Prevalence of proximal tubular dysfunction in ambulatory HIV infected clients on 1st line HAART attending the Mater Comprehensive care clinic.

DR Okanga N. Okanga | H58/69212/13 |

DECLARATION

This study proposal is my original work and to my best knowledge has not been submitted either wholly or in part to this university for the award of any degree or diploma.

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Prevalence of proximal tubular dysfunction in ambulatory HIV infected clients on 1st line HAART attending the Mater Comprehensive care clinic.

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LIST OF ABBREVIATIONS

AKI	Acute kidney injury.
ATV	Atazanavir
BMI	Body mass index.
CKD	Chronic kidney disease.
DHHS	Department of Health and Human Services
EFV	Efavirenze.
ESRD	End stage renal disease.
FEP	Fractional excretion of phosphate.
FEU	Fractional excretion of urate.
GFR	Glomerular filtration rate.
HAART	Highly active antiretroviral therapy.
HBV	Hepatitis B virus.
HCV	Hepatitis C virus.
HIV	Human immunodeficiency virus.
KNH	Kenyatta national hospital.
MtDNA	Mitochondrial deoxyribonucleic acid.
NtRTI	Nucleotide reverse transcriptase inhibitor
NSAIDS	Non steroidal anti-inflamatory drugs.
PTD	Proximal tubular dysfunction
TDF	Tenofovir disoproxil fumarate.
TRP	Tubular reabsorption of phosphate.
TRU	Tubular reabsorption of urate
WHO	World health organization.

DEFINITION OF TERMS

$FEU = \left[\frac{Urine\ uric\ acid\ x\ plasma\ creatinine}{Urine\ creatinine\ x\ plasma\ uric\ acid}\right]$ x 100 (≥15% indicates hyperuricosuria)

$FEPO4 = \left[\frac{Urine\ phosphate\ x\ Plasma\ creatinine}{Plasma\ phosphate\ x\ Urine\ creatinine}\right]$ X 100 (≥ 5% indicates renal phosphate wasting)

ABSTRACT

BACKGROUND

Tenofovir disoproxil fumarate (TDF), is now a widely used component of antiretroviral regimens owing to its high potency and acceptable safety profile. Multiple randomized clinical trials have shown low incidence of nephrotoxicity with use of TDF, though case reports and case series to the contrary abound. Apart from the costs involved in management of renal impairment, the risk of cardiovascular disease is increased significantly.

OBJECTIVE

To determine prevalence of proximal tubular dysfunction (PTD) in clients on 1st line highly active antiretroviral therapy (HAART) attending comprehensive care clinic at The Mater Misericordiae Hospital in Kenya.

METHODOLOGY

This was a cross sectional observational study conducted between August and November 2018 that enrolled clients who were 18 years and above and on HAART for at least two years. We used a structured questionnaire for both clinical and demographic data. Random blood and urine samples were analyzed for markers of proximal tubular dysfunction specifically fractional excretion of uric acid, fractional excretion of phosphate, proteinuria and normogylcemic glycosuria. Significant proximal tubular renal dysfunction was defined by the presence of at least two of the following; normoglycemic glycosuria, hyperphosphaturia, tubular proteinuria and hyperuricosuria with at least one cardinal feature of Fanconi syndrome (normoglycemic glycosuria,or hyperphophaturia).

RESULTS

284 out of 2780 clients on 1st line regimens were sampled consecutively. 237 were on TDF and 47 were on non TDF regimen. Average age was 43 and 44 years and mean CD4 level was 365 and 309 cells/ml for the TDF and non TDF groups respectively. Median duration since diagnosis of HIV infection was 7 (IQR 4-9) years for TDF group and 9 (IQR 7-11) years for non TDF group. Proximal tubular dysfunction as defined by occurrence of FEP > 20% with concomitant FEU > 15% was found in 17% of participants in the TDF group compared to 10% in the non TDF group. No preselected risk factors were found to have significant association with the presence of proximal tubular dysfunction on univariate analysis.

CONCLUSION

There was a high prevalence of PTD in patients on the two first line regimens suggesting a significant contribution from HIV virus rather than the regimen used.

CHAPTER 1 LITERATURE REVIEW

1.1 INTRODUCTION

The impact of HIV infection on life expectancy has been blunted by widespread access to highly active antiretroviral therapy (HAART). Patients who have received antiretroviral therapy for at least six years and have achieved a CD4+ lymphocyte level greater than 500cells/ml have an estimated mortality that is similar to that of the general population (1). In high income countries kidney, liver and cardiac disease are overtaking opportunistic infections as the leading causes of death in HIV infected patients (2).

HIV infected persons are at a higher risk of developing renal dysfunction compared to the general age matched population (3). In addition to traditional risk factors such as diabetes, hypertension and atherosclerosis; immune complex disease, thrombotic microangiopathy, HIV-associated nephropathy (HIVAN) and medication toxicity increase the susceptibility of the HIV infected cohort to development of renal impairment (1).

1.2 TDF – NEPHROTOXICITY AND CONTROVERSIES

TDF is a prodrug of tenofovir, an acyclic nucleotide analogue reverse transcriptase inhibitor (NtRTI) similar in structure to adefovir and cidofovir. It is a structural analog of deoxyadenosine-5-triphosphate terminating DNA synthesis from RNA-dependent DNA polymerase of HIV while weakly inhibiting host cell DNA α- and β-polymerases and mitochondrial DNA γ-polymerase (4).

Since its approval in 2001, TDF has become one of the most widely used antiretroviral agent owing to its high potency, good safety profile and convenient once a day dosing. The 2013 WHO guidelines recommend TDF in combination with emtricitabine (FTC) and efavirenze (EFV) as the preferred regimen for all HIV-infected patients with CD4 cell counts less than 500 cells/ml. Guidelines released by British HIV Association in 2016 favour inititation of HAART using a backbone of TDF/TAF + FTC. TDF is among

the drugs recommended by the United States DHHS as of 2018 to be used in combination with other antiretroviral drugs in the treatment of HIV. Kenya, adapting WHO guidelines has TDF in combination with Lamivudine (3TC) and Dolutegravir (DTG)/ EFV as its first line regimen.

Early randomized controlled studies of healthy HIV_+ persons with minimal risk factors for kidney disease supported renal safety of TDF(5). Indeed initial post marketing survey of 10343 patients demonstrated renal effects in only 0.5% of patients (6).Similarly observational data from the Development of Antiretroviral Therapy in Africa (DART) trial team in East Africa implies that TDF-based ART can be provided safely without close monitoring of renal function (7).

In a 2010 systematic review and meta-analysis on renal safety of TDF in HIV-infected patients by Ryan D. Cooper *et al* a total of 17 studies (most from high income countries, 9 studies were randomized controlled trials) were analyzed. There was significantly greater loss of kidney function among the TDF recipients, compared with control subjects (mean difference in calculated creatinine clearance, 3.92 mL/min; 95% confidence interval [CI], 2.13–5.70 mL/min), as well as a greater risk of acute renal failure (risk difference, 0.7%; 95% CI, 0.2–1.2) (8).

Case reports and case series linking TDF use to development of proximal tubular dysfunction, acute kidney injury and decline in kidney function abound. A cross-sectional study by Overton *et al* of 845 HIV-infected outpatients showed a prevalence of CKD higher than that of the general population (8% vs. 2%), and significant predictors of lower GFR in multivariate analyses were found to be use of were diagnoses of hypertension, hyper-lipidemia, proteinuria, use of TDF or stavudine, and lower viral load (9). A retrospective cohort analysis of 1,647 patients enrolled in the Kaiser Permanente Health Maintenance Organization during 2002 to 2005, linked TDF exposure to decline in GFR and proximal tubular dysfunction (10) while the Swiss HIV Cohort Study found a consistent evidence for a significant reduction in GFR associated with TDF use (11).

In the Euro-SIDA Cohort Study patients initially on TDF, who ceased using the drug continued to have a significant increased incidence of CKD during the first year after cessation, suggesting that the effect of the drug is not easily reversible (12).

