Changes in Glomerular Filtration Rate in HIV-infected Adolescents on Tenofovir

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine in the branch of Paediatrics

Declaration

I, Zoleka Josephine Ncanywa, declare that this Dissertation is my own, unaided work. It is being submitted for the Degree of Master of Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

_____

(Signature of candidate)

_____day of _____20_____in_____

Dedication

To my boys and husband, you were my pillar of strength during this journey. To my mom and my late brother ‘Ile’, thank you for always believing in me and for your prayers. We did it.

Presentations and publications arising from this research project

1. **Presentation:**

   Changes in glomerular filtration rate in HIV infected adolescents on tenofovir. Oral presentation. Wits Paediatric Research Day; 20 November 2020.

2. **Publications:**

   Nil to date.

Abstract

Introduction

From 2019, South African antiretroviral therapy (ART) guidelines for first-line therapy in adolescents over 10 years of age, weighing more than 35 kilograms, contains tenofovir (TDF) which has been reported to impact on kidney function.

Objectives

We assessed the change in estimated glomerular filtration rate (eGFR) over time in adolescents aged 10-19 years that were ever exposed to TDF at Harriet Shezi Children's Clinic, Chris Hani Baragwanath Academic Hospital, from 01 January 2010 to 31 December 2015.

Methods

The characteristics of adolescents with and without kidney dysfunction whilst on TDF-containing ART were compared. Generalised mixed methods regression models were used to identify patient characteristics associated with sustained kidney dysfunction at last follow-up visit. The incidence of sustained kidney dysfunction was estimated using survival analysis.

Results

Of the 346 adolescents receiving TDF-containing ART regimens during the study period, 117 (33.8%) met study inclusion criteria. Twenty (17.1%) developed sustained kidney dysfunction, contributing 246.0 person-years of follow-up; incidence of sustained kidney dysfunction was 8.1 per 100 person-years (95% confidence interval (CI): 5.2, 12.6). Those with sustained kidney dysfunction on TDF-containing ART had significantly (P<0.001) lower baseline eGFR compared to adolescents with normal kidney function at last visit (108.56 mL/min/1.73m^2 and 133.77

mL/min/1.73m^2, respectively). One (0.9%) adolescent required referral to the paediatric nephrology clinic for evaluation of their kidney dysfunction.

Conclusions

TDF appears safe in HIV-infected adolescents, however more studies are needed. Closer attention to monitoring of serum creatinine according to guideline recommendations is needed, particularly at TDF initiation and in older adolescents.

Acknowledgements

My supervisors were amazing, you walked with me throughout this journey. You pushed me and never gave up on me and your dedication is appreciated.

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Nomenclature

3TC	Lamivudine
ABC	Abacavir
ART	antiretroviral therapy
AZT	Zidovudine
BP	blood pressure
BMI	body mass index
CHBAH	Chris Hani Baragwanath Academic Hospital
d4T	Stavudine
ddI	Didanosine
DTG	Dolutegravir
EFV	Efavirenz
eGFR	estimated glomerular filtration rate
FDA	Food and Drug Administration
FTC	Emtricitabine
GFR	glomerular filtration rate
HIV	human immunodeficiency virus type 1
HREC	Human Research Ethics Committee
HSCC	Harriet Shezi Children's Clinic

kg	kilogram
LPV/r	Lopinavir/ritonavir
NDoH	National Department of Health
NHLS	National Health Laboratory Service
NIMART	Nurse Initiated and Management of Antiretroviral Therapy
NNRTI	nonnucleoside reverse transcriptase inhibitor
NRTI	nucleotide analogue reverse transcriptase inhibitor
NVP	Nevirapine
PI	protease inhibitor
TDF	Tenofovir
VL	viral load
WHO	World Health Organisation
Wits RHI	Wits Reproductive Health and HIV Institute

Chapter 1 INTRODUCTION

1.1 Antiretroviral Therapy (ART) for Paediatric HIV Infection

South Africa has the largest epidemic of human immunodeficiency virus type 1 (HIV) globally. It is estimated that over 8 million South Africans are HIV-infected, 360,000 of which are children under 15 years of age (1, 2). The South African Government launched a national strategy to address HIV in children and adults in 2003, with the rollout of antiretroviral therapy (ART) commencing in April 2004 (3).

As access to ART was initially relatively restricted, clinical criteria for initiating ART in children emphasised each patient's clinical and immunological status, which lead to deferred ART initiation in young children (4). Deferred access to ART frequently resulted in severe morbidity and mortality (3). By 2010, the ART rollout expanded to include all children <5 years regardless of immunologic status, and decreased the CD4 T-lymphocyte threshold for starting ART in those aged from 5-15 years to <350 cells/mm^3 (3). ART coverage for children in need increased from 13 to 54% between 2003 and 2010 (3). By 2010, the Nurse Initiated and Management of ART (NIMART) strategy was implemented to decentralise HIV treatment services and further increase the number of children on ART (3).

The implementation of ART in children has resulted in a decrease in mortality and morbidity related to HIV (5). This decline in mortality has led to HIV-infected infants and toddlers reaching adolescence and adulthood.

1.1.1 First- and Second-line ART Regimens in Children

The recommended first-line ART regimen in South Africa prior to 2019 consisted of two nucleoside reverse transcriptase inhibitors (NRTIs) and one nonnucleoside reverse

transcriptase inhibitor (NNRTI) or protease inhibitor (PI), depending on the age of the child (Table 1) (4, 6, 7).

In 2010, the South African Paediatric ART Guidelines promoted abacavir (ABC) over stavudine (d4T) in the NRTI component of first-line treatment (6). The first-line regimen in children ≥3 years of age and weighing >10 kilograms (kg) consisted of ABC, lamivudine (3TC) and efavirenz (EFV) and the adolescent group was started on tenofovir (TDF), lamivudine/emtricitabine (3TC/FTC) and efavirenz/nevirapine (EFV/NVP) (Table 1) (6). In children <10kg, or <3 years of age, lopinavir/ritonavir (LPV/r) was used, rather than EFV (7). Recommended second line ART regimens used in children or adolescents failing first-line therapy, if adherence to treatment issues had been resolved, consisted of zidovudine (AZT), didanosine (ddI) and LPV/r for persons failing ABC/3TC/EFV first-line ART (Table 1) (6). Those failing a first-line regimen consisting of AZT or ddI were changed to ABC/3TC/LPV/r (6).

The 2010 and 2015 guidelines promoted the use tenofovir disoproxil fumarate (TDF), 3TC/FTC and EFV (as a combination tablet, taken once a day) as first-line ART for adolescents ≥15 years and weighing ≥40kg (Table 1) (6, 7).

Dolutegravir (DTG), in combination with ABC and 3TC, is currently the preferred first-line regimen for children between 20kg and 35kg according to the 2019 National Department of Health (NDoH) guidelines (8). First-line therapy in individuals ≥10 years of age and weighing >35kg is a combination tablet of TDF, 3TC and DTG (Table 1) (8). TDF has therefore been used in the South African ART programme, either as part of first-line regimens in adolescents, or as part of second line therapy since 2010 (Table 1).

Table 1.1: First-line therapy in children initiating ART, and recommended second line ART options according to the South African ART guidelines

<table>
<tr><th>Line of Therapy</th><th>Guideline</th><th>Children <3 years and <10 kg</th><th>Children ≥3 years and ≥10 kg</th><th>Adolescents 10-15 years and <40 kg</th><th>Adolescents ≥15 years and ≥40 kg</th></tr>
<tr><td rowspan="5">First-line</td><td>South Africa 2004</td><td>d4T + 3TC + LPV/r</td><td>d4T + 3TC + EFV</td><td>d4T + 3TC + EFV/NVP</td><td>d4T + 3TC + EFV/NVP</td></tr>
<tr><td>South Africa 2010</td><td rowspan="3">ABC + 3TC + LPV/r</td><td colspan="2">ABC + 3TC + EFV</td><td>TDF + 3TC/FTC + EFV</td></tr>
<tr><td>South Africa 2015</td></tr>
<tr><td>South Africa 2019</td><td>If <20 kg: ABC + 3TC + LPV/r
If ≥20 kg: ABC + 3TC + DTG</td><td>If <20 kg: ABC + 3TC + LPV/r
If 20-35 kg: ABC + 3TC + DTG
If ≥35 kg: TDF + 3TC + DTG</td><td>Males: TDF + 3TC + DTG
Females of childbearing potential: TDF + 3TC/FTC + EFV</td></tr>
<tr><td rowspan="4">Second Line</td><td>South Africa 2004</td><td>AZT + ddI + NVP</td><td colspan="3">AZT + ddI + LPV/r</td></tr>
<tr><td>South Africa 2010</td><td rowspan="2">Refer to specialist</td><td>AZT + ddI + LPV/r</td><td>TDF + 3TC/FTC + LPV/r</td><td>AZT + 3TC + LPV/r</td></tr>
<tr><td>South Africa 2015</td><td colspan="3">AZT + 3TC + LPV/r</td></tr>
<tr><td>South Africa 2019</td><td>ABC or AZT + 3TC + LPV/r</td><td>If first-line regimen contained NNRTI: 2 NRTIs + DTG
or
2 NRTIs + LPV/r</td><td>If first-line regimen contained NNRTI: 2 NRTIs + DTG
or
2 NRTIs + LPV/r</td><td>If first-line regimen contained NNRTI: AZT + 3TC/FTC + DTG
or
AZT + TDF + 3TC/FTC + DTG
or
TDF + 3TC + LPR/r</td></tr>
</table>

Abbreviations: 3TC, lamivudine; ABC, abacavir; AZT, zidovudine; d4T, stavudine; ddI, didanosine; DTG, dolutegravir; EFV, efavirenz; FTC, emtricitabine; LPV/r, lopinavir/ritonavir; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NVP, nevirapine; TDF, tenofovir disoproxil fumarate.

Blocks highlighted in blue indicate situations in which recommended regimens contain TDF.

1.2 Adverse Effects of Tenofovir Disoproxil (TDF) on Kidney Function

TDF, a nucleotide analogue reverse transcriptase inhibitor (NRTI), was approved by the Food and Drug Administration (FDA) in the United States for use in adolescents from the age of 12 years in 2010, and in 2012 the age was lowered to 10 years (9). South African ART guidelines

reserve the use of TDF for children older than 10 years of age with virological failure (as part of combination second line ART), and for newly-diagnosed adolescents initiated on the first-line adult regimen which is TDF containing (8).

TDF is one of the most pharmacologically potent antiretroviral drugs, resulting in significant reductions in HIV viral load within 24 weeks of initiation of treatment (9). However, clinical trials have highlighted adverse effects of TDF on kidney function, with 17 to 22% of patients developing tubular dysfunction compared to 6 to 12% of TDF naïve children (9). Proximal tubular dysfunction is evidenced clinically by proteinuria, glycosuria and renal tubular acidosis (5). Proximal tubular toxicity precipitated by TDF-containing regimens has been shown to result in a decline in glomerular filtration rate (GFR) over time (2, 10, 11). TDF-induced nephrotoxicity is postulated to be secondary to the high concentration of mitochondria in renal tubular cells, which may be affected by certain NRTIs, and because HIV is known to compartmentalise in the kidneys (10). A systemic review by Hall et al (11) found that adult patients on TDF have small but significant declines in kidney function.

Kidney biopsies from TDF-treated patients show misshapen mitochondria, a reduced number of mitochondria, flattening of the renal tubular epithelium, and interstitial oedema in the vicinity of the proximal tubules, suggesting that mitochondria are a target of TDF toxicity (11). The relationship between subclinical proteinuria and the development of chronic kidney disease in TDF-treated patients is currently unknown. It has been suggested that excess delivery of protein to the distal nephron may have pathologic consequences (11). This has been supported by experimental evidence in rodents which showed that proteinuria had effects on the sodium channels in the collecting ducts (11). This hypothesis can be argued against when looking at hereditary forms of Fanconi's syndrome where significant proteinuria does not result in the deterioration of renal function (11). Factors that potentiate the decline

in GFR include pre-existing kidney disease, advanced HIV disease, underweight and concomitant use of TDF and protease inhibitor (PI) -containing regimens (10).

The paediatric and adolescent population is underrepresented in the current literature on the effect of TDF on kidney function compared to the adult population (12). There are studies that have determined a favourable safety profile for TDF in adults and children, whereas renal toxicity and bone density loss were reported by other studies both in adults and children (2). TDF has been noted to cause decline in renal function, hypophosphataemia and phosphaturia over time (9). It has been reported that children on TDF-containing ART have a higher rate of hypophosphataemia compared to those on non-TDF based regimens (9).

1.3 Measurement of kidney function

Measurement of the GFR is considered the best indicator of kidney function in health and disease (13, 14), and is useful in monitoring drug-induced nephrotoxicity, early chronic kidney disease, and the progression of kidney disease. Direct measurements of GFR are not easily performed in the clinical setting; therefore, formulas have been proposed to estimate GFR. In those formulas, serum creatinine is the most commonly used marker of kidney function (14). In adults, such formulas have been found to overestimate GFR, as they do not correct for body surface area; however, these formulas are still recommended in the paediatric population, albeit not having been validated in adolescent populations (14). The modified Schwartz formula (which uses the easily obtainable clinical parameters of height (in centimetres) and serum creatinine (in mg/dL) is widely used to calculate estimated GFR (eGFR) in children (15). The formula is expressed as follows:

$$eGFR = k \times \frac{Height\ (cm)}{serum\ creatinine\ (mg/dL)}$$

where k is a numerical constant that depends on muscle mass, set at 0.55 for adolescent girls, and 0.70 for adolescent boys (15) .

1.4 Summary of the Literature Review and Rationale for this Study

There is a large population of children and adolescents that are in care and receiving ART for the treatment of vertically-acquired HIV infection in South Africa. Since 2010, South African ART Guidelines have recommended TDF as part of first- or second line ART in children >10 years of age. As TDF is known to impact on kidney function in adults and current literature on the effect of TDF on the child and adolescent kidney is limited, this study was designed to explore the renal outcomes of children and adolescents enrolled in a large public sector ART service in South Africa.

