, death can occur within a few days.

The nephron is the kidney's primary functional unit. Each kidney in the human contains about 1 million nephrons (Arthur and John, 2006). Each nephron contains a filtering system known as a glomerulus, and a tubule, through which the filtered liquid passes (Wikipedia, 2007). Each glomerulus consists of a capillary network surrounded by a membrane called Bowman′s capsule. The afferent arteriole carries blood from the renal artery into the glomerulus, where it divides to form a circulatory network. At the distal end of the glomerulus, the capillaries rejoin to form the efferent arteriole through which blood leaves the glomerulus. Fluid filtered from the glomerular capillaries flows into Bowman′s capsule and then into the proximal tubule, which lies in the cortex of the kidney (Arthur and John, 2006).

From the proximal tubule, fluid flows into the loop of Henle, which dips into the renal medulla. Each loop consists of a descending and an ascending limb. The walls of the descending limb and the lower end of the ascending limb are very thin and therefore are called the thin segment of the loop of Henle. After the ascending limb of the loop has returned partway back to the cortex, its wall becomes much thicker, and it is referred to as the thick segment of the ascending limb.

At the end of the thick ascending limb is a short segment, which is actually a plaque in its wall, known as the macula densa (Arthur and John, 2006). Beyond the macula densa, fluid enters the distal tubule, which, like the proximal tubule, lies in the renal cortex. This is followed by the connecting tubule and the cortical collecting tubule, which leads to the cortical collecting duct. The initial parts of 8 to 10 cortical collecting ducts join to form a single larger collecting duct that runs downward into the medulla and becomes the medullary collecting duct. The collecting ducts merge to form progressively larger ducts that eventually empty into the renal pelvis through the tips of the renal papillae. In each kidney, there are about 250 of the vary larger collecting ducts, each of which collects urine from about 4000 nephrons (Arthur and John, 2006).

A large amount of circulating blood flows through the kidneys. Approximately 25% of the cardiac output or 1200 ml of blood per minute is received by the kidneys. One litre of urine is the end product of more than 1000 litres of circulating blood processed through the kidneys. Urine formation begins in the glomerular capillaries. With dissolved substances passing into the proximal tubule as a result of the force of blood pressure in the large afferent arteriole and the pressure in Bowman′s capsule (Wikipedia, 2007).

2.2.1 Functions of the Kidney

The functional unit in the kidney is the nephron. The kidney regulates extracellular fluid (ECF) volume and electrolyte composition to compensate for

wide daily variations in water and electrolyte intake. They form urine in which the potentially toxic waste products of metabolism are excreted (Allan *et al*, 1999). The functions of the kidney therefore include;

i) Excretion of waste products;- excretion of a variety of waste products of protein and nucleic acid metabolism: urea, creatinine, creatine, uric acid, sulphate, phosphate.

ii) Homeostasis;- the kidney is one of the major organs involved in whole-body homeostasis. Among its homeostatic functions are acid-base balance, regulation of electrolyte concentrations, control of blood volume, and regulation of blood pressure. The kidneys accomplish these homeostatic functions independently and through coordination with other organs, particularly those of the endocrine system. The kidney communicates with these organs through hormones secreted into the bloodstream.

   Acid-base balance: the kidneys regulate the pH of blood by adjusting hydrogen ion levels, referred as augmentation of mineral ion concentration, as well as water composition of the blood.

   Blood pressure: Sodium ions are controlled in a homeostatic process involving aldosterone which increases sodium ion reabsorption in the distal convoluted tubules.

Plasma volume: any significant rise or drop in plasma osmolality is detected by the hypothalamus, which communicates directly with the posterior pituitary gland. A rise in osmolality causes the gland to secrete antidiuretic hormone, resulting in water reabsorption by the kidney and an increase in urine concentration. The two factors work together to return the plasma osmolality to its normal levels.

iii) Hormone secretion;- the kidneys secrete a variety of hormones, including erythropoietin, urodilatin, renin, and 1,25 dihydroxy vitamin D (the most active form of vitamin D).

2.2.2 Classification of Kidney Failure

Kidney failure is basically classified into two:

2.2.2.1 Acute Renal Failure (ARF)

Acute renal failure (ARF) is a syndrome characterised by rapid decline in glomerular filtration rate (hours to days), retention of nitrogenous waste products, and perturbation of extracellular fluid volume and electrolyte and acid-base homeostasis. ARF is usually asymptomatic and diagnosed when biochemical monitoring of hospitalised patients reveals a recent increase in blood urea and creatinine concentrations. It may complicate a wide range of diseases, which for purposes of diagnosis and management are conveniently divided into three categories: i. Diseases that cause renal hypoperfusion without compromising the intergrity of renal parenchyma (prerenal ARF, prerenal

azotaemia) (55%). ii. Diseases that directly involve renal parenchyma (intrinsic renal ARF, renal azotaemia) (40%) and iii. Diseases associated with urinary tract obstruction (post renal ARF, post renal azotaemia) (5%) (See table 2.3). Most ARF is reversible, the kidney being relatively unique among major organs in its ability to recover from almost complete loss of function (Hugh and Barry, 2005).

2.2.2.2 Chronic Kidney Disease (CKD)

Chronic kidney disease (CKD) also known as chronic renal disease is a progressive loss of renal function over a period of months or years through five stages. Each stage is a progression through an abnormally low and deteriorating glomerular filtration rate, which is usually determined indirectly by the creatinine level in blood serum. The end result of progressive renal damage is the same no matter what the cause of the disease may have been. The major effects of renal failure all occur because of the loss of functioning nephrons (Allan *et al*, 1999).

Table 2.3: Causes of ARF;-

	Causes
1. Prerenal	Hypovolaemia (trauma, burns, surgery) Decreased effective plasma volume (nephritic syndrome, sepsis, shock) Decreased cardiac output (congestive cardiac failure, pulmonary embolism) Renovascular obstruction (atherosclerosis, stenosis) Interference with renal autoregulation (angiotensin converting enzyme inhibitors, cyclosporine)
2. Renal	Glomerular small vessel disease (aggressive glomerulonephritis) Interstitial nephritis (infection, infiltration, drugs/toxins) Tubular lesions (post ischaemic, nephrotoxins, rhabdomyolysis, Bence Jones protein, hypercalcaemia)
3. Post renal	Bladder outlet obstruction (prostatism, neurogenic bladder) Ureteric obstruction (stones, blood clots, tumours, radiotherapy, retroperitoneal fibrosis)

(Michael et al, 2006)

Signs and Symptoms of CKD;-

Initially, symptoms are non specific and can only be detected as an increase in serum creatinine or protein in the urine. As the kidney function decreases (Adrouge and Madias, 1981);

- Blood pressure is increased due to fluid overload and production of vasoactive hormones, increasing one′s risk of developing hypertension and/or suffering from congestive heart failure.
- Urea accumulates, leading to azotaemia and ultimately uraemia (symptoms ranging from lethargy to pericarditis and encephalopathy). Urea is excreted by sweating and crystallizes on skin (uraemic frost).
- Potassium accumulates in the blood (hyperkalaemia with a range of symptoms including malaise and potentially fatal cardiac arrhythmias)
- Erythropoietin synthesis is decreased (potentially leading to anaemia, which causes fatigue)
- Fluid volume overload (symptoms may range from mild oedema to life-threatening pulmonary oedema)
- Hyperphosphataemia, due to reduced phosphate excretion, associated with hypocalcaemia (due to vitamin D_3 deficiency). Later this progresses to tertiary hyperparathyroidism, with

hypercalcaemia, renal osteodystrophy and vascular calcification that further impairs cardiac function.

- Metabolic acidosis, due to decreased excretion of bicarbonate by the kidney. This may cause altered enzyme activity by excess acid acting on enzymes and also increased excitability of cardiac and neuronal membranes by the promotion of hyperkalaemia due to excess acid (acidaemia).

Stages of Chronic Kidney Disease (CKD);-

All individuals with a GFR < 60 ml/min for three (3) months are classified as having chronic kidney disease, irrespective of the presence or absence of kidney damage. The rationale for including these individuals is that reduction in kidney function to this level or lower represents loss of half or more of the adult level of normal kidney function, which may be associated with a number of complications (Wikipedia, 2007).See Table 2.4.

Table 2.4 : Stages of Chronic Kidney Disease (CKD)

Stages	Description	GFR (ml/min)	Management
1	Kidney damage with normal or increased GFR	>90	Observation and control of blood pressure
2	Mildly decreased GFR	60-89	Observation, control of blood pressure and cardiovascular risk factors
3	Moderately decreased GFR	30-59	Observation, control of blood pressure and cardiovascular risk factors
4	Severely reduced GFR	15-29	Planning for end stage renal failure
5	Kidney failure /End stage renal failure (ESRD)	<15	Transplant or Dialysis

(David and Christopher, 1986)

An estimated glomerular filtration rate of 60-90 ml/min in the absence of other evidence of kidney disease does not signify chronic kidney disease and does not indicate that further testing is required (Jamie *et al*, 2006).

Causes;-

The most common causes of CKD are diabetic nephropathy, hypertension and glomerulonephritis. Together, these cause approximately 75% of all adult cases. Certain geographic areas have a high incidence of HIV nephropathy (Perazella and Khan, 2006).

Historically, kidney disease has been classified according to the part of the renal anatomy that is involved, as:

- Vascular, includes large vessel disease such as bilateral renal artery stenosis and small vessel disease such as ischaemic nephropathy, haemolytic uraemic syndrome and vasculitis
- Glomerular, comprising a diverse group and subclassified into
  . Primary glomerular disease such as focal segmental glomerulosclerosis and IgA nephritis
  . Secondary glomerular disease such as diabetic nephropathy and lupus nephritis.

- Tubulointestitial including polycystic kidney disease, drug and toxin-induced chronic tubulointestitial nephritis and reflux nephropathy
- Obstructive such as with bilateral kidney stones and diseases of the prostate (Perazella and Khan, 2006).

2.2.3 Assessment of Renal Function

The kidney has a remarkable ability to dilute or concentrate urine, according to an individual′s changing physiological needs, and to regulate electrolyte excretion. Impaired renal function has adverse effects on blood chemistry, blood pressure, fluid balance, nutrient intake, and the person′s general state of health. When kidney function becomes compromised by disease, the processes of glomerular filtration and tubular reabsorption and secretion becomes affected. Blood and urine biochemical tests reflect the extent of this dysfunction. Renal function tests are used to screen the kidney disease, to help determine the cause of kidney disease, and to determine the extent of renal dysfunction.

Different parts of the nephrons are in close anatomical association and are dependant on a common blood supply. Renal dysfunction of any kind affects all parts of the nephrons to some extent, although sometimes either glomerular or tubular dysfunction is predominant. The net effect of renal disease on plasma

and urine depends on the proportion of glomeruli to tubules affected and on the number of nephrons involved.

2.2.3.1 Glomerular Dysfunction

The total amounts of urea and creatinine excreted are affected by the GFR. If the rate of filtration fails to balance that of production, plasma concentrations will rise.

Phosphate and urate (uric acid) are released during cell breakdown. Plasma concentrations rise because less than normal is filtered. Most of the reduced amount reaching the proximal tubule can be reabsorbed, and the capacity for secretion is impaired if the filtered volume is too low to accept the ions; these factors further contribute to high plasma concentrations.