In a cross-sectional study including 99 HIV-infected patients with serum creatinine levels < 150.2 umol/l and dipstick-negative proteinuria, subjects using TDF had increased urine retinol-binding protein/creatinine ratio and protein/creatinine ratio, confirming a subclinical renal tubulopathy (13). Labarga *et al* in a cross sectional study of 284 HIV-positive patients concluded that a significant correlation exists between exposure to TDF and tubular dysfunction in the absence of impaired glomerular function (14).

Kenneth Mugwanya et al studying sero-discordant heterosexual couples in both Kenya and Uganda in a large randomized, placebo-controlled trial, with median follow-up of 18 months and maximum follow-up of 36 months found that daily oral TDF-based preexposure prophylaxis (PrEP) resulted in a small but non-progressive decline in e-GFR that was not accompanied by a substantial increase in the risk of clinically relevant (≥25%) e-GFR decline (15). Similarly Tino Salome et al in Uganda in the year 2013 – 2014 found no differences in renal function among patients on TDF and non-TDF containing ART for almost a decade (16).

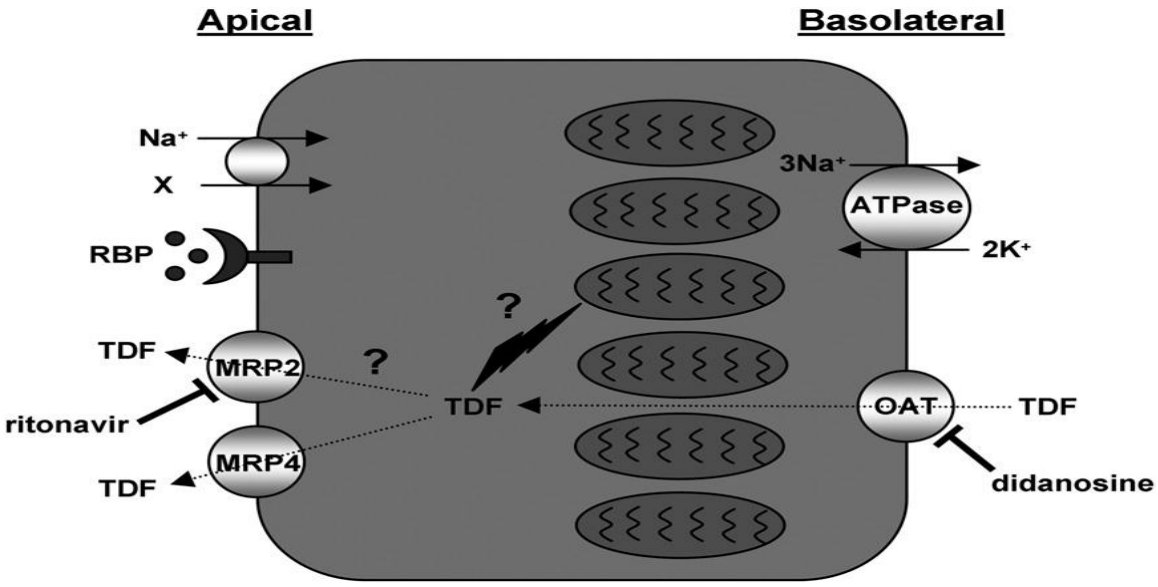
Real life patients unlike their trial counter parts may have associated conditions, medication or background that predisposes to TDF toxicity. Although clinical trials on safety of TDF report on the decline GFR only, proximal tubular dysfunction can occur without reduction in GFR.

1.2 MECHANISM OF TDF RENAL DYSFUNCTION

TDF is eliminated unchanged in urine by combination of glomerular filtration and tubular secretion. 20–30% of the drug is actively transported into renal proximal tubule cells by organic anion transporters (hOAT1, and to a lesser extent, OAT3) in the basolateral membrane(17), (18). Apical membrane transporters MRP-4 and MRP -2 (Multidrug resistance proteins, encoded by ABCC4 and ABCC2 genes) secrete TDF to the tubular lumen(19).

Rise in intracellular TDF concentration occurs when the plasma concentration of TDF increases or apical secretion is inhibited. The resultant partial inhibition of mitochondrial deoxyribonucleic acid γ(mtDNA), depletion in mtDNA and oxidative respiratory chain dysfunction leads to ATP shortage in proximal tubular cells(20). Ions and small molecules such as potassium, glucose, phosphate, uric acid, amino acids and b2-microglobulin appear in urine in abnormal quantities because of impaired reabsorption (Fanconi syndrome).

Figure 1 Mechanism of TDF nephrotoxicity



Tenofovir (TDF) transport in the renal proximal tubule. TDF enters proximal tubular cells across the basolateral membrane through organic anion transporters (OAT), where it competes for binding with didanosine, and exits the tubule across the apical membrane through the multidrug resistance transporter MRP4. Ritonavir is a substrate for MRP2 and may interact with TDF excretion through mechanisms that presently are unclear. The proximal tubule contains a high density of mitochondria, which lie in a basolateral striated distribution, and evidence suggests that these organelles are the target of TDF toxicity. A variety of solutes (X) are reabsorbed across the apical membrane through sodium (Na_)-mediated cotransport, which is driven by the Na_ gradients generated by the activity of the basolateral adenosine triphosphatase sodium-potassium pump (Na_-K_-ATPase). The proximal tubule also is responsible for the uptake of low-molecular-weight proteins (such as retinol-binding protein [RBP]) from the renal filtrate through receptor-mediated endocytosis. Mitochondrial toxicity in the proximal tubule leads to impaired reabsorption of low-molecular-weight proteins and other solutes, with urinary wasting and the clinical features of renal Fanconi syndrome.(21)

It is postulated that acute tubular necrosis results from apoptosis of epithelial cells through caspace pathway induced by the mitochondrial abnormalities(22).

Impaired tubular function leads to decreased 1-α hydroxylation of vitamin D and lower tubular reabsorption of vitamin D–binding protein(23). This may result in secondary

hyperparathyroidism, hypophosphatemia, osteomalacia, bone pain, decreased bone mineralization, and bone fractures(24), (25).

Nephrogenic diabetes insipidus occurs due to TDF induced reduction of aquaporin-2 expression in epithelial cells along the medullary collecting ducts(26).

1.3 RISK FACTORS FOR TDF NEPHROTOXICITY

Predictors of significant renal function decline in patients on TDF include concomitant use of protease inhibitors (PI)(27), polymorphism in genes encoding proximal tubular transporters(28),(29) low body mass index (BMI), older age, advanced HIV infection, concomitant hepatitis C virus (HCV) and concurrent use of other nephrotoxic drugs(6), (30)

Table 1 Prevalence of proximal tubular dysfunction in patients receiving TDF

Tubular alterations	**Prevalence**
Fanconi syndrome	0.3 – 2%(31),(5)
Proximal tubular renal dysfunction as defined by two or more tubular alterations	6- 15%(14), (32),(33)
Serum hypophosphatemia	4-31%(32), (34).
Decreased tubular phosphate reabsorption	4-50%(32),(35)(naïve vs. TDF treated)
Increased biomarkers B2M, RBP	9-71% (14),(35), (36)(naïve vs. TDF treated)

Adapted from Maria del Palacio, Sara Romero and Jose L. Casado(37)

β2M; Beta 2 microglubulin, RBP; Retinol binding protein, TDF; Tenofovir

1.4 MEASURING PROXIMAL TUBULAR DYSFUNCTION

Complete proximal tubular dysfunction results in a metabolic acidosis with normal plasma ion gap, hypophosphatemia, hyperphophaturia, hypokalemia, hypouricemia, urinary tubular protein waste, glycosuria with normal blood glucose and aminoaciduria. In isolation these alterations are of limited value in detecting tubular dysfunction since specificity is low.

