PREVALENCE AND FACTORS ASSOCIATED WITH RENAL DYSFUNCTION AMONG HIV INFECTED PATIENTS RECEIVING TENOFOVIR AT MULAGO COMMUNICABLE DISEASE CLINIC.

BY

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DECLARATION

I declare that the work submitted in this dissertation has been done by me and has not been submitted for any other degree award in any university

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DEDICATION

I dedicate this work to my lovely wife Susan; and our dear children Louise, Leticia (RIP), Lisa and Blessing.
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<tr>
<td>AIDS</td>
<td>Acquired immune deficiency syndrome</td>
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<tr>
<td>AKI</td>
<td>Acute kidney injury</td>
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<td>ART</td>
<td>Antiretroviral therapy</td>
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<td>CD4</td>
<td>Cluster cell differentiation</td>
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<td>CKD</td>
<td>Chronic kidney disease</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>EGFR</td>
<td>Estimated glomerular filtration rate</td>
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<td>FDC</td>
<td>Fixed drug combination</td>
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<td>HBV</td>
<td>Hepatitis B virus</td>
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<td>HIV</td>
<td>Human immunodeficiency virus</td>
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<td>HIVAN</td>
<td>Human immunodeficiency virus associated nephropathy</td>
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<td>HIVICK</td>
<td>Human immunodeficiency virus immune complex kidney disease</td>
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<td>IDI</td>
<td>Infectious disease institute</td>
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<tr>
<td>MRP</td>
<td>Multidrug resistant protein</td>
</tr>
<tr>
<td>MTDNA</td>
<td>Mitochondrial deoxyribonucleic acid</td>
</tr>
<tr>
<td>NRTIs</td>
<td>Nucleoside reverse transcriptase inhibitors</td>
</tr>
<tr>
<td>OAT</td>
<td>Organic anion transporter</td>
</tr>
<tr>
<td>PLWHA</td>
<td>People living with HIV/AIDS</td>
</tr>
<tr>
<td>PZA</td>
<td>Pyrazinamide</td>
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<td>RFTS</td>
<td>Renal function tests</td>
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<td>RIF</td>
<td>Rifampicin</td>
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<tr>
<td>ST</td>
<td>Streptomycin</td>
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<tr>
<td>TDF</td>
<td>Tenofovir disoproxil fumarate</td>
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<td>WHO</td>
<td>World Health Organisation</td>
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</table>
OPERATIONAL DEFINITIONS

Renal dysfunction for this study will be defined as any of the following; Calculated estimated glomerular filtration rate (eGFR) < 60ml/min/1.73m² or urine abnormalities manifesting as proteinuria of ≥ 2+ glycosuria with a normal blood sugar.

Tenofovir disoproxil fumarate - based regimen- Antiretroviral therapy regimen with one of the drugs as Tenofovir disoproxil fumarate (TDF)
ABSTRACT

**Background:** There is an increasing burden of non-communicable disease globally. Tenofovir disoproxil fumarate (TDF) is the most commonly prescribed antiretroviral drug globally. Studies show that patients receiving TDF are more prone to renal dysfunction at some point in time during treatment. Evaluation of kidney function is not routinely done in most HIV public clinics. Identification of renal dysfunction is key in resource constrained settings because managing patients with end stage renal disease is costly.

**Objectives:** To determine the prevalence, factors associated with renal dysfunction and electrolyte abnormalities among HIV infected patients receiving tenofovir disoproxil fumarate at Mulago Communicable Disease Clinic.

**Methods:** This was a cross-sectional study conducted at Mulago Communicable Disease Clinic between November 2017 and March 2018, a total of 305 patients were recruited. Eligible patients were aged ≥18 years, on TDF based ART for 6 or more months. Data on socio-demographics, clinical and laboratory characteristics were collected using interviewer-administered questionnaires. Blood collected for serum electrolytes and blood sugar was analysed by Abbott Architecture-plus©-Ci (Chemistry & immunochemistry analyser) machine. A dipstick was done on urine for presence of protein, glucose or haematuria. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-Epi formula. Renal dysfunction was defined as any of the following; either eGFR<60mL/min/1.73m², or proteinuria of ≥ 2+ on urine dipstick, glycosuria with normal blood glucose. Electrolyte abnormalities were reported as proportions and the factors associated with renal dysfunction were determined using multivariate logistic regression.
Results: We enrolled 278 participants. One hundred sixty nine (60.8%) were females, majority 234(84.2%) were < 50 years old, 205 (73.74%) were in WHO stage 1, most participants 271(97.5%) in addition to tenofovir disoproxil fumarate were receiving lamivudine/efavirenz. The median age was 37(IQR 29-45) years, median duration on ART was 36 (IQR 24-60) months. The prevalence of renal dysfunction was 2.52% (7/278). Most noted electrolyte abnormality was hypocalcaemia (15.44%). Factors associated with renal dysfunction could not be determined because of limited sample size.

Conclusion: The prevalence of renal dysfunction was low though some participants had hypocalcaemia. Use of tenofovir disoproxil fumarate in resource limited settings is safe to use with minimal need to monitor renal function rigorously.
CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

People living with HIV/AIDS (PLWHA) are living longer and as a result face a challenge of increasing morbidity from non-communicable diseases (NCDS) (Haregu, Oldenburg, Sestwe, Elliott, & Nanayakkara, 2012; Holmes et al., 2010). Kidney disease manifesting as renal dysfunction is one of the emerging NCDs in Sub-Saharan Africa and the world, with a prevalence of 13.9% (Stanifer et al., 2014) and 20-48.5% (Naicker, 2013) respectively. There is increasing concern that HIV being epidemic in this region, is contributing to the increasing prevalence of renal dysfunction in Sub-Saharan Africa (Stanifer et al., 2014). The aetiology of renal dysfunction includes; HIV-associated nephropathy (HIVAN), HIV immune complex kidney disease (HIVICK), drugs used for treatment of opportunistic infections, antiretroviral therapy (ART), use of non-steroidal anti-inflammatory drugs (NSAIDS), herbal medicines, other comorbid conditions namely diabetes mellitus, hypertension and Hepatitis B & C. The consequences of renal dysfunction range from acute kidney injury (AKI), chronic kidney disease (CKD), end stage renal disease (ESRD) or death (Campos, Ortiz, & Soto, 2016). In countries with limited resources curative services for NCDs are an obvious strain to government rendering prevention as the best strategy (Bastos & Kirsztajn, 2011; Naicker, 2013; Wouters, O'donoghue, Ritchie, Kanavos, & Narva, 2015). Prevention involves identifying those conditions early, continuous health education/screening of the infected/affected people and appropriate referral for proper management to delay complications.
The 90-90-90 UNAIDS strategy by 2020 (HIV/AIDS, 2014) is not far away, but with it more people will be on antiretroviral therapy (ART) coupled with the “test and treat approach”, whereby an individual testing positive for HIV is initiated on ART regardless of the CD4 or WHO clinical stage (Organization, 2017). HIV infected people will be on ART for a long time yet with advancing age renal dysfunction deteriorates (Kim et al., 2017). PLWHA will be on ART for life, live longer and probably suffer renal dysfunction at some point in their life. When ART programs started in Uganda, there was a lot of emphasis on initial evaluation of patients before starting treatment, but as the number of patients has increased this has not always been the case (DART, 2010). With regard to ART, patients on Tenofovir disproxil fumarate (TDF) based regimen from a number of studies are more prone to decline in kidney function. Other factors associated with renal dysfunction include, being older, being female, African-American ethnicity, low weight, low CD4 count, high viral load and having co-morbid conditions (Crum-Cianflone et al., 2010). The cost of managing a patient with ESRD is high yet early identification with simply estimating glomerular filtration rate and a urine dipstick can help. The problem is even made worse by presentation of renal dysfunction being asymptomatic, late patient presentation, having few nephrologists and few/costly renal replacement therapies (Osafo et al., 2016). There are few studies quantifying the magnitude of renal dysfunction among HIV positive patients in sub-Saharan Africa and no renal registries/inadequate data in the region (Odongo, Wanyama, Obol, Apiyo, & Byakika-Kibwika, 2015; SCarPino, 2015). This study was carried out to quantify the magnitude of renal dysfunction and its associated factors among HIV positive patients receiving TDF based ART.

1.2 Problem statement
Despite the increasing burden of non-communicable diseases in Uganda, assessment for kidney function is not routinely done in HIV government supported out-patient clinics, because of the cost implications, large patient numbers and lack of awareness (Campos et al., 2016; SCarPino, 2015). Laboratory tests are done for symptomatic patients and by this time irreversible damage may have already occurred. Majority of patients on ART are receiving TDF based therapy and the number is set to increase with a number of strategies aimed at this in place (HIV/AIDS, 2014; Organization, 2017). Patients with HIV especially those on TDF and Indinavir based therapy are more prone to renal dysfunction compared to other drugs (Cooper et al., 2010). If not identified early these patients may progress to ESRD and even death. Recognition of renal derangement is appropriate and saves resources in constrained health systems (Perico & Remuzzi, 2014; Wouters et al., 2015). Uganda has few nephrologists, few dialysis centres and is currently unable to carry out renal transplant. We undertook this study to evaluate the prevalence and factors associated with renal dysfunction among HIV infected patients receiving TDF based ART at Mulago Communicable Disease Clinic a government aided facility.

1.3 Justification of the study

Previous studies on renal dysfunction have been carried out in research settings which have meticulous follow up and monitoring of patient appointments and adverse events. These programs tend to have enough funds for evaluating even asymptomatic patients. This is not always the case for other HIV government aided outpatient clinics that may be overwhelmed by large patient numbers and few staff. Understanding the magnitude of renal dysfunction will provide evidence regarding need for rigorous evaluation of kidney function in HIV infected patients receiving TDF based therapy for six months or more. This study provides simple methods to assess renal dysfunction in a busy HIV outpatient clinic. This study was carried out
in an outpatient clinic embedded in a national referral hospital (public facility). The results of this study will provide data for future research on TDF and renal dysfunction in resource limited settings.
**Pre-existing kidney disease** should have been documented to include but not limited to any of the following; Chronic kidney disease, HIVAN, HIVICK, Glomerulonephritis etc.

**Factors studied:** Socio-demographic, clinical and drug factors though the data tool captured some information on some concurrent opportunistic infections.
1.4 **Research questions**

1. What is the prevalence of renal dysfunction among HIV infected patients receiving TDF based ART for at least six months at Mulago Communicable Disease Clinic?
2. What are the factors associated with renal dysfunction among HIV infected patients receiving TDF based ART for at least six months at Mulago Communicable Disease Clinic?
3. What electrolyte abnormalities are seen in HIV infected patients receiving TDF based ART for at least six months at Mulago Communicable Disease Clinic?

1.5 **Objectives of the study**

1.6 **General objective;**

To determine the prevalence, factors associated with renal dysfunction and describe the electrolyte abnormalities among HIV infected patients receiving TDF based ART for at least six months at Mulago Communicable Disease Clinic

1.6.1 **Specific objectives**

1. To determine the prevalence of renal dysfunction among HIV infected patients receiving TDF based ART for at least six months at Mulago Communicable Disease Clinic.
2. To determine the factors associated with renal dysfunction among HIV infected patients receiving TDF based ART for at least six months at Mulago Communicable Disease Clinic.
3. To describe the electrolyte abnormalities seen in HIV infected patients receiving TDF based ART for at least six months at Mulago Communicable Disease Clinic.
CHAPTER TWO

2.0 LITERATURE REVIEW

Renal dysfunction manifests itself as acute kidney injury (AKI) or chronic kidney disease (CKD) in HIV positive patients. AKI can adequately be managed when identified, but may develop into CKD which has become an important comorbidity among HIV-infected persons (Samir K Gupta et al., 2005). Since the introduction of antiretroviral therapy (ART), the number of deaths due to opportunistic infections has significantly declined, a greater proportion of patients are developing chronic conditions not traditionally related to HIV, such as cardiovascular, liver, and kidney disease (Palella Jr et al., 2006). As the prevalence of HIV infection increases, renal dysfunction is projected to increase (Samir K Gupta et al., 2005; Schwartz et al., 2005). Chronic kidney disease during the pre-ART period was largely a result of HIV-associated nephropathy (HIVAN), which was associated with African American ethnicity and low CD4 cell counts (Szczech et al., 2004). The introduction of ART has resulted in significant change in the epidemiology of renal disease among HIV positive patients, with a substantial reduction in the incidence of HIVAN (Lucas et al., 2004). In contrast with HIV immune complex kidney disease (HIVICK), these patients usually have high CD4 counts, low viral load, have high blood pressure and normal sized kidneys on ultra sound imaging. Despite the benefits of ART, renal dysfunction remains common among HIV patients (Scherzer et al., 2012). Contributing factors to renal disease among HIV patients includes; drugs, the ageing of the population, concurrent medical conditions such as diabetes mellitus, hypertension, and uncontrolled viremia.
2.1 Differential diagnosis of renal dysfunction in HIV infected patients

Renal dysfunction in HIV positive patients has a wide variety of causes and the clinician should consider a wide differential diagnosis including; AKI, CKD, common HIV non-specific glomerulopathies, drugs, fluid and electrolyte disorders (Campos et al., 2016). Acute kidney injury (AKI) is the most common presentation among these patients associated with increasing need for admission (Kalim, Szczech, & Wyatt, 2008; SCarPino, 2015). The causes are vast being divided into the prerenal (kidney hypo perfusion & ischemia), renal (acute tubular necrosis, acute interstitial nephritis, glomerulonephritis, rhabdomyolysis) and post renal (mainly obstruction). A number of drugs used in the management of these patients may cause AKI, such as ART (cotrimoxazole, Amphotericin-B and anti-TB medicines. AKI carries a risk of progression to end stage renal disease if not well managed. Treatment varies from addressing the underlying cause, stopping nephrotoxic medications and correcting any electrolyte abnormalities. Other possible causes of CKD include; Immune complex mediated/post infectious glomerulonephritis, Immunoglobulin A nephritis, mixed sclerotic/inflammatory disease, membranous glomerulopathy, Lupus-like disease and thrombotic microangiopathy (Campos et al., 2016). Common HIV non-specific glomerulopathies include; Hepatitis C virus related membranoproliferative glomerulonephritis/cryoglobulinemia, Amyloidosis, Diabetic nephropathy, minimal change disease and Nephroangiosclerosis. Fluid/electrolyte disorders manifest as problems of osmolality, potassium and acid-base.

2.2 ART and renal dysfunction

Renal impairment secondary to ART can present in different forms as acute kidney injury, acute tubular necrosis, kidney stones and CKD. Dose adjustments of ART are recommended for
patients with renal impairment. The most common drugs causing renal dysfunction are TDF and Indinavir but isolated cases of other drugs have been reported (Samir K Gupta et al., 2005).

2.3 TDF and the kidney

Tenofovir disoproxil fumarate is the first nucleotidic inhibitor of HIV reverse transcriptase and became available in 2001 (Tourret, Deray, & Isnard-Bagnis, 2013). Tenofovir disoproxil fumarate is an oral prodrug of tenofovir, a nucleotide (nucleoside monophosphate) analogue with activity against retroviruses, including HIV-1, HIV-2 and hepadnaviruses. It is widely used and is now the most prescribed antiretroviral drug. Its high antiviral activity and favourable metabolic profile are responsible for its success. It is taken once a day and therefore promotes adherence to medications. Following absorption, tenofovir disoproxil fumarate is rapidly converted to tenofovir, which is metabolised intracellularly to its active anabolite tenofovir diphosphate, which is a competitive inhibitor of HIV-1 reverse transcriptase and terminates the growing Deoxyribonucleic acid (DNA) chain. Tenofovir exerts antiviral effects in a variety of cell types, including resting cells. Tenofovir exhibits longer serum (17 hours) and intracellular (≥ 60 hours) half-lives than those of nucleoside analogues, which supports a flexible once-daily administration schedule (Kearney, Flaherty, & Shah, 2004).

ART improves renal function yet paradoxically is associated with nephrotoxicity; toxicity ranges from elevation in the serum creatinine to end stage renal dysfunction (SCarPino, 2015). Fears of potential nephrotoxicity that TDF would have in common with two other drugs from the same family (adefovir to treat Hepatitis B and Cidofovir used to treat cytomegalovirus infections) was alleviated by earlier clinical trials (Cooper et al., 2010). Yet in 2001 the first case of TDF-
induced acute nephrotoxicity was published. Numerous cases have been published since then and it is now established that TDF presents a tubular toxicity risk (Tourret et al., 2013). There is increasing use of TDF based therapy regimen in the east and central Africa as well; Data obtained from 29,507 patients from 146 facilities between 2007-2012 in Kenya, Uganda and Zambia demonstrated that the overall percentage of patients initiated on TDF-based therapy increased from 3% to 37% in Kenya, 2% to 34% in Uganda, and 64% to 87% in Zambia (Duber et al., 2015). Tenofovir disoproxil fumarate is associated with a 33% increased risk of CKD and urinary phosphate wasting reflective of proximal tubular dysfunction (Samir K Gupta, 2008; Menezes et al., 2011; Waheed et al., 2015).

A retrospective chart review in New York of HIV-infected patients started on either TDF or Abacavir (ABC) from 1998 to 2008, showed that long-term therapy with TDF is associated with mild to-moderate nephrotoxicity which is significantly higher than in ABC treated Patients with CKD stage 3 (30-59 ml/min) noted as 5.8% of patients on TDF and 0% for those on ABC (Monteagudo-Chu, Chang, Fung, & Bräu, 2012).

A number of case reports of TDF nephrotoxicity are increasingly being recorded (Cooper et al., 2010). In this systematic review and meta-analysis of comparative studies it was found that TDF use was associated with a statistically significant loss of renal function although the clinical magnitude of this effect was modest. TDF is also associated with Fanconi syndrome (FS), a proximal tubule disease characterized by proteinuria, hypophosphatemia, euglycemic glycosuria, hypouricemia, hypokalaemia and metabolic acidosis (Samir K Gupta et al., 2014). The typical presentation of TDF-associated kidney toxicity is proximal tubulopathy, likely related to the effect on mitochondrial DNA polymerase γ and decreased mitochondrial DNA replication leading to renal function impairment and proteinuria (Labarga et al., 2009). In a meta-analysis done of 11 studies, patients on TDF had a reduction in eGFR-these patients were on drugs for
less than a year on average (Tourret et al., 2013). Risk factors for TDF renal toxicity include; lower renal function at baseline, low body mass index (BMI), older age, diabetes, a lower CD4+ T-cell count, underlying CKD, Ritonavir-boosted protease inhibitors (PIs/r) co-current use and the presence of genetic polymorphisms in the genes encoding multidrug resistance protein (MRP) 2, MRP4 and MRP7 (Yombi et al., 2014).

### 2.4 The burden of renal dysfunction in HIV positive patients

Renal dysfunction among HIV positive patients tends to be asymptomatic and is usually not the primary focus of a visit to an HIV clinic; Up to 30% of HIV-infected patients have abnormal renal function and this has been correlated with enhanced progression to AIDS and death (S. K. Gupta et al., 2004; SCarPino, 2015). Non-AIDS-related diseases, such as malignancies, cardiovascular disease, bone and renal dysfunction, have emerged as the leading cause of morbidity and mortality among HIV positive patients (Castronuovo, Pinzone, Moreno, Cacopardo, & Nunnari, 2015).

Renal dysfunction may be associated with the HIV illness itself (HIVAN, HIVICK), drugs used for prophylaxis/treatment of opportunistic infections, ART medicines and comorbid conditions such as Diabetes, hypertension and Hepatitis C (Campos et al., 2016; Naicker, Han, & Fabian, 2006). Risk factors for renal dysfunction include older age, black race, diabetes, hypertension, hepatitis C coinfection, advanced HIV disease, high HIV plasma viral load (>4000 copies/mL) and lower baseline CD4+ T-cell count (<200 cells/µL) (Campos et al., 2016; Crum-Cianflone et al., 2010; SCarPino, 2015; Winston et al., 2008).
2.5 Renal dysfunction in developed countries

Early in the epidemic of HIV most patients were diagnosed as having HIV associated nephropathy (HIVAN), an aggressive form of collapsing focal segmental glomerulosclerosis caused by direct HIV infection of the kidney in genetically susceptible patients (Rao et al., 1984). Blacks are more predisposed to the disease and it had been found that the MYH9 associated gene is involved in the development of idiopathic HIV 1 related focal segmental glomerulosclerosis in people of African descent. It is characterized by nephrotic range proteinuria, renal failure and large echogenic kidneys on ultrasonography (Rao, Friedman, & Nicastri, 1987). Pre-ART HIVAN was associated with rapid progression to esrd and death. With the widespread use of ART, HIVAN prevalence has decreased markedly (Mayer & Szczech, 2001).

Overton and colleagues found out that 4% of patients who were HIV infected had mild to moderate renal dysfunction defined as estimated glomerular filtration rate (eGFR) less than 90ml/min/1.73m² and proteinuria, 5.3% of participants had CKD defined as eGFR less than 60ml/min/1.73m². In this study, TDF and stavudine were significantly associated with decline in renal function. ART generally consisted of three or more antiretroviral drug combinations and cumulative exposure to ART drugs might have caused the decline in renal function (Overton, Nurutdinova, Freeman, Seyfried, & Mondy, 2009).

The Manhattan HIV Brain Bank established in America was a prospective cohort of antiretroviral experienced patients and looked at histology specimens from those individuals who had consented to post mortem organ donation (Wyatt et al., 2009). Of the 89 kidney tissue donors, 27 had chronic renal disease based on the presence of proteinuria and an eGFR less than 60ml/min/1.73m² for at least 3 months. Most common diagnoses were arterionephrosclerosis,
HIVAN and glomerulonephritis. The prevalence of chronic renal disease was common among black female population (Wyatt et al., 2009). However, there was no association between chronic renal diseases or renal pathology and HIV related factors such as HIV viral load and CD4 counts. This contrasted with other studies that had shown that overt renal disease tends to be associated with low CD4 counts.

In a cross sectional study done in Brazil the prevalence of chronic renal dysfunction diagnosed on the basis of proteinuria and eGFR less than or equal to 60ml/min/1.73m² was 8.4%. The risk factors were hypertension, time on ART and Tenofovir exposure. They postulated that prolonged use of ART could be associated with greater long term renal toxicity as there was a 15% increase in the prevalence of chronic kidney disease per year of additional exposure to ART. However, this was a cross sectional study hence it might have included patients with reversible causes of renal dysfunction (Menezes et al., 2011).

In a prospective study to find an association between renal disease and outcomes among HIV infected women receiving or not receiving antiretroviral therapy, done in the United States of America, Szczech L.A and colleagues, found that proteinuria and / or an elevated creatinine level are associated with an increased risk of death among women before the widespread use of ART and after initiation of antiretroviral therapy (Szczech et al., 2004). The study was done before and after the widespread of ART and it was shown that high creatinine levels and presence of proteinuria increased risk of getting AIDS defining illness. Proteinuria has been demonstrated to be associated with more-advanced HIV infection manifested by a lower CD4 lymphocyte count and a higher viral load. Proteinuria may be a marker for diseases, such as HIVAN, that are associated with a reservoir of viral replication in the kidney and resultant systemic implications. Immune dysfunction associated with worsening renal disease could
contribute to the risk of AIDS defining illness among HIV-infected women with higher creatinine levels.

2.6 Renal dysfunction in Africa

More than two thirds of the world’s HIV infected individuals live in Africa but very few studies have been done to find the prevalence of renal dysfunction in patients on ART (Fabian & Naicker, 2009). In those few studies done, the prevalence is variable, thought to reflect a genetic heterogeneity across Africa as well as variability in access to care and different estimates of GFR used during the studies.

In Zambia, Mulenga and colleagues found a prevalence of 33.4% renal insufficiency at initiation of ART with increased mortality among those patients with more severe renal insufficiency within 90 days and also two year survival analysis after initiating ART (Mulenga et al., 2008). Renal dysfunction was characterized using the Cockcroft-Gault method with about 3.1% having severe renal dysfunction defined by a creatinine clearance of less than 30mL/min. In this study they did not do routine urinalysis; this might have missed some patients who present with proteinuria but with preserved creatinine clearance.

Emem and colleagues in a cross sectional study from Nigeria, found a prevalence of 38% renal dysfunction determined by at least 1+ dipstick albuminuria and/or raised serum creatinine concentration (greater than 132 micromoles/l) in 400 HIV-AIDS patients. They attributed the high prevalence to late presentation as evidenced by low CD4 counts in the affected patients (Emem et al., 2007).

A cross sectional study done in Ethiopia by Yewulsew Mekuria and colleagues at a referral hospital recruited 446 HIV positive patients, 223 ART naïve and 223 on ART. The prevalence of
renal impairment based on GFR calculated using the Cock-croft Gault method in ART naïve was 28.7%, and the prevalence of renal impairment in those on ART was 7.6% (ART improved renal dysfunction). The prevalence of renal impairment was lower in those on ART and this might be due to improved immune status and also improvement in weight as this was incorporated in calculating eGFR. For the patients on ART it was shown that patients aged 50 years or more and CD4 less than 200cells/ml had significant association with the prevalence of renal impairment (Mekuria, Yilma, Mekonnen, Kassa, & Gedefaw, 2016)

A study done in Zambia by Wantakisha and colleagues in 2017—a cross-sectional retrospective chart review, demonstrated a prevalence of renal dysfunction among patients on TDF based ART as 18.6%(Wantakisha, Chongwe, Munkombwe, & Michelo, 2017). This was concentrated in older patients with low CD4+ (≤ 350 cells) counts suggesting a need for close renal function monitoring in this population when initiating TDF-based treatment.

2.7 Renal dysfunction in Uganda

The DART trial conducted in Uganda and Zimbabwe, had an observational analysis within a randomized trial of ART management strategies (DART, 2010). The trial included 3316 participants with CD4 count less than 200cells/ml and creatinine levels less than 360umol/l at baseline. Renal function was monitored for up to 96 weeks while patients were on different ART regimens. Mild renal impairment was seen in 45% of patients based on eGFR 60-90ml/min/1.73m² at baseline. Patients with more severe decrease in GFR at baseline showed a greater increase over 96 weeks of follow up. Severe reductions in GFR were seen in 52 patients during the period with no relation to ART agents. The conclusion was that renal impairment was highly prevalent among patients with a low CD4 count and this tended to improve with ART.
However, the results cannot be generalized as patients with high creatinine (>360 µmol/l) were excluded from the study.

Philip et al., 2008 evaluated the importance of monitoring renal function in a rural population of patients on ART (Peters et al., 2008). Five hundred eight HIV positive patients with symptomatic disease or CD4 counts less than 250 cells/ml were recruited. Patients with an initial eGFR of less than 25 ml/min were excluded. They monitored patients for two years and these patients were on ART comprising of stavudine, lamivudine, nevirapine or efavirenz. Results showed that 8% had an elevated serum creatinine at baseline greater than 233 micromol/l. Of the 508 patients, 20% had a reduced baseline renal function with creatinine clearance of 2550 ml/min. There was a 16% decline in the median creatinine level during follow up, and the median creatinine clearance rose by 21% after 24 months of follow up. More marked improvements were noted among patients with more depressed renal function at baseline. From this study it was noted that ART resulted in the improvement of renal function. However, about 5% of the study population had a significant decline in renal function despite achieving viral load suppression. This decline was attributed to renal disease that does not improve with ART for example renal lesions other than HIVAN like immune complex glomerulonephritis, membranous nephropathy or diabetic nephropathy.

A study by Odongo et al in 2015 revealed that newly HIV infected adult patients had a high prevalence of renal impairment- 14.4% had eGFR of < 60 ml/min/1.73m² and 28.8% had severe impairment of < 30 ml/min/1.73m². From this study it was concluded that screening for renal disease be done at the time the diagnosis of HIV is made (Odongo et al., 2015).
2.8 Symptoms and signs of renal dysfunction

The clinical presentation of renal dysfunction ranges from patients with AKI, CKD and ESRD. Patients may be asymptomatic or present with complications of CKD (Campos et al., 2016). Symptomatic patients may present with symptoms such as reduced urine output (and other micturition problems-haematuria, dysuria or loin pains) and swelling of the body (initially beginning as bilateral lower limb swelling and later becomes generalized). They may present with various kidney syndromes, including various electrolyte and acid base disorders (FANCONI syndrome) (S. K. Gupta et al., 2014)

2.9 Factors associated with renal dysfunction in HIV positive patients

Renal dysfunction in HIV can be caused by HIV as a disease, OIs and their prophylaxis/treatment and ART medicines. The factors can be classified as those that are HIV-related and those that are not HIV-related. HIV related factors include, infections s such as; Cytomegalovirus, mycobacterium, cryptosporidium and malignancies like lymphoma & Kaposi’s sarcoma, poorly controlled HIV disease with longer ART duration, older age, ethnicity, CD4 nadir less than 200 cells/mm3, combined therapy with TDF & Protease inhibitors such as ritonavir (Overton et al., 2009). Non-HIV related factors include; hypertension, diabetes mellitus, pre-existing kidney disease, concomitant use of non-ART nephrotoxic drugs such as herbs, Hepatitis B & C, elevated baseline creatinine concentration, female gender, and African American ethnicity.

Aetiology of renal dysfunction in HIV infected patients is multifactorial. Volume depleting conditions are a major cause of AKI (pre-renal failure) in HIV patients with diarrheal illness and vomiting from any cause being the main cause of renal dysfunction. Franceschini and colleagues showed that acute renal failure (ARF) remains an important complication of HIV in
patients on ART (Franceschini & F., 2005) ARF most often resulted from pre renal causes or acute tubular necrosis and was associated with advanced HIV infection CD4 less than 200 cells/ml, viral load greater than 10,000 copies/ml, any ART use and Hepatitis C co-infection. More than half of ARF cases were due to underlying infections 76% of which were AIDS defining illnesses and almost 3% of them necessitated hospitalizations. Drug related complications accounted for nearly a third of cases and amphotericin B was the most common drug causing nephrotoxicity (Franceschini & F., 2005).

Race is an important risk factor for CKD with 30% of patients comprising black people according to United States of America renal data system. Young black males with HIV infection have an 11 fold increased risk of CKD compared to their white counterparts (Winston et al., 2008). The reason for this is not well understood but it may be due to greater risk of diabetes and hypertension, lower socioeconomic status and poorer access to health care. HIV increases the risk of renal dysfunction and patients with other comorbidities are at an increased risk of renal dysfunction.

Due to the use of ART people are now living longer and there is an increased risk of renal dysfunction as GFR also decreases with age. In the ART era traditional risk factors of hypertension, diabetes and ageing are more prevalent. In developed countries Hepatitis C virus (HCV) coinfection has been associated with increased risk of acute kidney injury and is an important cause of mortality and morbidity in patients with HIV (Perazella, 2000). In Sub-Saharan Africa hepatitis B has greater significance than hepatitis C in patients who are HIV infected. In a subgroup study done in the DART trial in Zimbabwe a high level of exposure to hepatitis B was observed as evidenced by 55.4% (DART, 2010). This might be attributed to the fact that HIV and hepatitis B are both sexually transmitted.
A study by Lopes demonstrated a multifactorial cause of renal dysfunction with sepsis being the most prevalent risk factor of developing acute renal failure (Lopes et al., 2011). This was a cohort study of 489 hospitalized HIV-infected patients to analyse the incidence, aetiology and risk factors of AKI, as well as its impact on in-hospital mortality. They found that 18% of patients developed AKI within the hospitalization. AKI was multifactorial in approximately one-half of cases, and the most common etiologist were sepsis, nephrotoxic drug administration, volume depletion and radiocontrast use. The development of AKI was associated with lengthened time of hospitalization and increased in-hospital mortality of those patients. Furthermore, there was a relationship between more severe AKI and increased in-hospital mortality.

2.10 Laboratory characteristics of renal dysfunction

Elevated urinary protein excretion indicates tubular damage and is evaluated qualitatively with the urine dipstick or quantitatively by spot urine protein/creatinine ratio. Among HIV-infected persons, the presence of proteinuria has been linked to an increased risk of CKD. All HIV positive patients should be assessed for existing kidney disease at the time of HIV diagnosis with a screening urinalysis; if there is no evidence of proteinuria at initial evaluation, patients should undergo annual screening for renal dysfunction, including risk assessment, eGFR and urine dipstick analysis (Clumeck, Pozniak, & Raffi, 2008). The main target of TDF toxicity is the proximal tubule, and the presence of tubular proteinuria is thought to be the most sensitive test for proximal tubule dysfunction (Labarga et al., 2009; Waheed et al., 2015). Urinalysis looking out for proteinuria & glycosuria and measurement of electrolytes is done to look out for all or some of the features of the Fanconi syndrome (hypokalaemia, hypouricaemia, aminociduria, glycosuria and hypophosphaturia). However, since the urine electrolytes measurement is expensive, serum electrolytes is often the alternative with specific focus on changes in potassium or Phosphate. Blood samples are also evaluated for creatinine and urea so as to be able to
estimate the glomerular filtration rate (eGFR) using the CKD-EPI formula (Stevens, Coresh, Greene, & Levey, 2006)

**Equation 1:** Chronic kidney disease epidemiology (CKD-EPI) collaboration equation

Creatinine = $A \times (\text{Scr}/B)^C \times 0.993^{\alpha \text{ge}} \times (1.159 \text{ if black})$,

This CKD-EPI equation calculator should be used when $S_{cr}$ is reported in mg/dL.

$$GFR = 141 \times \min \left(\frac{S_{cr}}{\kappa}, 1\right)^{\alpha} \times \max \left(\frac{S_{cr}}{\kappa}, 1\right)^{-1.209} \times 0.993^{\alpha \text{ge}} \times 1.018 \times 1.159 \times \text{[if female]} \times 1.159 \times \text{[if black]}$$

where:

- $S_{cr}$ is serum creatinine in mg/dL,
- $\kappa$ is 0.7 for females and 0.9 for males,
- $\alpha$ is -0.329 for females and -0.411 for males,
- min indicates the minimum of $S_{cr}/\kappa$ or 1, and
- max indicates the maximum of $S_{cr}/\kappa$ or 1

The equation does not require weight because the results are reported normalized to 1.73 m² body surface area, which is an accepted average adult surface area.
CHAPTER THREE

3.0 METHODOLOGY

3.1 Study design

This was a cross-sectional study with an analytical component.

3.2 Study setting

The study was carried out at the Mulago Communicable Disease Clinic (MCDC) under the directorate of medicine situated in Kampala, Uganda. Mulago-Kiruddu hospital is home to the directorate of medicine since May 2016; running different medical outpatient and inpatient clinics. The communicable disease clinic is supported by Makerere University Joint AIDS Program (MJAP), runs on Fridays as an outpatient clinic, and mainly attends to HIV positive patients. It was started in 2007; has close to 4,000 active patients enrolled in care. About 37.3% (1,490) are on ART, of these 808 patients (54%) are on TDF based regimen. The clinic operates as an outpatient clinic on Fridays only between 8am to 5pm, attending to approximately 120-240 patients per clinic day. The study was carried out from November 2017 to March 2018 (4 months). It is important to note that there was a one month interruption as a result of the industrial action by the health workers this delayed data collection for one month.

3.3 Population

3.3.1 Target population - All HIV positive patients receiving TDF based ART in Uganda

3.3.2 Accessible population - All HIV positive patients receiving TDF based ART for at least six months at Mulago Communicable Disease Clinic during the study period.
3.3.3 **Study population**- All HIV positive patients receiving TDF based ART for at least six months at Mulago Communicable Disease Clinic during the study period, who met the eligibility criteria and were enrolled.

3.4 **Selection/eligibility criteria**

3.4.1 **Inclusion criteria**

- HIV positive patients 18 years or older at Mulago Communicable Disease Clinic
- Had received TDF based ART for at least six months because this was considered adequate time for drug exposure
- Written informed consent

3.4.2 **Exclusion criteria**

- Patients with documented evidence of chronic kidney disease
- Pregnant women because proteinuria seen in them may not necessarily mean renal dysfunction.

3.5 **Sampling method**

Patients who met the inclusion criteria were recruited consecutively on Fridays, 9:00am to 3:00pm until the required sample size was attained.

3.5.1 **Sample size estimation**

*For objective 1*, the sample size was estimated using the Kish and Leslie formula (1965) for descriptive studies (Leslie, 1965).

**Equation 2: Keish and Leslie formula for sample size estimation**
\[
 n = \left[ \frac{Z^2}{2} \cdot \rho (1 - \rho) \right] \div \alpha^2
\]

Where;

\( n \) = Sample size

\( Z \) = Z score corresponding to 5% level of significance (1.96).

\( P \) = Prevalence 18.6% (Wantakisha et al., 2017)

\( \alpha \) = Precision of 0.05

\[
 n = (1.96)^2 \times 0.186 \times 0.814 = 232 \text{ patients.}
\]

\( (0.05)^2 \)

Considering a 10% non-response rate, total sample size came to 232+23.2 = 255 patients

\[
 N = 255 \text{ patients}
\]

**Objective 2**: Sample size calculation for determining associated factors

The sample size for factors associated with renal dysfunction was calculated using Kaplan formula

**Equation 3**: Formula for sample size calculations for comparisons between two proportions

\[
 n = \left[ \frac{z_\alpha + z_\beta}{2} \cdot \left( \rho_1 (1 - \rho_1) + \rho_2 (1 - \rho_2) \right) \right] \div [\rho_2 - \rho_1]^2
\]

**I. Diabetes Mellitus**

With a desired power 80% chance of detecting a significant difference at a two-sided 0.05 significance level, and using the formula above;

Where:
**II. CD4 < 200 cells/ml**

$Z_{\alpha/2}$, is the Z value corresponding to an $\alpha$ value of 0.05 (1.96)

$Z_{1-\beta}$, is the Z value corresponding to a power of 80% (0.84)

$p_1$ is the proportion of HIV positive patients with CD4 < 200 cells/ml but no renal dysfunction -76% -(Banda et al., 2010)

$p_2$ is the proportion of HIV positive patients with CD4 < 200 cells/ml with renal dysfunction – 83% (Banda et al., 2010)

\[
N (\text{Per group}) = (1.96+0.84)^2 \times (0.83 \times (1-0.83)) + (0.76 \times (1-0.76))
\]

\[
(0.83-0.76)^2
\]

$N (\text{Per group}) = 179 \text{ participants per group}$

The sample size for 2 proportions should be **358** participants.

**III. WHO stage IV**

$Z_{\alpha/2}$, is the Z value corresponding to an $\alpha$ value of 0.05 (1.96)

$Z_{1-\beta}$, is the Z value corresponding to a power of 80% (0.84)

$p_1$ is the proportion of HIV positive patients with WHO stage IV but no renal dysfunction -46% (Banda et al., 2010)
P₂is the proportion of HIV positive patients with WHO stage IV with renal dysfunction – 27 % (Banda et al., 2010)

\[
N \text{ (Per group)} = \frac{(1.96+0.84)^2 \times (0.27 \times (1-0.27)) + (0.46 \times (1-0.46))}{(0.27-0.46)^2}
\]

N (Per group) = 33.45 participants per group

The sample size for 2 proportions should be 67 participants.

From the above calculations the minimum sample size needed to answer all the objectives was 358 patients but 305 were recruited into the study.

3.6 Study protocol

3.6.1 Screening and recruitment of participants

The Principal Investigator and the research assistant screened patients from the Mulago Communicable Disease Clinic that run only Fridays between 9.00 am and 3.00 pm. Participants were conveniently selected, talked to individually explaining study objectives and responding to their questions. For the patients who were interested in participating, more information regarding the study including risks/benefits were given. Those willing to participate were requested to sign or provide thumb print on the informed consent form (Appendix 3). Pre-tested questionnaires were administered, a focused history done and a physical examination carried out. Patients were directed to the side laboratory to have blood drawn for renal function tests and blood sugar of about 10mls as per standard protocol (Appendix 6). On the same day, a sterile urine container was availed with instructions of how to collect a mid-stream urine sample of about 10-20mls as per standard protocol (Appendix 6)
Figure 2: Study flow diagram showing participant accrual

HIV positive patients on TDF based ART

Ineligible excluded from the study
- Patients with CKD
- Pregnant women

Sign informed consent form Clinical evaluation-biodata, history and physical
Examination entered into a questionnaire

Patients without renal dysfunction

Laboratory evaluation
- Blood- blood sugar, renal function tests & extended electrolytes
- Urinalysis on dip stick

Patients with renal dysfunction
Primary care giver notified
3.6.2 Data collection procedure

Patients were each assigned a study number for identification. A focused clinical assessment was done using a standardized pre-tested questionnaire (Appendix 4) in which the socio-demographic information, relevant history and physical examination findings were recorded. The CD4 Cell count and viral load results were obtained from patients’ records (MOH HIV ART blue card and electronic data base -OPEN- MRS). The most recent value for CD4 or Viral load was abstracted from the charts and noted in the study files. This clinic implemented the guidelines for viral load monitoring using a dried blood spot (DBS) so it was not surprising that some patients only had viral loads in their files as opposed to CD4 counts.

3.6.3 Study instruments used for data collection

All laboratory request forms were identified using the study numbers. Results of the laboratory tests were entered in patients’ questionnaires. The principal investigator (PI) and research assistant collected data using pre-tested standardized questionnaires to get information on demographics, past medical history, drug history, clinical findings, procedures, vacutainers, crial vials and sterile urine containers were available for the study. All laboratory request forms were identified using the study numbers. Results of the tests were entered in patients’ questionnaires.

3.6.4 Laboratory examination procedures

Procedure for blood draw and processing

Blood was drawn as per standard operating procedure (Appendix 6) in aseptic conditions, put in red and grey tops for renal function tests and blood sugar respectively. Ten mls of blood was drawn, 3mls put in grey top (for blood sugar), and the resultant 7 mls were placed in red top vacutainers. After centrifugation, 4 mls of supernatant were put in crial vials (this was to
determine Renal function and serum electrolytes). The grey tops and crial vials were adequately packaged in Ziplock bags and carrier. These were later in the day transported and analysed at Mulago National Referral Hospital clinical chemistry laboratory (old Mulago) which is open 7 days a week including weekends. Chemistry and blood sugar were carried out using an Abott Machine, Architect plus-Ci (Chemistry and immunochemistry analyser).

**Procedure for urine sample collection**

A sterile urine container was given to the patient with clear instructions of collecting an appropriate and good sample as per standard operating procedures. (Appendix 6). Urine was analysed immediately it was received in the side laboratory using a dipstick (details) giving information on protein, glucose, nitrites, pus cells and leucocytes. Results were documented in the questionnaire.

**3.7 Study variables**

The primary outcome was renal dysfunction among HIV positive patients receiving TDF attending Mulago Communicable Disease Clinic. This was determined by measuring the renal function tests and dipstick urinalysis. Based on these measurements, renal dysfunction was defined as

- Creatinine clearance of $< 60 \text{ ml/min/1.73m}^2$ (calculated from CKD-EPI) or
- Proteinuria of $\geq 2+$ on urine dipstick with glycosuria but normal blood sugar.

The predictor variables were the factors associated with renal dysfunction. These were determined by assessing the following variables:
3.7.1 Clinical variables

1) History

- Age – for this study we divided the age categories into two; < 50 years and those ≥ 50 years. This is because from previous studies patients over 50 are more prone to renal dysfunction (Kim et al., 2017).
- Symptoms of renal dysfunction – (bilateral swelling of the limbs, fatigue, anorexia, nausea, vomiting, abdominal pain, dizziness, itchy skin) and their duration.
- Past medical history- comorbid conditions such as Diabetes Mellitus, Hypertension, Hepatitis (B & C), Tuberculosis, any other Opportunistic infections or other chronic illness and past use of contrast studies.
- Recent drug history - e.g. ART, Cotrimoxazole, Dapsone, Amphotericin- B, anti-TB drugs, NSAIDs and herbal use
- Social history- occupation, educational level, alcohol/cigarette smoking / use of recreational drugs e.g. Cocaine. Marijuana

2) Physical examination;

- Body mass index was calculated from the formula

Equation 4: \( BMI = \frac{\text{Weight (kg)}}{\text{Height (m)}^2} \)

- Presence/absence of oedema
- Blood Pressure (BP)
- WHO HIV stage (Appendix 5)

3.7.2 Laboratory variables

- CD4 cell count
- Viral load
• Blood sugar
• Serum creatinine
• Serum electrolytes- Potassium, Phosphate & Calcium

Laboratory normal reference ranges
- Potassium with normal ranges of 3.5-5.1 mmol/l
- Phosphate with normal ranges of 0.97-1.45 mmol/l
- Calcium with normal ranges of 2.10-2.55 mmol/l

• Urinalysis- Using Dip stick to detect proteinuria, leucocytes, haematuria, glycosuria and nitrites.

3.8 Data management

Data was collected using a standardized questionnaire (Appendix 4) through a face-to-face interview. Individual patient records and forms were kept in files after data collection. Raw data was stored in a secure place and locked at the discretion of the principal investigator to ensure safety and confidentiality. Data cleaning and validation (for completeness and quality) was performed before analysis. Data collected was entered into the computer using EPI-DATA (version 3.2) software and then exported to STATA 14.0, for cleaning and analysis. Data was backed up and archived in both soft and hard copy to avoid losses.

3.8.1 Data analysis

Statistical analysis was performed using STATA 14.0 software package. Continuous variables were summarized as means and standard deviation for normally distributed data, medians and interquartile ranges for not normally distributed data. Categorical variables were summarized as frequencies, percentages and also in tables and figures.
3.8.2 Analysis of specific objectives

3.8.2.1 Objective 1; The prevalence of renal dysfunction was calculated as a percentage of the number of patients with renal dysfunction divided by the total number of study participants and then presented with its 95% confidence interval.

3.8.2.2 Objective 2;
To determine the factors associated with renal dysfunction, bivariate analysis was performed. Cross tabulations were done with Chi-square or Fischer’s exact test for categorical variables. The associations are presented with crude odds ratios, their 95% confidence intervals and the p-values. Factors with a p-value less than 0.05 were considered significant at 95% level of confidence.

At multivariate analysis, all factors which had a p-value less than 0.200 were considered for the model then used the backward method to reduce them depending on their p-values and the magnitude of the log likelihood. The results for the model are presented with adjusted odds ratios with their 95% confidence intervals-value and factors with a p-value less than 0.05 were considered significant at 95% level of confidence.

3.8.2.3 Objective 3; Electrolyte abnormalities are presented as proportions

3.9 Quality control

The principal investigator worked with two research assistants and one laboratory personnel, who were trained for a day to ensure accurate data is collected. Orientation of personnel on study protocol, ethics, and use of all data collection tools like the questionnaire, blood specimen collection and sample transportation to the laboratory prior to initiation of the study were done. The data forms were pre-tested on 5 patients for clarity and standardization to ensure internal
validity. The PI cross checked all data daily to ensure completeness. Laboratory investigations were performed by experienced technologists using standard operating procedures in a credible and certified laboratory.

3.9 Ethical considerations

Ethical approval to carry out the study was sought from the Department of Medicine of Makerere University College of health sciences, school of medicine research & ethics committee (SOMREC), Mulago hospital ethics committee and Uganda national council for science and technology (UNCST). All benefits and potential risks of the study were clearly explained to the participants before obtaining the recruitment consent. Written/thumb print informed consents were obtained from all participants before participation in the study. Participants were allowed to withdraw from the study at any time as they wished without impacting their care. All information given was kept confidential by use of patients’ identification numbers and not names. For purposes of clarification of raw data by the principal investigator, only the patient’s clinic file number will be recorded to assist in retrieving some laboratory results (CD4 and viral load). Access to data or patient information was limited to those directly involved in the study and the attending clinician. For patients who had renal dysfunction, a note and a copy of the results was availed to the primary care giver.

3.10 Benefits of the study to the participants

- Kidney function assessed for all study patients
- Laboratory tests were done at no cost to the patient.
- Results of all laboratory tests were availed to the clinician in charge of the patient as soon as they were available.
3.11 Risks from study

There were no added risks to the patients from this study. The procedures like blood draw carried minimal risk to the patients.

3.12 Dissemination of results

Our findings will be circulated to the Makerere University School of postgraduate studies, department of Medicine, Makerere University College of Health Sciences, Mulago Hospital, and Makerere University Joint AIDS Program, Sir Albert Cook College of health science library and Uganda Heart Institute and clinicians taking care of the study patients. We hope to submit these findings for publication in peer reviewed international journals.
CHAPTER FOUR

4.0 RESULTS

From November 2017 through March 2018, 808 patients were consecutively screened in Mulago Communicable Disease Clinic for study enrolment. Reasons for excluding patients included; duration on TDF < 6 months (375), pregnancy (21), < 18 years (1) and refusal to provide consent/participate in the study (98). Three hundred five patients met the eligibility criteria and were enrolled. Of these, 278 were entered into the final analysis and 27 patients were excluded due to incomplete data. One hundred sixty nine (60.7%) were female, majority 234 (84.4%) were < 50 years old. Median age was 37 (IQR 29-45) years. One hundred nineteen (42.81%) were married, 141 (50.72%) had a primary education, 250 (89.93%) had formal employment, 252 (83.45%) resided in Kampala and 81 (29.14%) consumed alcohol. (See Table 1)
Figure 3 Study recruitment diagram for the study participants receiving TDF ≥ 6 months at MCDC

4,000 HIV positive active patients attending the MCDC.

1,490 HIV positive on ART

808 HIV positive patients on TDF based ART screened over 4 months

503 Patients excluded
- 383 On TDF < 6 months
- 21 Pregnant
- 1 < 18 years
- 98 Refused to consent

305 HIV positive patients on TDF ≥ 6 months and eligible to participate in the study

27 patients enrolled but excluded from analysis due to incomplete data

278 patients enrolled and analysed
Table 1 Socio-demographic Characteristics of the study participants receiving TDF for at least six months at MCDC

<table>
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<tr>
<th>Socio-demographics</th>
<th>Frequency(n)</th>
<th>Percentage (%)</th>
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</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
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<tr>
<td>Female</td>
<td>169</td>
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<tr>
<td><strong>Age (Years) median,(IQR)</strong></td>
<td>37(29-45)</td>
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<tr>
<td>&lt; 50</td>
<td>234</td>
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<tr>
<td><strong>Marital status</strong></td>
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<td>Single</td>
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<tr>
<td>Married</td>
<td>119</td>
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<td>Separated</td>
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<tr>
<td>Yes</td>
<td>81</td>
<td>29.14</td>
</tr>
</tbody>
</table>
4.1 Clinical and laboratory characteristics

The median duration on ART was 36 IQR (24-60) months, 271 (97.48%) were on TDF/3TC/EFV, 152(72.38%) had CD4 ≥ 200 cells/ml, 109 (86.51%) had viral load of < 1000 copies/ml, 205(73.74%) were in WHO stage 1,155(55.76%) had a normal BMI, 38(13.67%) had systolic hypertension, 42(15.11%) had diastolic hypertension and 270(97.12%) had no co-current opportunistic infections. Three (1.08%) had random blood sugar ≥ 11.1 mmol/l, 10 (3.6%) had proteinuria of ≥ 2+, 4(1.44%) tested positive for nitrites, 7(2.52%) had haematuria and 2(0.72%) had glycosuria. Ten (3.59%) had hypophosphatemia, 11(4.44%) had hyperphosphatemia, 15(5.26%) had hyperkalaemia, 3(1.32%) had hypokalaemia, 43 (15.44%) had hypocalcaemia and 2 (0.67%) had hypercalcemia. The factor associated with renal dysfunction at Multivariate analysis was having diastolic hypertension (AOR: 8.566, 95%CI 1.718-42.70, p <0.009). (See Table 2)
Table 2 Clinical and laboratory characteristics of the study participants receiving TDF for at least six months six 6 months at MCDC

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Frequency(n)</th>
<th>Percentage (%)</th>
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<tbody>
<tr>
<td>BMI</td>
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<tr>
<td>18.5-24.9-Normal</td>
<td>155</td>
<td>55.76</td>
</tr>
<tr>
<td>Systolic hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>38</td>
<td>13.67</td>
</tr>
<tr>
<td>Diastolic hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>42</td>
<td>15.11</td>
</tr>
<tr>
<td>Most recent (CD4+ cell count cells/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>58</td>
<td>27.62</td>
</tr>
<tr>
<td>Viral load (copies/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥1000</td>
<td>17</td>
<td>13.49</td>
</tr>
<tr>
<td>ART Regimen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDF/3TC/EFV</td>
<td>271</td>
<td>97.48</td>
</tr>
<tr>
<td>Duration on ART (months)</td>
<td>36(IQR 24-60)</td>
<td></td>
</tr>
<tr>
<td>&gt;24</td>
<td>135</td>
<td>48.56</td>
</tr>
<tr>
<td>WHO stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>205</td>
<td>73.74</td>
</tr>
<tr>
<td>Concurrent opportunistic infections</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8</td>
<td>2.88</td>
</tr>
<tr>
<td>Random blood sugar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥11.1</td>
<td>3</td>
<td>1.08</td>
</tr>
<tr>
<td>Protein present in urine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 2+ (present)</td>
<td>10</td>
<td>3.6</td>
</tr>
<tr>
<td>Nitrites in urine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>4</td>
<td>1.44</td>
</tr>
<tr>
<td>Haematuria present</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>7</td>
<td>2.52</td>
</tr>
<tr>
<td>Glucose in urine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>2</td>
<td>0.72</td>
</tr>
<tr>
<td>Hyperphosphatemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>4.31</td>
</tr>
<tr>
<td>Hyperkalaemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>5.26</td>
</tr>
<tr>
<td>Hypocalcaemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>15.44</td>
</tr>
</tbody>
</table>
4.2 Prevalence of renal dysfunction among study participants

Out of the 278 subjects, 7 were found to have eGFR less than 60 ml/min/1.73m², ten patients had proteinuria (but no glycosuria), 2 patients had glycosuria (but no proteinuria). Therefore prevalence of renal dysfunction was 2.52% (7/278). (See table 3 below)
Table 3: EGFR calculated using CKD-EPI to determine renal dysfunction among study participants on TDF for at least six months at MCDC

<table>
<thead>
<tr>
<th>eGFR (CKD-EPI)</th>
<th>Frequency (n)</th>
<th>Percentage (%)</th>
<th>[95% Conf. Interval]</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;60</td>
<td>7</td>
<td>2.52</td>
<td>(1.19-5.21)</td>
</tr>
<tr>
<td>≥60</td>
<td>271</td>
<td>97.48</td>
<td>(94.72-98.80)</td>
</tr>
<tr>
<td>eGFR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-29</td>
<td>1</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>30-59</td>
<td>6</td>
<td>2.16</td>
<td></td>
</tr>
<tr>
<td>60-89</td>
<td>51</td>
<td>18.35</td>
<td></td>
</tr>
<tr>
<td>≥ 90</td>
<td>220</td>
<td>79.14</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4: The distribution of eGFR calculated from CKD-EPI among study participants on TDF for at least six months at MCDC.
4.3 Characteristics of the seven participants with renal dysfunction

All the patients were on TDF/3TC/EFV, median duration of ART 40 (IQR 24-72) months, majority were females (57.14%), 5 (71.43%) were ≥ 50 years old, 5 (71.43%) had CD4 ≥ 200 cells/ml, 3 (42.9%) had Viral load < 1000 copies/ml, 6 (85.7%) were employed and 2 (28.57%) had comorbidities (Hypertension & Diabetes Mellitus).

4.4 Factors associated with renal dysfunction among study participants

The second objective on factors associated with renal dysfunction was not analysable in this study as the sample size (7 participants with renal dysfunction) was too small to make accurate, interpretable and reliable conclusions. The findings cannot be compared to previous reports in which bigger sample size was used.
Table 4 Bivariate analysis of socio-demographic factors associated with renal dysfunction among study participants receiving TDF for at least six months at MCDC

<table>
<thead>
<tr>
<th>Socio-demographic factors</th>
<th>≥60</th>
<th>&lt;60</th>
<th>OR(95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>106(97.25)</td>
<td>3(2.75)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>165(97.63)</td>
<td>4(2.37)</td>
<td>0.39(0.04-3.35)</td>
<td>0.397</td>
</tr>
<tr>
<td><strong>Age (Years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-35</td>
<td>127(99.22)</td>
<td>1(0.78)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>35-49</td>
<td>105(99.06)</td>
<td>1(0.94)</td>
<td>1.20(0.07-19.57)</td>
<td>0.893</td>
</tr>
<tr>
<td>≥50</td>
<td>39(88.64)</td>
<td>5(11.36)</td>
<td>16.28(1.84-143.57)</td>
<td>0.012</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not married</td>
<td>155(97.48)</td>
<td>4(2.52)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>116(97.48)</td>
<td>3(2.52)</td>
<td>1.01(0.22-4.56)</td>
<td>0.998</td>
</tr>
<tr>
<td><strong>Education level</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>137(97.16)</td>
<td>4(2.84)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>113(98.16)</td>
<td>2(1.74)</td>
<td>0.61(0.10-3.37)</td>
<td>0.567</td>
</tr>
<tr>
<td>Tertiary</td>
<td>21(95.45)</td>
<td>1(4.55)</td>
<td>1.63(0.17-15.30)</td>
<td>0.668</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unemployed</td>
<td>27(96.43)</td>
<td>1(3.57)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>244(97.60)</td>
<td>6(2.40)</td>
<td>0.66(0.08-5.72)</td>
<td>0.709</td>
</tr>
<tr>
<td><strong>Address</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outside Kampala</td>
<td>46(100)</td>
<td>0(0)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Within Kampala</td>
<td>225(96.98)</td>
<td>7(3.02)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Alcohol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>191(96.95)</td>
<td>6(3.05)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>80(98.77)</td>
<td>1(1.23)</td>
<td>0.39(0.05-3.35)</td>
<td>0.397</td>
</tr>
</tbody>
</table>
Table 5 Bivariate analysis of clinical and laboratory factors associated with renal dysfunction among study participants receiving TDF for at least six months at MCDC

<table>
<thead>
<tr>
<th>Clinical and laboratory</th>
<th>≥60</th>
<th>&lt;60</th>
<th>OR(95%CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18.5</td>
<td>21(100)</td>
<td>0(0)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>18.5-24.9</td>
<td>151(97.42)</td>
<td>4(2.58)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>25-29.9</td>
<td>75(96.15)</td>
<td>3(3.85)</td>
<td>0.66(0.14-3.04)</td>
<td>0.596</td>
</tr>
<tr>
<td>&gt;=30</td>
<td>24(100)</td>
<td>0(0)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Systolic hypertension</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>236(98.33)</td>
<td>4(1.67)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>35(92.11)</td>
<td>3(7.89)</td>
<td>5.05(1.08-23.55)</td>
<td><strong>0.039</strong></td>
</tr>
<tr>
<td><strong>Diastolic hypertension</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>233(98.73)</td>
<td>3(1.27)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>38(90.48)</td>
<td>4(9.52)</td>
<td>8.17(1.76-37.97)</td>
<td><strong>0.007</strong></td>
</tr>
<tr>
<td><strong>Most recent (CD4+ cell count cells/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>55(94.83)</td>
<td>3(5.17)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>&gt;=200</td>
<td>148(97.37)</td>
<td>4(2.63)</td>
<td>0.49(0.11-2.28)</td>
<td>0.368</td>
</tr>
<tr>
<td><strong>Viral load (copies/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1000</td>
<td>106(97.25)</td>
<td>3(2.75)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>&gt;=1000</td>
<td>16(94.12)</td>
<td>1(5.88)</td>
<td>2.21(0.22-22.54)</td>
<td>0.504</td>
</tr>
<tr>
<td><strong>Duration on ART (months)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1year</td>
<td>50(98.04)</td>
<td>1(1.96)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>1-2years</td>
<td>91(98.91)</td>
<td>1(1.09)</td>
<td>0.54(0.03-8.97)</td>
<td>0.674</td>
</tr>
<tr>
<td>&gt;2years</td>
<td>130(96.30)</td>
<td>5(3.70)</td>
<td>1.92(0.21-16.87)</td>
<td>0.555</td>
</tr>
<tr>
<td><strong>WHO stage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>204(99.51)</td>
<td>1(0.49)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>57(90.48)</td>
<td>6(9.52)</td>
<td>21.47(2.53-182.01)</td>
<td><strong>0.005</strong></td>
</tr>
<tr>
<td>3</td>
<td>8(100)</td>
<td>0(0)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2(100)</td>
<td>0(0)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Concurrent opportunistic infections</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7(87.50)</td>
<td>1(12.50)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>264(97.78)</td>
<td>6(2.22)</td>
<td>0.16(0.01-1.51)</td>
<td>0.109</td>
</tr>
<tr>
<td><strong>Protein present</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(≥2+)</td>
<td>9(90.0)</td>
<td>1(10.0)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Nil (&lt;2+)</td>
<td>262(97.76)</td>
<td>6(2.24)</td>
<td>0.21(0.02-1.89)</td>
<td>0.163</td>
</tr>
</tbody>
</table>
Table 6 Logistic Regression of the factors associated with renal dysfunction among study participants receiving TDF ≥ six months at MCDC

<table>
<thead>
<tr>
<th>Factors associated with Renal dysfunction</th>
<th>Adjusted Odds Ratio</th>
<th>(95% Confidence Interval)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diastolic hypertension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8.566</td>
<td>(1.718-42.70)</td>
<td>0.009</td>
</tr>
<tr>
<td>Most recent (CD4+ cell count cells/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥200</td>
<td>0.425</td>
<td>(0.08-2.29)</td>
<td>0.320</td>
</tr>
<tr>
<td>Concurrent opportunistic infections</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>0.201</td>
<td>(0.01-3.29)</td>
<td>0.261</td>
</tr>
<tr>
<td>Protein present (≥2+)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nil (&lt;2+)</td>
<td>0.332</td>
<td>(0.02-6.32)</td>
<td>0.463</td>
</tr>
</tbody>
</table>
CHAPTER FIVE

5.0 DISCUSSION

This was a cross sectional study that determined the prevalence and factors associated with renal dysfunction among HIV infected patients receiving TDF based ART at Mulago Communicable Disease Clinic. The electrolyte abnormalities were also described. The prevalence of renal dysfunction was 2.52%, the factors associated with renal dysfunction were not assessed due to the small sample size. The commonest electrolyte abnormality noted was hypocalcaemia 43 (15.44%).

5.1 Prevalence of renal dysfunction

Prevalence of renal dysfunction in our study was 2.52% among HIV positive patients on TDF based ART attending an outpatient clinic. This is in agreement with other studies that show that use of TDF is associated with modest renal dysfunction. A study by Eon Jinn Kim et al 2017 done in Korea to determine prevalence and renal dysfunction (eGFR< 60 ml/min/1.73m² calculated using Modification of Diet in Renal Disease (MDRD) equation among HIV infected patients found a rate of 5.3%. They had a bigger sample size of 1,218 patients (Kim et al., 2017)

Kalyesubula et al 2017 in a community based study done in Uganda, found a prevalence of 2.4% (eGFR 30-59-stage 3 CKD), 0% (eGFR15-29-Stage 4 CKD) and 0.1 % (eGFR< 15 –stage 5 CKD). Therefore in patients with eGFR<60, prevalence was 2.5%. In this study it was noted that traditional risk factors such as diabetes mellitus, smoking and alcohol intake were not significantly associated with CKD(Kalyesubula et al., 2017)
Campos et al 2016 noted that prevalence of CKD ( < 60ml/min/1.73m^2) among HIV-infected persons in North America and Europe ranges from 4.7 to 9.7 % but was as high as 33% when defined by either reduced GFR or pathological proteinuria(Campos et al., 2016).

Odongo et al 2015 in a study done in Uganda noted a prevalence of 14.4 % with renal impairment among newly diagnosed HIV infected patients. These patients had eGFR< 60 ml/min/1.73m^2 assessed using CKD-EPI formula for black race(Odongo et al., 2015).These patients however were not on ART.

Menezes et al 2011 done in Brazil, noted a prevalence of CKD (defined as eGFR ≤ 60 ml/min/1.73m^2) as 8.4 % among HIV infected patients on ART for ≥ 12 months with undetectable viral load. Risk factors noted to be significant were Hypertension, time on ART and TDF exposure. In this study eGFR was assessed by the modification of diet in renal disease (MDRD)(Menezes et al., 2011).

Lynda A Szczech in 2008 noted that rates of TDF nephrotoxicity in retrospective cohort studies have been reported in general at approximately 2% (1.90%[Karras et al, Clin Infect Dis, 2003],1.60% [Padilla et al, AIDS Patient Care STDS, 2005], 0.80% [Jones, JAIDS,2004], 0.78% [Franceschini, Kidney Int, 2005], and 0.00% [Gallant et al,Clin Infect Dis, 2005]).Variation in incidence in these reports may be attributable to surveillance and recognition biases, lack of a standard definition of toxicity, and reporting of mean versus median renal function values(Winston et al., 2008).

Reid et al 2008 in a study (DART trial) done in Uganda and Zimbabwe, noted a prevalence of 7 % among patients initiating ART and followed up for 96 weeks, these patients had eGFR ≥ 30 but < 60 ml/min/1.73m^2(this was documented as moderate renal impairment).74% of patients were on TDF based ART regimen.GFR was assessed using the Cockroft gault formula (Reid et
al., 2008) These patients were closely monitored, being in a study setting and may not reflect the same picture in other settings.

Emem et al 2007 in a study done in Nigeria found a prevalence of 38% as patients with renal the definition of renal disease was consistent presence of at least 1+albuminuria and/or elevated serum creatinine (>132 µmol/l) as well as the absence of other identifiable causes of CKD. In this study eGFR was not calculated or assessed, this may have accounted for the higher percentage (Emem et al., 2007).

The low prevalence in our study could be explained by the small sample size, being an outpatient clinic receiving not very critically sick patients, the possible effect of ART improving kidney function (Peters et al., 2008) and use of the CKD-Epi formula which is a more accurate method of assessing eGFR in asymptomatic patients.

5.2 Electrolyte abnormalities among patients

We assessed three serum electrolytes namely; Potassium, Calcium and Phosphate. We noted mainly Hypocalcaemia (15.44%), Hyperkalaemia (15%) and hypophosphatemia (3.59%). For patients with hypophosphatemia, data is similar to studies elsewhere that demonstrate that TDF is associated with Proximal tubulopathy manifesting as Fanconi syndrome (Labarga et al., 2009; Mathew & Knaus, 2006). Rarely all the components of FS occur in one patient. Because of the wasting of Phosphate, potassium and amino acids in urine they tend to be low in serum. Hypophosphatemia could have a dual origin: decreased proximal reabsorption of phosphate and decreased vitamin D activation. Another possible cause of low phosphate in serum is the possibility of Vitamin D deficiency that is prevalent in HIV infected patients (McComsey et al., 2010; Tourret et al., 2013)
Hyperkalaemia may be explained by the possibility of these patients having had acute kidney injury, chronic kidney disease and medications e.g. Cotrimoxazole (Campos et al., 2016; Choi et al., 1993). In this study majority of the patients were asymptomatic, though not enough information as regards symptoms was collected.

Hypocalcaemia may be explained by the possibility of the patients having chronic kidney disease, later on manifesting with hyperphosphatemia but other causes such as vitamin D deficiency cannot be excluded.

5.3 Study limitations

- The study participants had no baseline renal function to assess change over time. The results we got will form a baseline for future reference.
- Missing information for some patients e.g. CD4 and Viral load, which were not done/document ed by primary health care giver.
- Transportation of blood samples drawn to final processing site may have given rise to errors and delay in results.
- The limited sample size led to the inability to study the second objective adequately.

5.5 Study strength

- We carried out the study in a free public facility outpatient clinic at Mulago National Referral Hospital therefore the information may be generalizable to the other government supported facilities in other parts of the country.
- We assessed renal dysfunction using eGFR calculated from CKD-Epi equation which is more accurate than Cockcroft-Gault formula.
CHAPTER SIX

6.1 Conclusion

- The prevalence of renal dysfunction among HIV positive patients on TDF based ART was low at 2.52%.
- Hypocalcaemia was the most common electrolyte abnormality noted among patients receiving TDF.

6.2 Recommendations

- Rigorous monitoring of renal function using renal function tests is not necessary in asymptomatic patients receiving TDF for at least six months.
- When renal function is assessed, extended electrolytes for example calcium should be assessed.
- Future studies should focus on identifying early renal dysfunction for patients receiving TDF based ART for at least six months.
REFERENCES


Leslie, K. a. (1965). Survey sampling


APPENDICES

Appendix 1: Budget

<table>
<thead>
<tr>
<th>No.</th>
<th>Item</th>
<th>Quantity</th>
<th>Unit Cost (Ugshs)</th>
<th>Total Cost (Ugshs)</th>
<th>Cost in USD (1USD @ 3600)</th>
<th>Justification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Box file</td>
<td>5</td>
<td>8,000</td>
<td>40,000</td>
<td>11.1</td>
<td>For safe custody of CRFs</td>
</tr>
<tr>
<td>2</td>
<td>Printing, Photocopying &amp; binding</td>
<td>1</td>
<td>500,000</td>
<td>500,000</td>
<td>138.9</td>
<td>For all paper work pertaining the study</td>
</tr>
<tr>
<td>3</td>
<td>Stapler</td>
<td>1</td>
<td>10,000</td>
<td>10,000</td>
<td>2.8</td>
<td>Keeping the documents orderly</td>
</tr>
<tr>
<td>4</td>
<td>Punching machine</td>
<td>1</td>
<td>10,000</td>
<td>10,000</td>
<td>2.8</td>
<td>To aid in orderly storage in box files</td>
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<tr>
<td>5</td>
<td>Registry book</td>
<td>1</td>
<td>15,000</td>
<td>15,000</td>
<td>4.2</td>
<td>To register study participants</td>
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<tr>
<td>6</td>
<td>ID labels</td>
<td>3</td>
<td>3,500</td>
<td>10,500</td>
<td>2.9</td>
<td>For patient numbers (names will not be used)</td>
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<tr>
<td>7</td>
<td>Pens</td>
<td>9</td>
<td>500</td>
<td>4,500</td>
<td>1.3</td>
<td>For documentation of all study information</td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>HUMAN RESOURCE</td>
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<td></td>
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<td></td>
<td></td>
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<td>8</td>
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<td>As an IRB requirement for research</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>SUPPLIES</td>
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<td>80,000</td>
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<td>To carry out blood sugar test</td>
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<tr>
<td>12</td>
<td>Glucose strips</td>
<td>360</td>
<td>40,000 for 100</td>
<td>144,000</td>
<td>40</td>
<td>Inserted in glucometer with patient’s blood to ascertain blood sugar level</td>
</tr>
<tr>
<td>13</td>
<td>Urine containers</td>
<td>360</td>
<td>100 for 20,000</td>
<td>72,000</td>
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<td>To collect participant’s urine</td>
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<tr>
<td>14</td>
<td>Vacutainers(Red purple tops)</td>
<td>360</td>
<td>100 for 40,000</td>
<td>144,000</td>
<td>40</td>
<td>To collect blood samples</td>
</tr>
<tr>
<td>D</td>
<td>LABORATORY TESTS</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Renal function tests</td>
<td>358</td>
<td>5,000</td>
<td>1,790,000</td>
<td>497.2</td>
<td>These tests will be carried out for all study participants to ascertain factors associated</td>
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<tr>
<td>16</td>
<td>Serum electrolytes</td>
<td>358</td>
<td>5,000</td>
<td>1,790,000</td>
<td>497.2</td>
<td></td>
</tr>
<tr>
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<td>Urinalysis</td>
<td>358</td>
<td>5,000</td>
<td>1,790,000</td>
<td>497.2</td>
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</table>

GRAND TOTAL (Ugshs/Us dollars)  7,200,000  2000 $
APPENDIX 2: WORK PLAN FOR THE RESEARCH PROJECT

Time frame (months)-April 2017-May 2018

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<th>Apr</th>
<th>May</th>
<th>Jun</th>
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<th>Nov</th>
<th>Dec</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
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</thead>
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<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Submission of the book</td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>
APPENDIX 3: CONSENT FORMS

Consent form in English

PREVALENCE AND FACTORS ASSOCIATED WITH RENAL DYSFUNCTION AMONG PATIENTS RECEIVING TENOFOVIR BASED ANTIRETROVIRAL THERAPY AT MULAGO-KIRUDDU INFECTIOUS DISEASE CLINIC

Purpose and Background

My name is Dr. Nyende Louis a Masters in medicine student at Makerere University. I am currently conducting research about kidney disease in patients living with HIV who are taking Tenofovir based antiretroviral therapy for six months or more.

The study involves knowing how patients with renal dysfunction present to the clinic, any findings on examination and requesting appropriate laboratory tests to confirm or rule out disease. You will be told about the study and if you wish to join, will be required to offer written verbal consent or thumb print which ever you prefer.

Your participation is very important because we want to learn more about renal dysfunction among HIV patients and the associated factors. We estimate that this interview will last about thirty minutes. I will ask you questions that are well outlined in a questionnaire. Everything that you answer will be completely confidential.

You will also be required to provide 20 mls of a mid-stream urine sample

What to encounter when participating in the study;

If you agree to participate in the study, then the following will happen:

You will document your consent, have a questionnaire filled out with details of your illness and blood, 10 mls will be drawn; A sterile container will be provided to you to collect your urine.

Risks of blood draw

Blood withdrawal carries a minimal risk of pain, swelling, bleeding or even infection at the site of injection. The pain is minimal, reducing a few minutes after the needle has been withdrawn. To minimise pain, bleeding and infection at the puncture site, blood will be taken
off by a skilled person using aseptic techniques ensuring that a clean swab is applied to the spot with gentle pressure. All attempts will be made to have blood drawn once to avoid pricking you again.

The blood draw is associated with pain at the punctured site and bleeding. About 10 mls of your blood will be taken off for analysis.

The samples will be transported and identified by a number and taken to the laboratory for analysis.

**Benefits of the study**

You will be able to know how your kidneys are functioning from the results of the blood and urine.

If you are found with renal dysfunction your primary care giver will be notified so as to refer you to see a kidney specialist.

This study will help us understand better about renal dysfunction among HIV positive infected patients on antiretroviral therapy.

**Rights to refuse or withdraw**

Your participation in this part of the study is entirely voluntary and if you choose not to do so, you will still get care and treatment from the clinic.

You are free to withdraw from the study any time.

**Confidentiality**

All documents and specimen will be given a participant ID number. This means that your name will not appear on any document.

All information and results will be kept confidential and safely.

**Questions**

In case of any questions or problems related to the study, you may ask or contact Dr Louis Nyende (the principal investigator) at the Department of Medicine, Mulago Hospital or on
mobile phone number 0758874234 / 0782063606 anytime during the study. For any questions regarding your rights as a patient, you may contact Professor OcamaPonsiano, the chairman school of medicine research ethics committee (SOMREC) on mobile telephone number 0772421190.

**Consent procedure and confidentiality**

You have been requested to participate in a study which involves knowing how common renal dysfunction is among HIV patients on TDF based ART and associated factors. You should only participate if you are entirely in agreement. Your answers will not be revealed to anyone without your consent. You reserve the right to consent or withdraw from this study at any point. No costs to participate in this study a part from your time of about 30 minutes. I am happy to answer any questions about this consent form or about the study.

If you agree to participate, we will obtain signed written consent from you.”

I agree to participate in this study.

Name of the provider /Signature of subject ………………………………………………………

Date………………………………………

Name of the witness/ Signature/thumbprint of witness…………………………………………………..

Date………………………………………

Name of Investigator or authorized representative……………………………………………………

Date……………………………………………………………………………………………

Contact telephone number in case of any queries: 0758874234 /0782063606
LUGANDA CONSENT FORM

Ekiwandiiko kyokukkiriza okwetaba mu kunoonyereza

OMUTWE GWOKUNOONYEREZA: OBUGI WAMU NENSONGA EZEKUUSA KU BULWADDE BWENSGI GO BALWADDE ABAFUNA EDDAGALAL LYA ‘TENOFOVIR’ KULWOBULANJABI KU KILINIKA Y’OBULWADDE.

Omugaso wamu nensibuko

Erinya lyange nze musawo Nyende Louis, omuyizi mu setendekero ya Makerere. Nkola okunoonyereza okukwata ku bulwadde bwensigo mu balwadde abalina obulwadde bwa siriimu abamila eddagala lya 'Tenofovir' kulwokunjaba mu myezi mukaaga oba ejisingawo.

Okunoonyereza kulimu okumanya butya abalwadde abalina obulwadde bwensigo gyebajja ku kilinika, ebiva mu kukebera wamu nokusaba okukebera okwa labalatole okusaana okukakasa oba okujjamu obulwadde. Ojja kugambibwa ebikwata ku kunoonyereza era singa oyagala okwetabamu, ojja kwetagisibwa okuwa okukkiriza okwetabamu oba ekinkumu, kyonna kyonayagala.

Okwetabamukwo kwa kyeyagalire kubanga tuyagala okuyiga ebisingawo ebikwata ku bulwadde bwensigo mu balwadde abalina akawuka akaleeta siliimu wamu nembeera ezikyekuusako. Tutebereza nti okubuuza kuno kuma edakiika ng’amakumi asatu. Nja kukubuuzayo ebibuuzo ebili mu kwetabamu, ojja kwetagisibwa okuwa okukkiriza okwetabamu oba ekinkumu, kyonna kyonayagala.

Okujjako omusaayi kulimu obulumi mu kifo ekifumitidwa wamu nokuvaamu omusaayi. Mililiita nga 10 ezomusaayigwo zijja kukujibwako kulwokukebelebwa.

Era ojja kwetagisibwa okuwa muliliita 20 eza sampolo yomusulo

Biki ebyokuyitamu mu kwetaba mu kunoonyereza kuno;

Bwokkiriza okwetaba mu kunoonyereza. Bino wammanga bijja kukolebwa:

Ojja kuvandiiika okukkirizakwo, ojuze ekiwandiiko kyebibuzo ebilimu ebikwata ku bulwaddebwo wamu nomusaayi, mililiita 10 zijja kujibwako. Akakebe akataliimu buwuka kajja kukuweebwa okukunganyizaamu omusulo.
Okujjako omusaayi kulimu obulabe butono obwobulumi oba okuzimba mu kifo awakubidwa empiso.

Sampolo zijja kutambuzibwa era zilagibwe ne namba era zitwalibwe mu labalatole kulwokwekebejebwa.

**Eddembe okugaana oba okuvaamu**

Okwetabakwo mu kunoonyereza kuno kwa kyeyagalire era singa olondawo obuteetabamu ojja kusigala ngofuna obulabilizi wamu nobujanjabi okuva mu kilinika.

Oliwaddembe okuva mu kunoonyereza akadde konna.

**Okukuuma ebyama**

Ebiwandiiko byonna wamu ne ‘specimen’ bijja kuweebwa namba yeyetabyamу. Kino kitegeeeza nti nambayo tejja kulabikila ku kiwandiiko kyonna.

Obubaka bwonna wamu nebivaamu bijja kukuumibwa nga byakya era bulungi.

**Ebibuuzo**

Singa wabaawo ebibuuzo byonna oba ebizibu ebikwata ku kunoonyereza, oyunza okubuuza kati oba notuukirira musawo Louis Nyende ku kitongole kyebyeddagala, mu dwaliro ly’ e Mulago oba ku ssimu 0758874234/0782063606 akadde konna mu biseera byokunoonyereza. Kulwebibuuzo byonna ebikwata ku ddembelyo ngomulwadde, oyunza okutuukirira sabakenkufu Ocama Ponsiano, sentebe wakakiiko akakwasisa empisa mu kunoonyereza, mu kitundu kyebyeddagala ku ssimu +256414-530 020.

**Emitendera gyokukkiriza wamu nekyama**

Bwokkiriza okwetabamu, tujja kufuna okukkirizakwo.”

Nzikiriza okwetaba mu kunoonyereza.

Erinya lyawa okukkiriza/Omukono .................................

Enaku zomwezi........................................

Erinya lyomujulizi/Omukono/Ekinkumu kyomujulizi .................................

Enaku zomwezi........................................

Erinya lyanoonyereza oba ayambako akkilizidwa...............................

Enaku zomwezi................................................

Namba yessimu singa wabaawo ebibuuzo: 0758874234 /0782063606
Appendix 4: Data collection tool (Questionnaire)
A study to determine the prevalence and associated factors of renal dysfunction among patients on TDF based ART at Mulago-Kiruddu infectious disease clinic.

PTID __________

A. INFORMATION

1. Gender  [ ] Male  [ ] Female
2. Age (in years)  [ ]  [ ]  [ ]
3. Address__________________________
5. Education level  [ ] Primary  [ ] Secondary  [ ] Tertiary
6. Occupation ________________________________
7. Alcohol use (CAGE)________________________

B. CLINICAL DATA

8. General condition  [ ] Good  [ ] Wasted
9. Weight (Kg)  [ ]  [ ]  [ ]  Height (cm)  [ ]  [ ]  [ ]  [ ]
   BMI__________
10. Blood pressure__________________________mmHg
11. Any other important /abnormal findings__________________________________________

**B. HIV DATA AT TIME OF PRESENTATION**

12. WHO stage  

- [ ] I  
- [ ] II  
- [ ] III  
- [x] IV

13. Most recent CD4+ cell count  

- [ ]  
- [ ]  
- [x]  
- [x] cells/ml  

- [x] date  
- [ ]  
- [ ]  
- [ ]  
- [x] missing

14. Viral load (if available)  

- [ ]  
- [ ]  
- [ ]  
- [ ]  
- [ ] copies/ml  

- [x] date  
- [ ]  
- [ ]  
- [ ]  
- [ ] missing

15. ART Regimen  

- [ ] TDF/3TC/EFV  
- [x] TDF/FTC/EFV  
- [ ] TDF/3TC/ATV/r  
- [ ] TDF/3TC/LPV/r  

- [ ] Other TDF based regimen____________________________________________________-

16. Duration on ART (months) ______________________

17. Concurrent opportunistic infections  

- [ ] Yes  
- [x] No
If yes  TB  CCM  Toxoplasmosis  
others____________________

18. Comorbidities  Hepatitis B  Diabetes  Hypertension  
   Others______________________________________________________

19. Other drugs currently taking (within a month)____________________

C. LABORATORY PARAMETERS

20. Recent serum creatinine (Cr)  .  μmol/l  Date  .  .

21. Estimated GFR using (Cockcroft gault formula) ml/min/1.73 m²

22. Random blood sugar mmol/l

Serum electrolytes

23. Potassium mmol/l

24. Phosphate mmol/l

25. Calcium mmol/l
Urinalysis

26. Macroscopy Appearance

27. Protein present (≥2+) Nil (<2+)

28. Nitrites Pos Negative

29. Leucocytes

30. Hematuria present Nil

31. Glucose present Nil
Appendix 5: WHO staging of HIV/AIDS for adults and adolescents (2005)

Primary HIV infection

Asymptomatic

Acute retroviral syndrome

Clinical stage 1

Asymptomatic

Persistent generalized lymphadenopathy

Clinical stage 2:

- Moderate and unexplained weight loss (<10% of presumed or measured body weight)
- Recurrent respiratory tract infection (such as sinusitis, bronchitis, otitis media, pharyngitis)
- Herpes Zoster
- Recurrent oral ulcerations
- Papular pruritic eruptions
- Angular cheilitis
- Seborrheic dermatitis
- Fungal finger nail infections

Clinical stage 3
Conditions where a presumptive diagnosis can be made on the basis of clinical signs and simple investigations

- Unexplained chronic diarrhea for longer than one month
- Unexplained persistent fever (intermittent or constant for longer than one month)
- Severe weight (>10% of presumed or measured body weight)
- Oral candidiasis
- Oral hairy leukoplakia
- Pulmonary tuberculosis (TB) diagnosed in the last 2 years
- Severe presumed bacterial infections (e.g. pneumonia, empyema, meningitis, bacteria, pyomyositis, and bone or joint infections.
- Acute necrotizing ulcerative stomach, gingivitis or periodontitis

Conditions where confirmatory diagnostic testing is necessary

- Unexplained anaemia (<80g/l), and or neutropenia (<500/ul) and/or thrombocytopenia (<50,000/ul) for more than one month.

Clinical Stage 4

Conditions where a presumptive diagnosis can be made the basis of clinical signs and simple investigations

- HIV wasting syndrome
- Pneumocystis pneumonia
- Recurrent severe or radiological bacterial pneumonia
- Chronic herpes simplex infection (orolabial, genital or anorectal of more than one month’s duration)
- Oesophageal candidiasis
- Extra pulmonary tuberculosis
- Kaposi’s sarcoma
- Central nervous system toxoplasmosis
- HIV encephalopathy

**Conditions where confirmatory diagnostic testing is necessary**

- Extrapulmonary cryptococcosis including meningitis
- Progressive multifocal leukoencephalopathy
- Candidiasis of the trachea, bronchi or lungs
- Cryptosporidiosis
- Isosporiasis
- Visceral herpes simplex infection
- Cytomegalovirus (CMV) infection (retinitis or organ other than the liver, spleen or lymph nodes
- Any disseminated mycosis (e.g. histoplasmosis, coccidioimycosis, peniciliosis)
- Recurrent non-typhoidal salmonella septicemia
- Lymphoma (cerebral or B cell Non-Hodgkin’s)
- Invasive cervical carcinoma
- Visceral leishmaniasis
Appendix 6: Standard operating procedures (SOPs)

**SCREENING/RECRUITMENT SOP VERSION 5.0**

- Study participants will be identified from the infectious disease clinic on the days it runs (Monday, Tuesday, Wednesday and Thursday)
- Screening will be done by PI or designated research assistant through administering the inclusion criteria initially to identified files and then these are tagged to the participants
- Participants that do not meet inclusion criteria will be explained to and excluded
- Participants that are eligible for the study will then undergo informed consent process
- Participants will then sign or thumb print the consent in presence of witnesses
- A standard questionnaire will then be administered to capture the study variables

**INFORMED CONSENT SOP VERSION 5.0**

Purpose; to ensure protection of participants and follow ethical principles during the study

Responsibility; Principal investigator / designated research assistant

- This process has no designated time line but will not take less than 30 minutes.
- This will be read to the participant in Luganda or English depending on their preference
- The participant may interrupt the session at any time during the process for clarification
- The participant’s understanding will be checked through questioning about what they have been told
- Once the participant has fully understood, she/he should document this by appending a signature or thumb print on the informed consent form.
- The date when this is read will be documented next to signature/thumb print.
• A witness will also append signature/thumb print as well as the PI/Research assistant
• The participant will be given an opportunity to choose whether to go with a copy of the
  signed document or not.

**BLOOD DRAW SOP VERSION 5.0**

Purpose; to ensure timely and sterile technique in collecting and transporting blood specimens

Responsibility; Principal investigator/ Designated Research assistant

• Tests to be carried out ; Renal function tests including serum electrolytes (Potassium,
  Phosphate ,Calcium) and random blood sugar
• Ensure participant has consented to participate in the study before blood draw is done.
• Explain procedure to the participant and amount of blood to be taken off (approximately
  10 mls)
• Ensure appropriate equipment- syringes (10mls), vacutainers and cotton swabs are
  readily available (Red tops), well labelled with participant number & age before blood
  draw.
• Wash area to be punctured with soap and water to remove visible dirt
• Wash your hands with antiseptic and water, dry them with soft tissue or clean cloth
• Apply tourniquet gently just above one of the cubital fossae
• Wear sterile gloves, clean area with alcohol (70%) on cotton swab in concentric circles
  away from the puncture site for at least 30 seconds, then apply Iodine for 30 seconds. If
  patient reacts to Iodine, use only Alcohol.
• Ask patient to flex hand at the wrist, while identifying the vessel to with draw blood
• Use sterile syringe to withdraw 10mls of venous blood, apply pressure with cotton swab and withdraw needle gently.
• Quickly open red vacutainer and insert the collected blood.
• Reserve a few drops of blood in the syringe to measure the blood sugar using a glucometer
• Close the vacutainer and swirl it to avoid clotting
• Put container in collecting box ready for transportation to the laboratory for analysis in not more than one hour.

**URINE COLLECTION SOP VERSION 5.0**

Purpose; to collect good and adequate sample

Responsibility; Principal investigator /designated research assistant

• Tests to be done; urine dipstick and microscopy
• Ensure participant has consented to join the study
• Ensure appropriate sterile or clean container is avaied to the participant
• This container should be well labelled with participant number, age and sex
• Avail participant with 3 clean cotton swabs as well
• Explain procedure of collection
• Participant should clean urethral opening with these 3 swabs before passing urine.
• The appropriate sample is collected using the sterile/clean container from mid- stream urine, approximately 20-30mls.
• Sample is then transported and stored in refrigerator awaiting analysis