ACUTE KIDNEY INJURY IN TENOFOVIR EXPOSED PATIENTS IN HIV INFECTED INDIVIDUALS ADMITTED AT GROOTE SCHUUR HOSPITAL, CAPE TOWN AND LIVINGSTONE HOSPITAL, GQEBERHA, SOUTH AFRICA

By

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MZNSIM003

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Dedication

This dissertation would not have been possible without the unwavering support of my sister, Thandiwe Mazondwa, and the rest of my family. To my late grandmother, Esther Nopati Mazondwa, and my late grandfather, Johnson Mazondwa; I will forever be grateful to you for instilling the desire to always seek.

Declaration

I, ***Simthandile Fiona Mazondwa***, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgement indicate otherwise) and that neither the whole work nor part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Date: 07 JUNE 2021

Plagiarism declaration

I confirm that this is my own work, is not copied from any other person's work (published or unpublished)

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List of Abbreviations

AKI: Acute kidney injury

ART: Anti-retroviral therapy

ATN: Acute tubular necrosis

CD4: Cluster of differentiation 4

CKD: Chronic kidney disease

eGFR: Estimated glomerular filtration rate

FEPi: Fractional excretion of phosphate

GSH: Groote Schuur hospital

HIV/AIDS: Human Immune deficiency virus/acquired immune deficiency syndrome

HPT: Hypertension

KDIGO: Kidney Disease Improving Global Outcomes

LTFU: Lost to follow up

LVH: Livingstone hospital

MCS: Microscopy, culture, and sensitivity

MDRD: Modification of Diet in Renal Disease

MRP: Multidrug resistant protein

NHLS: National Health Laboratory System

OAT: Organic anion transporter

PI: Protease inhibitor

SA: South Africa

T2DM: Type 2 diabetes mellitus

TAF: Tenofovir alafenamide

TB: Tuberculosis

TDF: Tenofovir disoproxil fumarate

TFV: Tenofovir

uPCR: Urinary protein creatinine ratio

WHO: World Health Organization

Chapter 1: Journal ready manuscript

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Abstract

Introduction:
Tenofovir disoproxil fumarate (TDF) is vastly used in South Africa (SA) as a first line agent for the treatment of human immunodeficiency virus (HIV). TDF is known to be associated with nephrotoxicity with identified risk factors. This study aimed to describe the demographics, clinico-biochemical features, kidney function and mortality outcomes in TDF exposed patients with acute kidney injury (AKI) in two tertiary centres in SA.

Method:
This observational cohort study reviewed all HIV infected in-patients presenting with AKI referred to the nephrology units at both Groote Schuur Hospital, Cape Town and Livingstone hospital, Gqeberha. Baseline characteristics, contributory factors to the AKI, associated clinical and biochemical features were recorded. Where a kidney biopsy was indicated, histological features were documented. Kidney and mortality outcomes of the enrolled patients were assessed over a 1-year period.

Results:
There were 213 patients enrolled from 1 August 2013 to 30 September 2016, 114/213 (51.8%) of the patients were TDF-exposed and 99/213 (45%) were TDF-unexposed. The median age was 37 years (IQR: 31 – 45yrs). The TDF-exposed were significantly older, 40 years versus 34 years ($p<0.01$). The TDF-unexposed group had a higher prevalence of hypertension: 21/99 (21.2%) versus 11/114 (9.7%), ($p=0.02$). The median creatinine at referral was 642 µmol/L (IQR: 340 – 1116 µmol/L) and 96/210 (45.7%) required dialysis. HIV/tuberculosis (TB) coinfection was common, 119/199 (59.8%). There was significant exposure to nephrotoxic drugs and drugs associated with idiosyncratic drug reactions in both groups, with anti-tuberculous treatment being the most common. Rifampicin was used by 51/212 (24.1%) [TDF-exposed 31/114 (27.2%) and TDF-unexposed 20/98 (20.4%), $p=0.25$]. There were no differences in serum and urinary biochemical features between the TDF-exposed and unexposed groups. Of the enrolled patients, 57/213 (26.8%) underwent a kidney biopsy. On histology, the incidence of acute tubular necrosis (ATN) was higher in TDF-exposed individuals (TDF-exposed: 47% versus TDF-unexposed: 22% $p=0.05$) whilst in TDF-unexposed, HIV associated nephropathy was most common. In the total cohort, chronic kidney disease (CKD) developed in 22/212 (10.4%) and the mortality was 62/213 (29.1%). There were no significant differences between the TDF-exposed and non-exposed cohorts in terms of CKD or mortality.

Conclusion:
This study demonstrated that hospitalized people living with HIV in SA have a high rate of tuberculosis co-infection and significant drug exposures. The clinical characteristics, severity of AKI and outcomes were similar in TDF-exposed and -unexposed. TDF exposure was associated with a greater degree of ATN on kidney biopsy. AKI in this HIV infected cohort carried a high mortality, regardless of the aetiology.

(410 words)

Introduction

In 2009, the World Health Organization (WHO) introduced tenofovir disoproxil fumarate (TDF) as a first line drug in the management of human immune deficiency virus/ acquired immune deficiency syndrome (HIV/AIDS).(1) In South Africa (SA) the transition to fixed dose combinations, containing TDF, occurred in 2013 thereby improving the pill burden.(2) The emergence of these combinations (*Odimune®,Tribus® and Atripla®*) has led to a significant rise in TDF use.

