SERUM ELECTROLYTE CHANGES ASSOCIATED WITH KIDNEY AND LIVER FUNCTION MARKERS IN HIV-INFECTED INDIVIDUALS ATTENDING JARAMOGI OGINGA ODINGA TEACHING AND REFERRAL HOSPITAL, KISUMU COUNTY, KENYA

BY

OPIYO WILLIAM OUMA

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MASENO UNIVERSITY

DECLARATION

This work is original and has not been presented for examination in any other university.

Date:……..Signature:……

Candidate's Name: Opiyo William Ouma

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

1. Prof. Ayub V.O. Ofulla, PhD.

   Biomedical Science & Technology Programme

   School of Public Health & Community Development

   Maseno University, Private Bag, Maseno

   Kenya.

Date:……..Signature:……

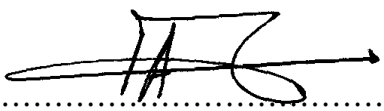
2. Dr Ng'wena G. Magak, PhD.

   Department of Medical Physiology

   Maseno University, Private Bag, Maseno

   Kenya

Date:…….Signature:……



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DEDICATION

I dedicate this work to my late parents Mrs. Risper Onyango Omuga and Mr. Benson Omuga Oloo for their vision and unrelenting patience inculcated in me the value of hard work.

ABSTRACT

Serum electrolytes disorders in HIV patients in addition to resulting from disease induced fluids losses or accumulation could be attributed to a wide range of structural defects of cellular apparatus, tissue or organs of regulation. Most routine clinical investigation of impaired serum electrolytes in HIV infection limit attribution to body fluids charges and to primary organs of regulations. Such investigations do not address the likelihood of existence of multiple regulatory organs defects, the contribution of secondary regulatory organs and of non elemental electro chemical forces in establishing observed serum electrolytes states in HIV infection. This study investigated the association of electrolytes levels with kidney and liver functions in HIV infection in order to explore the extent of contribution of the renal and gastrointestinal primary regulatory organs to existing serum electrolytes disorders in light of the extended range of HIV impact on multiple organs of electrolytes regulation. This was a hospital based cross sectional study enrolling consecutive attendants of the PSC at Jaramogi Oginga Odinga Teaching and Referral Hospital. 800 HIV-infected and 406 seronegative controls were enrolled. Biochemical analysis was done of serum levels of major electrolytes (Na+, K+ and Cl-), markers of kidney function (creatinine and urea) and liver pathology (bilirubin, albumin, total protein and enzymes) and related body fluids parameters (osmolality and pressure). Frequency counts and measures of central tendency and dispersion around normal reference values were used to assess the distribution of analytes in the population. Associations of HIV status, CD4 count, ARV use, age and gender with electrolytes and fluid parameters were tested using, t-tests and Chi-square and regression logistics (r and r^2) and significance levels assigned using $\alpha = 0.05$. Female gender, increasing age and CD4<200cells/mm^3 emerged as determinants of occurrence of kidney disorders which were observed in 54% of the seropositive individuals. HIV infection conduced significant reduction in mean eGFR (88.1mls/min v/s 95.5mls/min, t=3.1, p=0.001). Therefore creatinine and urea imbalance were more prevalent in seropositive than healthy controls (26.1% vs11.8%; p<0.0001, 4.4% vs 0.5%, p<0.0001). liver function indicators; albumin, total proteins, bilirubin and enzymes (AST and ALT), were significantly impaired in seropositive than seronegative individuals; (32.8g/l vs 34.5g/l, t=5.3,p<0.0001); 64g/l vs 67.1g/l, t=6.7,p<0.0001); (6.2vs5, t=5.7,p<0.0001) AST;45.1U/l vs 36.9U/l, t=10.3,p<0.0001 and ALT;36.5U/l vs 30.7U/l, t=7.2,p<0.0001). Serum Na+, K+ and Cl- ion imbalance were observed in 26.1%, 27.4% and 17.3% of HIV+ individuals respectively with only the prevalence of sodium imbalance being significantly more in HIV+ than HIV- individuals (26.1% vs 17.7%, χ^2 =10.6, p = 0.001). Using ARVs was accompanied with significant reduction in prevalence of Na+ ion imbalance (24.6%vs32.3%, χ^2 =3.98, p=0.046). Rates of electrolytes imbalance in HIV+ infection did not differ with or without kidney disorders and only 0.1% Na+, 0.01 % K+ and 0.3 % Cl- imbalance were attributable to kidney defects. Lower prevalence of K+ imbalance (OR=0.6, χ^2 =10.5,p=0.001; OR=0.6, χ^2 =6.7,p=0.01) were associated with albumin and total protein depletion while higher rates of Na+ (55.5%vs25.4%, χ^2 =8.3,p=0.004) and K+(38.9% vs 16.8%, χ^2 =6,p=0.01) imbalance were associated with hyperbilirubinemia. Nonetheless co-variation between electrolytes imbalance and liver markers were minimal. Thus impaired liver and kidney functions did not sufficiently explain occurrence of the multiple electrolytes imbalance in HIV infection. Therefore diagnostic and management practices of electrolytes disorders in HIV-infected individuals need to expand to include comprehensive biochemical assessment of probable causes to reach as many as the likely elemental contributors as possible.

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ABBREVIATIONS AND ACRONYMS

ECF	Extracellular fluid
LFTs	Liver function tests
KFTs	Kidney function tests
IVF	Intravascular fluid
ISF	Interstitial fluid
CNS	Central nervous system
HIV	Human immunodeficiency virus
CD-4	Cluster of differentiation -4
BUN	Blood urea nitrogen
PCV	Packed cell volume
mEq	MilliEquivalents
mmol	millimoles
GIT	Gastrointestinal tract
GFR	Glomerular filtration rate
EPO	Erythropoietin
SG	Specific gravity
WHO	World health organization
KAIS	Kenya AIDS indicator survey
CDC	Center for disease control and prevention
F&E	Fluids and electrolytes
eGFR	Estimated glomerular filtration rates
LEE	Liver enzyme elevation

DEFINITION OF TERMS

AIDS Acquired immunodeficiency syndrome, a stage in HIV natural history characterized with depletion of CD4 lymphocyte count below 200 cells/µl.

Albuminuria Presence of albumin proteins in urine

Anion gap The difference between concentrations of ignored cations and ignored anions in body fluids. Normal anion gap range is 9-12meq

Alkalosis Abnormally elevated body fluid pH. Normal blood pH is 7.35-7.45. Alkalosis exists when blood pH levels exceeds 7.45

Fanconi syndrome A disease of the proximal renal tubules of the kidney in which glucose, amino acids, uric acid, phosphate and bicarbonate are passed into urine, instead of being reabsorbed

Glycosuria Presence of glucose in urine

Hyponatremia Abnormally low serum sodium levels, below 135mEq/L. Sodium reference values range between 135-145 mEq/L.

Hypernatremia Abnormally raised serum sodium levels above 145mEq/L.

Hypokalemia Abnormally low serum potassium levels, below 3.8mEq/L. Potassium reference values range between 3.8-5.0mEq/L

Hyperkalemia Abnormally elevated serum potassium levels above 5.0mEq/L

Hematocrit The fraction of whole blood in percentage occupied by formed elements. Normal levels are; men 40-45%; women 37-47%; Children 1-14years (32-43%)

Homeokinesis	The physiological process by which the internal systems of the body are maintained at equilibrium, despite variations in the external environment
Humoral	Secretory elements circulating in the bloodstream and other fluid compartments of the body
Hypoxia	Diminished partial pressure of arterial oxygen below normal levels
Metabolic acidosis	Lowering of pH of body fluids attributable to over production of non-volatile acids. This exists when pH falls below 7.35
Respiratory acidosis	Lowering of pH of body fluids attributable to respiratory failure
Nephropathies	Diseases and disorders afflicting the kidney
Non electrolyte	These are mainly organic macromolecules such as proteins dissolved or suspended in various body fluids
Na+/H+ exchanger	Trans-membrane anti-port transport protein molecule resident on the luminal tubular epithelial membrane that transports sodium ions and hydrogen ions in opposing directions
Osmolarity	The aggregate concentration of solutes dissolved in body fluids (290 mosm/L)
Proteinuria	Presence of proteins in urine
Pre-renal/extra-renal organs	Organs with a role in regulation of body fluids and electrolytes balance and whose regulatory roles impact on fluid and electrolytes levels independent of the influence of the role of kidneys in regulating these parameters

Steady state	The optimal level or quantity of physiologic or biochemical parameters that is essential and sufficient to sustain normal biological processes
Uremia	The presence of excessive amounts of urea and other nitrogenous waste compounds in the body
Ultrafiltrate	The portion of plasma filtered through the glomerular filtration barrier into the Bowman's space

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CHAPTER ONE: GENERAL INTRODUCTION

1.1. Background Information

The role of regulation of body fluids and electrolyte (F&E) parameters is undertaken concertedly by several organs employing various mechanisms which are divergent at their onset but convergent at the end. This physiologic regulatory model constrains periodic oscillatory deviations of levels of vital parameters thereby preventing wide fluctuations from the normal steady state levels (Boron, 2005). Designing multiple mechanisms to control one parameter (homeostatic redundancy) is therefore a common theme around such vital parameters whose alterations are sensitively responded to by other organs and systems and whose malfunction can precipitate a cascade of deleterious malfunctions in other systems of the body (Moore, *et al.*, 1952).

Within the body's fluid compartments, besides the quantitative levels of individual inorganic and organic substrates, there are several vital fluid parameters dependent on electrolyte levels such as blood volume, blood pressure, plasma and urine osmolality, urine specific gravity, and plasma and urine pH (Krupp, 1990). The dynamic steady states of these essential parameters though intimately linked, are regulated by complex and different homeostatic pathways anchored principally on the respiratory system, the renal system, the gastrointestinal system and the neuro-endocrine system (Glenn and Carol, 2007). These organs occupy unique loci in various homeostatic loops, all of whose design compose of receptors as entry points linked by sensory pathways, to the central nervous system which in turn is linked to effector entities by effector pathways.

Prevailing plasma fluid and electrolytes content is a consequence of the balance between individual and cumulative fluid and electrolytes load added to this compartment by input regulatory organs in relation to fluid and electrolytes load removed from it by excretory regulatory organs. The principal excretory organ for fluid and electrolytes from plasma is the kidney, while majority of the other regulatory organs contribute to plasma fluid and electrolyte load balance by replenishing its content and in some instances by serving as out put routes. As such between plasma and the exterior environment exchange of fluids and electrolytes occur at two functionally distinct interfaces which have been defined as; (a) the renal fluid and electrolytes regulatory interface (zone) in which the prominent interface organ is the kidney and; (b) the pre-renal (extra-renal) fluid and electrolytes regulatory zone functionally composed of other organs

Generally infectious diseases that interfere with fluids and electrolytes (F&E) balance affect specific organs in isolation and thereby alter only the affected organ's contribution to the regulation mechanism (Macleod, 1977). However, even though fluid, electrolyte and mineral perturbations are features of such diseases, these electrolyte and mineral perturbations are transient and quickly resolve when the offending disease is controlled (Sitprija, 2008). Routinely, analysis of electrolytes disorders in such transient episodes of disease and of electrolytes balance impairment, involves point- of care analysis of the abberated electrolytes and of the possible regulatory organ (often the kidneys) whose functional performance has occasioned the electrolyte perturbations (Smellie, 2007).

Investigating electrolytes disorders associated with these diseases therefore involve assessment of the magnitude of deviation of the electrolytes in serum prior to ascertaining the underlying clinical episodes that occasion these perturbations (Mount, 2013). Often the clinical events are fluids overload or depletion due to vomiting, diarrhea or renal insufficiency. Given the brief nature of these events, investigations centered on the actual organ structural defects leading to reduced or loss of organ performance that in turn results in the observed electrolytes disorders are rarely undertaken. Due to the selective impact of these diseases on organs regulating electrolytes levels, detected electrolytes disorders are routinely in clinical set up associated with the clinical episodes that are the immediate causes (fluids changes) of the electrolytes defects and with organs whose defects may have precipitated the body fluids changes. Assumptions of association between electrolytes imbalance and organ functions at point of care are often reached on the basis of matching impaired electrolytes levels to observed impaired levels of serum markers of organ function. Thus without further evidence attesting to the strength of the association and neither the magnitude of contribution of individual organs being taken into account.

HIV, contrary to these diseases can induce widespread pathology in different organ systems of the body including those responsible for electrolytes regulation. In addition it is a chronic disease whose impact on organs of electrolytes regulation is prolonged and sustained, with the type and extent of organ afflicted changing with advancing disease (Perazzela and Brown, 1994). The virus has been reported to impair functions of the central nervous system (CNS), the gastro-intestinal tract (GIT), the cardiovascular system, the genito-urinary system, the skin and the respiratory systems all of which are essential in regulating fluid and electrolyte levels

(Kaplan, *et al.,* 1987). Various pathological effects of HIV infection and antiretroviral drugs on several organs including the liver, the lungs, the gastrointestinal tract (GIT), the adrenal glands and the kidneys have been linked to body fluids volume losses and electrolyte alterations (Berggren and Batuman, 2005). Peter (1991) reported the prevalence of hyponatremia in HIV patients as 28.4%, of hypokalemia at 17.3% and hyperkalemia at 4.9%. Manfro, *et al.,* (1993) on the other hand reported the prevalence of hyponatremia to be 45%, hypokalemia to be 23.1% and metabolic acidosis to be 20.1% in HIV patients at admission

HIV- infected individuals are therefore prone to defects of multiple regulatory organs, and as a consequence multiple electrolytes disorders (Hollander, 1987). Despite these various possible outcomes associated with HIV on regulatory organs, point of care investigations of electrolytes disorders in HIV-infected individuals proceed with the routine practice of associating observed electrolytes changes to underlying immediate clinical episodes (diarrhea or vomiting or fever) and to observed aberrations of levels of pathological markers of functions of organs of the gastrointestinal system (GIT) and renal system considered to be primarily responsible for the fluids alterations associated with the observed electrolytes disorders. Equally, most studies report electrolytes disorders in isolation often without relating them to the possible range of contributing organ defects. These assumptions are often based on matching the observed imbalance in levels of electrolytes to levels of existing pathological markers of organ function without taking into account the magnitude of contribution of each organ and of the fact that HIV can induce pathology beyond these usual suspect organs. Due to the prolonged sustained assault by the HIV virus and opportunistic infections associated with it on a range of organs of regulation, such investigations do not consider the likelihood of

existence of a wider range of organs as sources of electrolytes defects in HIV infection and the magnitude that each contributes to observed electrolytes defects. This approach therefore does not probe the multiple organ involvement in HIV infection and essentially does not demonstrate the holistic or overall pattern of the spectrum of fluid and electrolyte imbalances existing in HIV patients.

Various factors can influence the outcomes and pattern of fluids and electrolyte imbalances following HIV infection. These include infection related factors such as the viral strain, viral load and CD4 lymphocyte cell depletion (Alcamo, 2002). Treatment factors such as types of anti-retroviral drugs (ARVs) used could also modify fluid and electrolyte imbalances in HIV infection (Butera, 2005). On the other hand patient factors such as gender, genetic variability and age, can also determine the extent, pattern, and form of final fluid and electrolyte imbalances that develops due to HIV infection (Kimmel, *et al.*, 2003).

The interaction of these factors and their ultimate impact depend to a large extent on the level of intervention availed to HIV patient in institutions of care. Reports concerning the association or impact of these factors on HIV infection and its related outcomes are based on the HIV populations from the Western world endowed with advanced resources for managing the disease. There is scarce knowledge concerning the impact of these factors in resource poor settings witnessed in the developing societies such as is in western Kenya.

Physiologically, cellular mechanisms based on membrane channels and pumps employ electrochemical diffusion forces to couple the distribution of electrolytes in order to attain

compartment specific electrolytes concentrations (McDonough, 2010). Sustained injury to cellular moieties decouples these distribution events and may lead to prolonged skewed electrolytes compartmentalization which in turn constrains the influence of the electrochemical forces essential for determining the overall and specific body fluids electrolytes concentrations that arises from the closely coordinated physiological distribution of electrolytes in body fluids compartments (Kaplan, *et al.*, 1996). Most studies and routine clinical electrolytes assays portray the occurrence of individual electrolytes disorders as independent events without establishing the possible range of sources of imbalance and the magnitude to which each source contributes to the existing electrolytes defects such as the subtle electrochemical forces reliant on the electrical and chemical gradients to move ions between the body fluids compartments.

1.2. Problem Statement

Most diseases transiently perturb electrolytes balance via fluids overload or depletion, and rarely by way of actual structural damage to underlying organs of electrolytes regulation. HIV has been reported to not only have the ability to induce changes in body fluids which influence electrolytes levels but in addition, the virus can directly or indirectly assault the parenchymal structure of several organs of electrolytes regulation. Furthermore due to its chronic nature the extent of the damage and the type of organs damaged vary with progression of the disease. As such sources of electrolytes imbalance in HIV infection can be multiple and not limited to effect of disease on body fluid volume.

Routine clinical investigations that peg electrolytes imbalance to the immediate clinical causes of fluids overload or depletion and to the frequently altered kidney function or gastrointestinal function may suffice for the assessment and management of electrolytes disorders that are transient in nature in transient diseases. However such investigation may not sufficiently explore electrolytes imbalance in a chronic disease with actual organic injury as HIV does. Studies limited to highlighting the prevalence of electrolytes disorders in HIV likewise do not explore exhaustively the contribution of underlying structural damage of organs to the existing electrolytes imbalance. These practices do not explore the fact that in HIV infection; the individual is prone to multiple electrolytes regulatory organ defects, existence of which may imply that individual electrolytes disorders may result from multiple sources. They also do not explore the possibility of co–occurrence of multiple electrolytes imbalance and the magnitude that each of the defective organs contributes to the observed electrolytes disorders in HIV infection.

Demographic, treatment and disease factors can modify the impact of the HIV virus on the regulatory organs' health. These factors are not adequately explored in studies that highlight the prevalence of electrolytes imbalance in isolation and in routine clinical investigations that are suited for assessing transient electrolytes perturbations. Chronically skewed distribution of electrolytes concentrations in the extracellular fluid compartment resulting from chronic electrolytes imbalance may alter bulk charge distribution between the two major fluids compartments (extracellular and intracellular fluids). Bulk charge distribution is an essential factor utilized by the physiological electrolytes coupling mechanisms which redistribute electrolytes between body fluids compartments using electrochemical forces to attain

compartment specific electrolytes concentrations. The existence and extent of contribution of these secondary influences to electrolytes levels in serum are not explored in routine investigation. Similarly, these secondary infections are not espoused in studies centered on associating observed serum electrolytes disorders based on underlying immediate fluid depletion or overload, or to pathological markers of GIT or renal organs routinely associated with impaired electrolytes levels.

1.3. Objectives

1.4. Broad Objective

To assess the association between serum electrolytes levels and kidney and liver function markers in HIV infection in adult patients attending Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH), western Kenya.

1.4.1. Specific Objectives

i. To determine serum levels of kidney and liver function markers (BUN, creatinine, protein, albumin, liver enzymes and bilirubin) in HIV-infected individuals and HIV negative persons attending Patient Support Center of JOOTRH,

ii. To determine levels of serum inorganic electrolytes (sodium, chloride, and potassium) in HIV-infected individuals and HIV negative persons attending Patient Support Center of JOOTRH,

iii. To investigate the magnitude of serum electrolytes alterations associated with levels of pathological markers of kidney and liver function and the influence of CD4+ lymphocyte levels, gender, age and anti-retroviral drugs use on the relationship of both variables in HIV-infected individuals

iv. To define the concurrent variations in distribution of the major serum electrolytes (Na+, K+, Cl-) in seropositive individuals

1.5.Research Questions

i. What are the levels of markers of kidney and liver function in HIV-infected and HIV negative patients?

ii. What are the levels of inorganic electrolytes, in HIV-infected and HIV negative patients?

iii. What is the association between markers of kidney and liver function and levels serum electrolytes in HIV patients as disease progresses through different CD4 cell levels and the impact on this association by ARV use?

iv. Which serum electrolytes exhibit concurrent variations within body fluids in HIV infected individuals?

1.6. Significance of the Study

The prevalence of HIV among adult Kenyans gas been reported as 7.4% (KNBS&KF Marco, 2010) translating into 1.4 million persons living with HIV/AIDS. There are regional disparities with Nyanza 15.3%, Nairobi 9%, Coast 7.9% and Rift valley 7% and the least affected being the North Eastern region (KAIS, 2012). HIV infection impacts on the longevity and quality of life and therefore good care of persons afflicted with the disease should be multi pronged. Attention to the management of the impact of HIV infection on body fluids and electrolytes is one such approach since if not attended to appropriately, subjects the

patient to several morbid conditions including enhancing the likelihood of mortality (Manfro, *et al.,* 1993).

In Africa, various socio economic factors which impinge on systems of health care delivery are likely to influence endemic HIV induced opportunistic infections and morbidities that can result in patterns of fluid and electrolyte disorders that are uniquely different from other settings (Cohen and Kimmel, 2007). Therefore it is prudent to establish knowledge concerning the status of fluid and electrolytes in HIV patients. This would facilitate identification of risk factors associated with their disorders and to design effective management strategies for precluding complications arising from their defects. By highlighting the prevalence of disorders of hydration and electrolytes among local HIV populations, the study addressed the foregoing knowledge gap.

The current study quantified the burden of electrolytes, kidney and liver disorders in the HIV population in Kisumu County western Kenya, a finding which points at an existing health care need. By highlighting the most common forms of fluid and electrolytes imbalances in HIV patients attending Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH) in Kisumu County, the study has pointed a health need in this group. The study also has shown that routine clinical practices may be falling short of elucidating all probable causes of serum electrolytes disorders observed in HIV patients. This was indicative of the need to overhaul the management of electrolytes and body fluids disorders in this group of patients beginning with a reassessment of the modalities of diagnosing these defects.

1.7. Delimitations/scope of the Study

- Whereas there are other methods of assessing the immune status of HIV patients, this study relied only on the CD4 cell count
- There are several indicators of kidney health and function but the current study relied on serum creatinine and urea levels and the Cockroft –Gault formula for calculating eGFR
- Liver function has several markers but the current study used liver enzymes, total protein, albumin and bilirubin levels only.
- The study focused on HIV patients registered and attending the Patient Support Center at the Jaramogi Oginga Odinga Teaching and Referral Hospital.

CHAPTER TWO: LITERATURE REVIEW

2.1. The Epidemiology of HIV-infection

The spread of HIV pandemic in the last two decades has shown disparity between the developed and developing nations, with decline of the incidence of AIDS mainly in Western societies, due to advances in research, diagnosis and treatment of HIV infection (Cock, 2000). On the other hand, with approximately 10% of the world's population, sub Saharan Africa accounts for two thirds of the world's HIV infections (Cohen and Kimmel, 2007). Lack of adequate resources for management of HIV infection and for research tailored to the mode of pathophysiology of the disease in indigenous African populations in order to delineate underlying risk factors both genetic and environmental, are largely responsible for the state of HIV infection in the African context (Cock, 2000). Adult HIV prevalence in Kenya, Tanzania, and Uganda are 6.3%, 6.2%, and 5.4%, respectively (USAID, 2011). However, hotspots within countries are common and prevalence rates can vary as much as 15-fold across different provinces in Kenya for instance, from 0.9% in North Eastern province to 13.9% in Nyanza province (USAID, 2011).

2.2. Pathogenesis of HIV- infection

Human immunodeficiency virus infection is a contagious chronic disease acquired through sexual intercourse, contact with infected fluids and through vertical transmission from mother to child (Ugwuja and Eze, 2006). The natural history of HIV infection is composed of a prolonged incubation period, a prodromal period and finally the stage of full blown illness referred to as AIDS. Progression of HIV infection is characterized by progressive depletion of CD4-lymphocytes, and therefore CD4+ lymphocyte count is a particularly preferred indicator

for monitoring and determining the clinical spectrum of the disease from incubation, through prodromal to AIDS stages (Schoub, 1994). Based on CD4+ cell count, the magnitude of immune damage and illness caused by the HIV virus has been classified into three categories as follows; stage 1 HIV infection is associated with CD-4 cell count of 500 cells/mm^3 or more, while stage 2 is associated with a CD4+ count of 200 to 499 cells/mm^3 and stage 3 with a CD4+ count of 200 cells/mm^3 and below (CDC, 1993). Alcamo (2002) defined AIDS as being the stage when CD4+ cells' count is below 200 cells per cubic millimeter of blood. At this point the immune system is defective and immune protection deficient and victims suffer from frequent opportunistic infections.

2.3. Effects of HIV on Organs Regulating Fluids and Electrolytes Balance

Human immunodeficiency virus infection and its associated opportunistic infections and morbidities as opposed to other diseases, expose the body to several concomitant health risk factors that can adversely affect all or several of the organs in systems that play some role in homeostasis of fluid and electrolytes balance as the disease progresses (Hollander, 1987). The virus is known to cause pathology in and interfere with the functions of the central nervous system (CNS), the gastro-intestinal tract (GIT), the cardiovascular system, the genito-urinary system, the skin and the respiratory systems all of which are essential in regulating fluid and electrolyte levels (Kaplan, *et al.,* 1987). This multi-organ involvement implies that the HIV virus in effect can interfere with several safeguards designed to cushion periodic changes in fluid and electrolytes balance, leaving the body vulnerable when it is unable to marshal all available homeostatic pathways in order to effectively respond to such challenges (see Fig 2.1).

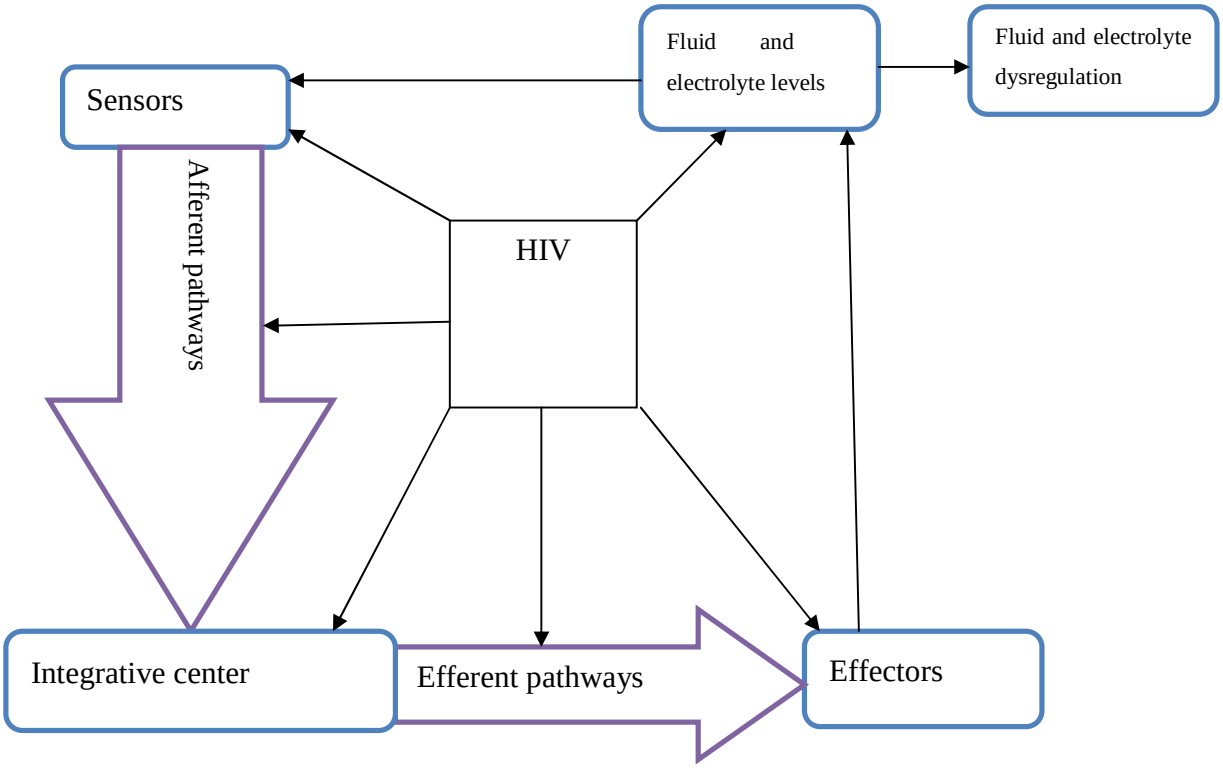


Figure 2.1: HIV Targets in the System Regulating Fluid and Electrolytes Levels

Glatt and colleagues (1988) similarly noted that certain common pulmonary and intracranial diseases in HIV patients have been associated with fluid and electrolyte imbalances. Sande and Volberding (1997) also reported that antiretroviral drugs and the HIV virus have been found to be associated with disorders of the kidneys, liver and cardiovascular systems which occupy significant positions within various homeostatic loops that regulate some of the intravascular parameters. The advent of HIV infection has therefore occasioned unprecedented challenge to the well established multiple pathway approach to defense against aberration of fluid and electrolyte (F & E) balance. This is because HIV infection can break

the homeostatic loop at any point or at multiple points by affecting the performance of physiologic sensors, relay pathways to and from the central nervous system (CNS), the integrative centers and the effector organs (Kaplan, *et al.*, 1987). Intrinsic and external factors constantly influence and challenge the functional viability of various pathways of fluid and electrolyte homeostatic balance and the range of output delivered at the end. Failure initiated at any point in the system manifests as dysregulation of the concerned parameter (Ganong, 2005). Effectively therefore, in HIV-infected individuals multiple electrolytes regulatory organs are prone to developing defective performance either concurrently or at different stages of the disease. Thus in HIV infection sources of electrolytes disorders can be multiple and can vary between different stages of the disease. Besides documenting the organs whose pathology possibly lead to the occurrence of fluids and electrolytes disorders, these studies fail to explore the likelihood of occurrence of multiple contributors of observed electrolytes disorders and quantify the magnitude of fluids and electrolytes disorders attributable to each of the likely sources of imbalance. This is essential for prioritizing intervention strategies which provide maximum impact to afflicted individuals.

2.4 Kidney Function in HIV Infection

Proportionately, the role of kidney in stabilizing levels of fluid and electrolyte parameters in blood supersedes that of other organs. This is because molecular specializations within kidney nephrons confer to it the unique property of being the organ whose functional capacity can periodically be altered by a diverse range of signals from, endocrine, paracrine, autocrine, and neuronal signals (Seldin and Giebisch, 2000). Furthermore, it is the organ to which effector signals emanating from the various homeostatic pathways in response to changes in fluid and

electrolyte load in plasma ultimately terminate. Subsequently by tailoring the kidney's performance to serum fluid and electrolytes load, physiologic processes periodically offset imbalances in plasma by correlating the load of fluid and electrolytes discharged in urine to changes in levels of these parameters in plasma (Kumar and Clark, 2005). Thus urine load is mainly predetermined and correlated to levels of plasma fluid and electrolytes, with the kidney serving as the site where the load to retain and that to offload are finely calibrated in normal health (Fray and Goodman, 2000).

These harmonious oscillations of urine and plasma fluid and electrolyte loads can be retained in disease states which spare kidneys despite having deleterious effects on other organs important to fluid and electrolytes balance (extra-renal anomalies) (Braam, *et al.*,1994). This is because in such instances, kidneys' performance flexibility remains intact and therefore they continue to match urine load to disease induced changes in plasma fluid and electrolyte levels. However, diseases that directly affect the kidneys by injuring renal parenchyma, will either dampen or render the kidneys insensitive to incoming effector signals thereby leading to loss of performance flexibility (Hakim and Lazarus, 1988). Renal diseases can also damage molecular moieties that drive the subcellular regulatory activities thereby leading either to retention of electrolytes whose only path of exit from the body is the kidney (creatinine) or lead to leakage of electrolytes when reabsorptive mechanisms stall (proteinuria, glycosuria) (Giebisch and Windhager, 2005).

The common feature among the various HIV induced kidney conditions that have been described is that they damage kidney parenchyma (Szczech, 2002). Studies have shown that

the HIV virus causes renal injury via various mechanisms. It has been reported that HIV-1 infects lymphocytes and macrophages which upon entry into the kidney might release inflammatory lymphokines or cytokines thereby causing renal injury (Kinter, *et al.,* 2000). Furthermore, the HIV-1 proteins may also directly injure renal parenchymal cells. Available evidence has shown direct HIV-1 infection of kidney parenchymal cells which is a potential cause of cytopathic effects including proliferation and/or apoptosis (Conaldi, *et al.,* 1998). Both HIV-1 protein and nucleic acid have been detected in podocytes and tubular epithelium by the use of sensitive *in situ* hybridization techniques (Kimmel, *et al.,* 1993; Bruggeman, *et al.,* 2000). Therefore HIV-associated kidney diseases have emerged as major outcomes of direct viral infection and/or anti-retroviral as well as non-ARVs drug toxicities (Kimmel, 2000). There are mainly three types of chronic kidney diseases caused by HIV infection; HIV-associated thrombotic microangiopathies, HIV immune-mediated renal diseases, and classic HIV-associated nephropathy (Weiner, *et al.,* 2003).

Destruction of kidney parenchyma of any form can lead to functional impairment which in turn can augment the dysregulatory effects of the various HIV co-morbidities on the physiology of fluid and electrolyte parameters (Glassock, *et al.,* 1990). Normal glomerular filtration rate coupled with carefully regulated secretion and reabsorption within the kidney nephron, determine the amount of electrolytes and fluids in plasma to meet demand and offset fluctuations occasioned by extrinsic and intrinsic causes (Cockcroft and Gault, 1976). Impaired kidney function can emanate from pathological events at any of these functional sites of the nephron as expounded in the sub-sections that follow.

2.5. Glomerular Diseases in HIV Infection

The glomeruli provide a large surface (glomerular filtration barrier) across which filtration occurs. This ultrafiltration separates plasma water and crystalloids from blood cells and protein macromolecules, which remain in the glomerular circulation (Hunley and Ichikawa, 2009). Out of total renal plasma flow (RPF) of 600ml per minute, glomerular filtration rate (GFR) forms at the rate of 125mls per minute when all the nephrone are functioning in parallel (Giebisch & Windhager, 2005). This translates to filtering the entire extracellular fluid volume through the nephrons 10 times a day for scrutiny.

Evidence has been adduced showing that HIV-1 virus replicates in the kidneys and targets the glomeruli cells which get injured in the process (Salifu, *et al*., 2009). Injury to the glomerulus may breach the integrity of the glomerular filtration barrier resulting in total collapse of whole glomeruli units or partial loss of function of the affected glomeruli (Giebisch and Windhager, 2005). In the former, a whole nephrone is lost and it no longer contributes to filtration and urine formation in that kidney. In the later, partial loss of function may be attributed to loss of permselectivity of the filtration barrier due to loss of its overall negative charge. It manifests as reduced glomerular filtration rate with or without anomalous solute sieving abilities resulting in abnormally high levels of certain solutes in glomerular filtrate or appearance in the filtrate of solutes not normally filtered into it (Giebisch and Windhager, 2005). Han, *et al*., (2006) demonstrated that diseases that induce glomerulonephritis lead to loss of the overall negative charge of the glomeruli filtration barrier and enhance the passage of hitherto restricted negatively charged molecules such as albumins resulting in albuminuria. In their

study, (Han, *et al.*, 2006) found that 6% of HIV patients displayed persistent proteins in urine while 36% had microalbuminuria.