Significant proximal tubular dysfunction is considered present if two or more of these alterations are present with at least one of them being Fanconi syndrome defining alteration (normoglycemic glycosuria, hyperphophaturia or aminoaciduria)(38).

Table 2 Methods for assessing kidney function in patients at risk of drug induced renal FS

Method	Requirement	Comment
Serum creatinine/eGFR	Single blood sample	Measures of glomerular function, not sensitive markers of tubular function. Mild creatinine rises may occur due to impaired tubular secretion
Fractional excretion or maximal tubular reabsorption of phosphate	Matched blood and urine samples of phosphate and creatinine	Phosphate wasting is an important complication of FS.
Metabolic acidosis	Single blood sample	Usually mild, unless distal tubular urinary acidification is also impaired
Urinary albumin/creatinine ratio	Spot urine sample	Predominantly a marker of glomerular disease, not sensitive for proximal tubular dysfunction
Urinary PCR	Spot urine sample	Not specific for tubular disease, but typically increased in FS.
Tubular proteinuria (e.g. retinol-binding protein)	Spot urine sample	The most sensitive marker of PT dysfunction.
Amino aciduria and organic ciduria	Spot urine sample	Typically increased in FS, but not usually used for monitoring purposes
Dipstick glycosuria	Spot urine sample	Marker of PT dysfunction, but may also be caused by hyperglycaemia.

(39)

CHAPTER 2: RESEARCH JUSTIFICATION

TDF now forms back bone of most HAART regimens in Kenya and considering the data linking TDF with decline in GFR, it is plausible to expect a rise in renal dysfunction in the HIV population. Local studies using a creatinine cut off of 60ml/min/1.732m2 have concluded that nephrotoxicity of TDF is a rare event. None of these studies investigated proximal tubular dysfunction which may occur without such drastic decline in GFR and for which the risk may vary among different ethnicities. Proximal tubular dysfunction may antedate decline in GFR. The findings of this study will be an addition to the growing body of evidence on the renal safety of TDF.

2.1 RESEARCH QUESTION

What is the burden of proximal tubular dysfunction in clients on 1st line HAART attending Mater Misericordiae Hospital Comprehensive Care Clinic?

2.2 OBJECTIVES

2.2.1 Primary objective

To determine the prevalence of proximal tubular dysfunction clients on 1st line HAART attending MCCC.

2.2.2 Secondary objective

Explore risk factors for proximal tubular dysfunction in patients on 1st line HAART attending MCCC.

CHAPTER 3 MATERIALS AND METHODS

3.1 STUDY DESIGN

Hospital based cross sectional observational study.

3.2 STUDY SITE

The Mater Comprehensive Care Clinic located at the Sisters' of Mercy run Mater Hospital in South B area of Nairobi has slightly above 3000 clients on follow up. Enrollment and follow up is free of charge. The catchment area for the clinic include Nairobi South, Nairobi West and the city center. The clinic opens from 0700hrs to 1630hrs on weekdays. On average 75 to 120 clients are seen daily. It is manned by a medical officer working closely with two nurses, five clinical officers, counselor and a nutritionist. An internist attends complicated cases as the need arises.

3.3 STUDY POPULATION.

HIV positive persons 18 years and above on follow up at the Mater Comprehensive Care Clinic.

Inclusion Criteria

All persons age 18 and above receiving HAART for more than 24 months, have medical data for review and have consented to participate in the study.

Exclusion criteria

Current or prior history of renal disease and pregnant persons in 2^{nd} and 3^{rd} trimesters.

3.4 SAMPLING PROCEDURE

1. **Sample size for estimation of prevalence in each group**

There are an estimated number of 2780 clients receiving 1st line HAART in Mater Hospital. Representative samples will be drawn from the two populations and the sample size calculation will be obtained using a formula for finite population (less than 10,000). The calculation will be as follows:

$$n' = \frac{NZ^2P(1-P)}{d^2(N-1)+Z^2P(1-P)}$$

Where

n' = sample size with finite population correction,

N = size of the target population

Z = Z statistic for 95% level of confidence = 1.96

P = Estimated prevalence of proximal tubular dysfunction in HIV patients

d = margin of error = 5%

The minimum sample sizes required for the two groups will be as follows:

Table 3 Sample size

	Ist line HAART
N	2780
P	22% (Labarga et al, 2009)
n'	241

3.5 SAMPLING METHOD

Subjects meeting inclusion criteria were selected consecutively during the study period until the target sample size was reached.

3.6 CLINICAL METHODS

All HIV-infected participants above 18yrs on follow-up and who obtain drugs from the hospital pharmacy were invited to participate in the study.

Age, sex, risk group, weight, chronic viral hepatitis and history of hypertension or diabetes mellitus, and HIV status (plasma viral load and CD4 T-lymphocyte count) was recorded at the time of recruitment in the study by the principal investigator or reasearch assistants. Duration of exposure to the various antiviral agents was obtained from the

pharmacy records. A questionnaire was issued to participants inquiring on the use of drugs with nephrotoxic potential, particularly nonsteroid anti-inflammatory drugs, valproic acid, co-trimoxazole, pentamidine, angiotensin converting enzyme inhibitors/ angiotensin receptor blockers, amphotericin B and gancyclovir.

Blood pressure was measured using a calibrated digital blood pressure machine with patient seated arm supported and with recommended cuff size for the arm.

3.7 LABORATORY METHODS

On recruitment renal parameters were determined in blood sample and spot urine. Sodium and potassium was measured in venous blood while creatinine, phosphorus, uric acid, glucose albumin and total protein were measured in both urine and venous blood using DIRUI 4000 automated spectrophotometric analyser.

Participants with glycosuria were advised to come the following day for fasting blood sugar test.

Creatinine clearance was calculated using the CKD EPI.

Hyperphosphaturia was defined by a fractional excretion of phosphorus of more than 18%, hyperuricosuria by fractional excretion of uric acid more than 15%. Tubular injury was defined as uPCR ≥200 mg/g with uAPR < 0.4.

$FEU = \left\lfloor \frac{Urine\ uric\ acid\ x\ plasma\ creatinine}{Urine\ creatinine\ x\ plasma\ uric\ acid} \right\rfloor$ x 100 (≥15% indicates hyperuricosuria)

$FEPO4 = \left\lfloor \frac{Urine\ phosphate\ x\ Plasma\ creatinine}{Plasma\ phosphate\ x\ Urine\ creatinine} \right\rfloor$ x 100 (≥ 18% indicates renal phosphate wasting)

$uAPR = \frac{urine\ albumin}{urine\ total\ protein}$ (Values < 0.4 suggest tubular proteinuria)

3.8 CASE DEFINITION

Participants were considered hypertensive if they had a history of the disease regardless of being treated for it or if they had a resting systolic blood pressure higher than 140mmgHg or diastolic pressure higher than this 90 mmHg or both on more than two occasions six hours apart.

Clients with diabetes were identified as those receiving antidiabetic drugs or had a fasting sugar of more than 7mmol/l.

Significant proximal tubular renal dysfunction was defined by the presence of at least two of the following; normoglycemic glycosuria, hyperphosphaturia, tubular proteinuria and hyperuricosuria with at least one cardinal feature of Fanconi syndrome (normoglycemic glycosuria,or hyperphophaturia).

3.9 QUALITY ASSURANCE MEASURES

Research assistants were registered clinical officers. A week prior to conducting the study the principal investigator took the research assistants through the process of proper administration of consent and data collection to ensure adherence to laid out procedure.

The principle investigator provided direct supervision throughout the study period. Recommended procedures of specimen collection (aseptic technique, labelling and storage) were adhered to. Laboratory tests were done at the KNH lab which has put in place the following quality assurance measures:

- Qualified staff licensed by the Kenya Medical Laboratory Technicians and Technologist Board.
- Periodic internal audits and reviews
- Readily accessible operation manuals for the various machines.
- Periodic calibrations as per manufacturer’s recommendations.
- ISO 9001:2008 certification of the Laboratory.