TDF is an acyclic nucleotide analogue reverse transcriptase inhibitor. It is freely filtered by the glomerulus and 20-30% is secreted by the proximal tubule. TDF is secreted from the kidney via organic anion transporters-1 and -3 (OAT-1 and OAT-3) on the basolateral membrane and multidrug resistant proteins -2 and -4 (MRP-2 and MRP-4) on the apical membrane.(3-5)

TDF has been associated with nephrotoxicity.(6) Mocroft *et al*. has reported 2.1% (468/20 603) progressing from eGFR of ≥90 mL/min to a confirmed eGFR of ≤70 mL/min (incidence rate, 4.78 cases/1000 person-years of follow-up.(7) Identified risk factors associated with TDF nephrotoxicity include older age, prolonged exposure to TDF, low creatinine clearance at therapy initiation, the combination of TDF with a protease inhibitor (PI), use of cotrimoxazole, MRP-2 and MRP-4 polymorphisms, and other comorbidities (including diabetes mellitus, hepatitis B/C co-infection and hypertension).(8-10) Apart from risk factor identification, biomarkers of proximal tubular injury have been explored to screen for early TDF nephrotoxicity. Markers of proximal tubular damage, include β_2-microglobinuria, uricosuria and an increased fractional excretion of phosphate.(3, 11)

TDF nephrotoxicity should be considered in the clinical setting of proximal tubular dysfunction (phosphaturia, glycosuria, and amino aciduria), acute kidney injury (AKI) or the development of chronic kidney disease (CKD) associated with TDF exposure.(12, 13) However, the definitive diagnosis is based on the presence of typical histopathological changes on kidney biopsy.

The diagnostic histological characteristics of TDF nephrotoxicity include varying degrees of proximal tubular injury and associated interstitial fibrosis. Light microscopy reveals 1) acute tubular necrosis (ATN) with luminal ectasia, 2) hyper-eosinophilia with prominent eosinophilic intracytoplasmic inclusions, 3) irregular luminal contours with prominent nucleoli and loss of the brush border. Electron microscopy demonstrates acute proximal tubular degeneration with depleted and dysmorphic mitochondria.(14, 15)

There are limited data on TDF nephrotoxicity in SA. An observational cohort study in a South African out-patient setting showed a small but significant decline in eGFR over time in PLWH on TDF.(16) Data is particularly limited for hospitalized patients with AKI, where there is a high burden of HIV/ tuberculosis (TB) co-infection. Wearne *et al*. looked at kidney disease in Africans with HIV and TB; in the Cape Town cohort, renal TB was diagnosed in 43/70 (61%).(17) This study therefore aimed to firstly, determine the severity of TDF associated AKI in an HIV positive cohort at 2 tertiary referral centers, Groote Schuur Hospital (GSH), in Cape Town and Livingstone Hospital (LVH), in Gqeberha. Secondly it aimed to describe the clinical, biochemical, and histological features associated with TDF nephrotoxicity and report on kidney and mortality outcomes.

Methods

Study population

This was a prospective, observational cohort study that recruited patients from two South African sites GSH, Cape Town and LVH, Gqeberha. All HIV positive patients referred to the nephrology units for assessment of kidney dysfunction from 1 August 2013 to 30 September 2016 were considered for the study. The study was approved by the Human Research Ethics Committee of the University of Cape Town (HREC 620/2016). Informed consent was taken prior to data collection.

AKI was defined according to the KDIGO definition and staging.(18) As the measurement of urine output was unreliable, only the increase in creatinine from baseline was used to define and stage AKI. In cases where baseline creatinine was not available, other features supportive of AKI were used to assist with the diagnosis such as normal kidney sizes and normal echogenicity on ultrasound as well as improvement of kidney function to normal during the study period. In cases where a kidney biopsy was done, the findings were also taken into diagnostic consideration in support of AKI. The inclusion criteria included age > 18 years with positive HIV status (HIV positivity was based on a positive ~~e~~nzyme-linked immunosorbent essay, a known self-reported HIV positive status or a positive rapid HIV diagnostic test). Patients were classified as TDF-exposed if they had received TDF within 2 months of the onset of renal dysfunction. Those fulfilling the inclusion criteria were consented and enrolled. Patients were excluded from the study if they were known to have CKD [i.e. a sustained abnormal creatinine over a 3-month period on the National Health Laboratory System (NHLS)]. Other exclusion criteria included age <18 years and inability to give informed consent.

Data collection and management

Baseline demographic data (age and gender) and a detailed drug history were obtained at the time of nephrology referral. History of drugs with potential to be nephrotoxic (e.g., TDF) and those commonly associated with idiosyncratic drug reactions (e.g., Rifampicin) were documented. Specifically, current or recent TDF use, current antiretroviral therapy (ART) regimen, anti-tuberculosis treatment, and use of any of the following drugs: aminoglycosides, penicillin, diuretics, angiotensin converting enzyme inhibitors or angiotensin receptor blockers, sulfamethoxazole/ trimethoprim, and amphotericin B.

Data of co-morbid illnesses (TB, hypertension, diabetes mellitus, recent or current sepsis, and hepatitis B status) were documented. Patients were assessed for the likelihood of sepsis and concurrent illnesses. Specific enquires were made regarding gastro-enteritis, dehydration, and hypotension. Sepsis was determined by suspicion of the treating physician at the time of assessment based on history or presence of a systemic inflammatory response syndrome, raised white cell count and/or raised C-reactive protein. Kidney biopsies were performed if there was a clinical indication. The routine indications for biopsy included unexplained renal impairment, proteinuria and/ or haematuria. All biopsies were reviewed by a panel of nephrologists and a single histopathologist.

For each enrolled patient, baseline creatinine i.e. the last normal recorded creatinine (µmol/L) and estimated glomerular filtration ratio (eGFR) (ml/min/1.73m^2) on the NHLS in the past year prior to nephrology referral was documented. [eGFR was documented using the MDRD formula as per laboratory reporting at time of the study]. Biochemical data were collected at the time of nephrology referral, including creatinine, eGFR, ferritin (ug/L), phosphate (mmol/L) and uric acid (mmol/L). A full blood count with differential count, particularly looking for eosinophilia (10^9/L), was noted. CD4 count (cells/mm^3) and need for dialysis was documented.

The urine was analyzed at the time of nephrology referral and included urine dipsticks, protein/creatinine ratio (uPCR, g/mmol), microscopy, culture, and sensitivity (MCS) and urinary phosphate (mmol/L) and creatinine. Proximal tubular dysfunction was evaluated by collecting serum phosphate (mmol/L) in comparison to urine phosphate, from which the fractional excretion of phosphate was calculated [FEPi = $\frac{PO4(U)\times Cr\ (S)}{PO4(S)\times Cr\ (U)} x100$]. Urinary lipoarabinomannan (LAM) was performed to investigate for renal TB. Where a kidney biopsy was performed, histology including light and electron microscopy was documented. The primary histological diagnosis was described as the most significant diagnosis as described by the histopathologist.