Glomerular diseases that destroy whole or portions of the capillary tuft as seen in sclerosing glomerulonephritis also have direct impact on the glomerular filtration rate (GFR) by reducing the net capillary surface area available for ultrafiltration (Valeri, *et al.*, 1996). This is because the amount of fluid filtered through the glomerular capillaries as in any other capillaries depends on the net surface area of the capillaries perfused or available for filtration to occur through, in addition to hydraulic conductivity and the Starling forces (Giebisch and Windhager, 2005) as depicted by the formula that follows:

GFR (glomerular filtration rate) = Kf . [(PGC – PBS) - (πGC – πBS)] where [(PGC – PBS) – (πGC – πBS)] = PUF.

PGC is the hydrostatic pressure in the glomerular capillary favouring ultrafiltration, PBS is the hydrostatic pressure in Bowman's space opposing ultrafiltration, πGC is the oncotic pressure in the glomerular capillary opposing ultrafiltration, and πBS is the oncotic pressure in the Bowman's space favouring ultrafiltration. Kf, is the ultrafiltration coefficient, and PUF, is the net driving force favouring ultrafiltration (Adopted from Boron & Boulpaep: Medical Physiology: Philadelphia Elsevier Saunders, pp. 763, 2005).

According to the formula, when the net capillary surface area reduces due to collapse and reduction of the number of viable glomeruli capillaries, the glomerular filtration rate (GFR) reduces and in the long run this leads to accumulation of constituents such as creatinine and

electrolytes in the extracellular fluid (ECF) compartment. Reduced ultrafiltration rate also leads to reduced reabsorption, secretion and ultimately urine production downstream. The more nephron are damaged, the greater the impact on regulatory capacity and urine production (Giebisch and Windhager, 2005). Hence the volume of urine production in HIV patients needs to be monitored to ascertain its sufficiency.

2.6. HIV Induced Tubular Diseases

The epithelial cells of the tubules undertake two functions, namely secretion and reabsorption. Epithelial plasma membrane associated molecular mechanisms of transport regulation, confer the ability to match secretion and reabsorption rates to prevailing physiologic demands of the body (McDonough, 2010). The Human immunodeficiency virus and antiretroviral drugs have been associated with direct injury to tubular epithelial cells (Salifu, *et al.*, 2009). Functional damage due to such injuries arise as a result of damage to the said molecular components such as the sodium – proton (Na+ / H+) exchanger responsible for the various transport functions that constitute secretion and reabsorption (Heyns and Fisher, 2006). Extracellular cell volume, circulating blood volume, and blood pressure are interdependently regulated by the rate of sodium transport at the proximal segment by the Na+/H+ exchanger and injury of this segment by the HIV would interfere with these fluid parameters (McDonough, 2010). It also interferes with secretion of ammonium ion (NH4+) which affects acid-base balance (Kassirer, 1985).

Glatt, *et al.,* (1988) asserted that proximal tubular dysfunction associated with drugs such as tenofovir and amphotericin B cause an electrolyte wasting state that can lead to life threatening hypokalemia. Winston *et al.,* (2009) also reported that tenofovir has been associated with proximal renal tubule damage characterized by proteinuria, glycosuria, hypokalemia, and hypophosphatemia (Fanconi syndrome). Injury to the loop of Henle interferes with the urine concentrating capacity of the kidney. Pathologic damage to interstitial cells of the kidney has also been associated with electrolyte disturbance (Sellmeyer and Grunfeld, 1996). It may also interfere with the kidney's ability to secrete erythropoietin (EPO) thereby reducing erythropoiesis (Fray and Goodman, 2000).

2.7 Kidney Function Markers in HIV Infection

The varying array of kidney pathology that are associated with HIV infection imply that impaired balance of biomolecules in urine (proteinuria), systemic acid-base disturbance and physical fluid parameters perturbations (blood pressure and osmolality) can serve to indicate impaired kidney performance in HIV infection (Salifu, *et al.,* 2009). However serum urea and creatinine levels which depend on the balance between their production and excretion are the most routinely used markers in clinical care and in most studies (Provan and Krentz, 2003). Urea a product of amino acid breakdown in the liver is excreted mainly through the kidneys. A significantly high serum urea levels usually indicates impaired kidney function.

Daily production of creatinine mainly as an end-product of metabolism of muscle creatinine is fairly constant and its only route of excretion from plasma is the kidney (Widmaier, *et al.,* 2004). Kidneys match the amount of creatinine excreted in 24 hours to that produced in the

same period so that the level of serum creatinine remains fairly constant at 60- 125 mmol/l (Provan and Krentz, 2003). Creatinine excretion in the kidneys is by both ultrafiltration and secretion and it is not reabsorbed at the tubules. Therefore its elevation in plasma is often used as an indicator of kidney function disorder (Szczech 2009). Several studies have reported creatinine clearance anomalies following the institution of antiretroviral drugs; Gallant, *et al.*, (2005) found that a year after patients were started on a tenofovir-containing regimen, they had a 10% decline in their creatinine clearance compared with a 6% decline in creatinine clearance observed in patients started on other nucleoside reverse transcriptase inhibitors (NRTI)-containing regimens. Lao, and Colleagues (2011) established that elevated baseline serum creatinine was a potential predictor for tenofovir discontinuation in their study population. However in clinical practice, creatinine-based equations, such as the Cockcroft-Gault equation that calculates creatinine clearance or the Modification of Diet in Renal Disease (MDRD) equation for estimated GFR (eGFR) are frequently used to estimate renal function, instead of serum creatinine measurement alone (Franceschini, *et al.*, 2005).

2.8. Liver Function in HIV Infection

The liver contributes to serum electrolytes balance by way of production of angiotensinogen and by its role as the main point of synthesis of serum proteins (Sherlock and Dooley, 2002). Angiotensinogen is the precursor of angiotensin II which triggers the secretion of aldosterone essential for regulating sodium and potassium excretion by the kidneys (Gines, *et al.*, 2003). Proteins are the most abundant organic molecules in blood and contribute significantly to effective intravascular osmolality in form of oncotic pressure a parameter essential for maintain intravascular volume and consequently systemic blood pressure (Macleod, 1977) .

Oncotic pressure is an essential component of the Starling forces that determine fluid exchanges between blood and the tissue spaces (Boulpaep, 2005). Of the proteins, the most abundant are the albumins, globulins and fibrinogen in decreasing order (Mader, 2007). Liver diseases that impair the ability to synthesize angiotensinogen and proteins have profound effects on the liver's role in regulation of serum fluids and electrolytes balance (Green and Flamm, 2002).

Common infectious diseases frequently affect liver health only transiently leading to minimal impact on the liver's role in electrolytes regulation. However chronic impairment of synthetic functions of the liver due to disease, such as occurs in HIV infection or its co-infection/co-morbid factors, could reduce circulating total protein levels sufficiently enough, to lower overall oncotic pressure and trigger a cascade of compensatory events that eventually impact on blood pressure, and overall body fluids and electrolytes balance (Schuppan and Afdhal, 2008). On the other hand, liver disease that culminates in liver cirrhosis could lead to portal hypertension resulting in shunting of hepatic blood into systemic circulation in response to which, nitric oxide and other vasodilators cause splanchnic vasodilatation and fluid retention in the abdominal region (ascites) (Rodriguez and Krowka, 2008). Pronounced splanchnic vasodilatation in turn precipitates a fall in systemic arterial blood pressure, which activates the rennin-angiotensin system and also triggers increased sympathetic activity as well as alters the activity of the Kallikrein-Kinin system (Kumar and Clark, 2005). These responses have the effect of leading to salt and water retention, alteration of capillary permeability and resulting in impairment of elements responsible for sustaining body fluids and electrolytes balance.

The liver is one of the organs mostly structurally and functionally afflicted by gastrointestinal diseases in HIV-infected individuals, besides impacts on the bowel, and the oesophagus (Jablonowski, 1995). Besides predominantly infecting the CD4+ lymphocyte cells a number of studies have elucidated HIV infection of non hemopoietic cells, including the liver cells (Cao, *et al.,* 1992). HIV infection of liver cells such as the primary Kupffer cells, differentiated tissue macrophages, sinusoidal cells and hepatocytes proceeds by means of several mechanisms. It has been demonstrated that Kupffer cells, sinusoidal cells and hepatocytes can be infected by the HIV virus *in vivo* (Hufert, *et al.,* 1993). As hepatocyte cell lines do not express CD4 cell surface markers, it is thought that their infection with the HIV virus is non CD4 dependent (Berger *et al,* 1999). Hepatocytes may act as transient reservoirs of the HIV, but in some instances infection causes apoptosis of hepatocytes which may precipitate pro-fibrotic activity of stellate hepatic cells (Macias, *et al.,* 2005).

Liver histology in HIV patients have shown a common pattern of portal vein occlusion, peri-portal or peri-sinusoidal fibrosis, low grade inflammation and steatosis (Kovari, *et al.,* 2009). The HIV virus causes deterioration of liver health and function by directly infecting liver cells (Kupffer cells, sinusoidal cells and hepatocytes) and by indirect injurious mechanisms (Hufert, *et al.,* 1993). Infected liver cells often undergo apoptosis which is subsequently followed by fibrosis. Thus liver histology in these individuals have shown portal vein occlusion, peri-portal or peri-sinusoidal fibrosis, low grade inflammation and steatosis (Kovari, *et al.,* 2009). However besides direct HIV pathogenetic involvement in liver disease, the most common cause of liver disease in HIV- infected patients is hepatitis (Smith, *et al.,* 2010). In the absence of Hepatitis C (HCV) and hepatitis B (HBV), the various forms of liver

lesions associated with HIV infection that have been reported include; liver decompensation, with and without evidence of cirrhosis on biopsy, to non-alcoholic liver disease (NAFLD) and its more severe form, non-alcoholic steatohepatitis (NASH) and hepatocellular cancer (HCC), (Chang, *et al.,* 2009); (Crum-Cianflone, *et al.,* 2009). Some studies have posited that chronic liver enzyme elevations (LEEs) have been associated with steatosis and steatohepatitis (Patrick, *et al.,* 2009). Studies have shown that liver abnormalities are common findings in HIV infected patients (Kalafateli, *et al.,* 2012) and factors ranging from alcoholism, diabetes, hepatitis, neoplasm, drug toxicity and direct HIV pathology have been listed as determinants of liver health and function impairment in HIV infection (Crum-Cianflone, *et al.,* 2009).

Various forms of liver diseases have therefore been described among HIV-infected patients including non-alcoholic fatty liver diseases (NAFLD), non-alcoholic steatohepatitis (NASH), hepatocellular cancer (HCC) and liver decompensation. Decompensated portal hypertension in HIV patients presents with manifestations such as ascites and bleeding esophageal varices without cirrhosis at histology (Vispo, *et al.,* 2010).

In addition to direct adverse effects of the HIV, a variety of factors have been identified as being associated with damage to liver structure and function in HIV patients including: antiretroviral and non-antiretroviral drugs toxicities; tumors (lymphoma and Kaposi sarcoma); and opportunistic infections such as cytomegalovirus or mycobacterium and co-infection with Hepatitis B virus (HBV) or Hepatitis C virus (HCV) (Vogel, 2007).

Antiretroviral hepatotoxicity has been attributed to nucleoside reverse transcriptase inhibitors (NRTIs), protease inhibitors (PIs) and non-nucleotide reverse transcriptase (NNRTIs) (Núñez, 2010). These drugs afflict liver health and function by way of hypersensitivity, direct mitochondrial toxicity and /or disturbance of lipid or sugar metabolism (Coffie, *et al.,* 2010). As a result ARV- hepatotoxicity is a major challenge that strains medical budgets due to repeated hospital visits or admissions in order to manage the disorders resulting from it. There is therefore need to investigate the burden, pathogenesis and determinants of liver diseases in HIV patients, as a part of comprehensive HIV management strategy in every health setting.

In a Swiss cohort of 2365 HIV –infected patients not co-infected with HBV or HCV, in whom levels of alanine aminotransferase (ALT) was monitored, 16% had chronically elevated levels of ALT (defined as >2x upper limit of normal (Kovari, *et al.,* 2010). Determinants described as risks associated with these elevations were HIV RNA, prolonged exposure to antiretroviral treatment (ART), high body mass index (BMI), alcohol, and increasing age. In a study involving a Polish cohort of 182 HIV– infected individuals on HAART followed for eight years, Anita and colleagues (2009) established that the most common clinical presentation was asymptomatic enzyme elevation, usually <10times the upper limit of the normal range. Lucien *et al* (2010) monitored the levels of transaminases in HIV patients attending Central Hospital Yaounde, Cameroon, involving 150 participants over a three year period, and established that 54% and 24.6% had elevated levels of AST and ALT respectively.

2.9. Liver Function Markers in HIV Infection

Tests of liver performance efficiency carried out on biomolecules associated with the liver and found circulating in blood fall into two categories ;(1) liver function tests which utilize serum albumin and prothrombin as the markers of liver functional integrity; and (2) liver biochemistry which assess serum AST and ALT to evaluate hepatocellular damage. Liver biochemistry also assesses serum alkaline phosphatases and γ-glutamyl transpeptidase reflecting cholestasis and also involves investigation of serum total protein levels (Colledge, *et al.,* 2010).

The multiplicity of liver pathology in HIV infection have occasioned various types of abnormalities of liver function tests (LFTs) (Ejilemele, *et al,* 2007). These include signs or symptoms of hepatocellular injury, such as increases in serum liver enzyme levels (Saves *et al*, 1999). Research by Zucker, *et al.*, (2001) demonstrated that indinavir (a protease inhibitor) directly inhibits the activity of the hepatic enzyme UDP-glucuronosyl transferase (UGT), leading to the development of a reversible, asymptomatic, indirect elevated serum bilirubin (hyperbilirubinemia). In HIV infection, hypergammaglobulinemia and hypoalbuminemia are also common findings indicative of liver function compromise (Salifu, *et al.*, 2009).

Most studies outline kidney and liver functions and their impairment in isolation in HIV infection as in other common diseases. However due to the prolonged history and sustained effects of HIV and associated morbidities, these organs together with others that play crucial roles in electrolytes balance can be functionally impaired concurrently or in overlapping periods of the HIV disease progression. The current study therefore undertook to explore the

prevalence of these organs' defects in HIV-infected individuals and the likelihood of their co-occurrence in the history of the disease. The delineation of the occurrence of multiple regulatory organ defects implies that the range of possible sources of electrolytes defects in HIV infection is broader than the organs frequently targeted as major sources of electrolytes imbalance in routine investigations during most studies and for clinical purposes.

2.10. Serum Electrolytes

Electrolytes are electrically charged minerals dissolved in the two major body fluids compartment, the extracellular and intracellular body fluids. Serum the fluid component of whole blood is a sub compartment of the extracellular fluid. Quantitatively there are two categories of electrolytes in serum; the major serum electrolytes (sodium, potassium, chloride and bicarbonate ions) and the minor serum electrolytes including magnesium and calcium among others (Rose and Fost, 2001). Generally electrolytes help move nutrients into and wastes out of the body's cells, maintain a healthy water balance and help stabilize the body's acid level (Steel and Hediger, 1998). Specifically, sodium predominantly an extracellular electrolyte, helps to regulate the amount of water in the body. Potassium mainly an intracellular electrolyte is important in regulation of the heart's rhythm, cell membrane resting potential and muscle cell contractility (Kaplan, *et al.,* 1996). Chloride ions are dominant extracellular anions which move in and out of the cells to help maintain electrical neutrality and its level usually mirrors that of sodium. Bicarbonate, which is released and reabsorbed by the kidneys, helps maintain a stable extracellular fluid pH level (Alper, 1991).

Mechanisms of Regulation of serum electrolytes aim at maintaining a physiologically optimal level of each of the electrolytes by balancing input with output. Broadly, these mechanisms can be subdivided into two main categories; external regulation and internal regulation (Seldin and Giebisch, 2000). External regulation involves mechanisms which aid exchange of electrolytes between serum and the body's external environment. The main organs of external regulation are the GIT, the cardiovascular, the respiratory, the renal and the integumentary systems (see Fig 2.2).

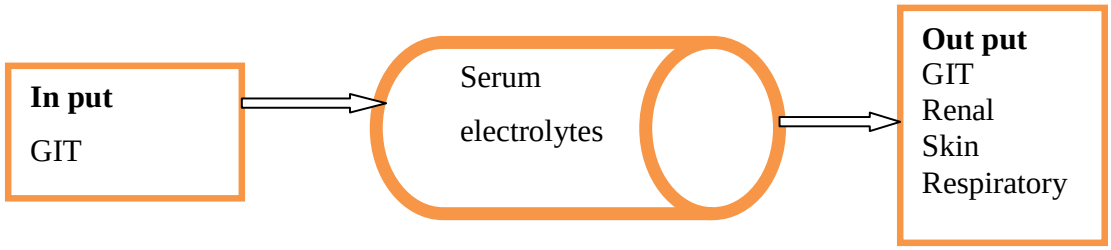


Figure 2.2: Components of External Regulation of Serum Electrolytes

Here serum is sandwiched between two functional groups of organs of electrolytes regulation namely, the input and the output organs. The former aid delivery of electrolytes into serum from the external environment with the GIT being the prominent organ. The later aid exit of electrolytes from serum into the external environment the main player being the kidneys and the respiratory system (Seldin and Giebisch, 2000). These elements of external regulation contribute to establishing and maintain serum electrolytes levels by whole organ performances with their activities closely coordinated by the neuroendocrine systems enabling output organs to match the magnitude of electrolytes that they void from serum to the magnitude taken into the body through the input organs (Fitzsimons, 1998).

However input and output of electrolytes into and from serum also occurs via the elements that constitute the internal regulation mechanisms. Internal regulation is mainly undertaken by cellular components and mechanism which aid exchange of electrolytes between serum and the intracellular fluids (Wingo and Cain, 1993) (see Fig 2.3).

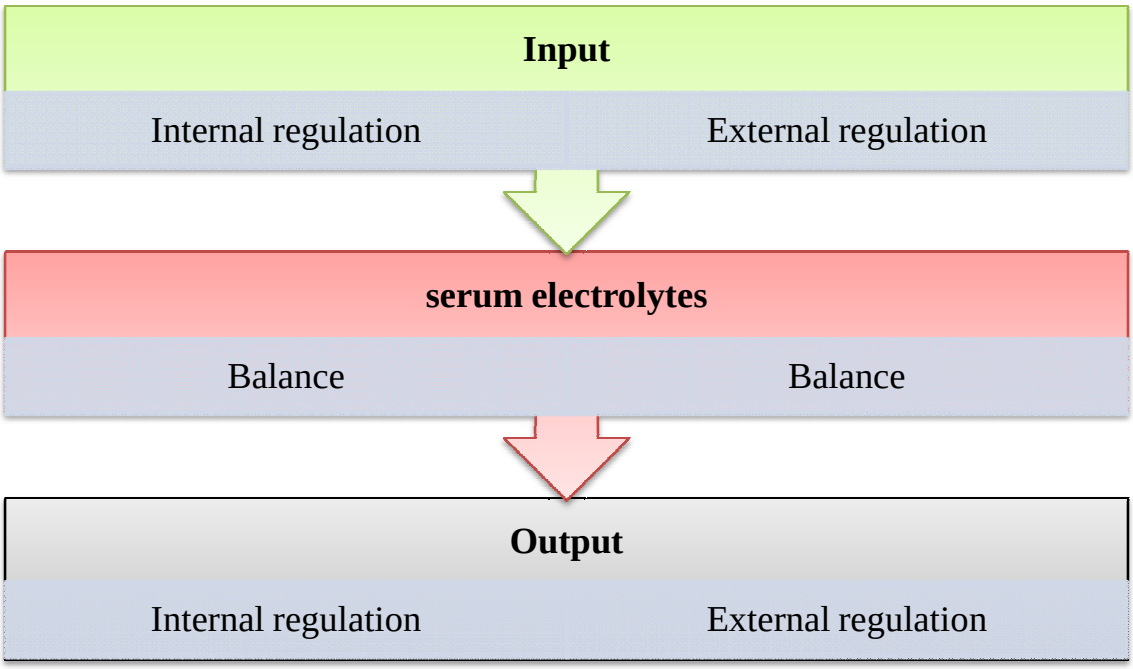


Figure 2.3: Comprehensive Regulation of Serum Electrolytes

Comprehensive regulation of serum electrolytes therefore involves input events from both external and internal regulation arms and output events from both external and internal regulation arms. Whereas input and output events of the external regulation arm depend on whole organ performance of the concerned organs, these events in the internal regulation arm rely heavily on the individual molecular moieties mainly clustered within the individual cell membrane such as the ubiquitous sodium-potassium pump, sodium channels, potassium channels and chloride channels (Wakabayashi, *et al.*, 1997).

2.11. Serum Electrolytes in Systemic Diseases

Systemic diseases often affect the respiratory system, the gastrointestinal system, the central nervous system or the renal system in isolation and occasionally concomitantly (Kumar and Clark, 2005). The resultant hemodynamic alterations (fluid depletion or accumulation), fever, nitrogen wasting, and changes in membrane transport and acid-base balance contribute to frequent serum electrolytes perturbations (Lee, 2010).

Based on the foregoing, there are various possible permutations of regulatory organs' injuries that can affect the established cooperativity and hence the direction, extent and efficiency of plasma and urine fluid and electrolyte balance. Each type is associated with specific outcomes in fluid and electrolyte imbalance. When disease specifically injures pre-renal organs and spares the renal component, the nature of the regulatory cooperativity process is altered by losing the additive mechanisms partially or fully, while it retains competent output mechanisms resulting in characteristic impact on levels of fluid and electrolytes balance in plasma and urine (Mitch and Wilcox, 1982). On the other hand, when disease injures renal regulatory capacity but spares pre-renal organs, cooperativity loses the output mechanisms partially or fully while it retains the additive mechanism. However, in the case of renal injuries, the derangements in output mechanisms can arise as a consequence of one of the following four histological injuries to the kidney nephron; (a) there can exist impairment of the glomerular filtration efficiency as seen in glomerulopathies; (b) impairment of tubular reabsorption efficiency in tubular injuries; (c) it can result from impairment of tubular secretion ability in tubular injuries; or (d) from impairment of both glomerular and tubular

functional capacities. In addition, there can occur any two combinations of the above renal histopathologies, or all these injuries can present concomitantly.

Depending on the site of disease impact therefore, different forms of fluid, electrolyte and mineral perturbations can be observed as features of infectious diseases. The common hemodynamic changes that arise in infectious diseases include decreased systemic vascular resistance, increased cardiac output and increased renal vascular resistance (Scott *et al*, 2006). Blood volume is initially increased, but it decreases as disease progresses. The hyponatremia frequently observed in infectious diseases results from increased levels of antidiuretic hormone (vasopressin), entry of sodium into cells, sodium loss by diarrhea and resetting of osmoreceptors (Wakil, 2011). While hypokalemia caused by hyperventilation is often observed, in severe infective episodes, hyperkalemia arises from intravascular hemolysis or rhabdomyolysis, and occasionally from decreased activity of Na+,K+-ATPase (Acker, *et al*, 1998).

These electrolyte and mineral perturbations are transient and quickly resolve when the disease is controlled. In these diseases the contribution of elements of internal regulation to fluids and electrolytes balance are barely given the attention they deserve since the mechanisms by which these diseases trigger electrolytes disorders are transient and often when the infection is treated the functionally altered elements recover their viability and resume playing their roles in electrolytes balance. But in HIV which is a longstanding infection, these impacts are sustained and the contribution of these other sources to fluids and electrolytes imbalance may be as eminent as that of the organs usually suspected as the main culprits in the development

of electrolytes disorders. In addition to the role played by the hemodynamic changes in development of electrolytes disorders in infectious diseases, the sustained assault by the chronic nature of HIV infection and recurrent opportunistic infection induce direct structural damage to organs and cellular elements regulating electrolytes levels (Bailey, *et al.*, 2004). These can occur from the molecular levels through to whole organs structures.

2.12. Electrolytes in HIV Infection

Literature has shown that patients with HIV infection can develop a variety of fluid and electrolytes disorders, some attributable to the HIV, while others arising as a result of illnesses associated with AIDS or as toxic side effects to regulatory organs by antiretroviral medications (Perazella and Brown, 1994). Pathological effects of HIV infection and antiretroviral drugs on several organs including, organs of the gastrointestinal system (liver), the adrenal glands and the kidneys have been linked to volume losses and electrolyte alterations (Berggren and Batuman, 2005). Glatt, *et al.*, 1988, reported that infection with the Human immunodeficiency virus is associated with a wide range of the morbidities that may lead to changes in fluid and electrolyte composition. Bruggeman and Kalayjian (2009) asserted that higher rates of diabetes, hypertension and hepatitis C co-morbidities are common findings in HIV patients. Sande and Volberding (1997) reported that tuberculosis and pneumonia are common opportunistic infections in people with HIV and AIDS and that gastrointestinal conditions related to HIV infections including dysphagia, vomiting, anorexia and diarrhea are almost universal at some point as the diseases develops. If these conditions are acute or become prolonged, they can severely hamper the ability of the lungs, liver and GIT systems to undertake their normal functions including the roles they play in fluid and

electrolyte balance (Macleod, 1977). In a study by Peter (1991), the prevalence of hyponatremia in HIV patients was reported at 28.4%, of hypokalemia at 17.3% and hyperkalemia at 4.9%. Manfro, *et al.,* (1993) on the other hand reported the prevalence of hyponatremia to be 45%, hypokalemia to be 23.1% and metabolic acidosis to be 20.1% in HIV patients at admission.

2.12.1. Body Water Composition and Pathophysiology in HIV Infection

Total body water is affected by numerous diseases, leading to depletion or intoxication (Stevens 2007). Primary water depletion though rare, occurs from reduced intake or unusual losses. Reduced intake is likely when the patient is obtunded, unconscious, disabled or cannot ingest water due to interference with swallowing or oesophageal obstruction (Glatt, *et al.*, 1988). In HIV patients, water depletion can arise due to causative factors that commonly lead to water depletion in other disease conditions namely dysphagia, coma, depression or apathy (Elenberg and Vellaichamy, 2009). HIV linked oro-pharyngeal candidiasis and oesophageal Kaposi sarcoma can induce dysphagia or obstructive lesions of the oesophagus (Willis, 2002). Glatt, *et al.,* (1988) reported that volume depletion in HIV patients is also a common consequence of diarrhea and vomiting. According to (Kalim, *et al.*, 2008), HIV patients are at risk of developing volume depletion resulting from salt wasting, poor nutrition, nausea, or vomiting. WHO (2009) reported that the protease inhibitors (PIs) are associated with diarrhea as a side effect.

Water intoxication (dilution syndrome) produces an expansion of the extracellular water (ECW) and intracellular water (ICW), with a corresponding decrease in solute concentration

(hyposomolality) (DeFronzo and Thier, 1980). It occurs in patients whose illness restricts their ability to dilute urine leading to intolerance to water loading (Jamison and Oliver, 1982). Decreased renal perfusion can lead to water retention especially in the presence of the following states; acute and chronic renal diseases, nephrotic syndrome, adrenocortical insufficiency, hepatic cirrhosis, and congestive cardiac failure (DeFronzo and Thier, 1980). Cooke (1979) also stated that renal retention of water occurs in the presence of the syndrome of inappropriate antidiuretic hormone secretion (SIADH). The syndrome of inappropriate antidiuretic hormone secretion is associated with pulmonary diseases (pneumonia, lung abscess, tuberculosis, mycoses), central nervous system disease (encephalitis, tumor), malignant tumours (Kaposi sarcoma), and a wide variety of drugs (Kinzie, 1987). All these diseases are likely eventualities of HIV infection, and therefore predispose the HIV patient to water accumulation.

2.12.2. Body Sodium Changes in HIV Infection

Normal blood sodium level is 135 - 145 milliEquivalents /liter (mEq/L) and is essential in determining extracellular osmolality, and in ensuring the myriad of transport mechanisms across the cell membranes in various tissues (Agata, *et al.,* 2008). Excessive accumulation of sodium in blood or hypernatremia occurs whenever there is excess sodium in relation to water. There are numerous causes of hypernatremia; these may include kidney disease, too little water intake, and loss of water due to diarrhea and/or vomiting (Alcázar, 2008). These conditions frequently afflict HIV infected patients.

Sodium depletion in blood (hyponatremia) occurs whenever there is increase in the amount of body water relative to sodium. This happens with some diseases of the liver and kidney which are pathologies that have been reported in HIV infections (Alcázar, 2008). Glatt, *et al.*, (1988) intimated that HIV patients frequently suffered hyponatremia as a consequence of diarrhea, vomiting and the syndrome of inappropriate antidiuretic hormone (SIADH). According to Agrawal, *et al.*, (1988), the prevalence of hyponatremia ranges from 30-60% in HIV patients.

2.12.3. Chloride Changes in HIV Infection

Because it readily diffuses from extracellular to intracellular fluid and vice versa, chloride ions are essential in helping to balance the levels of anions in various body fluid compartments as seen in chloride shift which transpires between the intracellular fluid in red blood cells and plasma when carbon dioxide concentrations increase or decrease (Tortora and Grabowski, 1996). Chloride is the most abundant anion in the extracellular space and its serum concentration ranges from 95 to 103 mEq per liter. Low chloride levels can arise from vomiting, heavy sweating and from adrenal glands or kidney diseases and on the other hand, excessive accumulation of chloride ions in blood is at times a sequel of certain kidney diseases, or diarrhea (Robert and Abbas, 2009; Mahendra, *et al*., 2009). Dehydration, vomiting and renal failure are not uncommon in HIV patients and as such chloride disorders are likely occurrences in these patients (Glatt *et al.*, 1988).

2.12.4. Potassium Changes in HIV Infection

Potassium is the most abundant cation intracellularly and it serves to establish cells' resting membrane potential. It is important in the dynamics of action potential in muscles and

neurons, and plays a role in pH regulation in that it is readily exchangeable with H+ ions in either direction of the cytosol (Tortora and Grabowski, 2003). Due to its abundance inside the cell, potassium is the main determinant of intracellular osmolality (Maxwell and Kleeman 1987). The normal serum levels of potassium are 3.8 to 5.0 mEq per liter. The steady state levels of potassium are maintained by oral intake balanced by excretion of excess through GIT and kidneys. Disorders such as acute or chronic diarrhea, hypoxia, water depletion and acidosis encourage transfer of potassium out of cells into the extracellular space and lead therefore to increased urinary loss and ultimate depletion of potassium (Macleod, 1977). Glatt, *et al.*, (1988) asserted that gastrointestinal infections leading to diarrhea or vomiting and certain drugs used for treating HIV or HIV related opportunistic infections can result in hypokalemia. On the other hand, hyperkalemia in HIV patients is seen to be associated with adrenal insufficiency, disorders that decrease the function of the kidneys, the syndrome of hyporeninemic hypoaldosteronism and the use of drugs such as trimethoprim-sulfamethoxazole or intravenous pentamidine (Glenn and Carol, 2007).

2.12.5. Acid – base dysregulation in HIV infection

The pH of blood is slightly alkaline at between 7.35 and 7.45 and it depends mainly on the concentrations of carbonic acid and bicarbonates in blood (Garret and Grisham, 2008). The concentration of carbonic acid in turn depends on the partial pressure of carbon dioxide in the alveoli. The alveoli partial pressure of carbon dioxide is itself maintained by the equality between its rate of production by the tissues and the rate at which ventilation eliminates it from the body (Boulpaep, 2005). On the other hand, the concentration of the bicarbonates is regulated by the kidney tubular epithelium (Bailey, *et al.*, 2004). Thus the lungs and kidneys

play essential roles in maintaining the body fluid's pH, and diseases which limit the functional efficiency of these organs can lead to pH imbalance. Glatt, *et al.*, (1988) reported that non-anion gap and high anion gap metabolic acidosis in HIV patients were precipitated by varying causes. The former is mainly associated with intestinal losses of bases caused by diarrhea and renal acidosis resulting from adrenal insufficiency, the syndrome of hyporeninemic hypoaldosteronism, or drug toxicity (e.g, amphotericin B toxicity) (Smellie, 2007). The later form of metabolic acidosis in HIV is mainly associated with chronic kidney disease, type A lactic acidosis caused by tissue hypoxia, and type B lactic acidosis (Garret & Grisham, 2008). Type B lactic acidosis has been linked with use of nucleoside reverse transcriptase inhibitors such as zidovudine, didanosine, zalcitabine, lamivudine, and stavudine (Bonnet, *et al.*, 2011). Prolonged or severe vomiting which are common morbidities in HIV patients predispose to metabolic alkalosis as in other disease states (Huang, 2010). On the other hand, respiratory alkalosis and respiratory acidosis in HIV patients have been associated with opportunistic infections of the lungs or central nervous system (Glatt, *et al.*, 1988).

The difference in findings of prevalence of body fluids and electrolytes disorders could be attributed to study settings and this informed the need to conduct and establish prevalence of electrolytes disorders in local HIV populations. With these possible permutations of fluids and electrolytes disorders, the empirically observed fluids and electrolytes states in HIV patients is a culmination of numerous probable regulatory disorders. Merely pointing the existence of aberration in fluids and electrolytes disorders does not serve as an adequate pointer to the proximal contributors to the observed imbalance. Efforts aimed at attributing to specific regulatory elements quantifiable magnitudes of observed F&E disorders are therefore

necessary when the HIV infection exists as the background disease. Also point of care investigations of electrolytes disorders which associate these states with immediate underlying clinical episodes such as fluid depletion or overload do not explore the contribution of actual structural defects in regulatory organs and cellular elements to the electrolytes imbalance in HIV infection as opposed to the transient perturbation of organ performance that are usually the means by which other diseases occasion electrolytes defects.

2.13 Effect of HIV on Cell Plasma Membrane

The cellular plasma membrane maintains cellular material and ionic gradients necessary for the proper functioning of the cell (Boulpaep, 2005). The ability to alter the cell's intracellular ion concentration is necessary for many of the animal viruses in their life cycle and for cytolysis as a means of extruding the virons after host assembly (Dalgeshi, *et al*, 1984). Studies have elucidated several viral proteins responsible for the increased membrane permeabilities and ion transport including viroporins, glycoproteins and proteases is a common feature of viral infection (Dalgeshi, *et al.*, 1984).

HIV mediated plasma membrane perturbation can affect membrane permeability leading to altered transmembrane gradients of cations and small molecules (Fermin and Garry, 1992). HIV Viroporin proteins have been found to form channels in the lipid layer of plasma membrane (Coffin, 1995). This channel is less discriminating than the highly selective channels for bacteria and eukarya (Foster, *et al.*, 1980). Viroporins are not the only means by which the HIV alters plasma membrane permeability and the virus employs other mechanisms such as generalized membrane destabilization and alteration of existing ion channels and

pumps or of their expression (Gallo and Montagnier, 2003). This it does using other HIV proteins which have been shown to interfere with cellular electrophysiology. These include, HIV-1 Nef which induces alteration in intracellular potassium ion concetrations doing so indirectly by interacting with plasma membrane ion channels to modify their conductance properties (Kort and Jalonen, 1998). The HIV Vpr which has been observed to cause a large inward current and cell death in hippocampal neurons (Piller *et al,* 1996). It has also been demonstrated that the HIV Vpu protein forms channels and induces potassium ion conductance in Xenopus oocytes (Schubert *et al,* 1996). On the other hand the HIV Tat blocks L-type calcium ion channels in dendritic cells (Poggi, *et al.*, 1998).

The Surface glycoproteins (SU) of HIV activates the Na+/H+ antiport and K+-conductance in astrocytes (Bubein, *et al.*, 1995). As a result it has been shown that HIV infection leads to increases in intracellular concentration of monovalent cations (Na+ and K+) (Carrasco, 1995). This influx of osmotically active ions enhances water influx into cells thereby expanding the cell volume (Voss, *et al.*, 1996). The HIV virus also alters the normal functions of the sodium-potassium-chloride (Na+/K+/Cl) co-transporter during its control of cell volume (Makutonina, *et al.*, 1996). Therefore, the alteration of membrane ion transport and permeability are important mechanism for HIV cytopathogenesis. These events continue during the sustained viral infection and have long term effect on the cells ability to participate in proper redistribution of electrolytes between the extracellular and intracellular fluids.