A large proportion of the reduced amount of filtered sodium is reabsorbed by isosmotic mechanisms; less than usual is then available for exchange with hydrogen and potassium ions distally. This has two outcomes;

i) Reduced hydrogen ion secretion throughout the nephron: bicarbonate can only be reclaimed if hydrogen ion is secreted; plasma bicarbonate concentrations will fall;
ii) Reduced potassium secretion in the distal tubule, with potassium retention (potassium can still be reabsorbed proximally).

If there is a low GFR accompanied by a low renal blood flow:

i) Systemic aldosterone secretion will be maximal; in such cases, any sodium reaching the distal tubule will be almost completely reabsorbed in exchange for hydrogen ion and potassium ion, and urinary sodium concentration will be low;

ii) ADH secretion will be increased: ADH acting on the collecting ducts allows water to be reabsorbed in excess of solute, further reducing urinary volume and increasing urinary osmolality well above that of plasma. This high urinary osmolality is mainly due to substances not actively dealt with by the tubules. If the capacity of the proximal tubular cells to reabsorb solute, and therefore water, is normal, a larger proportion than usual of the reduced filtered volume will be reclaimed by isosmotic processes, thus further reducing urinary volume.

2.2.3.2 Tubular Dysfunction

Damage to tubular cells impairs adjustment of the composition and volume of the urine. Impaired solute reabsorption from proximal tubules reduced isosmotic water reabsorption. Counter-current multiplication may also be affected, and therefore the ability of the collecting ducts to respond to ADH is reduced. A large volume of inappropriately dilute urine is produced.

The tubules cannot secrete hydrogen ions and therefore cannot reabsorb bicarbonate normally or acidify the urine.

The response to aldosterone and therefore the exchange mechanism involving reabsorption of sodium are impaired; the urine contains an inappropriately high concentration of sodium for the renal blood flow.

Potassium reabsorption from the proximal tubule is impaired and plasma potassium concentrations may be low.

Reabsorption of glucose, phosphate, magnesium, urate and amino acids is impaired. Plasma phosphate, magnesium and urate concentrations may be low. There may also be tubular proteinuria (Martin, 2006).

The reliable measurement of renal excretory function is of great importance in clinical practice and in research. The introduction of routine reporting of estimated glomerular filtration rate and a new definition of chronic kidney disease has renewed interest in methods of measuring renal function. Accurate measurement is methodologically difficult so surrogate measures such as serum creatinine levels and prediction formulas (based on factors such as the patient's age, sex, and serum creatinine level) are more commonly used in routine practice (Jamie *et al*, 2006).

The kidney has several functions. These depend on glomerular filtration rate, the unit measure of kidney function. GFR can be defined as the volume of plasma cleared of an ideal substance per unit of time (usually expressed as

ml/min). The ideal substance is one that is freely filtered at the glomerulus and neither secreted nor reabsorbed by the renal tubules (Jamie *et al*, 2006).

2.2.3.3 Creatinine

Creatinine is the closest to an ideal endogenous substance for measuring GFR. Plasma creatinine is almost exclusively a product of the metabolism of creatine and phosphocreatine in skeletal muscle, although ingestion of meat may also contribute slightly. In patients with stable renal function, serum creatinine levels are usually constant, with variability daily of about only 8%. Creatinine is freely filtered at the glomerulus and is not reabsorbed but up to 15% is actively secreted by the tubules.

2.2.3.3.1 Creatinine Clearance (CrCl)

Measuring the creatinine clearance using serum creatinine level and a timed urine collection gives an estimate of glomerular filtration rate.

CrCl = (Urine creatinine x Volume) / Serum creatinine

As a result of tubular secretion of creatinine, creatinine clearance tends to over estimate true glomerular filtration rate. This is a systemic error of fairly stable magnitude, however, until advanced renal failure is reached, allowing creatinine clearance to be a reasonable method of following changes of renal function in patients. The main problem with creatinine clearance is the requirement for urine collection over 24 hours; patients find this inconvenient

and therefore collections are often inaccurate. Also a 25% daily variation in the values obtained using this method has been reported. Creatinine clearance is therefore no longer much used in clinical practice (Jamie *et al*, 2006).

2.2.3.4 Urea

Serum urea is a less reliable marker of glomerular filtration rate than creatinine because levels are more vulnerable to change for reasons unconnected to glomerular filtration rate. A high protein diet, tissue breakdown, major gastrointestinal haemorrhage, and corticosteroid therapy can lead to an increase in plasma urea whereas a low protein diet and liver disease can lead to a reduction. Also 40-50% of filtered urea may be reabsorbed by the tubules, although the proportion is reduced in advanced renal failure (Jamie *et al*, 2006).

2.2.3.5 Prediction Formulas

To circumvent the practical difficulties of formal measurement of clearance, several prediction formulas have been published. The most commonly used are the Cockcroft and Gault equation and formulas based on the Modification of Diet in Renal Disease (MDRD) study.

2.2.3.5.1 Cockcroft and Gault Equation

The Cockcroft and Gault equation, which estimates creatinine clearance on the basis of serum creatinine level, age, sex, and weight, was one of the earliest prediction formulas and is still widely used. It was based on

creatinine excretion in men with normal renal function with a correction for women, based on three other studies. It tends to over estimate renal function at lower levels, particularly when obesity or fluid overload is present, as the resultant increase in weight does not reflect an increase in muscle mass. However, as with creatinine clearance, this is largely a systematic error and the equation remains useful for following changes in renal function in a patient (Jamie *et al*, 2006).

The formula is as follow;-

eCrCl = (140 – Age (yr)) x Wt (Kg) x 1.2 / Cr (µmol/L) …. For male

= (140 – Age (yr)) x Wt (Kg) x 1.2 x 0.85 / Cr (µmol/L)…..For female

The equation is named after the scientists who first published the formula (Cockcroft and Gault, 1976). The equation is popular because it is easy to calculate.

2.2.3.5.2 MDRD Formula

The most recently advocated formula for calculating the GFR is the one that was developed as a result of the modification of diet in renal disease study (Levey *et al*, 1999). The formula is as follow;-

eCrCl = 186.3 x (Cr/88.4)–1.154 x (Age)–0.203 x constant

The constant is 1 for a White male, and is multiplied with 0.742 for females and multiplied with 1.21 for Blacks.

A more elaborate version of the MDRD equation also includes serum albumin and serum urea nitrogen levels (Wikipedia, 2007):

GFR = 170 x (PCr)–0.999 x (Age)–0.176 x (0.762 if patient is female) x (1.180 if patient is black) x (SUN)–0.170 x (Alb)+0.318

(PCr) = Serum creatinine concentration (mg/dl)

(SUN) = Serum urea nitrogen concentration (mg/dl)

(Alb) = Serum alb concentration (g/dl).

The routine investigation of an HIV- infected person include tests for sodium, potassium, calcium, phosphate (every three months) and creatinine (creatinine clearance). The urine should be tested for glucosuria, proteinuria, erythrocyturia and leukocyturia every three (3) months (Ansgar, 2007). However, creatinine clearance is not done in most patients with HIV/AIDS.

An asymptomatic, slight proteinuria with no rise in creatinine in untreated patients is normally a consequence of the infection of the glomerulus or tubular apparatus with HIV and should be monitored quarterly (Ansgar, 2007).

2.3 HIV AND RENAL FUNCTION

A quarter of the cardiac output is consigned to the perfusion of the kidneys, even though the kidneys amount to just 0.5% of the total body weight. Approximately every 20 minutes, ie 70 times a day the entire blood plasma is

filtered by the kidneys. Therefore, kidney glomeruli are target organs for every haematogenous infection. Viral infection can cause primary glomerulonephritis, whereas an immune reaction can lead to secondary glomerulonephritis. HIV infection, hepatitis B and C as well as bacterial infections are all typical causes of renal disease. Nephrotoxic agents precipitate renal diseases that affect the interstitium and the tubular apparatus in particular, and these have to be differentiated from glomerulonephritis (Ansgar, 2007).

Both viral infections and nephrotoxic agents can cause renal impairement and can lead to end-stage renal disease. While HIV-associated nephropathy (HIVAN) is common in Afro-Americans (80-85%), in the HAART era the greater task will be to examine the renal safety of antiretroviral agents. However, only a small amount of literature is available on this subject to date (Ansgar, 2007).

HIVAN is diagnosed in cases of nephritic syndrome with oedema, hypoalbuminaemia, hyperlipidaemia and proteinuria of more than 3.5 g/day. However, even a mild proteinuria is possible. The occurrence of proteinuria and erythrocyturia is pathognomonic for glomerulonephritis (GN) and, together with nephritic sediment, usually confirms the diagnosis.

Patients with HIV-1 infection are at risk of developing renal diseases with diverse aetiologies. Acute renal failure occurs in up to 20% of hospitalised patients with HIV infections, and chronic renal diseases of diverse etiology have

been reported (Wiston and Klotman, 1996; Abobott *et al*, 2001; Paul and Paul, 2004). The single most common cause of chronic renal insufficiency in HIV-1 patients is HIVAN.

2.3.1 Pathogenesis of Renal Disease in HIV/AIDS

The kidneys, like other major organ systems are also affected by infection with HIV. This affectation can result from common complications of the disease like dehydration and sepsis or nephrotoxic drugs commonly used in the management of AIDS (Berns *et al*, 1991). Other evidence suggests a direct role of the virus resulting in a distinct entity termed HIV-associated nephropathy (HIVAN) (Pardo *et al*, 1984; Pardo *et al*, 1987).

Until recently, it was unknown whether HIV-1 infection of renal parenchymal cells caused HIVAN directly or whether HIVAN was an indirect response to HIV-induced immune dysregulation (Cohen *et al*, 1989; Schwartz and Klotman, 1998). The primary reason for this uncertainty was the presence of conflicting data regarding the presence of HIV-1 in renal parenchymal cells in clinical HIVAN specimens. Studies using HIV-1 transgenic mice model of HIVAN have provided important insight into HIVAN pathogenesis. Mice transgenic for a replication defective HIV-1 reconstruct lacking the gag and pol genes, expressed under control of the viral promoter (Long terminal repeat or LTR), developed proteinuria, renal failure, and histologic renal disease identical to HIVAN (Kopp *et al*, 1992; Bruggeman *et al*, 2001). The pattern of HIV-1

infection of renal tubules is focal and may involve epithelial cells from multiple nephron segments, including proximal tubule, thick ascending loop of Henle and collecting duct (Bruggeman *et al*, 2000).

The mechanism by which HIV-1 gains entry into renal epithelial cells is unknown. CD4, the receptor for HIV-1, and CCR5 and CXCR4, the major co-receptors for HIV-1 are not expressed in most normal renal epithelial cells (Frank *et al*, 2000). Several other co-receptors (CXCR6/Bonzo and CCR8) for HIV-1 have been recently identified, but whether they are expressed in renal epithelial cells remains to be determined (Paul and Aine, 2002).

Hanna *et al* (2001) reported that mice transgenic for HIV-1 expressed under the control of the human CD4 regulatory sequences develop an AIDS-like illness as well as renal disease. They also generated several transgenic lines with mutations in one or more HIV genes and found that expression of *nef* was necessary and sufficient to produce their renal phenotype.
Nef has been shown to be capable of building to *Hck* as well as several other *Src* family of tyrosine kinases *in vitro* (Collette *et al*, 2000). To determine whether *Hck* was important in modulating the renal disease in the *nef* transgenic mice, they cross bred the transgenic mice with *Hck* knocked out mice and found that the development of renal disease was delayed; suggesting *Hck* may play a role in the pathogenesis of HIV-related renal disease (Greenway *et al*, 1999; Collette *et al*, 2000).