Follow up data were collected at routine clinic follow up visits in a dedicated clinic. Creatinine and eGFR were recorded at 1, 3, 6, 9, and 12 months. Renal recovery was defined as an eGFR >60ml/min/1.73m^2. Once a patient had achieved a normal kidney function, follow-up was discontinued. Kidney dysfunction persisting for longer than three months was staged for CKD according to KDIGO guidelines.(19) Mortality and cause of death were documented from patient notes or from electronic records.

Statistical analysis

Patients' demographic and clinical characteristics were summarized using descriptive statistics and were disaggregated by TDF exposure for presentation. Categorical variables were presented as frequencies/proportions, and continuous variables were presented as median with interquartile range or mean with standard deviation depending on data normality. Mann-Whitney U test or student t-test were used to compare continuous variables and either the Chi-squared or Fisher's exact test was used to compare categorical variables dependent on the large sample size assumption. Graphical presentation of the data was done using Microsoft Excel and Stata v14,2 (StataCorp, Texas, USA).

Survival probabilities for time to normal renal function and time to death were calculated overall and compared based on TDF-exposure using the log rank test. Survival was analyzed up to one year of follow-up and all patients were included for analysis. A p value less than or equal to 0.05 was interpreted as statistically significant. Statistical analysis was performed using Stata v14,2 (StataCorp, Texas, USA).

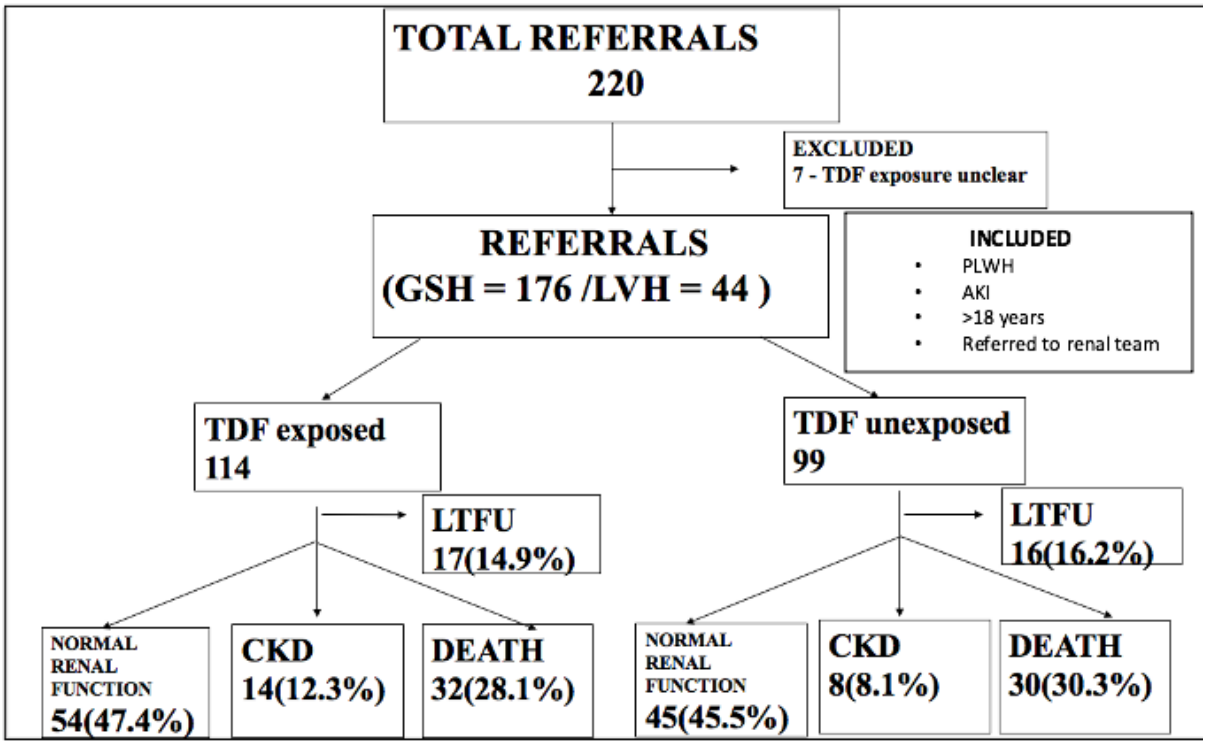
Results

During the study period, 1 August 2013 to 30 September 2016, a total of 213 people living with HIV (PLWH) were enrolled. GSH, Cape Town enrolled 176 and LVH, Gqeberha, 44 patients. Of the enrolled patients, 114/213 (53.5%) were TDF-exposed. This was in combination with either emtricitabine 79/114 (69.3%) or lamivudine 36/114 (31.6%) with efavirenz 108/114 (94.7%). The TDF-unexposed were 99/213 (46.5%). In the TDF-

unexposed, 83/99 (83.4%) were not on ART with the remainder on ART including either stavudine, zidovudine, and abacavir. Lopinavir /ritonavir as a second line agent was used in 5/213 (2.4%) of the enrolled patients.

Figure 1 illustrates the flow diagram of the outcomes of the TDF-exposed and TDF-unexposed study cohorts. Table 1 describes the demographic and biochemical features (blood and urinary) at the time of nephrology referral.