This implies that as opposed to the transient effects of infectious disease which abate when the infectious disease is handled, actual structural damage of a wide array of cellular and

tissue elements are relevant to establishing electrolytes disorders in HIV. Therefore focusing on whole organs alone in investigating the sources of electrolytes defects in HIV may not sufficiently explain the observed electrolytes defects. Furthermore few studies have explored whether and how prolonged skewed electrolytes distribution constrains the influence of the electrochemical forces essential in determining the overall and specific body fluids electrolytes levels given the closely coordinated physiological distribution of electrolytes in body fluids compartments. Most studies portray the occurrence of individual electrolytes disorders as independent events without establishing the possible range of sources of imbalance and the magnitude to which each source contributes to the existing electrolytes defects such as the subtle electrochemical forces reliant on the electrical and chemical gradients to move ions between the body fluids compartments. This study aimed at demonstrating that the coupling of electrolytes distribution between the body fluids compartments is weakened by the prolonged skewed distribution of ions due to these viral interferences with plasma membrane permeabilities.

2.14. Routine Clinical Electrolytes Assays

At analytical level whole organ performance is readily quantifiable through assessment of organ performance markers or indicators while measurement of performance levels of molecular moieties is a daunting task. Therefore most methods of assessing body fluids electrolytes balance and imbalance while relying on direct measurement of these electrolytes levels in serum, end at the level of assigning attribution to organs in the external regulation arm, and less so the molecular moieties in the internal regulation arm. Thus assays of major serum electrolytes (sodium, potassium, chloride and bicarbonate CO2) are mainly used to

evaluate presence of underlying heart, liver , respiratory and kidney health and functional aberrations as the causes of these electrolytes disorders secondary to infectious illnesses (Smellie, 2007). This has led to development of point of care investigations limited to evaluating the performance of a confined range of organs whenever serum electrolytes imbalance is detected to serve as the exclusive explanation for the observed electrolytes imbalance. Most studies duly rely on these organs too when searching for explanation for physiological electrolytes states observed to be abberated.

The current study postulates that in HIV infection which has the ability to structurally afflict multiple organs and cellular regulatory apparatus as well as those that play coordinative role (neuroendocrine elements), confining attribution to these routinely assessed organs might not be sufficient for explaining observed electrolytes disorders in this disease. That is, to sufficiently explain electrolytes imbalance in HIV patients may require comprehensive review and attribution of existing electrolytes imbalances to multiple sources rather than to individual sources as is routinely practiced in most studies and in routine clinical investigations. This is because while in ordinary physiological states each of these organs are necessary individually for explaining electrolytes balance or imbalance each might not be the only source of electrolytes defects in HIV infection. The current study was therefore designed to explore the short comings or weaknesses of confining attribution of electrolytes disorders in HIV to a narrow range of the usual suspect organs within the renal and GIT systems. This may help to highlight that existing electrolytes disorders in HIV infection are not fully accounted for by investigating and confining attribution to a narrow range of organs individually as is the usual routine point–of care practice.

2.15. Back Ground Factors

Certain factors constrain the impact of HIV virus on the overall health of body organs and consequently on the capacity of the regulatory organs to maintain electrolytes balance. These factors could either enhance the deleterious effects of the virus or ameliorate the effects of the virus on the health of these organs. These factors include but are not limited to demographic variables of the affected population, socio- economic variables, and genetic disposition of the individuals with the disease.

2.15.1 Outcomes Associated with CD4 Depletion

Selective depletion of the CD4 lymphocyte and is accomplished by direct cytopathic effects and also by way of immune dysregulation (Costin, 2007; Nishimura, *et al.,* 2007). The CD4 count is the number of CD4 lymphocytes per microliter (µL) of blood and it is used to mark the degree of immune suppression or immunocompromise (immune status). Clinically, CD4 lymphocyte count is used to stage the patient's disease, determine the risk of opportunistic illnesses, assess prognosis, and guide decisions about when to start antiretroviral therapy (ART) (Hogg, *et al.,* 2008). When homeostatic mechanism that replenish the CD4 lyphocytes collapse this leads to immune system failure (Gary, 1989). Increased depletion of CD4 lymphocytes leaves the body with fragile immune defense which in turn conduces to opportunistic infection, pathologic conditions and malignancies of various organs including those that are vital in the regulation of body fluids and electrolytes levels (CDC, 2009). Reports documenting the relationship between changes in CD4 lymphocyte count to changes in fluids and electrolytes states in local HIV populations are not sufficient. This study therefore intended to investigate the association between progressive immune depletion (CD4

lymphocyte count) and the status of fluid and serum electrolytes balance in HIV patients in Kisumu County, western Kenya.

2.15.2. Age and Associated Changes in HIV-infection

Human immunodeficiency virus causes a chronic disease and therefore its pathologic impact in the body is bound to be complicated by age related health defects such as diabetes, high blood pressure, arthritis and malignancies (Magalhães, *et al.,* 2007). Conversely the outcomes associated with geriatric diseases in immune defective HIV-infected individuals provide unique challenges to heath hitherto not associated with these diseases in seronegative persons (Choi, *et al.,* 2011). Studies have shown that HIV-infected individuals have higher prevalence of cerebrovascular risk factors (including myocardial infarction, coronary heart disease, diabetes, hypertension, obesity, and atherosclerosis) than their seronegative counterparts (Lorenz, *et al.,* 2008). In addition, older patients demonstrate accelerated disease progression compared with younger patients and recovery of CD4 lymphocyte levels in older patients has also been found to be less robust in response to ART (Butt, *et al.,* 2001; MacArthur, *et al.,* 1993). This study therefore also investigated the influence of age on the serum electrolytes changes associated with HIV infection in Kisumu County.

2.15.3. Anti-retroviral Treatment and Associated Health Outcomes in HIV Patients

Antiretroviral therapy (ART) has been shown to be effective in slowing down the progression of AIDS and in reducing HIV-related illnesses and death (Palella, *et al.,* 2003). However, the best time to start antiretroviral therapy in people with HIV infection, who have not received antiretroviral treatment before and who do not have any symptoms of HIV illness remains

debatable (Palella, *et al.,* 2003). Guidelines issued by various agencies provide different initiation recommendations according to resource availability (Hammer, *et al.,* 2008). This can be confusing for clinicians and policy-makers when determining the best time to initiate therapy. Optimizing the initiation of ART is clearly complex and must, therefore, be balanced between individual and broader public health needs. Traditionally, therapy is administered based on a patient's CD4 count, rather than on viral load, a marker of virologic replication (Hammer, *et al.,* 2008). Patients with a CD4 cell count of 500 cells/μL are considered healthy enough not to need ARVs but when the CD4 cell count reaches 200 cells/ μL, the immune system is severely weakened and ART is necessary (WHO, 2009). Nonetheless a patient with advanced symptoms receives treatment regardless of CD4 count. Coupled with this, is the observation that ARTs induce toxic damage to various organs among them those that are vital for fluid and electrolytes regulation (Hammer, *et al.,* 2008); (Reilly, *et al.,* 2001). Due to these twin challenges in relation to ARV use, this study investigated the association between ARVs use and the status of fluid and electrolytes in plasma in HIV patients in Kisumu County. The aim was to determine whether ARVs usage attenuate the negative impact of HIV infection on F&E balance.

2.15.4. Gender and HIV Infection

Women have been reported as being vulnerable to HIV infection than men due to various biological and cultural determinants (Turmen, 2003). Biologically the rate of transmission of the virus from men to women is two to four fold higher than the rate of transmission from women to men (Mocroft, *et al.,* 2000). Furthermore the presence of underlying sexually transmitted diseases increase the likelihood of contracting HIV infection yet often times

sexually transmitted infection remain asymptomatic in women and therefore present challenges in their diagnosis and early treatment (Prins, *et al* 1999). On the other hand in resource poor settings because of social power strata women have limited access to high quality health care.

HIV/AIDS progression in the different gender groups show certain gender specific traits. In a longitudinal study Anastos and colleagues (1999) observed that women experienced a more rapid decline in CD4 cell count than men even though their HIV-1 RNA load was 32% to 50% lower than in men at CD4 counts >200cells/mm^3 but not in CD4 count<200cells/mm^3. In their study Sandra and Colleagues, (1999) found that seropositive women were at a higher risk of developing bacterial pneumonia than HIV-infected men. On the other hand Nicastri, *et al.,* (2005) observed that long-term clinical, virological and immunological outcomes associated with ARVs use revealed that women had fewer AIDS defining illnesses, higher CD4 cell counts and lower viral load as a response to medication use. Nonetheless Kuyper, *et al.,* (2004) noted that upon poor adherence to medication women were more likely to experience HIV-1 viral load rebound than men. These findings shows that women in HIV infection experience multiple challenges as it has also been reported that in resource poor settings they are less able than men to follow medication instructions (Valverde, *et al.,* 2009). These varying HIV/AIDS outcomes could impact on the influence that the disease has on fluids and electrolytes states in the different gender categories.

2.16. The Conceptual Framework

Literature has shown that the influence of HIV infection on the state of serum electrolytes is multilayered. To begin with HIV as other infectious diseases can precipitate fluid dynamics that leads to fluid depletion (vomiting, diarrhea and fever) or fluid accumulation (Schroeder, *et al.*, 1990). These fluid dynamics can impact on body fluids electrolytes concentration by leading to relative or absolute dilution or hyper concentration of the serum electrolytes. Hemodynamic events accruing from the body fluid volume changes like circulatory collapse can also trigger processes that destabilize the regulation of serum electrolytes balance (Elenberg and Vellaichamy, 2009). On the other hand the HIV virus is associated with prolonged and sustained injury of cellular apparatus which actively redistribute electrolytes between body fluids compartments (Gallo and Montagnier, 2003).

This has two pronged effects; systemically it leads to skewed distribution of electrolytes between intracellular and extracellular fluids compartments by way of intracellular sequestration of electrolytes or by way of extravasation of electrolytes into the extracellular space in extensive cytolysis episodes. Secondly injuries to cells of organs specialized for regulation of electrolytes cumulatively result in defects in these organs' performance and as a consequence lead to deficiency that perturb electrolytes balance as influenced by the organ involved. Prolonged injury of CD4 cells leads to progressive reduction of their circulating numbers which critically impairs immune protection (Hogg, *et al.,* 2008). Opportunistic infections that arise due to this outcome compound the effects of HIV infection on electrolytes states by either inducing fluid and hemodynamic dysregulation (vomiting, diarrhea or hyperventilation) or by directly injuring cells as well. Changes of serum

electrolytes levels resulting from these predictors can either be depletion or accumulation of electrolytes.

Antiretroviral drugs attempt to reduce the viral load permitting the repair of damage that it may have been occasioned (Palella, *et al.,* 2003). When this is effective, recovery or protection of regulatory apparatus allows restoration of the processes that safeguard electrolytes balance to undertake their roles sufficiently. This can improve any prior existing electrolytes imbalance. However certain antiretroviral drugs especially the protease inhibitors have been associated with injuries to organs that regulate body fluids electrolytes levels (Kalyesubula and Perazella, 2011). This can compound already existing impacts of HIV infection on electrolytes levels or institute their own electrolytes imbalance where none existed before.

Advanced age coupled with the Human immunodeficiency virus infection introduces additional challenges linked to age related health determinants including vulnerability to non-communicable diseases such as cancers, auto-immune diseases such as arthritis, endocrine disorders such as diabetes, cardiovascular conditions in addition to increased vulnerability to co-infections such as tuberculosis and pneumonia (Choi, *et al.*, 2011). In addition socio-economic challenges might impact on their ability to access sustained quality health care. Male and female infected with the HIV virus have been shown to manifest unique responses as the disease progresses (Nicastri *et al,* 2005). These responses influence the intensity of the disease which in turn impacts differently on the extent to which the virus can afflict the

apparatus that regulates serum electrolytes in. Figure 2.2 gives a graphical representation of the conceptual framework.

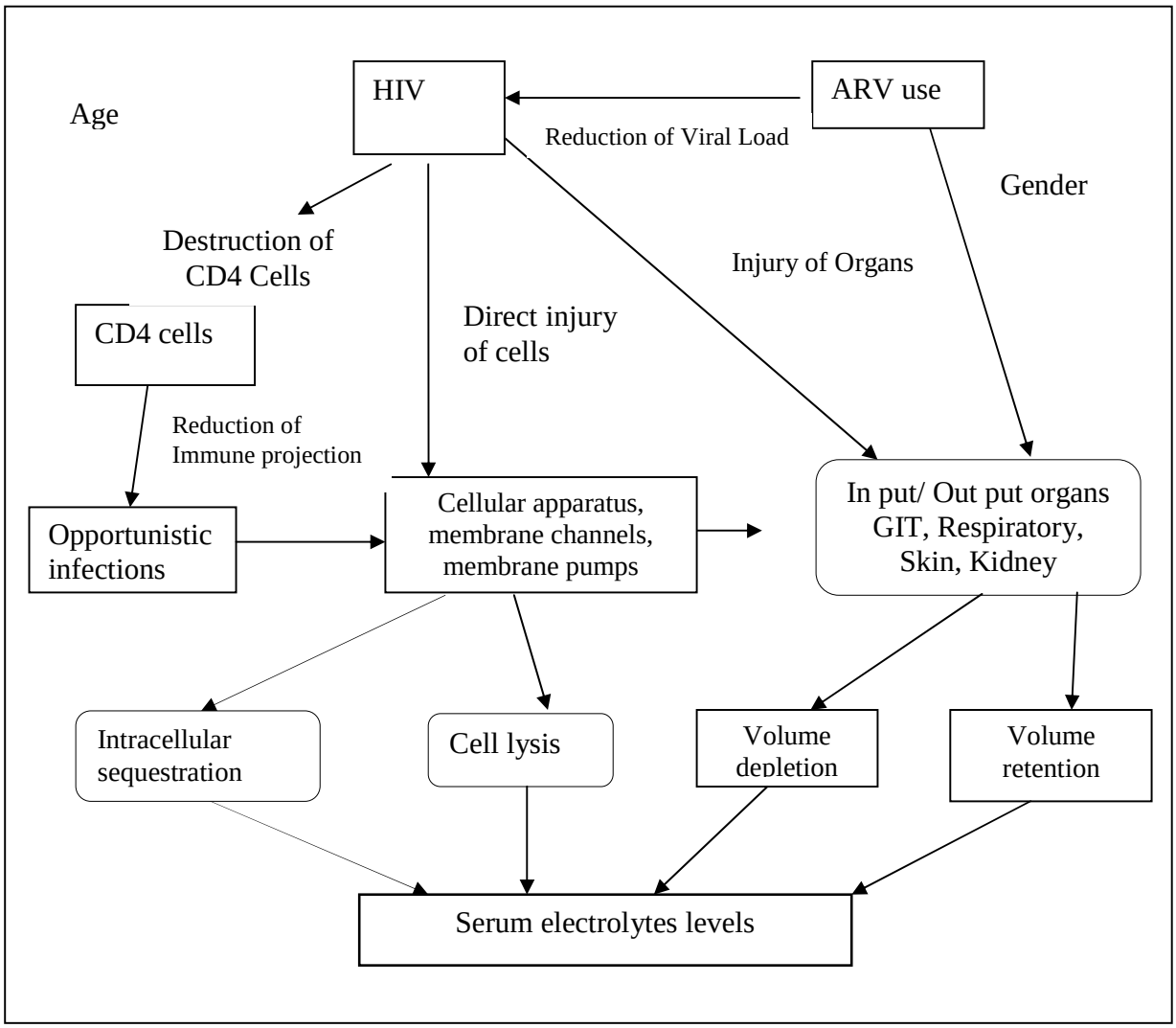


Figure 2.4: Conceptual Framework

This study was therefore designed to explore and highlight the actual magnitude of electrolytes disorders attributable to two organs which are part of the two systems (GIT and renal) frequently associated with fluid disturbance (depletion or overload) which in turn are the most immediate causes of serum electrolytes imbalance in most infectious diseases. This was so as to determine whether defects in the performance of the two organs are sufficient to explain observed co-existing serum electrolytes imbalance in light of the extended range of impact which the virus has on multiple organs essential for regulating serum electrolytes levels. Body fluids disturbance though frequent in HIV infection are by no means the only contributors of electrolytes disorders in the course of the disease. The HIV virus has been shown to have deleterious effects on the substantive structure of cells, tissues and organs and can induce electrolytes imbalance at all these levels (Kaplan, *et al.,* 1996). Routine analysis associating electrolytes disorders in HIV to immediate fluid disturbance could therefore be missing out other contributors and this study intended to determine whether all existing serum electrolytes disorders could be attributed to the two systems as represented by the two organs or only part of the electrolytes disorders could be explained by the defects in the two systems and what amount of that electrolytes imbalance it is.

CHAPTER THREE: MATERIALS AND METHODS

3.1. Study Area

The study was carried out in the Patient Support Center of Jaramogi Oginga Odinga Teaching and Referral Hospital of Kisumu County, Western Kenya. Kisumu County is bordered by Siaya County to the West, Vihiga County to the North, Nandi County to the North West, Kericho County to the East, Nyamira to the South and Homabay County to the South West. Its longitude is 34^{o} 45'E and latitude is 00^{o} 03'S. Kisumu County's mean rainfall is 1280 mm and its mean annual temperature ranges between 200C and 300C. The main occupation in is fishing, small scale farming, general trade and light industry. HIV prevalence in this region is about 15.3%, the highest in the country (KAIS, 2012). The doctor to patient ratio stands at 1:5379 and it is estimated that 80% of households have access to health care services (Kisumu District Development Plan, 2002).

3.2. Target Population

The target population was persons living with HIV in Kisumu County. From this, the population studied was male and female persons 18 years of age and above both living with HIV and attending Patient Support Center of Jaramogi Oginga Odinga Teaching and Referral Hospital in Kisumu County of western Kenya. There were 5408 patients registered at the PSC at the beginning of the study. The final sample of the study was based on 3200 patients. The sampling unit was an adult patient either male or female aged 18 and above as indentified from the hospital records.

3.3. Inclusion Criteria

To be enrolled in the study, the potential participant had to be able and willing to provide informed consent and be above the legal age of 18 years with confirmed HIV and AIDS status, whether symptomatic or asymptomatic and whether receiving HAART or not. HIV negative persons with no history of diabetes, hypertension, respiratory infection and gastro-intestinal infection were recruited as control participants in the study.

3.4. Exclusion Criteria

Individuals with overt or confirmed conditions such as diabetes, hypertension, acute or chronic respiratory infection or any other conditions with a bearing on body fluids and electrolytes states were excluded. Those using non-antiretroviral drugs which impact on body fluids electrolytes such as diuretics were excluded from the study. Those eligible and not willing to give consent were also excluded from the study.

3.5. Study Design

This was a hospital laboratory based cross sectional study in which consenting consecutive participants were enrolled into the study.

3.6. Sample Size and Sampling Procedure

Given the proportion of electrolytes disorders was used predominantly to compare the two populations, the minimum sample size required for each group was calculated using the formula for finding a minimum sample size to estimate proportions as described below (Larson, 2000).

n =$pq(\frac{Z_c}{E})^2$; where p = sample proportion; q = (1-p); Z_c = standard value at confidence level and E= maximum error of estimated proportion from the real population proportion.

Given the proportion of fluids and electrolytes disorders in local populations (healthy or with HIV) are not documented, the minimum sample size for either group was calculated as follows;

$$n = 0.5 \times 0.5 \left(\frac{1.96}{0.05}\right)^2$$

= 385

To determine the actual sample size, consideration was given to the following factors: the size of the targeted HIV population, the sampling procedure used, the nature of the primary variables, the variability of the attribute to be measured in the target population, the number of subgroups to be assessed and the variability of the measured attribute in every subgroup, the kind of statistical analysis to be used, the margin of error allowed as well as the level of precision of the study (Krejcie & Morgan, 1970).

The target population was HIV infected individuals in Kisumu county where prevalence of HIV infection is reported as 15.3% (KAIS, 2007). Therefore the estimated HIV population in Kisumu County was = Kisumu population x prevalence of HIV in Kisumu

= 535,164 x .15 = 80, 275

The sampling technique used was consecutive sampling and the primary variables were ordinal variables. There is no information concerning the specified levels of variability of body fluids and electrolytes disorders in HIV population in Kisumu County. The margin of error for the study was set at ±5% with a 95% precision level.

Therefore, use was made of published tables and imitation of sample size from a previous similar study (Cochran, 1977). Considering that the HIV population in Kisumu exceeds 10,000 people, then according to Anderson *et al.,* (1995) when using covariance analysis, one needs a sample size between 200 – 500. In the published tables by Cochran (1977), to draw a sample from a population more than 10,000 in a study intended to have a margin of error of 5% and a precision of 95% where the primary variables are ordinal, one needs at least 370 participants. In a study of lactic acidosis in HIV patients, (Boubaker, *et al.,* 2001). Eight hundred and eighty (880) participants were enrolled in a cross sectional study to determine the prevalence of this electrolyte disorder in the study population. Kuehn, *et al.,* (1999) in a study of hypocalcaemia in HIV infection used 828 seropositive and 549 healthy controls. The current study therefore pegged its sample size on the two studies above and used eight hundred seropositive individuals and additional four hundred HIV negative were recruited as controls for the study to carter for laboratory analytical shortcomings.

Consecutive sampling technique was employed using the list of daily patient attendants to recruit consecutive participants until the requisite sample size was obtained. The consenting participants were enlisted and investigated for the requisite parameters. Between November 2012 and April 2013, 800 HIV+ patients from PSC of JOOTRH and 406 HIV negative

controls were enrolled into the study. Enrolled participants in the control group were enlisted from among consenting ministry of health staff who in the period of the study were due for and underwent routine laboratory profiling at JOOTRH, as a pre-condition for hepatitis vaccination.

3.7. Laboratory Methods and Specimen Handling

Personnel in the PSC undertook routine medical review and care of HIV patients and introduced them to study. Common attributes recorded for both groups included age, gender, blood pressure, body weight, random blood sugar, kidney and liver functions parameters. Additional attributes obtained for the HIV infected individuals were ARV use and CD4 lymphocytes levels.

Volunteers were then informed of consent provisions and consenting patients sent to the hospital's laboratory where assigned hospital laboratory personnel collected blood and urine samples for analysis. Five millilitres of blood was collected using sterile disposable non-pyrogenic syringes (CATHY YOUGO®, France) into dry vacutainer tubes from each of the participants.

The serological status of PSC participants was confirmed from hospital records while for control was determined using collected blood samples. For members of either group serological analysis followed three standard procedures as stipulated by the Ministry of Public Health. This entailed the use of two rapid screening tests and a confirmation test. To begin with the samples were tested using Determine TM (Abbot Laboratories, Japan) HIV strips to

screen for the presence of HIV antibodies. Positive samples were further screened with Unigold (PDS-Orgenics, Israel). Positive cases in either or both of the screening tests were then subjected to a final ELISA (U.K.) assay as the tie-breaker with the outcome here used as the confirmed serological status.

Total CD4+ cell count was ascertained by flow cytometry using FACS caliber (Becton Dickinson, USA, 2006) using the procedure described by the manufacturer. Essentially, into the trucount tubes was added 20 µl of multi-test reagent containing the CD3/CD5/CD 4-flourochrome labeled antibodies that bind specifically to antigens on the surface of lymphocytes. Thereafter, 50 µl of whole blood was added to the trucount tube containing the multi-test reagent. The tube contents were mixed gently for homogenization and incubated in the dark at room temperature for 15 min. After incubation, 450 µl of FACS lysing solution was added to the contents of the tube which was then vortexed gently and the mixture analyzed using FACS caliber counter and the results printed automatically. Results obtained for the CD4 counts were grouped as follows: Counts < 500 cells/µL; >500cells/µL (WHO, 2004).

Collected blood samples were then prepared and assayed for the levels of the following analytes; urea, creatinine, sodium, potassium, chloride, total protein, albumin, random sugar, direct bilirubin, liver transaminases (alanine amino-transferase and aspartate amino-transferase), and total bilirubin. Before measurements, the machine was calibrated using a standard solution provided by the company, and quality control sera. These blood samples were separated to produce serum using a centrifuge at 3000 rpm for 3 to 5 min and the serum components were subjected to immediate analysis. Serum creatinine, urea, total protein,

albumin, total and direct bilirubin, and transaminases were assayed using colorimetric method with wavelength range of 340-670 nm (Eurolyser CCA180, Eurolyser Diagnostica GmbH, Austria, 2006). Analysis of this group of serum components was done either by means of enzyme kinetics or end-point substrate measurements. Enzyme kinetic procedure was done as listed for the following the components;

- **BUN**- 200µl of reagent-1 was pipetted into 2 µl of sample, incubated for 300 seconds; then 50 µl of reagent 2 was added and incubated for 240 seconds and finally optical density measured at 450nm within 1minute
- **ALT** - Pipette 200 µl of reagent-1, pipette 50 µl of sample, incubate for 300 seconds; pipette 50 µl of reagent 2, incubate for 90 seconds and then measure optical density at 520nm within 10 seconds.
- **AST** - Pipette 200 µl of reagent-1, pipette 50µl of sample, incubate for 300 seconds; pipette 50 µl of reagent 2, incubate for 180 seconds and finally measure optical density at 520nm within 10 seconds
- **Creatinine** - Pipette 200µl of reagent-1, pipette 10µl of sample, incubate for 300 seconds; pipette 50µl of reagent 2, incubate for 10 seconds and finally measure optical density at 520 nm within 180 seconds
- Creatinine clearance (eGFR) calculated from Cockcroft-Gault equation was used to estimate glomerular filtration rate, as follows;

  $$\text{Ccr (ml/min)} = \frac{(140\text{-age}) \times \text{mass (in Kg)} \times \text{constant}}{\text{serum creatinine (in } \mu\text{mol/l)}}$$

Where constant is 1.23 for men and 1.04 for women as adopted from Cockcroft and Gault, (1976). Values estimated for glomerular filtration rate were grouped as follows: Normal

values (males >70 to 140 ml/min, females >60 to 128 ml/min), mild decrease in CC hence, GFR (males >40 to 70 ml/min, females >40 to 60 ml/min), moderate decrease in GFR (>10 to 40 ml/min), severe decrease in GFR (<10 ml/min) such patients are usually on dialysis (Szczech, 2002).

Fixed end point analysis was done as listed for the following components;

- **Total protein** - Pipette 200µl of reagent-1, pipette 4µl of sample, incubate for 300 seconds; pipette 50µl of reagent 2, incubate for 300 seconds and measure optical density at 540nm immediately.
- **Albumin** - Pipette 200µl of reagent-1, pipette 2µl of sample, incubate for 300 seconds; and then measure optical density at 630nm immediately
- **Total bilirubin**- Pipette 200µl of reagent-1, pipette 20µl of sample, incubate for 300 seconds; pipette 50µl of reagent 2, incubate for 300 seconds and then measure optical density at 540nm immediately
- **Direct bilirubin** - Pipette 200µl of reagent-1, pipette 4µl of sample, incubate for 180 seconds; pipette 50µl of reagent 2, incubate for 150 seconds and instantly measure optical density at 540nm.

3.8. Data Collection Tools

The research used approved laboratory request forms in which data concerning variables measured from the participants was entered in duplicate, one for the health care records of the institution of care and one for the principal investigators file. An accompanying proforma was used to enter demographic data. To ensure confidentiality patient identity was screened and only the file numbers recorded. Data was entered by the hospital's laboratory technicians and

stored by the principal investigator. Patient files were retained at the institution of care under the mandated health records officers.

3.9. Data Presentation and Analysis

Data was entered in Microsoft Excel. Statistical data was generated using SPSS software (SPSS version 22, Chicago, IL). P value of <0.05 was considered statistically significant. Independent variables used were HIV status, age, anti-retroviral drugs and CD4-cell counts, as well as kidney and liver function. Dependent variables were markers of organ pathology, electrolytes and related parameters. The distribution of analytes in both groups was determined using frequency counts, measures of central tendency (mean, mode and median), and measures of dispersion (range, standard deviations and quartiles). The mean serum levels of electrolytes in either group were compared with the standardized reference range of these electrolytes in serum to assess the likelihood of existing imbalance. Rates of occurrence of electrolytes imbalance were depicted using proportions. Determination of differences in rates of electrolytes imbalance between groups and the association between these imbalances with independent variables was established using the t-tests, Chi-square and/or Fisher's exact tests as well as Spearman's correlation coefficients.

3.10. Ethical Considerations

Approval by the Nyanza Provincial Hospital's Research Committee was obtained prior to commencement of the study. The research restricted recruitment of investigators to the pool of health care workers routinely assigned to handle the patients of interest, to avoid introducing new procedural dynamics, avoid further psychological alienation of the

participants, and enable the test and study findings to be incorporated into the system for routine care of the HIV patients. Participants were enrolled on voluntary basis only after the nature and purpose of the study has been explained to enable them make informed consent as a basis for enrollment. Qualified institutional staffs were responsible for administering consent, obtaining and investigatively handling the samples. The patients were informed about the people who will subsequently handle the results of the tests and the clinical and research uses to which the results will be put for their benefit. Test results with significant clinical implication were relayed to respective institutional health care providers for further clinical intervention.

Handling of the patients was done as part of the routine care in their normal appointment schedules. Requisite institutional routine procedures preceded the request for the test samples. Testing and reporting of the findings were expedited to enable results to be used for procedural management of the patient besides being entered as data for the study.

The researcher only opened the data during indexing in order to be statistically analyzed. Results obtained was used as inference in reporting the study outcome. For purposes of verification in put records (laboratory forms and demographic proforma) was retained by the researcher until the thesis is accepted and defended. The Nyanza Provincial Hospital will be informed of study outcomes.

3.11. Electrolytes Assay Techniques

Within serum two different electrolytes quantities are determined during clinical chemistry: 1) Electrolyte concentration (total) in serum (S) e.g. S-sodium (mmol/l), S-calcium (mmol/l).

2) Electrolyte concentration (ionized) in serum water [S(W)] e.g. S(W)-sodium, ionized (mmol/kg), S(W)-calcium, ionized (mmol/kg) ad 1) (Burnett *et al,* 2000). For the determination of the concentration of the ionized or free fraction of sodium, potassium, chloride, calcium, and magnesium in serum water (or the extracellular water phase of whole blood) the only applicable method is Ion selective electrode assays without dilution of the sample (Koryta, 1991). Ion-selective electrodes (ISEs) respond to ion-activity instead of sensing substance concentration directly. For routine clinical purposes results of ISE measurements of sodium, potassium and chloride in undiluted plasma are reported in terms of substance concentration (mmol/l) (Plambeck, 1982). In specimens with normal concentrations of plasma water, total CO2, lipids, protein and pH, the values obtained with ISE will concur with the total substance concentration as determined by flame atomic emission spectrometry (FAES) or ISE measurements on diluted samples (Turner, *et al,* 1987). In specimens with abnormal concentrations of plasma water, measurements of sodium, potassium and chloride by ISE in the undiluted sample more appropriately reflects the activity of sodium, potassium and chloride and are therefore clinically more relevant than the determination in diluted samples (Koryta, 1980). The current study therefore opted to use ISE to assay the electrolytes levels in the undiluted samples obtained for this superior reason.

3.12. Assay of Kidney and Liver Functional Markers in Serum

Measuring the concentration of proteins provides information regarding disease states in different systems. Even though total protein content provides some information regarding a patient's general status, clinically useful data are obtained from fractionating the total protein. The most commonly used means for further fractionating serum proteins is electrophoresis

(Hames, 1990). During electrophoresis, protein solutions in appropriate buffered solvents are placed on a medium such as paper or starch blocks and exposed to an electrical current. Differences in their electrical charge cause the protein components to migrate at different rates toward the anode or cathode. (Savory and Hammond 1980).

Albumin the most abundant of protein fractions, is synthesized in the liver at a constant rate in normal individuals of 150 to 250 mg/kg/day, resulting in the production of 10 to 18 g of albumin daily in a 70-kg man (Dumas *et al*, 1997). Albumin constitutes more than half of the total protein present in serum and is the major protein providing the critical colloid osmotic or oncotic pressure that regulates passage of water and diffusable solutes through the capillaries (Dumas *et al*, 1997). Albumin is generally measured by a dye-binding technique that utilizes the ability of albumin to form a stable complex with bromocresol green dye (Bollag and Edelstein, 1991). The BCG-albumin complex absorbs light at a different wavelength from the unbound dye. On the other hand the most widely used method of measuring serum total protein is the biuret reaction (Harris and Angal, 1989). The principle of this reaction is that serum proteins react with copper sulfate in sodium hydroxide to form a violet biuret complex. The intensity of the violet color is proportional to the concentration of protein. The current study used these techniques for assaying total protein and albumin, where the end point substrate obtained from these reactions were subjected to spectrophotometry to determine the concentration of the two molecular entities in serum.

Serum creatinine is derived from the breakdown of tissue creatine at a constant rate and is excreted by filtration by the kidneys with no reabsorption. Decline in kidney functional efficiency is often accompanied by a steady rise in the serum creatinine levels hence this

biomolecule is a reliable marker of kidney function pathology (Zamora, *et al.*, 2007). The Jaffe reaction using alkaline picrate method remains the cornerstone of most routine creatinine assays (Parvesh, *et al.,* 1981). Improvement to Jaffe assay have attempted to improve specificity and these have included the isolation of creatinine from common interfering substances by adsorption on to aluminium silicates such as Lloyd's reagent, the removal of interfering agents, dialysis, varying pH, and kinetic measurements (Avinash, *et al.,* 2013). Other assay methods of creatinine include use of reagents such as 3.5-dinitrobenzoic acid (Langley and Evans, 1936 and Benedict and Behre, 1936), 3,5-dinitrobenzoyl chloride (Parekh, *et al.,* 1976 and Critique, *et al.,* 1977) as well as the use of methy, 3,5-dinitrobenzioate in a mixture of dimethyl sulfoxide, methanol, and tetra methyl ammonium hydroxide (Sims and Parekh, 1977).

Automation has provided opportunity for efficient processing of increasing workloads, and incorporation of on-line dialysis to remove protein as well as bilirubin, important interfering substances in the Jaffe assay. Modification of the Jaffe method using multi -enzyme systems to improve the specificity of creatinine determination a method which under optimal conditions gives accurate results (Moss, *et al.,* 1975). These enzymatic techniques involve assaying the sample before and after enzymatic treatment with creatininase and creatinase for 1 hr. The current study employed enzyme kinetic assays since they provide similar accuracy as high performance liquid chromatography (HPLC) and currently form the basis of the most specific routine assays of creatinine levels.

Bilirubin is one of the most commonly used tests to assess liver function. Approximately 85% of the total bilirubin produced is derived from the heme moiety of hemoglobin when old red blood cells are broken down and processed by liver cells (hepatocytes). The resultant bilirubin is rapidly taken up by hepatocytes where it is conjugated with glucuronic acid to produce mono- and diglucuronide, which are excreted in the bile (Jansen *et al*, 1986). Diseases affecting the hepatobiliary system can interfere with one or more of the steps involved in the production, uptake, storage, metabolism, and excretion of bilirubin. This can lead to elevated bilirubin levels in blood (Bilirubinemia). The most widely used techniques for the assay of bilirubin in serum employ a coupling reaction with various diazo dyes in the presence of an accelerator (Westwood, 1991). The direct reaction in the absence of the accelerator gives a precise estimate of the conjugated and protein bound bilirubin. However other methods including enzymatic assays based on bilirubin oxidase, HPLC or dry-slide techniques have been employed. Enzymatic assyas appear to give results that favorably approximating the diazo dyes techniques (Kurosaka, *et al.,* 1998). Spectrophotometric techniques are also in use but with varying outcomes. The current study employed enzyme based assays for their suitability in clinical use.