1.5 Aims and Objectives

The aim of this study was to describe the demographic profile, clinical characteristics, and outcomes of adolescents on TDF-containing ART regimens treated at Harriet Shezi Children's ART Clinic (HSCC), Chris Hani Baragwanath Academic Hospital (CHBAH), Soweto, Gauteng Province.

1. To elucidate the number of children that were on TDF-containing regimens at HSCC in 2010 through 2015, their age range, and reasons for initiating TDF-containing therapy;
2. To describe the clinical, virological and immunological outcomes of children treated with TDF-containing regimens;
3. To describe the changes in kidney function (using the Schwartz formula to calculate the eGFR) in children on TDF-containing regimens attending HSCC;

4. To establish indications for referral to nephrologist in children treated with TDF-containing regimens who develop kidney dysfunction;
5. To describe the eGFR at 'end-point' in children that were treated with TDF-containing regimens in 2010 through 2015;
6. To describe ART regimen switches, and reasons for making such switches, in children on TDF-containing regimens.

Initially the study design was a case control aiming at comparing patients on TDF versus not on TDF, however protocol assessor committee highlighted the point that routine renal function is inconsistently measured in patients who are not on TDF. This would have resulted in having significantly fewer controls.

Chapter 2 METHODS

2.1 Study Design and Site

This retrospective study was conducted in Soweto, the most densely populated township in South Africa, which has an estimated population of over 1.27 million persons (16). HSCC offers services to over 7,000 HIV-infected paediatric patients, including over 1,000 adolescents (personal communication, Dr Sipambo). The clinic caters for the needs of HIV-infected outpatients from birth to >20 years of age, with the majority of its attendees aged 1 to 14 years. Paediatric patients newly diagnosed with HIV infection during an admission episode to the CHBAH paediatric wards are generally managed, subsequent to discharge from hospital, at the HSCC as outpatients if they have challenging underlying medical conditions requiring paediatric infectious diseases follow-up. Additionally, children developing virological failure while on ART at other Soweto clinics are referred to HSCC for evaluation, adherence intervention and switch to second or third line ART as necessary.

The study period under review was from 01 January 2010 through 31 December 2015. Data were retrieved, with permission, from the HSCC electronic database, which was managed by the Wits Reproductive Health and HIV Institute (Wits RHI) at the time of data extraction.

2.1.1 Inclusion and Exclusion Criteria

Inclusion Criteria

1. Adolescents 10 to 19 years of age attending HSCC during the study period were treated using TDF-containing ART regimens;
2. Adolescents on either a first- or second-line TDF-containing regimens;
3. Adolescents with available serum creatinine results were included.

Exclusion Criteria

1. Adolescents in whom a pre-TDF creatinine test result was not available;
2. Adolescents on TDF-containing ART for less than 3 months;
3. Adolescents with no available creatinine results post-initiation of TDF-containing ART.

2.2 Data Collection

Data that were collected included: age at ART initiation and at TDF-containing ART initiation; clinical, immunological and virological status at baseline and at TDF-initiation; nutritional status (weight, height and body mass index (BMI)); previous and current ART regimen. Important clinical parameters included serial creatinine results, urine dipstick and serial blood pressure readings.

Clinical, immunological and virological status were categorised using WHO classification guidelines (17). Baseline immunological status classification was done according to age (<5 years or >5 years), as either severe (<15%, <200 cells/µL absolute count), moderate (15-<25%, 201-349 cells/µL absolute count) or mild (>25%, 350-499 cells/µL absolute count). Virological classification was defined as virological suppression (<50 HIV RNA copies/mL), low-level viraemia (50-1,000 HIV RNA copies/mL) or virological failure (>1,000 HIV RNA copies/mL in 2 or more consecutive months, after previous suppression) (17, 18).

Nutritional status was categorised using WHO Growth Standards. BMI was classified as normal (18.5-24.5 kg/m^2), underweight (<18.5 kg/m^2), overweight (25-29.9 kg/m^2) and obese (≥30 kg/m^2). Height-for-age Z-scores were defined as normal (between -2 and +2 Standard Deviations (SD)), moderate stunting (between -2 and -3) and severe stunting (below -3) (19).

The eGFR was calculated using the modified Schwartz's formula (14, 15), using the *transplantr* R package (20) and was categorised according to the chronic kidney disease system (21), as follows:

1. Stage 1 kidney damage with normal or high eGFR of >90mL/min/1.73m^2;
2. Stage 2 mild reduction in eGFR (60-90mL/min/ 1.73m^2);
3. Stage 3a mild to moderate reduction in eGFR (45-59mL/min/1.73m^2);
4. Stage 3b moderate to severe reduction in eGFR (30-44mL/min/1.73m^2);
5. Stage 4 severe reduction in eGFR (15-29mL/min/1.73m^2);
6. Stage 5 kidney failure (eGFR <15mL/min/1.73m^2).

Sustained kidney dysfunction was defined as eGFR categorised in any of the above-listed kidney stages other than Stage 1 at the time of last available serum creatinine measurement. Study subject time of observation (person-time) was censored at the end of study period (31 December 2015), at date of transfer out to other clinics, at date on which subjects were lost to follow up, or date of death, whichever came first.

A folder review was conducted in order to extract study-specific indices that may not have been routinely recorded in the HSCC electronic database. Study variables obtained for the analysis are listed in Appendix 1.

2.3 Statistical Analysis

Stata software version 13.0 (StatCorp, College Station, TX) and R version 4.0.2 (23) were used for analysis. Sociodemographic, anthropometric and clinical characteristics at ART initiation, TDF-initiation and during follow-up between adolescents with and without sustained kidney dysfunction were compared using descriptive statistics: means and standard deviations (SD) were used for normally distributed data and were compared using the Student's t-test or Fisher's exact test, as appropriate, while medians and interquartile ranges (IQRs) were used

for non-normally distributed data, and were compared using the Wilcoxon rank sum test. P-values of <0.05 were considered statistically significant.

Linear mixed effects regression was used to describe inter- and intra-participant changes in kidney function over time, using the *lme4* package in R (24), with P-values derived using the *jtools* package (25). Choice of candidate variables to be considered in the linear mixed effects regression models was based on plausibility and prior knowledge. A multimodel inference approach was used to determine the regression models most likely associated with change in eGFR over time by selecting out those models with the lowest Akaike Information Criterion (AIC) (26). The candidate models are listed in Appendix 2.

Survival analysis was used to determine the incidence of renal dysfunction, using the *survival(27)*(28) *survminer* (29) and *mStats* (28) R packages. Person-time commenced at date of TDF initiation. Multivariate Cox proportional hazards modelling was done using the *M.stepwise* R package, using an iterative forwards and backwards stepwise approach (30). Adolescents contributed towards person-time at risk until the end of follow-up, or occurrence of sustained kidney dysfunction, with censoring due to death, transfer out, loss-to-follow-up or study time frame cut-off date (31 December 2015).

Advanced statistical analyses were done with the assistance of the study supervisors.

2.4 Ethical Considerations

The study was approved by the Human Research Ethics Committee of the University of Witwatersrand (clearance number M160854; Appendix 3). As this was a retrospective study, permission was granted for a waiver of informed consent. Each participate was assigned a unique study number, and identifying parameters were removed ahead of data analysis. The plagiarism report for this dissertation in included in Appendix 4.

Chapter 3 RESULTS

3.1 Baseline Characteristics of Study Participants

There were 1,734 patients between the ages of 10 and 19 years that attended HSCC between 04 January 2010 and 29 December 2015. Three hundred and forty-six (20.0%) adolescents ever received TDF. Of the 346 adolescents that had ever received TDF, 337 (97.4%) had available eGFR results (Figure 3.1). Of the 337 adolescents that had ever received TDF and who had eGFR results, 160 (47.5%) had eGFR determinations done within 12 months prior to starting TDF. Furthermore, 129 adolescents had more than one measured eGFR, and 123 had been on TDF-containing regimens for more than 3 months. Six adolescents had renal dysfunction prior to TDF initiation, and are excluded from the analysis; hence, 117 adolescents that were exposed to TDF and had baseline eGFR results were known not to have kidney dysfunction at TDF initiation. These 117 adolescents form the basis of the rest of the analyses: 97 (82.9%) had normal renal function, and 20 (17.1%) had renal dysfunction at last recorded clinic visit met the criteria for renal dysfunction during their follow up period (Figure 3.1).

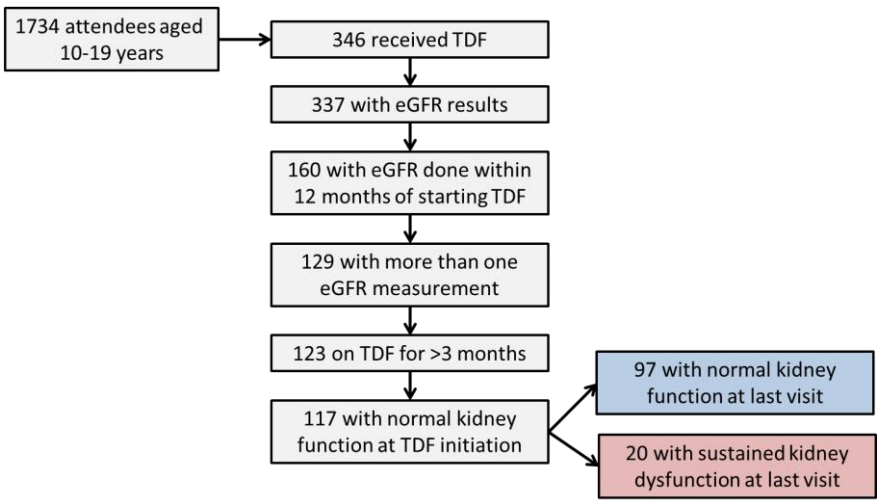


Figure 3.1: Flow of participant selection in the study

Comparisons were made between the 20 adolescents with sustained renal dysfunction on TDF-containing ART, and the 97 adolescents that did not have sustained kidney dysfunction while on TDF-containing ART. Baseline characteristics of the 117 adolescents that were included for further analysis are presented in Table 3.1.

Table 3.1: Baseline characteristics of the 117 adolescents included in this analysis

	LEVEL	NO KIDNEY DYSFUNCTION	SUSTAINED KIDNEY DYSFUNCTION	P-VALUE
N		97	20	
MEDIAN AGE (MONTHS) [IQR]		12.7 [11.6, 13.6]	13.1 [12.4, 13.6]	0.323
SEX (%)	Male	58 (59.8)	8 (40.0)	0.168
	Female	39 (40.2)	12 (60.0)	
BASELINE CD4 CATEGORY (%)	<200	11 (19.0)	1 (6.7)	0.133
	200-349	12 (20.7)	2 (13.3)	
	350-499	15 (25.9)	9 (60.0)	
	≥500	20 (34.5)	3 (20.0)	
BASELINE VL CATEGORY (%)	<50	5 (8.6)	0 (0.0)	0.725
	50-999	2 (3.4)	0 (0.0)	
	≥1000	51 (87.9)	14 (100.0)	
MEDIAN BMI Z-SCORE [IQR]		-0.5 [-1.2, 0.3]	-0.4 [-0.9, 0.5]	0.492
WHO CLINICAL STAGE AT BASELINE (%)	I	11 (16.9)	2 (16.7)	0.918
	II	16 (24.6)	2 (16.7)	
	III	24 (36.9)	6 (50.0)	
	IV	**14 (21.5)**	**2 (16.7)**	

For continuous variables (baseline BMI): all participants had available baseline BMI scores. For categorical variables, the number of participants with missing data can be inferred by comparing the sum of the available data (which are tabulated) against the total number of participants in each group.

Median age at first HSCC visit (baseline) was similar in adolescents that had sustained kidney dysfunction on TDF-containing ART and those with normal kidney function (13.1 months and 12.7 months, respectively; P=0.323). Most of the adolescents had high-level HIV viraemia at baseline, and immunological status was similar in both groups (Table 3.1). Although a higher proportion of adolescents amongst those that developed sustained kidney dysfunction on TDF-containing ART were classified at baseline as having WHO clinical stage III or IV disease (66.7% versus 58.4%), this was not statistically significant (Table 3.1).

3.2 Characteristics at TDF Initiation

At TDF initiation, the median age of the study participants was approximately 15.2 years in both groups (Table 3.2). The majority (84.5%) of patients in the normal renal function group were put on TDF as a second regimen and 89.5% in the renal dysfunction group. An unanticipated finding at this time point was that none of the adolescents had data relating to blood pressure captured, even though they were about to be initiated onto a TDF-containing ART regimen (Table 3.2). At the time of TDF initiation, most of the adolescents were on second-line or third-line ART regimens (Table 3.2).