Also, the spectrum of an allergic or autoimmune reaction in the kidney of HIV-infected patient is no different from the skin. Reactions can be humoral or T-cell mediated and can lead to renal insufficiency. The spectrum ranges from the type I immune reaction (acute interstitial nephritis after exposure to medication) to the type IV T-cell mediated reaction (special forms of a chronic interstitial nephritis). It is, therefore, important to know that even the one-off use of an analgesic (eg ibuprofen) can lead to renal failure (Ansgar, 2007).

Antiretroviral drugs commonly associated with renal insufficiency include Indinavir which was established in 1996 and administered at the high dosage of 3 x 800 mg, the renal side effects ranged from asymptomatic crystalluria to renal failure. Another one with tubulotoxic side effect suggesting Fanconi′s syndrome is Tenofovir and according to the cases available at present, it can be assumed that tubular acidosis, hypokalaemia, hypophosphataemia and glucosuria will all recede after Tenofovir has been discontinued. A recently published overview concerning changes in the renal function under antiretroviral therapy saw no advantage of one particular therapy with regard to nephrotoxicity. Therefore, only careful monitoring of serum creatinine, proteinuria, and erythrocyturia and serum phosphate is advised (Ansgar, 2007).

2.4 RENAL DISEASE IN HIV/AIDS

HIV-related renal diseases are increasingly prevalent and are associated with proteinuria and rapid progression to end-stage renal failure (CKD stage 5) (Gupta *et al*, 2004). HIV infected individuals with worsening renal function has an increasing risk of progression to AIDS and death. The prevalence and predictors of chronic kidney disease (CKD) in HIV-infected individuals have not been well defined (Ronald *et al*, 2005).

In a study by Agaba *et al* (2003) in Jos, central Nigeria in a hospital based prospective case control study of 79 AIDS patients and 57 HIV negative healthy controls reported that renal disease was present in 41 (51.8%) of the patients in the AIDS group and 7 (12.2%) of controls. And also, that the GFR was significantly higher in controls compared to the study group (103.30 ± 37.78 and 68.03 ± 37.55 respectively). Anteyi *et al* (2005) in Abuja reported HIVAN in four (28.5%) of 14 HIV-positive patients on their study. Also Pedro (2005) in Ife reported HIV- associated nephropathy (HIVAN) in 152 (38%) of 400 patients who were diagnosed HIV positive.

In another study by Gupta *et al* (2004) in Indiana University school of medicine, Wishard Hospital Indianapolis, USA reported that of 487 subjects with initially normal renal function, 10 (2%) developed CKD and thus concluded that a small, but potentially clinically meaningful proportion of HIV-infected patients develop CKD. Kara *et al* (2007) reported that of the 373 stable

HIV-infected Kenyan outpatients (antiretroviral naïve), 11.5% had eCrCl less than 60 ml/min and 4.8% less than 50 ml/min using Cockcroft and Gault equation.

Stephen Becker of Pacific Horizon medical group, San Francisco reported that more than 10% of patients in his study were found to have renal insufficiency consistent with the National Kidney Foundation′s definition of stage 3 CKD determined by decreased glomerular filtration rate, and that only about 2% would have been identified if serum creatinine alone were used. He then concluded that the use of a more sensitive marker, like GFR (MDRD, Cockcroft-Gault equation), may be warranted for early detection of renal impairement (Stephen, 2005).

Ronald *et al* (2005) of Institute of Human Virology Maryland, USA, also found that HIV-infected men on HAART regimens were more likely to have stage 3 kidney disease (5.3%) than were HIV-infected men not on HAART (1.8%), based on GFR. Also in another study by Susman (2005), 172 HIV patients were treated with regimens that contained Tenofovir. Of these, 55.8% had GFR below 90 ml/min (CKD stage 2), while 44.2% of those patients had higher rates. The prevalence of renal disease was found to be 48.5% (145/299) and 0.3% of them had HIVAN, according to a study by Andia *et al* (2005) in Mbarara, Uganda, this was based on calculated GFR below 80 ml/min in 299 patients.

In a similar study by Chi Yuen *et al* (2007) among Chinese HIV-infected patients in Hong Kong, it was found that of 322 patients recruited (301 patients 93.5% received HAART), 16.8% had CKD and 18 patients (5.6%) had eGFR < 60 ml/min. Chioma *et al* (2008) in Ile-Ife Nigeria reported the prevalence of renal disease (proteinuria and/or elevated serum creatinine) to be 38% (152) of the 400 HIV/AIDS patients studied.

CHAPTER THREE

3.0 SUBJECTS AND METHODS

3.1 BACKGROUND OF STUDY AREA

This is a cross-sectional descriptive study, which was conducted at Ahmadu Bello University Teaching Hospital Shika, Zaria. The predominant occupation of the people of Zaria is Farming but civil servants and businessmen also form a sizable part of the population. The main religions are Islam and Christianity. Zaria has a tropical climate with a temperature range from 15.3-36.2 ^{0}C and a rainfall that ranges from 0-816 mm/month. Zaria has two main seasons, the rainy season from April to October and a dry season from November to March.

3.2 STUDY POPULATION (SUBJECTS)

For the purpose of this study, renal function of one hundred and one (101) randomly selected HIV (I & II) positive patients confirmed with Western blot on highly active antiretroviral therapy (HAART) attending antiretroviral (ARV) clinic in ABUTH shika, Zaria and one hundred (100) apparently healthy controls was assessed. These controls were recruited from population of staff and students.

3.2.1 Inclusion Criteria

(i) HIV – seropositive between the ages of 15-50 years

(ii) Patients on antiretroviral drugs

(iii) Patients had no hypertension, diabetes mellitus, or malignancy prior to the diagnosis of HIV infection.

(iv) Apparently healthy subjects between the ages of 15-50 years (controls).

3.2.2 Exclusion Criteria

(i) Pregnant patients

(ii) Presence of congestive heart failure, hypertension, diabetes mellitus, or tuberculosis.

(iii) Presence of urinary tract infection.

3.2.3 Consent

Fully informed written consent was obtained from each patient and control in the study.

3.2.4 Sample size

The sample size (n) was determined using the formula;-

$$n = Z\alpha/2^2 \times P(1-P)/d^2$$

Where $Z\alpha/2^2 = (1.96)^2$

P = Prevalence rate of disease (4.4%)

d = Tolerable sampling error (0.04)

$= (1.96)^2 \times 0.044 \times 0.956/0.0016 = 100.99$

Approximately 101 patients.

3.2.5 Ethical Approval

This was obtained from Ethical and Scientific Committee of the Hospital before embarking on the study.

3.3 STUDY PROTOCOL

A questionnaire (attached to this thesis) was administered to all participants. This contained the biodata, laboratory results, family history of hypertension, diabetes mellitus and renal disease, and also drug history of study subjects.

The weight (kg) of all patients was recorded to the nearest 0.5 kg using Hansons standard bathroom (standing) weighing scale with subjects wearing light clothing. Blood pressure was taken on the dominant arm of the patient using Accosons sphygmomanometer with an appropriate standard cuff of 12-13 cm by 55 cm size.

The following biochemical investigations were performed on all study subjects. Viz: Serum electrolytes, urea, creatinine, uric acid, phosphate, calcium and urinalysis for glucose, protein, blood, ketone, nitrite, blood, specific gravity and pH.

Estimated Creatinine clearance (eCrCl) was calculated by the use of prediction equation using Cockcroft & Gault formula (Cockcroft and Gault, 1976).

$$eCrCl = (140 - Age\ (yr) \times Wt\ (kg) \times 1.2 / Cr\ (\mu mol/l) \quad \text{for male}$$

$$= (140 - Age\ (yr) \times Wt\ (kg) \times 1.2 \times 0.85 / Cr\ (\mu mol/l) \quad \text{for female}$$

3.4 SPECIMEN COLLECTION AND PROCESSING

3.4.1 Blood

A tourniquet was applied and then removed immediately after blood collection when necessary a few centimeters above the antecubital fossa to distend veins. The antecubital fossa was cleaned with methylated spirit and allowed to dry. Blood was then taken using a sterilized 10 ml syringe and 21 G needle. This was transferred into a plain bottle and allowed to stand for about 30minutes for it to clot. This was then centrifuged for 5 minutes at 1000 rpm. The serum was separated from the cells and transferred into Bijou (sample) bottle and then frozen at -20^{o}C until the time for analysis which was within 48 hours of serum separation.

3.4.2 Urine

About 10 – 15 ml of random urine was obtained for analysis from each subject.

3.5 CHEMICALS

The reagents used for the measurement of serum electrolytes, urea, and creatinine were procured from BDH Chemicals Limited (Poole Dorrest,

England) and M&B Limited (England). The kits used for measurement of serum calcium, phosphate, and uric acid were procured from Agappe Diagnostics Limited (India). All the chemicals and kits were of analytical grade or higher.

3.6 EQUIPMENT

Hettich Universal 32 Centrifuge (Germany) was used to spin the blood specimens. Beckman Coulter DU-20 General purpose UV/Vis Spectrophotometer (Germany) was used for the measurement of absorbance of the analytes. Also Corning 400 Flame Photometer was used for sodium and potassium analysis.

Other equipment that was used, especially for the estimation of chloride and bicarbonate are micropipettes, graduated pipettes, volumetric flask, test tubes, and test bottles.

3.7 ANALYTICAL METHODS

Choice of analytical methods was based on routine methods available in Chemical Pathology laboratory of ABUTH, Zaria.

3.7.1 Creatinine

Creatinine was measured by the method of Rehbery-Folin, 1886.

3.7.1.1 Principle

Creatinine gives a red colour with alkaline solutions of picric acid (Jaffes reaction). The red colour is measured in the presence of high background (yellow colour) and the solutions appear orange at low creatinine concentrations.

3.7.1.2 Procedure

Into 3 set of test tubes, labelled test, standard and control; 3 ml of distilled water was added to test tube labelled test containing 1ml of serum and 4 ml to blank while the standard tube was left empty. Sodium tungstate (10%) and 2⁄3N sulphuric acid (1 ml each) were added to test and blank tubes, mixed and centrifuged for 10 min. Supernatants 3 ml from the test tube labelled test above was transferred to a new glass tube and 3 ml of standard solution was also added into the test tube labelled standard. An addition of 1ml each of picric acid and 0.75N sodium hydroxide was followed with incubation for 15 min at room temperature. The absorbance was read at 520 nm.

3.7.1.3 Calculation

Creatinine (µmol⁄L) = $A_{test}/A_{std} \times C_{std}$

Where A = Absorbance, C = Concentration, std = standard

3.7.2 Urea

Urea was measured using Diacetyl monoxime method (Natelson, 1951 modified by March *et al*, 1965).

3.7.2.1 Principle

When urea is heated in strongly acidic conditions with substance such as diacetyl monoxime, yellow condensation compounds are formed. The reaction is intensified by the presence of polyvalent ions such as ferric ions, which form a red coloured complex. The intensity of the red complex is measured colorimetrically at 520nm and is proportional to the concentration of the urea in the sample.