Most enzymes occur in cells at higher concentrations than in plasma. During ordinary cell turnover, plasma enzyme levels reflect the balance between the enzyme's synthesis and release during and its catabolism and excretion (Eisenthal and Danson, 1992). An increase in the rate of the cells turnover or rate of cell damage usually results in raised plasma enzyme levels. Within hepatocytes the AST is predominantly a mitochondrial enzyme found in hepatocytes while ALT is predominantly a cytosolic enzyme. Serum ALT and AST is

elevated in diseases that destroy the liver cells among other tissues. Enzyme concentration is usually measured in terms of enzyme activity. This is done by measuring either the concetrations of the substrate used in a reaction or the concentration of a product formed (Bergmeyer and Gawehn, 1978). These can take one of the following methods; fluorimetric methods, potentiometric methods, microcalorimetric methods or spectrophotometric methods (Palmer, 1985). Fluorimetric methods use fluorescent substrate or products that permit sensitive kinetic measurement of enzyme reactions to be done, and is particularly useful for measuring enzyme activity of nucleotide co-enzymes (Bergmeyer and Gawehn, 1978). The disadvantage of this method is that many fluorescent compounds are unstable and that many substances often present as impurities in reagents or solvents can quench the radiation emitted by the fluorescent substance. In potentiometric methods, measurement of pH is done using glass electrodes in reactions from which either an acid or a base is produced (Palmer, 1985).

This method is typically useful for measuring the activity of hydrolytic enzymes. Microcalorimetris methods are founded on the basis that almost all enzyme catalyzed reactions are exothermic and involve the release of heat energy (Eisenthal and Danson, 1992). The temperature increase that results provides a non-specific method of measuring enzyme activity. Due to the miniscule heat changes microcalorimetric methods are useful when assays are done in excess enzymes and not for routine enzyme assays. Spectrophotometry involves the use of a chemically modified product to produce a substance with particular spectral properties (Palmer, 1985). It is a method that is convenient due to its simplicity and the precision possible with it. The current study employed automated spectrophotometric technique to assay the liver enzyme activity for these aforementioned qualities.

CHAPTER FOUR: RESULTS

4.1. Study Participants' Characteristics

Between the month of November 2012 and April 2013, 800 HIV positive participants and 406 HIV seronegative participants were enrolled into the study, with most of the participants in either group being females (see Table 4.1).

Table 4.1 Characteristics of Study Participants

CHARACTERSITICS OF PARTICIPANTS IN THE STUDY POPULATION		
	HIV Positive (n=800)	HIV negative (n=406)
	n (%)	n %
Males	331(41.4)	42(32)
Females	469(58.6)	364(68)
Old (≥50yrs)	120(15)	67(16.5)
Young (<50yrs)	680(85)	339(83.5)
Using ARV	639(79.9)	
ARV naïve	161(20.1)	
CD4+ <500	473(59.1)	
CD4+≥500	327(40.9)	

Key: CD4+-lymphocyte sub-group bearing CD4 membrane markers; ARV- anti-retroviral drugs

While the age range of participants spanned from 18 to 84 years, there was no significant disparity between the mean age of HIV infected participants and that of HIV negative individuals (38.1 v/s 38.0 years; p=0.865). Majority of the participants in either HIV+ or HIV- group were females 469 (58.6%) and 364 (68%)respectively. a greater proportion of the HIV-infected individuals had CD4 lymphocyte counts below 500cell/mm^3 (59.1%) and those using antiretroviral drugs were more (79.9%) than those not using the drugs. The least CD4 levels

recorded in HIV-infected participants was 1 lymphocyte cell/mm^3 and the highest was 11745cell/mm^3, with a mean CD4 count of 474.3 cells/mm^3 (Figure 4.1).

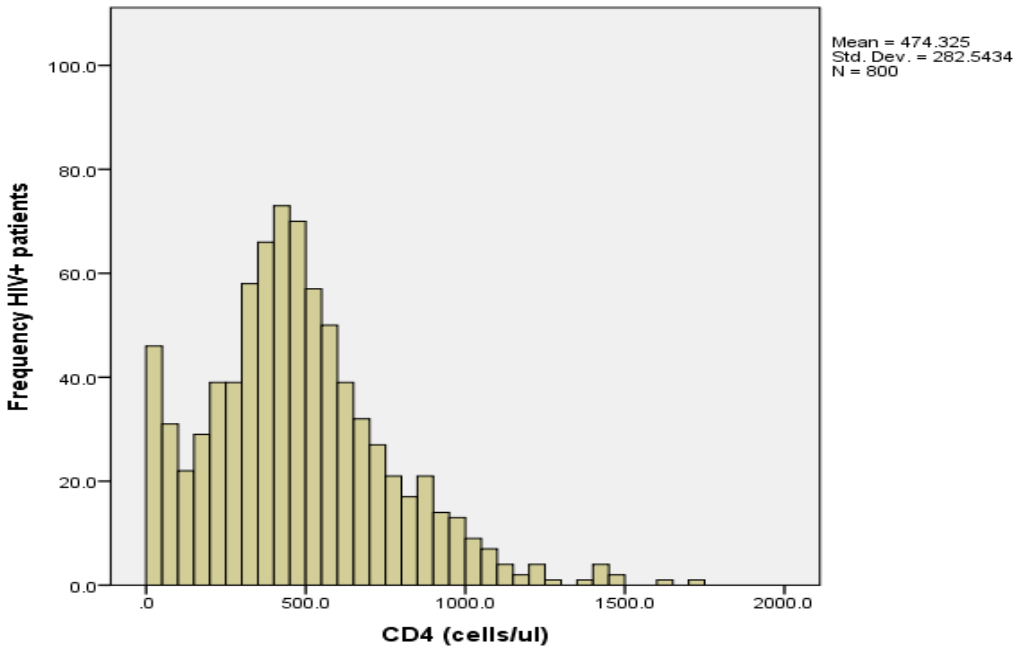


Figure 4.1: Distribution of CD4 Lymphocyte Count in HIV-infected Individuals

However 50% participants had CD4 levels ranging from 291 to 620 lymphocytes /mm^3. Majority of enrolled HIV infected individuals (79.9%) were using anti-retro viral drugs.

4.2. Kidney Function in HIV Infection

HIV-infection was associated with increased risk of decline in glomerular filtration rate compared to healthy participants (OR = 2.22; χ^2 = 44.7; p<0.0001). Overall therefore with a mean estimated glomerular filtration rate (eGFR) of 95.5mls/min, HIV negative individuals displayed more robust kidney function than participants with HIV infection whose mean eGFR was lower (88.1mls/min; t=3.1, p = 0.001). Similarly, the proportion of participants with normal eGFR (>90mls/min) was significantly higher in seronegative individuals than those afflicted with the HIV (55.9% v/s 36.4%, χ^2 = 55.1, p<0.0001). Mild decline in eGFR (60-90mls/min) was most common (40%) followed by moderate impairment of kidney function (eGFR; 30-60mls/min) while severe kidney function loss (eGFR<30mls/min) occurred least in seropositive individuals (see Figure 4.2).

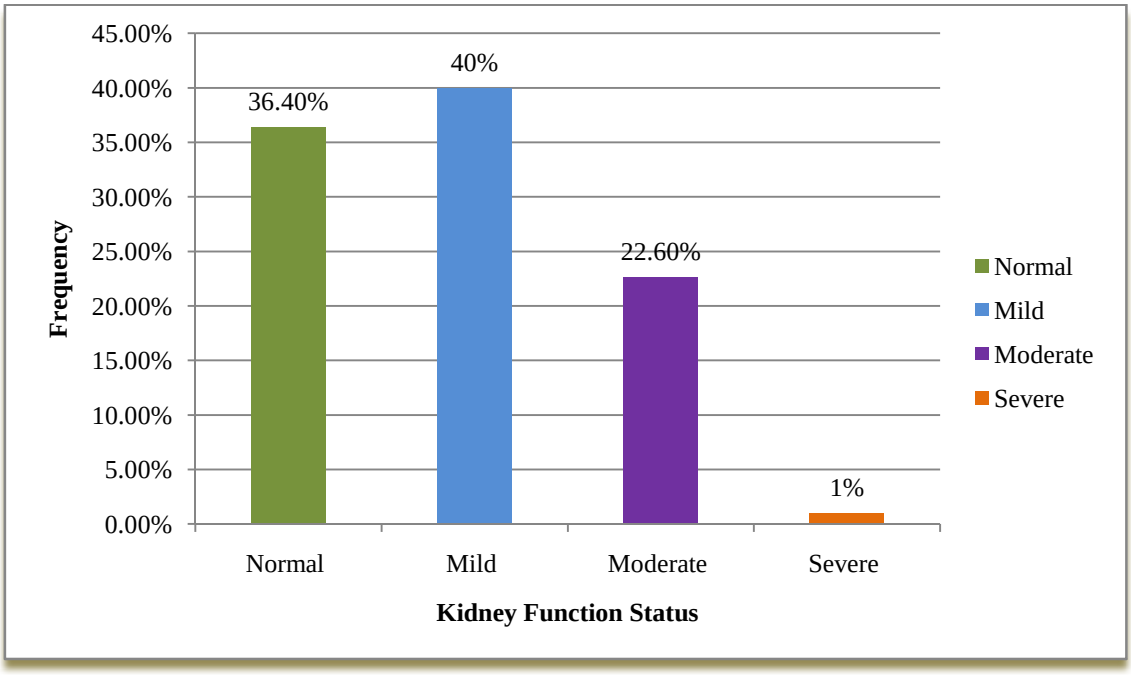


Figure 4.2: Kidney Function States in HIV-infected Individuals

Females with HIV infection had a higher tendency of developing disorders of glomerular filtration rate compared to HIV negative females (OR = 1.7; χ^2 = 10.4; p = 0.001). This was exemplified by the low mean glomerular filtration rates in females with HIV infection compared to seronegative females (82.2mls/min v/s 92.1mls/min; t = 3.1; p = 0.002) as well as a higher prevalence of impaired glomerular filtration rates (<90mls/min) in the former than the later category (69.1% v/s 56.6%; χ^2 = 24.6; p<0.0001). Equally, HIV+ males were more susceptible to developing impaired eGFR than seronegative males (OR = 2.8; p<0.0001). However among the former, only the rates of occurrence of eGFR disorders was more (55.9% v/s 31.6%; χ^2 = 34.9; p<0.0001) but mean glomerular filtration rates did not differ markedly from that of seronegative males (96.3 mls/min v/s 99.0mls/min; t = 0.826; p = 0.409).

The likelihood of developing eGFR disorders was indistinct in old patients irrespective of serological status (OR = 1.2; χ^2 = 0.3; p = 0.617) which was further highlighted by insignificant difference in prevalence of eGFR anomalies (72.8% v/s 69.7%; χ^2 = 4; p = 0.265) and their mean estimated glomerular filtration rate which were both lower than the normal physiological range in either group (76.9mls/min v/s 78.8mls/min; t = 0.5; p = 0.637). Among those participants below 50 years, similarly, marked contrasts were observed in kidney function states of those with HIV infection compared to those without HIV infections. The predisposition to developing glomerular filtration disorders was profoundly elevated in young HIV participants with HIV infection (OR = 2.9; χ^2 = 63.8; p<0.0001) and accordingly the prevalence of kidney function anomalies in participants below 50 years with HIV infection (62.3%) was more than the prevalence of such disorders in young HIV negative individuals (36%; χ^2 =76.1; p<0.0001). Similarly mean glomerular filtration rates in non-

infected young individuals was contrastingly higher than that of HIV infected participants of the same age category (100.9 mls/min v/s 89.7mls/min; t = 4.6; p <0.0001) Table 4.2.

Table 4.2: Kidney Function in Various Serological States (n=1206)

N		Mean eGFR (mls/min)	t	p-value	Rates of impaired e GFR	χ^2	p-value	Reference range (mls/min)
800	**HIV+**	88.1	3.1	0.001	(507)63.4	55.1	<0.0001	Males >70-140
406	**HIV-**	95.5			(179)44.1			Females >60-128
331	**Male HIV+**	96.3	0.8	0.409	(185)55.9	34.9	<0.0001	
130	**Male HIV-**	99	3		(50)36.1			
469	**Female**	82.2	3.1	0.002	(324)69.1	24.6	<0.0001	
276	**Female HIV-**	92.1			(156)56.6			
680	**<50 yrs**	89.7	4.6	<0.0001	(423)62.3	76.	<0.0001	
339	**<50 yrs HIV-**	100.9			(122)36			
120	**≥50yrs HIV+**	76.9	0.5	0.637	(87)72.8	4	0.265	
67	**≥50yrs HIV-**	78.8			(47)69.7			

However within HIV-infected individuals, males displayed significantly stable kidney function than HIV+ females (eGFR 96.3mls/min v/s 82.2mls/min, t = 4.3, p<0.0001). As such a lower prevalence of impaired kidney function was observed in seropositive males compared to seropositive females (55.9% v/s 63.4%; χ^2 = 34.7; p <0.0001). Age difference among HIV-infected individuals, was also associated with marked disparities in kidney function with patients above 50 years having a significantly lower mean eGFR (78.4mls/min) than patients younger than 50 years (89.8mls/min, t = 2.7, p = 0.007) while the prevalence of eGFR disorders was also higher in old than in young patients (72.5% v/s 62.1%; χ^2 = 15.1; p = 0.002).

Mean eGFR was not affected by ARV use (89mls/min v/s 84.6mls/min; p = 0.272) and neither did the rates of occurrence of impaired kidney function improve in patients using ARVs compared to those not on ARVs (63.6% v/s 65.8%; $\chi^2 = 2.7$; p = 0.434). Initial decline in CD4 immune levels was accompanied with gradual rise in levels of mean GFR. However in patients with profound immune depletion (<500 cell/mm^3), there was associated steady and eventual significant decline in mean glomerular filtration rate. Indeed 5 out of 8 cases recorded with severe reduction in eGFR were participants with CD4 levels below 200cells/mm^3 (Figure 4.3).

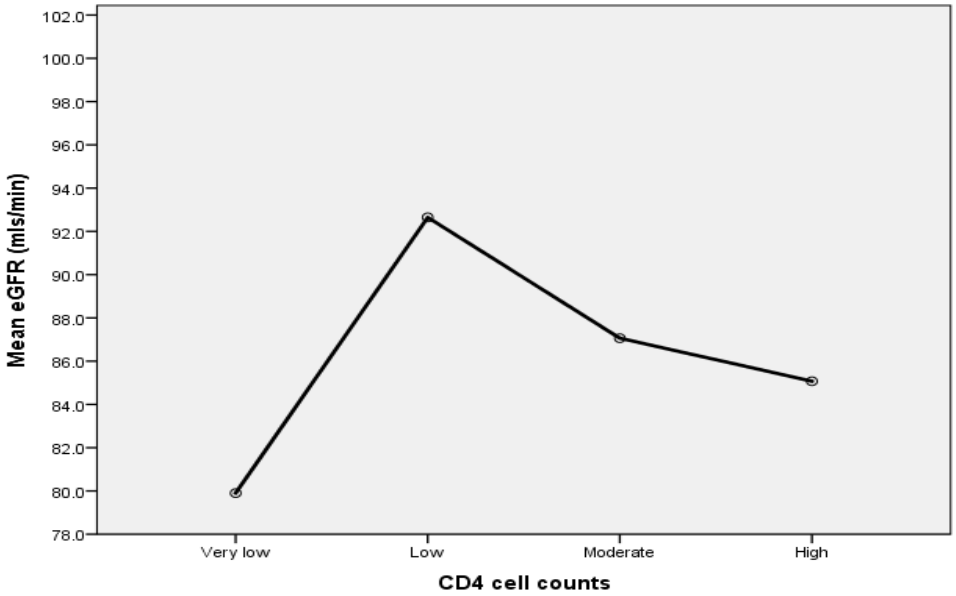


Figure 4.3: Mean Glomerular Filtration Rate at Different CD4 Cell Levels

4.3. Kidney Function Markers

Serum creatinine and urea's intricate co-variance with glomerular function were further explored as markers of kidney function in order to elaborate the relationship between kidney health and function to body fluids and electrolyte. The range of serum creatinine levels in HIV+ individuals was 11.5 – 410.5µmol/l, with a mode of 98µmol/l, and a mean =95.2µmol/l ±SD(35). On the other hand creatinine levels in healthy controls was 40–173.1 µmol/l, with a mode of 89.6µmol/l and a mean =86.2µmol/l ±SD(20.4). Hence whereas in seropositive individuals, levels of serum creatinine fell on either side of the normal physiological reference range (26 - 97µmol/l), for the healthy controls there was no value that fell below the lower limit. The creatinine levels that exceeded values above the upper limit of the reference range were the only impaired values observed in the healthy controls.

There was a higher tendency of developing serum creatinine anomalies in HIV-infected individuals than seronegative individuals (OR = 2.64; χ^2 = 32.9; p<0.0001). As such a higher proportion of HIV infected patients (26.1%) were observed with serum creatinine disorder (defined as < 40µmol/l or > 120µmol/l) than the control population (11.8%), χ^2 = 32.6, p<0.0001). Mean serum creatinine levels in HIV positive participants was also significantly raised than that in the seronegative group (95.2µmol/l v/s 86.2µmol/l, p<0.0001) see Figure 4.4.

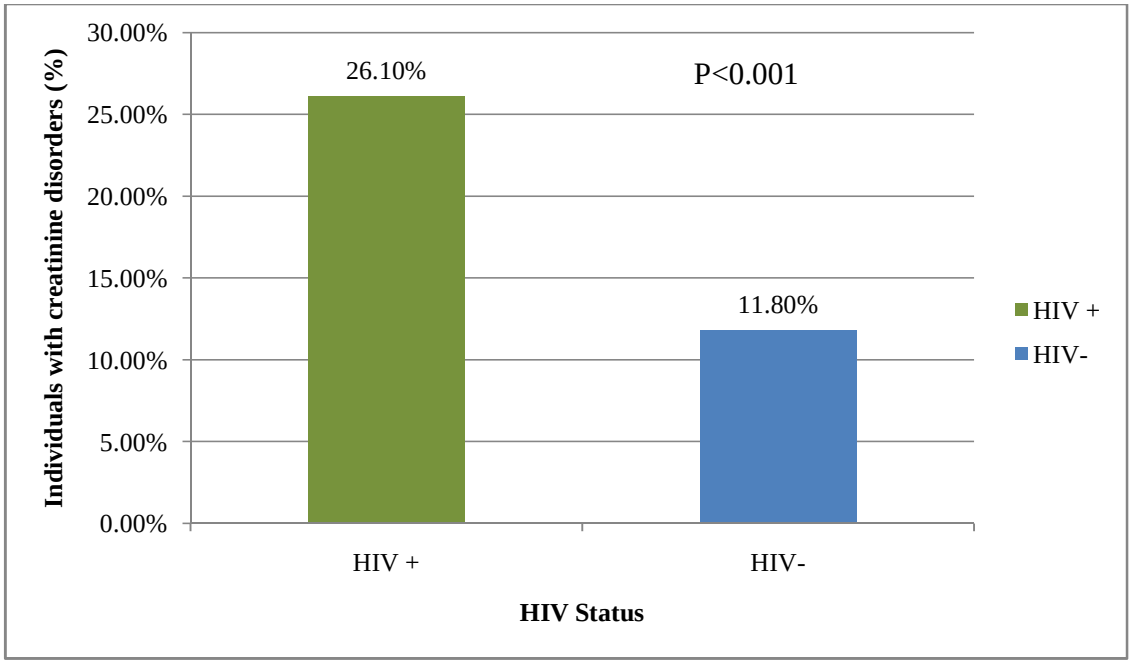


Figure 4.4: Prevalence of Creatinine Disorders by HIV Status

HIV infection was also associated with significant differential impacts on serum creatinine levels in gender and age categories between the two study groups. Males with HIV infection manifested a higher propensity of developing creatinine disorders than males without HIV infection (OR = 2; χ^2 = 4.6; p = 0.03) and similarly females with HIV infection were more likely to develop creatinine disorders than females without HIV infection (OR = 3.2; χ^2 = 34.04; p<0.0001). The prevalence of serum creatinine disorders in HIV-infected males (18.1%) was therefore significantly more than in HIV negative males (10%; p = 0.031) even though their mean serum creatinine levels were indistinct (94.9µmol/l v/s 89.8µmol/l, p = 0.07). On the other hand, mean creatinine level in seropositive females was significantly

raised than in uninfected females (95.4µmol/l v/s 84.6µmol/l, p<0.0001), as was the prevalence of abnormal serum creatinine states (31.8% v/s 12.7%, p<0.001).

In the different age categories, the impact of HIV infection on serum creatinine was more pronounced in participants below 50 years compared to those above 50 years. HIV infected individuals below 50 years were more likely to develop creatinine disorders than uninfected participants within the same age category (OR = 2.94; χ^2 = 33.4; p<0.0001). In contrast, HIV infected participants above 50 years did not depict a higher likelihood of developing creatinine disorders than uninfected individuals within the same age bracket (OR = 1.5; 1; p = 0.320). In younger participants (<50 years) mean creatinine level was accordingly noticed to be significantly higher in infected as opposed to uninfected counterparts (95.1µmol/l v/s 85.8µmol/l, p<0.0001) as was the prevalence of anomalous creatinine levels (27.1% v/s 11.2%, χ^2 = 33.4, p<0.0001). However the proportion with abnormal creatinine levels among old HIV infected patients (20.8%) was not different from old HIV negative participants (14.9%), p = 0.321 and neither was the mean creatinine level (95.8µmol/l v/s 88.5µmol/l, p = 0.09) Table 4.3.

Table 4.3: Serum creatinine in Various Study Population Attributes

N		Mean creatinine (µmol/l)	t	p-value	Creatinine disorder N (%)	χ^2	p-value	Reference range
800	**HIV+**	95.2	5.6	<0.0001	(209)26.1	32.6	<0.0001	26 - 97µmol/l
406	**HIV-**	86.2			(48)11.8			
331	**Male HIV+**	94.9	1.8	0.07	(60)18.1	4.6	0.031	
130	**Male HIV-**	89.8			(13)10			
469	**Female HIV+**	95.4	5.6	<0.0001	(149)31.8	34	<0.0001	
276	**Female HIV-**	84.6			(35)12.7			
680	**<50 yrs HIV+**	95.1	5.3	<0.0001	(184)27.1	33.4	<0.0001	
339	**<50 yrs HIV-**	85.8			(40)11.2			
120	**≥50yrs HIV+**	95.8	1.5	0.09	(25)20.8	0.986	0.321	
67	**≥50yrs HIV-**	88.5			(10)14.9			

Among HIV infected individuals 26.1% of participants had abnormal levels of creatinine out of which majority were hypercreatininemia (91%) while 9% were hypocreatininemia. HIV afflicted females had a higher predisposition of developing creatinine disorders than HIV positive males (OR = 1.8; χ^2 = 13; p = 0.0003), hence females with HIV had a higher prevalence of serum creatinine disorders than their male counterparts (31.8% v/s 18.1%, χ^2 = 18.7, p<0.0001) despite their mean creatinine levels being indistinct (95.4µmol/l v/s 94.9µmol/l, p = 0.850).

The risk of developing creatinine disorders was different between HIV infected participants above 50 years compared to younger HIV infected participants (OR = 0.7; χ^2 = 2.05; p = 0.152). HIV + patients not using ARVs were as equally prone to developing creatinine disorders as those using ARVs (OR = 1.4; χ^2 = 2.54; p = 0.132). Hence there was no significant contrast in the prevalence of serum creatinine disorders in HIV afflicted

individuals using ARVs compared to prevalence of creatinine disorders in HIV infected participants not using ARVs (24.9% v/s 31.1%, p = 0.111) and neither was the mean serum creatinine levels in both groups (94.7µmol/l v/s 97.1µmol/l, p = 0.438).

Decline in CD4 cell below 500 cells/mm3, was associated with a steady rise in serum creatinine levels surpassing pre-immune depletion levels (see Figure 4.5).

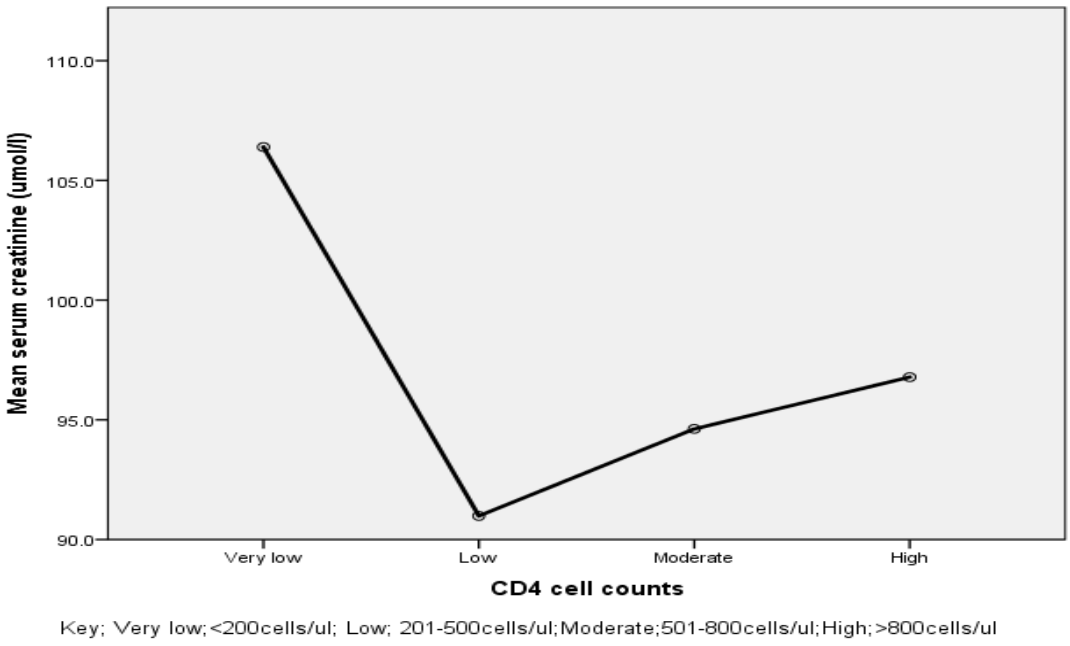


Figure 4.5: Mean Serum Creatinine Levels with Progressive Immune Depletion

The range of serum urea in HIV + and HIV – spanned beyond either side of the normal reference range of 1.7 – 6.5µmol/l. However the mean and mode values for both groups fell within the normal physiological range. Nonetheless the mean serum urea values in HIV-infected participants (4.6mmol/l) was significantly raised than in HIV negative control (4.1mmol/l; p<0.0001) as was the proportion with abnormal urea levels (4.4% v/s 0.5%, χ^2 = 13.7, p<0.0001). Therefore, the risk of developing anomalously altered serum urea was

observed to be higher in HIV positive individuals compared to uninfected counterparts (OR = 9.24; χ^2 = 13.65; p = 0.0002).Comparison of similar gender by HIV status, revealed that among HIV infected males, the prevalence of abnormal urea levels was demonstratively higher than uninfected males (6% v/s 0%, χ^2 = 8.2, p = 0.004) despite mean urea level insignificantly changing in the former (4.6mmol/l v/s 4.3mmol/l, p = 0.07). On the other hand HIV+ females presented with markedly different outcomes in both mean urea levels (4.6mmol/l v/s 4.1mmol/l, p<0.0001) and prevalence of abnormal urea levels (3.2% v/s 0.7%, χ^2 = 4.8, p = 0.029) from uninfected females. Similar outcomes were observed in younger infected participants (<50 years) among whom mean urea level was significantly higher than in uninfected counterparts (4.6mmol/l v/s 4.1mmol/l p<0.0001) as well as the prevalence of abnormal creatinine states, (4.6% v/s 0.6%, χ^2 = 11.4, p = 0.001). However in old participants (>50 years) HIV infection did not herald significant alteration in both mean urea levels (4.5mmol/l v/s 4.2mmol/l, p = 0.142) and prevalence of anomalous urea states (3.3% v/s 0%, p = 0.131).

Of HIV infected participants, 4.4% had serum urea disorders (defined as <2mmol/l or >7.5mmol/l). Majority of the urea anomalies (65.7%) were due to accumulation of urea in circulation (uremia) while a few (34.3%) were hypouremia. HIV-infected males had a higher likelihood of developing abnormally raised serum urea than seropositive females (OR = 2.0; χ^2 = 3.8; p = 0.05). Rates of occurrence of urea disorders in HIV infection was therefore more in males compared to females (6% v/s3.2%, p = 0.05), despite their mean urea levels remaining indistinct (p = 0.856). Occurrence of disorders of urea states was however evenly

distributed between older and younger seropositive individuals (OR = 0.722; χ^2 = 0.4; p = 0.543).

Initial decline in CD4 cell lymphocyte levels was accompanied with corresponding decline in serum urea levels but CD4 cells within AIDS defining range were associated with steady rise in serum urea levels attaining levels higher than pre-immune depletion states (see Figure 4.6). Anti-retroviral therapy was not a determinant of impaired serum urea states in HIV infection (OR = 0.5; χ^2 = 1.7; p = 0.190).

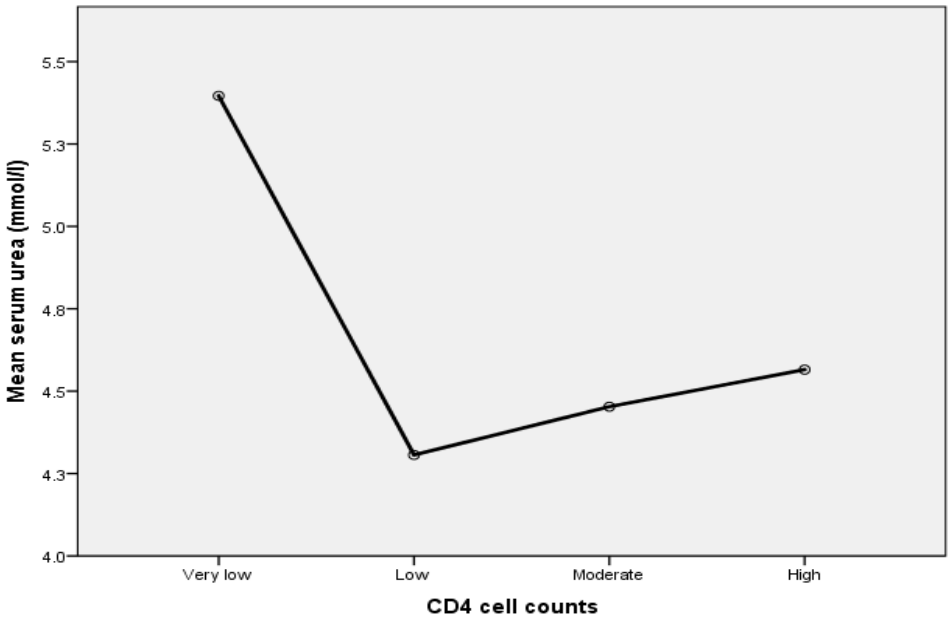


Figure 4.6: Mean Serum Urea Levels at Different CD4 Cell Levels

4.4. Liver Function Markers in HIV infection

Several liver function markers were used to delineate the state of liver function in HIV infection.

4.4.1. Liver transaminases

Data on liver function indicators showed that seropositive participants were more likely to present with abnormally altered serum aspartate-aminotransferase (AST) and serum alanine-aminotransferase (ALT) levels compared to seronegative participants (OR= 4.6; χ^2 = 56.9; p<0.0001 and OR = 6.2; χ^2 = 11.7; p = 0.001, respectively). Accordingly, HIV positive individuals had a higher prevalence of elevated AST activity (defined as >50U/L) than HIV negative participants (24.5% v/s 6.7%, χ2 = 56.9; p<0.0001) as well as prevalence of elevated ALT activity (> 65U/l) was higher in HIV-infected participants (4.4% v/s 0.7%, χ^2 = 11.7, p = 0.001). Mean ALT and AST activity were also noted to be elevated among seropositive participants than their seronegative counterparts (36.5 U/L v/s 30.7 U/L, p<0.0001 and 45.1U/L v/s 36.9U/L, p<0.0001, respectively) see Table 4.4.

Table 4.4: Distribution of AST by Serological Status

N		AST levels (U/L)	t	p-value	AST disorders N (%)	χ^2	p-value	Reference range
800	**HIV+**	45.1	10.3	<0.0001	(196)24.5	56.9	<0.0001	0 – 50U/l
406	**HIV-**	36.9			(27)6.7			
331	**Male HIV+**	46	5.7	<0.0001	(90)27.2	19.1	<0.0001	
130	**Male HIV-**	37.2			(11)8.5			
469	**Female HIV+**	44.4	8.7	<0.0001	(106)22.6	35.8	<0.0001	
276	**Female HIV-**	36.8			(16)5.8			
680	**<50yrs HIV+**	45.3	7.8	0<.0001	(167)24.6	47.1	<0.0001	
339	**<50yrs HIV-**	36.9			(23)6.8			
120	**≥50yrs HIV+**	43.6	3.6	<0.0001	(30)24.2	9.8	0.002	
67	**≥50yrs HIV+**	37.1			(4)6			

Key; ALT-Alanine-aminotransferase, AST-Aspartate aminotransferase

Between the two study groups, females with HIV infection had a higher propensity of developing AST and ALT anomalies than females without HIV infection (OR = 4.8; χ^2 = 35.8; p<0.0001 and OR = 15.5; χ^2 = 12.7; p<0.0001, respectively). Thus, 22.6% of seropositive females had deranged AST activity which was significantly more than the prevalence of abnormal AST activity in the HIV negative females (5.8%, p<0.0001). Similarly, 5.4% of HIV- infected females had clinically elevated ALT levels as opposed to 0.4% of HIV-negative females (p<0.0001). There was also lack of parity of mean AST and ALT levels in females with and without HIV infection (44.4 U/L v/s 35.8 U/L, t = 8.7; p<0.0001and 35.8 U/L v/s 30.2 U/L; t = 6.3; p<0.0001). However, among males the risk noted was of developing elevated AST and not ALT activity in HIV –infected individuals compared to HIV negative counterparts (OR = 4.0; χ^2 = 19.1; p<0.0001). Correspondingly, only the prevalence of AST anomalies in HIV- infected males (27.2%) was significantly higher than the prevalence of AST anomalies in HIV negative males (8.5%; χ^2 = 19.2; p <0.0001) while rates of ALT anomalies were not different between males with and without

HIV infection. However, at 46 U/L and 37.8 U/L mean AST and ALT in HIV seropositive males were significantly dissimilar from 37.2 U/L and 31.9 U/L observed in males in the control group ($t = 5.7$; $p<0.0001$ and $t = 3.3$; $p = 0.001$, respectively).

In the different age categories, the impact of HIV infection on liver enzyme activity was more pronounced in younger (<50 years) than among the older (≥50 years) individuals. Younger HIV infected patients were more susceptible to AST and ALT disorders than individuals in the same age category who were seronegative (OR = 4.5; $\chi^2 = 47.1$; $p <0.0001$ and OR= 5.7; $\chi^2 = 10.5$; $p = 0.001$). Occurrence of elevated AST and ALT activity in HIV- infected young patients was therefore significantly more frequent than in HIV negative participants of the same age category (24.6% v/s 6.8% , $\chi^2 = 47.1$; $p<0.0001$ and 4.9% v/s 0.9%; $\chi^2 = 10.5$; $p = 0.001$, respectively). Mean AST and ALT levels in HIV- infected participants below 50 years was also significantly raised than in young HIV- negative participants ($t = 9.6$; $p <0.0001$ and $t = 6.5$; $p <0.0001$, respectively). Among older participants on the other hand, whereas HIV infection was accompanied with significant changes in mean serum AST and ALT ($t = 3.6$; $p <0.0001$ and $t = 2.9$; $p = 0.004$), only AST activity was significantly raised in those infected compared to seronegative counterparts (24.2% v/s 6%, $\chi^2 = 9.8$; $p = 0.002$). The prevalence of ALT anomalies was not different between those with HIV compared to those without ($\chi^2 = 1.13$; $p = 0.288$) (Table 4.5).