3.3 Characteristics at Last Visit

Characteristics at last visit are presented in Table 3.3. Participants' median age was similar (17.0 years and 17.6 years in adolescents that did not develop sustained kidney dysfunction and those that did, respectively; P=0.122). All study participants were included in the study only if they had normal kidney function at baseline. Thereafter, they were stratified as to whether they had "no kidney dysfunction" or "sustained kidney dysfunction" at last clinic follow-up. "Sustained kidney dysfunction" was defined as eGFR categorised in kidney stages 2 to 5 (i.e. eGFR <60 mL/min/1.73m^2) at the time of last available serum creatinine measurement (see Section 2.3 in the dissertation). The group with sustained kidney

Table 3.2: Participant characteristics at TDF initiation

	LEVEL	NO KIDNEY DYSFUNCTION	SUSTAINED KIDNEY DYSFUNCTION	P-VALUE
N		97	19	
MEDIAN AGE (MONTHS) [IQR]		15.2 [14.1, 16.1]	15.1 [14.6, 15.4]	0.777
MEDIAN PRIOR CD4 COUNT [IQR]		566.0 [442.5, 756.2]	618.0 [498.0, 721.0]	0.790
PRIOR HIV VL CATEGORY (%)	<50	22 (52.4)	3 (60.0)	1.000
	50-999	6 (14.3)	0 (0.0)	
	≥1000	14 (33.3)	2 (40.0)	
MEDIAN BMI Z-SCORE [IQR]		-0.4 [-1.2, 0.5]	-0.7 [-0.9, 0.3]	0.976
WHO CLINICAL STAGE (%)	I	11 (11.3)	2 (10.5)	0.275
	II	4 (4.1)	0 (0.0)	
	III	38 (39.2)	9 (47.4)	
	IV	23 (23.7)	1 (5.3)	
BP CLASSIFICATION (%)	BP not measured	97 (100.0)	19 (100.0)	-
REGIMEN (%)	3TC Monotherapy	1 (1.0)	0 (0.0)	0.949
	ABC, 3TC, EFV	28 (29.2)	6 (31.6)	
	ABC, 3TC, LPV/r	2 (2.1)	0 (0.0)	
	D4T, 3TC, EFV	50 (52.1)	12 (63.2)	
	D4T, 3TC, LPV/r	1 (1.0)	0 (0.0)	
	Other	11 (11.5)	1 (5.3)	
	TDF Containing	3 (3.1)	0 (0.0)	
MEDIAN DURATION ON ART (MONTHS) [IQR]		25.0 [6.0, 58.0]	46.0 [32.5, 55.0]	0.133
FIRST REGIMEN (%)	NO	82 (84.5)	17 (89.5)	0.735
	YES	15 (15.5)	2 (10.5)	

For continuous variables (prior CD4, BMI and duration on ART): 65 (67.0%) and 14 (73.7%) of participants without and with sustained kidney dysfunction had missing prior CD4 results; all participants had available BMI and duration of ART data. See note under Table 3.1 for missing values relating to categorical variables.

Table 3.3: Characteristics at last visit

	LEVEL	NO KIDNEY DYSFUNCTION	SUSTAINED KIDNEY DYSFUNCTION	P-VALUE
N		97	20	
MEDIAN AGE (MONTHS) [IQR]		17.0 [16.2, 18.0]	17.6 [17.2, 18.3]	0.122
MEDIAN PRIOR CD4 COUNT [IQR]		503.0 [342.0, 711.0]	459.0 [176.8, 666.5]	0.489
PRIOR HIV VL CATEGORY (%)	<50	37 (38.1)	7 (35.0)	0.965
	50-999	28 (28.9)	6 (30.0)	
	≥1000	32 (33.0)	7 (35.0)	
MEDIAN BMI Z-SCORE [IQR]		-0.5 [-1.1, 0.1]	-0.4 [-1.0, 0.1]	0.635
REGIMEN (%)	3TC Monotherapy	2 (2.1)	1 (5.0)	0.272
	Other	2 (2.1)	1 (5.0)	
	TDF Containing	93 (95.9)	18 (90.0)	
MEDIAN PRIOR EGFR [IQR]		112.9 [102.7, 129.3]	83.2 [79.7, 86.7]	**<0.001**
SUMMARY EGFR OVER PERIOD OF FOLLOW-UP [IQR]		118.9 [108.4, 133.0]	92.0 [85.8, 99.4]	**<0.001**
STAGE OF KIDNEY DISEASE (%)	Stage 1	97 (100.0)	0 (0.0)	**<0.001**
	Stage 2	0 (0.0)	20 (100.0)	
BP CLASSIFICATION (%)	BP not measured	82 (84.5)	18 (90.0)	0.930
	Normal	8 (8.2)	1 (5.0)	
	Elevated	3 (3.1)	1 (5.0)	
	Stage 1 hypertension	4 (4.1)	0 (0.0)	
MEDIAN DURATION ON ART (MONTHS) [IQR]		82.0 [59.0, 111.0]	105.0 [80.2, 112.7]	0.140
MEDIAN DURATION OF TDF (MONTHS) [IQR]		18.0 [11.0, 34.0]	32.0 [17.8, 41.5]	**0.038**
PARTICIPANT STATUS (%)	Active	50 (51.5)	12 (60.0)	0.694
	Defaulted	2 (2.1)	0 (0.0)	
	Died	1 (1.0)	0 (0.0)	
	LTFU	11 (11.3)	1 (5.0)	
	TF OUT	32 (33.0)	6 (30.0)	
	UNKNOWN	1 (1.0)	1 (5.0)	

For continuous variables (prior CD4, BMI, prior and summary eGFR, duration on ART and duration on TDF): all participants had available data. See note under Table 3.1 for missing values relating to categorical variables.

dysfunction had a significantly (P<0.001) lower median prior eGFR of 83.2 mL/min/1.73m^2 (IQR, 79.7-86.7) compared to those that did not develop sustained kidney dysfunction (112.9 mL/min/1.73m^2; IQR, 102.7-129.3). We observed that 4 patients in the normal renal function and 2 in the sustained renal dysfunction group were not on TDF at the last visit. Four patients in the normal renal function group were switched from TDF due to virological failure of which 2 were put on a 3TC monotherapy holding regimen. One of the patients in the sustained renal dysfunction group was on the 3TC monotherapy holding regimen.

The median duration of ART was longer in adolescents that developed sustained kidney dysfunction compared to those that did not (105.0 versus 82.0 months), this was not statistically significant; duration of TDF-containing ART regimens was significantly (P=0.038) longer in adolescents that developed sustained kidney dysfunction, however (Table 3.3). At last clinic follow-up appointment, all of the adolescents that developed sustained kidney dysfunction were classified as having Stage 2 kidney dysfunction (i.e., eGFR 60-90 mL/min/ 1.73m^2; Table 3.3).

3.4 Adverse Outcomes on TDF-containing ART

3.4.1 Kidney disease classification summary

Of the 20 adolescents that developed kidney dysfunction on TDF-containing ART regimens, most developed Stage 2 kidney disease and only one (Subject 31) developed Stage 3a kidney disease, which recovered to Stage 2 kidney disease over time (Figure 3.2). All had normal kidney function at TDF-initiation. The median eGFR in this groups of adolescents was 90.6 mL/min/ 1.73m^2 (range 52.8 to 148.0 mL/min/ 1.73m^2) over the total period of observation.

3.4.2 Referral to the Paediatric Nephrology Clinic

One (0.9%) of the 117 patients (Subject 71) was discontinued off TDF-containing ART due to renal effects and required assessment at the Paediatric Nephrology Clinic; her clinical course

is summarised in Table 3.4. She was 13.3 years at the first HSCC visit during the study period, and had been on ART for 33 months. She had normal kidney function at TDF initiation, aged 15.2 years, but developed proteinuria attributed to a tubulopathy which necessitated discontinuation of the TDF-containing regimen. The lowest eGFR which she attained was 64.6 mL/min/ 1.73m^2, which occurred at 18 months on TDF-containing ART. At last clinic follow-up appointment (aged 19.0 years) her eGFR was 86.5 mL/min/ 1.73m^2 (Table 3.4).

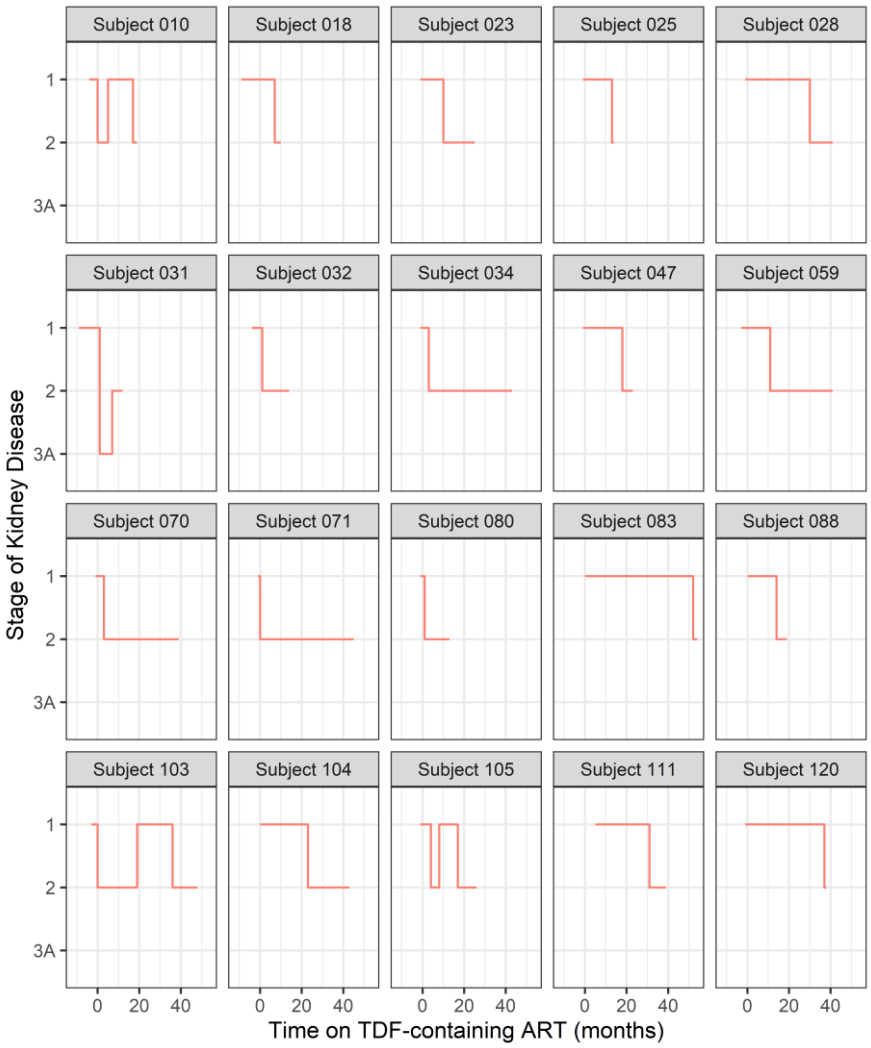


Figure 3.2: Kidney function over time in adolescents that had sustained kidney dysfunction on TDF-containing ART (n=20)

Table 3.4: Characteristics of Subject 71, who was referred to the renal clinic

	BASELINE	FIRST STUDY VISIT	TDF INITIATION	LAST STUDY VISIT
WHO STAGE	IV			
AGE (YEARS)	10.5	13.3	15.2	19.0
LOG_{10} VIRAL LOAD	6.32	-	4.04	4.31
ABSOLUTE CD4 COUNT	207	295	-	-
BMI Z-SCORE	-	-0.85	-0.99	-0.98
REGIMEN	-	ABC/3TC/ EFV	TDF-containing	AZT/3TC/LPV/r
DURATION ON ART (MONTHS)	0	33	56	102
EGFR	-	-	123.4	86.5
STAGE OF KIDNEY DISEASE	-	-	1	2

3.5 Linear Mixed Effects Regression Analysis of Change in eGFR over Time

Trends in eGFR over time for each of the 117 adolescents included in the analysis are graphically depicted in Appendix 5 (for children without sustained kidney dysfunction during the course of TDF treatment) and Appendix 6 (for those with sustained kidney dysfunction). Univariate linear mixed effects analysis of independent factors associated with change in eGFR over time are shown in Table 3.5. Time on ART, time on TDF, the interaction between time on TDF and baseline HIV viral load, and increasing age were significantly associated with declining eGFR in study participants (Table 3.5).

Independent characteristics with a P-value of <0.2 were considered for inclusion in the multivariate linear mixed effects regression model. The multivariate model which best described the data (Model 9; Appendix 2), was described by the following equation:

$$y_{ij} = \beta_0 + \beta_1(sustained\ kidney\ disease) + \beta_2(sex) + \beta_3(age\ months) + \beta_4(duration\ on\ ART)(baseline\ log_{10}\ VL) + \beta_5(time\ on\ TDF) + \beta_6(BMI) + \beta_7(baseline\ CD4) + e_{ij}^*$$

Table 3.5: Univariate and interaction term linear mixed effects regression analysis

	ESTIMATE	95% CI	P-VALUE
SUSTAINED KIDNEY DYSFUNCTION	-27.30	-33.87, -20.73	**<0.001**
SEX (FEMALE)	-6.83	-13.96, 0.30	0.060
AGE (MONTHS)	-0.34	-0.46, -0.22	**<0.001**
DURATION OF ART (MONTHS)	-0.19	-0.29, -0.09	**<0.001**
TIME ON TDF (MONTHS)	-0.25	-0.41, -0.09	**0.001**
BMI Z-SCORE	1.54	-0.58, 3.66	0.155
BASELINE ABSOLUTE CD4 COUNT	0.01	-0.01, 0.03	0.608
BASELINE LOG_{10} HIV VIRAL LOAD (VL)	-0.54	-4.21, 3.13	0.775
INTERACTION TERM 1			
BASELINE ABSOLUTE CD4 COUNT	0.02	-0.04, 0.08	0.625
BASELINE LOG_{10} HIV VL	0.04	-7.06, 7.14	0.991
BASELINE ABSOLUTE CD4 × BASELINE LOG_{10} VL	0.00	-0.02, 0.02	0.768
INTERACTION TERM 2			
TIME ON TDF (MONTHS)	0.57	-0.08, 1.22	0.080
BASELINE LOG_{10} HIV VL	1.01	-3.01, 5.03	0.623
TIME ON TDF × BASELINE LOG_{10} VL	-0.18	-0.32, -0.04	**0.012**
PRIOR CD4 COUNT	0.00	0.00, 0.00	0.684
PRIOR LOG_{10} VL	3.56	1.58, 5.54	**<0.001**
INTERACTION TERM 3			
PRIOR CD4	0.00	-0.02, 0.02	0.697
PRIOR LOG_{10} VL	2.04	-1.61, 5.69	0.271
PRIOR CD4 × PRIOR LOG_{10} VL	0.01	0.01, 0.01	**0.039**

where y_{ij} is the change in eGFR over time, and β_n are the coefficients associated with each of the included parameters. A summary of the multi-model comparison which illustrates that Model 9 has the lowest Akaike Information Criterion (AIC) and is therefore best suited to describe the available data, is presented in Appendix 2. Model 9 coefficients are detailed in Table 3.6, below as well as in Figure 3.3. In summary, the baseline eGFR was 133.77 mL/min/1.73m^2 in adolescents that did not develop sustained kidney dysfunction on TDF-containing ART, compared to a baseline eGFR of 108.56 mL/min/1.73m^2 (i.e. 133.77 – 25.21 mL/min/1.73m^2) in adolescents with sustained kidney dysfunction; P<0.001 (Table 3.6).