Figure 1: Flow diagram of the study



GSH- Groote Schuur Hospital
LVH- Livingstone hospital
TDF- Tenofovir disoproxil fumarate
LTFU-Lost to follow-up
CKD- Chronic kidney disease

Table 1: Baseline characteristics at the time of Renal Unit Referral

Demographics at presentation	**Total (n=213)**	**TDF-exposed (n=114)**	**TDF-unexposed (n=99)**	**p-value**
Age in years, median (IQR), n=213	37 (31 – 45)	40 (35 – 46)	34 (30 – 41)	<0.01
Gender, n (%) Female Male	 99/213 (46.5) 114/213 (53.5)	 54/114 (47.4) 60/114 (52.6)	 45/99 (45.5) 54/99 (54.5)	 0.78
CD4 count (cells/mm3), median (IQR) n=212	145 (58 – 273)	148 (56 – 287)	139 (58 – 239)	0.66
Comorbidities				
T2DM, n (%)	10/213 (4.7)	7/114 (6.1)	3/99 (3.0)	0.29
HPT, n (%)	32/213 (15.0)	11/114 (9.7)	21/99 (21.2)	0.02
Tuberculosis, n (%) No TB Probable/Definite TB	 80/199 (40.2) 119/199 (59.8)	 46/105 (43.8) 59/105 (56.2)	 34/94 (36.2) 60/94 (63.8)	 0.27
Hepatitis B surface antigen positive, n (%)	4/213 (1.9)	1/114 (0.9)	3/99 (3.0)	0.25
Biochemical results at time of nephrology referral				
Baseline creatinine * (µmol/L), median (IQR), n=140	71 (57-84)	71 (56-84)	71 (59-83)	0.85
Referral Creatinine (µmol/L), median (IQR), n=213	642 (340 – 1116)	698 (333 – 1150)	562 (340 – 1009)	0.56
Referral eGFR (mL/min/1.73 m²), median (IQR), n=213	9.2 (5.0 – 19.9)	8.9 (4.9 – 22.0)	10.3 (5.0 – 19.5)	0.78
KDIGO AKI classification, n (%) ** 1 2 3	 7/140 (5.0) 9/140 (6.4) 124/140 (88.6)	 6/99 (6) 6/99(6) 87/99 (87.9)	 1/41 (2.4) 3/41 (7.3) 37/41 (90.2)	 0.65
Ferritin (ng/mL), median (IQR), n=96	1227 (638 - 2459)	966 (612 – 2459)	1328 (705 – 2518)	0.48
Serum Phosphate (mg/dL), median (IQR), n=198	1.8 (1.3 – 2.5)	1.6 (1.2 – 2.5)	1.9 (1.3 – 2.5)	0.33
Eosinophil count, median (IQR), n=166	0.07 (0.02 – 0.3)	0.07 (0.03 – 0.30)	0.07 (0.02 – 0.35)	0.69
Positive Urine dipstick findings:				
Glucose, n (%)	41/193 (21.2)	23/105 (21.9)	18/88 (20.5)	0.81
Protein, n (%)	154/193 (79.8)	80/105 (76.2)	74/88 (84.1)	0.17
Blood, n (%)	150/193 (77.7)	80/105 (76.2)	70/88 (79.5)	0.58
Leucocytes, n (%)	63/192 (32.8)	34/105 (32.4)	29/87 (33.3)	0.89
uPCR, g/mmol, n=188	0.21 (0.14 – 0.35)	0.20 (0.14 – 0.31)	0.22 (0.13 – 0.38)	0.35
Urinary phosphate, mEq/L, n=149	6.8 (2.6 – 9.8)	6.7 (2.8 – 10.0)	6.9 (2.3 – 9.4)	0.75
FEPi , n=128	0.3 (0.1 – 0.5)	0.3 (0.2 – 0.6)	0.3 (0.1 – 0.5)	0.17

*(**Baseline creatinine)** Last normal recorded creatinine and eGFR on National Health Laboratory System in the past year prior to renal referral, (**AKI)** Acute Kidney Injury**, (CD4 count**) cluster of differentiation 4 count**, (KDIG**O) Kidney Disease: Improving Global Outcomes, **(eGFR**) estimated glomerular filtration rate, **(IQR**) interquartile range, **(FEPi**) fractional excretion of phosphate, **(uPCR**) Urine protein creatinine ratio, **(TB**) tuberculosis, **(T2DM**) Type 2 Diabetes mellitus, ****AKI KDIGO** classification using change in creatinine in comparison to the baseline.

The TDF-exposed were significantly older, 40 years versus (vs.) 34 years (p=<0.01) with no other significant differences in demographics. The TDF-unexposed group had a greater prevalence of hypertension 21/99 (21.2%), vs. 11/114 (9.7%), (p=0.02) with no other significant differences in comorbidities. Both groups had a normal mean baseline creatinine. However, 73/213 (34%) of the enrolled patients had no recorded baseline creatinine retrievable from the NHLS and 14/73 (19.2%) were TDF-exposed. Of this group, 14/73(19.2%) were lost to follow up (LTFU) and 23/73(37.5%) died.

Both TDF-exposed and unexposed groups had comparable degrees of AKI at the time of nephrology referral. Of the 141 patients with a baseline creatinine where AKI could be staged; 133(94.3%) had moderate to severe AKI (stage 2 or 3 AKI).(18) There was no difference in serum phosphate or fractional excretion of phosphate between cohorts. Due to missing data, the fractional excretion of phosphate was calculated in 128 (60.1%) of the total cohort. There were no differences in the presence of proteinuria, glycosuria, or hematuria.

Table 2 describes the suspected contributing factors for AKI at the time of nephrology referral. These included sepsis, diarrhoea, dehydration, and toxins/ drugs. There were no differences between the two groups at the time of referral. The nephrotoxic drugs to which the two groups were exposed are shown in table 2. Anti-TB therapy constituted the most common drug associated with idiosyncratic drug reaction in this cohort. Rifampicin was used by 51/212 (24.1%) and isoniazid in 46/212 (21.7%), and there was no difference in exposure between the groups (p=0.25 and p=0.28 respectively). Of note, there was also no difference in PI exposure (p = 0.77). Kidney sizes on ultrasound were preserved and similar between both groups. However, echogenicity was greater in the TDF-unexposed group with increased echogenicity in 48/51 (94.1%) compared to 51/65 (78.5%) of the TDF-exposed patients, (p=0.02).