Table 4.5: Distribution of ALT Levels by Serological Status

N		ALT levels (U/L)	t	p-value	ALT imbalance n (%)	χ^2	p-value	Reference range (U/L)
800	**HIV+**	36.5	7.2	<0.0001	(35)4.4	11.7	0.001	Males; 0-70
406	**HIV-**	30.7			(0)0			Females :0-65
331	**Male HIV+**	37.8	3.3	0.001	(10)3	0.8	0.368	
130	**Male HIV-**	31.9			(2)1.5			
469	**Female HIV+**	35.8	6.3	<0.0001	(25)5.4	12.7	<0.0001	
276	**Female HIV-**	30.2			(1)0.4			
680	**<50yrsHIV+**	36.6	6.5	0<.0001	(33)4.9	10.5	0.001	
339	**<50yrs HIV-**	30.8			(3)0.9			
120	**≥50yrsHIV+**	36.1	2.9	0.004	(2)1.7	1.13	0.288	
67	**≥50yrsHIV-**	30.2			(0)0			

Key; ALT- Alanine aminotransferase; AST- Aspartate aminotransferase

Among HIV afflicted participants, the tendency to develop abnormally raised serum ALT and AST was not distinctly disparate between the two gender categories either (OR = 0.6; χ^2 = 2.5; p = 0.116 and OR = 1.3; χ^2 = 2.21; p = 0.137, respectively). Thus the proportion with abnormally elevated AST and ALT activities in males (27.2% and 3%), was not dissimilar from that of females (22.6% and 5.3%; p = 0.137 and p = 0.116, respectively). Similarly mean AST and ALT level in HIV- infected males (46 U/L and 37.6 U/L), was comparable to that of females, 44.4 U/L and 35.8 U/L; p = 0.226 and 0.129, respectively.

Table 4.6: Prevalence of Liver Enzymes Imbalance in HIV Patients (n=800)

N	Patient	Liver enzymes					
		AST			ALT		
		N (%)	χ^2	p-value	N (%)	χ^2	p-value
331	**Male**	(90)27.2	2.2	0.137	(10)3	2.5	0.116
469	**Female**	(106)22.6			(25)5.3		
680	**<50 yrs**	(165)24.2	0.008	0.927	(14)2	2.5	0.116
120	**>50yrs**	(30)24.6			(6)4.9		
639	**ARV users**	(159)24.9	0.251	0.616	(28)4.4	<0.0001	0.985
161	**ARV naive**	37)23			(7)4.3		

Key; ARV-antiretroviral drugs, AST- Aspartate aminotransferase, ALT- Alanine aminotransferase

In the different age categories, older HIV- infected participants (>50 years) did not exhibit a higher predisposition of developing abnormal AST and ALT states than younger HIV infected participants (OR = 1.8; χ^2 = 0.01; p = 0.920 and OR = 0.33; χ^2 = 2.38; p = 0.115, respectively). Correspondingly mean AST (43.6 U/L) and ALT (36.1 U/L) in HIV positive over 50 years were not significantly higher than 45.3 U/L and 36.6 U/L recorded in HIV infected participants below 50 years (t = 0.967; p = 0.334 and t = 0.33; p = 0.741). Indeed there was no correlation between age and AST level or ALT (r = 0.018, p = 0.613 and r = 0.003, p = 0.924) and virtually no variation in AST or ALT level could be attributed to age change (r^2 <0.0001) (Figure 4.7).

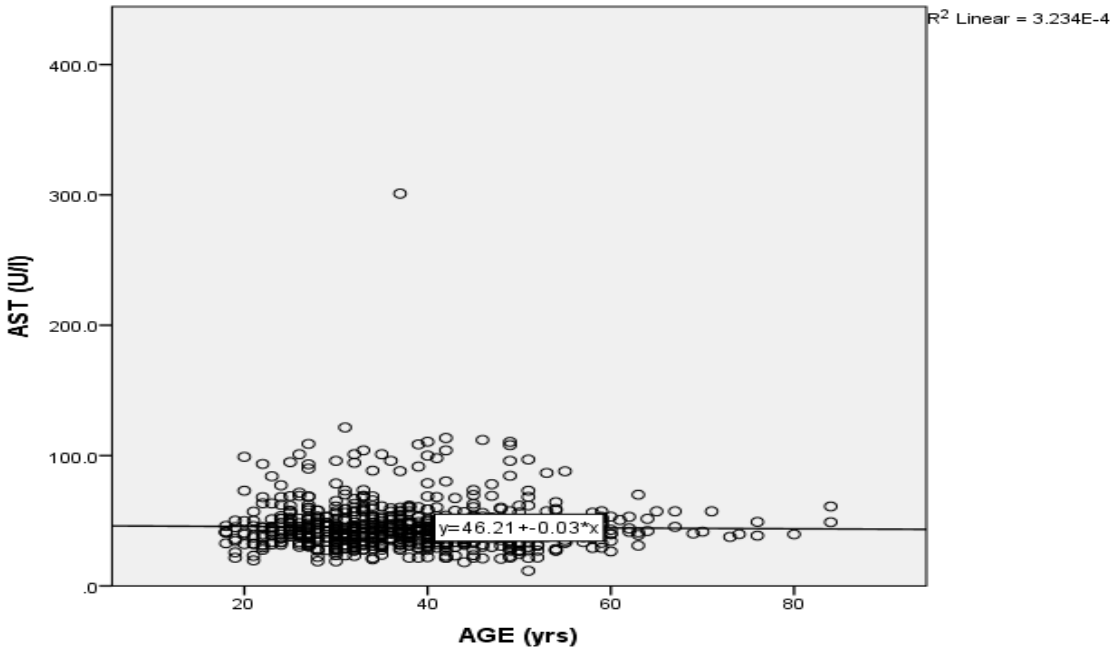
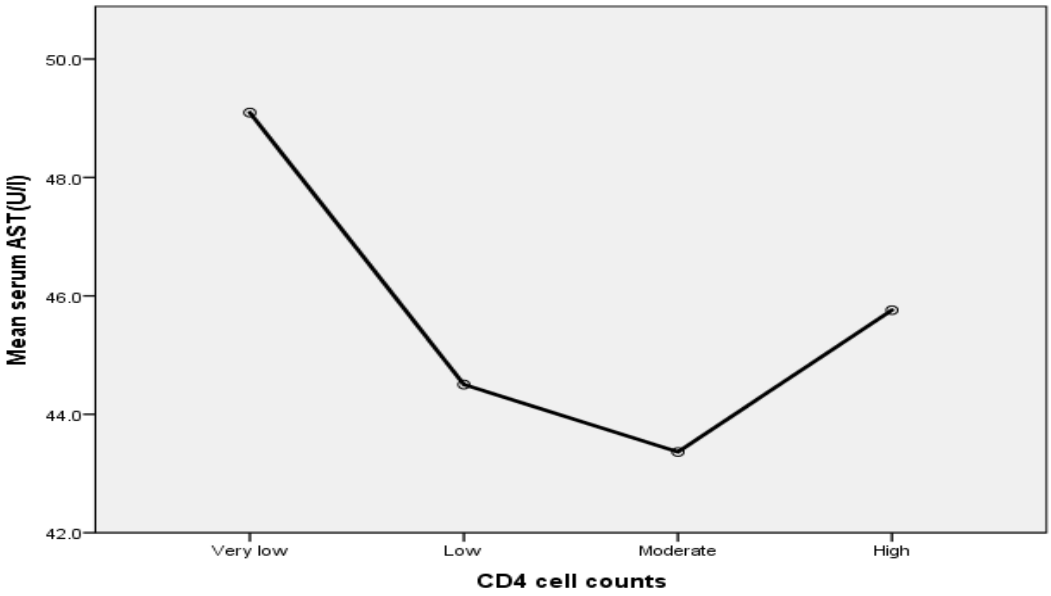


Figure 4.7: Correlation of AST with age in HIV Infected Patients (r = 0.018)

Though mean AST and ALT activity did not fall out of the normal physiological range in all CD4 categories, depletion of CD4 lymphocytes below 800 cells/mm3 was accompanied with steady rise in mean activity levels of both liver enzymes (see Figure 4.8).



Key; Very low;<200cells/ul; Low; 201-500cells/ul;Moderate;501-800cells/ul;High;>800cells/ul

Figure 4.8: Mean serum aspartate- aminotransferase at different CD4 cell levels

The risk of developing anomalously elevated AST and ALT activity was not significantly lowered in patients using ARVs either (OR= 0.9; χ^2 = 0.25; p = 0.617 and OR = 0.99; p = 1). Correspondingly, mean AST and ALT activity in HIV infected patients using ARVs was not different from mean AST and ALT activity in HIV infected patients not using ARVs (44.3 U/L v/s 45.3 U/L, t = 0.615; p = 0.538 and 36.2 U/L v/s 36.6 U/L; t = 0.329; p = 0.742, respectively).

4.4.2. Bilirubin levels in HIV infection

Proclivity of impairment of serum bilirubin (direct and total) levels was higher in HIV-positive participants than HIV negative participants (OR = 1.32; χ^2 = 4.96; p = 0.026 and p = 0.002, respectively). In effect therefore, observed prevalence of bilirubin disorders (total and

direct) in HIV positive participants was more than in HIV negative controls (2.3% v/s 0%, χ^2 = 9.3, p =0.002 and 43.1% v/s 36.5%, χ^2 = 4.96, p = 0.026) (see Table 4.7 for direct bilirubin values).

Table 4.7: Distribution of direct Bilirubin by Serological Status (n =1206)

N	Demographic characteristics	D/Bilirubin levels (µmol/l)	t	p-value	D/Bilirubin disorders N (%)	χ^2	p-value	Reference range
800	**Hiv+**	4.9	3.7	<0.0001	(345)43.1	4.9	0.026	0- 4.2µmol/l
406	**Hiv-**	4.2			(148)36.5			
331	**Male HIV+**	6.3	3.2	0.002	(138)41.7	1.2	0.275	
130	**Male HIV-**	5			(47)36.2			
469	**Female HIV+**	4.9	4.2	<0.0001	(207)44.1	4.1	0.04	
276	**Female HIV-**	4.1			(101)36.6			
680	**<50 yrs HIV+**	4.8	4.6	<0.0001	(293)43.1	3.96	0.046	
339	**<50 yrs HIV-**	4.2			(124)36.6			
120	**≥50yrs HIV+**	5.2	1.4	0.158	(52)43.3	1.0	0.316	
67	**≥50yrs HIV+**	4.1			(24)35.8			

By serological status, the propensity of developing direct bilirubin anomalies was greater in females afflicted with HIV than females without HIV infection (OR = 1.4; χ^2 = 4.1; p = 0.04). There was therefore manifest differences of mean direct bilirubin in HIV+ females and uninfected females (4.9 µmol/l v/s 4.1µmol/l; t = 4.2; p<0.0001) and a higher prevalence of direct bilirubin disorders (>4.2µmol/l) in the former than the later group (44.1% v/s 36.6; p = 0.04). 6.1 v/s 4.9; t = 5; p<0.0001). Similarly, serum total bilirubin disorders (defined as >19µmol/l) were mostly prevalent in seropositive females than in seronegative females (2.6% v/s 0%; p = 0.007). However, among males despite the mean direct and total bilirubin levels being significantly higher in HIV infected than uninfected individuals (4.9µmol/l v/s 4.2µmol/l; t = 2.6; p = 0.01 and 6.3µmol/l /v/s 5µmol/l; t = 3.2; p = 0.002), the prevalence of bilirubin disorders (total and direct) was not different between those with HIV infection

compared to those without HIV infection (1.8% v/s o%; χ^2 = 2.4; p = 0.122 and 41.7% v/s 36.2% ;χ^2 = 1.2; p = 0.275, respectively). Within the same age categories, the impact of HIV infection on bilirubin was comparatively more pronounced in younger than among the old participants. Mean serum total and direct bilirubin was raised in young HIV infected patients compared to their uninfected individuals within the same age bracket (6.1µmol/l v/s 5µmol/l; t = 5.5; p<0.0001 and 4.8µmol/l v/s 4.2µmol/l; t = 4.6; p<0.0001). Equally, the frequency of bilirubin disorders was higher in HIV infected young participants than in uninfected individuals of the same age group (2.2% v/s 0%; p = 0.006 and 43.1% v/s 36.6%; p = 0.046). On the other hand, among participants above 50 years HIV infection was accompanied with significant alteration of the mean of serum total bilirubin (6.7µmol/l v/s 5µmol/l; p = 0.03; see Table 4.8) and not of serum direct bilirubin levels.

Table 4.8: Total Bilirubin Levels in Participants (n=1206)

N		T/Bilirubin levels (µmol/l)	t	p-value	T/Bilirubin disorders N (%)	χ^2	p-value	Reference range (µmol/l)
800	**HIV+**	6.2	5.7	<0.0001	(18)2.3	9.3	0.002	0-17
406	**HIV-**	5			(0)0			
331	**Male HIV+**	6.3	3.2	0.002	(6)1.8	2.4	0.122	
130	**Male HIV-**	5			(0)0			
469	**Female HIV+**	6.1	5	<0.0001	(12)2.6	7.2	0.007	
276	**Female HIV-**	4.9			(0)0			
680	**<50 yrs HIV+**	6.1	5.5	<0.0001	(15)2.2	7.6	0.006	
339	**<50 yrs HIV-**	5			(0)0			
120	**≥50yrs HIV+**	6.7	2.2	0.04	(2)1.7	1.2	0.286	
67	**≥50yrs HIV+**	5			(0)0			

Elevated serum bilirubin were the only the forms of bilirubin disorders displayed in seropositive individuals. Among HIV afflicted participants, the susceptibility to developing abnormally raised serum direct bilirubin and total bilirubin was not dissimilar between the

two gender categories (OR = 0.7; χ^2 = 0.5; p = 0.483 and OR = 0.9; χ^2 = 0.5; p = 0.492 respectively). Therefore the proportion with abnormally elevated total bilirubin and direct bilirubin activities in males (1.8% and 41.7%), was not different from that of females (2.6% and 44.1%; p = 0.491 and p = 0.492 respectively). Similarly mean total bilirubin and direct bilirubin level in HIV- infected males (6.3µmol/l and 4.9 µmol/l), were not distinct from that of females, 6.1µmol/l and 4.9µmol/l; p = 0.613 and 0.805 respectively (see Table 4.9).

Table 4.9: Prevalence of Bilirubin Disorders in HIV Infected Individuals (n=800)

N	Patient attributes	Serum bilirubin					
		Impaired Total bilirubin			Impaired Direct bilirubin		
		N (%)	χ^2	p-value	N (%)	χ^2	p-value
331	Male	(6)1.8	0.491	0.484	(138)41.7	0.473	0.492
469	Female	(12)2.6			(207)44.1		
680	<50 yrs	(15)2.2	0.040	0.841	(293)43.1	0.002	0.960
120	>50yrs	(3)2.5			(52)43.3		
639	ARV users	(83)13	0.671	0.413	(280)43.8	0.623	0.430
161	ARV naive	(8)5			(65)40.4		

Key; ARV- antiretroviral drugs

The likelihood of occurrence of total and direct bilirubin was not aggravated either in older HIV positive individuals than in young HIV infected participants (OR = 1.14; 95% C.I. (0.3 – 3.99) and OR = 1.01; p = 1.0, respectively). No correlation was demonstrated between age and total bilirubin level or direct bilirubin (r = 0.018; p = 0.613 and r = 0.003; p = 0.924) and virtually no variation in total bilirubin and direct bilirubin level could be attributed to age change (r^2 <0.0001).

The likelihood of occurrence of impaired circulating bilirubin (total and direct) levels was not different in HIV infected participants using or not using ARVs (OR= 1.54; 95% C.I. (0.5 –

4.4) and OR = 0.9; χ^2 = 0.6; p = 0.432). Correspondingly, mean total bilirubin and direct bilirubin level in HIV- infected patients using ARVs was not significantly different from mean total and direct bilirubin in HIV infected patients not using ARVs (6.2µmol/l v/s 6.1µmol/l, t = 0.261; p = 0.794 and 4.9µmol/l v/s 4.8µmol/l; t = 0.306; p = 0.760, respectively). Even though the prevalence of clinically elevated total bilirubin and direct bilirubin states was not related to changes in CD4 lymphocytes count (χ^2 = 3.3, p = 0.342 and χ^2 = 1.4, p = 0.711, respectively), extreme CD4 depletion (<200cell/mm3) was associated with highest mean serum total and direct bilirubin levels (Figure 4.9).

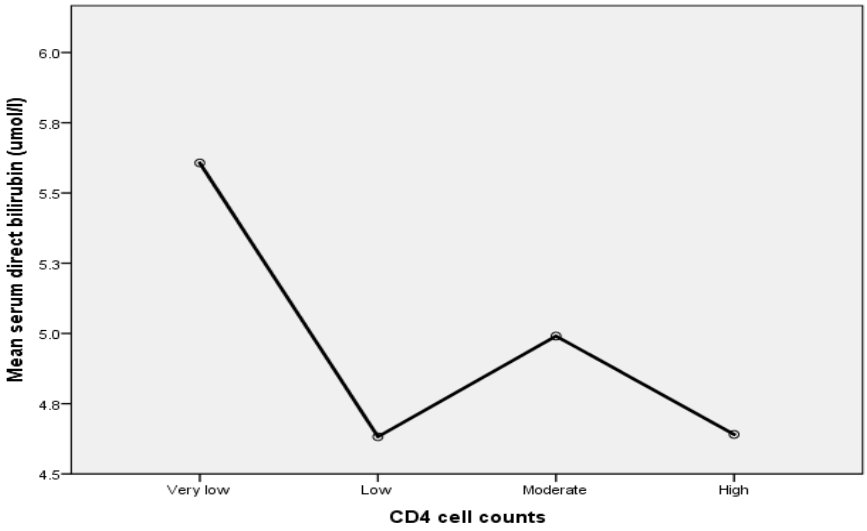


Figure 4.9: Mean Serum Direct bilirubin levels by Immune Status

4.4.3. Serum total Protein and Albumin states in HIV infection

Infection with HIV virus was accompanied with a higher susceptibility of developing pathologically altered albumin and total protein levels (OR = 1.38; χ^2 = 6.89; p = 0.009 and OR = 1.99; χ^2 = 30.5; p<0.0001, respectively). In effect therefore, the proportion with deranged protein levels (defined as <65g/l or >80g/l) and albumin levels (<35g/l or >50g/l),

was higher in HIV- infected patients than their HIV negative counterparts (52.8% v/s 36%, χ^2 = 30.5, p<0.0001 and 60.1% v/s 52.2%, χ^2 = 6.9, p = 0.009, respectively). Mean total protein (64g/l) and albumin (32.8g/l) in the HIV- infected individuals declined substantially below those of HIV negative controls (67.1g/l., p<0.0001 and 34.5g/l, p<0.001, respectively) (see Table4.10 for albumin values).

Table 4.10: Serum Albumin Levels by Serological Status

N		Albumin levels (g/l)	t	p-value	Albumin disorders N(%)	χ^2	p-value	Reference range g/l
800	**HIV+**	32.8	5.3	<0.0001	(481)60.1	6.9	0.009	35-50
406	**HIV-**	34.5			(212)52.2			
331	**Male HIV +**	32.5	3.8	<0.0001	(209)63.1	4	0.047	
130	**Male HIV -**	34.3			(69)53.1)			
469	**Female HIV+**	33	4.4	<0.0001	(272)58	2.7	0.101	
276	**Female HIV-**	34.5			(143)51.8			
680	**<50yrs HIV+**	32.6	6	<0.0001	(419)61.6	6.3	0.01	
339	**50 yrs HIV-**	34.4			(181)53.4			
120	**≥ 50 yrs HIV-**	33.7	1.2	0.254	(62(51.7)	0.501	0.479	
67	**≥ 50 yrs HIV-**	34.6			(31)46.3)			

Within the same gender category, HIV infected females had a higher propensity of developing disorders of total protein than females without HIV infection (OR = 1.9; χ^2 = 16.9; p<0.0001). This was not however reflected in occurrence of serum albumin anomalies in females irrespective of serological status (OR = 1.3; χ^2 = 2.7; p = 0.101). Accordingly the prevalence of serum total protein anomalies in HIV + females (51.4%) was significantly higher than in HIV negative females (35.9%; χ^2 = 16.9; p<0.0001), though the rates of occurrence of

albumin anomalies was not different between them (58% v/s 51.8%, p = 0.101). In contrast at 64.1g/l and 33g/l, mean total protein and albumin in seropositive females were both significantly lower than mean total protein (67.2g/l) and albumin (34.5g/l) in seronegative females (p <0.0001). HIV infected males also presented with significantly higher tendency of developing both serum total protein and albumin disorders than seronegative males (OR = 2.1; χ^2 = 12.8; p <0.0001 and OR = 1.5; χ^2 = 4; p = 0.05). Correspondingly therefore, the prevalence of total protein and albumin disorders in HIV+ males (54.7% and 63.1%;) was substantially more than in HIV negative males (36.2%; p <0.0001 and 53.1%; p = 0.047). Mean total protein and albumin levels in HIV + males (63.9g/l and 32.5g/l) were also significantly below that of seronegative males (66.9g/l; t = 3.9; p<0.0001 and 34.3g/l; t = 3.8; p <0.0001).

In the different age categories, the impact of HIV infection on total protein and albumin levels was more pronounced in young participants. Thus whereas the likelihood of developing total protein and albumin disorders was higher in young HIV + compared to young HIV – (OR = 2.1; χ^2 = 30.3; p <0.0001 and OR = 1.4; χ^2 = 16.3; p = 0.01), this was not the case in old participants (OR = 1.4; χ^2 = 1.2; p= 0.266 and OR = 1.2; χ^2 = 0.5; p = 0.480). Accordingly, this study observed that HIV infected participants below 50 years presented with higher rates of occurrence of protein and albumin disorders than young HIV negative participants (54.3% v/s 36% , χ^2 = 11.4; p<0.0001 and 61.6% v/s 53.4%; χ^2 = 6.3; p = 0.01, respectively). Mean protein and albumin levels in seropositive participants in this age category (63.8g/l and 32.6g/l) was also considerably lower than their age correlates who were not infected (66.9g/l; t = 6.5; p <0.0001 and 34.4g/l; t = 6; p <0.0001, respectively). On the other hand among the

old, whereas HIV infection was accompanied with changes in mean serum total protein compared to that of HIV negative participants of the same age bracket (65.4g/l v/s 67.9g/l; t = 2; p =0.05), there was no significant change in levels of albumin associated with HIV infection (see Table 4.11 for protein values).

Table 4.11: Distribution of Serum Total Protein Levels by Serological Status

N		Protein levels	t	p-value	Protein disorders	χ^2	p-value	Physiological range
800	**HIV+**	64	6.7	<0.0001	(422)52.8	30.5	<0.0001	65-80
406	**HIV-**	67.1			(146)36			
331	**Male**	63.9	3.9	<0.0001	(181)54.7	12.8	<0.0001	
130	**Male**	66.9			(47)36.2			
469	**Female**	64.1	5.4	<0.0001	(272)58	16.9	<0.0001	
276	**Female**	67.2			(142)51.4			
680	**<50yrs**	32.6			(244)35.9			
339	**<50yrs**	63.8	6.5	<0.0001	(184)54.3	11.4	<0.0001	
120	**≥50yrs**	65.4			(53)44.2			
67	**≥50yrs**	67.9	2	0.05	(24)35.8	1.2	0.265	

Among HIV afflicted participants, the disposition to developing protein and albumin disorders was indistinct between the two gender categories (OR = 1.1; χ^2 = 0.85; p = 0.357 and OR = 1.2; χ^2 = 2.14; p = 0.144, respectively). Thus the proportion with protein and albumin disorders in males (54.7% and 63.1%) were not significantly dissimilar from that of females (51.4% and 58%; p = 0.358 and p = 0.143, respectively). Similarly there was no significant contrast between mean protein and albumin levels in males (63.9g/l and 32.5g/l) and that of females (64.1g/l; p = 0.712 and 33g/l, p = 0.312). (see Table 4.12).

Table 4.12: Prevalence of serum protein and albumin disorders in HIV infection (n = 800)

N	Patient attributes	Impaired Total protein			Impaired Albumin		
		N (%)	χ^2	p-value	N (%)	χ^2	p-value
331	**Male**	(181)54.7	0.846	0.358	(209)63.1	2.1	0.143
469	**Female**	(272)51.4			(272)58		
680	**<50 yrs**	(244)54.3	4.2	0.041	(419)61.6	4.2	0.04
120	**>50yrs**	(53)44.2			(62)51.7		
639	**ARV users**	(336)52.6	0.036	0.850	(378)59.2	1.3	0.264
161	**ARV naïve**	(86)53.4			(103)64		

Key; ARV- Antiretroviral drugs

Old HIV infected participants displayed a lower proclivity of developing abnormal protein and albumin states than younger HIV infected participants (OR = 0.67; χ^2 = 4.17; p = 0.04 and OR = 0.7; χ^2 = 4.2; p = 0.04, respectively). Thus the rates of occurrence in old participants of protein disorders (44.2%) and albumin disorders (51.7%) was significantly lower than in young HIV + patients (54.3%; χ^2 = 4.2; p = 0.04 and 61.6%; χ^2 = 4.2; p = 0.04, respectively).

The likelihood of occurrence of protein and albumin disorders in HIV infected patients was not differentially affected by using ARVs (OR = 0.5; p = 1.90 and OR = 1.2; p = 0.264). As such patients using ARVs did not present with distinctly altered mean serum protein and albumin levels than those not using ARVs (64g/l v/s 64.2g/l; p= 0,342 and 32.8g/l v/s 32.9g/l; p = 0.837).

Extreme immune, depression CD4 depletion (<200 cell/mm3) was associated with lowest mean serum total protein and albumin (Figure 4.10).

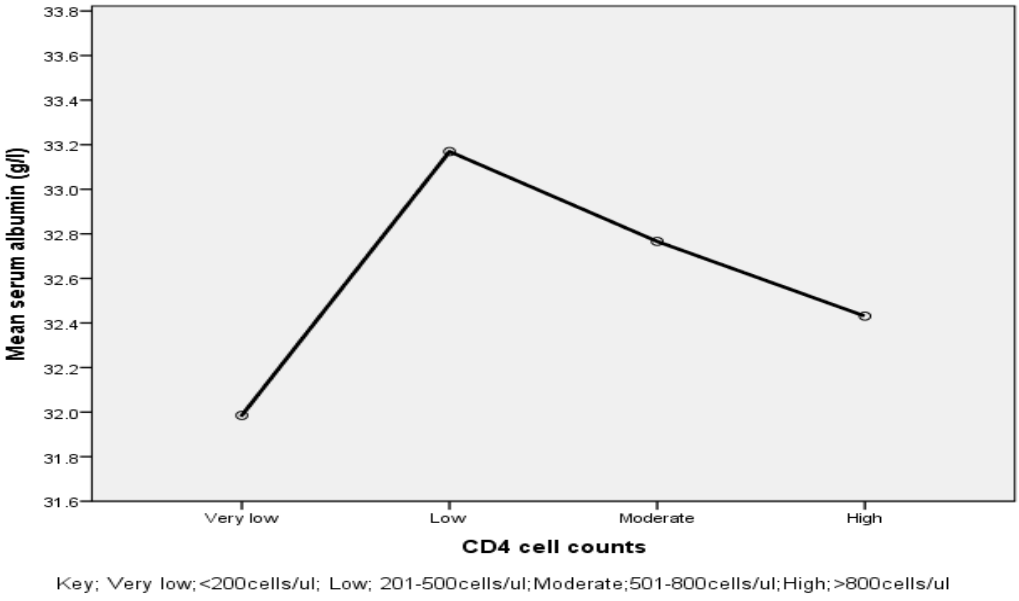


Figure 4.10: Mean Serum albumin at different CD4 cell levels

4.5. Body Fluids parameters in HIV infection

During the period of the study three successive blood pressure measurements were recorded at intervals of one month and compared with baseline blood pressure at the time the participant was diagnosed with HIV infection. Mean of the successive blood pressures measurements showed that in later stages of the disease, majority of HIV- infected individuals' blood pressure remained within the normal range (defined as <139/89 mmHg) and did not differ significantly from the proportion with normal levels of blood pressure at diagnosis (97.6% v/s 95.5%; p=1.0). Similarly mean serum osmolality in HIV- infected individuals was 294.9 mmol/l which was not significantly altered from the mean serum osmolality of 294.8 mmol/l in HIV negative participants. Table 4.13 details the levels of various biochemical components observed during the study.

Table 4.13: Distribution of Serum Biochemical Parameters by HIV Status

		HIV STATUS							
Analytes		**HIV Positive (n=800)**				**HIV Negative (n=406)**			
		Mean	**Std**	**Min.**	**Max.**	**Mean**	**Std**	**Min**	**Max.**
Creatinine	µmol/l	95.2	35.	11.5	410.5	86.2	20.4	40	173.1
Potassium	mmol/l	4.2	0.7	2.8	6.4	4.2	0.7	2.9	6
Sodium	mmol/l	138.9	6	127	160	139.1	4.9	130	153
Chloride	mmol/l	100.2	3.9	90	139	99	3.2	90	108
Urea	mmol/l	4.6	2.1	1.5	36.7	4.1	1.0	1.9	8.8
Protein	g/l	64	8.4	40	89	67.1	6.9	42	93
Albumin	g/l	32.8	5.8	15.5	47.2	34.5	3.9	22.7	43.3
ALT	U/l	36.5	16	11	210	30.7	11.6	11.5	145
AST	U/l	45.1	18	11.6	301	36.9	9.6	16.3	104
Total Bilirubin	µmol/l	6.2	5.5	1.9	70	5	1.6	2.2	14.1
Direct bilirubin	µmol/l	4.9	4	1.5	62.8	4.2	1.3	2	10.8
Glucose	mmol/l	4.2	0.9	2.1	8.6	4.1	0.9	2	6.7

Key: ALT-alanine-aminotransferase; AST- aspartate –aminotransferase; Min.- minimum; Max.-maximum

4.6. Serum Electrolytes Levels in HIV infection

The distribution of the three major serum electrolytes Na+, K+ and Cl- were investigated in HIV infection.

4.6.1 Sodium ion Levels in HIV Infection

Serum sodium levels in either seropositive individuals or the healthy controls were found to be distributed on either side of the normal physiological reference range of 135mmol/l to 155mmol/l. Thus serum sodium levels in HIV- infected individuals ranged from as low as 127 mmol/l to as high as 160 mmol/l with a mean of 138.9 mmol/l ±SD(6.0) while in seronegative participants it ranged from 130 mmol/l to 153 mmol/l with a mean of 139.1 mmol/l ±SD(4.9).

The most frequent values for serum sodium levels in HIV+ (136mmol/l) and HIV- individuals (138mmol/l), fell within the physiological reference range of sodium in the intravascular fluid compartment. Whereas the rates of occurrence of sodium disorders (<135mmol/l or >155mmol/l) among HIV-infected participants (26.1%) was significantly higher than in uninfected group (17.7%; χ^2 = 10.6, p = 0.001 see Fig 4.2), mean sodium level did not shift significantly in the former compared to the later group (138.9mmol/l v/s 139.1mmol/l, p = 0.516).

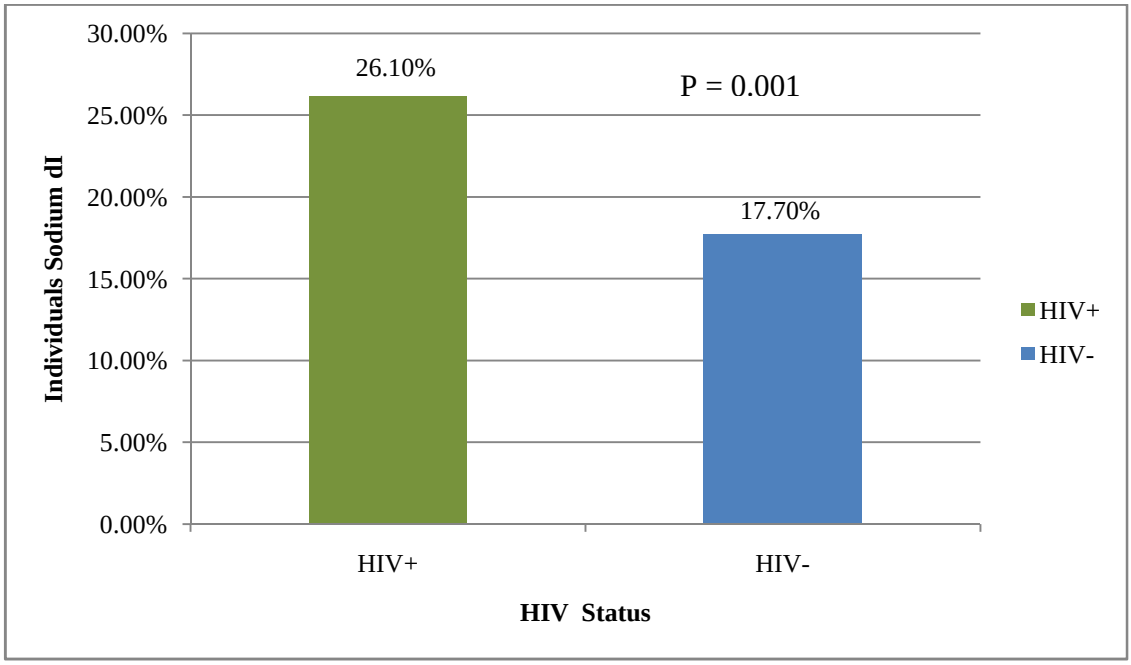


Figure 4.11: The Prevalence of Sodium Disorders in HIV+ and HIV- Individuals

Comparison of sodium levels in similar gender groups by HIV status, revealed that sodium disorders in HIV-infected females (27.9%) was more than in uninfected females (18%, p = 0.003) though their mean sodium levels were indistinct (138.8mmol/l v/s 139.2mmol/l, p = 0.329). In HIV afflicted males however mean serum sodium (139.1mmol/l), was not significantly higher than that of uninfected males (139.0 p = 0.840) as was neither the

prevalence of serum sodium disorders (23.6% v/s 16.9% p = 0.119). Within the same age category, HIV-infected patients above 50 years, did not display significantly dissimilar sodium outcomes compared to HIV negative counterparts above 50 years in both mean sodium levels (139.8mmol/l v/s 138.6mmol/l p = 0.143), and prevalence of sodium anomalies (23.3% v/s 20.9%, p = 0.702). However among participants below 50 years, HIV infection was associated with a higher prevalence of abnormal sodium levels compared to those without HIV infection (26.6% v/s 17.1%, χ^2 = 11.4, p=0.001), despite there being no difference between their mean sodium levels (138.7mmol/l v/s 139.2mmol/l, p = 0.182) see Table 4.14.

Table 4.14: Serum Sodium Ion States and HIV Status

N		**Mean sodium (mmol/l)**	**t**	**p-value**	**Sodium imbalance N (%)**	χ^2	**p-value**	**Reference range** mmol/l
800	**HIV+**	138.9	0.650	<0.516	(209)26.1	10.6	0.001	135-155
406	**HIV-**	139.2			(72)17.7			
331	**Male**	139.1	0.202	0.840	(78)23.6	2.4	0.119	
130	**Male**	139.0			(22)16.9			
469	**Female**	138.8	0.978	0.329	(131)27.9	9.1	0.003	
276	**Female**	139.2			(50)18.1			
120	**≥50yrs**	139.8	1.5	0.143	(28)23.3	0.147	0.702	
67	**≥50yrs**	138.6			(14)20.9			
680	**<50yrs**	138.7	1.3	0.182	(181)26.6	11.4	0.001	
339	**<50yrs**	139.2			(58)17.1			

There were 207 out of 209 HIV infected participants found with serum sodium disorders presented with hyponatremia (<135mmol/l) while 2 cases were due to hypernatremia (>155mmol/l). Gender and age were not associated with significant changes in sodium outcomes among HIV- infected participants as mean sodium levels in HIV infected males (139.1mmol/l) was not markedly different from that in HIV infected females (138.8mmol/l), p = 0.421. Similarly the proportion of infected males with impaired sodium levels (23.6%) was not different from that of females with abnormal sodium levels (27.9%, p = 0.166). Mean

sodium levels in HIV+ patients over 50 years (139.8mmol/l) was also not different from that of seropositive patients below 50 years of age (138.7mmol/l), p = 0.072 and neither was the prevalence of anomalous sodium levels (23.3% v/s 26.6%, p = 0.450). Generally HIV-infected participants not using ARVs had a higher prevalence of serum sodium disorders (32.3%) than those using ARVs (24.6%), χ^2 = 3.98, p = 0.046. However, among gender groups the impact of anti-retro viral treatment on serum sodium ion states was mainly evident in females among whom those using ARVs had a higher mean sodium level (139.2mmol/l v/s 137.5mmol/l, p = 0.007) and a lower prevalence of sodium anomalies (24.3% v/s 38.2%, χ^2 = 8.8, p = 0.003), than those not using ARVs. In HIV-infected males, ARV use was not associated with significant changes in sodium outcomes. Similarly whereas in HIV- infected patients over 50 years, ARV use had no significant impact on sodium states, in younger HIV infected patients (<50 years) those using ARVs had a higher mean sodium level (139mmol/l v/s 137.6mmol/l, p = 0.013) and a lower prevalence of sodium anomalies (24.3% v/s 36%, χ^2 = 7.7 p = 0.005) than those not using ARVs. Immune depletion was accompanied with rise in mean sodium levels that constituted two phases; a modest rise associated with initial CD4 lymphocyte decline up to 800 cells/ mm3 and a steep rise associated with fall in CD4 cell count below 800 cells /mm3. However, mean sodium did not fall out of the normal reference physiological range (135 – 155mmol/l) across the CD4 lymphocyte count ranges (Figure 4.12).

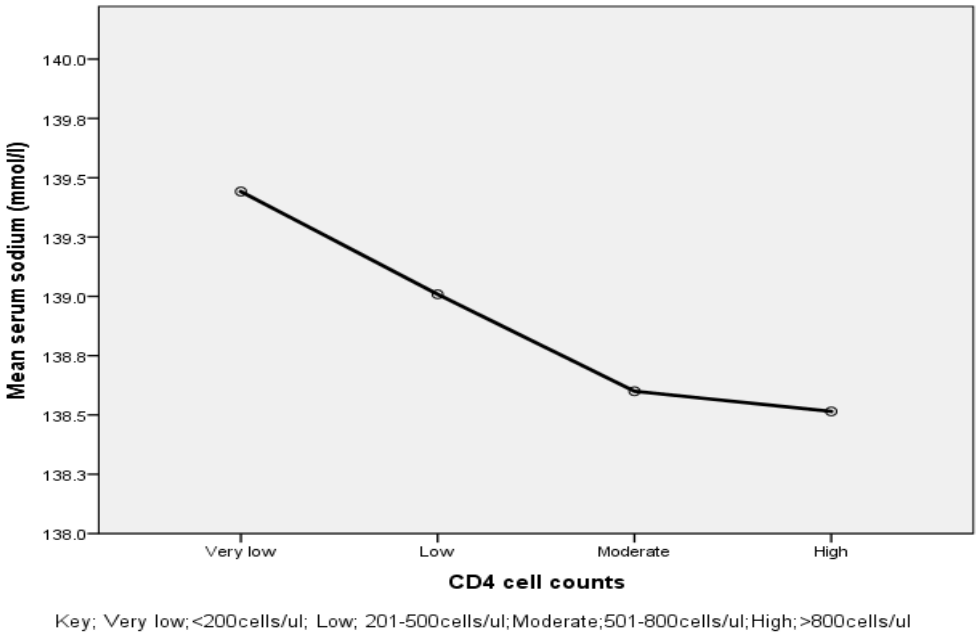


Figure 4.12: Mean serum Sodium at different CD4+ Cell Levels

4.6.2 Serum Chloride ion States in HIV Infection

Serum chloride ion levels in both study groups were distributed on either side of the optimal physiological reference range of 98mmol/l to 106mmol/l. Thus HIV-infected individuals were detected with serum chloride levels ranging from 90 mmol/l to 139 mmol/l with a mean of 100.2 mmol/l ±SD(3.9) while seronegative participants' chloride levels ranged from 90 mmol/l to 108 mmol/l with a mean of 99.9 mmol/l ±SD(3.3). The most frequently observed value of serum chloride ion levels in HIV + individuals (96mmol/l) was below the lower reference range indicating that chloride ion depletion was a common finding in these individuals. However among the healthy controls the most frequently observed value 99mmol/l was within the normal serum chloride ion levels.

The proportion with Cl- ion disorders (<98 or >106mmol/l) in the study population, was 27.4% with 87.2% of these being due to depletion of serum chloride content (hypochloremia; <98mmol/l) and 12.8% hyperchloremia (>106mmol/l). Mean chloride in HIV+ participants (100.2mmol/l) was not significantly different from that of HIV-negative participants (99.9mmol/l, p = 0.252) and neither was the prevalence of serum chloride disorders (27.4% v/s 25.9%, p = 0.575). However, among males, HIV infection was associated with higher mean chloride levels compared to uninfected males (100.2mmol/l v/s 99.5mmol/l, p = 0.047). Nonetheless, the prevalence of chloride anomalies in HIV+ males was not different from that of uninfected males (29.3% v/s 26.2%, p = 0.5). There was no noticeable contrast among HIV-infected and uninfected females, in mean chloride levels (p = 0.968) and prevalence of abnormal chloride states (26% v/s 25.7%, p = 0.931) see Table 4.15.

Table 4.15: Distribution of Serum Chloride ion by Serological Status

N		**Mean chloride (mmol/l)**	**t**	**p-value**	**Chloride imbalance N(%)**	**χ^2**	**p - value**	**Physiological range (mmol/l)**
800	**HIV+**	100.2	1.2	<0.252	(219)27.4	0.314	0.575	90-139
406	**HIV-**	99.9			(105)25.9			
331	**Male HIV +**	100.2	2.0	0.047	(97) 29.3	0.456	0.5	
130	**Male HIV -**	99.5			(34)26.2			
469	**Female HIV+**	100.1	0.04	0.968	(122)26	0.008	0.931	
276	**Female HIV-**	100.1			(71)25.7			
120	**<50yrs HIV+**	100.3	1.3	0.206	(35)29.2	0.112	0.738	
67	**50 yrs HIV-**	99.6			(19)28.3			
680	**≥ 50 yrs HIV-**	100.1	0.662	0.508	(184)27.1	0.226	0.635	
339	**≥ 50 yrs HIV-**	100.0			(90)26.6			

Gender difference among HIV-infected individuals was not associated with differences in serum chloride outcomes. Mean chloride levels in seropositive males (100.2mmol/l) was not dissimilar from seropositive females (100.1mmol/l, p = 0.816) and neither was the prevalence of chloride disorders (29.3% v/s 26%, p = 0.304). Age of HIV infected participants was also not a determinant of differences in serum chloride outcomes. Thus, HIV infected individuals over 50 years did not have contrasting mean chloride levels form HIV infected participants below 50 year (100.3mmol/l v/s 100.1mmol/l, p = 0.657) and neither did the prevalence of serum chloride pathological states differ between them (29.2% v/s 27.1%, p = 0.633). Use of ARVs was also not accompanied with significant changes in mean serum chloride levels compared to non-ARV use (100.2mmol/l v/s 99.9mmol/l, p = 0.270). Similarly the proportion using ARVs with anomalous chloride levels (27.5%) was not different from those not using ARVs (26.7%, p=0.832). Decline in CD4 cell count was accompanied with mixed outcomes in serum chloride levels (see Figure 4.13).

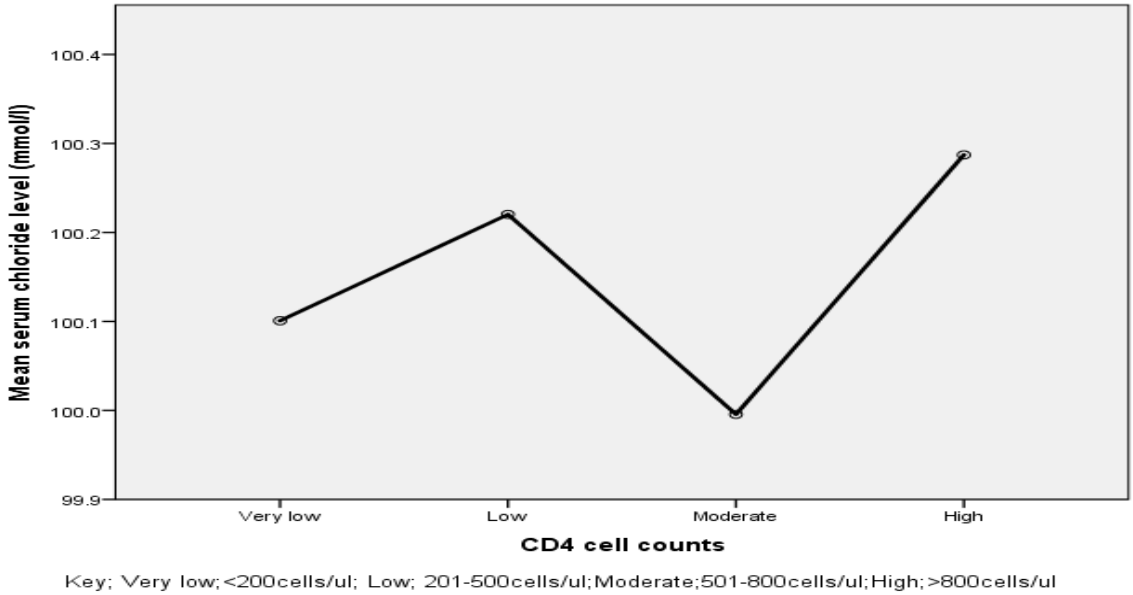


Figure 4.13: Mean Serum Chloride Ions at different Immune states

4.6.3 Serum Potassium Levels in HIV Infection

Serum potassium levels in both groups was distributed on either side of the physiological reference range. Hence serum potassium levels detected in HIV-infected participants fluctuated between 2.8mmol/l and 6.4mmol/l with a mean of 4.2 mmol/l ±SD(0.7) (Table 4.16).

Table 4.16: Serum Potassium States by HIV Status

N		Mean potassium mmol/l)	t	p-value	Potassium imbalance N(%)	χ^2	p-value	Reference Range (mmol/l)
800	**HIV+**	4.2	0.132	0.895	(138) 17.3	1.2	0.274	3.4-5.3
406	**HIV-**	4.2			(60)14.8			
331	**Male HIV+**	4.1	0.182	0.334	(57)17.2	1.7	0.194	
130	**Male HIV-**	4.2			(16)12.3			
469	**Female HIV+**	4.2	0.917	0.359	(81)17.3	0.220	0.639	
276	**Female HIV-**	4.1			(44)15.9			
120	**≥50yrs HIV+**	4.1	0.0350	0.727	(28)23.3	3.6	0.058	
67	**≥50 yrs HIV-**	4.1			(8)11.9			
680	**≤ 50 yrs HIV+**	4.2	0.037	0.971	(119)16.2	0.119	0.731	
339	**≤ 50 yrs HIV-**	4.2			(52)15.3			

On the other hand seronegative individual manifested a mean potassium of 4.2mmol/l ±SD(0.7) and a range of 2.9mmol/l to 6mmol/l. The most frequent serum potassium values observed in either group was 3.6mmol/l which fell within the optimal physiological range (3.4 – 5.3 mmol/l). Comparatively therefore, mean potassium level in HIV-infected participants was not significantly different from that of HIV negative individuals (4.2mmol/l v/s 4.2mmol/l, p = 0.895) and neither was the prevalence of serum potassium disorders (17.3% v/s 14.8%, p = 0.274).

Among similar gender groups, HIV afflicted males, did not have significantly altered serum potassium level (4.1mmol/l v/s 4.2mmol/l, p = 0.334) or rates of occurrence of potassium disorders (17.3% v/s 12.3%, p = 0.194) than uninfected male counterparts. Similarly HIV+ females were not observed with a significant change in mean potassium level compared to uninfected females (4.2mmol/l v/s 4.2mmol/l, p = 0.971) as well as in prevalence of potassium disorders (16.2% v/s 15.3%, p = 0.731). Within the same age categories, rates of occurrence of potassium disorders in HIV+ individuals over 50 years was not dissimilar from that of seronegative participants of the same age (23.3% v/s 11.9%, p = 0.058). Younger (<50 years) HIV+ participants equally had no contrasting mean potassium level (4.2mmol/l) from that of younger uninfected individuals (4.2mmol/l, p = 0.97) and neither was the prevalence of anomalous potassium states between them disparate (16.2% v/s 15.3%, p = 0.731).

Among seropositive participants 17.3% had potassium disorders majority (77.5%) of which were due to hypokalemia (<3.4mmol/l). Mean potassium levels in HIV+ males and females were not markedly different (4.1mmol/l v/s 4.2mmol/l p = 0.333) as was neither the prevalence of potassium disorders (17.2% v/s 17.3%, p = 0.985). Similarly, age difference among infected individuals, did not herald different physiological and pathological potassium outcomes. Anti-retroviral therapy was not associated with contrasting difference in potassium outcomes irrespective of age and gender of the HIV infected participants. Mean potassium level among infected participants using ARVs (4.1mmol/l) did not shift significantly from that of infected participants not using ARVs (4.2mmol/l, p = 0.101). Prevalence of anomalous potassium states was also not significantly different between those on ARVs compared to those not using ARVs (18.2% v/s 13.7%, p = 0.178). Initial decline in CD4+ immune levels

was associated with decline of serum potassium level up to moderate CD4+cell count. Beyond this CD4+ level, serum potassium level rose up to low CD4+ levels. In very low CD4+ levels serum potassium declined again (Figure 4.14).

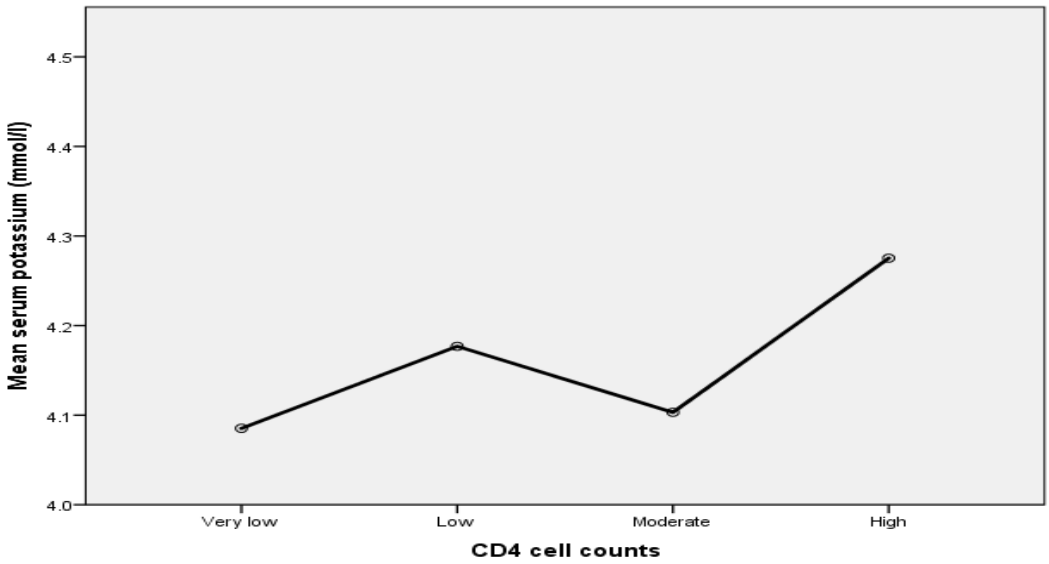


Figure: 4.14 Mean serum Potassium at different CD4 + cell levels

4.7. Association between Serum Electrolytes Levels with Kidney and Liver Function Markers

The association and level of co-variation exhibited between kidney and liver function markers were assessed to delineate whether any co-existing electrolytes imbalance could be attributed to underlying kidney or liver defects and the magnitude of the electrolytes disorders that could be explained by the concurrent kidney or liver defects.

4.7.1. Kidney Function Markers and Associated Electrolytes Changes

The risk of developing aberrations in serum electrolytes was indistinct between HIV-infected patients with and without abnormal creatinine states (OR = 0.98; p = 0.888, OR = 0.953; p =

0.823 and OR = 0.993; p = 1, respectively). This was exemplified in mean electrolyte levels (sodium, potassium and chloride) in HIV infection with or without impaired serum creatinine levels (139.1mmol/l v/s 138.8mmol/l; t = 0.605; p = 0.545, 4.1mmol/l v/s 4.2mmol/l; p = 0.752 and 100.2mmol/l v/s 100.1mmol/l; p = 0.781, respectively).

In both gender, where HIV infection was complicated with underlying creatinine disorders, the susceptibility of developing Na+, K+ and Cl- disorders was not dissimilar between males and females (OR = 0.7, p = 0.383, OR = 1.2, p = 0.699 and OR = 0.96, p = 0.888 respectively). But where creatinine disorders existed in both age categories, it was noted that whereas mean sodium remained unchanged, mean potassium levels was higher in younger HIV positive individuals than in old HIV-infected individuals (4.2mmol/l v/s 3.9mmol/l; t = 2.3; p= 0.03) while chloride level was lower in younger HIV infected persons than in older HIV positive individual (99.6mmol/l /v/s 102mmol/l; t = 2.7; p = 0.01). Among the HIV positive patients with abnormal creatinine levels, those using ARVs did not manifest a significant proclivity of developing abnormal serum fluid electrolytes levels (Na^+, K^+, Cl^- ; OR = 0.58,p = 0.198; OR = 1.77, p = 0.398 and OR = 1.16, p = 0.729 respectively).

4.7.2. Electrolytes and Metabolites Changes Attributable to Creatinine Levels

A strong positive correlation existed between creatinine levels and urea (r = 0.953; p<0.0001) with over 90% of changes in urea associated with changes in creatinine (r^2 = 0.908; p<0.0001), among seronegative study individuals. In this group weaker correlation also existed between creatinine and totalbilirubin (r = 0.11; p = 0.02) as well as direct bilirubin (r = 0.103; p = 0.04) but with virtually no co-variation between them (r^2 = 0.014 and r^2 = 0.011,

respectively). Among HIV-infected individuals, a strong positive correlation persisted between creatinine and urea in those with normal creatinine states (r = 0.656; p<0.0001) and those with abnormally altered creatinine levels (r = 0.715; p<0.0001). There was also 43% and 51.1% co-variation respectively between creatinine and urea in the two HIV infected categories (Figure 4.15).

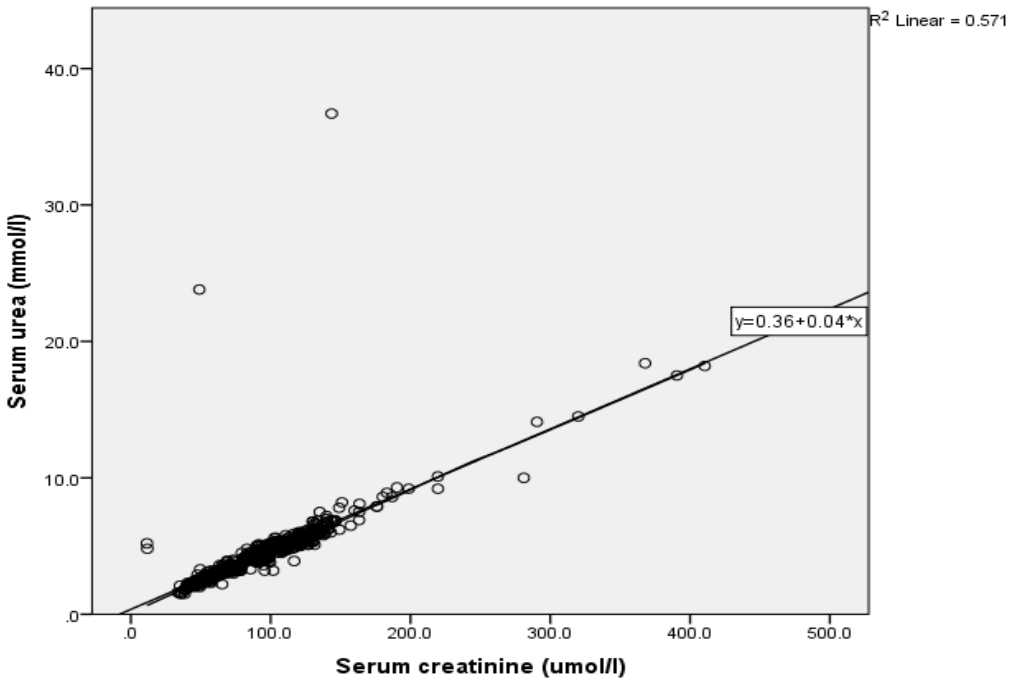


Figure 4.15 Correlation of Serum Creatinine Levels and Urea in HIV Infection (r = 0.715)

Among seropositive participants creatinine also displayed weaker correlation with, total bilirubin, directbilirubin, glucose, ALT, AST and potassium, but with no observable co-variation between them. Correlation and co-variation between creatinine and urea was also co-directional with regard to development of pathological clinical states. This was

exemplified by the fact that 91.4% of urea disorders were in HIV positive participants with creatinine disorders which was contrastingly higher than the proportion of urea disorders found in HIV positive participants with normal creatinine levels (8.6%; $\chi^2 = 80.9$; $p<0.0001$). Moreover, the type of urea anomaly found in the later group matched the type of creatinine disorder existing in the group of patients. Notably 22 out of 23 hyperuremia cases were found in hypercreatinine cases, while 10 out of 12 hypouremia cases were in HIV infected patients also with hypocreatinine states. In patients with creatinine retention disorders (hypercreatininemia) the concomitant urea disorder predominantly exhibited in them therefore was urea retention disorders and conversely patients with creatinine depletion disorders (hypocreatininemia) preferentially tended to have urea depletion disorders. Alternately, no patient with creatinine retention disorders had urea depletion disorders and no patient with creatinine depletion disorders had urea retention disorders. Such co-directional shifts between urea and creatinine, were not exhibited between creatinine and any of the other electrolytes with or without marginal correlation between them.

4.7.3. Serum Electrolytes States Associated with Urea Levels in HIV Infection

In general, HIV- infected patients with abnormal urea states did not display a higher tendency to develop abnormally altered serum sodium, potassium, and chloride levels than their counterparts with normal urea levels (OR = 1.12, p = 0.74, OR = 1.99, p = 0.07 and OR = 1.6, p = 0.185, respectively). Thus the prevalence of sodium, potassium and chloride anomalies in HIV- infected patients was not associated with underlying urea disorders. Mean sodium, potassium and chloride levels in HIV- infected patients with urea disorders was equally noted to be unchanged from that of HIV infected patients without urea disorders (139.1mmol/l v/s

138.9 mmol/l; t = 0.213; p = 0.831; 4.1 mmol/l v/ 4.2 mmol/l; p = 0.361 and 100.7 mmol/l v/s 100.1 mmol/l; t = 0.885; p = 0.376, respectively). In addition, where there was impaired urea levels in both gender categories, the likelihood of developing serum sodium, potassium and chloride disorders in males with abnormal urea levels was not different from that of HIV+ females with urea disorders (OR = 2.2; 95% C.I. (0.5 – 10.3); OR = 0.4 955 C.I. (0.08 – 1.09) and OR = 0.8; p = 0.764). Existence of underlying urea disorders in either age categories did not as well increase their predisposition to developing serum electrolytes disorders. Using antiretroviral drugs did not contribute to improving the risk of developing body fluids and electrolytes disorders in seropositive individuals with abnormally altered urea levels.

4.7.4. Serum Analytes Levels Attributable to Urea States

Urea levels in seronegative individuals had a strong positive correlation to creatinine (r = 0.953; p < 0.0001) with 90.7% co-variation between them (r^2 = 0.907; p < 0.0001). In the same participants, urea also showed a weak correlation with totalbilirubin (r = 0.102; p = 0.04) with nonexistent co-variation between them (r^2 = 0.008). Among HIV infected individuals with normal urea states, a strong correlation was retained between urea and creatinine (r = 0.943; p< 0.0001) with 88.9% co-variation between them (r^2 = 0.889; p< 0.0001). Urea also exhibited minimal correlation with potassium but without co-variation between them in this group of participants (r^2 = 0.004). However, in seropositive participants with impaired urea states, a significant positive correlation only existed with creatinine (r = 0.576; p< 0.0001) with 31.2% co-variation between them.

4.7.5. Glomerular Filtration Rates and Associated Electrolytes Changes

Overall among HIV positive individuals, the likelihood of occurrence of serum sodium, potassium and chloride imbalances was not elevated in individuals with impaired eGFR compared to those with unimpaired kidney function (OR = 1.03, p = 0.864; OR = 1.44, p = 0.07 5 and OR = 1.105, p = 0.546 respectively). Similarly, where both gender were seropositive and had underlying kidney function disorders as well, the propensity to develop serum electrolytes disorders was not distinct between them. The rates of occurrence of sodium, potassium and chloride disorders in males with underlying eGFR disorders (22.2%, 20.5%, and 32.4%) were not in contrast from the rates observed in female participants with eGFR disorders (28.7%, p = 0.107; 18.2%, p = 0.520 and 25.6%; p = 0.100). In both age categories, complication of HIV infection with kidney function disorders was accompanied with indistinct rates of occurrence of serum sodium, potassium and chloride anomalies (27.3% v/s 21.8%, p 0.297; 17.8% v/s 25.3%, p = 0.104 and 27.7% v/s 29.9%, p = 0.683).

Glomerular filtration rate in HIV-infected individuals with normal kidney function, had a significant positive correlation with creatinine (r = 0.590; p<0.0001) as well as urea (r = 0.160; p = 0.006). This correlation was accompanied with co-variation between creatinine and glomerular filtration rate (r^2 = 0.345) but less so between glomerular filtration rate and urea (r^2 = 0.022) within the same participants (Table 4.17).

Table 4.17: Correlation of Glomerular Filtration Rate and Serum Biochemical Substrates (n=800)

	HIV+ normal eGFR			HIV+ deranged eGFR		
	r	**p-value**	**r^2**	**r**	**p-value**	**r^2**
Creatinine	0.590	<0.0001	0.345	0.598	<0.0001	0.598
ALT	0.006	0.916	<0.0001	0.021	0.629	<0.0001
AST	0.033	0.579	0.001	0.084	0.058	0.005
Urea	0.160	0.006	0.022	-0.452	<0.0001	0.203
Glucose	0.004	0.947	<0.0001	0.080	0.07	0.005
Protein	0.013	0.826	<0.0001	0.016	0.723	<0.0001
Albumin	0.013	0.825	<0.0001	<0.0001	0.994	<0.0001
Sodium	0.013	0.828	<0.0001	0.031	0.491	0.001
Potassium	0.032	0.591	0.001	0.008	0.864	<0.0001
Chloride	0.064	0.275	0.004	0.072	0.103	0.003
TTBIL	0.060	0.309	0.004	0.234	<0.0001	0.053
DRCTBIL	0.069	0.239	0.001	0.249	<0.0001	0.062

Key: TTBIL- Total bilirubin; DRCTBIL- Direct bilirubin, AST-Aspartate aminotransferase, ALT- Alanine aminotransferase, eGFR- Estimated glomerular filtration rate

On the other hand, in HIV positive individuals with impaired glomerular filtration rate, eGFR correlated also with creatinine ($r = 0.598$; $p < 0.0001$), urea ($r = 0.452$; $p<0.0001$), total bilirubin ($r = 0.234$; $p<0.0001$) and direct bilirubin ($r = 0.249$; $p<0.0001$). Nonetheless in these individuals, strong co-variation was only observed between glomerular filtration rate and creatinine ($r^2 = 0.393$; $p<0.0001$) as well as urea ($r^2 = 0.203$; $p<0.0001$) (see Figure 4.16).

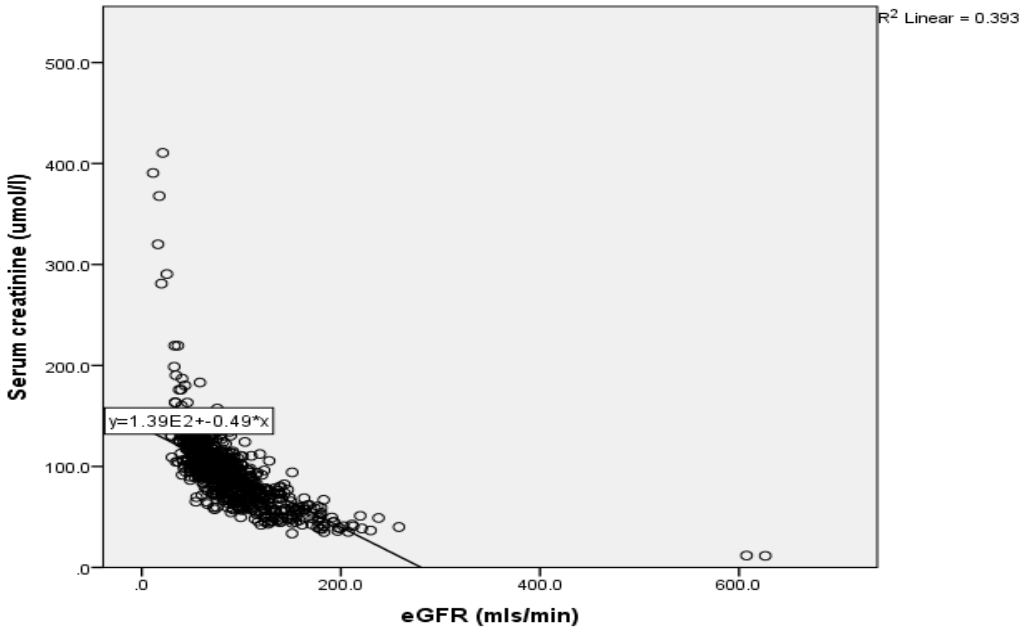


Figure 4.16: Correlation of Serum Creations Levels and Glomerular Filtration Rate (r = -0.872)

Co-directional shift towards abnormal levels was exemplified between glomerular filtration rate and creatinine as well as urea. Hence whereas 7.2% of HIV + individuals with normal eGFR, had abnormal creatinine states, this proportion increased to 34% in cases with mild reduction of glomerular filtration rate (<90mls/l) and to 60.2% in moderate reduction of eGFR (<60mls/min). On the other hand 4.5% of seropositive individuals with normal eGFR had urea disorders which increased to 8.8% in individuals with moderate fall of eGFR and 75% in patients with severe reduction of glomerular filtration rate (<30mls/min). Whereas the rates of occurrence of potassium disorders increased significantly with severe reduction in kidney function (50% v/s 21.5% in moderate and 16.9% in mild kidney dysfunction, p = 0.02), the rates of occurrence of serum sodium and chloride disorders remained unchanged with declining kidney function (p = 0.948 and p = 0.690, respectively). It was observed therefore

that the mean serum sodium levels, did not shift beyond the normal physiological range as was also mirrored by the other inorganic electrolytes investigated (Figure 4.17).

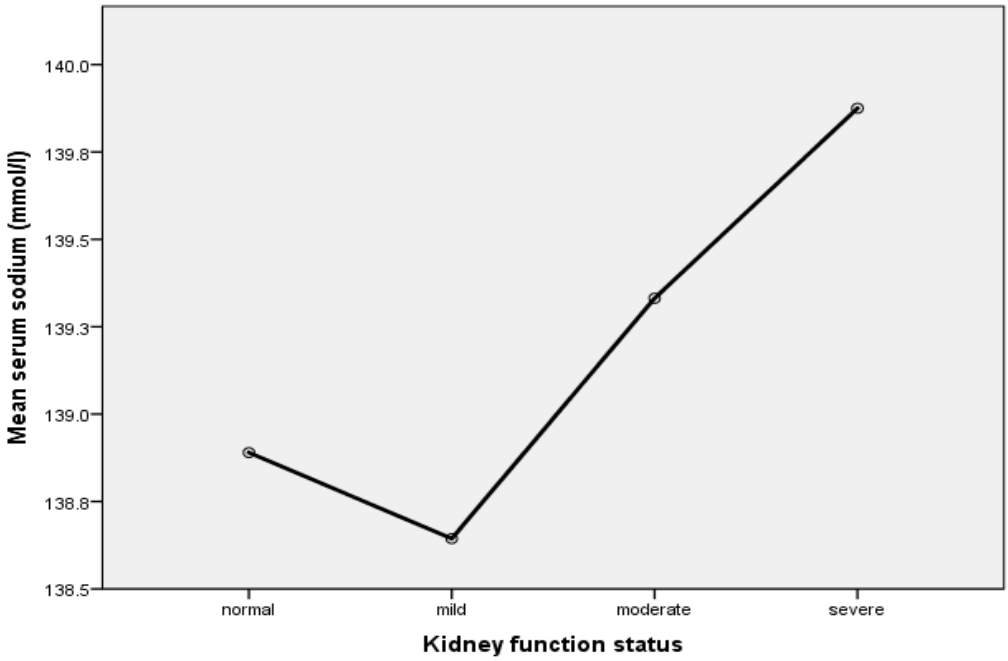


Figure 4.17: Mean Serum Sodium Levels at Different Kidney Function States

4.7.6. Electrolytes States Associated with Liver Enzymes Activity in HIV Infection

In general, the prevalence of serum sodium imbalance manifested in equal magnitude in HIV infected patients irrespective of existence of altered liver enzyme levels. Presence of underlying AST disorders in HIV infected patients was therefore not associated with a higher proclivity to developing abnormally altered serum Na+, K+, and Cl- levels than in HIV+ individuals with normal AST levels (OR = 1.3, p = 0.203, OR = 0.9, p = 0.689 and OR = 1.1, p = 0.663, respectively). As a result the prevalence of sodium, potassium and chloride

disorders in HIV- infected patients with AST disorders(29.6%, 16.3% and 28.6%) was not different from that of HIV- infected patients with normal AST states (25%; p = 0.204, 16.3%; p = 0.694 and 27%; p = 0.665, respectively). Mean sodium, potassium and chloride levels in HIV- infected patients with AST disorders was equally affected by complication of HIV infection with elevated AST activity (138.8mmol/l v/s 139 mmol/l; t = 0.363; p = 0.717; 4.2mmol/l v/ 4.1mmol/l; p= 0.496 and 99.9mmol/l v/s 100.2mmol/l; t = 1.1; p = 0.293, respectively).