3.7.2.2 Procedure

Three test tubes were labelled as test, standard and blank. Into test and standard tubes 10 ml of distilled water was added followed by 100 µl of sample and standard respectively with vigorous mixing. From the above preparations 1 ml was then pipetted into new tubes labelled as sample and standard. Distilled water (1 ml) was added into the tubes labelled sample and standard while 2 ml of distilled water into a tube labelled blank. Working reagent and mixed acid reagent (2 ml) was added sequentially into the tubes and mixed thoroughly. The tubes were then placed into a hot block at 100 ºC for 20 minutes, cool and the absorbance read at 520 nm.

3.7.2.3 Calculation

Urea (mmol/L) = $A_{test} / A_{std} \times C_{std}$

Where A = Absorbance , C = Concentration , std = standard

3.7.3 Sodium and Potassium

Sodium and Potassium were estimated using Flame photometric method.

3.7.3.1 Principle

Solution of electrolytes under carefully controlled condition when finely sprayed into a burner leaves a solid salt which dissociates to give atoms at neutral ground state. Some of these atoms are excited to the higher energy level; when these excited atoms fall back to the ground state – light of characteristic wavelength is emitted (590 nm for sodium and 770 nm for potassium). This light then passes through a suitable filter onto a photosensitive element and the amount of current thus produced is measured which is proportional to the amount of sodium or potassium present in sample.

3.7.3.2 Procedure

A one in hundred dilution (0.1 ml of sample + 9.9 ml of deionised water) of the original sample was made. About 10 ml of the working standard and distilled water as blank was placed into 2 separate containers. The appropriate filters for

sodium or potassium were used. The gas chamber was ignited. The galvanometer reading was blanked using distilled water and the galvanometer reading set to 80 (sodium) or 70 (potassium) using the working standard and reset back to zero. The samples were then read while checking the standard after 2-3 test readings.

3.7.3.3 Calculation

Sodium mmol/L = galvanometer reading x 2

Potassium mmol/L = galvanometer reading x 0.1

3.7.4 Chloride

Chloride was estimated by the method of Schales and Schales, 1971.

3.7.4.1 Principle

When mercuric nitrate solution is added to a solution containing chloride, unionised but soluble mercuric chloride is formed. At the end point, the first excess mercuric ions combine with diphenylcarbazone (indicator) to give a violate-blue coloured complex.

3.7.4.2 Procedure

To 2 ml of deionised water, 200 µl of sample was added with 2 drops of the indicator and then mixed. Titration was done against mercuric nitrate solution using 2 ml pipette (with 0.01 ml graduation).

3.7.4.3 Calculation

Chloride conc. (mmol / L) = titre of test / titre of std x conc. of std (100 mmol / L)

3.7.5 Bicarbonate

Bicarbonate was measured by the method of Scribner-Caillouette, 1964.

3.7.5.1 Principle

When diluted HCl is added to the serum containing bicarbonate, carbondioxide is released and the excess non-reacted HCl is titrated with sodium hydroxide solution using phenol red as indicator.

3.7.5.2 Procedure

Into a conical flask bottle, 200 µl of serum sample was added followed by the addition of 1 ml of 0.01N HCl. Sample was shaken followed by the addition of 2 ml of carbondioxide free water and then titrated against 0.01N sodium

hydroxide, after the addition of 1 drop of phenol red as an indicator, to a faint pink colour end point.

3.7.5.3 Calculation

1- titre x 50 mEq / L x 1 (mmol/L).

3.7.6 Calcium

Calcium was measured using the method of Baginski *et al*, 1973.

3.7.6.1 Principle

Calcium in a sample solution reacts with a solution containing o-cresophthalein complexone and 8-hydroxy-quinoline in dilute hydrochloric acid. A coloured complex is formed between calcium and the cresolphthalein-complexone at a pH between 10 and 12, which is maintained by the addition of diethylamine buffer. The absorbance is then measured at 575 nm. The 8-hydroxyquinoline contained in the reagent binds magnesium and prevents the interference from this ion.

3.7.6.2 Procedure

Three test tubes were prepared and labelled as blank, test and standard. Cresolphthalein-complexone, 2.5 ml was dispensed into each test tube and 50 µl

of distilled water, serum and standard was added into test tubes labeled blank, test and standard respectively. Di-ethylamine buffer (2.5 ml) was added to each test tube. The content of the test tubes was thoroughly mixed and read at 575 nm using the blank to zero the instrument. The concentration of serum calcium was then obtained as follows;

3.7.6.3 Calculation

Serum calcium (mmol/L) = $A_{test}/A_{std} \times C_{std}$

Where A = Absorbance, C = Concentration, std = standard

3.7.7 Inorganic Phosphorus

Inorganic phosphorus was measured by the method of Gomori, 1942.

3.7.7.1 Principle

The filtrate obtained after removing protein by means of trichloroacetic acid (T.C.A) is treated with an acid-molybdate reagent which reacts with inorganic phosphate to form phosphomolybdic acid. The hexa-valent molybdenum of the phosphomolybdic acid is then reduced by means of metol to give blue compound the intensity of which is proportional to the concentration of inorganic phosphorus present in the sample and which is estimated colorimetrically.

3.7.7.2 Procedure

Two cleaned centrifuge tubes were labeled test and control.10% T.C.A (3.8 ml) was dispensed into each tubes. Serum (200 µl) and control sample (200 µl) was added to tubes labelled test and control respectively. The content was thoroughly mixed and centrifuged for 5 min at 1,000 rpm. Four cleaned test tubes were then prepared and labeled blank, test, control, and standard. Sample supernatant (2.5 ml) and control supernatant (2.5 ml) was dispensed into test tubes labeled test and control respectively. 10% T.C.A (2.5 ml) was dispensed into test tube labeled blank. Standard (25 µl) and 2.25 ml of 10% T.C.A was dispensed into test tube labelled standard. Ammonium molybdate and metol (0.5 ml each) was added to all the 4 test tubes. The content was mixed thoroughly, incubated for 30 minutes at room temperature and read at 680 nm using blank to zero the instrument. The concentration of serum phosphorus was then obtained as follows;

3.7.7.3 Calculation

Serum phosphorus (mmol/L) = $A_{test} / A_{std} \times C_{std}$

Where A = Absorbance, C= Concentration, std = standard

3.7.8 Uric Acid

Uric acid was measured using Caraway method, 1955.

3.7.8.1 Principle

Phosphotungstic acid is reduced by uric acid in the presence of sodium carbonate to give a blue complex which can be measured colorimetrically

3.7.8.2 Procedure

Serum (0.5 ml) was pippetted into a test tube, 0.5 ml of sodium tungstate and 4.0 ml of 2/3N sulphuric acid was added. Mixed and centrifuged for 10 minute. Supernatant (3 ml), standard (3 ml), and distilled water (3 ml) was transferred into test tubes labeled test, standard, and blank respectively. An addition of 1 ml each of sodium carbonate and phosphotungstate was followed with incubation for 15 minute at room temperature. The reading of absorbance was made at 680 nm.

3.7.8.3 Calculation

Uric acid (µmol/L) = $A_{test}/A_{std} \times C_{std}$

Where A = Absorbance, C = Concentration, std = standard

3.7.9 Quality Control

Adequate quality control was observed when carrying out the analysis of the samples to ensure that the results obtained are reliable. This was done by analysing the samples in a batch together with quality control sera for specific analytes. The quality control sera are commercially prepared from Agappe Diagnostics Limited (India) and are of high grade.

3.8 URINALYSIS

Medi-Test Combi 10 strip was used.

The followings were tested using the strip – urobilinogen, glucose, bilirubin, ketones, specific gravity, occult blood, pH, protein, nitrite, and leucocytes.

3.8.1 Principles

3.8.1.1 Urobilinogen

Modified Ehrlichs reaction. Urobilinogen present react with Ehrlichs reagent to form a red coloured compound. Colour changes from light orange –pink to dark pink.

3.8.1.2 Glucose

Glucose oxidase catalysis the oxidation of glucose to form hydrogen peroxide. The hydrogen peroxide thus formed then oxidises a chromogen (4- amino phenazone) on the reaction pad by the action of peroxidase. Colour changes from white to blue or brown.

3.8.1.3 Bilirubin

Azo-coupling reaction of bilirubin with a diazonium salt in an acid medium to form an azo-dye. Colour changes from light tan to light brown or light pink.

3.8.1.4 Ketones

Legals test nitroprusside reaction. Acetoacetic acid in an alkaline medium reacts with nitroferricyanide to produce a colour change from light brown to purple.

3.8.1.5 Specific Gravity

Ionic solutes present in the urine cause protons to be released from a polyelectrolyte. As the protons are released the pH decreases and produces a colour change of brothymol blue from blue-green to yellow-green.

3.8.1.6 Occult Blood

The occult blood test is based on the pseudo-peroxidase activity of the haem moiety of haemoglobin. The chromogen is oxidised by a hydroperoxide in the presence of haem and changes colour from yellow to blue.

3.8.1.7 pH

Double indicator system. Indicators methyl red and bromothymol blue are used to give distinct colour changes from orange to green to blue (pH 5.0 to 9.0).

3.8.1.8 Protein

The urine protein test is based on the "protein error" principle of indicators. When pH is held constant by a buffer, indicator dyes release H ions because of the protein present and change colour from yellow to blue green.

3.8.1.9 Nitrite

The nitrite test is based on the diazotisation reaction of nitrite with an aromatic amine to produce a diazonium salt. It is followed by an azo-coupling reaction of this diazonium salt with an aromatic compound on the reaction pad. The azo-dye produced causes a colour change from white to pink.

3.8.1.10 Leucocytes

This test pad contains an indoxyl ester and diazonium salt. It is followed by an azo-coupling reaction of the aromatic amine formed by leucocyte estrase with a diazonium salt on the reaction pad. The azo-dye produced causes a colour change from light brown to violet.

3.8.2 Procedure

The test strip was dipped into the urine sample for not more than 1 sec. Excess urine was removed by drawing the strip along the brim of the vessel avoiding contact with the test area and onto an absorbent to remove the remaining urine. After exactly 60 sec., under a good light the strip was read by comparing it against the provided colour chart for each analytes.

3.9 STATISTICAL ANALYSIS

The data were analysed using statistical programme for the social sciences 11.0 (SPSS 11.0) for windows (SPSS inc. Chicago, 16). Anthropometric measurements, estimated creatinine clearance (eCrCl), serum electrolytes, calcium, phosphate, uric acid, urea, and creatinine obtained from the patients were compared with those of the controls using the two tailed student's t-test.

Correlation between duration on HAART and eCrCl, body mass index (BMI), serum electrolytes, calcium, phosphate, uric acid, urea and creatinine was carried out in the patients. Also correlations between BMI and eCrCl, and serum creatinine and eCrCl were carried out in both patients and controls using Pearson′s linear correlation analysis.

A p-value of equal to or less than 0.05 ($p \leq 0.05$) was considered as statistically significant.

Also the qualitative results from urinalysis between the patients and the controls were compared.