Table 2: Potentially contributory factors to AKI

	Total	**TDF-exposed**	**TDF-unexposed**	**p-value**
Potentially contributing factors to AKI	**(n=213)**	**(n=114)**	**(n=99)**	
Sepsis, n (%)	106/213 (49.8)	55/114 (48.3)	51/99 (51.5)	0.63
Diarrhoea, n (%)	49/213 (23.0)	25/114 (21.9)	24/99 (24.2)	0.69
Dehydration, n (%)	53/213 (24.9)	27/114 (23.7)	26/99 (26.3)	0.66
Herbal/Traditional, n (%)	4/213 (1.9)	2/114 (1.8)	2/99 (2.0)	1.00
Drug exposure				
Rifampicin, n (%)	51/212 (24.1)	31/114 (27.2)	20/98 (20.4)	0.25
Isoniazid, n (%)	46/212 (21.7)	28/114 (24.6)	18/98 (18.4)	0.28
Bactrim, n (%)	37/212 (17.5)	24/114 (21.1)	13/98 (13.3)	0.14
Penicillin, n (%)	9/212 (4.3)	4/114 (3.5)	5/98 (5.1)	0.57
Furosemide, n (%)	13/212 (6.1)	7/114 (6.1)	6/98 (6.1)	0.10
Enalapril, n (%)	16/212 (7.6)	7/114 (6.1)	9/98 (9.2)	0.40
Aminoglycoside, n (%)	5/212 (2.4)	4/114 (3.5)	1/98 (1.0)	0.23
Protease inhibitor, n (%)	5/213 (2.4)	3/114 (2.6)	2/99 (2.0)	0.77
KUB Ultrasound findings				
Increased echogenicity of the kidney, n (%)	99/116 (85.3)	51/65 (78.5)	48/51 (94.1)	0.02
Mean right kidney size(cm), mean (±SD) n = 113	11.8 (1.7)	11.9 (1.8)	11.7 (1.6)	0.45
Mean left kidney size(cm), mean (±SD) n = 117	11.6 (1.6)	11.8 (1.5)	11.4 (1.6)	0.24

(**AKI**) acute kidney injury, **(SD)** standard deviation, **(eGFR)** estimated glomerular filtration rate, (**cm**) centimeter

Table 3: Outcome

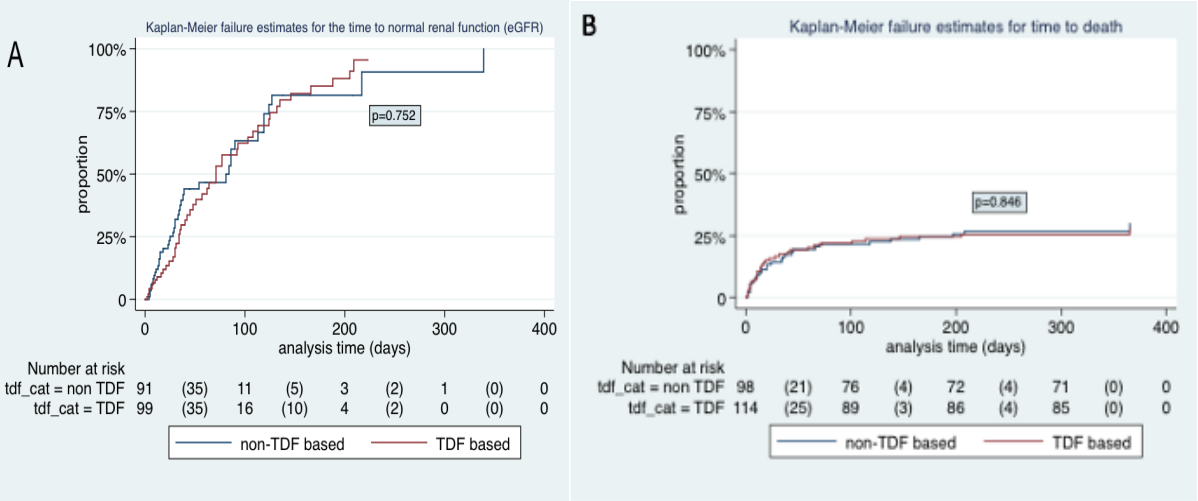
Outcomes	**Total**	**TDF-exposed**	**TDF-unexposed**	**p-value**
Last Creatinine (µmol/L), median (IQR)	84 (71-91)	84 (75.0-95.5)	83.5 (70-91)	0.49
Last eGFR (mL/min/1.73 m²), median (IQR)	100.6 (89.8 – 118.1)	100.0 (89.8 – 114.4)	104.3 (88.9 – 126.1)	0.39
Time to resolution of AKI, (days) median (IQR)		21 (7 – 62)	25 (10 – 60)	0.40
Dialysis for AKI, n (%)	96/210 (45.7)	49/111 (44.1)	47/99 (47.5)	0.63
LTFU, n (%)	33/212 (15.6)	17/114 (14.9)	16/98 (16.3)	0.78
Length of hospital stay, n (%)				0.94
0-3 days	43/213 (20.2)	23 (20.2)	20 (20.2)	
4-7 days	25/213 (11.7)	12 (10.5)	13 (13.1)	
8-14 days	53/213 (24.9)	29 (25.4)	24 (24.2)	
15-21 days	36/213 (16.9)	18 (15.8)	18 (18.2)	
>21 days	56/213 (26.3)	32 (28.1)	24 (24.2)	
Length of hospital stay, median (IQR)	13 (5-22)	13 (5-23)	12 (4-20)	0.53
CKD, n (%)	22/212 (10.4)	14/114 (12.3)	8/98 (8.2)	0.33
Death, n (%)	62/213 (29.1)	32/114 (28.1)	30/99 (30.3)	0.72

(AKI) acute kidney injury, **(IQR)** interquantile range, **(LTFU)** lost to follow up, (**CKD**) chronic kidney disease (eGFR <60)

Outcomes of enrolled patients are also demonstrated in Table 3. During hospitalization there was no difference between the two groups in the need for dialysis (p=0.63). There was a median duration of 13 days of hospitalization, calculated from the time of nephrology referral with no significant time differences between the groups. After 12 months, 22/213 (10.4%) developed CKD; 14/114 (12.3%) in the TDF-exposed group and 8/98 (8.2%), in TDF-unexposed, (p = 0.33). Unfortunately, 33/213 (15.6%) patients were lost to follow up with no significant differences between the groups, (p=0.78). The mortality was high in the total cohort: 62/213 (29.1%). Furthermore, of the 73/213 (34%) patients with no recorded baseline creatinine, 27/73(37.0%) had resolution of AKI and 9/73(12.3%) developed CKD.