3.10 ETHICAL CONSIDERATION AND CONSENTING PROCEDURE.

Study approval was sought from Kenyatta National Hospital – University of Nairobi Research and Ethics Committee and The Mater Misericordiae Hospital Ethics Committee. There were no inducements offered to subjects who agreed to participate in the study. Subjects were recruited after a written consent. The consent procedure was as described here after. All potential participants were:

- Informed of the purpose and procedure of the study.
- Assured that participation was voluntary and no medical attention would be denied if they declined to participate.
- Assured that confidentiality would be strictly maintained and that all data would be securely stored and only revealed upon a need-to-know basis.
- Assured that participation in the study was free of charge.
- Expected to carry on with their clinic visits and receive the standard care offered.
- Expected to sign consent form after full explanation to signify their understanding and acceptance to participate in the study.

3.11 DATA STORAGE

All the raw data and the questionnaire forms in this study were filed in a box file and stored in a lockable cabinet accessible to the principal investigator only. All the sheets were checked to confirm completeness before filing.

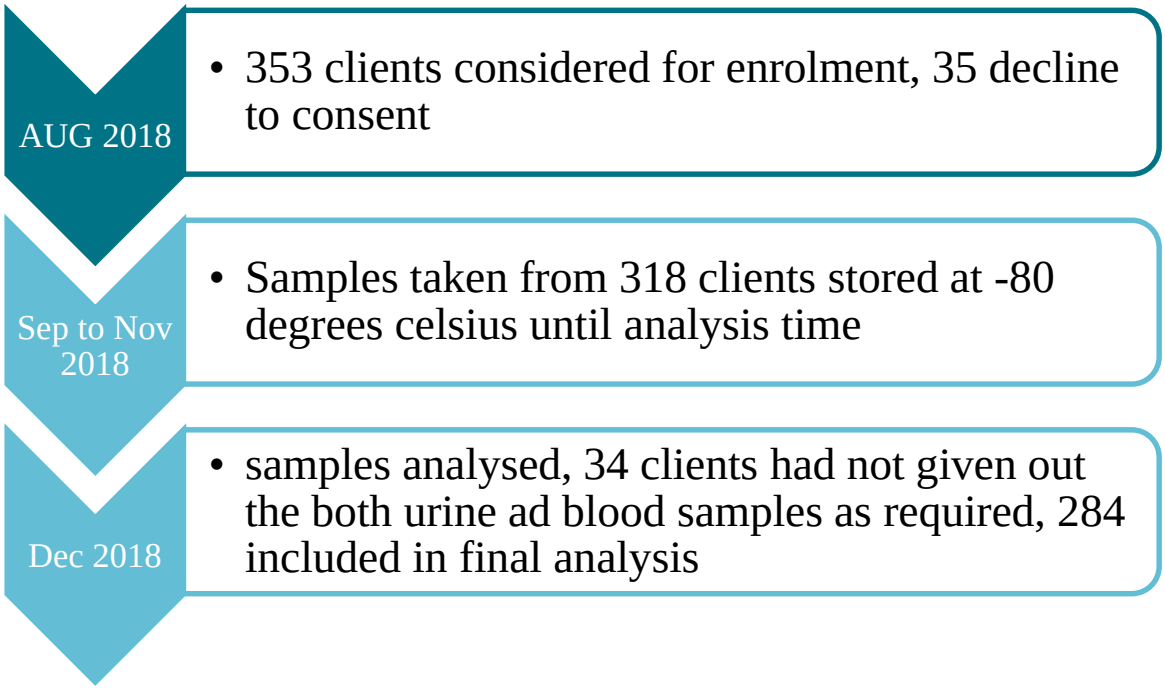
3.12 STATISTICAL ANALYSES

Data was entered and managed in Microsoft Excel spreadsheet coded with validation checks to ensure high quality data. Data cleaning was done continuously during data entry. Statistical analysis was done in SPSS version 21.0. The study population was described using demographic and clinical characteristics. Categorical variables were presented as percentages and continuous data analyzed as means and medians. Renal parameters were analyzed appropriately as means/ medians for the numerical parameters and percentages for categorical ones. Prevalence of proximal tubular dysfunction was calculated as a proportion with 95% confidence interval. Prevalence of proximal tubular dysfunction was associated with ART regimen type using chi square test and tested 5% level of significance.

CHAPTER 4 RESULTS AND ANALYSIS

During the study period extending from August to October 2018 records of 532 ambulatory clients attending the Mater CCC were perused and 353 considered for enrolment into the study. Out of these 284 met the inclusion criteria and were enrolled.

Figure 2 Flow chart for client enrollment



4.1 CHARACTERISTICS OF PARTICIPANTS

4.1.1 SOCIO-DEMOGRAPHIC CHARACTERISTICS

The two groups were comparable in age, BMI, race and gender proportion. One third of participants in each of the two groups had no formal education. Table 4 below summarizes the demographic features of the study participants.

Table 4 Demographic characteristics

Variable	
Mean age (SD)	43.8 (17.5)
Gender	
Male	89 (31.4)
Female	195 (68.6)
Employment	
Unemployed	23 (8.1)
Retired	4 (1.4)
Employed	257 (90.5)
Education level	
None	82 (28.8)
Primary school	12(4.2)
High school	81 (28.5)
College	109 (38.3)

4.1.2 CLINICAL CHARACTERISTICS

Median duration since HIV diagnosis was 7 IQR (4-11yrs) while median CD4 count at initiation of HAART was 303 (IQR 166 – 480). None of the participants were co infected with hepatitis B or hepatitis C. About a tenth of participants were hypertensive. Most of the participants were on Cotrimoxazole prophylaxis. Clients on TDF had a higher body weight compared to clients on AZT. Table 5 next page summarizes the clinical characteristics of the clients in the study.

	TDF	**AZT**
Number of patients (%)	237	47
Females	167 (70.4%)	24 (74.1%)
Mean HAART duration in years	7 (4-9)	7 (7-11)
Median CD 4 (IQR)	365 (168-485)	309 (150-468)
Median body weight in Kg(IQR)	69.75 (60-79)	63 (53-71)
Low BMI (%)	4 (1.7%)	1 (2.1%)
High BMI	119 (50.2%)	15 (31%)
HTN	15 (6.3%)	3 (6.3%)
Diabetes	2	0
Detectable viral Load	12 (5.1%)	3(6.3%)
HBsAg	0	0
HCV	0	0

Table 5Baseline clinical characteristics are comparable

4.1.3 RENAL PARAMETERS

Glomerular function as assessed by plasma creatinine was impaired in both categories of participants. More than 30% in each category were in CKD stage 3. A total of 45 (16.7%) participants fulfilled criteria for proximal tubular dysfunction defined as FEP >20% and FEU>15%. No participant had normoglycemic glycosuria. Hypokalemia was rare in both categories. Table 6 below summarizes the renal parameters of the participants.

	TDF	**AZT**
Number of patients (%)	237	47
Plasma creatinine Median (IQR)	101 (90-115)	103 (90-121)
e GFR Median (IQR)	65 (56.3-73)	62 (52-71)
e GFR < 60ml/min	**76 (32.1%)**	**19 (40.4%)**
Median serum uric acid mmol	264 (218-310)	272 (202-316)
Median serum phosphate mmol (I)	0.96 (0.84-1.07)	0.975(0.87-1.125)
Low phosphate <0.81mmol/l	45 (19.3%)	6 (12%)
Low Potassium	2	1
Normoglycemic glycosuria	0	0
FEU > 15%	104	21
FEP > 20%	42	9
PCR >20mg/mmol	**84 (35.4%)**	**19 (40%)**
PTD* (FEP >20%, FEU >15%)	**40 (16.8%)**	**5 (10%)**

Table 6 Comparable renal parameters

Only one patient in the TDF group nearly satisfied the criteria for Fanconi syndrome; he had low serum uric acid, hypophosphatemia, FEU > 15% and FEP > 20%. The serum potassium level was however normal.

4.1.4 PROXIMAL TUBULAR DYSFUNCTION

In clients with proximal tubular dysfunction, TDF use was associated with proteinuria.

Table 7 below summarizes the characteristics of clients with PTD.

Proximal tubulopathy FEU >15%+FEP >20%	**TDF**	**Non TDF**	**Odds ratio (95% CI)**
Number of patients	40	5	
Females	23 (57.5%)	4 (80%)	0.3 (0.0-3.3)
Haart duration in months	62	93	-
Mean CD 4 (SD)	366.6 (259.1)	230 (158.8)	-
Mean BMI (SD)	26.3 (4.8)	24.3 (4.6)	-
Low BMI	2	0	-
HTN	2	0	-
e GFR < 60ml/min	**7(17.5%)**	1 (20%)	0.8 (0.1-8.8)
Mean serum uric acid mmol (SD)	239.4 (71.4)	263.4 (96.2)	-
Mean serum phosphate mmol (SD)	1.04 (0.34)	1.01 (0.20)	-
			0.4 (0.0-5.0)
Detectable viral Load	4	1	0.4 (0.0-5.0)
Low phosphate <0.81mmol/l	4	1	-
Low uric acid	1	0	-
Low Potassium	0	0	4 (0.4-39.0)
PCR >20mg/mmol	20 (50%)	0	-
Normoglycemic glycosuria	0	0	

Table 7Proximal tubular dysfunction.

4.2 DISCUSSION

TDF is now a widely used molecule in the management of chronic HBV and HIV infections. Because of its association with renal impairment, its use is avoided in patients with renal disease and dose adjusted once creatinine clearance reduces to 30ml/min. The proximal tubular dysfunction it causes can however precede decline in e-GFR. Although long term effects of minor tubular abnormalities is currently unknown severe tubulopathy can results in osteomalacia and fractures. In most developing countries routine monitoring of tubular function for patients on TDF is hardly done hence the burden is a mystery. Consensus statements from various organizations recommend at least annual evaluation of renal function including tubular parameters in patients on HAART(40), (41).

We assessed tubular function among ambulatory patients on 1st line HAART attending The Mater Comprehensive Care Clinic using three readily measured parameters in our set up. The overall prevalence of PTD was 16% (TDF, 17% AZT 10%). The only potentially nephrotoxic medication used by study participants was Co- trimoxazol.

Clients on AZT based regimen were much older and were on HAART for a longer duration than patients on TDF based regimen (9 vs. 7yrs,) because AZT was introduced to the country much earlier than TDF. More over only 16% of patients on AZT regimen had BMI above 25, compared to 50% of patients on TDF regimen; a plausible explanation being the lipodystrophic side effects of AZT.

Over a third of clients in both groups had PCR of more than 20mg/mmol. Since we did not measure albumin levels in urine, we were unable to ascertain whether the proteinuria was tubular or glomerular in origin. Although the study was cross sectional, the high proteinuria levels suggest increased cardiovascular and all-cause mortality in the population studied.

Nearly a third of clients on TDF were already in CKD stage three and therefore should have been switched to a different molecule as per the current guidelines. This probably reflects over reliance on absolute creatinine levels instead of e GFR calculation for monitoring clients on TDF. Majority of clients on TDF with PTD had e-GFR above

60ml/min, affirming that tubular dysfunction may occur independent of severe impairment in glomerular function.

No participant was found to have normoglycemic glycosuria. The similar rates of PTD and the high rates of proteinuria suggests a high rate of background tubulopathy attributed to antiretroviral therapy in the population studied although the possibility of toxic effects of HIV virus on the tubules cannot be entirely excluded (42). Addition of a control group of HIV infected HAART naïve could have shed more light. The low levels of hypokalemia noted could be due to the potassium sparing effect of co-trimoxazole.

Our study was not sufficiently powered to explore predictors for development of PTD. No trend was noted regarding age, sex, and BMI.

Mugwanya et al in a multicenter study published in 2015 found that the prevalence of proximal tubular dysfunction among healthy volunteers taking TDF/FTC for pre exposure prophylaxis for 24 months was a paltry 1.7% (15) In our study mean duration of TDF exposure was 62 months and all our participants on HAART for treatment not prophylaxis. The higher prevalence in our study alludes to the contribution of HIV virus to the develpoment of tubular dysfunction or the longer duration on HAART in our cohort.

In contrast Chadwick et al in a single center study conducted in Ghana involving HIV-infected patients on HAART found 35% prevalence of tubular dysfunction among TDF uses compared to only 6% among non TDF uses(43). However, the duration on HAART among the two groups of participants was quite short compared to our study (median TDF exposure 20 months IQR 12 – 14 and 24 months IQR 15–48 for non TDF group). Proteinuria (PCR 200g/g) occurred in 37% of the entire cohort studied comparing well with our study in which 35% in the TDF group and 40% in the non TDF group had proteinuria reflecting a high background rate of tubulopathy.

Although several case studies report onset of tubolupathy within six months of initiating TDF(44), (45) a Kaplan-Meier estimate in study by Mukdaporn et al revealed that of all the patients who develop tubulopathy after initiation of TDF, 10% do so by the 24th month, and this increases to 50% by the 72nd month(46). Chadwick's findings may imply a greater predisposition to toxic tubular effects of TDF in the West African population.

Pablo Labarga et al in Madrid found 22% prevalence of PTD among 154 patients on TDF HAART compared to 7% among 49 patients on non TDF HAART(14). Median duration of TDF exposure was 36 months (IQR 14-48) compared to 55 months (IQR 35-91) for Non TDF HAART exposure. Patients on TDF were had initially been on Non TDF HAART for a median of 59 months unlike our cohort. Criterion for diagnosing PTD in their study was exhaustive; six parameters considered, unlike our study that used FEP and FEU only. Hypophosphatemia was observed in 9.8% on TDF group and 6.7% on non TDF group compared to 19% and 10% respectively in our study. In both studies differences in the serum phosphate level between the two groups was not statistically significant and normoglycemic glycosuria rare. It is difficult to extrapolate these findings to ours since CKD risk factors and underlying kidney disease etiologies are different nevertheless it is fair to assume our prevalence could have been much higher had all markers of PTD been tested.

Salome Tino et al compared renal parameters between 385 patients on non-TDF and 568 on TDF-ART regimens in Uganda (16). More than 60% of participants both arms had been on medication for more than 5 years. Slightly above 10% of the participants have viral load >1000. Dip stick proteinuria occurred in 78% of participants in the TDF group compared to 63% in the non TDF group. Impaired fractional reabsorption of phosphate occurred in less than 5% of participants in both groups (cut off 82%). Hypophosphatemia was more prevalent in the non TDF group 16.6% compared to the TDF group (10.6%). This study differs from ours because apart from fractional reabsorption of phosphate, other specific markers of proximal tubulopathy were not actively sought. Dipstick

assesses mainly albumin and hence a reflection of glomerular rather than tubular dysfunction. It is noteworthy that impaired fractional reabsorption of phosphate was a paltry 5%. Uganda's banana based low phosphate diet compared to Kenya's corn based high phosphate diet may account for the disparity.

A major limitation of our study is the omission of other key indicators of PTD such as b2-microglobulinuria, hyperaminoaciduria and tubular proteinuria. Because definition of PTD has not been standardized, inter-study comparison becomes a dicey affair. With the snap shot nature of the study time frame for development of tubulopathy remains unknown and long term trends are difficult to predict, indeed a longitudinal study has shown that PTD fluctuates over time (47). A change in the national guidelines directing a switch from AZT/3TC/EFV to DTG/3TC/TDF reduced the number of clients on AZT in the final analysis.

Going forward, larger longitudinal studies incorporating data on bone density, Vitamin D, parathyroid hormone levels and a control group of HAART naïve HIV positive participants will offer insight on the clinical significance of impaired tubular parameters in the setting of HIV infection. More over once Tenofovir Alefenamide becomes widely available a study of its renal safety in our setup would be highly desirable.

In conclusion we found a high prevalence of PTD in clients on both TDF and non TDF regimens. Decline in e GFR was not a predictor of PTD. With its clinical significance still unknown it may be prudent to discontinue TDF in patients who meet criteria for PTD on multiple occasions especially in the background of risk factors for development of renal disease.

6 REFERENCES

1. HIV-infected adults with a CD4 cell count greater... - Google Scholar [Internet]. [cited 2017 Nov 9]. Available from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=HIV-infected+adults+with+a+CD4+cell+count+greater+than+500+cells%2Fmm3+on+long+term+combination+antiretroviral+therapy+reach+same+mortality+rates+as+the+general+population.+Lewden+C%2C+Chene+G%2C+Morlat+P+et+al.+s.l.+%3A+J+Acquir+Immune+Defic+Syndr+%2C+2007%2C+Vols.+46%3A72-7.&btnG=

2. Trends in diseases reported on U.S. death certificates that mentioned HIV infection, 1987-1999. - Abstract - Europe PMC [Internet]. [cited 2017 Nov 9]. Available from: http://europepmc.org/abstract/med/11917243

3. Reid A, Stöhr W, Walker AS, Williams IG, Kityo C, Hughes P, et al. Severe Renal Dysfunction and Risk Factors Associated with Renal Impairment in HIV-Infected Adults in Africa Initiating Antiretroviral Therapy. Clin Infect Dis. 2008 Apr 15;46(8):1271–81.

4. Birkus G, Hitchcock MJ, Cihlar T. Assessment of mitochondrial toxicity in human cells treated with tenofovir: comparison with other nucleoside reverse transcriptase inhibitors. Antimicrob Agents Chemother. 2002;46(3):716–723.

5. Izzedine H, Isnard-Bagnis C, Hulot J-S, Vittecoq D, Cheng A, Jais CK, et al. Renal safety of tenofovir in HIV treatment-experienced patients. Aids. 2004;18(7):1074–1076.

6. Nelson MR, Katlama C, Montaner JS, Cooper DA, Gazzard B, Clotet B, et al. The safety of tenofovir disoproxil fumarate for the treatment of HIV infection in adults: the first 4 years. Aids. 2007;21(10):1273–1281.

7. Routine versus clinically driven laboratory monitoring of HIV antiretroviral therapy in Africa (DART): a randomised non-inferiority trial. The Lancet. 2010 Jan 9;375(9709):123–31.

8. Cooper RD, Wiebe N, Smith N, Keiser P, Naicker S, Tonelli M. Systematic review and meta-analysis: renal safety of tenofovir disoproxil fumarate in HIV-infected patients. Clin Infect Dis. 2010;51(5):496–505.

9. Overton E, Nurutdinova D, Freeman J, Seyfried W, Mondy K. Factors associated with renal dysfunction within an urban HIV-infected cohort in the era of highly active antiretroviral therapy. HIV Med. 2009 Jul 1;10(6):343–50.

10. Horberg M, Tang B, Towner W, Silverberg M, Bersoff-Matcha S, Hurley L, et al. Impact of tenofovir on renal function in HIV-infected, antiretroviral-naive patients. JAIDS J Acquir Immune Defic Syndr. 2010;53(1):62–69.

11. Fux CA, Simcock M, Wolbers M, Bucher HC, Hirschel B, Opravil M, et al. Tenofovir use is associated with a reduction in calculated glomerular filtration rates in the Swiss HIV Cohort Study. Antivir Ther. 2007;12(8):1165.

12. Mocroft A, Kirk O, Reiss P, De Wit S, Sedlacek D, Beniowski M, et al. Estimated glomerular filtration rate, chronic kidney disease and antiretroviral drug use in HIV-positive patients. Aids. 2010;24(11):1667–1678.

13. Hall AM, Edwards SG, Lapsley M, Connolly JO, Chetty K, O'Farrell S, et al. Subclinical tubular injury in HIV-infected individuals on antiretroviral therapy: a cross-sectional analysis. Am J Kidney Dis. 2009;54(6):1034–1042.

14. Labarga P, Barreiro P, Martin-Carbonero L, Rodriguez-Novoa S, Solera C, Medrano J, et al. Kidney tubular abnormalities in the absence of impaired glomerular function in HIV patients treated with tenofovir. Aids. 2009;23(6):689–696.

15. Mugwanya KK, Wyatt C, Celum C, Donnell D, Mugo NR, Tappero J, et al. Changes in glomerular kidney function among HIV-1–uninfected men and women receiving emtricitabine–tenofovir disoproxil fumarate preexposure prophylaxis: a randomized clinical trial. JAMA Intern Med. 2015;175(2):246–254.

16. Salome T, Kasamba I, Mayanja BN, Kazooba P, Were J, Kaleebu P, et al. The effect of Tenofovir on renal function among Ugandan adults on long-term antiretroviral therapy: a cross-sectional enrolment analysis. AIDS Res Ther. 2016;13(1):28.

17. Cihlar T, Ho ES, Lin DC, Mulato AS. Human renal organic anion transporter 1 (hOAT1) and its role in the nephrotoxicity of antiviral nucleotide analogs. Nucleosides Nucleotides Nucleic Acids. 2001;20(4–7):641–648.

18. Ray AS, Cihlar T, Robinson KL, Tong L, Vela JE, Fuller MD, et al. Mechanism of active renal tubular efflux of tenofovir. Antimicrob Agents Chemother. 2006;50(10):3297–3304.

19. Kohler JJ, Hosseini SH, Green E, Abuin A, Ludaway T, Russ R, et al. Tenofovir renal proximal tubular toxicity is regulated by OAT1 and MRP4 transporters. Lab Invest. 2011;91(6):852–858.

20. Côté HC, Magil AB, Harris M, Scarth BJ, Gadawski I, Wang N, et al. Exploring mitochondrial nephrotoxicity as a potential mechanism of kidney dysfunction among HIV-infected patients on highly active antiretroviral therapy. Antivir Ther. 2006;11(1):79.

21. Hall AM, Hendry BM, Nitsch D, Connolly JO. Tenofovir-Associated Kidney Toxicity in HIV-Infected Patients: A Review of the Evidence. Am J Kidney Dis. 2011 May;57(5):773–80.

22. Ortiz A, Justo P, Sanz A, Melero R, Caramelo C, Guerrero MF, et al. Short communication Tubular cell apoptosis and cidofovir-induced acute renal failure. Antivir Ther. 2005;10:185–190.

23. Fernandez-Fernandez B, Montoya-Ferrer A, Sanz AB, Sanchez-Nino MD, Izquierdo MC, Poveda J, et al. Tenofovir nephrotoxicity: 2011 update. AIDS Res Treat. 2011;2011.

24. Parsonage MJ, Wilkins EGL, Snowden N, Issa BG, Savage MW. The development of hypophosphataemic osteomalacia with myopathy in two patients with HIV infection receiving tenofovir therapy. HIV Med. 2005;6(5):341–346.

25. Perrot S, Aslangul E, Szwebel T, Caillat-Vigneron N, Le Jeunne C. Bone pain due to fractures revealing osteomalacia related to tenofovir-induced proximal renal tubular dysfunction in a human immunodeficiency virus-infected patient. JCR J Clin Rheumatol. 2009;15(2):72–74.

26. Rodriguez-Novoa S, Labarga P, Soriano V. Pharmacogenetics of tenofovir treatment. Pharmacogenomics. 2009;10(10):1675–1685.

27. Zimmermann AE, Pizzoferrato T, Bedford J, Morris A, Hoffman R, Braden G. Tenofovir-associated acute and chronic kidney disease: a case of multiple drug interactions. Clin Infect Dis. 2006;42(2):283–290.

28. Rodríguez-Nóvoa S, Labarga P, Soriano V, Egan D, Albalater M, Morello J, et al. Predictors of kidney tubular dysfunction in HIV-infected patients treated with tenofovir: a pharmacogenetic study. Clin Infect Dis. 2009;48(11):e108–e116.

29. Nishijima T, Komatsu H, Higasa K, Takano M, Tsuchiya K, Hayashida T, et al. Single nucleotide polymorphisms in ABCC2 associate with tenofovir-induced kidney tubular dysfunction in Japanese patients with HIV-1 infection: a pharmacogenetic study. Clin Infect Dis. 2012;55(11):1558–1567.

30. Madeddu G, Bonfanti P, Giuseppe V, Carradori S, Grosso C, Marconi P, et al. Tenofovir renal safety in HIV-infected patients: results from the SCOLTA Project. Biomed Pharmacother. 2008;62(1):6–11.

31. Jones R, Stebbing J, Nelson M, Moyle G, Bower M, Mandalia S, et al. Renal dysfunction with tenofovir disoproxil fumarate-containing highly active antiretroviral therapy regimens is not observed more frequently: a cohort and case-control study. JAIDS J Acquir Immune Defic Syndr. 2004;37(4):1489–1495.

32. Tenofovir and PI Use Are Associated with an Increased Prevalence of Proximal Renal Tubular Dysfunction in the Swiss HIV Cohort Study: boosted PI+TDF associated with renal function [Internet]. [cited 2017 Nov 10]. Available from: http://www.natap.org/2009/CROI/croi_144.htm

33. Dauchy F-A, Lawson-Ayayi S, de La Faille R, Bonnet F, Rigothier C, Mehsen N, et al. Increased risk of abnormal proximal renal tubular function with HIV infection and antiretroviral therapy. Kidney Int. 2011;80(3):302–309.

34. Day SL, Date HAL, Bannister A, Hankins M, Fisher M. Serum hypophosphatemia in tenofovir disoproxil fumarate recipients is multifactorial in origin, questioning the utility of its monitoring in clinical practice. JAIDS J Acquir Immune Defic Syndr. 2005;38(3):301–304.

35. Kinai E, Hanabusa H. Renal tubular toxicity associated with tenofovir assessed using urine-beta 2 microglobulin, percentage of tubular reabsorption of phosphate and alkaline phosphatase levels. Aids. 2005;19(17):2031–2033.

36. Kinai E, Hanabusa H. Progressive renal tubular dysfunction associated with long-term use of tenofovir DF. AIDS Res Hum Retroviruses. 2009;25(4):387–394.

37. Del Palacio M, Romero S, Casado JL. Proximal tubular renal dysfunction or damage in HIV-infected patients. AIDS Rev. 2012;14(3):179–87.

38. Izzedine H, Launay-Vacher V, Isnard-Bagnis C, Deray G. Drug-induced Fanconi's syndrome. Am J Kidney Dis. 2003;41(2):292–309.

39. Hall AM, Bass P, Unwin RJ. Drug-induced renal Fanconi syndrome. QJM Int J Med. 2013;107(4):261–269.

40. Willem D.F. Venter,June Fabian, Charles Feldman. An overview of tenofovir and renal disease for the HIV-treating clinician. South Afr J HIV Med [Internet]. Available from: https://sajhivmed.org.za/index.php/hivmed/article/view/817

41. José L. Górriz1*, Félix Gutiérrez2*, Joan C. Trullas3*, Piedad Arazo4, José R. Arribas5, Guillermina Barril6, Miguel Cervero7, Frederic Cofán8, Pere Domingo9, Vicente Estrada10, Xavier Fulladosa11, María J. Galindo12, Silvia Gràcia13, José A. Iribarren14, Hernando Knobel15, José López-Aldeguer16, Fernando Lozano17, Alberto Martínez-Castelao11, Esteban Martínez8, María A. Mazuecos18, Celia Miralles19, Rosario Montañés13, Eugenia Negredo20, Rosario Palacios21, María J. Pérez-Elías22, Joaquín Portilla23, Manuel Praga24, Carlos Quereda22, Antonio Rivero25, et al. Executive summary of the consensus document on the management of renal disease in HIV-infected patients [Internet]. Nefrologia 2014;34(6):768-88; Available from: doi:10.3265/Nefrologia.pre2014.Sep.12745

42. Mikulak J, Singhal PC. HIV-1 and Kidney Cells: Better Understanding of Viral Interaction. Nephron Exp Nephrol. 2010 Jun;115(2):e15.

43. Tenofovir is associated with increased tubular proteinuria and asymptomatic renal tubular dysfunction in Ghana | BMC Nephrology | Full Text [Internet]. [cited 2018 Nov 30]. Available from: https://bmcnephrol.biomedcentral.com/articles/10.1186/s12882-015-0192-4

44. Lify B, Dabo G, Nascimento O, Iraqui S, Elkhayat S, Zamd M, et al. Fanconi syndrome induced by tenofovir: A case report. Saudi J Kidney Dis Transplant. 2016 Jul 1;27(4):808.

45. Pascale R, Guardigni V, Badia L, Volpato F, Viale P, Verucchi G. Early Onset of Tenofovir-Related Fanconi Syndrome in a Child with Acute Hepatitis B: A Case Report and Systematic Review of Literature [Internet]. Case Reports in Hepatology. 2017 [cited 2019 Jan 14]. Available from: https://www.hindawi.com/journals/crihep/2017/3921027/

46. Mukdaporn Pichpattana, M.D., , Danaband Phiboonbanakit, M.D., Ph.D.1, , Kitti Trakulhun, M.D.,Ouppathum Supasyndh, M.D. Incidence of Tenofovir Disoproxil Fumarate Induced Proximal Tubulopathy in HIV-Infected Patients. J INFECT ANTIMICROB AGENTS. (2016;33:33-55.).

47. Longitudinal assessment of proximal tubular dysfunction in HIV seropositive and seronegative persons: correlates and implications [Internet]. [cited 2018 Nov 30]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5388566/

7. APPENDICES

7.1 APPENDIX I: STATEMENT OF INFORMATION FORM

STATEMENT OF INFORMATION FOR PATIENTS PARTICIPATING IN THE STUDY

Purpose of the study:

I, DR. Okanga N Okanga, a post-graduate student in Internal Medicine at the University of Nairobi, would like to introduce you to a study I will be undertaking entitled **Prevalence of proximal tubular dysfunction in HIV infected clients on 1st line HAART attending the Mater Comprehensive Care Clinic.**

HIV infected persons are at a higher risk of developing kidney complications. This study compares kidney function in patients on various antiretroviral regimens. Early detection of kidney impairment reduces the risk of progression and improves patient outcomes and quality of life. The study is being conducted at the Mater comprehensive care clinic with cooperation from the staff and permission from the hospital administration.

Procedures:

You are being asked to participate in a survey that will take 45 to 60 minutes. If you agree to participate, it will involve checking of your medical records before I ask you questions and note your responses in writing in a questionnaire. I will then take your weight, height, waist and hip measurements and blood pressure. Afterwards, I will examine you thoroughly before drawing about 8 ml of venous blood for lab analysis to assess your renal function, protein, uric acid, phosphate and fasting blood sugar. In addition 10ml urine sample will be required for analysis of creatine, protein, uric acid, glucose and phosphate. All the information you provide and the results will remain confidential but a copy of the lab results will be placed in your file for continued care.

The risk to you as a participant in this study includes:

- Pain in the cubital region on your arm upon blood sample removal.
- Swelling at the venepuncture site may appear due to collection of blood under the skin (haematoma).

NB: should any of the above happen to you; please feel free to contact Dr. Okanga N Okanga for examination and management.

The benefit to you as a participant includes:

- Free evaluation of your serum creatinine, fasting blood sugar and kidney parameters.
- Free copy of your results will be availed to you on request or placed inside your file.
- The findings of this study will inform on the renal safety of Tenofovir in management of HIV.

Right to refuse or withdraw:

Your participation in this research is voluntary. Feel free to decline answering any question. You are also free to withdraw from the study at any time and this will not affect your care or treatment in any way. You are free to ask questions before signing the consent form. If you agree to participate in the study, please sign the consent form.

Thank you for your cooperation

NB Principal investigator or research assistant will show the potential participant how much 8ml and 10mls amount to using a 10cc hypodermic syringe.

7.2 APPENDIX II: CONSENT FORM

Names.....

I, the above named have been requested to take part in a study on the prevalence of proximal tubular dysfunction in clients on 1st line HAART at The Mater Hospital. This involves obtaining history, general examination and laboratory tests. Kidney function tests, phosphate, uric acid and fasting glucose will be estimated from a sample of 8ml of my blood. Around 10ml of urine will be required for estimating protein, creatinine, uric acid and phosphate. I have been shown how much 8ml and 10mls amounts to. Costs for laboratory expenses will be met by the investigator. All the results obtained will remain confidential.

I also understand that this consent is voluntary and that I can withdraw from the study at any time without any penalties.

I therefore consent to be recruited into the study.

Signature of patient/guardian: .....

Date: .....

If you have any question during the course of the study, you may contact the following:

DR Okanga N. Okanga 0712453213/0780453218

OR

RESEARCH AND ETHICS COMMITTEE

KENYATTA NATIONAL HOSPITAL

020-2726300/0722829500/0733606400 EXT 44102

OR

RESEARCH AND ETHICS COMMITEE

THE MATER HOSPITAL

BOX 30325 NAIROBI, TEL 254-20531199/0722828629

Investigator's statement:

I the investigator have educated the research participant on the purpose and implication of this study.

Signed: ..... Date: .....

7.3 MAELEZO KWA WANAOSHIRIKI UTAFITI

7.3.1 Lengo la Utafiti

Jina langu ni Daktari Okanga Nashon Okanga, mwanafunzi katika Chuo Kikuu cha Nairobi. Ningependa kukueleza kuhusu utafiti ninaoufanya kuhusu madhara ya madawa yanayotumika kupunguza makali ya virusi vya ukimwi, kwa kiungo cha figo**.**

Wanaoishi na virusi vya ukimwi wanahatari ya kupata madhara ya figo. Utafiti huu unalinganisha vipimo vya figo za wanaotumia madawa tofauti za kupunguza makali ya virusi vya ukimwi. Kutambulika mapema kwa kuharibika kwa figo hupunguza hatari ya maendeleo na kuboresha matokeo ya mgonjwa na ubora wa maisha. Utafiti utafanyika katika hospitali ya Mater kwa ushirikiano kutoka kwa wafanyakazi na kibali kutoka kwa utawala wa hospitali.

Utaratibu

Utafiti utachukua kama saa moja hivi. Nitachunguza kumbukumbu yako ya matibabu kabla ya kuuliza maswali yalioko kwenye dodoso. Nitakupima urefu, uzito na upana wa kiuno, halafu baada ya kukuchunguza mwili, nitakutoa damu kidogo kama mililita nane na nitakuomba utoe mkojo kama mililita kumi niipeleke ipimwi. Vipimo vyenyewe ni viwango vya Sodium, potassium, protein, phophate, uric acid,creatine and sukari. Maelezo yote utakayotoa na matokeo ya vipimo yatabaki siri lakini nakala ya matokeo ya maabara zitawekwa katika faili yako kwa huduma unaoendelea.

Hatari kwako kama mshiriki katika utafiti huu ni pamoja na

1. Maumivu katika sehemu za kiwiko juu ya mkono wako wakati wa kutolewa sampuli ya damu .
2. Uvimbe sehemu ya kutolewa damu.

.

NB: Tafadhali jisikie huru kuwasiliana na Daktari. Okanga N Okanga kwa ajili ya uchunguzi na matibabu iwapo umeathirika wakati sampuli ya damu inatolewa.

Faida kwako kama mshiriki ni pamoja na

1. Kufanyiwa uchunguzi bila malipo yoyote.

2. Utapewa nakala ya matokeo yako itatumiwa kwako kwa ombi au kuwekwa ndani ya faili yako.

3. Matokeo ya utafiti huu yatasema juu ya usalama wa figo wa Tenofovir katika kupunguza makali ya virusi vya ukimwi.

Haki ya kukataa au kuondoka

Kushiriki kwako katika utafiti huu ni kwa hiari. Uko huru kukataa kujibu swali lolote. Wewe pia ni huru kujiondoa kwenye utafiti wakati wowote na hii haiathiri huduma yako au tiba kwa namna yoyote. Wewe ni huru kuuliza maswali kabla ya kusaini fomu ya ridhaa. Ikiwa unakubali kushiriki katika utafiti huo, tafadhali ishara fomu ya kibali.

Asante kwa ushirikiano wako.

7.3.2 IDHINI

Mimi ..... natoa idhini mwenyewe bila kushurutishwa kivyovyote kushiriki katika utafiti kuhusu madhara ya madawa ya kupunguza makali ya virusi vya ukimwii kwa kiungo cha figo.

Nimeelezewa kikamilifu kwamba habari za kibinafsi kama vile makao yangu zitachukuliwa na vile vile mililita nane za damu na mkojo zitachukuliwa kwa madhumuni ya vipimo. Nimeonyeshwa kiwango cha mililita nane na mililita kumi.

Nimeelezewa kuwa naweza kujiondoa wakati wowote iwapo nitabadilisha mawazo.

Sahihi ya mshiriki.....

Sahihi ya shahidi.....

Tarehe .....

Ukiwa na swali au jambo lolote unaitaji kuelezwa zaidi, tafadhali wasiliana na DKT. Okanga N. Nambari ya simu ni: 0712453213

Ama:

MWENYE KITI WA CHAMA CHA UTAFITI NA MAADILI.

HOSPITALI KUU YA KENYATTA/CHUO KIKUU CHA NAIROBI.

SIMU: 020-2726300/0722829500/0733606400 EXT 44102.

AMA MWENYE KITI WA CHAMA CHA UTAFITI NA MAADILI

HOSPITALI YA MATER

BOX 30325 NAIROBI, TEL 254-20531199/0722828629

INVESTIGATORS STATEMENT

I, the investigator have educated the research participant on the purpose and implications of this study.

Signed .....Date.....

7.4 APPENDIX III: STUDY PROFORMA

Prevalence of proximal tubular dysfunction in ambulatory HIV infected clients on 1st line HAART attending the Mater Comprehensive care clinic.

Study No..... **Date.....**

(A) HISTORY

1. Socio-demographic data

Hospital No. _____

Age _____Sex _____

1 = Male ☐ 2 = Female ☐

Usual Occupation _____ Current Employment Status _____

1 = employed ☐ 2= self employed ☐ 3 = retired ☐ 4 = Other ☐

Residence (province) _____

Education attainment

1 = No formal Education ☐ 2 = Primary ☐ 3 = High School ☐

4 = College/University ☐ 5 = Other (specify) ☐

2. Year of HIV diagnosis _____

3. Date of HAART initiation_____

4. TDF exposure duration in months _____

5. CD 4 at initiation _____

6. Current viral load and CD 4 _____

7. HBsAg results_____, **HCV RNA** _____

7. Current medication

Class	Name	Date of commencement	Dose
Nephrotoxic drugs			
Antihypertensive			
Antidiabetic			
Prophylaxis			
Others			

(B) PHYSICAL EXAMINATION

1. Anthropometric data

Weight (kg) _____

Height (cm) _____

Waist circumference (cm) _____

Hip circumference (cm) _____

BMI _____

2. Vital signs

SBP (mmHg) First reading _____ Second reading _____ Average _____

DBP (mmHg) First reading _____ Second reading _____ Average _____

C. Lab results

Parameter	Blood	Urine
Pre HAART Cr		
Egfr		
Current Cr		
Current eGFR		-
K		-
Na		
PO4		
Uric acid		
Glucose		
Total protein		
Albumin		
FEUa		
FEPO4		
uAPR		
UPCR		
Dipstick gylcosuria		