Table 3.6: Model 9 coefficients describing the change in eGFR over time in adolescents with and without sustained kidney dysfunction on TDF-containing ART

VARIABLE	ESTIMATE	95% CI	P-VALUE
(INTERCEPT)	133.77	79.87, 187.67	**<0.001**
SUSTAINED KIDNEY DYSFUNCTION (YES)	-25.21	-34.39, -16.03	**<0.001**
SEX (FEMALE)	-1.67	-10.47, 7.13	0.706
AGE (MONTHS)	-0.38	-0.61, -0.14	**0.002**
DURATION ON ART (MONTHS)	0.82	0.22, 1.42	**0.007**
BASELINE LOG_{10} HIV VL	11.92	3.75, 20.09	**0.004**
TIME ON TDF (MONTHS)	0.06	-0.21, 0.34	0.641
BMI Z-SCORE	-0.70	-3.67, 2.27	0.637
BASELINE ABSOLUTE CD4 COUNT	0.01	-0.01, 0.03	0.259
INTERACTION TERM			
DURATION ON ART × BASELINE LOG_{10} HIV VL	-0.18	-0.29, -0.06	**0.003**

Sex, time on TDF, BMI Z-score and baseline CD4 count did not impact on eGFR trends over time in this Model; however, increasing age (decline in eGFR by 0.38 mL/min/1.73m^2 with each additional month of age), and the interaction between duration on ART (in months) and baseline HIV VL (decline in eGFR by 0.18 mL/min/1.73m^2 for each additional month on ART) were associated with significant declines in the eGFR over time in both study groups (i.e. those with and without renal dysfunction at last visit).

Model 9 parameters are depicted graphically in Figure 3.3. Each panel of the plot depicts one of the patient characteristics that were included in Model 9 and shows how that parameter was affected holding each of the other parameters constant in the Model. These plots help to contextualise the model coefficients shown in Table 3.6: sustained kidney disease, age in months, and the interaction terms describing duration on ART (in months) and baseline HIV VL describe steep (i.e. statistically significant) slope gradients of the change in eGFR in response to the variables included in Model 9.

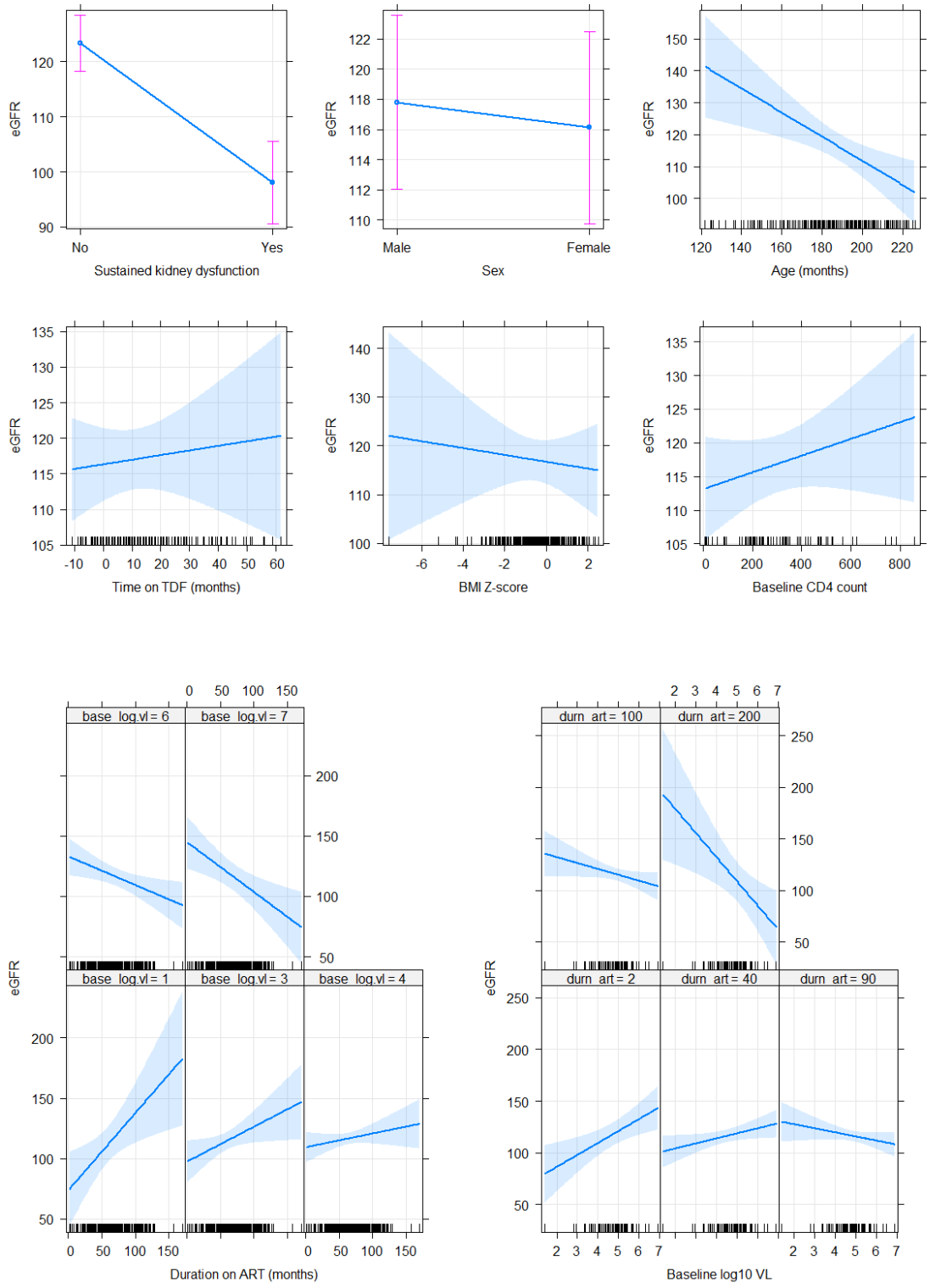


Figure 3.3: Model 9 predictor effects plots of the effect of each of the model parameters on eGFR

The last two plots illustrate the interactions between duration on ART (durn_art) and baseline $\log_{10}$ viral load (base_log.vl) in lattice plot layout. Each panel in these plots show how eGFR changed in response to length of time on ART and the patient's HIV viral load at baseline.

Of note in the interaction between baseline HIV VL and duration on ART, adolescents with the highest baseline HIV VL levels (log_{10} 6 and log_{10} 7) had declining eGFR with each additional month on ART, whereas adolescents with baseline HIV VL of log_{10} 3 or less had increasing eGFR measurements as duration on ART increased (lower left panel in Figure 3.3).

3.6 Survival Analysis

For all survival analyses, the start of observation time (T_0) was commencement of a TDF-containing ART regimen.

3.6.1 Incidence of sustained kidney dysfunction on TDF-containing ART

Overall, there were 246.0 person-years observed in the 117 patients from initiation of TDF-containing ART to last visit. The incidence of sustained kidney disease amongst the 117 adolescents was 8.1 (95% CI, 5.2-12.6) per 100 person-years of follow-up.

3.6.2 Cox proportional hazards analysis

In univariate analysis, the only patient characteristic that was associated with development of sustained kidney dysfunction was receipt of 3TC-containing ART (hazard ratio (HR) 2.53; 95% CI, 1.04, 6.18; P=0.041) (Table 3.7).

In a final multivariate model that was constructed using iterative forwards and backwards selection, receipt of 3TC was associated with the development of sustained kidney dysfunction on TDF-containing ART; Table 3.8.

Table 3.7: Univariate Cox proportional hazards analysis of patient characteristics potentially associated with kidney dysfunction at last follow-up visit

	HR	95% CI	P-VALUE
SEX (MALE)	0.44	0.18, 1.07	0.070
RECEIVED LOPINAVIR-RITONAVIR	1.47	0.49, 4.46	0.494
RECEIVED LAMIVUDINE	2.53	1.04, 6.18	**0.041**
RECEIVED ZIDOVUDINE	1.34	0.31, 5.83	0.701
RECEIVED EFAVIRENZ	0.72	0.21, 2.52	0.608
WHO STAGE I	0.51	0.12, 2.20	0.363
BASELINE CD4 CATEGORY <200	0.54	0.07, 4.07	0.552
BASELINE HIV VL <50	0.00	0.00, INF	0.997
PRIOR HIV VL <50	1.59	0.63, 4.03	0.331
ON FIRST ART REGIMEN	1.57	0.36, 6.86	0.552
NON-TDF CONTAINING REGIMEN AT LAST VISIT	0.85	0.19, 3.70	0.823
ON 3-DRUG ART REGIMEN AT LAST VISIT	1.17	0.27, 5.11	0.832
ON TDF AND PI-CONTAINING REGIMEN AT LAST VISIT	1.36	0.49, 3.76	0.559

Table 3.8: Final Stepwise Model

	ADJUSTED HR	95% CI	P-VALUE
SEX (MALE)	0.42	0.17, 1.03	0.059
RECEIVED LAMIVUDINE	**3.67**	**1.35, 9.98**	**0.011**
ON TDF AND PI-CONTAINING REGIMEN AT LAST VISIT	2.49	0.81, 7.65	0.111

Likelihood ratio test: 9.94 on 3 degrees of freedom, P=0.020; Wald test = 9.76 on 3 degrees of freedom, P=0.020; Score (logrank) test = 10.30 on 3 degrees of freedom, P=0.020. Chosen significance levels for entry and retention into the final model was set as a P-value of ≤0.15.

3.6.3 Kaplan-Meier survival plots

Kaplan-Meier plots for the study participants (combined and stratified by lamivudine exposure) are presented in Figure 3.4. The overall median time of observation for all 117 adolescents included in the study was 1655 days (95% CI, 1337 to >2000 days) after commencement of TDF-containing ART. Median time to development of sustained kidney

dysfunction was 994 days (95% CI, 590 to 1316 days) after commencement of TDF-containing ART in those who developed sustained kidney dysfunction.

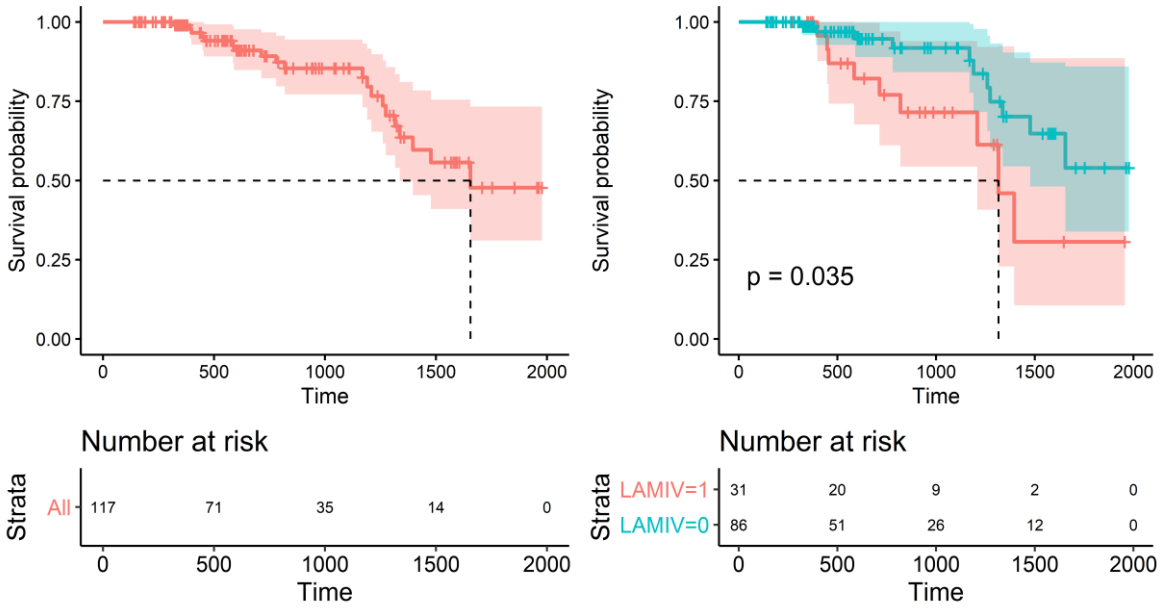


Figure 3.4: Kaplan-Meier survival curves for the study cohort overall (left), and stratified by receipt of 3TC-containing ART regimens (right)

Chapter 4 DISCUSSION

4.1 Key findings from this Study

This study presents an analysis of eGFR trends in adolescents attending a large hospital-based paediatric and adolescent ART clinic in South Africa, and suggests that TDF is safe to use in adolescents with 17.1% (20 of 117) developing sustained renal dysfunction and 0.9% (1 of 117) patients with documented normal kidney function at the TDF-initiation visit requiring discontinuation of TDF due to renal adverse effects. The incidence of sustained kidney dysfunction in adolescents that received TDF-containing ART was 8.1 (95% CI 5.2-12.6) per 100 person-years. The size of the sample (117 patients) included in this study is comparable to other published studies conducted among adolescents on TDF, where sample sizes ranged from 26 to 198 patients (31-34). The estimated eGFR at baseline was 133.77 mL/min/1.73m² in adolescents without sustained kidney dysfunction, and 108.56 mL/min/1.73m² in those that developed sustained kidney dysfunction; P<0.001.

The most important finding of the analysis was that kidney function, as evaluated through serial measurement of eGFR over time, declined with increasing age and the interaction between duration of ART and baseline HIV VL, regardless of whether they developed sustained kidney dysfunction on TDF-containing ART or not. We established that patients with high viral load at baseline and longer duration of treatment, developed significantly lower kidney function indices (as measured by eGFR) compared to children with lower HIV VL at baseline (Table 3.6 and Figure 3.3). The majority (84.5%) of patients in the normal renal function group were put on TDF as a second regimen and 89.5% in the renal dysfunction group (Table 3.2). At first study visit and at the time of TDF initiation, there were no significant differences in clinical characteristics. Median age at TDF-initiation was approximately 15.2 years. Of concern was that blood pressure measurements were poorly captured at the time of TDF initiation and at

follow-up on TDF-containing ART (Tables 3.2 and 3.3). Elevated blood pressure is a sign of renal deterioration and therefore has clinical significance. There is a need to strengthen routine blood pressure monitoring in adolescent patients on TDF-containing ART regimens in our setting. Urine analysis using a dipstick shows early signs of renal toxicity and has clinical significance; however in this study population there was inconsistent documentation of urine dipstick results, so dipstick parameters did not form part of the analysis.

4.1.1 Findings in the context of current South African ART guidelines

Serial measurement of creatinine levels is advocated in patients on TDF-containing ART regimens. Literature has highlighted that TDF adverse effects include renal toxicity and therefore patients who are receiving it should be monitored using eGFR for renal deterioration (11). The current South African ART guidelines recommends that kidney function should be evaluated using serum creatinine levels at baseline, 3 months, 6 months and 12 months in patients on TDF-containing ART, with an annual repeat test to assess trends in eGFR (8). Of all 346 adolescents that received TDF-containing ART, only 117 (33.8%) had baseline and follow-up creatinine results and were thus eligible for inclusion in the current analysis. This indicates that monitoring of serum creatinine in TDF-exposed adolescents needs to be strengthened in our setting.

One in five adolescents had severe immunodeficiency at baseline, and almost half of the patients had virological failure at TDF initiation. These findings could reflect the historical aspects of paediatric ART management over time, which was guided by serially updated South African ART management policies. The South African ART roll-out programme commenced in 2004, with focus on treating children based on immunological or clinical stage of disease and a “wait and watch” approach for children with less advanced HIV infection. At that time, older children were presenting for ART, at a greater degree of immunosuppression and the first-line

ART regimen of choice consisted of stavudine, lamivudine and efavirenz (in children >10 kg and >3 years of age) (3, 4, 6). As TDF-containing regimens were recommended as part of second-line ART in adolescents that were failing ART, it is expected that a high proportion of adolescents had virologic failure at TDF initiation.

4.1.2 Study findings in the context of previous studies in adolescents and adults

There are limited published studies which have evaluated trends in kidney function over time in response to TDF in the adolescent population as compared to adults. A previous study which evaluated the outcomes of children that started ART at Harriet Shezi Children's Clinic (HSCC) gives a good indication of patient characteristics in the first four years after the governmental ART roll-out, from 2004 through 2008 (35). At that time, the median age at ART initiation was 4.3 years (IQR, 1.6-7.5 years), and 78% of those starting ART had advanced disease (WHO clinical stage III and IV). Outcomes on ART included: a doubling of the mean CD4 of percentage, from 12.7% to 25.1%, in 12 months; excellent weight gain from -2.4 to -1.4 Z-scores; and viral suppression rates of 59.4% in the first 6 months, which improved to 96.2% by 24 months (35). In a South African multicentre study of 6,078 paediatric patients, there were substantial improvements in immunological status and HIV viraemia in response to ART, which was most pronounced in the first 12 months on treatment (36). In contrast to previous descriptions of favourable clinical responses to ART in the afore-mentioned studies, the subset of children and adolescents included in the current study reflect a complicated subset of HSCC attendees, with immunological suppression (median CD4 absolute 566 and 618 cells/µL in adolescents without kidney dysfunction and those with sustained kidney dysfunction on TDF-containing ART, respectively) and virological failure (33% and 40% in children without kidney dysfunction and those with sustained kidney dysfunction on TDF-containing ART, respectively) at the time

of TDF initiation; Table 3.2. This could be explained by the fact that HSCC is a referral centre and manages complicated patients.

In a South African adult study, there was a small but significant decline of eGFR (categorised by drop to <90 mL/min/m²) over time in the TDF-exposed group (37). The increased risk of kidney dysfunction was most marked in the first 6 months of TDF-containing ART in the older age group, those that were severely immunodeficient and those that were underweight. In our study we found that time on TDF, poor nutritional status and severe immunodeficiency were not significantly associated with changes in eGFR in the multivariate linear mixed effects regression model (Table 3.6). Protease inhibitor and TDF-containing regimens were associated with and increased risk of kidney dysfunction when duration of therapy was more than 6 months in adult patients, in studies from Zambia and South Africa (37, 38). In univariate survival analysis, coadministration of TDF and protease inhibitors was not associated with sustained kidney dysfunction in our study (Table 3.7).

Current literature which evaluates eGFR trends in patients on TDF-containing ART regimens focuses predominantly on adults (39), and most describe outcomes in patients from the developed world (2) . Previous studies done in children and adolescents included less than 200 participants, and mostly evaluated eGFR trends over time periods <60 months (2, 31), although one study followed patients up for a period of 132 months (40). Of four paediatric studies which included fewer than 100 participants (2, 33, 40, 41), one study found a significant decline in eGFR after 2 years of treatment (2). That study analysed data from 49 patients with a median age of 13.6 years at TDF initiation, half of which were virologically suppressed and 39% of which had advanced disease (2). Judd et al. (31) who evaluated 131 participants, with a median time on TDF of 2.1 years, did not demonstrate significant differences in kidney function before and after TDF exposure in their study. A cross sectional

paediatric study from Zimbabwe demonstrated that 35.9% of children on TDF-containing ART developed an eGFR of <90mL/min/1.73m² (34). Children on TDF-containing ART for >60 months had a 3.5-fold greater risk of declining eGFR than those not exposed to TDF. Advanced disease and stunting, and co-treatment with protease Inhibitors, were associated with a decrease in eGFR (34).

In the cohort of patients presented in this study, median age at TDF initiation (15.2 years) was similar to other adolescent studies in which median age at TDF-initiation ranged from 10.1 years to 17.3 years (2, 32, 33, 40). A cross-sectional study of 198 adolescent patients from Zimbabwe reported a prevalence of malnutrition (underweight or severely underweight for age) of 12.6% at ART initiation (34). In our cohort, median BMI Z-score was normal (-0.4; IQR, -0.9, 0.5) at baseline in patients who developed sustained renal dysfunction and remained normal (-0.4; IQR, -1.0, 0.1) at last visit (Tables 3.1 and 3.3). Patients presented in the current study had a similar prevalence of advanced disease classified as WHO Stage III or IV (39.3%) at first visit (Table 3.1) compared to study participants from other African countries and developed world cohorts (22%-39% with WHO III or IV disease) (2, 32, 34). At the time of TDF-initiation in the group that developed sustained renal dysfunction, the median CD4 count of 618 cells/µL (IQR, 498-721) was similar to those observed in other studies where mean CD4 count was >500 cells/µL (565, 763, 796 and 847 cells/µL respectively) (2, 40, 41). Between 50% and 60% of the participants in both groups initiated onto TDF-containing ART in the current study were virologically suppressed at the time of commencement of TDF, which was similar to other studies, in which 55%-100% were virologically suppressed (2, 40, 41).

Multivariate linear mixed effects regression model outputs from this study indicate that the association between ART duration and baseline viral load and increasing age are independently associated with declining eGFR in TDF-exposed patients, regardless of whether

they developed sustained kidney dysfunction or not. A study of eGFR in living kidney donors confirms that eGFR declines with advancing age, a reductions in eGFR of (6.6 – 7.7 mL/min/1.73 m^2) per decade (42); hence, there is a well-described physiological decline in eGFR with advancing age over time.

There was an association with development of sustained kidney dysfunction in patients receiving 3TC-containing ART (hazard ratio (HR) 2.53; 95% CI, 1.04, 6.18; P=0.041) (Table 3.7). This association has not been described in other studies, and warrants further exploration in future studies. A possible explanation for this finding is that children and adolescents on 3TC-monotherapy as a holding regimen who frequently develop worsening virologic and immunologic control of their HIV infection, may develop renal dysfunction secondary to sustained viraemia and immunoparesis.

Increasing time on ART was independently associated with increasing eGFR (0.82 mL/min/1.73 m^2; 95% CI, 0.22 to 1.42) per month on ART in the current analysis. However, when taking the interaction between baseline HIV VL into account, duration on ART was associated with significant declines in eGFR (Table 3.6 and Figure 3.2). Patients with high viral load at baseline and a longer duration of treatment had a significantly low eGFR. In this interaction, there was a 0.18 mL/min/1.73m^2 (95% CI, -0.29 to -0.06) reduction in eGFR with each additional month on ART, which was most pronounced in adolescents who had a higher baseline HIV VL. Intuitively, it is not surprising that patients who had higher HIV VL levels at baseline should develop kidney dysfunction compared to those whose HIV VL was lower at baseline (Figure 3.3): HIV has been shown to be tropic to renal tissues (43), which suggests that higher baseline HIV VL could impact more substantially on outcome kidney function. Significant reductions of eGFR related to length of time on ART, controlling for age as was done in the current analyses,

has also been described previously, with 3 to 6 mL/min/1.73m^2 per year decline in eGFR being observed in some cohorts (2, 41, 44).

A systemic review of 31 studies that cumulatively evaluated over 100,000 adults attending ART Clinics in Africa highlighted that in 15 (48.4%) of the studies, TDF was not associated with significant reductions in renal function (39). In the 16 studies that reported reduced renal function in patients on TDF-containing regimens, risk factors for renal dysfunction on TDF included impaired renal function at baseline, older age, and female sex (39). One study included in the review suggested that renal dysfunction was more likely to occur during the first year on TDF-containing ART (38). The finding that baseline kidney function is an important predictor of development of kidney dysfunction in response to TDF-exposure is corroborated by our study, in which adolescents who developed sustained kidney dysfunction – but who had normal kidney function at TDF-initiation – had significantly lower eGFR at TDF initiation compared to the group of adolescents that did not develop sustained kidney dysfunction (Table 3.6). This speaks to the importance of obtaining baseline creatinine levels, and determination of eGFR, prior to initiation of TDF-containing ART regimens, and to follow a rigorous approach, as outlined by the current ART guidelines, in terms of monitoring creatinine levels serially, especially in adolescents with borderline kidney function at baseline.

Complication rates were extremely low in the cohort of adolescents described in this study, with 1 (0.9%) of 117 TDF-exposed adolescents requiring referral to Nephrology Services and discontinuation of her TDF-containing regimen. This finding suggests that TDF-containing regimens can safely be used in adolescent HIV-infected patients attending public sector ART clinics in South Africa, despite having some level of sustained kidney dysfunction. Current ART Guidelines recommend the use of TDF-containing ART as first-line therapy in children >10 years and weighing ≥35kg. In children between 10 years and 16 years, the acceptable eGFR is

>80 mL/min/1.73m² (calculated using the Counahan Barratt formula) (8). For adolescents >16 years, an eGFR of >50 mL/min/1.73m² is acceptable (using the MDRD equation). It is noteworthy that in all 20 adolescents that developed sustained kidney dysfunction while on TDF-containing ART in our study, all had Stage 2 kidney disease (i.e. eGFR 60-90mL/min/ 1.73m^2) at last clinic assessment. Monitoring of the renal function is to be done at 3 months, 6 months, 12 months and yearly thereafter according to current Guidelines (8).

4.2 Study Strengths and Limitations

This study represents a comparable sample size of the effects of TDF-containing ART regimens on child and adolescent kidney function as compared to other published studies. HSCC down-referred patients who were between the age of 5 years and 9 years who were stable on ART (virological suppression and mild immunodeficient) which limited the population of adolescents that could be included in this analysis. Two-thirds of the patients receiving TDF did not form part of the analysis due to missing eGFR determinations, which unfortunately limited the size of the study cohort further. Patients that were virologically suppressed and with less severe immunodeficiency on TDF-containing ART were often down-referred in order to decentralise treatment, and long-term outcomes of such patients could not be evaluated. The indication for changing to TDF-containing ART was not always apparent from the clinical notes, however most of the patients had virological failure and therefore the switch may have been due to a need to switch to second-line ART to achieve virologic suppression. A reduction in eGFR is a late sign of kidney dysfunction related to TDF (11). Early signs, such as proteinuria on dipstick check, were not documented routinely during clinic visits, and the prevalence of this finding in children and adolescents receiving TDF could not be evaluated.

Despite these limitations, the study objectives were met. We used robust analytic techniques to evaluate changes in eGFR over time and were able to control the analytic

outcomes using clinical parameters that were easily obtainable and plausibly associated with changes in eGFR over time.

4.3 Conclusion

The study demonstrated that the incidence rate of sustained kidney dysfunction in adolescents on TDF-containing ART was 8.1 (95% CI, 5.2-12.6) per 100 person-years of follow-up. TDF appears safe to use in paediatric and adolescent patients when adhering to recommended dosing regimens. Our data suggests that patients that are older, and those with lower eGFR prior to TDF-initiation, should be closely observed for renal deterioration. Similarly, patients who have been on ART for a longer duration of time, particularly those with higher baseline HIV VL, should be observed closely, as they are at increased risk of renal dysfunction. In patients on TDF-containing ART, blood pressure and urine dipstick analysis should be incorporated as part of the routine clinical evaluation at outpatient visits.

In the current South African ART Guidelines, TDF is part of first-line drug regimen (8). This will increase the number of children and adolescents receiving this drug. Patients should be followed up closely according to Guideline recommendations. Although kidney function would be expected to be stable, based on the findings of the current study, should kidney function deteriorate on TDF-containing ART, renal subspecialist input and consideration of the use of other antiretroviral agents should be considered.