Figure 2A demonstrate the Kaplan Meier estimates for resolution of AKI. The median time to AKI resolution was longer amongst TDF-unexposed patients (25 days; IQR: 10-60) compared to TDF-exposed patients (21 days; IQR: 7-62), however the difference was not significant (p-value=0.40). There were no significant differences in the time to death regardless of TDF exposure. The Kaplan Meier estimates for time to death shows that the first month had a high mortality rate in both cohorts (Figure 2B).

Figure 2: Kaplan-Meier estimates of time to resolution of AKI (A) and time to death (B)



(**TDF**) Tenofovir disoproxil fumarate, (**eGFR**) estimated glomerular filtration rate

Figure 3 shows the histological results of the kidney biopsy performed. Of the enrolled patients, 57/213 (26.8%) underwent a kidney biopsy, 34/57 (59.6%) were TDF-exposed and 23/57(40.4%) were TDF-unexposed.

Figure 3: Kidney biopsy histological diagnosis in the TDF-exposed and TDF-unexposed groups

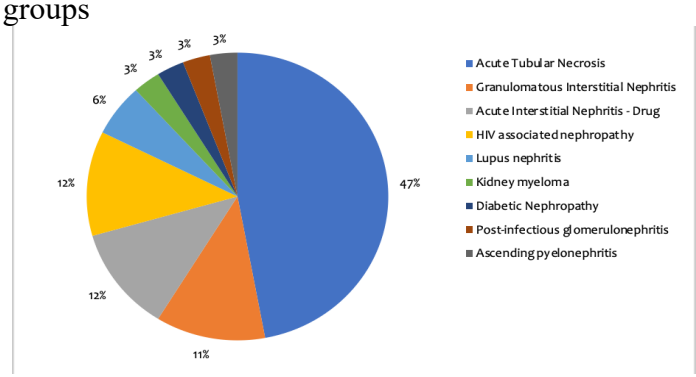


Diagram A: Primary diagnosis* in the TDF-exposed group (n=34)

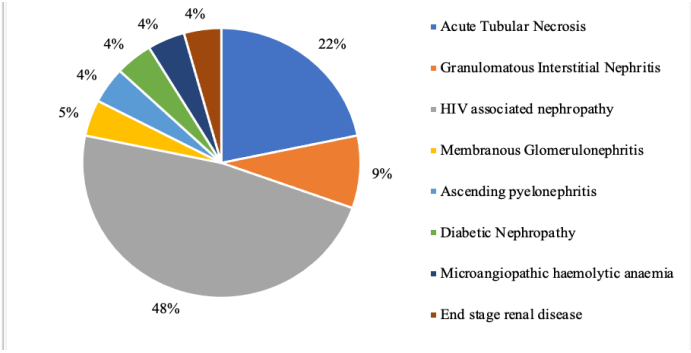


Diagram B: Primary diagnosis* in the TDF-unexposed group (n=23)

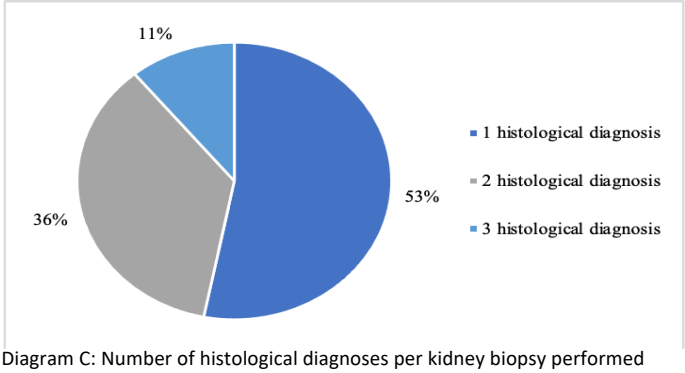


Diagram C: Number of histological diagnoses per kidney biopsy performed in TDF-exposed group on biopsy (n=64)

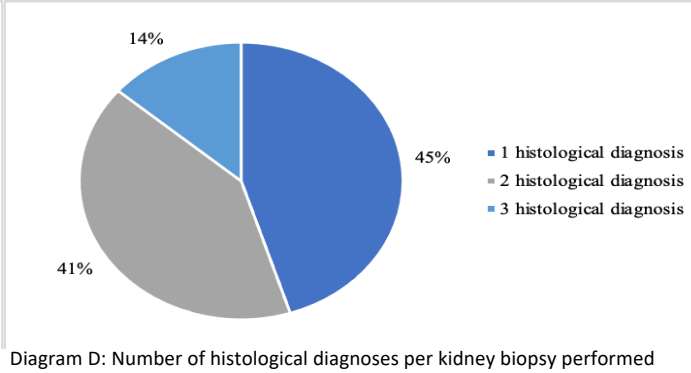


Diagram D: Number of histological diagnoses per kidney biopsy performed in TDF-unexposed group on biopsy (n=51)

*(**Primary diagnosis**) the most significant diagnosis as described by the histopathologist, (**TDF**) Tenofovir disoproxil fumarate

Figure 3 A demonstrates that 47% of patients who were TDF-exposed had a primary diagnosis of ATN. This was higher in TDF-exposed individuals (TDF-exposed: 47% versus

TDF-unexposed: 22%, p=0.05). The next commonest diagnoses were HIV-associated nephropathy (HIVAN) (12%), acute interstitial nephritis (AIN) secondary to drugs (12%) and granulomatous interstitial nephritis (GIN) (11%). In the kidney biopsies from the TDF-unexposed PLWH, the leading diagnosis was HIV associated nephropathy HIVAN (48%), followed by ATN (22%) and granulomatous interstitial nephritis (GIN) (9%).(Figure 3B) Figure 3C and D, depict the number of histological diagnoses on biopsy per person for the TDF-exposed and TDF-unexposed, respectively. For both groups, a high number of patients (46.8% for TDF-exposed and 54.9% for TDF-unexposed) had 2 to 3 diagnoses on kidney biopsy.