Similarly anomalously elevated ALT activity was also not a predictor of developing serum electrolytes (sodium, potassium and chloride) disorders (OR = 1.95, p= 0.056; OR = 0.792, p = 0.632 and OR = 1.23, p = 0.583, respectively). However, in HIV infected patients with elevated ALT activity mean serum sodium level was lower than in HIV infected patients with normal ALT activity (136.6mmol/l v/s 139mmol/l, t = 2.3, p = 0.019). Potassium and chloride levels were however not found to be significantly changed in HIV infected participants with abnormal ALT activity than in those with normal ALT states 4.3mmol/l v/s 4.2mmol/l; p= 0.135 and 99 mmol/l v/s 100.2mmol/l, p = 0.082, respectively.

In addition, where liver enzyme elevation was present in both gender groups, neither females nor males displayed a contrastingly increased susceptibility to developing serum electrolytes disorders than the other. The risk of developing serum sodium, chloride and potassium anomalies in HIV-infected males with AST disorders was therefore not different from that of HIV infected females with AST disorders (OR =1.14, p 0.671, OR = 1.7, p = 0.09 and OR= 1.22, p = 0.61, respectively). Similarly in abnormal ALT states, the risk of developing serum

sodium, potassium and chloride disorders was not significantly higher in males compared to females (OR = 1.14, p = 0.671; OR= 1.2, p = 0.610 and OR = 1.7, p = 0.09, respectively). Where AST and ALT anomalies was present in both age categories, neither age group displayed a higher tendency to developing serum electrolytes disorders that the other. Thus the likelihood of developing serum sodium, potassium and chloride disorders in old HIV infected patients was not different from that of young patients with abnormal liver AST (OR = 0.7, p = 0.484, OR = 0.8; 95% C.I. 0.3 – 2.5; and OR = 1.7, p = 0.227).

Among HIV- infected patients with abnormally elevated AST, using anti-retroviral therapy did not lower comprehensively the likelihood of developing serum sodium, potassium and chloride disorders than absence of treatment (OR = 0.8, p = 0.639, OR = 1.7, p = 0.327 and OR = 1.9, p = 0.160). Similarly in HIV- infected patients with abnormal ALT states, no marked improvement was registered in the risk of developing plasma sodium and chloride disorders among those using ARVs compared to those not using ARVs (OR = 1.9; 95% C.I. (0.3 – 11.4 and OR= 3.3; 95% C.I. (0.4 – 31.8).

4.7.7. Electrolytes and Metabolites Changes Attributable to Liver Enzymes Alterations

In HIV negative individuals, AST showed strong correlation with ALT (r = 0.721; p <0.0001) with 52% of variations that occurred in ALT associated with variations occurring in AST (r^2 = 0.520; p<0.0001). Among the HIV negative participants, AST displayed an additional mild correlation with glucose (r = 0.102; p = 0.04) but only a dismal 1% of their values shared common variation trends. Among HIV+ patients with normal AST levels, strong correlation was observed between AST levels and ALT (r = 0.701; p<0.0001) with 49.2% co-variation

them. In addition, in this group AST also depicted correlation with chloride ion levels (r = 0.084, p = 0.039), but only 0.5% co- variation between them.

However in HIV positive participants with elevated AST activity (>50U/L), an even stronger correlation was exemplified with ALT (r = 0.784, p<0.0001) with a higher proportion (71.8%) of ALT changes associated with changes in AST levels (r^2 = 0.718; p<0.0001) See Figure 4.18.

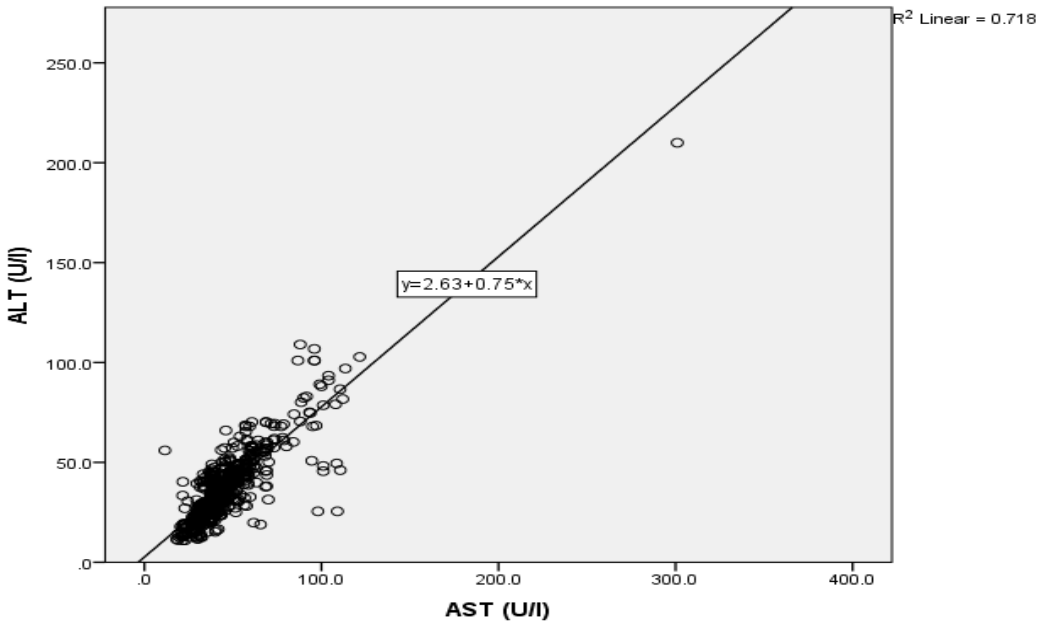


Figure 4.18: Correlation of ALT and AST in HIV-Infected Individuals (r = 0.784)

Additionally in this group with elevated AST levels, correlation was observed between AST and seven other analytes namely; creatinine, urea, directbilirubin, totalbilirubin, sodium, chloride and albumin (Table 4.18).

Table 4.18: Correlation of AST Levels with Other Serum Substrates and Electrolytes (n = 1206)

	AST in HIV negative			HIV positive-AST normal (< 50U/L)			HIV positive–elevated AST (>50U/L)		
	r	p	r^2	r	p	r^2	r	P	r^2
ALT	0.721	<0.0001	0.520	0.701	<0.0001	0.492	0.784	<0.0001	0.613
Creatinine	0.057	0.254	0.003	0.054	0.189	0.003	0.470	<0.0001	0.221
Chloride	0.021	0.668	<0.0001	0.084	0.039	0.007	0.172	0.016	0.030
Sodium	0.008	0.880	<0.0001	0.031	0.441	0.001	0.222	0.002	0.049
Potassium	0.057	0.250	0.003	0.060	0.141	0.004	0.051	0.474	0.003
Urea	0.039	0.433	0.002	0.028	0.496	0.001	0.304	<0.0001	0.092
Glucose	0.102	0.039	0.010	0.076	0.063	0.006	0.127	0.075	0.016
Total /Bil	0.035	0.484	0.001	0.003	0.950	<0.0001	0.227	0.001	0.051
Direct/Bil	0.064	0.195	0.004	0.001`	0.981	<0.0001	0.268	<0.0001	0.072
Protein	0.050	0.311	0.003	0.008	0.845	<0.0001	0.120	0.094	0.014
Albumin	0.046	0.630	0.002	0.020	0.627	<0.0001	0.156	0.029	0.024

The proportion of alteration in these seven analytes that portrayed common variation trend with AST was however dismal, with exception of creatinine where 21.7% of changes in its level were accounted for by changes in AST levels. A strong co-directional shift in levels of ALT and AST was apparent where AST was regressed against clinically deranged ALT cases or where ALT was regressed against cases with elevated AST activity. Thus in HIV infected patients with abnormal AST states, 97.1% also had abnormal ALT levels whereas only 2.9% of patients with normal AST states had abnormal ALT levels (χ^2 = 104.4; p <0.0001). Thus where the strength of correlation was considerably large, it was observed that shifts in the AST levels that predominantly culminated in development of abnormal AST levels (>50U/L), were accompanied by similar shifts in the proportion of the co-variant electrolytes towards abnormal level.

4.7.8. Association of Electrolytes Levels with Bilirubin Levels in HIV

Generally, sodium and potassium imbalance manifested in HIV-infected patients with total bilirubin anomalies (OR = 3.7; 95% C.I. (1.4 – 9.4) and OR = 3.2; 95% C.I. (1.3 – 4.2). Thus in HIV infection, developing total bilirubin disorders was associated with significantly raised rates of occurrence of sodium and potassium disorders (55.6% v/s 25.4%; χ^2 = 8.3; p = 0.004 and 38.9% v/s 16.8%; χ^2 = 6.04; p = 0.01). In contrast, occurrence of direct bilirubin disorders in HIV infected patients did not impact on the risk of occurrence of sodium, potassium and chloride disorders compared to those with normal direct bilirubin levels (OR = 0.945; p = 0.729, OR = 0.989; p = 0.92 and OR = 1.1; p = 0.64, respectively).

Where elevated serum bilirubin was present in both gender groups neither females nor males comparatively displayed a higher tendency of developing serum electrolytes disorders than the other gender. The risk of developing serum sodium, chloride and potassium anomalies in HIV infected males with total bilirubin disorders was as such not different from that of HIV infected females with total bilirubin disorders (OR = 0.7; 95% C.I. (0.09 – 5.1), OR = 0.2 ;95% C.I. (0.01 – 2.3) and OR= 0.3; 95% C.I. (0.02 – 3.2), respectively). Similarly in abnormal direct bilirubin states the risk of developing serum sodium, potassium and chloride disorders was not significantly different in males compared to females (OR = 0.7, p = 0.118; OR = 0.86, p = 0.603 and OR = 1.16, p = 0.554, respectively). Similarly with underlying total bilirubin age difference was not associated with elevated vulnerability to developing serum electrolytes as exemplified in risks related to sodium, and potassium disorders (OR = 0.3; 95% C.I. (0.11 – 2.9), and OR = 4; 95% C.I. (0.7 – 5.9). In HIV -infected patients whose serum total bilirubin levels was abnormally elevated, the predisposition to developing serum

electrolytes (sodium, potassium and chloride) disorders was not ameliorated by anti-retroviral therapy (OR = 0.2; 95% C.I. (0.02 – 2.5), OR = 3.4; 95% C.I. (0.3 – 39.6) and OR = 0.7 95% C.I. (0.08 – 5.7). Similarly in HIV- infected patients with abnormal direct bilirubin states, no marked improvement was registered in the risk of developing serum electrolytes disorders among those using ARVs compared to those not using ARVs (Sodium- OR = 6, p = 0.09 and Chloride-OR 1.3, p = 0.403). In this group, the prevalence of serum potassium disorders was significantly elevated in HIV positive individuals with abnormally elevated direct bilirubin and using ARVs compared to those with abnormal direct bilirubin and not using ARVs (OR = 2.5, p = 0.04).

4.7.9. Correlation Electrolytes and Metabolites in Serum with Bilirubin Levels

Direct bilirubin in healthy controls was strongly correlated with total bilirubin levels (r = 0.925; p<0.0001) with 92.5% of variations in levels of either electrolyte tied to variation in level of the other. Among the same participants, direct bilirubin also showed a weak correlation to creatinine (r = 0.103) with only 1.4% of alterations in levels of either electrolytes associated with changes in levels of the other. Total bilirubin in this group had a weak correlation with creatinine and urea (r = 0.116; p = 0.019 and r = 0.102; p = 0.04, respectively). In HIV infected participants with normal direct bilirubin levels, correlation was only noted between direct bilirubin and total bilirubin (r = 0.752, p<0.0001) with 56.5% co-variations existing between them (r^2 = 0.565). However, in HIV- infected patients with clinically elevated direct bilirubin states (>4.2µmol/l), there was increased positive correlation between direct bilirubin and total bilirubin (r = 0.958; p<0.0001) with 92.7% of changes in level of total bilirubin associated with changes in levels of direct bilirubin (Figure 4.19).

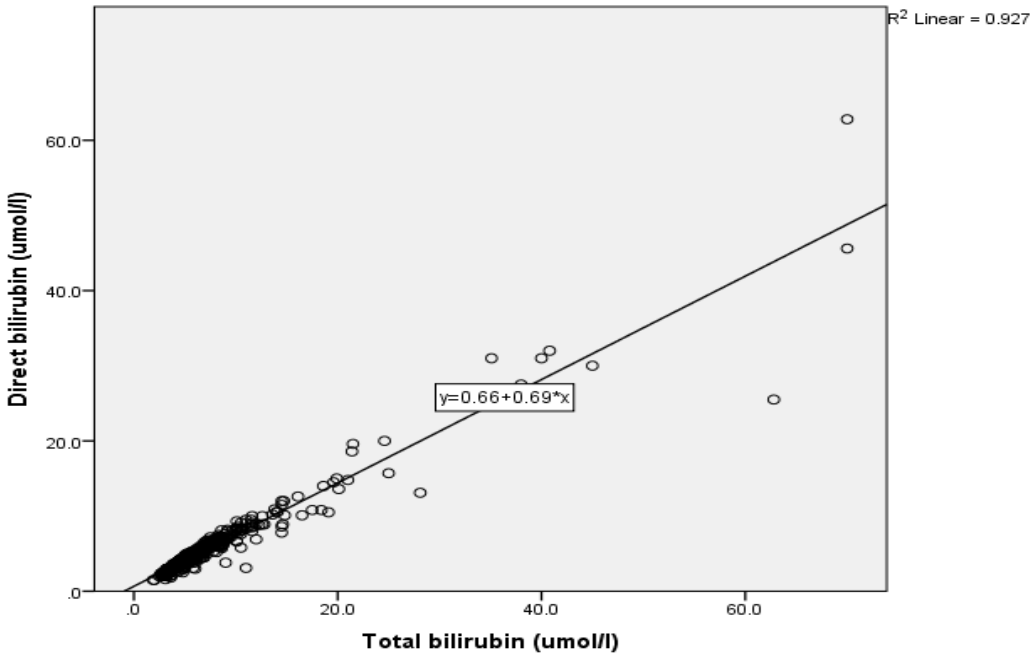


Figure 4.19: Correlation of Direct and Total Bilirubin In HIV-Infected Individuals (r = 0.958)

In addition four other metabolites showed increased correlation with direct bilirubin in HIV-infected patients with underlying direct bilirubin disorders; these electrolytes included creatinine ($r = 0.412$, $p<0.0001$), urea ($r = 0.362$, $p<0.0001$), AST ($r = 0.212$, $p<0.0001$) and glucose ($r = 0.139$, $p = 0.010$). However the proportion of changes of levels of these later four analytes that could be associated with changes in levels of direct bilirubin was only noticeably moderate for urea (12.9%) but remained dismally low for the rest. Furthermore only total bilirubin was seen to have co-directional shift with direct bilirubin levels in such patients, as no patient with normal direct bilirubin had total bilirubin disorders while all total bilirubin disorders occurred only in patients with abnormal direct bilirubin disorders.

On the other hand in HIV- infected patients with raised total bilirubin (>19µmol/l), correlation existed between total bilirubin and direct bilirubin only ($r = 0.875$, $p<0.0001$) with 76.6% co-variation between them. Pathological shift in levels of bilirubin (direct and total) were also in tandem, with all the patients with abnormal levels of total bilirubin also having abnormal levels of direct bilirubin while none of the patients with normal levels of direct bilirubin had abnormal levels of total bilirubin. On the other hand the strength of correlation between direct bilirubin and creatinine in HIV infected patients with abnormal levels of direct bilirubin was a pointer to co-existence rather than co-directional shift in magnitude of pathological alterations of both. Thus even though there was 17% co-variation between direct bilirubin and creatinine in HIV patients with abnormal direct bilirubin levels, the prevalence of abnormal creatinine in them (27.5%) was not significantly different from the proportion with abnormal creatinine level in HIV infected patients with normal direct bilirubin levels (25.1%, $p = 0.429$). Urea levels in HIV infected patients with abnormal direct bilirubin also portrayed similar outcomes as creatinine with a weak correlation not sufficient to engender co-directional pathological shift.

4.7.10. Total protein and Albumin States and Related Electrolytes Levels

Among HIV infected patients, underlying protein anomalies was not a predictor of pathogenesis of serum sodium and chloride anomalies (OR= 1.3, $p = 0.08$, and OR = 1.3, $p = 0.07$, respectively) but was associated with lower risk of developing serum potassium anomalies (OR = 0.6, $p = 0.01$). In effect, HIV infected participants with protein disorders, manifested lower rates of occurrence of potassium disorders than HIV infected patients with normal protein levels (14% v/s 20.9%, $\chi^2 = 6.7$; $p = 0.01$). However where both males and

females had abnormal protein levels, males displayed a lower risk of developing serum sodium anomalies than females (OR = 0.5, p = 0.005) while the propensity to develop chloride and potassium anomalies was not distinctly incongruent between the two gender groups (OR = 1.34, p = 0.294 and OR = 1.03, p = 0.920). However where protein anomalies was present in both age categories the risk of developing sodium, potassium and chloride anomalies was not dissimilar between old HIV patients and younger HIV patients with protein disorders (OR = 0.6; 1.9; p = 0.173, OR = 1.5; 1.2; p = 0.273 and OR = 1.2; 0.43; p = 0.512).

Occurrence of underlying serum albumin disorders was accompanied with outcomes that mirrored those observed in HIV infected patients with impaired serum total protein levels in that albumin disorders in HIV infection did not conduce serum sodium and chloride anomalies (OR 1.1, p = 0.699 and OR = 1.04, p = 0.823) but was associated with lower risk of developing potassium anomalies (OR = 0.6, p = 0.001). Hence lower rates of occurrence of potassium disorders was noted in HIV infection with underlying albumin anomalies (13.7% v/s 22.6%, p = 0.001) there was no difference between rates of occurrence of sodium and chloride anomalies irrespective of existing anomalous albumin levels. In the event of occurrence of albumin imbalance in both gender, males depicted lower likelihood of developing sodium disorders than females (OR = 0.519, p = 0.002) but potassium and chloride disorders were not differentially prevalent in one group more than the other. Similarly HIV infection complicated with underlying albumin anomalies were not associated with contrasting outcomes in serum electrolytes levels in either age categories. On the other hand the likelihood of developing sodium, potassium and chloride ion disorders was not

lowered by use of ARVs in HIV infected patients with concomitant albumin disorders (OR = 0.85, p = 0.532, OR = 2.1, p = 0.08 and OR= 1.2, p = 0.446).

4.7.11. Correlation of Total Proteins with Electrolytes and Metabolites in Serum

Despite the observed association of total protein and albumin with certain serum electrolytes states, changes in none of the serum electrolytes was significantly correlated with changes in either albumin or total protein levels. Indeed albumin and total protein mainly co-varied significantly between them as markers of liver disease. In HIV negative controls, total protein levels showed strong correlation to albumin ($r = 0.867$; $p<0.0001$) with 75.2% of variations that occurred in albumin associated with variations occurring in protein. Similarly, significant correlation between protein and albumin levels ($r = 0.705$; $p<0.0001$) existed in HIV -infected participants with normal protein levels, with 49.6% variations in albumin accounted for by variation in protein levels. However, among HIV infected patients with protein disorders (<65g/l or >80g/l), correlation existed between protein and albumin ($r = 0.872$; $p<0.0001$) as well as protein and creatinine levels ($r = 0.108$; $p = 0.03$). Whereas in this group there was strong co-variation between protein and albumin ($r^2 = 0.759$), virtually no co-variation was exemplified between protein and creatinine ($r^2 = 0.009$) despite the correlation noted above.

Albumin on the other hand showed significant correlation with protein only ($r = 0.867$, $p <0.0001$) with 75.1% of changes in protein levels explained by changes in albumin levels in seronegative. Similarly among HIV infected patients with normal albumin states, correlation was exhibited between albumin and protein levels ($r = 0.738$; $p <0.0001$) with 54.3% of changes in protein levels here associated with changes in albumin levels.

On the other hand in HIV infected patients with underlying albumin disorders (<35g/l or >50g/l), correlation was noted between albumin and protein (r = 0.841; p <0.0001; see Figure 4.20), total bilirubin (r = 0.171; p<0.0001), direct bilirubin (r = 0.161; p<0.0001), AST (r = 0.097; p = 0.03) and creatinine (r = 0.130; p = 0.004).

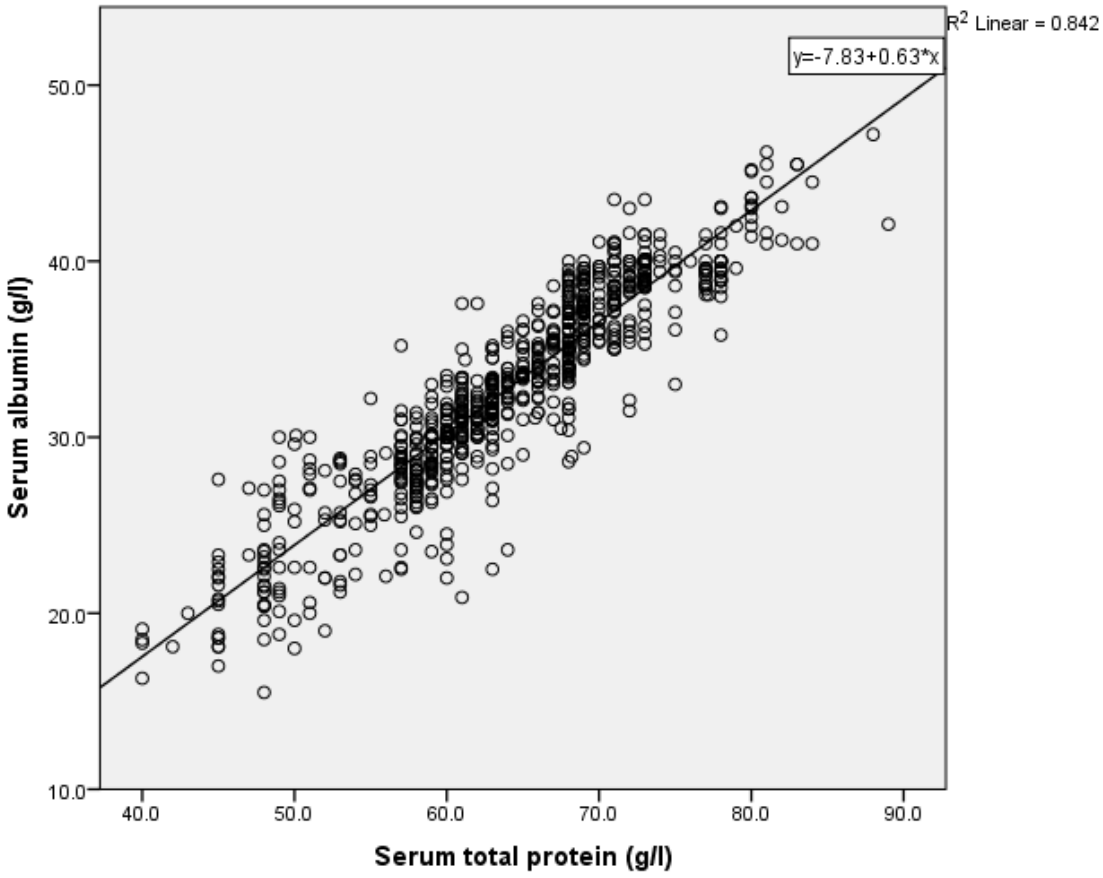


Figure 4.20: Correlation of Serum Albumin and Total Protein (r= 0.841)

However among these participants, despite the range of correlation observed between albumin and several analytes, significant co-variation was only present between albumin and protein

where 70.6% of variations in protein levels were explained by changes in albumin levels (Table 4.19).

Table 4.19 : Correlation of Albumin with Other Serum Biochemical Components

(n = 1206)

	Albumin(HIV-)			**Normal Albumin (HIV+) (35-50g/l)**			**Impaired albumin(HIV+) (<35g/l;or>50g/l)**		
	r	**P**	**r^2**	**r**	**P**	**r^2**	**r**	**P**	**r^2**
ALT	0.075	0.181	<0.0001	0.075	0.181	0.003	0.06	0.164	0.004
Creatinine	0.000	0.994	0.001	0.0001	0.994	<0.0001	0.13	0.004	0.017
Chloride	0.061	0.275	0.001	0.061	0.275	0.004	0.04	0.380	0.002
Sodium	0.042	0.453	0.004	0.042	0.453	0.002	0.06	0.163	0.004
Potassium	0.037	0.514	0.006	0.037	0.514	0.001	0.00	0.863	<0.00
Urea	0.017	0.767	0.001	0.017	0.767	<0.0001	0.05	0.262	0.003
Glucose	0.084	0.134	<0.0001	0.084	0.134	0.007	0.02	0.599	0.001
Total/Bil	0.008	0.886	0.001	0.008	0.886	<0.0001	0.17	<0.0001	0.027
Direct/Bil	0.003	0.961	<0.0001	0.003`	0.961	<0.0001	0.16	<0.0001	0.026
Protein	0.738	<0.000	0.751	0.738	<0.0001	0.543	0.84	<0.0001	0.706
AST	0.048	0.395	0.002	0.048	0.395	0.002	0.09	0.03	0.007

Key; AST – Aspartate aminotransferase, ALT- Alanine aminotransferase, Bil -bilirubin

Most of protein anomalies were protein depletion states (defined as < 65g/l) with only 18 HIV infected patients with protein retention states (defined as > 80g/l). However all albumin anomalies were due to depletion states (defined as < 35g/l). Co-variation in protein and albumin levels also displayed co-directional trend as 94.3% of patients with albumin disorders also had protein disorders while only 5.7% of patients with normal albumin states displayed abnormal protein states (χ^2 = 435.4; p<0.0001). Evidence of co-directional shift in protein and albumin levels was further adduced from the fact that 97.6% of patients with protein depletion

states had concomitant albumin depletion disorders. Such manifest co-directionality was not observed between protein changes or albumin changes and any other analytes.

4.8. Comparative Risks of Developing Serum Electrolytes Impairment in Renal and Pre-Renal Disorders

Despite the prominent role of kidneys in regulating body F&E states, comparatively the risk to serum electrolytes imbalances attributable only to kidney disorders (eGFR<90mls/min) in HIV infected individuals was not raised beyond the risk of impairment of these electrolytes attributable to only liver function disorders (albumin <35g/l) (OR = 1.2; 0.42; p = 0.517, OR = 1.6; 2.32; p = 0.128 and OR = 1.05; 0.03;p = 0.863 for sodium, potassium and chloride levels, respectively). On the other hand, concomitant occurrence of kidney and liver function defects in a patient, did not also result in significantly elevated risk of serum electrolytes disorders than in individuals with only kidney function disorders (OR = 1.03; = 0.01; p = 0.920, OR = 1.7; = 2.2; p = 0.137 and OR 1.12; = 0.12; p = 0.729) for risk to sodium, potassium and chloride levels, respectively). Similarly the likelihood of occurrence of electrolytes disorders (sodium, potassium and chloride) was not enhanced in states of contemporaneous kidney and liver anomalies compared to where only liver functional anomalies existed (OR = 1.01; p = 1, OR = 1.1; 0.11; p = 0.740 and OR = 1.1; = 0.07; p = 0.791). Nonetheless mean electrolytes levels in any of these kidney or liver function states did not rise or fall beyond the normal physiological ranges.

4.9. Correlation between Electrolytes in Intravascular Compartment

Sodium levels correlated mainly with potassium and chloride levels in all categories of the participants. In HIV negative participants sodium levels had modest positive correlation with chloride levels ($r = 0.446$, $p<0.0001$) with 19.7% co-variation between them. There was also a noticeable correlation between sodium and potassium levels ($r = 0.308$, $p<0.0001$) but with minimal co-variation between their level ($r^2 = 0.092$). In HIV-infected participants with normal sodium levels a similar trend was observed with sodium retaining a noticeable correlation with chloride levels ($r = 0.427$) and 18.1% co-variation between them. In this group, correlation also existed between sodium and potassium ($r= 0.244$, $p<0.0001$) but with diminished co-variation between their levels ($r^2 = 0.058$). However in HIV infected individuals with impaired serum sodium levels, there was correlation between sodium and three analytes i.e. chloride, potassium and glucose. Nonetheless co-variation diminished progressively between sodium and chloride levels ($r^2 = 0.088$), potassium levels ($r^2 = 0.022$) and glucose levels ($r^2 = 0.043$) in infected patients with sodium disorders.

Chloride co-variance with sodium levels tended to show general co-directional anomalous shift as HIV infected patients with normal sodium levels had significantly less concurrent chloride disorders (16.2%) compared to HIV infected individuals with abnormal sodium levels (58.9%, $\chi^2 = 141$, $p<0.0001$). However potassium levels had no co-directional shift with sodium, as the proportion with abnormal potassium levels in HIV afflicted participants with abnormal and normal sodium levels were not distinct (16.7% v/s 17.4%, $p = 0.823$).

Correlation was seen between chloride and potassium levels in these three categories of participants. In healthy individuals chloride correlated with potassium ($r = 0.186$, $p<0.0001$) but with minimal co-variation ($r^2 = 0.032$). Among HIV-infected participants with normal chloride levels chloride correlated with potassium ($r = 0.173$, $p<0.0001$) with more diminished co-variation between them ($r^2 = 0.028$) but in HIV infected individuals with abnormal chloride levels, chloride correlated with potassium ($r = 0.372$) but with a stronger co-variation ($r^2 = 0.135$).

Potassium levels exhibited different correlation trends in HIV-infected individuals. Among seropositive participants with normal potassium levels, correlation was observed between potassium levels and sodium ($r = 0.254$, $p<0.0001$), chloride ($r = 0.210$, $p<0.0001$), creatinine ($r = 0.102$, $p = 0.009$) and urea ($r = 0.084$, $p = 0.032$). Nonetheless there was no co-variation between potassium and these analytes. On the other hand, in HIV-infected participants with serum potassium disorders, correlation between potassium and sodium ($r = -0.491$, $p<0.0001$) and chloride ($r = -0.401$, $p<0.0001$), were accompanied with slightly raised co-variation between them at 2.43% and 1.6%, respectively.

Table 4.20: Correlation of Serum Electrolytes (n=1206)

	Na+ (HIV-)			**Normal Na+ (HIV+)**			**Impaired Na+(HIV+)**		
	r	p-value	r^2	r	p-value	r^2	r	p-value	r^2
K+	-0.308	P<0.0001	0.092	-0.244	P<0.0001	0.058	-0.346	P<0.0001	0.022
Cl-	0.446	P<0.0001	0.197	0.427	P<0.0001	0.181	0.526	P<0.0001	0.088

Key; Na+ - sodium ion, K+- Potassium ion, Cl- Chloride ions

CHAPTER FIVE: DISCUSSION

5.1 Introduction

This study investigated the impact of HIV infection on body fluids and electrolytes parameters. A secondary though related aim was to determine which organ functions' impairment contribute to more profound pathophysiological states of body fluids and electrolytes and whether age, CD4 cell count and gender or ARV use were determinants of existing fluids and electrolytes states in HIV-infected individuals.

5.2. Kidney Function Markers in HIV Infection

Normal glomerular filtration rate coupled with carefully regulated secretion and reabsorption within the kidney nephron, determine the amount of electrolytes and fluids in plasma to meet demand and offset fluctuations occasioned by extrinsic and intrinsic causes (Rolls and Rolls, 1982). Impaired kidney function can emanate from pathological events at any of these functional sites of the nephron. This study estimated glomerular function and investigated the relationship between altered glomerular function and body fluids and electrolytes and whether age and gender or ARV use were determinants of existing disturbances in kidney functions in HIV-infected individuals.

The current study established that HIV-infection was associated with increased risk of decline in glomerular filtration rate compared to healthy participants. The results of this study indicated that 54% of HIV infected individuals had reduced glomerular filtration rate. Several studies in agreement, have reported renal insufficiency in HIV infection at 51.8% (Agaba *et al*, 2003), 55.8% (Susman, 2005) and 48.5% (Andia, *et al.*, 2005). Other literature observed

kidney disorders in 4 - 30% of HIV infected individuals (Gupta, *et al.,* 2004). The prevalence of kidney function impairment in the current study was pegged on the calculation of eGFR using serum creatinine levels which could explain the difference between the rates observed in the study from other studies which reported specific kidney disorders based on clinical and histological investigations. Mild reduction in eGFR (60-90mls/min) was most the common followed by moderate reduction (eGFR; 30-60mls/min) while least common was severe forms glomerular filtration decline (eGFR<30mls/min). This was in agreement with Cara, *et al.,* (2009) and Chi, *et al.,* (2007) who reported low prevalence of severe kidney function impairment in their study cohort.

Causes of renal disease in HIV-infected patients are multi-factorial reflecting an interplay between the host (genetics and ageing) and viral factors, as well as exposure to nephrotoxic agents and so include HIV infection itself, co-infections, and anti-retroviral treatments (Roling, *et al.,* 2006). This study elucidated that advanced age, female gender and profound immunosuppression marked by low CD4 levels are risk factors of impaired kidney function in HIV-infected individuals. This is partly in agreement with Cara and colleagues (2009) account that extreme immune depletion, age and male gender are predictors of reduced eGFR. Differences in findings concerning the role of gender could be attributed to selection bias in the study as most of the participants were female gender. Studies have documented direct HIV virus infection and residency in podocytes and tubular epithelial cells, subsequently triggering proliferative disorders and in some cases apoptosis (Ross, *et al.,* 2001). In some cases HIV-infects lymphocytes and macrophages which then infiltrate the kidneys releasing inflammatory cytokines and lymphokines that cause renal injury (Bruggeman, *et al.,* 2000).

The current study consequently investigated the distribution of biochemical markers closely associated with glomerular filtration functional status and possible risk factors that influence the observed kidney function states

5.3. Creatinine in HIV Infection

The prevalence of impaired serum creatinine levels in HIV infected individuals was 26.1% which reflected findings of up to 30% prevalence reported by Fernando, *et al.,* (2008). Majority of creatinine disturbances in the current study is attributable to reduced creatinine clearance rates leading to elevation of serum creatinine levels. The main elimination route for creatinine in circulation is the kidney via its urine forming processes which are anchored on a sound glomerular filtration rate (Stevens, *et al.,* 2008). Normal GFR is directly proportional to the total number of glomeruli that are active. In HIV infection studies have demonstrated that HIV infection is associated with several variants of injuries to glomeruli some leading to collapse and obliteration of individual units of these essential structures of the nephron (Szczech, 2002). Progressive loss of glomeruli leads to reduction of the surface area available for filtering plasma and thus affects clearance of elements such as creatinine which rely heavily on this route as a means of excretion.

This study observed that female gender and depleted CD4 levels (<200cells/mm3) are risk factors of serum creatinine disorders in HIV–infected participants. Other investigations reported that in HIV–infected patients' impaired creatinine clearance is associated with older age, female gender, hepatitis B and C infections, diabetes, hypertension, and ART exposure (Lucas *et al.,* 2007). Obirikorang *et al.,* (2014) reported that ARV use was not a risk factor of

reduced creatinine clearance in HIV infected patients which concurred with the findings of this study. Reid and colleagues (2013), intimated that low CD4 cell count, is a determinant of elevated serum creatinine level as observed in our study.

5.4. Urea

HIV infection was associated with significantly impaired serum urea levels than in healthy controls in all study categories apart from individuals above 50 years of age. Within the HIV–infected individuals serum urea imbalance was observed in 4.4% of the population with majority being accumulation disorders (uremia). Male gender was predictive of serum urea perturbations in the seropositive individuals while age and ARV use were not. AIDS defining CD4 cell levels was associated with significantly raised serum urea levels.

5.5. Liver Function Markers and HIV Infection

5.5.1. Liver Enzymes

Findings of the current study reflected previous observation that HIV infection is a risk factor of developing anomalies in liver transaminases as mean serum liver transaminases levels was higher in HIV infected individuals than the healthy controls. Ignatius, *et al.,* (2009) reported that mean liver transaminases' activity levels are higher in HIV patients compared with similarly matched subjects not infected with HIV. In the current study 24.6% of HIV-infected participants had transaminitis which did not differ considerably from earlier reports of chronically elevated ALT in 16% of a Swiss cohort (Kovari, *et al*., 2009). Streling, *et al.*, (2008) similarly observed that liver enzyme abnormalities were common among HIV

infection. They reported the prevalence of elevated AST to be 20% and of ALT to be 15% in a cross-sectional study involving HIV –infected individuals.

Liver enzyme abnormalities in HIV infection have been attributed to several factors ranging from, immunoallergic reactions, anti-retroviral drugs and direct HIV-viral damage of hepatocytes (Stanislas, *et al.*, 2008). On the other hand Ejilemele, *et al.*, 2007, in a study of HIV –infected individuals found that 87.6% of the participants all of whom were ARV naïve had abnormal liver function biochemistry. These findings could be attributable to the broad range of parameters used to define liver function anomalies and the fact that only 129 participants were involved in the study. The current study established that AST levels correlated with highly ALT levels which concurred with findings by Sterling and colleagues, (2008).

5.5.2. Serum Albumin and HIV Infection

The current study established that save for individuals over 50 years, HIV infection of the younger individuals and in both gender was accompanied with significant risk of depletion of serum albumin levels than the healthy control with similar demographic characteristics. However among the HIV-infected individuals, only younger age and CD4 cell count below 200 cells/mm^3, were risk factors for developing depleted serum albumin levels. Prevalence rate of serum albumin disorders in HIV- infected individuals was 60.1% with majority of these being hypoalbuminemia. Previous studies have also demonstrated that hypoalbuminemia (<35g/l) is a consequence associated with HIV infection and that it is associated with faster disease progression and increased mortality after seroconversion

(Mehta, *et al.,* 2006). Onwuliri (2004), simiallry reported that HIV patients had lower mean serum albumin levels than healthy controls. Serum albumin reduction in HIV as in other diseases that affect the liver is normally due to reduction in synthesis from infected and injured hepatocytes (Núñez, 2010). As such with progress of HIV and continued destruction of hepatocytes, investigations have revealed that serum albumin level is closely correlated to progression of HIV towards AIDS and conversely changes in its level closely mirror changes in CD4 level encountered in successful remission of disease in patients responding positively to anti-retroviral treatment (Olawuni, 2006). The assertions above concurred with observations from the current study that CD4 depletion and younger age are predisposing factors among HIV individuals for impaired albumin states.

5.5.3. Total Protein and HIV Infection

The current study established that apart from individuals over 50 years, HIV infection of the younger individuals and both gender was accompanied with significant risk of depletion of serum total protein levels than the healthy control with similar demographic characteristics. However among the HIV-infected individuals, younger age and CD4 cell count below 200 cells/mm^3, were risk factors for developing depleted serum total protein levels reflecting the outcomes observed in albumin levels. Total protein was lower than the physiological reference range in 52.8% of the HIV infected study participants. This concurred with findings from Okpa, *et al.,* (2015) and Audu, *et al.,* (2004), who observed that total protein in serum are often depleted below the physiological range. Scrimshaw and SanGiovanni, (1997) also showed that in HIV positive individuals, serum total protein was significantly lower than healthy controls. Similarly Ugwuja and Eze (2006) reiterated that HIV infected individuals

have lower mean serum total protein than health controls in their study. Increased losses or increased catabolism, reduction in intake and/or absorption due to sores in the mouth, pharynx and/or eosophagus, depression and side effects of medications have been listed as the some of the causes of decreased serum total protein in HIV infection (Macallan, 1999). However on their part, Hunziker and Colleagues (2003), and Audu, *et al.*, (2004) observed that total serum protein was elevated in HIV infection than in controls. These differences in findings could be attributed to the fact that the population of the current study included a wide range of patients at different stages of the disease while the later study observed a limited group of individuals with possibly different disease characteristics. Audu and colleagues (2004) attributed their findings to hypergammaglobulinemia an immune dysregulation state in HIV infection.

Despite their limited diagnostic value, total serum protein are physiologically essential in determining overall intravascular oncotic pressure. Equally important is the fact that by the volume they occupy within intravascular compartment and their overall charge, they impart significant impact on the distribution of anions and cations between interstitial fluid and plasma compartment (Aronson, *et al.,* 2005). The commonest form of protein balance perturbations in the current study was hypoproteinemia which if pronounced frees more proportion of the intravascular space which is taken up by solute free water (Vitting, *et al.,* 1990). This lowers the overall intravascular oncotic pressure allowing more fluid exudates to filter out of capillaries into the interstitial space accompanied with more electrolyte solutes.

The current study elucidated younger age and advanced immune depletion as predictors of serum protein disorders, a situation also reported by Echevrria, *et al.,* (1999). Egwuja and Eze

(2006) observed that serum total protein was significantly raised in HIV patients using ARTs than in HIV individuals not using Arts but failed to link serum protein decline to CD4 cell levels. This contrasted with the findings of the current study possibly that ARTs are not predictors of serum protein disorders possibly due to the sample size differences between the two study population and methodological differences in study design.

5.5.4. Bilirubin levels in HIV infection

The current study established that hyperbilirubinemia was the significantly higher in HIV infection compared to HIV negative controls irrespective of the gender or age of the individuals. Ranjit and colleagues (2015) and Onwuliri (2004) reported similar findings that HIV was a predictor to development of hyperbilirubinemia in HIV infected individuals. Free and conjugated serum bilirubin assays serve as sensitive indicators of liver disease (Boyer *et al,*1992). This study established that clinically elevated levels of direct bilirubin was present in 2.3% and of total bilirubin in 43.1% of the HIV infected individuals. Bilirubin, the principal product of heme catabolism, is cleared from the circulation by the liver microsomal enzyme UDP-glucuronosyltransferase (UGT), which conjugates it with glucuronic acid to form water-soluble metabolites destined for secretion into bile (Oude Elferink *et al,* 1995). Besides liver cell injury, inhibition of UGT by some ARVs has been reported as an explanatory mechanism that results in increased circulating bilirubin levels in HIV- infected patients (Stephen *et al.,* 2001).

Whereas the current study observed that CD4 lymphocyte depletion was associated with elevation of serum bilirubin levels among HIV- infected participants, age and ARV were not

elucidated as risk factors of developing impaired serum bilirubin levels. This is in contrast with other studies that have demonstrated that age, gender and ARVs are determinants of elevated serum bilirubin in HIV- infected individuals (Stephen, *et al.*, 2001). Findings of the current study may be attributed to use of ARTs with sparse hepatotoxicity in the HIV population that was recruited in the study.

5.6. Serum Electrolytes in HIV infection

Results of the study revealed mixed outcomes for the major serum electrolytes investigated.

5.6.1. Sodium

Results of the current study revealed that HIV infected individually generally had a higher prevalence of serum sodium impairment (26.1%) than the healthy counterparts. Specifically HIV infected females and younger individuals were noticed to have significantly elevated risk of developing serum sodium imbalance than healthy counterparts with similar demographic characteristics while the same was not reflected among males and individual of increasing age. Within the HIV infected individuals however rates of occurrence of serum sodium abnormalities were not significantly different with age or between gender groups. Sundaram and Colleagues (2010) noted that 67.2% of HIC infected individuals in their study had impaired serum sodium levels. Among their study population 59.9% had CD4 levels below 200cells/mm^3 with 20.4% having AIDS defining illness which could account for the difference noted.

Physiologically the regulation of sodium balance is pivoted on its being the major cation in extracellular fluid, and consequently is essential in determining a wide array of physiological parameters including, ECF volume, hence plasma volume, blood pressure and resting membrane cell membrane potential (Field, 2010). Balance of sodium ions within the various body fluid compartments is also critical in enabling occurrence of a wide range of membrane transport of various bio-molecules in and out of the cells, while in neuronal and muscle cells it is a major driver of electrical potential changes essential for communication and contraction activities in these cells (Rodriguez-Boulan & Powell, 1992). Several interrelated mechanisms serve to regulate total body sodium content and balance, by matching urinary sodium excretion to sodium intake. Monitoring of and afferent signaling about fluctuations of body sodium content is undertaken by receptors and mechanisms based within the cardiovascular system (volume receptors and pressure receptors), the kidneys (pressure receptors and renin hormone secretion), the liver (angiotensinogen) and the respiratory system (angiotensin converting enzyme) (Giebisch and Windhager, 2005). The main effector mechanism for correcting imbalances in body sodium content however, is the osmotic mechanisms controlling water intake due to the fact that sodium imbalance physiologically manifests in form of altered ECF volume (Walser, 1985). Some or all of organs and structures in all these systems have been reported in literature to be susceptible to pathological injuries either by the human immune deficiency virus or the co-infections/co-morbidities accruing out of long term HIV infection (Keating and Bjarnason, 1995).

The most common form of sodium disorders in the current study was hyponatremia (<135mmol/l) with hypernatremia observed in only two of the participants a finding which

concurred with findings of Alcazar (2008). Hyponatremia has been reported in 23.5% of non-hospitalized and 75% of hospitalized HIV positive individuals (Vitting, *et al.,* 1990) which the current study concurred with. Other studies have similarly reported that 35-55% of HIV-infected patients suffer from hyponatremia (Tang, *et al.,* 1993). Hyponatremia occurs frequently in infectious conditions, as a result of entry of sodium into cells, or sodium loss, as well as increased levels of vasopressin and resetting of osmoreceptors (Sitprija, 2008). However in HIV infection, studies have reported that occurrence of hyponatremia is commonly a consequence of three disorders all related to impaired water excretion namely; syndrome of inappropriate ADH secretion, water depletion and adrenal insufficiency (Vitting, *et al.,* 1990). The frequent type of hyponatremia in HIV infection is hypovolemic hyponatremia, due to water and salt losses and the syndrome of inappropriate antidiuretic hormone secretion (Hoen, *et al.,* 1991).

Hyponatremia, threatens optimal maintenance of aforementioned body parameters and processes which if extreme can be life threatening. In attempt at mitigating these deleterious outcomes, the body mainly deploys negative feedback mechanisms to stem further loss of sodium (Green and Giebisch, 1989). These include and are not limited to responses by the renal system, the cardiovascular system, as well as the central nervous system.

Responses by the renal system institute sodium and water retaining mechanisms underlied by changes in glomerular filtration rate and alteration of tubular handling of water and sodium ions (Wilcox, *et al.,* 1992). These changes in turn may alter other parameters downstream resulting in pathological consequences. Lowering glomerular filtration in response to

hyponatremia not only compounds HIVAN associated depression of GFR where it exists, but would in isolation or in both circumstances result in elevation of serum creatinine and serum urea (Giovanni, *et al.*, 2008). Sustained sodium and water retention by the kidneys, eventually results in increased urine osmolality and depletion of urine sodium concentration. Within the ECF rapid hyponatremia presents the special challenge of instigating flow of water from within cell cytoplasm leading to loss of cell volume a situation that is especially inimical to neurons within the central nervous system (Mason, 1980). However, prolonged hyponatremia predisposes to plasma volume depletion accompanied with lowered blood pressure (Guyton, *et al.,* 1974). Among physiological responses to these haemodynamic outcomes are renal events that mirror the same activities outlined above i.e. salt and water retention mechanisms with attendant downstream consequential changes affecting serum creatinine, urea and urine osmolality.

Anti-retroviral drugs were determinants of occurrence of serum sodium disorders with those using the drugs experiencing significantly lower rates of serum sodium impairment. The beneficial impacts of ARVs was mainly evident among the HIV infected females and those below 50 years of age.

5.6.2. Serum Potassium

Overall the prevalence of potassium disorders in HIV positive individuals was 17.3% and the most common form of serum potassium perturbation in the current study was hypokalemia (77.5%) with few individuals noted with hyperkalemia (22.5%). Musso (2004) similarly found that 19% of HIV –infected individuals had serum potassium imbalance. Peter, (1991)

also noted in his study that hypokalemia was more prevalent than hyperkalemia in HIV-infected individuals. The current study elucidated that as reported by Onwuliri (2004) the mean serum levels of potassium in HIV positive individual was not significantly different from that of HIV negative controls and neither was the rates of occurrence of impaired potassium levels. However Ugwuja and Eze (2006) reported that serum potassium levels were noted to be significantly lower in HIV positive individuals than healthy controls. On the other hand Sundaram, *et al,* (2010) observed that 60% of their study participants had impaired serum potassium levels and most of whom had AIDS defining characteristics with 53.6% having CD4 cells below 200 cells/mm^3 and 28.4% having AIDS defining illnesses. These differences suggest that potassium perturbations become more eminent in later disease stages which most of the study participants had not experienced.

Potassium, largely an intracellular electrolyte is essential in establishing resting membrane potential and overall in functions of excitable cells (Ho, *et al.,* 1993). A high intracellular concentration of potassium ions is also essential in maintaining cell volume, regulation of intracellular pH, controlling intracellular enzyme functions, DNA and protein synthesis as well as cell growth (Wingo & Cain, 1993). Whereas the kidneys play a crucial role in regulating body fluids potassium balance, the most important factor in acute or chronic adjustment of total body potassium load is the aldosterone hormone (Malnic, *et al.,* 1964). Aldosterone secretion is especially triggered by elevated plasma potassium ion concentration (hyperkalemia). Surge in plasma aldosterone increases the excretion of K+ by the principal cells of renal collecting duct which by negative feedback leads to fall of plasma potassium levels back to normal.

Unique to potassium however, is that factors influencing distribution of potassium between ICF and ECF in disease situations can redistribute the concentration of potassium between compartments resulting in depletion from one and accumulation in the other compartment without any overall change in total body potassium load. Thus studies have reported that potassium is driven into cells by alkalosis, and hormones (insulin, catecholamines and aldosterone) to the extent of culminating in hypokalemia. On the contrary extracellular acidosis, lack of insulin or of aldosterone can precipitate hyperkalemia by a reverse mechanism (Agata, *et al.*, 2008). These pathological conditions resulting in redistribution are frequently observed in HIV- infected patients. Deleterious impact by either the HIV virus or co-morbid conditions on the kidney or liver (angiotensinogen) or the lungs can therefore adversely affect potassium balance. Lately however, a putative mechanism behind potassium disorders in HIV infection has been posited as being impairment of transmembrane potassium channels resulting in alteration of transmembrane potassium transport (Carlos, *et al.*, 1999). In addition to impacting on the ability of cells to conduct electrical impulse along their membrane, chronic K+ depletion leads to a variety of metabolic disturbances including; disabling the urine concentrating ability of kidneys, tendency to develop metabolic alkalosis and subsequently acid-base disturbance, as well as precipitating enhanced renal ammonium excretion (Jamison, 1987).

Gender, age, CD4 lymphocyte count and ARVs were not associated with the risk of developing adverse disturbances in serum potassium levels in the current study. Obirikorang and colleagues (2014) similarly observed that use of ARVs was not a predictor of the rates of

occurrence of potassium impairment. However Coca and Perazella (2002) observed that anti-retroviral therapy particularly tenofovir is associated with hypokalemia. Sundaram, *et al.*, (2010) also intimated that younger age was a determinant of development of serum potassium impairment in HIV infection. These differences suggest that disease characteristics particularly the history of the disease play an important role in development of serum potassium perturbations.

5.6.3. Serum Chloride Ion Levels

The distribution of chloride ions within the body fluids compartments mirrors that of sodium ions. However unexpectedly, the current study observed that the levels of chloride ions and the prevalence of its imbalance in HIV infected individuals were not significantly different from the state of serum chloride ions in the HIV negative controls. Nonetheless the prevalence of chloride ions imbalance in seropositive individuals was 27.4% with majority being hypochloremia in tandem with the sodium ion states in the same group. Onwuliri (2004) also established that serum chloride ion levels were not found to be significantly different between HIV + and HIV – individuals in their study. On their part Ugwuja and Eze (2006) reported that HIV positive individuals had significantly lower serum chloride ion levels than their healthy controls a fact that could be attributed to the sample selection. Sundaram and colleagues (2010) however noted that 68.7% of their participants had impaired serum chloride ion levels. Majority of their study participants had AIDS defining parameters which could explain the higher rates of occurrence of serum chloride imbalance than in the current study. Among the HIV + individuals age, gender, ARV use or CD4 levels were not determinants of impaired serum chloride levels.

5.7. Serum Electrolytes changes associated with Pathological Markers of Kidney and Liver function.

Kidney function insufficiency was significantly correlated with urea and creatinine levels in serum. Similarly creatinine and urea levels showed significant correlation with each other. However whereas the presence of underlying kidney disorders in HIV positive individuals was associated consistently with shifting prevalence in the levels of kidney functional markers imbalance, it had inconsistent association with changes in rates of occurrence of serum electrolytes disorders. Accordingly the prevalence of creatinine imbalance in HIV + individuals with normal eGFR was 7.2%, but this proportion increased to 34% in cases with mild reduction of glomerular filtration rate (<90mls/l) and to 60.2% in moderate reduction of eGFR (<60mls/min). On the other hand 4.5% of seropositive individuals with normal eGFR had urea disorders which increased to 8.8% in individuals with moderate decline of eGFR and 75% in patients with severe reduction of glomerular filtration rate (<30mls/min).

On the other hand rates of occurrence of potassium disorders increased significantly but with severe reduction in kidney function (50% v/s 21.5% in moderate and 16.9% in mild kidney dysfunction, $p = 0.02$), while the rates of occurrence of serum sodium and chloride disorders remained unchanged with declining kidney function ($p = 0.948$ and $p = 0.690$, respectively). Glassock and Colleagues (1990), also observed that serum potassium imbalance are more likely to develop in extremely morbid HIV patients. However overall where there was underlying kidney disorders the magnitude of existing electrolytes imbalance that could be explained by the underlying kidney functional impairment were 0.1% for Na+ ions, 0.3% for

Cl- and 0.01% for K+ ions respectively. Similarly, the magnitude of electrolytes imbalance attributable to the underlying liver defect irrespective of the indicator of function was less than 1% of the observed overall electrolytes disorders, despite the fact that low albumin and total protein levels and hyperbilirubinemia were apparently accompanied with co-existing serum electrolytes imbalance.

This could either be due to the fact that even though kidneys are essential for F&E regulation, the magnitude and / or types of structural injuries in kidneys due to HIV infection are not extensively deleterious to mechanisms by which the kidneys are involved in regulating body fluids and electrolytes or adjustment of regulatory performance by pre-renal regulatory organs, effectively remedied the negative impacts of renal disorders on body fluids and electrolytes levels. In addition this could be explained by the fact that existing electrolytes perturbations could have developed as a result of disorders in the ignored regulatory organs and cellular elements. Impaired adrenal glands or pituitary glands for instance could induce disturbance in the regulation of electrolytes even when the kidneys are healthy by cutting the link of hormonal events that trigger appropriate responses to altered electrolytes levels (Seldin and Giebisch, 2000). Thus potential sources of serum electrolytes disorders are possibly unaccounted for when routine analysis of observed electrolytes disorders in HIV are confined to probable kidney disorders only.

Mean serum electrolytes levels in HIV infected individuals with impaired liver enzyme levels were all within the normal physiological reference range with exception of HIV+ participants with abnormal serum ALT levels whose mean serum sodium levels was significantly lower

than those with normal serum ALT levels. In HIV positive individuals with impaired albumin or total proteins levels the rates of occurrence of serum potassium disorders was significantly lower than that for of seropositive individuals with normal albumin or total protein levels. On the other hand in HIV–infected individuals with hyperbilirubinemia the prevalence of Na+ and K+ ion disorders were seen to be significantly higher than in seropositive patients with normal total bilirubin levels. These findings suggested that liver disorders are determinants of certain serum electrolytes disorders. However correlation of markers of liver function with electrolytes revealed that changes in serum electrolytes levels attributable to the underlying imbalance in liver function markers only accounted for a fraction and not all of the magnitude of existing serum electrolytes disorders. Thus in HIV+ individuals with underlying serum albumin impairment only 0.2% of Na+ ,0.3 % of K+ and 3% of Cl- perturbations in serum could be attributed to the causes that triggered the serum albumin defects. This implies that the observed association if the sole basis relied upon to explain the development of serum electrolytes defects would ignore other possible underlying causes. This is a possible weakness in the currently practiced routine clinical studies of electrolytes disorders in HIV patients.

The range of sources of electrolytes defects in HIV infection are diverse and are largely classified as disturbance due to increased losses, reduced intake/absorption and alterations in metabolism (Ugwuja and Eze, 2006). Injuries to the gastro intestinal epithelial lining in HIV infection have been documented to induce increased intestinal permeability in this group (Williams, *et al.,* 2011). Other possible causative influences on electrolytes levels in HIV infection include, opportunistic infections, inflammatory conditions, and oncological variables

as well as a variety of medical interventions (ARVs, antibiotics, and anti-neoplastic agents) (Musso, *et al.,* 2016). These factors impact on a wide range of structures of electrolytes regulation from the neuroendocrine, cardiovascular to cellular elements. Studies have intimated that intracellular escape of electrolytes in certain HIV conditions influence observed electrolytes states (Voss, *et al.,* 1996). However Voss and Colleagues (1996) also identified cytotoxic events as being associated with extrusion of intracellular electrolytes into the intravascular compartment leading to elevation especially of K+ ion levels.

5.8. Distribution of Major Electrolytes within Body Fluids Compartments

The levels of the major serum electrolytes displayed significant correlation in every category of the individuals (healthy and HIV+). However the strength of this correlation diminished in HIV- infected patients with abnormal electrolytes states. This could be explained by the fact that existing electrolytes imbalance if chronic could impair the physiological capacity to redistribute major electrolytes to attain the optimal concentrations of sodium predominantly in the extracellular space together with chloride and potassium predominantly concentrated in the intracellular fluid.

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The purpose of this study was to assess the association of serum electrolytes with kidney and liver function in HIV –infected individuals This was intended to explore and highlight the actual magnitude of electrolytes disorders attributable to these two organs which are part of the two systems (GIT and renal) frequently associated with fluid disturbance (depletion or overload) fluid volume changes (depletion or overload). Fluid volume changes are the most immediate causes of serum electrolytes imbalance in most infectious diseases. It was hoped that local health care systems could use the findings to improve the effectiveness of diagnosis, evaluation and management of body fluids and electrolytes states in this group of patients. The study established that;

1. The prevalence of renal insufficiency was 54% of the HIV- infected individuals in the study which was significantly higher than the rates of occurrence of these conditions in the healthy population. Female gender, increasing age and immune depletion (CD4<200cells/mm^3) were determinants of rates of occurrence of kidney function impairment in HIV+ persons.
2. Accordingly levels of kidney function markers in serum (creatinine and urea) were found to be impaired in 26.1% and 4.4% of the HIV –infected persons respectively
3. Rates of occurrence of liver function impairment were observed to be significantly enhanced in seropositive individuals compared to seronegative persons. Markers of liver function insufficiency such as transaminitis, depletion of total protein and albumin as well as hyperbilirubinemia were markedly more prevalent in seropositive individuals than the healthy population

4. There were perturbations of the balance of sodium, potassium and chloride ion levels in 26.1%, 17.3%, and 27.4% of the HIV- infected individuals. Majority of the imbalance involved serum electrolytes depletion disorders
5. Whereas observed electrolytes levels exhibited limited association with kidney or liver function states, underlying kidney or liver function impairment were not found to be sufficient explanation for the co-existing major serum electrolytes disorders in HIV – infected individuals.
6. The levels of the major electrolytes displayed consistent correlation with sodium disorders matched with chloride disorders (26.1% v27.4%) and K+ ion levels inversely correlated with sodium levels. However in instances where there was impaired sodium levels in HIV-infection, the levels of Cl- ions and K+ ions showed weak correlation suggesting impaired physiologically coupled distribution between the fluid compartments. Impaired compartment specific distribution of electrolytes could be one of the sources of serum electrolytes imbalance in HIV infection.

6.2 Recommendations

In view of the results from the study the following are recommended so as to improve the among the HIV positive patients

1. The high prevalence of kidney insufficiency and liver function defects in the local HIV population requires dedicated attention to highlight their determinants and address their impact in the disease process. At risk groups such as female gender, patients with extreme immune depletion and increasing age need to have special programs addressed towards their health challenges.
2. The prevalence of electrolytes imbalance shows that HIV + individuals in the region are prone to multiple electrolytes disorders and there is need to roll out health care programmes that address these challenges concurrently
3. Neither kidney defects nor liver defects could fully explain the existing electrolytes disorders. Therefore laboratory, diagnostic and management practices that limit their focus on immediate clinical episodes and the primary regulatory organs defects to explain observed electrolytes imbalance in HIV patients do not explore all possible causes of electrolyte disorders in HIV infection.
4. Full metabolic panels investigative approaches practiced in resource endowed settings need to be developed to address their costs and avail them universally as a means of addressing electrolytes disorders in HIV –infected individuals.

6.3 Suggestions for Further Studies

In view of the scope of this study there are important issues that were not addressed and are recommended for further research in the area of HIV and electrolytes

1. A longitudinal study to delineate the histopathological nature of kidney and liver diseases that prevail in the local HIV population and any changing trends in their manifestation as the HIV infection progresses
2. The role of secondary fluids and electrolytes regulatory organs in development of serum electrolytes imbalance in HIV –infected individuals need to be studied further
3. Studies need to be undertaken to investigate the relationship between hyperbilirubinemia and serum sodium and potassium levels as this study found a higher prevalence of disorders of these electrolytes in impaired bilirubin levels.

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APPENDICES

APPENDIX I: CONSENT FORM

EFFECT OF HIV INFECTION ON FLUID AND ELECTROLYTE STATUS IN PLASMA AND URINE OF ADULT PATIENTS ATTENDING NYANZA GENERAL HOPSITAL OF KISUMU COUNTY, WESTERN KENYA.

I am a doctorate student in Medial Physiology at the Maseno University, School of Public Health and Community Development (ESPUDEC). My study above will investigate the state of body fluids and electrolytes and their correlation to various HIV factors. You are voluntarily requested to participate by donating requisite samples of blood and urine, which will be tested for the levels of the electrolytes. This study is designed without risks to the patient and the information obtained will be handled with complete confidentiality. If you have any question be free to consult with the attending investigator for clarification.

You are entitled to withdraw from participation at any stage without prejudice. Any result obtained is confidential.

Participant Sign: _____ Date: _____

Investigator Sign: _____ Date: _____

LUO VERSION OF CONSENT

Ma en nonro ma ng'iyo kaka teko pi kod chumbi romo e dend ng'atno ma osedak gi kute mag ayaki kendo osetiyo gi yedhe mag ayaki kuom kinde mang'eny. Ikwayi mondo ichiw remo gi lach mondo onon. Ibiro miyi duoko ma oyudi, kendo ka oyudi ni nitie ranyisi madwaro thieth to ibiro miyi thieth. Janono onge kod thuolo mar tiyo gi duoko no eyo maopogre gi gima ne onyis jatuo ni ibiroti kode, ma jatuo ok ochiwo thuolo makamo.

Ka ineno ni idwaro weyo nonro ekinde moro amora to in thuolo mar timo kamano. Janono ok bitmare mondo idhi nyime kod yudo thieth moro amora nikuop iweyo bedo achiel kuom jogo ma itimo negi nonro.

Jatuo Sign: _____ Date: _____

Janono Sign: _____ Date: _____

The study is authorized by the Ethical Review Committee of the Nyanza Provincial General Hospital whose telephone number is provided below should you need to clarify certain concerns with them.

ERC telephone number; 057- 2020801/2020803/2020321

APPENDIX II: PATIENT CLINICAL/DEMOGRAPHIC DATA

1. File number _____
2. Age_____
3. Sex _____
4. Date _____
5. Weight _____
6. Blood pressure _____
7. Clinical fluids and electrolytes symptoms present; oedema ☐

Type of oedema (generalized, localized, ascites) _____

Mucous membranes _____

8. Date first diagnosed with HIV infection _____
9. Date of initiation of ARVs _____ ARV types used currently _____

10. Have you ever stopped using ARVs; YES_____ NO _____

If yes; How many times have you had to change drugs _____

Why did you change the ARV drugs?

(a) Adverse/toxic reactions_____

(b) Resistance confirmed by deterioration of CD4 levels or escalating viral load levels in the course of drug use _____

(c) Non compliance _____

11. Have you ever changed the combination of ARVs; YES _____ NO _____

If Yes; (a) From which drugs previously used?_____

(b) To which combination of drugs ? _____

12. Which non –antiretroviral drugs are you currently receiving

Drugs	**Type of medication** (e.g. anti-inflammatory, antibiotics, analgesics)
_____	_____
_____	_____
_____	_____

LABORATORY DATA

1. **BLOOD**

Site of sample_____

Amount_____

Time taken _____

CD4 Lymphocyte count_____

Hemoglobin_____

Hematocrit _____

pH _____

Osmolality _____

Sodium _____

Potassium _____

Chloride _____

Bicarbonates _____

Creatinine _____

Protein _____

Albumin _____

ALT _____

SGOT _____

BUN _____

Glucose _____

URINE (random)

Physical characteristics _____

pH _____

Specific gravity _____

Glucose _____

Creatinine _____

Sodium _____

Potassium _____

Bicarbonates _____

Chlorides _____

Protein _____

Albumin _____

Hematuria _____

APPENDIX III: LABORATORY REFERENCE INTERVALS

JARAMOGI OGINGA ODINGA TEACHING & REFERRAL HOSPITAL
P.O BOX 849-40100 KISUMU
LABORATORY REFERENCE INTERVALS
CLINICAL CHEMISTRY DEPARTMENT

TEST	CHILDREN 0-10 YRS	ADULT- FEMALE	ADULT-MALE	PANIC VALUES
T. Bilirubin	0.0-19.0µmol/l**	0.0-19.0µmol/l	0.0-19.0µmol/l	≥30.0
D. Bilirubin.	0.0-4.2µmol/l	0.0-4.2µmol/l	0.0-4.2µmol/l	≥10.0
T. Protein.	56.0-80.0g/l	65.0-80.0g/l	65.0-80.0g/l	≤40.0
Albumin	35.0-50.0g/l	35.0-50.0g/l	35.0-50.0g/l	≤20.0
A.L.P	100-500U/L	0-240U/L	0-270U/L	≥350
A.L.T	0-65 U/L	0-65 U/L	0-70U/L	≥80
A.S.T	0-50U/L	0-50U/L	0-50U/L	≥60
Gamma GT	0-49U/L	0-49U/L	0-49U/L	≥60
Urea.	1.7-6.5mmol/l	2.0-7.0mmol/l	2.1-7.5mmol/l	≥8.0
Creatinine	26-97µmol/l	40-107µmol/l	45-120µmol/l	≥130
Sodium	135-155mmol/l	135-155mmol/l	135-155mmol/l	≥165
Potassium.	3.4-5.3mmol/l	3.4-5.3mmol/l	3.4-5.3mmol/l	≥5.8
Chloride.	98-106mol/l	98-106mol/l	98-106mol/l	≥110
Phosphorus.	0.8-1.45mmol/l	0.8-1.45mmol/l	0.8-1.45mmol/l	≥1.6
Calcium.	2.2-2.6mmol/l	2.2-2.6mmol/l	2.2-2.6mmol/l	≥2.8
Uric acid.		155-357µmol/l	200-400µmol/l	≥480
Cholesterol	5.2-6.2mmol/l	5.2-6.2mmol/l	5.2-6.2mmol/l	≥6.7
Cholesterol HDL	0.9-1.4mmol/l	0.9-1.4mmol/	0.9-1.4mmol/	≥1.6
Cholesterol LDL	0-3.3mmol/l	0-3.3mmol/l	0-3.3mmol/l	≥4.1
Triglycerides	2.3-4.5mmol/l	2.3-4.5mmol/l	2.3-4.5mmol/l	≥5.0

Glucose.(RBS)	3.2-8.3mmol/l	3.2-8.3mmol/l	3.2-8.3mmol/l	≥10
Glucose.(FBS)	2.1-6.6mmol/l	2.1-6.6mmol/l	2.1-6.6mmol/l	≥7.0
L.D.H	204-414U/L	204-414U/L	204-414U/L	≥450
Lipase	7-58U/L	7-58U/L	7-58U/L	≥75
α-Amylase	22-80U/L	22-80U/L	22-80U/L	≥100
B.U.N.				
Iron.	50-120mcg/dl	60-150mcg/dl	60-17-mcg/dl	≥190
Magnesium.	1.7-2.2mg/dl	1.7-2.2mg/dl	1.7-2.2mg/dl	≥2.5
C.S.F Glucose.	2.1-5.1mmol/l	2.1-5.1mmol/l	2.1-5.1mmol/l	≥7.0
C.S.F Protein.	15.0-40.0g/l	15.0-40.0g/l	15.0-40.0g/l	≥100.0
P.S.A.	0-4 ng/ml	0-4 ng/ml	0-4 ng/ml	
H.PYLORI	0-5 U/ml (NEGATIVE)	5-10 U/ml(Equivocal)	>10 U/ml (POS)	
A.F.P.	0.2-8.5 ng/ml	0.2-8.5 ng/ml	0.2-8.5 ng/ml	
T.S.H.	0.32-5.2 mIU/L	0.32-5.2 mIU/L	0.32-5.2 mIU/L	
T3.	0.92-3.23 Umol/l	0.92-3.23 Umol/l	0.92-3.23 Umol/l	
T4.	4.7-12.5 ug/dl	4.7-12.5 ug/dl	4.7-12.5 ug/dl	
C.E.A.		0.3-5.0 ng/ml (NON-SMOKING)	0.4-8.5 ng/ml (SMOKING)	
B-H.C.G.	<10.0 IU/L (NEGATIVE)	10.0-25.0 IU/L (SUSPECTED)	>25.0 IU/L (POSITIVE)	
PROLACTIN	31-433 (MALE)	33-413 (FEMALE PRE-MENOPAUSE)	118-555 (FEMALE POST-MENOPAUSE)	

** Total Bilirubin in Neonates is up to ≤130mmol/l

APPENDIX IV: LABORATORY RESULTS

JARAMOGI OGINGA ODINGA TEACHING & REFERRAL HOSPITAL

P.O BOX 849-40100 KISUMU

LABORATORY RESULTS

CLINICAL CHEMISTRY DEPARTMENT

PATIENT ID: …06/11036… AGE: … YRS…SEX: …FEMALE…

TEST: LFT, U/E/C DEPT: …PSC…

				REFERENCE RANGES		
PARAMETER	RESULT	UNITS	FLAG	MALE	FEMALE	CHILD
CD4	382	Cells/uL		410-1590	410-1590	<25%
T.BILIRUBIN	4.1	mmol/L		0-17.0	0-17.0	0-130.0
D.BILIRUBIN	3.6	mmol/L		0-4.0	0-4.0	0-4.0
ALBUMIN	39.2	g/L		35-50	35-50	35-50
T PROTEIN	68.0	g/L		65-80	65-80	65-80
AST	48.3	U/L		0-50	0-50	0-50
ALT	40.5	U/L		0-70	0-65	0-65
UREA	3.6	mmol/l		2.5-7.5	2.0-7.0	1.6-6.5
CREATININE	79.8	Umol/l		45-120	40-107	26-97
SODIUM	145.0	mmol/l		135-155	135-155	135-155
POTTASSIUM	4.3	mmol/l		3.4-5.5	3.4-5.5	3.4-5.5
CHLORIDE	98.0	mmol/l		98-106	98-106	98-106
GLUCOSE.(RBS)	4.8	mmol/l		3.2-8.3	3.2-8.3	3.2-8.3

PERFORMED BY: …MOSES……SIGNATURE: ……DATE: ……

REVIEWED BY: …MBAJA……....SIGNATURE: ……DATE: ……..

APPENDIX V: MAP OF THE STUDY AREA