APPENDIX 1: DATA COLLECTION SHEET

Form 1: Baseline Characteristics

DEMOGRAPHICS	
Age	
Gender	
Race	
At ART initiation	
Date of visit	
Weight (kg)	
Height (cm)	
Date of ART initiation	
1st Regimen	
WHO clinical staging	
Viral load (including 6 months prior to initiation)	
CD4 (including 6 months prior to initiation)	
Blood Pressure	
Urine dipstick:	
- Blood	
- Protein	
- Glucose	
- Nitrite	
At TDF initiation	
Date of visit	
Weight (kg)	
Height (cm)	
WHO clinical staging	
Viral load (at least 12 months prior to TDF initiation)	
CD4 (at least 12 months prior to TDF initiation)	
Urea (at least 6 months prior to initiation)	
Creatinine (at least 6 months prior to initiation)	
Urine dipstick:	
- Blood	
- Protein	
- Glucose	
- Nitrite	
Abbreviations: ART, antiretroviral therapy; TDF, tenofovir; WHO, World Health Organization	

Form 2: Follow-up Visit Characteristics

Visit Number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Etc.**
Date of visit											
Weight (kg)											
Height (cm)											
Blood Pressure											
Viral load											
CD4											
ART Regimen											
Urea											
Creatinine											
Urine dipstick											
- Blood											
- Protein											
- Glucose											
- Nitrite											
Age at referral for Renal consult											
Age of renal intervention											
Age at renal outcome											
Last visit date											
Last visit status											
Date stopped TDF											
Abbreviations: ART, antiretroviral therapy; TDF, tenofovir											

APPENDIX 2: CANDIDATE MODELS USED IN THE LINEAR MIXED EFFECTS REGRESSION ANALYSIS

$y_{ij} = 1 + e_{ij}^*$	(Model 0)
$y_{ij} = \beta_0 + \beta_1(sustained\ kidney\ disease) + e_{ij}^*$	(Model 1)
$y_{ij} = \beta_0 + \beta_1(sustained\ kidney\ disease) + \beta_2(sex) + e_{ij}^*$	(Model 2)
$y_{ij} = \beta_0 + \beta_1(sustained\ kidney\ disease) + \beta_2(sex) + \beta_3(age\ months) + e_{ij}^*$	(Model 3)
$y_{ij} = \beta_0 + \beta_1(sustained\ kidney\ disease) + \beta_2(sex) + \beta_3(age\ months) + \beta_4(duration\ on\ ART) + e_{ij}^*$	(Model 4)
$y_{ij} = \beta_0 + \beta_1(sustained\ kidney\ disease) + \beta_2(sex) + \beta_3(age\ months) + \beta_4(duration\ on\ ART) + \beta_5(time\ on\ TDF) + e_{ij}^*$	(Model 5)
$y_{ij} = \beta_0 + \beta_1(sustained\ kidney\ disease) + \beta_2(sex) + \beta_3(age\ months) + \beta_4(duration\ on\ ART) + \beta_5(time\ on\ TDF) + \beta_6(BMI) + e_{ij}^*$	(Model 6)
$y_{ij} = \beta_0 + \beta_1(sustained\ kidney\ disease) + \beta_2(sex) + \beta_3(age\ months) + \beta_4(duration\ on\ ART) + \beta_5(time\ on\ TDF) + \beta_6(BMI) + \beta_7(baseline\ CD4) + e_{ij}^*$	(Model 7)
$y_{ij} = \beta_0 + \beta_1(sustained\ kidney\ disease) + \beta_2(sex) + \beta_3(age\ months) + \beta_4(duration\ on\ ART) + \beta_5(time\ on\ TDF) + \beta_6(BMI) + \beta_7(baseline\ CD4) + \beta_8(baseline\ log_{10}\ VL) + e_{ij}^*$	(Model 8)
$y_{ij} = \beta_0 + \beta_1(sustained\ kidney\ disease) + \beta_2(sex) + \beta_3(age\ months) + \beta_4(duration\ on\ ART)(baseline\ log_{10}\ VL) + \beta_5(time\ on\ TDF) + \beta_6(BMI) + \beta_7(baseline\ CD4) + e_{ij}^*$	(Model 9)
$y_{ij} = \beta_0 + \beta_1(sustained\ kidney\ disease) + \beta_2(sex) + \beta_3(age\ months) + \beta_4(duration\ on\ ART)(baseline\ log_{10}\ VL) + \beta_5(time\ on\ TDF) + \beta_6(BMI) + \beta_7(baseline\ CD4)(baseline\ log_{10}\ VL) + e_{ij}^*$	(Model 10)
$y_{ij} = \beta_0 + \beta_1(sustained\ kidney\ disease) + \beta_2(sex) + \beta_3(age\ months) + \beta_4(duration\ on\ ART)(baseline\ log_{10}\ VL) + \beta_5(time\ on\ TDF) + \beta_6(baseline\ CD4)(baseline\ log_{10}\ VL) + e_{ij}^*$	(Model 11)
$y_{ij} = \beta_0 + \beta_1(sustained\ kidney\ disease) + \beta_2(sex) + \beta_3(age\ months) + \beta_4(duration\ on\ ART)(baseline\ log_{10}\ VL) + \beta_5(time\ on\ TDF) + \beta_6(BMI) + \beta_7(prior\ CD4) + e_{ij}^*$	(Model 12)
$y_{ij} = \beta_0 + \beta_1(sustained\ kidney\ disease) + \beta_2(sex) + \beta_3(age\ months) + \beta_4(duration\ on\ ART)(baseline\ log_{10}\ VL) + \beta_5(time\ on\ TDF) + \beta_6(BMI) + \beta_7(prior\ \log_{10} VL) + e_{ij}^*$	(Model 13)
$y_{ij} = \beta_0 + \beta_1(sustained\ kidney\ disease) + \beta_2(sex) + \beta_3(age\ months) + \beta_4(duration\ on\ ART)(baseline\ log_{10}\ VL) + \beta_5(time\ on\ TDF) + \beta_6(BMI) + \beta_7(prior\ CD4) + \beta_8(prior\ \log_{10} VL) + e_{ij}^*$	(Model 14)
$y_{ij} = \beta_0 + \beta_1(sustained\ kidney\ disease) + \beta_2(sex) + \beta_3(age\ months) + \beta_4(duration\ on\ ART)(baseline\ log_{10}\ VL) + \beta_5(time\ on\ TDF) + \beta_6(BMI) + \beta_7(prior\ CD4)(prior\ \log_{10} VL) + e_{ij}^*$	(Model 15)

In each of the proposed models, y_{ij} is the change in eGFR over time, and β_n are the coefficients associated with each of the included parameters. Results of the multimodel comparison are shown in Appendix 2 Table 1, below.

Appendix 2 Table 1: Summary of multi-model comparison to select the linear mixed effects regression model which best described changes in eGFR over time

MODEL	K	AICC	DELTA AICC	MODEL LIKELIHOOD	AICC WEIGHT	E-RATIO
MODEL 0	3	4596	1984.60	0.00	0.00	INF
MODEL 1	6	4557	1945.80	0.00	0.00	INF
MODEL 2	7	4554	1942.35	0.00	0.00	INF
MODEL 3	8	4534	1922.02	0.00	0.00	INF
MODEL 4	9	4538	1926.68	0.00	0.00	INF
MODEL 5	10	4540	1928.55	0.00	0.00	INF
MODEL 6	11	4540	1928.74	0.00	0.00	INF
MODEL 7	12	2904	292.10	0.00	0.00	INF
MODEL 8	13	2615	3.05	0.22	0.18	4.58
MODEL 9	**14**	**2612**	**0.00**	**1.00**	**0.81**	**1.00**
MODEL 10	15	2621	9.79	0.01	0.01	133.00
MODEL 11	14	2622	10.38	0.01	0.00	179.00
MODEL 12	14	2744	132.30	0.00	0.00	5.35E+28
MODEL 13	14	2708	96.14	0.00	0.00	7.54E+20
MODEL 14	15	2716	104.57	0.00	0.00	5.09E+22
MODEL 15	16	2727	114.85	0.00	0.00	8.70E+24

Model 9 (highlighted) best described change in eGFR over time in the available dataset, as evidenced by the low corrected Akaike Information Criteria (AICc), and the high weight of evidence of feasibility of the model (81%). The E-ratio (the evidence ratio), expressed by the equation:

$$Evidence\ ratio = \frac{AICc\ of\ the\ best\ fitting\ model}{AICc\ of\ the\ model\ being\ compared\ to\ the\ best\ fitting\ model}$$

shows that only Models 8, 10 and 11 had evidence to support their adequacy in describing the available data to a similar degree of accuracy as Model 9. The higher the evidence ratio, the less feasible a model is to adequately describe the data.

APPENDIX 3: ETHICS CLEARANCE CERTIFICATE

R14/49 Dr Zoleka Ncanywa and Prof Udai Kala

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M160854

NAME: (Principal Investigator)	Dr Zoleka Ncanywa and Prof Udai Kala
DEPARTMENT:	Paediatrics Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Changes in Glomerular Filtration Rate in HIV-Infected Adolescents on Tenofovir
DATE CONSIDERED:	26/08/2016
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr David Moore
APPROVED BY:	Prof A Woodiwiss, Co- Chairperson, HREC (Medical)
DATE OF APPROVAL:	12/12/2016

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in August and will therefore be due in the month of August each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX 4: TURNITIN REPORT

Changes in Glomerular Filtration Rate in HIV-infected Adolescents on Tenofovir

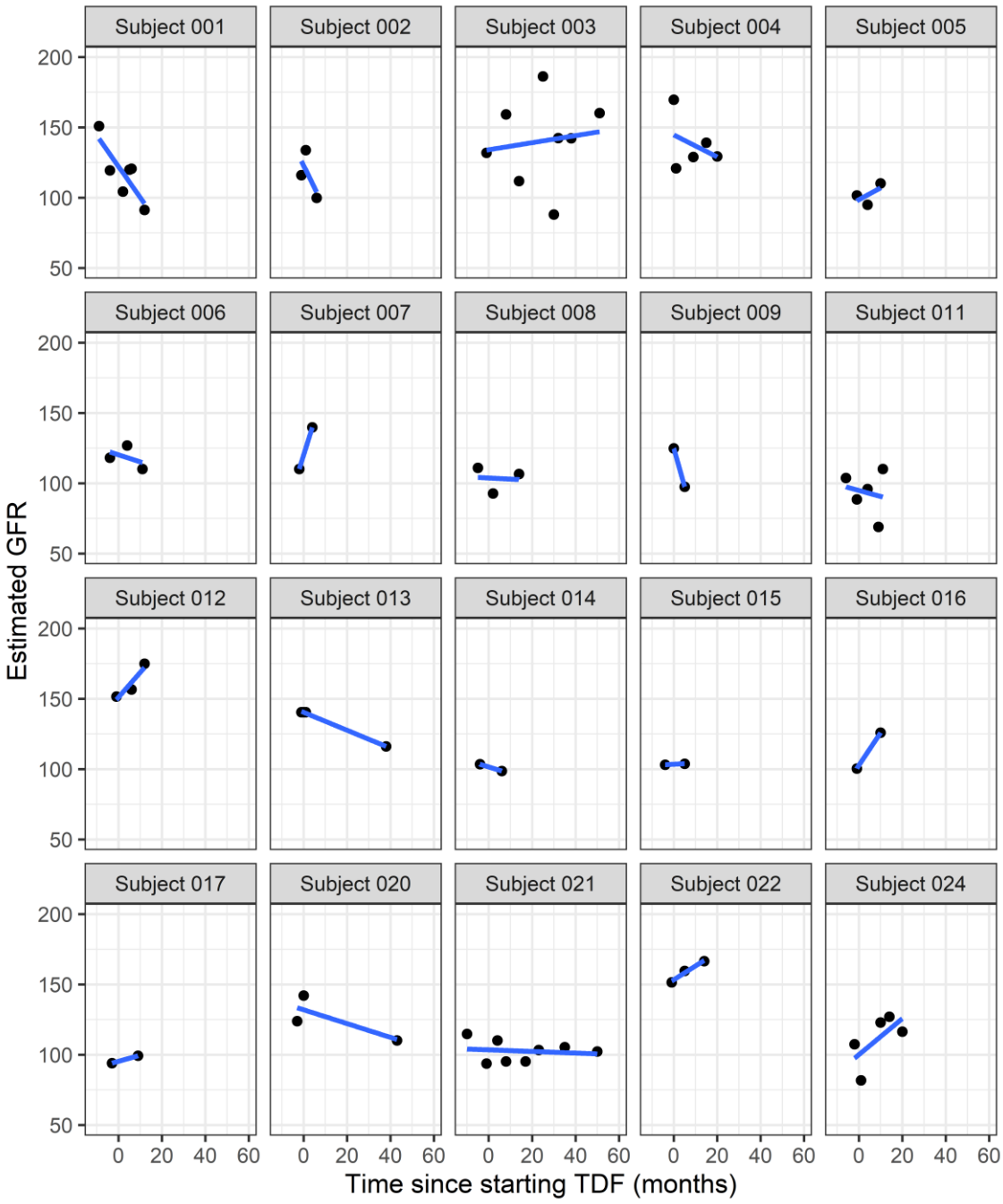
ORIGINALITY REPORT

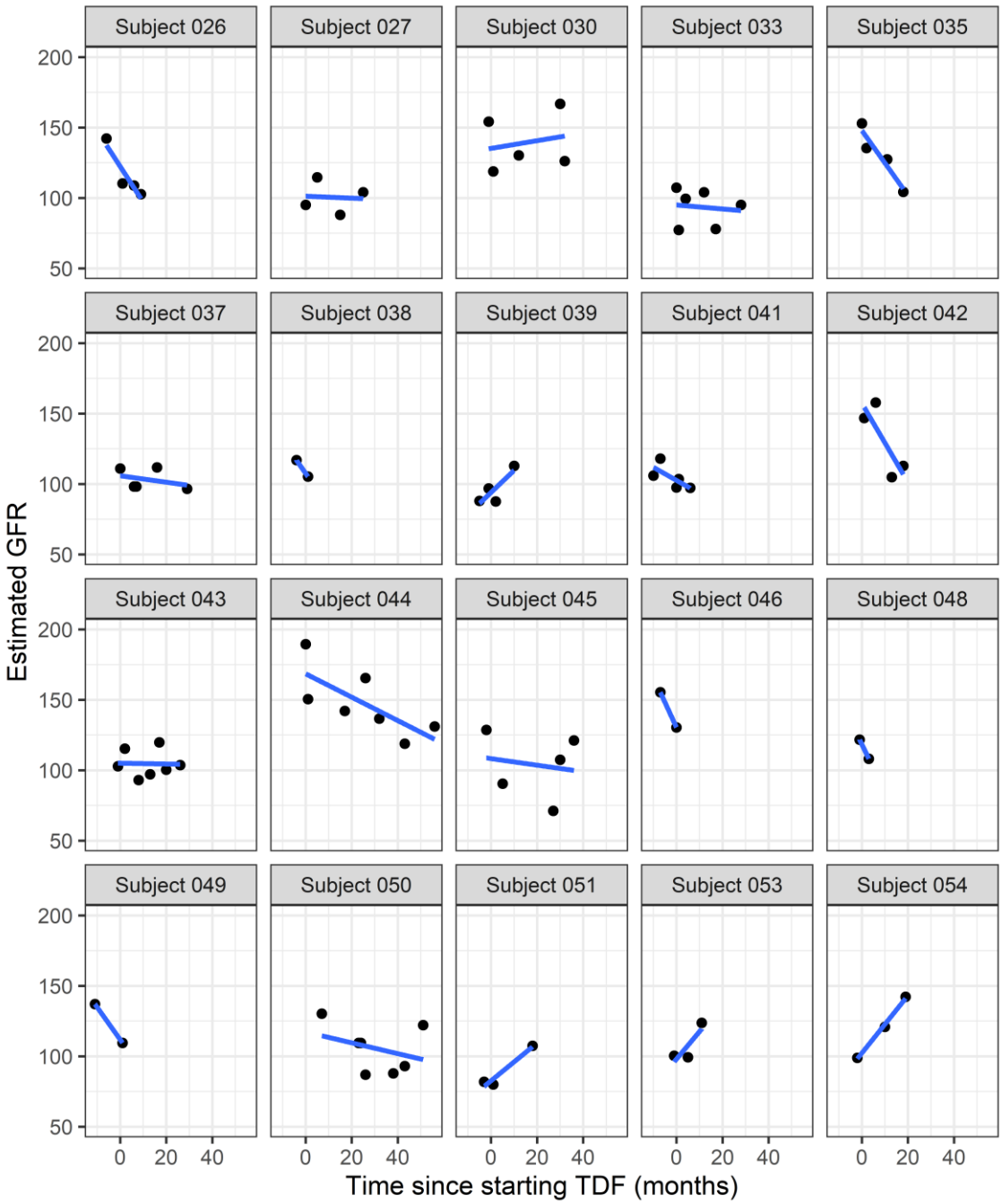
13%	10%	9%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	hdl.handle.net Internet Source	1%
2	onlinelibrary.wiley.com Internet Source	1%
3	Submitted to London School of Hygiene and Tropical Medicine Student Paper	1%
4	Submitted to Aspen University Student Paper	<1%
5	academic.oup.com Internet Source	<1%
6	pure.uva.nl Internet Source	<1%
7	wiredspace.wits.ac.za Internet Source	<1%
8	stacks.cdc.gov Internet Source	<1%

APPENDIX 5: SERIAL KIDNEY FUNCTION TRENDS IN CHILDREN WITHOUT SUSTAINED KIDNEY DYSFUNCTION (N=97)

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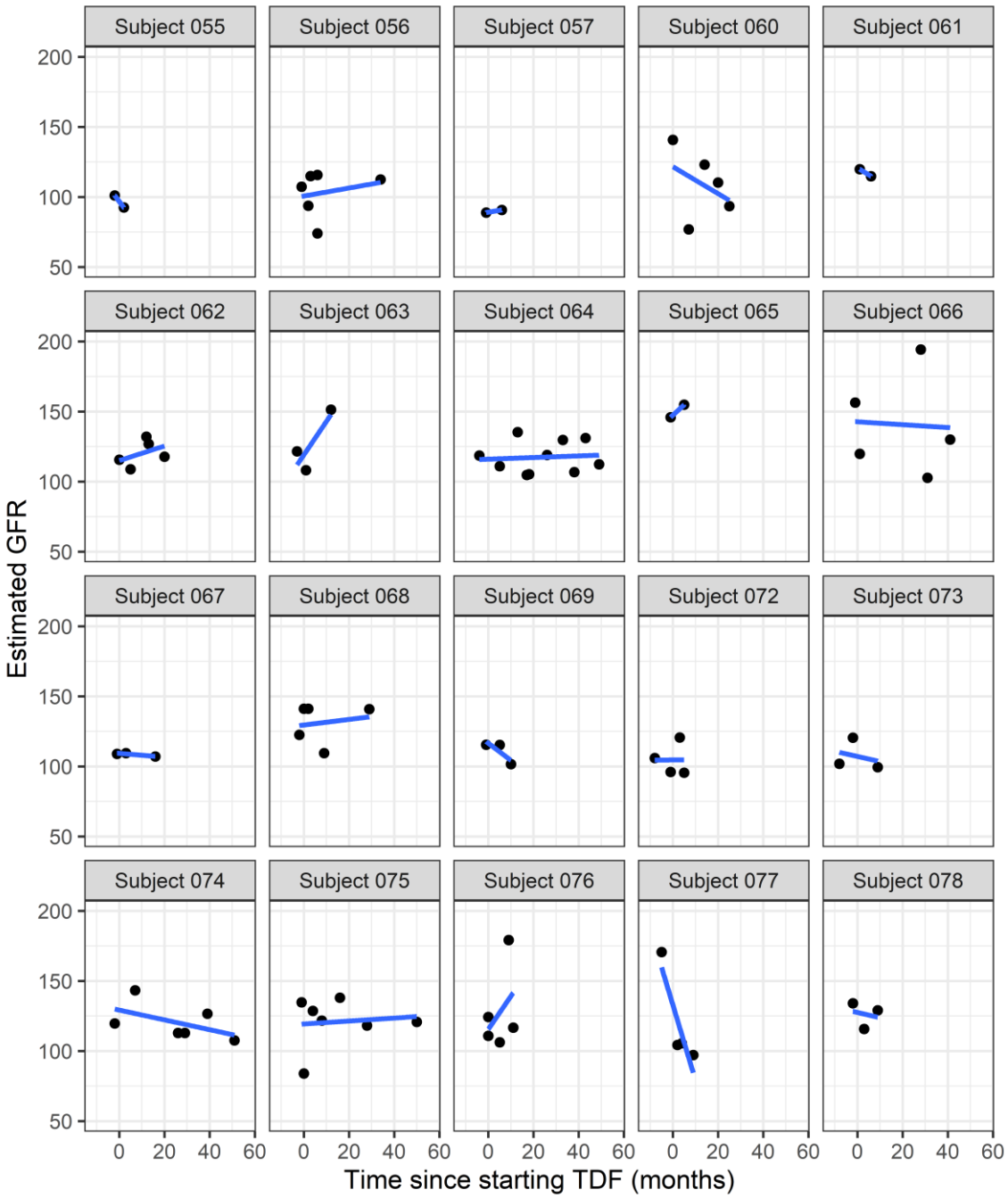
Subject 039
Subject 041
Subject 042
Subject 043
Subject 044
Subject 045
Subject 046
Subject 048
Subject 049
Subject 050
Subject 051
Subject 053
Subject 054
Estimated GFR
200
150
100
50
0
20
40
Time since starting TDF (months)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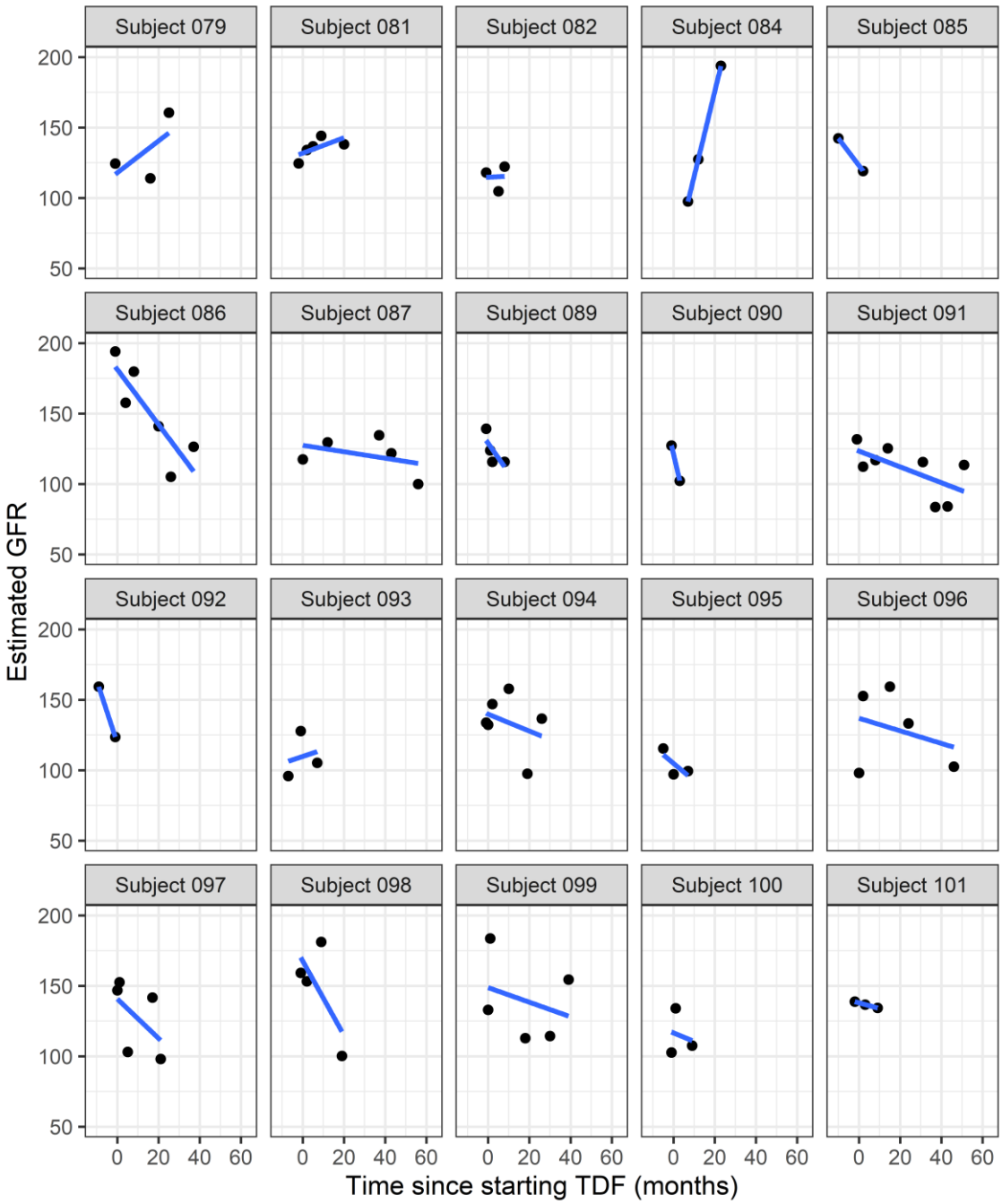
Subject 069
Subject 072
Subject 073
Subject 074
Subject 075
Subject 076
Subject 077
Subject 078
Estimated GFR
50
100
150
200
0
20
40
60
Time since starting TDF (months)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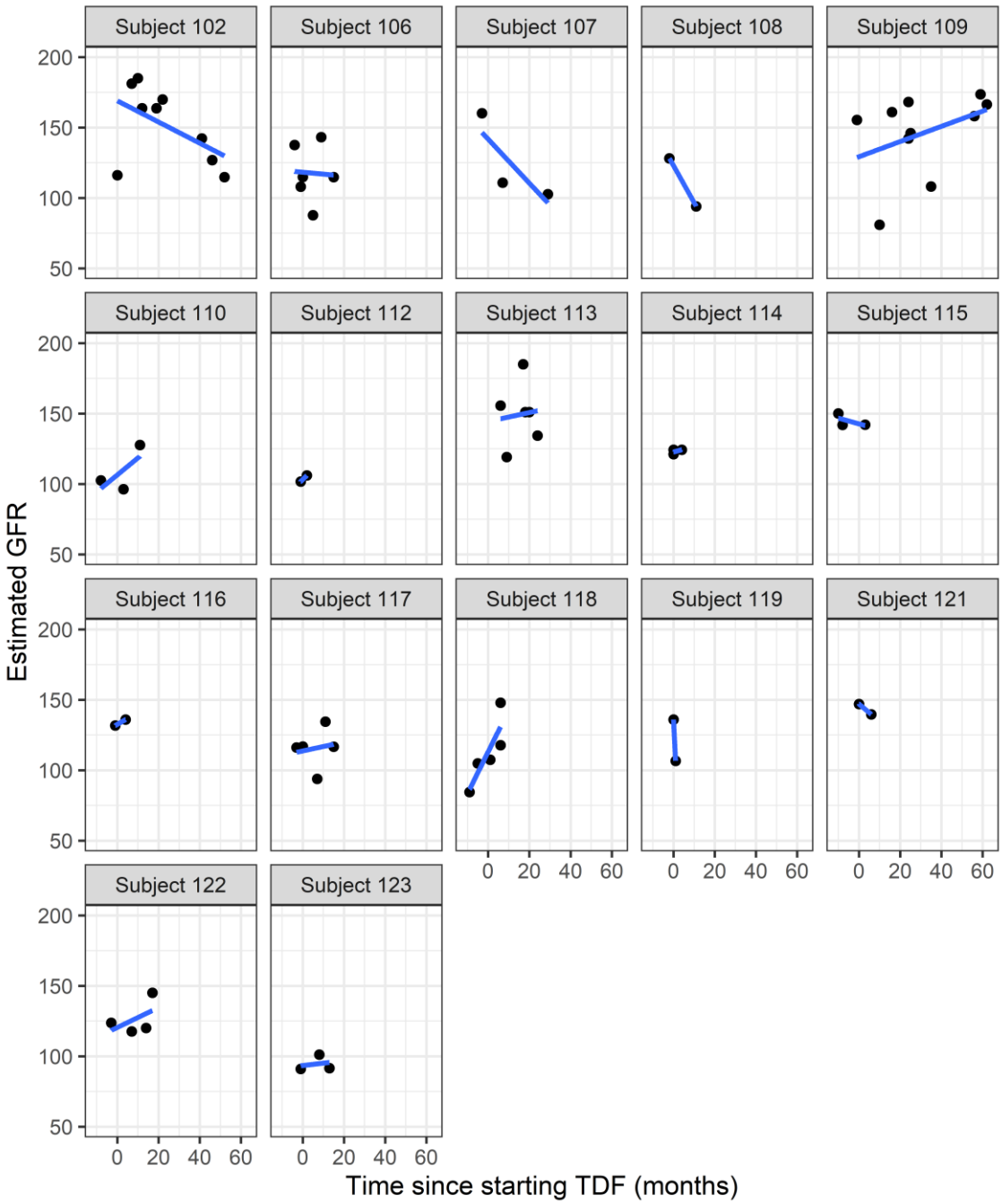
Subject 095
Subject 096
Subject 097
Subject 098
Subject 099
Subject 100
Subject 101
Estimated GFR
200
150
100
50
0
20
40
60
Time since starting TDF (months)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Subject 115
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Subject 117
Subject 118
Subject 119
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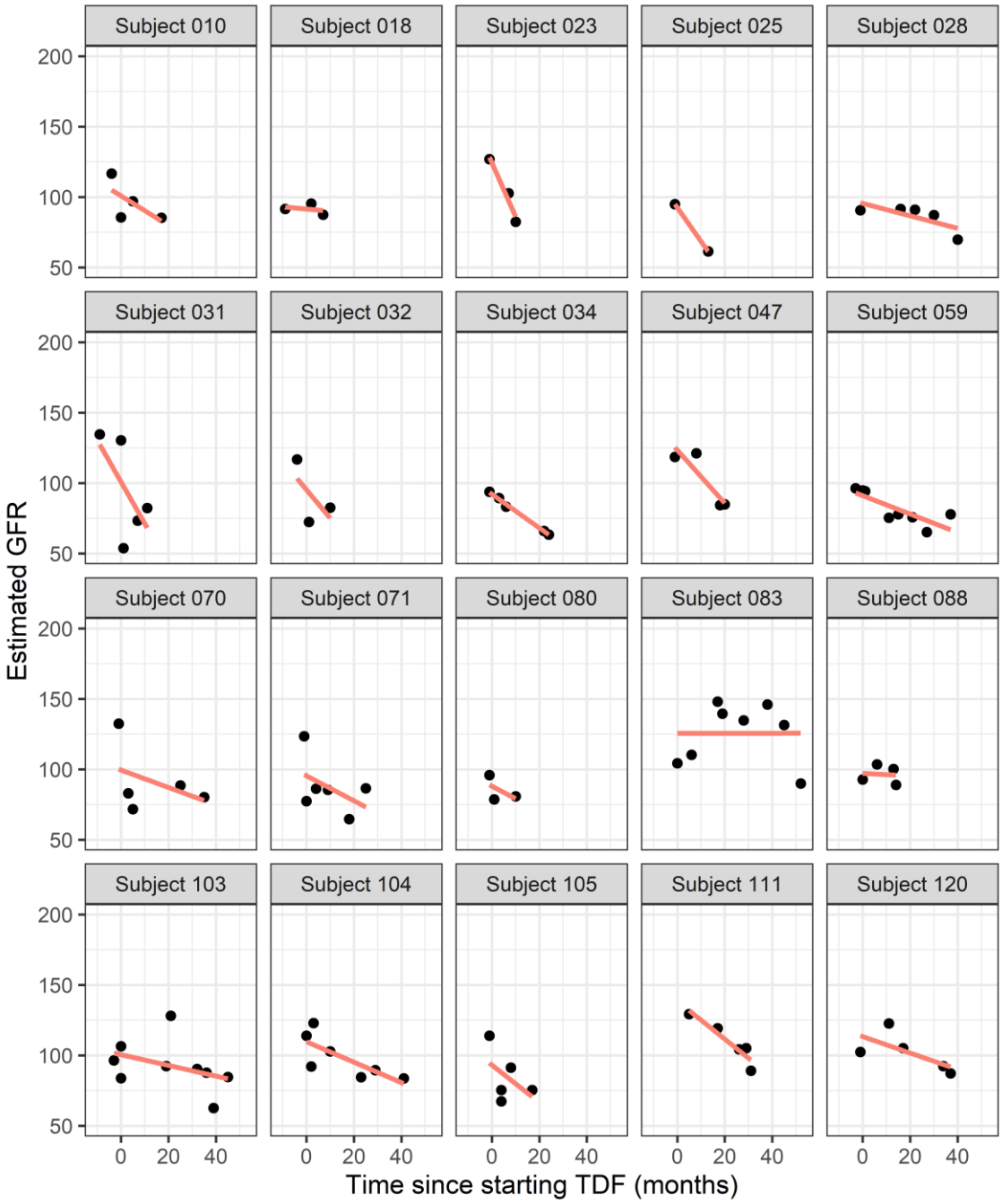
Subject 122
Subject 123
Estimated GFR
200
150
100
50
0
20
40
60
Time since starting TDF (months)

APPENDIX 6: SERIAL KIDNEY FUNCTION TRENDS IN CHILDREN WITH SUSTAINED KIDNEY DYSFUNCTION DURING THE COURSE OF FOLLOW-UP (N=20)



REFERENCES

1. UNAIDS. Country Factsheet South Africa. United Nations Programme on HIV/AIDS; 2018.
2. Pontrelli G, Cotugno N, Amodio D, Zangari P, Tchidjou HK, Baldassari S, et al. Renal function in HIV-infected children and adolescents treated with tenofovir disoproxil fumarate and protease inhibitors. BMC infectious diseases. 2012;12:18.
3. Patel SD, Larson E, Mbengashe T, O'Bra H, Brown JW, Golman TM, et al. Increases in pediatric antiretroviral treatment, South Africa 2005-2010. PloS one. 2012;7(9):e44914.
4. South African National Department of Health. National Antiretroviral Treatment Guidelines. South African National Department of Health, editor. Pretoria: Department of Health; 2004. 26-51 p.
5. Burrage A, Patel M, Mirkovic K, Dziuban E, Teferi W, Broyles L, et al. Trends in antiretroviral therapy eligibility and coverage among children aged< 15 years with HIV infection—20 PEPFAR-supported sub-Saharan African countries, 2012–2016. Morbidity and Mortality Weekly Report. 2018;67(19):552.
6. South African National Department of Health. Guidelines for the Management of HIV in Children. In: South African National Department of Health, editor. Pretoria: South African National Department of Health; 2010. p. 1-138.
7. National Department of Health. National consolidated guidelines for the prevention of mother-to-child transmission of HIV (PMTCT) and the management of HIV in children, adolescent and adults. In: Health Do, editor. Pretoria, South Africa: National Department of Health; 2015. p. 1-136.
8. National Department of Health. ART Clinical Guidelines for the Management of HIV in Adults, Pregnancy,Adolescents, Children, Infants and Neonates. In: Health Do, editor. Pretoria, South Africa: Department of Health; 2019. p. 1-26.
9. Havens PL, Essajee S. Technical Update on Treatment Optimization: Use of Tenofovir in HIV-infected Children and Adolescents: a Public Health Perspective: World Health Organization; 2012.
10. Fernandez-Fernandez B, Montoya-Ferrer A, Sanz AB, Sanchez-Nino MD, Izquierdo MC, Poveda J, et al. Tenofovir nephrotoxicity: 2011 update. AIDS research and treatment. 2011;2011:354908.
11. Hall AM, Hendry BM, Nitsch D, Connolly JO. Tenofovir-associated kidney toxicity in HIV-infected patients: a review of the evidence. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2011;57(5):773-80.
12. Della Negra M, De Carvalho AP, De Aquino MZ, Pinto JA, Da Silva MT, Andreatta KN, et al. Long-term efficacy and safety of tenofovir disoproxil fumarate in HIV-1-infected adolescents failing antiretroviral therapy: the final results of study GS-US-104-0321. The Pediatric infectious disease journal. 2015;34(4):398-405.
13. Schwartz GJ, Work DF. Measurement and estimation of GFR in children and adolescents. Clinical journal of the American Society of Nephrology : CJASN. 2009;4(11):1832-43.
14. Hoste L, Dubourg L, Selistre L, De Souza VC, Ranchin B, Hadj-Aissa A, et al. A new equation to estimate the glomerular filtration rate in children, adolescents and young adults. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2014;29(5):1082-91.
15. Schwartz GJ, Munoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, et al. New equations to estimate GFR in children with CKD. Journal of the American Society of Nephrology. 2009;20(3):629-37.

16. Statistics South Africa. My settlement. 2011. Contract No.: December 05, 2020.
17. Ryscavage P, Kelly S, Li JZ, Harrigan PR, Taiwo B. Significance and clinical management of persistent low-level viremia and very-low-level viremia in HIV-1-infected patients. Antimicrobial agents and chemotherapy. 2014;58(7):3585-98.
18. World Health Organization. WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. Geneva: World Health Organization; 2007.
19. Nuttall FQ. Body Mass Index: Obesity, BMI, and Health: A Critical Review. Nutr Today. 2015;50(3):117-28.
20. Transplantr. Audit and Research Functions For Transplantation. 0.2.0 ed2020. p. A set of vectorised functions to calculate medical equations used in transplantation, focused mainly on transplantation of abdominal organs.
21. Eknoyan G, Lameire N, Eckardt K, Kasiske B, Wheeler D, Levin A, et al. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int. 2013;3(1):5-14.
22. Whyte DA, Fine RN. Chronic Kidney Disease in Children. Pediatrics in Review. 2008;29(10):335-41.
23. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2020.
24. Bates D, Machler M, S W. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software. 2015;67(1):1-48.
25. Long JA. _jtools: Analysis and Presentation of Social Scientific Data. 2020;R package version 2.1.0.
26. Long JD. Longitudinal Data Analysis for the Behavioral Sciences Using R. 1st ed: SAGE Publications, Inc.; 2012.
27. Terry M. Therneau PMG. Modeling Survival Data: Extending the Cox Model.

. New York: Springer; 2000.
28. Oo MM. Epidemiological Data Analysis. 3.4.0 ed2020.
29. Alboukadel Kassambara MK, Przemyslaw Biecek, Scheipl Fabian. Drawing Survival Curves using 'ggplot2'. 0.4.8 ed2020.
30. International-Harvard Statistical Consulting Company. My.stepwise: Stepwise Variable Selection Procedures for Regression Analysis. 2017.
31. Judd A, Boyd KL, Stohr W, Dunn D, Butler K, Lyall H, et al. Effect of tenofovir disoproxil fumarate on risk of renal abnormality in HIV-1-infected children on antiretroviral therapy: a nested case-control study. Aids. 2010;24(4):525-34.
32. Riordan A, Judd A, Boyd K, Cliff D, Doerholt K, Lyall H, et al. Tenofovir use in human immunodeficiency virus-1-infected children in the United kingdom and Ireland. The Pediatric infectious disease journal. 2009;28(3):204-9.
33. Lim Y, Lyall H, Foster C. Tenofovir-associated nephrotoxicity in children with perinatally-acquired HIV infection: a single-centre cohort study. Clinical Drug Investigation. 2015;35(5):327-33.
34. Mashingaidze-Mano R, Bwakura-Dangarembizi MF, Maponga CC, Morse GD, Monera-Penduka TG, Mtisi TJ, et al. Proximal renal tubular function in HIV-infected children on tenofovir disoproxil fumarate for treatment of HIV infection at two tertiary hospitals in Harare, Zimbabwe. PloS one. 2020;15(7):e0235759.
35. Meyers T, Yoteieng M, Kuhn L, Moultrie H. Antiretroviral therapy responses among children attending a large public clinic in Soweto, South Africa. The Pediatric infectious disease journal. 2011;30(11):974.

36. Davies M-A, Keiser O, Eley B, Rabie H, van Cutsem G, Giddy J, et al. Outcomes of the South African national antiretroviral treatment programme for children: the IeDEA Southern Africa collaboration. South African medical journal. 2009;99(10).
37. De Waal R, Cohen K, Fox MP, Stinson K, Maartens G, Boulle A, et al. Changes in estimated glomerular filtration rate over time in South African HIV-1-infected patients receiving tenofovir: a retrospective cohort study. Journal of the International AIDS Society. 2017;20(1):21317.
38. Mulenga L, Musonda P, Mwango A, Vinikoor MJ, Davies M-A, Mweemba A, et al. Effect of baseline renal function on tenofovir-containing antiretroviral therapy outcomes in Zambia. Clinical infectious diseases. 2014;58(10):1473-80.
39. Mtisi TJ, Ndhlovu CE, Maponga CC, Morse GD. Tenofovir-associated kidney disease in Africans: a systematic review. AIDS research and therapy. 2019;16(1):12.
40. Giacomet V, Nannini P, Vigano A, Erba P, Benincaso A, Bedogni G, et al. Long-term renal effects of tenofovir-disoproxil-fumarate in vertically HIV-infected children, adolescents, and young adults: a 132-month follow-up study. Clinical drug investigation. 2015;35(7):419-26.
41. Grignolo S, Tatarelli P, Gustinetti G, Viazzi F, Bonino B, Maggi P, et al. Trend of eGFR in an Italian cohort of mother-to-child HIV-infected patients exposed to tenofovir for at least 2 years. European Journal of Pediatrics. 2015;174(6):843-6.
42. Fenton A, Montgomery E, Nightingale P, Peters AM, Sheerin N, Wroe AC, et al. Glomerular filtration rate: new age-and gender-specific reference ranges and thresholds for living kidney donation. BMC nephrology. 2018;19(1):1-8.
43. Bruggeman LA, Nelson PJ. Controversies in the pathogenesis of HIV-associated renal diseases. Nature reviews Nephrology. 2009;5(10):574-81.
44. Laprise C, Baril J-G, Dufresne S, Trottier H. Association Between Tenofovir Exposure and Reduced Kidney Function in a Cohort of HIV-Positive Patients: Results From 10 Years of Follow-up. Clinical Infectious Diseases. 2013;56(4):567-75.