Discussion

This prospective, cohort study of AKI in HIV infected patients admitted to 2 tertiary referral centres adds to limited existing literature on TDF-exposure and AKI in an African in-patient setting. This study demonstrated the following: (i) at baseline the TDF-unexposed cohort had a higher prevalence of HPT and a younger mean age, (ii) the degree of AKI in the overall cohort was severe, with 45.7% requiring dialysis, (iii)TB co-infection was significant, (iv) creatinine normalised within 3 months in 75% of the patients with renal recovery irrespective of TDF exposure, (v) the aetiology of AKI was multifactorial in the majority of cases, however more ATN was seen in the setting of TDF exposure, p=0.05 (vi) the AKI event predisposed to the development of CKD (10,4%), and (vii) mortality is high at 29.1% in the overall cohort.

The 2019 UNAIDS estimates for SA reported that 7 200 000 adults (15 and older) are living with HIV. The highest is amongst men and women aged 15 to 49.(20) In keeping with SA demographic data in PLWH, the patients were young, with a median age of 37 years and there was a high prevalence of HIV/TB co-infection. Additionally, in SA, the incident TB cases in PLWH in 2014, was 179 756.(21) Furthermore, Wearne *et al*., reviewed kidney disease in Africans with HIV/TB co-infection. Kidney biopsy database at GSH between 2015 and 2017 showed multiple renal diseases with renal TB in 61%, and HIVAN, ATN, and pyelonephritis in 41, 69, and 17%, respectively.(17)

AKI was advanced at presentation with 134/213 (62.9%) classified as KDIGO 2 and 3 in those with recorded baseline creatinine on NHLS in the cohort. TDF containing ART regimens are reported to be associated with higher risk of AKI.(22) Seedat *et al*., assessed AKI in hospitalized PLWH stratified by TDF exposure and reported TDF group to have a higher median admission creatinine.(23) This however has not been consistent across studies in literature. In a systemic review and meta-analysis, TDF exposure was associated with a higher risk of AKI, albeit not significant.(22) In our cohort, there was no difference in the creatinine and eGFR at the time of nephrology referral between the TDF-exposed and TDF-unexposed groups. The median creatinine in the TDF-exposed group was 698 µmol/L (333-1150µmol/L), vs. 562 µmol/L (340-1009µmol/L) in the TDF-unexposed group, (p = 0.56). There was a high prevalence of dialysis requirement with 96/210 (45.7%) patients receiving dialysis. This is a marker of severe AKI and a reflection of how critically ill this population was at presentation.

In an out-patient setting in South Africa, Brennan *et al*., showed that 21/890 (2.4%) of the patients on TDF developed AKI. Furthermore, HIV-infected patients with AKI receiving

TDF-based ART had more severe AKI compared to non-TDF-based ART. TDF was (24)delayed renal recovery. However, the main limitation of this study was that patients were only followed up for 3 months and were well enough to be treated as out-patients.(25) Comparatively, we looked at assessing acutely unwell PLWH requiring hospital admission in a tertiary institution. A prospective, observational study from GSH showed an AKI prevalence of 3.4% in all patients admitted over a 1year period (366/10750). HIV was a risk factor in 75/366 (20.5%) patients. Of the HIV infected patients, 6.3% had recent use of TDF, which was presumed to have contributed to the AKI.(26)

Potentially contributing factors for the development of AKI were numerous. In those who were TDF-unexposed the prevalence of hypertension was higher, which may have increased their risk of AKI. Overall, the median CD4 count was 145 cells/mm^3. It was surprising that the CD4 count was similar in both groups as the majority of the TDF-unexposed PLWH were not on ART. It is likely that the acute illness contributed to the low CD4 count in those on ART. There was high exposure to multiple nephrotoxic drugs. This reflects the high disease burden and multiple comorbidities. The most common nephrotoxic agents/ agents associated with idiosyncratic reactions were anti-TB drugs. Rifampicin was used by 51/212 (24.1%), isoniazid by 46/212 (21.7%), and co-trimoxazole by 37/212 (17.5%). There was no difference in drug exposure between the TDF-exposed and unexposed groups.

Data on renal recovery post AKI and TDF exposure, is limited. PLWH with AKI had a prolonged hospital stay, this is attributed to the burden and severity of disease and diagnostic complexity in many patients. Length of stay was similar in both groups. Despite this, there was a favourable recovery with 75% of the patients with renal recovery, improving to normal kidney function within the first 3 months, regardless of aetiology. In a single-centre in the United States of America, a retrospective chart review of 62 patients in an out-patient setting showed recovery to occur in most patients, with 78.5% achieving at least 80% of the baseline kidney function and nearly half of these patients achieving 100% recovery. The majority of kidney function recovery occurred within the first 6 month.(27)

Development of CKD post TDF nephrotoxicity has been described. In our study, 22/212 (10.4%) developed CKD with TDF-exposed higher at 14/114 (12.3) albeit not significant. In data obtained from a large Cohort Study from the UK, 1173/3088 (38%) did not improve to within 5% of the baseline creatinine after discontinuation of TDF following nephrotoxicity.(28) Mocroft *et al*., reviewed participants from the international Data Collection on Adverse events of Anti-HIV Drugs study and demonstrated a small but significantly increased incidence of CKD in patients exposed to TDF suggesting cumulative toxic effects of the drug. Patients never exposed to TDF had an incidence of CKD of 0·84 per 1000 person-years of follow-up, increasing to 4·84 per 1000 person-years of follow-up in individuals with more than 6 years of TDF exposure.(7) A single-centre cohort study of PLWH in Tokyo showed that TDF use was associated with the development of CKD (OR, 1.8; 95% CI, 1.00–3.13; $p = 0.052$), and the adjusted mean loss of eGFR continuously increased during the 8-year observation period (from −3.8 mL/min/1.73 m^2 at 1 year to −9.0 mL/min/1.73 m^2 at 8 years of TDF exposure).(29)

TDF nephrotoxicity has specific histological characteristics described on kidney biopsy.(14) The most common primary histological diagnosis on biopsy was ATN in the TDF-exposed group (47%) versus TDF-unexposed (22%), p=0.05. This is in contrast to the most common primary diagnosis of HIVAN (48%) in the TDF-unexposed group.