CHAPTER FOUR

4.0 RESULTS

4.1 ANALYSIS OF STUDY POPULATION

One hundred and one HIV/AIDS patients on HAART aged between 15 to 50 years were recruited for the study. These were made up of 43 males (42.57%) aged 31 to 48 years (mean age 39 years) and 58 females (57.43%) aged 19 to 48 years (mean age 32 years). The patients were also divided into three different age groups: group I (15 to 26 years, 16 patients), group II (27 to 38 years, 63 patients), group III (39 to 50 years, 22 patients). The mean duration on HAART was 19.88 ± 1.71 months.

One hundred apparently healthy subjects aged between 15 to 50 years were also recruited for the study as controls. These were made up of 68 males (68.00%) aged 20 to 45 years (mean age 26 years) and 32 females (32.00%) aged 19 to 43 years (mean age 26 years). These apparently healthy controls were also grouped into three different age groups to tally with the patients: group I (15 to 26 years, 68 subjects), group II (27 to 38 years, 26 subjects), group III (39 to 50 years, 6 subjects).

4.2 ANTHROPOMETRIC MEASUREMENTS IN HIV/AIDS PATIENTS ON HAART AND CONTROLS

Anthropometric parameters in HIV/AIDS patients on HAART and controls are presented in Table 4.1. The weight and height were significantly lower ($p<0.01$ and $p<0.001$ respectively) in patients than in controls.

The BMI was not statistically different between patients and controls ($p>0.05$), though of the patients, 8 (7.9%) are underweight, 34 (33.6%) are overweight and 14 (13.9%) have mild obesity with only 45 (44.6%) having normal weight. In the controls, 20 (20.0%) are overweight, 4 (4.0%) have mild obesity and 2 (2.0%) have moderate obesity with majority of them ie 74 (74.0%) having normal weight (table 4.2). These are based on the WHO classification (see Appendix1).

Table 4.1: Anthropometric measurements (Mean ± SEM) in Study Population.

Subjects	n	Weight (kg)	Height (m)	BMI (kg/m^2)
Patients	101	62.17±1.29	1.60±0.01	24.41± 0.46
Controls	100	66.42±0.92	1.68±0.01	23.47±0.32
p-value		<0.01	<0.001	>0.05

SEM, standard error of the mean; **n**, sample size; **BMI**, Body mass index.

Table 4.2 : Distribution of Body Mass Index (BMI) in Study Population.

Subject	n	Classification of Body Mass Index					
		Underweight	Normal weight	Overweight	Mild Obesity	Moderate Obesity	Morbid Obesity
P	101	8 (7.9%)	45 (44.6%)	34 (33.6%)	14 (13.9%)	-	-
C	100	-	74 (74.0%)	20 (20.0%)	4 (4.0%)	2 (2.0%)	-

KEY

Class of BMI	**BMI (Kg/m^2)**
Underweight	< 18.5
Normal weight	18.5 – 24.9
Overweight	25.0 – 29.9
Mild Obesity	30.0 – 34.9
Moderate Obesity	35.0 – 39.9
Morbid Obesity	≥ 40.0

4.3 SERUM ELECTROLYTES IN HIV/AIDS PATIENTS ON HAART AND CONTROLS

The concentrations of serum electrolytes in HIV/AIDS patients and apparently healthy controls are presented in Table 4.3. There are no statistically significant differences in the concentrations of serum sodium, bicarbonate and phosphorus ($p>0.05$) between the patients and controls. The differences in concentrations of serum potassium, chloride and calcium are statistically significant ($p<0.05$, $p<0.05$ and $p<0.001$ respectively) with serum potassium higher in controls and serum chloride and calcium higher in patients.

Table 4.3: Serum electrolytes (Mean ± SEM) in HIV/AIDS patients on HAART and apparently healthy controls

Subjects	N	Na+ (mmol/L)	K+ (mmol/L)	HCO_3- (mmol/L)	Cl- (mmol/L)	Ca2+ (mmol/L)	$PO4^{2-}$ (mmol/L)
Patients	101	138.97±0.43	3.83±0.05	25.28±0.26	100.02±0.42	2.62±0.03	1.21±0.03
Controls	100	138.84±0.26	3.96±0.04	25.78±0.16	98.90±0.35	2.31±0.02	1.22±0.02
p-value		>0.05	<0.05	>0.05	<0.05	<0.001	>0.05

SEM, standard error of the mean; **n,** sample size**; Na+,** Sodium; **K+,** Potassium; **HCO_3-,** Bicarbonate; **Cl-,** Chloride; **Ca2+,** Calcium; **$PO4^{2-}$,** Inorganic phosphorus.

4.4 ESTIMATED CREATININE CLEARANCE (eCrCl), SERUM CREATININE, UREA AND URIC ACID IN HIV/AIDS PATIENTS ON HAART AND CONTROLS.

Table 4.4 shows the results of estimated creatinine clearance and serum concentrations of creatinine, urea and uric acid of HIV/AIDS patients on HAART and controls. The differences in concentrations of serum creatinine, urea and uric acid were statistically significant ($p<0.01$, $p<0.01$ and $p<0.001$ respectively) with higher values of serum creatinine and urea in controls and higher value of uric acid in patients.

The differences in value of eCrCl between patients and controls were not statistically significant ($p>0.05$), though 26 (25.7%) patients had reduced eCrCl as compared to 12 (12.0%) controls. Out of the 26 (25.7 %) patients with reduced eCrCl, 19 (18.8 %) of them had eCrCl of < 90 ml/min (stage II CKD) with the remaining 7 (6.9 %) having eCrCl of < 60 ml/min (stage III CKD). None of the 12 controls had eCrCl of <80 ml/min but 11 of the patients with stage II CKD had eCrCl of <80 ml/min (Table 4.5).

Table 4.4: eCrCl, Serum Creatinine, Urea and Uric acid (Mean ± SEM) in HIV/AIDS patients on HAART and apparently healthy controls

Subjects	n	Creatinine (µmol/L)	eCrCl (ml/min)	Urea (mmol/L)	Uric acid (µmol/L)
Patients	101	65.29±2.61	121.52±4.95	4.27±0.20	258.62±8.32
Controls	100	74.46±1.33	121.04±2.62	5.02±0.09	204.00±5.35
p-value		<0.01	>0.05	<0.01	<0.001

SEM, standard error of the mean; **n**, sample size; **eCrCl,** Estimated creatinine clearance.

Table 4.5: Prevalence of CKD in study population.

Subject	n	Stages of CKD					Normal
		I	II	III	IV	V	
P	101	-	19 (18.8 %)	7 (6.9 %)	-	-	75 (74.3 %)
C	100	-	12 (12.0 %)	-	-	-	88 (88.0 %)

P, Patient; **C,** Control; **n,** sample size; **CKD,** Chronic Kidney Disease

KEY:

Stages of CKD	eCrCl (ml/min)
I	> 90
II	60 – 89
III	30 – 59
IV	15 – 29
V	< 15

4.5 RELATIONSHIP BETWEEN SERUM ELECTROLYTES AND DURATION ON HAART IN HIV/AIDS PATIENTS.

There was statistically significant positive correlation between serum potassium and duration on HAART (r=0.209, p=0.036) as shown in figure 4.1, positive and significant correlation between serum chloride and duration on HAART (r=0.232, p=0.019) as shown in figure 4.2, and also negative and significant correlation between serum phosphorus and duration on HAART (r= –0.396, p=0.000) as shown in figure 4.3. There was no correlation between serum sodium and duration on HAART (r=0.186, p=0.063), serum bicarbonate and duration on HAART (r=0.179, p=0.074), and also between serum calcium and duration on HAART (r= –0.176, p=0.078).

4.6 RELATIONSHIP BETWEEN DURATION ON HAART AND eCrCl, SERUM CREATININE, UREA AND URIC ACID IN HIV/AIDS PATIENTS.

There was no significant correlation between eCrCl and duration on HAART (r= –0.056, p=0.577), serum creatinine and duration on HAART (r=0.114, p=0.255), serum urea and duration on HAART (r= –0.097, p=0.332), and also between uric acid and duration on HAART (r= –0.151, p=0.132).

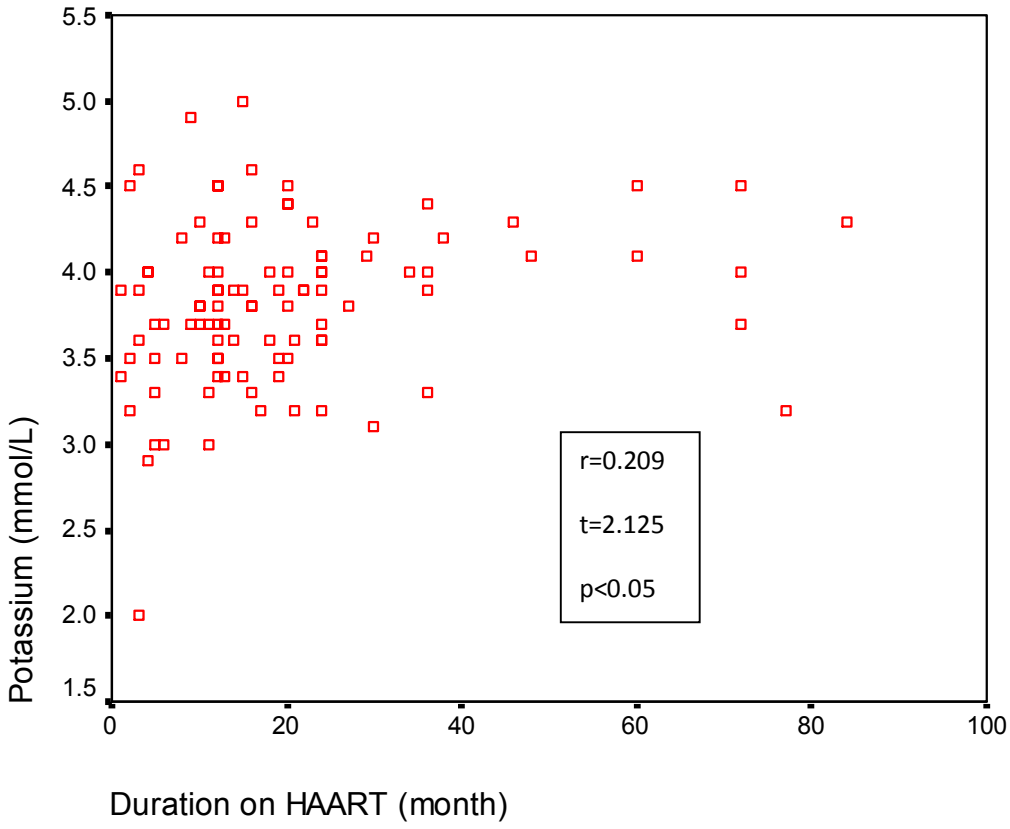


Figure 4.1. Relationship between serum potassium and duration on HAART in HIV/AIDS patients.

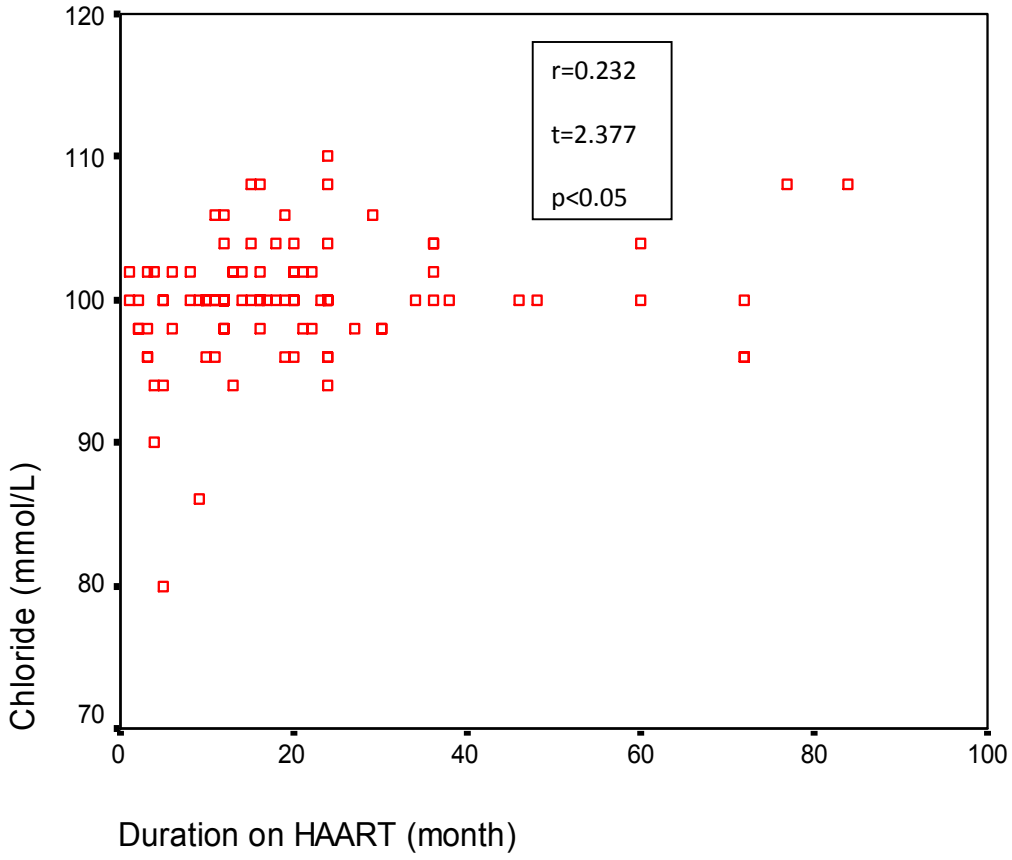


Figure 4.2. Relationship between serum chloride and duration on HAART in HIV/AIDS patients.

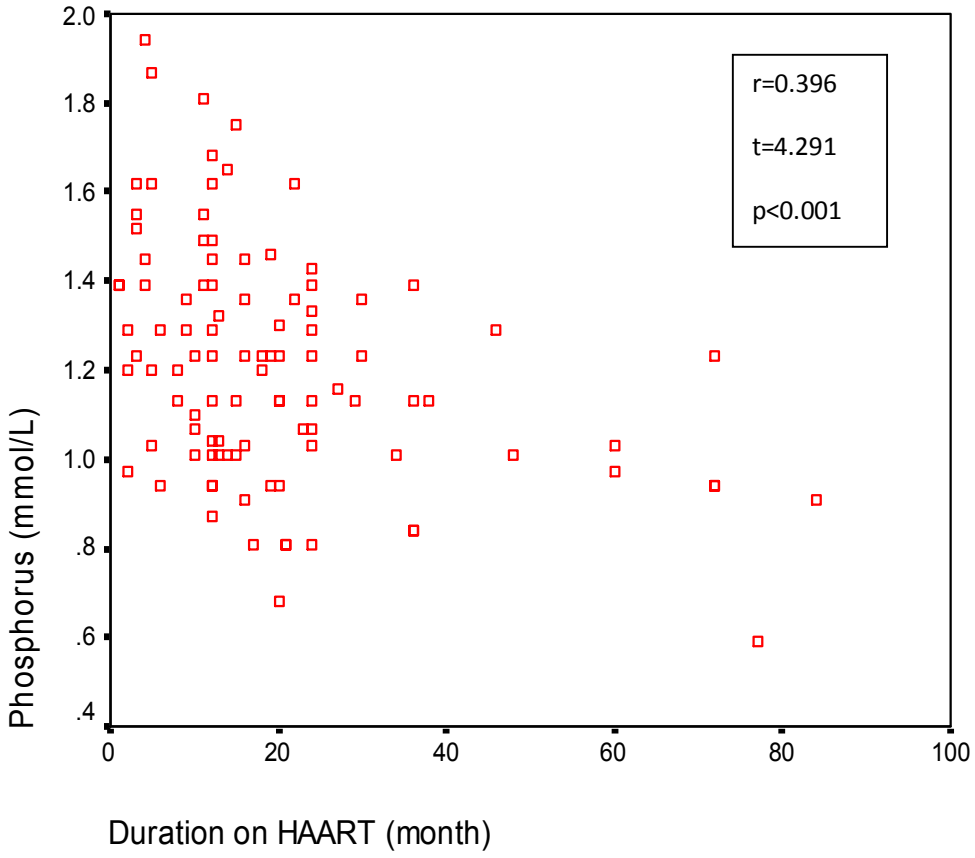


Figure 4.3. Relationship between serum phosphorus and duration on HAART in HIV/AIDS patients.

4.7 RELATIONSHIP BETWEEN DURATION ON HAART AND BODY MASS INDEX (BMI) IN HIV/AIDS PATIENTS.

There was a statistically significant positive correlation between duration on HAART and BMI (r=0.205, p=0.039) in HIV/AIDS patients as shown in figure 4.4.

4.8 RELATIONSHIP BETWEEN eCrCl AND BMI IN HIV/AIDS PATIENTS AND CONTROLS

There was a positive correlation which is statistically significant between BMI and eCrCl in both patients (r=0.273, p=0.006) (figure 4.5) and controls (r=0.416, p=0.001) (figure 4.6).

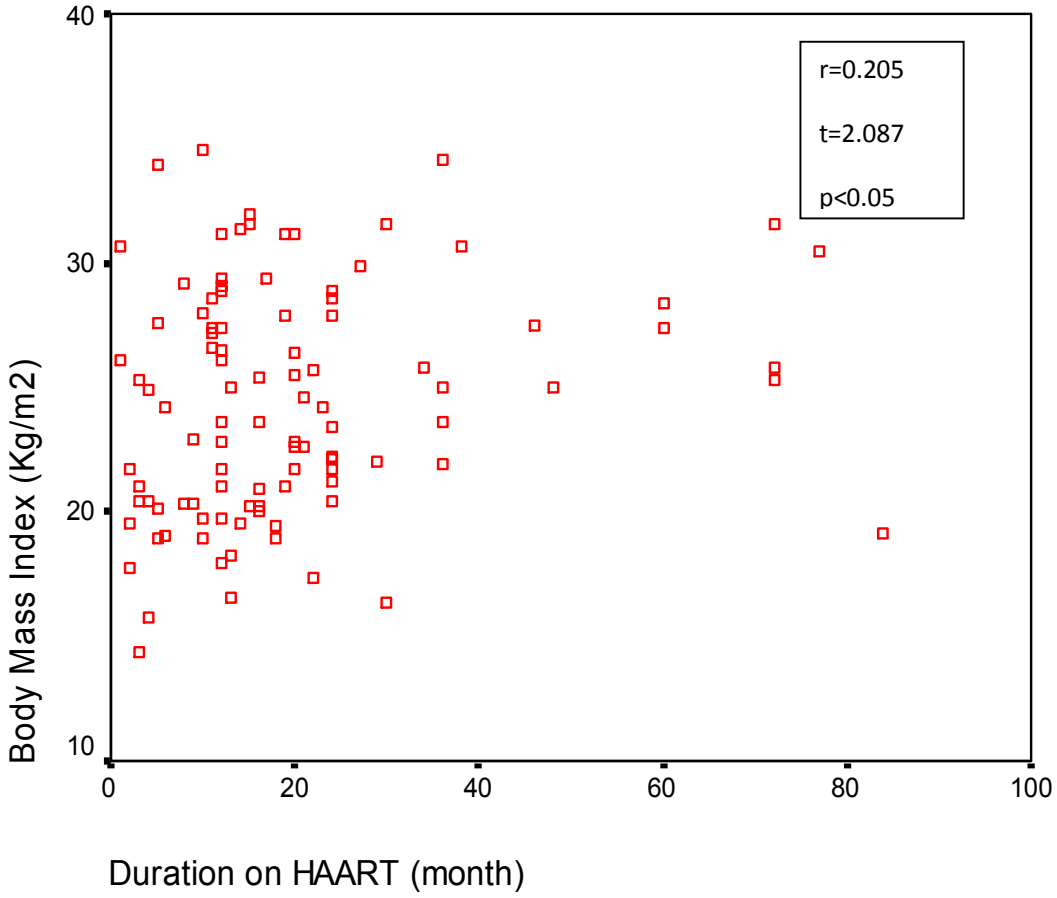


Figure 4.4. Relationship between body mass index and duration on HAART in HIV/AIDS patients.

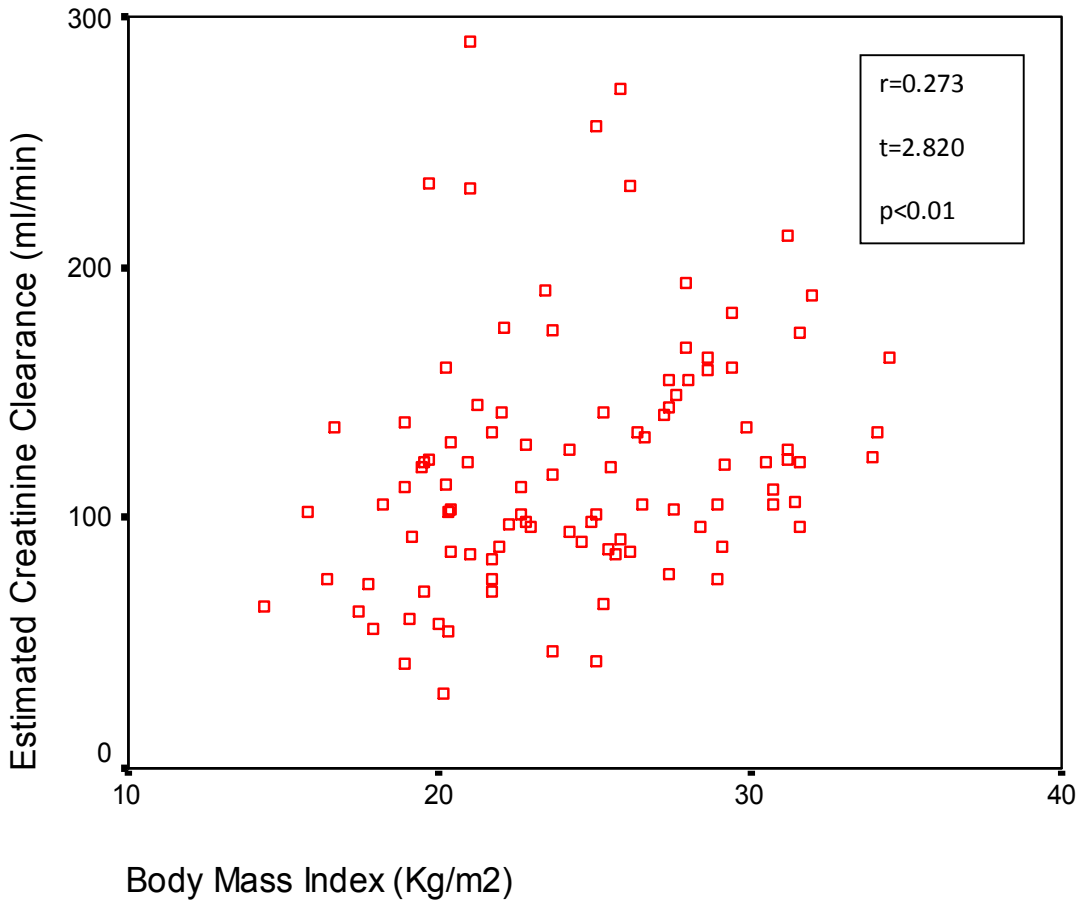


Figure 4.5. Relationship between Estimated creatinine clearance and body mass index in HIV/AIDS patients on HAART.

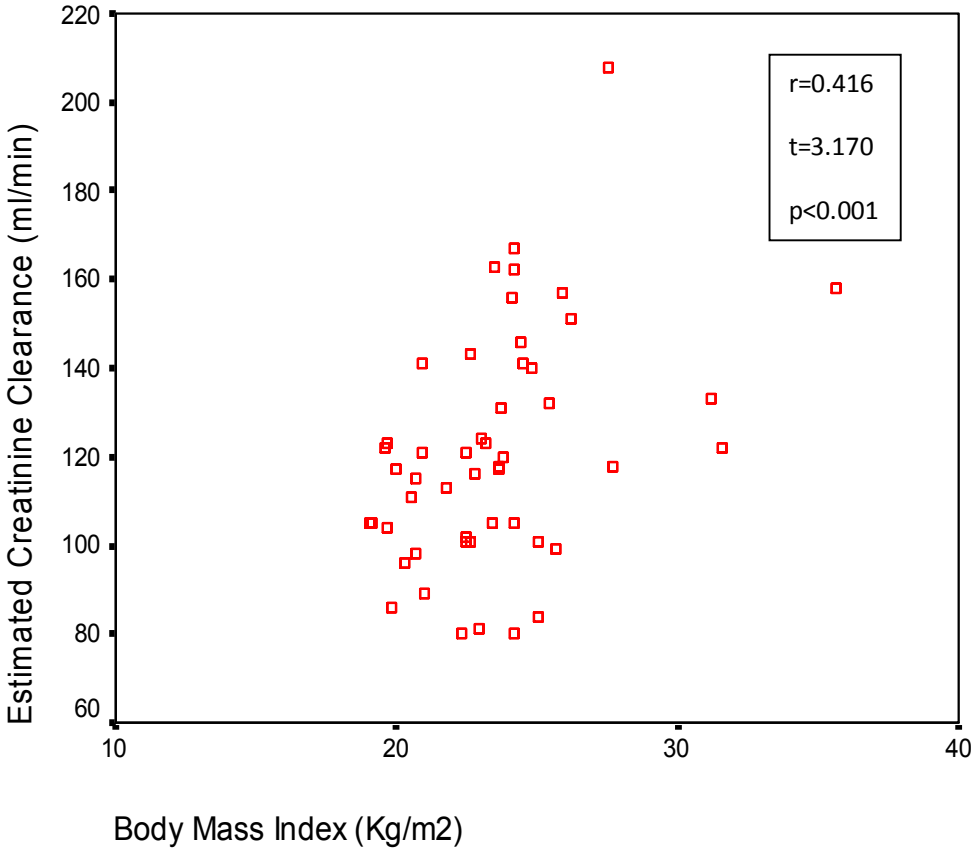


Figure 4.6. Relationship between Estimated creatinine clearance and Body mass index in apparently healthy subjects (controls).

4.9 RELATIONSHIP BETWEEN eCrCl AND SERUM CREATININE IN HIV/AIDS PATIENTS AND CONTROLS.

There was a negative correlation which is statistically significant between eCrCl and serum creatinine in both patients (r= –0.761, p=0.000) (figure 4.7) and controls (r= –0.659, p=0.000) (figure 4.8).

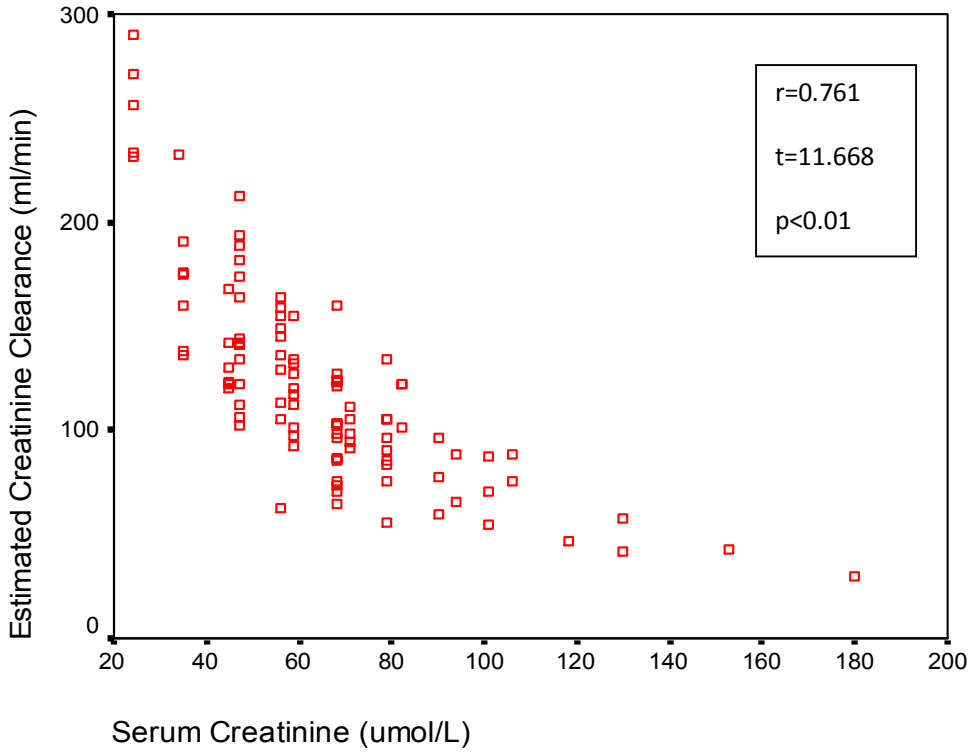


Figure 4.7. Relationship between Estimated creatinine clearance and serum creatinine in HIV/AIDS patients on HAART.

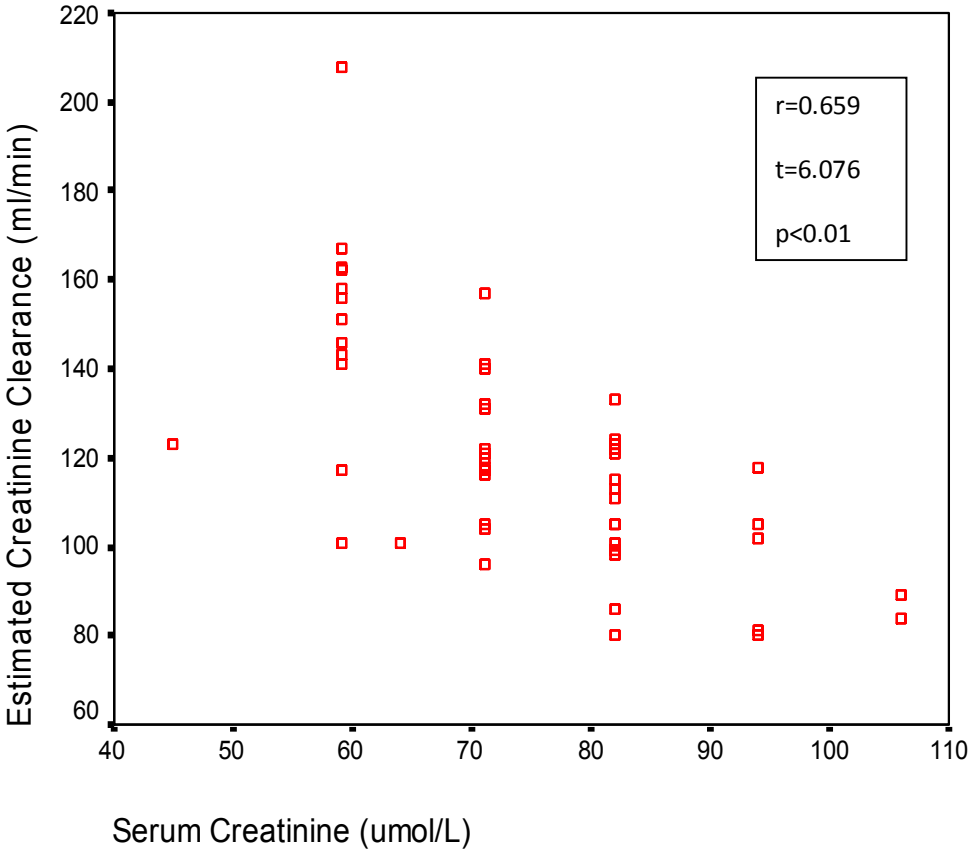


Figure 4.8. Relationship between Estimated creatinine clearance and serum creatinine in apparently healthy subjects (controls).

4.10 URINALYSIS IN THE STUDY POPULATION

Table 4.6 shows the pattern of urinalysis in HIV/AIDS patients on HAART and apparently healthy controls. The incidence of proteinuria, erythrocyturia and leukocyturia is higher in patients than in controls.

Table 4.6: Pattern of Urinalysis in study population

		Proteinuria		**Glucosuria**		**Erythrocyturia**		**Leucocyturia**		**Ketonu**	
Subject	n	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-
P	101	15	86	-	101	6	95	8	93	-	1
		(14.85%)	(85.15%)		(100%)	(5.94)	(94.06%)	(7.92%)	(92.08%)	-	(1
C	100	6	94	-	100	2	98	-	100	-	1
		(6.0%)	(94.0%)		(100%)	(2.0%)	(98.0%)		(100%)		(10

P, Patient; **C**, control; **n**, sample size; **+ve**, Positive; **-ve**, Negative.

CHAPTER FIVE

5.0 DISCUSSION

In the present study, the age distribution of patients showed that 27-38 years age group forms the predominant age group followed by 39-50 years age group. This finding is consistent with a previous observation in this environment (Babadoko, 2005), though it contradicts earlier reports in sub-saharan Africa, where 74% of those infected with HIV were in the age group 15-49 years (Fausi, 1999; Gayle and Hill, 2001). This was attributable to high rates of multiple non-marital sexual partnerships in this region. The finding in this present study may be due to the middle age group involvement in economically productive ventures, coupled with physical well being with a quest for sexual adventure. Also early marriages, which are the norms in this environment prevents early and multiple sexual exposures. The least number of patients were found in the age group 15-26 years.

Females formed the majority of the patients and constitute 57.43% of the total study group. This finding is in agreement with the increasing rate of women living with HIV globally from 25% in 1992, rose to 43% in 1999, also consistent with a similar finding in this environment of 53.2% (Babadoko, 2005). Certain traditional practices can augment this risk such as female genital cutting and polygamy (UNAIDS, 2003). Also, poverty and other socio-economic conditions served as serious driving forces for the infection in

females through increased commercialisation of sex (Federal Ministry of Health sentinel survey, 2003).

The differences in mean of body mass index (BMI) between the patients and the controls as demonstrated by this study is not statistically significant but most of the patients have abnormal BMI, with 48 (47.5%) of them being overweight to mild obesity and only 8 being underweight. This finding can be attributed to lipodystrophic (fat accumulation and fat atrophy) effects of some antiretroviral drugs, the Nucleoside Reverse Transcriptase Inhibitors (NRTIs) especially Stavudine, and to a lesser extent Zidovudine which are included in the HAART combination these patients were taking leading to weight gain and consequently increase BMI (Moro *et al*, 2007). This is supported by the finding of a positive and significant correlation between duration on HAART (mean duration on HAART was 19.88 ± 1.71 months) and BMI in this study.

The finding in this study showed that the difference in value of eCrCl between patients and controls was not statistically significant but 26 (25.7%) patients had reduced eCrCl (eCrCl of <90 ml/min), 19 (18.8%) of them had eCrCl of 60-89 ml/min (stage 2 CKD) with the remaining 7 (6.9%) having eCrCl of <60 ml/min. The finding of 6.9% of the patients having eCrCl of <60 ml/min which is consistent with stage 3 kidney diseases is in agreement with findings in other parts of the world (Gupta *et al*, 2004; Ronald *et al*, 2005; Stephen, 2005; and Chi Yuen *et al*, 2007). The finding in this study of 25.7% as

having renal insufficiency (based on eCrCl of <90 ml/min) is lower than findings of a higher prevalence of 51.8% by Agaba *et al* (2003), 55.8% (GFR <90 ml/min) by Susman (2005), 48.5% by Andia *et al* (2005) and 38.0% (based on proteinuria and/or elevated serum creatinine) by Chioma *et al* (2008). Of all these studies, only report by Ronald *et al* (2005) and Chi Yuen *et al* (2007) stated that the patients were on HAART as in the present study.

The decline in eCrCl found in 26 (25.7%) patients is likely to be as a result of invasion of the glomeruli by the HIV and not as a result of side-effect of HAART. This can be proven by the fact that all patients in the study were not on the two antiretroviral drugs implicated as causing nephrotoxicity (Tenofovir) and nephrolithiasis (Indinavir) which can lead to CKD and subsequently decreased GFR. Also, no correlation was found between duration of patients on HAART and eCrCl.

Also noteworthy is the fact that of the 26 (25.7%) patients with reduced eCrCl only 4 (4.0%) would have been detected as having renal insufficiency using serum creatinine with or without proteinuria as criteria. This finding is consistent with study by Stephen (2005) who found 10% of patients in his study having renal insufficiency as against 2% that would have been identified if serum creatinine alone were used. Therefore eCrCl proved to be more sensitive than serum creatinine in assessing GFR.

In keeping with previous reports by Klotman (1999) and Pardo *et al* (1987), a relatively high proportion of the HIV/AIDS patients on HAART had chronic renal insufficiency although the mean value of eCrCl was similar in the patients and controls. This is likely to have resulted from hyperfiltration associated with proteinuria in renal disease, as more patients (14.85 %) than controls (6.0 %) had proteinuria in this study.

A positive correlation which is statistically significant was found between BMI and eCrCl (i.e. increase in eCrCl with increasing BMI), and also a statistically significant negative correlation between serum creatinine and eCrCl (i.e. increase in serum creatinine with decreasing eCrCl) in both patients and controls, this is in agreement with established knowledge that serum creatinine usually increases with decreasing glomerular filtration rate (GFR), and also, increases with increasing weight or BMI (Cockcroft and Gault, 1976).

Concentrations of serum sodium, chloride and calcium were higher in patients than controls and serum potassium, bicarbonate, and phosphorus were lower in patients. These differences were only significant in serum potassium, chloride and calcium. This finding is consistent with the finding of lower serum concentrations of potassium, bicarbonate and phosphorus in HIV-infected patients on HAART by Izzedine and Deray (2007). However, the finding is not in agreement with finding of previous studies (Bourgoignie *et al*, 1988; Agaba *et al*, 2003) in which their findings of significantly lower serum concentrations

of sodium and bicarbonate were attributed to syndrome of inappropriate antidiuretic hormone (SIADH) secretion as a result of pulmonary tuberculosis which is a complication of HIV and present in 70% of their patients. The findings in the present study may be as a result of stability of the patients' health, absence of complication such as pulmonary tuberculosis, and also the fact that HAART has been found to lead to marked and prolonged improvements in renal function of HIV/AIDS patients (Michiel and Dominique, 2002).

Positive correlations which are statistically significant were found between duration on HAART and serum potassium and chloride (i.e. increase in serum potassium and chloride with increasing duration on HAART), and a statistically significant negative correlation between duration on HAART and serum phosphorus (i.e. decrease in serum phosphorus with increasing duration on HAART). This finding is in agreement with findings of higher serum concentration of potassium and chloride and lower serum concentration of phosphorus in HIV/AIDS patients on HAART compared to patients not on HAART (Ugwuja and Eze, 2007). The cause of this is poorly understood and thus worth further study.

In the present study serum urea and creatinine were significantly lower in patients compared with controls; this may be due to inadequate nutrition and

probably decreased muscle mass in patients as a consequence of wasting associated with HIV/AIDS.

Serum uric acid level is significantly higher in patients, this is consistent with the finding of previous studies (Walker *et al*, 2006; Izzedine and Deray, 2007; Moro *et al*, 2007). This is attributable to increased cell turnover as a result of HIV-infection (Walker *et al*, 2006) or as a result of side-effect of Stavudine which causes uric acid retention probably by decreasing renal function (Moro *et al*, 2007).

The pattern of urinalysis result in the study population shows more patients with proteinuria (14.85%), erythrocyturia (5.94%) and leukocyturia (7.92%). This finding is in agreement with a similar study by Alo *et al* (2007). The proteinuria is probably the first and one of the main presentations of nephropathy and it signifies glomerular damage. The presence of polymorphonuclear leucocytes in the urine is characteristic of inflammatory process associated mainly with pathogenic infection. All these are likely complications of either direct invasion of the kidney by the HIV or as a result of secondary bacterial or opportunistic infections.

CONCLUSION

It can be concluded from the findings of the present study that:

1. Age group 27-38 years forms the predominant age group attending antiretroviral clinic in Ahmadu Bello University Teaching Hospital (ABUTH) Zaria, with more females than males attending the clinic.

2. BMI rises with increasing duration on HAART in HIV/AIDS patients with 47.5 % of them being overweight or obese.

3. Prevalence of renal insufficiency in HIV/AIDS patients on HAART is 25.7 % with 18.8 % of them having stage 2 chronic kidney disease (eCrCl <90 ml/min) and 6.9 % having stage 3 chronic kidney disease (eCrCl <60 ml/min).

4. eCrCl rises with increasing BMI and decreases with increasing concentration of serum creatinine in both patients and controls.

5. Concentration of serum potassium is significantly lower while that of chloride and calcium were significantly higher in patients.

6. Concentrations of serum potassium and chloride rises while that of serum phosphorus decreases with advancing duration on HAART in patients.

7. Concentrations of serum creatinine and urea were significantly lower while concentration of serum uric acid is significantly higher in HIV/AIDS patients on HAART.

RECOMMENDATIONS

Considering the findings of the present study, it can be recommended that:

1. HIV/AIDS patients before being placed on a treatment regimen should be evaluated for kidney disease using a sensitive marker (eCrCl) in order to establish a baseline as both the infection and the HAART drugs can affect the renal function.

2. Renal function of HIV/AIDS patients on HAART should be closely monitored using the renal function test parameters and most especially regular assessment of GFR using eCrCl (Cockcroft and Gault equation) at least every 3 months.

3. Body Mass Index (BMI) of the patients should be monitored regularly to prevent overweight/obesity and its attendant complications by avoiding the implicating drugs (eg Stavudine) if possible.

4. Microalbuminuria should be tested for among the patients in subsequent research on this topic, this will give a better and an early index of glomerular function than the eCrCl (as eCrCl is based on accurate age and weight) and the dip stick method used for proteinuria in this study.

5. A follow-up study involving a long term monitoring of renal status of HIV/AIDS patients on specific combinations of HAART is needed to fully ascertain the effect of different combinations of HAART on renal function.

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APPENDIX I

Table 4.1b: WHO Classification of Body mass index (BMI)

BMI (Kg/m2)	Classification
>18.5	Underweight
18.5 – 24.9	Normal
25 – 29.9	Overweight
30 – 34.9	Mild obesity
35 – 39.9	Moderate obesity
≥40	Morbid obesity

(Martin A. Crook, 2006)

APPENDIX II

CONSENT FORM

Serial No. ……..

Hospital No. …….

Age …….

Consent form to participate in a study.

1. This study is about the Assessment of renal function in HIV/AIDS patients on highly active antiretroviral therapy in ABUTH. The procedure involves blood and urine tests.
2. The result from the study will be treated with strict confidentiality.
3. You are free to participate or decline in this study without any consequence.
4. The study will hopefully improve your present health status and that of others in the future.

If you desire to participate in this study kindly sign below attached form.

Thank you.

APPENDIX III

INFORMED WRITTEN CONSENT

I …….. of ……(address) agree to participate in the study of Assessment of renal function in HIV/AIDS patients on highly active antiretroviral therapy in ABUTH. The full procedure before and after the test have been explained to me.

I understand that a sample of my blood and urine will be taken for tests and that if I so wish the results will be communicated to me in confidence.

I make this consent wilfully without being subjected to any pressure.

Participants Name…… Signature……

Witness Name……. Signature……

Researchers Name…… Signature…….

APPENDIX IV

PROFORMA

S/No……..

Hosp No……

Age…… Sex……

Weight……..

Height…….. Blood pressure……

Education Primary□ Secondary□ Tertiary□

Participants' Occupation…….

Underlying medical condition(s)…….....

……

Drugs…….

When the drugs are commenced……..

RESULTS

Urea (mmol/L)……. Uric acid (µmol/L)……

Creatinine (µmol/L)…… eCrCl (ml/min)……

Electrolytes (mmol/l);-

Na^+…… K^+……. HCO_3^-……..

Cl^-…… Ca^{2+}…… PO_4^{2-}…….

Urinalysis ;-

……..

……..

……..

……

APPENDIX V

POSTGRADUATE OFFICE

FACULTY OF MEDICINE
AHMADU BELLO UNIVERSITY TEACHING HOSPITAL
SHIKA - ZARIA, NIGERIA.

P. M. B. 06 Samaru Zaria
Phone: 069 - 551399
Fax: 069 - 555001
E - mail: abuthpg@yahoo.com

Your Ref: _____

Our Ref: ABUTH/PGO/COMM/9

Date: 18 April 2008

Dr Rasheed Yusuf
Department of Chemical Pathology
Ahmadu Bello University Teaching Hospital
Zaria

Dear Sir

Re: RESEARCH PROPOSAL

Your research proposal "ASSESSMENT OF RENAL FUNCTION IN HIV/AIDS PATIENTS ON HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) IN ABUTH, SHIKA, ZARIA", refers.

Approval is hereby given for commencement of the research.

You are required to keep this office informed of the progress being made on the research.

Yours sincerely

Dr A G Bakari
Assistant Dean, Postgraduate Studies/
Residency Training Co-ordinator