In our study there was a high mortality rate of 29.1% recorded. Similarly, Lopes *et al*., assessed long-term risk of mortality for AKI in PLWH and found 81 patients (18.7%) died. The probability of death significantly differed among patients with AKI and without AKI during previous hospitalization. At 1, 2 and 5 years of follow-up, the cumulative probability of death of patients with AKI was 21.2, 25 and 31.3%, respectively, as compared with 10, 13.3 and 16.5% in patients without AKI (log-rank, P = 0.011). In multivariate analysis AKI was associated with increased mortality (adjusted HR 1.7, 95% CI 1.1-3; P = 0.049).(30)

The study was limited by only including patients referred to the nephrology service and thence excluded those managed in the medical department without the consultation of the nephrology teams; this likely influenced the severity of AKI that was noticed. Because we only enrolled PLWH with AKI, we could not determine the AKI prevalence in this population. Of the included patients, missing data was noted, with many patients not having a baseline creatinine recorded and this limited the certainty in establishing whether this was AKI; in such cases, other markers suggestive of AKI i.e. kidney ultrasound and resolution of AKI were used. Importantly, features of proximal tubulopathy could not be reported such as serum glucose in comparison to glycosuria. Furthermore, not all patients could have fractional excretion of phosphate calculated due to missing data. Importantly, the non-TDF PLWH were mostly not on ART which skewed the comparison to ART vs. non-ART. Lastly, during the follow up period, some patients were lost to follow up and as a result could not track their progression. Importantly, definitive diagnosis for TDF nephrotoxicity requires a kidney biopsy, this was a limiting factor as only 57/213 met the indication and proceeded to have a kidney biopsy.

Conclusion

In conclusion, this study assessed PLWH in a South African tertiary hospital setting. At presentation, the AKI was advanced with many patients requiring dialysis and a prolonged hospital stay. TB co-infection was high. There was no difference in clinical presentation and biochemical changes in patients who were TDF-exposed and TDF-unexposed. Kidney biopsy is invaluable in determining the aetiology and often demonstrates multiple diagnoses. TDF exposure was associated with more ATN on biopsy. CKD developed in 10% of the entire cohort. Lastly, AKI in HIV infected individuals in this cohort carried a high mortality rate of 29%.

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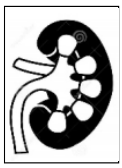

RENAL UNIT: HIVAKI study

STUDY NUMBER: ___

CONSENT TAKEN: ☐

DATE SEEN: dd / mm / yyyy **DATE FOLLOW UP (@1 month):** dd / mm / yyyy

Name: _____
DOB: dd / mm / yyyy
Age: _____ yrs Gender M F
Folder #:_____

Ward:
Phone number #1: _____
Phone number # 2:_____
Email:_____

Medical background

Cause of renal failure: Drugs ☐ Sepsis ☐ Chronic ☐ Other ☐ Specify: _____

Co-morbid illness: DM ☐ HPT ☐ Hepatitis ☐ Other: _____

Receiving Dialysis: Yes ☐ No ☐ **On Ferritin** Yes ☐ No ☐

TB: Yes ☐ No ☐ Possible ☐ **Confirmed by:** TB Blood culture ☐ _____

Sputum Gene Xpert ☐ _____ Sputum AFBS ☐ _____ Sputum TB culture ☐ _____

Urine LAM ☐ _____ with grading _____ Sputum AFBS ☐ _____ Renal biospsy ☐ attach results

ARV regimens

Initial ARV regimen (dd / mm / yyyy**)**: DDI ☐ TDF-based ☐ non-TDF-based ☐ or Specify: _____

Regimen change (dd / mm / yyyy**): :** Yes ☐ No ☐

Current regimen (dd / mm / yyyy**): :** DDI ☐ TDF-based ☐ non-TDF-based ☐ or Specify:

Nephrotoxins in last month

Rifampicin ☐ Isoniazid ☐ Bactrim ☐ Amlotericin ☐ Penicillin/Cephalosporin ☐

Furosemide/ hydrochlothiazide ☐ Enalapril ☐ Aminoglycoside ☐

Other ☐ Specify: _____

Bloods

Creatinine	GFR	CD4	VL	Eosino-phils	Phos	Ferritin	Uric acid
1. dd / mm / yyyy	1. dd / mm / yyyy						
2. dd / mm / yyyy	2. dd / mm / yyyy						
3. dd / mm / yyyy	3. dd / mm / yyyy						

Cr key: 1. Baseline Cr 2. Cr at start of HAART 3. Admission peak Cr

PTO

Urine							
Urine PO4	Urine Cr	Urine PCR	Urine culture	Urine dipstix			
				Blood	Protein	Glucose	Leuco-cytes

EXIT CODE/STATUS (tick appropriate box)			
Normal Cr & GFR ☐	Death	LTFU	Other:
Cr dd / mm / yyyy _____ GRF dd / mm / yyyy _____	DOD: dd / mm / yyyy Cause of Death:	Date last seen: @_____f/up	

FOLLOW UP

FOLLOW UP @ 1 months	Date: dd / mm / yyyy	
Cr dd / mm / yyyy _____ GRF dd / mm / yyyy _____	Requires FU: Yes ☐ No ☐ Reason: Date of FU:	Notes:

FOLLOW UP @ 3 months	Date: dd / mm / yyyy	
Cr dd / mm / yyyy _____ GRF dd / mm / yyyy _____	Requires FU: Yes ☐ No ☐ Reason: Date of FU:	Notes:

FOLLOW UP @ 6 months	Date: dd / mm / yyyy	
Cr dd / mm / yyyy _____ GRF dd / mm / yyyy _____	Requires FU: Yes ☐ No ☐ Reason: Date of FU:	Notes:

FOLLOW UP @ 9 months	Date: dd / mm / yyyy	
Cr dd / mm / yyyy _____ GRF dd / mm / yyyy _____	Requires FU: Yes ☐ No ☐ Reason: Date of FU:	Notes:

FOLLOW UP @ 12 months	Date: dd / mm / yyyy	
Cr dd / mm / yyyy _____ GRF dd / mm / yyyy _____	Requires FU: Yes ☐ No ☐ Reason: Date of FU:	Notes: