RESEARCH ARTICLE

Proximal renal tubular function in HIV-infected children on tenofovir disoproxil fumarate for treatment of HIV infection at two tertiary hospitals in Harare, Zimbabwe

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Abstract

Background
Renal abnormalities in HIV infected children may be due to the HIV infection or treatment among other factors. Tenofovir disoproxil fumarate (TDF) is associated with proximal renal tubular dysfunction, proteinuria and decrease in glomerular function. Studies in developed countries have shown variable prevalence of proximal renal tubular dysfunction in children on TDF. There are no known studies in developing countries, including Zimbabwe, documenting the proximal tubular function in HIV infected children on TDF. The aim of this study was to assess renal and proximal renal tubular function in HIV infected children receiving TDF and determine factors associated with proximal tubular dysfunction.

Methods
A descriptive cross-sectional study was conducted in HIV infected patients below 18 years of age attending outpatient clinics at two tertiary hospitals in Harare, who received a TDF-containing antiretroviral regimen for at least six months. Dipstick protein and glucose, serum and urine phosphate and creatinine levels were measured. Fractional excretion of phosphate was calculated. Estimated glomerular filtration rate (eGFR) was calculated using the Schwartz formula. Tubular dysfunction was defined by at least two of the following characteristics: normoglycaemic glycosuria, hypophosphatemia and fractional excretion of phosphate > 18%.
Findings
One hundred and ninety-eight children below 18 years of age were recruited over a period of six months. The prevalence of tubular dysfunction was 0.5%. Normoglycaemic glycosuria occurred in 1 (0.5%), fractional excretion of phosphate >18% in 4 (2%), and hypophosphatemia in 22 [11.1%] patients. Severe stunting was associated with increased risk of hypophosphatemia (OR 9.31 CI (1.18, 80.68) p = 0.03). Reduction in estimated glomerular filtration rate (eGFR) < 90ml/min/1.73m² and proteinuria was evident in 35.9% and 32.8% of children, respectively. Concurrent TDF and HIV-1 protease inhibitor-based regimen was the only independent factor associated with reduction in GFR (OR 4.43 CI (1.32; 4.89) p = 0.016).

Conclusion
Tubular dysfunction was uncommon in Zimbabwean children on a TDF-based ART regimen. Hypophosphatemia, proteinuria and reduction in eGFR were common. Reassessing renal function using more sensitive biomarkers is needed to examine the long-term tolerance of TDF.

Introduction
Tenofovir disoproxil fumarate (TDF), a nucleotide reverse transcriptase inhibitor approved by the World Health Organization (WHO) for the management of HIV infection in children is associated with nephrotoxicity [1]. Toxicity can manifest as proximal tubular or glomerular dysfunction, acute kidney injury, chronic kidney disease and end stage renal disease [2]. Mitochondrial damage to the proximal tubule leads to impaired reabsorption of low molecular weight proteins and other solutes resulting in urinary wasting. TDF-associated tubular dysfunction may affect the serum bicarbonate and glucose concentration and in severe cases lead to Fanconi syndrome, a generalized proximal tubular defect with hypokalemic metabolic acidosis associated with vitamin D resistant metabolic bone disease [3, 4].

Advanced HIV infection and HIV-1 protease inhibitor (PI) containing therapy, are among factors associated with TDF renal impairment [5]. Biomarkers used to assess proximal renal tubular function include; urinary low molecular proteins serum phosphates, phosphaturia, normoglycaemic glycosuria, as well as urinary and serum electrolytes [5]. In Zimbabwe TDF is recommended as part of the first line regimen for adolescents and children weighing at least 25kg [7]. Ideally, children on TDF-based regimen should be monitored for creatinine clearance [8] but in most developing countries such as Zimbabwe, that recommendation is constrained by limited resources. Studies describing renal tubular function in children on TDF are therefore limited to those mostly assessing glomerular function in ART-naïve cases [9, 10]. This study aimed to assess the prevalence and factors associated with proximal renal tubular dysfunction in children receiving TDF. It was assumed that about 80% of the children had vertical transmission of HIV based on a study by Ferrand et al [11].

Methods
Study design and participants
A cross-sectional study was conducted between May and November 2016 at two Paediatric HIV Clinics in Harare, Zimbabwe. The Institutional Review Boards of: Harare Central...
Hospital Ethics Committee (Ref: HCHEC 140916/62), the Joint Research Ethica Committee For The University of Zimbabwe College of Health Sciences and Parirenyatwa Group of Hospitals (JREC), (Ref: JREC66/16) and the Medical Research Council of Zimbabwe (MRCZ), (Ref: MRCZ/B/1053) approved the study. Written informed consent was acquired from caregivers and assent obtained from children aged six years and older. Children less than 18 years who weighed at least 25 kg and were on a TDF based regimen for at least six months were recruited during routine clinic visits, conducted on Mondays to Thursdays inclusive. A convenience sampling method was used. Patients were recruited in the outpatients clinic as they came either for doctor’s review, medicine pickup or blood collection. Those patients with pre-existing diabetes or hypertension were excluded from the study. The Dobson formula below was used to calculate sample size:

\[ n = \left( \frac{Z_{\alpha/2}}{d} \right)^2 p(1 - p) \]

where \( Z_{\alpha/2} \) is the standard normal value corresponding to the desired level of confidence (95%), 
\( d \) is the maximum allowable error, (0.05) or 5% (width of the confidence intervals)
\( p \) is the estimated prevalence of renal tubular dysfunction in children on TDF based regimen The calculated sample size was 196 participants based on a study in Ghana which found a prevalence \( p \) of proximal renal tubular dysfunction of 15% [12]. The precision for the sample size was 5%.

**Clinical assessment**

Demographic data, baseline CD4 count and World Health Organization (WHO) clinical staging were captured from participants and medical records respectively prior to highly active antiretroviral therapy (HAART) initiation. Blood pressure was measured in a sitting position using an appropriate size cuff (Dynamap V100™ manufactured by Menhold South Africa). Weight and height measurements were taken by a physician or trained clinic nurse. Weight was measured using a calibrated scale (Seca, model 8811021659) estimated to the nearest 0.1kg. Height was measured using a standardized wall mounted stadiometer with inbuilt millimeter ruler and estimated to the nearest 0.1cm. Body mass index calculation was done using the WHO AnthroPlus calculator (2009) [13]. The WHO growth charts (2007) were used to determine nutritional status of the study participants [14–16].

**Laboratory assessment**

Spot urine samples were collected during the clinic visits. The urine was immediately checked for presence of glucose and protein using the 10 parameter reagent strips (Uricheck M10, Omnipharm). Patients with positive urine glucose (> +1), had a capillary blood glucometer performed immediately (Glucoplus™). Qualitative proteinuria was reported on dipstick as negative, 1+(30mg/dL), 2+ (100mg/dL), 3+(300mg/dL) or 4+(1000mg/dL) [17]. Urine protein/creatinine ratios (mg/dL:mg/dL) was further performed on all urine samples. Proteinuria was defined as: normal range proteinuria if urine protein/creatinine <0.2g, further classified into intermediate proteinuria (0.2g - 3.0g) and nephrotic range proteinuria (>3.5g) [18]. The rest of the urine samples including glucose or protein negative dipstick were kept in a carrier cooler bag lined with ice packs between +2°C and +8°C then transported to the University of Zimbabwe, Department of Chemical Pathology laboratory within four hours of collection, for assessment of urine phosphate, creatinine and protein. Blood samples for measurement of serum creatinine and phosphate were collected and transported to the laboratory in carrier bags lined with ice packs maintaining temperature between +2°C and +8°C. Specimens were
processed within same day of collection. Urine and serum phosphorus concentration was measured using the Phosphomolybdate method [19]. The pyrogallol red method was used to measure urine protein concentration [20]. Serum and urine creatinine were measured using the modified Jaffe method [21, 22]. Estimated glomerular filtration rate (eGFR) was calculated using the Schwartz formula [23, 24]. A Mindray BS200E Chemistry analyser (Mindray, Shenzhen, China) was used to determine the concentrations of creatinine, phosphate and protein for both urine and serum samples. Calibration of the machine was done every fortnight for creatinine and monthly for phosphate and protein. Proximal tubular function was assessed using normoglyacemic glycosuria, fractional excretion of phosphate (FEP) >18% and hypophosphatemia. The FEP was calculated using a standard formula [25]. Hypophosphatemia was graded according to the Division of Acquired Immunodeficiency Syndrome (DAIDS) toxicity grading [26, 27]. Glomerular dysfunction as indicated by a low eGFR was classified as mild (60–89 ml/min/1.73 m²), moderate (30–60 ml/min/1.73 m²), and severe impairment (15–30 ml/min/1.73 m²) [8, 28]. The full laboratory protocol is found at protocols.io: https://protocols.io/view/pone-d-19-35784-proximal-renal-tubular-function-in-bfzxjp7n [dx.doi.org/10.17504/protocols.io.bfzxjp7n]

Table 1. Demographic and clinical characteristics of HIV infected children, on TDF for at least 6 months, <18 years old, (n = 198).

| Variable            | Frequency n = 198 (%) |
|---------------------|-----------------------|
| Gender              |                       |
| Female              | 89 (44.9)             |
| Male                | 109 (55.1)            |
| Age                 |                       |
| Median age [years]  | 15 (IQR 13–16)       |
| CD4 count ul/l      |                       |
| ≤ 200               | 63 (31.8)             |
| 201–500             | 25 (12.6)             |
| 501–800             | 24 (12.1)             |
| ≥ 800               | 11 (5.6)              |
| WHO Clinical Stage  |                       |
| Stage 1             | 41 (20.7)             |
| Stage 2             | 32 (16.1)             |
| Stage 3             | 61 (30.8)             |
| Stage 4             | 13 (6.6)              |
| Nutritional Status  |                       |
| "BMI for age [kg/m²] |                     |
| < -2SD              | 14 (7.0)              |
| -2SD -3SD           | 11 (5.6)              |
| ≥ 1SD               | 10 (5.1)              |
| ≥ 2SD               | 3 (1.5)               |
| ≥ 2SD               | 160 (80.8)            |
| Stunting "[HFA]     |                       |
| < -2SD              | 26 (13.1)             |
| -2SD -3SD           | 18 (9.1)              |
| > -2SD              | 154 (77.8)            |

*BMI = Body mass index, HFA = Height for age, SD = Standard deviation.

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Data analysis

Data were analyzed using Epi Info version 7. Descriptive statistics with median and interquartile range (IQR) for continuous, non-nominal variables and percentages for categorical variables were generated. Univariate and multivariate logistic regression analyses were performed to identify factors associated with proximal renal tubular dysfunction. Factors with \( p < 0.25 \) in the univariate analysis were entered into a stepwise multivariate analysis model. The results of the logistic regression analyses were expressed as odds ratios (ORs) with 95% confidence intervals (CIs). The MDB file data sets can be found at https://datadryad.org/stash/share/pRmK7SDWzk5Jnd3-usw2hKCGVe71OksxVKbxfTZv5PE and DOI (doi:10.5061/dryad.2fzp612mf).

Results

One hundred and ninety-eight children were enrolled into the study. The demographic and (clinical characteristics are summarized in Tables 1 and 2. The median age of participants was 15 years (IQR 13–16; Range 6–17.11). The median duration on a TDF based regimen was 37 months (IQR 16–52; Range 6–120). Baseline CD4 count was documented in 123 /198 children (62.1%). Fourteen (7%) participants’ BMI was classified as thin, 9 (5.6%) as severe thinness and 13 (6.6%) as overweight. Severe stunting (height for age < -3 standard deviation) was recorded in 18 (9.1%).

Table 3 below shows the frequency of abnormal renal function tests. These tests include serum phosphate decrease, fractional excretion of phosphate >18%, normoglycaemic glycosuria, reduction in glomerular filtration rate and proteinuria.

Proximal renal tubular function

\( \text{FEp} > 18\% \) was detected in 4 (2%), hypophosphatemia in 22 (11.1%) and normoglycaemic glycosuria in one (0.5%) participant. Mild to moderate decrease in serum phosphate was detected in 16 (8%) of the children while 6 (3%) had severe to life threatening hypophosphatemia. Tubular dysfunction was only detected in one male patient who had \( \text{FEp} > 18\% \) and hypophosphatemia. This patient was older than 14 years and had been on first line ART for 55 months. Severe stunting was associated with increased risk of hypophosphatemia in children younger than 14 years (OR 9.31 CI (1.18, 80.68) \( p = 0.03 \)). There were no significant factors associated with hypophosphatemia in children older than 14 years (Tables 4 and 5 below).

Table 2. ARV exposure of HIV infected children, on TDF for at least 6 months, < 18 years old, (n = 198).

|                        | N 198 (%) |
|------------------------|-----------|
| ART experienced prior to TDF use | 107 (54) |
| ART- naïve prior to TDF use       | 91 (46)  |
| No. of patients First Line Therapy | 165 (83.3) |
| No. of patients on Second Line Therapy | 33 (16.7) |
| Current ART regimen           |          |
| TDF/3TC/EFV                | 139 (70.2) |
| TDF/3TC/NVP                | 26 (13.1)  |
| TDF/3TC/bPI*               | 33 (16.7)  |
| Median duration on TDF (months) | 37 (IQR 16–52) |
| Range (months)             | 6–120     |

\*bPI is either Lopinavir/ritonavir or Atazanavir/ritonavir.

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Proteinuria

Proteinuria with at least 1+ of protein was detected in 31 (15.7%) of study participants on dipstick urinalysis. Only one participant had glycosuria on dipstick. Of the 31 participants with positive dipstick proteinuria only 7 (3.6%) were confirmed by spot urine protein:creatinine ratio. Sixty-five of the 193 samples were positive for proteinuria increasing positive to 33.7% (65/193), despite negative dipstick urinalysis in most of the participants. Nephrotic range proteinuria was detected in only 2 (1%) of the children. A protease inhibitor (PI) containing ART regimen was significantly associated with proteinuria in both univariate (OR 3.60 CI (1.62, 7.99) p = 0.002) and multivariate analysis (OR 3.75 CI (1.59; 8.86) p = 0.003), Fig 1. Duration on TDF was not significantly associated with proteinuria. Children with moderate and severe stunting were more likely to have proteinuria though not statistically significant (Fig 1).

Glomerular filtration rate

Estimated GFR > 90ml/min/m² was in 122 (64.2%) of the participants. Among the children with decreased eGFR, 67 had mild, three moderate and one had severe reduction. Exposure to a protease inhibitor (PI) was associated with reduction in eGFR and this was statistically significant (OR 6.08 CI (2.56, 14.45) p < 0.001), Fig 2. On multivariate analysis, exposure to PI regimen was the only independent factor associated with reduction in eGFR (p = <0.016). Patients with severe stunting, height for age (HFA) < -3 were more likely to have reduction in eGFR (OR 2.69 CI (1.00, 7.24) p = 0.051). Although age had a p-value greater than 0.25 in the univariate model it was included in the multivariate model to account for any confounding effects age may have on eGFR.

Discussion

This study uniquely describes the prevalence of proximal renal tubular dysfunction in paediatric patients on TDF based ART regimen in a resource constrained clinical setting.

Renal tubular function and dysfunction

There was a low prevalence of proximal tubular dysfunction found in the study population. Similar findings were reported in two studies of children treated with TDF in two developed countries, where renal tubular function remained stable throughout the study period [29, 30]. However, finding in this study were much lower than what was reported in a multicenter study in Spain where tubular dysfunction measured by reduction in tubular phosphate reabsorption (TPR) was observed in 74% and proteinuria 89% of study participants [31]. The study design, definitions and parameters of proximal tubular dysfunction used in the Spanish study may explain the differences. In a study by Chadwick et al and Labarga et al in Ghana, despite
assessing similar parameters for proximal tubular renal function used in our study, the prevalence was higher though. However the study population involved older patients compared to this study [12, 32]. Simultaneous measurement of urine albumin and protein may help differentiate tubular from glomerular proteinuria. Urine albumin/total protein ratio (uAPR) < 0.4 identifies tubular pathology in proteinuric patients and this was not measured in our study [33]. Although increased fractional excretion of phosphate, hypophosphatemia and normoglycemic glycosuria are established markers of proximal tubular dysfunction and are easy to screen for, they are less sensitive than tubular protein excretions (NGAL, B2M and RBP) and have been suggested to be the most appropriate alternatives when these are not available [6, 34, 35]. In addition better markers for proximal tubular function (Retinol-binding protein, β-2 microglobulin and N-acetyl-β-d-glucosaminidase) could not be used due non-availability of tests and cost constraints. Lack of these more sensitive tests might have affected the results and

| VARIABLE                        | CATEGORY | YES | NO | OR (95%CI) | P-VALUE |
|---------------------------------|----------|-----|----|------------|---------|
| Gender                          | Female   | 5 (31.3) | 34 (46.6) |            |         |
|                                 | Male     | 11 (68.7) | 39 (53.4) | 0.52 [0.17, 1.70] | 0.263 |
| Current ART Regimen.            | TDF/3TC/EFV | 10 (62.5) | 54 (74) | 1          |         |
|                                 | TDF/3TC/NVP | 2 (12.5) | 8 (11) | 1.35 [0.24, 7.32] | 0.728 |
|                                 | TDF/3TC/PI | 4 (25) | 11 (51.1) | 1.93 [0.52, 7.41] | 0.451 |
| ART Regimen                     | 1st Line | 13 (81.2) | 63 (86.3) |            |         |
|                                 | 2nd Line | 2 (18.8) | 10 (13.7) | 1.03 [0.2, 5.27] | 1 |
| Duration on current regimen in months | 6–12 | 4 (25) | 9 (12.3) | 1          |         |
|                                 | 13–60 | 12 (75) | 63 (86.3) | 2.33 [0.62, 8.82] | 0.212 |
|                                 | ≥61 | 0 | 1 (1.4) | - | - |
| W.H.O Staging                   | Stage 1 | 5 (45.5) | 15 (24.2) | 1          |         |
|                                 | Stage 2 | 2 (18.2) | 15 (24.2) | 0.40 [0.06, 2.39] | 0.416 |
|                                 | Stage 3 | 4 (36.4) | 27 (43.5) | 0.44 [0.10, 1.91] | 0.289 |
|                                 | Stage 4 | 0 | 5 (8.1) | - | - |
| *BMI                            | Normal | 12 (75) | 60 (82.2) | 1          |         |
|                                 | < -2SD | 2 (12.5) | 6 (8.2) | 1.67 [0.30, 9.27] | 0.623 |
|                                 | < -3SD | 1 (6.3) | 4 (5.5) | 1.25 [0.13, 12.19] | 1 |
|                                 | > 1SD | 0 | 2 (2.7) | - | - |
|                                 | > 2SD | 1 (6.3) | 1 (1.4) | 5.0 [0.29, 85.60] | 0.322 |
| Stunting                        | > -2SD | 11 (68.8) | 67 (91.8) | 1          |         |
|                                 | < -2SD | 2 (12.5) | 4 (5.5) | 3.05 [0.05, 18.67] | 0.231 |
|                                 | < -3SD | 3 (18.8) | 2 (2.7) | 9.31 [1.18, 80.68] | 0.03* |
| Baseline CD4 count              | 0–200 | 2 (25) | 10 (18.5) | 1          |         |
|                                 | 201–500 | 5 (62.5) | 28 (51.9) | 0.89 [0.159, 5.35] | 1 |
|                                 | 501–800 | 0 | 9 (16.7) | - | - |
|                                 | ≥801 | 1 (12.5) | 7 (87.5) | 0.71 [0.05, 9.50] | 1 |
| Proteinuria                     | Yes | 7 (43.8) | 19 (26.8) |            |         |
|                                 | No | 9 (56.2) | 52 (73.2) | 2.13 [0.70, 6.52] | 0.180 |
| *eGFR < 90m/min/1.73m²²         | Yes | 8 (50) | 21 (28.8) |            |         |
|                                 | No | 8 (50) | 52 (71.2) | 2.48 [0.82, 7.46] | 0.101 |
| *FPe > 18%                      | Yes | 1 (6.2) | 1 (1.4) |            |         |
|                                 | No | 15 (93.8) | 72 (98.6) | 4.80 [0.28, 81.10] | 0.233 |

*FPe = Fractional excretion of phosphate, *eGFR = estimated glomerular filtration rate, *BMI = Body mass Index.
patients with proximal tubular dysfunction missed. A future prospective study on beta-2 microglobulin, aminoaciduria, hypercalciuria and NGAL as markers of tubular function is therefore recommended.

The prevalence of fractional excretion of phosphate >18% was low in this study compared to a study by Chadwick et al, in which 7% of the participants had $\text{FEP}\text{>}18\%$. However, their sample size was smaller and included adults [12]. In a Swiss Cohort study, also done in adults, $\text{FEP}\text{>}20\%$ occurred in 11.5% of study patients which is much higher than our study [36]. Fractional excretion of phosphate varies with age with lower levels in children. In our study the $\text{FEP}\text{>}18\%$ was used to define pathological fractional excretion of phosphate. It is possible that the age difference could explain the different findings [37, 38].

Hypophosphatemia reported in 8% of the participants was at least Division of Acquired Immunodeficiency Syndrome (DAIDS) toxicity grade 2. The prevalence of hypophosphatemia was higher than reported in other studies [39]. The long term consequence of this

### Table 5. Factors associated with hypophosphatemia in children $\geq$ 14 years of TDF regimen for at least 6 months.

| Variable         | Category | Hypophosphatemia | OR(95%CI) | p-value |
|------------------|----------|------------------|-----------|---------|
|                  |          | Yes              | No        |         |
| Sex              | Female   | 2(33.3)          | 46(47.9)  |         |
|                  | Male     | 4(66.7)          | 50(52.1)  | 0.54(0.10–3.11) 0.487 |
| Current ART Reg. | TDF/3TC/EFV | 6(100)          | 68(70.8)  |         |
|                  | TDF/3TC/NVP | 0               | 13(13.5)  |         |
|                  | TDF/3TC/PI | 0               | 15(15.6)  |         |
| ART Regimen      | 1st Line  | 6(100)           | 84(87.5)  |         |
|                  | 2nd Line  | 0               | 12(12.5)  |         |
| Duration of Current Reg. | 0–12 | 1(16.7)          | 5(5.2)    |         |
|                  | 13–60     | 5(83.3)          | 85(88.5)  |         |
|                  | 61 plus   | 0               | 6(6.3)    |         |
| W.H.O Staging    | Stage 1   | 0               | 19(29.7)  |         |
|                  | Stage 2   | 1(25)            | 14(21.9)  |         |
|                  | Stage 3   | 3(75)            | 24(37.5)  |         |
|                  | Stage 4   | 0               | 7(10.9)   |         |
| BMI              | Normal    | 4(66.7)          | 78(81.3)  |         |
|                  | <-2SD     | 0               | 5(5.2)    |         |
|                  | <-3SD     | 1(16.7)          | 5(5.2)    |         |
|                  | >1SD      | 1(16.7)          | 7(7.3)    |         |
|                  | >2SD      | 0               | 1(1.0)    |         |
| Stunting         | >-2SD     | 5(83.3)          | 66(68.8)  |         |
|                  | <-2SD     | 0               | 18(18.8)  |         |
|                  | <-3SD     | 1(16.7)          | 12(12.5)  |         |
| Baseline CD4 Count | 0–200   | 2(50)            | 10(18.2)  |         |
|                  | 201–500   | 1(25)            | 27(49.1)  |         |
|                  | 501–800   | 1(25)            | 15(27.3)  |         |
|                  | 801 plus  | 0               | 3(5.5)    |         |
| Proteinuria      | Yes       | 3(50)            | 32(34.4)  |         |
|                  | No        | 3(50)            | 61(65.6)  | 1.91(0.36–9.99) 0.358 |
| **eGFR < 90ml/min/1.73m²** | Yes | 3(50)            | 36(37.9)  |         |
|                  | No        | 3(50)            | 59(62.1)  | 1.64(0.31–8.56) 0.426 |

* eGFR = estimated glomerular filtration rate, * BMI = Body mass Index

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hypophosphatemia in patients in the current study was unknown. In one study in children on TDF the phosphorous level normalised in four patients who had DAIDS toxicity grade 1 or 2 without discontinuation of therapy [39]. In a follow up study of 26 children for 132 months, hypophosphatemia occurred 72 months after commencing TDF but was of no clinical significance [40]. In a case series in adult patients on TDF, hypophosphatemia and osteomalacia necessitated discontinuation of therapy [41].

Patients in this study might still be at risk of rickets and or osteomalacia as they grow and continue taking TDF, hence we recommend continued clinical monitoring and follow up of patients with hypophosphatemia. Participants with stunting were more likely to have hypophosphatemia. However the numbers were small to make a general conclusion. The study cannot conclude on the contribution of malnutrition to hypophosphatemia rather than TDF. Future studies following up patients with hypophosphatemia who are stunted and on TDF may answer this question. A longitudinal prospective study of progressive impairment of renal function normalized by nutrition and HIV and phosphate excretion estimated on (a) timed (not ‘spot’ specimens) and (b) best expressed as tubular maximum of phosphate factored by GFR (i.e. TmP/GFR) is recommended for future studies. Normoglycaemic glycosuria was reported in only one child. This is much lower than reported in other studies in adults [12, 32]. Genetic polymorphism in the ATP binding cassette subfamily C2 (ABCC2) a gene that codes for organic anion transporters in the kidney has been reported as a risk factor for TDF.
associated nephrotoxicity in previous studies [42–44]. This was not investigated in this study that may also contribute to a lower prevalence of tubular dysfunction.

Proteinuria

Urinary dipstick was positive for protein in 15.7% of participants and this doubled on urine protein/creatinine ratio. Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification, and Stratification (KDOQI) guidelines recommend spot urine dipstick and urine protein/creatinine ratio to confirm proteinuria in children [8, 28]. Although a 24 hour urine collection is considered the gold standard for proteinuria, urine dipstick proteinuria with confirmatory urine protein creatinine ratio is also acceptable [45]. Urine albumin/total protein ratio ($\text{uAPR}) < 0.4 identifies tubular pathology in proteinuric patients and this was not measured in our study [46]. The prevalence of proteinuria was much higher than what was reported in an earlier study in ART-naïve children in Zimbabwe, where persistent proteinuria was reported in 5% of the study population [10]. The difference in findings could be because the study by Dondo et al was on ART naïve younger patients (2–12 years). The prevalence is also lower than what was reported in studies elsewhere [12, 47]. Longer duration on TDF was a negative predictor of detection of proteinuria. In this study, the odds of having proteinuria in patients on TDF for $\geq 60$ months was 0.69 CI (0.10; 4.52) compared to those with shorter duration. This was different to findings by Purswani et al, where TDF duration greater than three years was the single predictor of proteinuria [48]. Exposure to PI regimen was a positive predictor for having proteinuria. The findings are similar to other studies which showed the combination of a PI and TDF was associated with proteinuria [49, 50]. Since no other causes of proteinuria were assessed the authors do not conclude that PI is the cause of the proteinuria.

### Table: Factors associated with reduction in eGFR in HIV-infected children, on TDF for at least 6 months, $< 18$ years old, $(n=190)$. Children less than 18 years who weighed at least 25 kg and were on a TDF based regimen for at least six months were eligible for the study. Participants with pre-existing diabetes or hypertension were excluded from the study. Following serum creatinine determination estimated glomerular filtration rate (eGFR) was calculated using the Schwartz formula. $^\dagger$8 samples not processed due to a technical fault at laboratory. $^\ddagger$Statistically significant at $\alpha = 0.05$. $^\circ$Only age had a p-value greater than 0.25 in the univariate model but it was also included in the multivariate model to account for any confounding effects age may have on eGFR.

| Variable         | Category | eGFR<90ml/min/1.73m² | Univariate          | Multivariate $^\circ$ |
|------------------|----------|----------------------|---------------------|-----------------------|
|                  |          | Yes                  | No                  | OR (95% CI) p-value   | OR(95% CI) p-value |
| Age (years)      | 0 - 14   | 29 (42.6)            | 60 (49.2)           | 0.77 [0.42, 1.40] 0.387 | 0.78[0.33; 1.84] 0.574 |
|                  | $\geq$ 15| 39 (57.4)            | 62 (50.8)           | 1                     1 |
| Gender           | Female   | 23 (33.8)            | 64 (52.5)           | 2.16 [1.17, 3.99] 0.014* | 1.32[0.57; 3.08] 0.517 |
|                  | Male     | 45 (66.2)            | 58 (47.5)           | 1                     1 |
| Current ART Regimen | TDF/3TC/EFV | 38 (55.9)            | 99 (81.1)           | 1                     1 |
|                  | TDF/3TC/NVP | 9 (13.2)           | 14 (11.5)           | 1.67 [0.67, 4.19] 0.270 | 1.05[0.29; 3.82] 0.937 |
|                  | TDF/3TC/PI | 21 (30.9)          | 9 (7.4)             | 6.08[2.56, 14.45]<0.001* | 4.43[1.32; 4.89]* 0.016* |
| Stunting         | $>-2$SD  | 47 (69.1)            | 101 (82.8)          | 1                     1 |
|                  | $<-2$SD | 11 (16.2)            | 13 (10.7)           | 1.82 [0.76, 3.46] 0.180 | 1.56[0.41; 6.00] 0.518 |
|                  | $<-3$SD | 10 (14.7)            | 8 (6.6)             | 2.69 [1.00, 7.24] 0.051 | 2.17[0.49; 9.50] 0.305 |
| Baseline CD4 Count | 201 – 500 | 18 (40)            | 42 (56.8)           | 1                     1 |
|                  | $\leq$200 | 8 (17.8)           | 16 (21.6)           | 1.19 [0.43, 3.28] 0.731 | 1.16[0.40; 3.36] 0.788 |
|                  | $\geq$800 | 11 (24.4)         | 13 (17.6)           | 2.02 [0.76, 5.35] 0.156 | 1.71[0.60; 4.87] 0.313 |

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Gender and age were not predictors of proteinuria. Patients with advanced disease on this study were less likely to get proteinuria. This was different from findings by Dondo et al., where WHO stage 3 or 4 disease was associated with proteinuria [10]. This could be due to the difference in study population. This study used the WHO clinical stage recorded at HAART initiation.

Glomerular filtration rate
The prevalence of decreased eGFR was, comparable to an earlier study in ART naïve children in Zimbabwe [10]. However, studies in adult African patients had variable prevalence, 7.5% -86.5% [51–53]. The differences may be due to the different study methods, populations, adults compared to children and definition and cutoff of reduction in eGFR < 60ml/min/1.73m$^2$ versus < 90ml/min/1.73m$^2$. Combination with a PI was significantly associated with reduction in eGFR. On multivariate analysis use of PI was the only independent factor associated with reduction in eGFR. These findings were similar to other studies in children elsewhere [36, 54]. Advanced HIV disease, at least WHO stage 3 diseases was also associated with reduction in eGFR. Similar findings were reported by Dondo et al., though patients were younger and ART naïve [10]. Severe stunting was significantly associated with reduction in eGFR. TDF may need to be used with caution in these children. Children on TDF for more than 60 months were 3.5 times likely to have reduction in eGFR. Cianflone et al., reported an increase in reduction in GFR with longer duration of TDF in adult patients initiating HAART [55]. The World health organisation has now included tenofovir alafenamide (TAF) for children greater than 6 years weighing at least 25kg. The use of TAF versus TDF and effect of glomerular function will pave way for future studies [56].

Conclusions
Proximal tubular dysfunction was uncommon among HIV infected children on TDF based regimen attending the outpatient HIV clinics at two tertiary hospitals in Harare. Hypophosphatemia was common and prevalence of proteinuria was high. Severe stunting was associated with hypophosphatemia. Combination with protease inhibitor was a risk factor for proteinuria. Reduction in eGFR was reported in children on a PI and those severely stunted. Combination with a PI drug regimen was the only independent factor associated with reduction in eGFR on multivariate analysis. Tenofovir alafenamide (TAF) that has different metabolism to TDF and is less nephrotoxic may be replacing the latter in the future [57] TAF’s unique pharmacokinetic profile enables provision of lower required doses for antiviral efficacy. Lower concentrations reach renal tubules minimizing intracellular accumulation and mitochondrial damage hence less nephrotoxicity compared to TDF [58]. With future guidelines moving towards the use of integrase inhibitors the interaction with TDF/TAF and renal function is an area of future research. Children on TDF for longer duration for > 60 months may benefit from glomerular filtration measurement to assess their renal function. Further studies in children with hypophosphatemia and malnutrition may be of use in future. Patients with concomitant use of TDF and a PI regimen may benefit from targeted monitoring of glomerular function in resource-limited settings.

Study limitations
This study was a cross-sectional study hence causality could not be determined. This study only looked at patients who were on TDF based regimen without comparing with patients who were on non–TDF. Future studies comparing proximal renal tubular function in children on both TDF and non-TDF based regimen are recommended to ascertain the causality of
proximal renal tubular dysfunction by TDF as this was not studied in the current study. Viral load was not done due to financial constraints but this could have helped to relate tubular and glomerular dysfunction to viral load.

Supporting information

S1 File. Original tables for Figs 1 and 2. (DOCX)

S2 File. Laboratory protocol can be found at protocols.iohttps://protocols.io/view/pone-d-19-35784-proximal-renal-tubular-function-in-bfzxjp7n and DOI: dx.doi.org/10.17504/protocols.io.bfzxjp7n. (DOCX)

S3 File. MDB file for the data sets https://datadryad.org/stash/share/pRmK7SDWzk5Jnd3-usw2hKCGVe71OksxVKbxfTZv5PE and DOI (doi:10.5061/dryad.2fqz612mf). (ACCDB)

S4 File. Definition of terms. (DOCX)

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References

1. Organization WH. Technical update on treatment optimization: use of tenofovir in HIV-infected children and adolescents: a public health perspective. 2012.

2. Nelson MR, Katiama C, Montaner JS, Cooper DA, Gazzard B, Ciotet B, et al. The safety of tenofovir disoproxil fumarate for the treatment of HIV infection in adults: the first 4 years. Aids. 2007; 21(10):1273–81. https://doi.org/10.1097/QAD.0b013e3280b7b33 PMID: 17545703

3. Elias A, Ijeoma O, Edikpo NJ, Oputiri D, Geoffrey O-BP. Tenofovir Renal Toxicity: Evaluation of Cohorts and Clinical Studies Part 2. Pharmacology & Pharmacy. 2014; Vol.05 No.01:15.

4. Elias A, Ijeoma O, Edikpo NJ, Oputiri D, Geoffrey O-BP. Tenofovir Renal Toxicity: Evaluation of Cohorts and Clinical Studies Part One. Pharmacology & Pharmacy. 2013; Vol.04 No.09:12.

5. Kalyesubula R, Perazella MA. Nephrotoxicity of HAART. AIDS research and treatment. 2011; 2011:562750–9. https://doi.org/10.1155/2011/562750 PMID: 21860787

6. Fiseha T, Gebreweld A. Urinary Markers of Tubular Injury in HIV-Infected Patients. Biochemistry research international. 2016; 2016:1501785–. https://doi.org/10.1155/2016/1501785 PMID: 27493802

7. Guidelines for Antiretroviral Therapy for the Prevention and Treatment of HIV in Zimbabwe. 2017. Available from: [www2.kidney.org/professionals/kdoqi/guidelines_ckd/p5_lab_g5.htm].

8. NKF KDOQI GuidelinesAccessed 15 June 2017. Available from: [http://www2.kidney.org/professionals/kdoqi/guidelines_ckd/p5_lab_g5.htm].

9. Iduoriyekemwen NJ, Sadoh WE, Sadoh AE. Prevalence of renal disease in Nigerian children infected with the human immunodeficiency virus and on highly active antiretroviral therapy. Saudi journal of kidney diseases and transplantation: an official publication of the Saudi Center for Organ Transplantation, Saudi Arabia. 2013; 24(1):172–7.

10. Dondo V, Mujuru HA, Nathoo KJ, Chirehwa M, Mufandaedza Z. Renal abnormalities among HIV-infected, antiretroviral naive children, Harare, Zimbabwe: a cross-sectional study. BMC Pediatr. 2013; 13:75. https://doi.org/10.1186/1471-2431-13-75 PMID: 23663553

11. Ferrand RA, Munawa L, Matseketane J, Bandason T, Nathoo K, Ndhlou CE, et al. Undiagnosed HIV infection among adolescents seeking primary health care in Zimbabwe. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2010; 51(7):844–51.

12. Chadwick DR, Sarfo FS, Kirk ESM, Owusu D, Bedu-Addo G, Parris V, et al. Tenofovir is associated with increased tubular proteinuria and asymptomatic renal tubular dysfunction in Ghana. BMC Nephrology. 2015; 16(1):195.

13. Blossner M, Siyam A, Borghi E, Onyango A, de Onis M, Borghi E, et al. WHO AnthroPlus for Personal Computers Manual: Software for Assessing Growth of the World’s Children and Adolescents. 2009.

14. WHO. The WHO Child Growth Standards. 2009.

15. WHO. Height-for-age (5–19 years) [Internet]. WHO. [cited 2017 Jun 12] Available from: http://www.who.int/growthref/who2007_height_for_age/en/.

16. WHO. BMI classification tables for children 5–19 years to BMI who—Google Search. Internet[cited 2017 Feb 26] Available from: https://www.google.com/search?q=Table++The+International +Classification+of++5-19+yeaarsadolescents++underweight%2C+overweight+and+obesity +according+to+BMI&ie=utf8&q=Table++The+International +Classification+of++5-19+yeaarsadolescents++underweight%2C+overweight+and+obesity +according+to+BMI&oe=utf8#safe=active&q=BMI+classification+tables+for+children+5-19 +years+++to+BMI+who. 2017.

17. Simerville JA, Maxted WC, Pahira JJ. Urinalysis: a comprehensive review. American family physician. 2005; 71(6):1153–62. PMID: 15791892

18. JT D. Handbook of Chronic Kidney Disease Management. 2012:656.

19. VK B. Serum Inorganic Phosphorus. Clinical Methods: The History, Physical, and Laboratory Examinations. Walker HK HW, Hurst JW, editors, editor. Boston: Butterworths; 1990: C. 198.

20. Orsonneau JL, Douet P, Massoubre C, Lustenberger P, Bernard S. An improved pyrogalol red-molybdate method for determining total urinary protein. Clin Chem 1989; 35:2233–6 PMID: 2582622

21. Peake *Michael WM. Whiting M. Measurement of Serum Creatinine–Current Status and Future Goals. Clin Biochem Rev 2006; 27:173–84 PMID: 17581641

22. Ou M, Song Y, Li S, Liu G, Jia J, Zhang M, et al. LC-MS/MS Method for Serum Creatinine: Comparison with Enzymatic Method and Jaffe Method. PLOS ONE 2015: 10:e0133912 https://doi.org/10.1371/journal.pone.0133912 PMID: 26207996
23. Berg UB, Nyman U, Bäck R, Hansson M, Monemi KÅ, Herthelius M, et al. New standardized cystatin C and creatinine GFR equations in children validated with inulin clearance. Pediatr Nephrol 2015; 30:1317–26. https://doi.org/10.1007/s00467-015-3060-3 PMID: 25903639

24. Inker LA WC, Creamer R, Hellinger J, Hotta M, Leppo M, Levey AS, et al. Performance boosted PI +TDF associated with renal function. Conference on Viruses and opportunistic infections:8–11 February 2009; Montreal, Canada.

25. Gaasbeek A, Edo Meinders A. Hypophosphatemia: An update on its etiology and treatment. Am J Med 2005; 118:1094–101. https://doi.org/10.1016/j.amjmed.2005.02.014 PMID: 16194637

26. DAIDS adverse event clarification memo adult and pediatric adverse events grading the severity—table_for_grading_severity_of_adult_pediatric_adverse_events. 2004 Accessed 3 March 2016. Available from: http://src.tech-res.com/document/safetyandpharmacovigilance/table_for_grading_severity_of_adult_pediatric_adverse_events.pdf.

27. Grade ES.2004 Accessed 23 January 2017. Available from: https://www.ucdmc.ucdavis.edu/clinicaltrials/StudyTools/Documents/DAIDS_AE_GradingTable_FinalDec2004.pdf.

28. Levey AS, Eckardt K-U, Tsukamoto Y, Levin A, Coresh J, Rossert J, et al. Definition and classification of chronic kidney disease: A position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney International. 2005; 67(6):2089–100. https://doi.org/10.1111/j.1523-1755.2005.00365.x PMID: 15882252

29. Vigano A, Zuccotti G, Martelli L, Giacomet V, Cafarelli L, Borgonovo S, et al. Renal Safety of Tenofovir in HIV-infected Children. Clin Drug Investig 2007; 27:573–81. https://doi.org/10.2165/00044011-200727080-00006 PMID: 17638398

30. Vigano A, Zuccotti GV, Martelli L, Giacomet V, Cafarelli L, Borgonovo S, et al. Renal safety of tenofovir in HIV-infected children: a prospective, 96-week longitudinal study. Clinical drug investigation. 2007; 27(8):573–81. https://doi.org/10.2165/00044011-200727080-00006 PMID: 17638398

31. Labarga P, Barreiro P, Martin-Carbonero L, Rodriguez-Novoa S, Solera C, Medrano J, et al. Kidney tubular abnormalities in the absence of impaired glomerular function in HIV patients treated with tenofovir. AIDS. 2009; 23(6):689–96. https://doi.org/10.1097/QAD.0b013e32832ddde4 PMID: 19263355

32. Samarawickrama A, Cai M, Smith E, Nambiar K, Sabin C, Fisher M, et al. Simultaneous measurement of urinary albumin and total protein may facilitate decision-making in HIV-infected patients with proteinuria. HIV Med 2012; 13:526–32. https://doi.org/10.1111/j.1468-1293.2012.01003.x PMID: 22413854

33. Perazzo S, Soler-García AA, Hathout Y, Das JR, Ray PE. Urinary Biomarkers of Kidney Diseases in HIV-infected children. Proteomics Clin Appl 2015; 9:490–500. https://doi.org/10.1002/prca.201400193 PMID: 25764519

35. Post FA, Wyatt CM, Mocroft A. Biomarkers of impaired renal function. Curr Opin HIV AIDS 2010; 5:524–30. https://doi.org/10.1097/COH.0b013e328339203e PMID: 20978396

36. Fux C, Opravil M, Cavassini M, Calmy A, Flepp M, Gurtner-Delafuente V, et al. Tenofovir and PI Use Are Associated with an Increased Prevalence of Proximal Renal Tubular Dysfunction in the Swiss HIV Cohort Study: boosted PI+TDF associated with renal function. Conference on Viruses and opportunistic infections:8–11 February 2009; Montreal, Canada.

37. Cheng S, Vijayan A. The Washington Manual of Nephrology Subspecialty Consult. Lippincott Williams & Wilkins; 2013.

38. Schrier R. Diseases of the Kidney and Urinary Tract.: Lippincott Williams & Wilkins; 2007.

39. 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 Infect Dis 2012; 12:18. https://doi.org/10.1186/1471-2334-12-18 PMID: 22269183

40. Giacomet V, Nannini P, Vigano A, Erza P, Benincasa 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. Clin Drug Invest 2015; 35:419–26 https://doi.org/10.1007/s40261-015-0293-7 PMID: 26013475

41. Tjen-A-Looi A, Naseer SN, Worthing AB, Timpone JG, Kumar PN. Hypophosphatemic Osteomalacia Associated with Tenofovir Use in HIVInfected Patients: A Case Series and Review of the Literature. J AIDS Clin Res 2012; 01:2155–6113.

42. Salvaggio SE, Giacomelli A, Falvela FS, Oreni ML, Meraviglia P, Atzori C, et al. Clinical and genetic factors associated with kidney tubular dysfunction in a real-life single centre cohort of HIV-positive patients. BMC Infectious diseases. 2017; 17(1):396--. https://doi.org/10.1186/s12879-017-2497-3 PMID: 28583112
43. Dahlin A, Wittwer M, de la Cruz M, Woo J, Bam R, Scharen-Guivel V, et al. A pharmacogenetic candidate gene study of tenofovir-associated Fanconi syndrome. Pharmacogenetics and genomics. 2015; 25(2):82–92. https://doi.org/10.1097/FPC.0000000000000110 PMID: 25485598

44. Nishijima T, Komatsu H, Higasa K, Takano M, Tsuichiya K, Hayashida T, et al. Single Nucleotide Polymorphisms in ABC22 Associate With Tenofovir-Induced Kidney Tubular Dysfunction in Japanese Patients With HIV-1 Infection: A Pharmacogenetic Study. Clinical Infectious Diseases. 2012; 55(11):1558–67. https://doi.org/10.1093/cid/cis772 PMID: 22955427

45. Estrella MM, Fine DM. Screening for chronic kidney disease in HIV-infected patients. Adv Chronic Kidney Dis. 2010; 17(1):26–35. https://doi.org/10.1053/j.ackd.2009.07.014 PMID: 2005486

46. Samarakramma A, Cai M, Smith E, Nambiar K, Sabin C, Fisher M, et al. Simultaneous measurement of urinary albumin and total protein may facilitate decision-making in HIV-infected patients with proteinuria. HIV Medicine. 2012; 13(9):526–32. https://doi.org/10.1111/j.1468-1293.2012.01003.x PMID: 22413854

47. Izzedine H, Hulot JS, Vittecoq D, Gallant JE, Staszewski S, Launay-Vacher V, et al. Long-term renal safety of tenofovir disoproxil fumarate in antiretroviral-naive HIV-1-infected patients: Data from a double-blind randomized active-controlled multicentre study. Nephrol Dial Transplant. 2005; 20:743–6

48. Purswani M, Patel K, Kopp JB, Seage GR, Chernoff MC, Hazra R, et al. Tenofovir Treatment Duration Predicts Proteinuria in a Multi-Ethnic United States Cohort of Children and Adolescents with Perinatal HIV-1 Infection. Pediatr Infect Dis J 2013; 32:495–500 https://doi.org/10.1097/INF.0b013e31827f4eff PMID: 23249917

49. Cao Y, Gong M, Han Y, Xie J, Li X, Zhang L, et al. Prevalence and risk factors for chronic kidney disease among HIV-infected antiretroviral therapy-naive patients in mainland China: a multicenter cross-sectional study. Nephrol Carlton Vic 2013; 18:307–12

50. Izzedine H, Harris M, Perazella MA. The nephrotoxic effects of HAART. Nat Rev Nephrol 2009; 5:563–73. https://doi.org/10.1038/nrneph.2009.142 PMID: 19776778

51. Fana GT, Ndhlovu CE. Renal dysfunction among antiretroviral therapy naive HIV infected patients in Zimbabwe. Cent Afr J Med 2011; 57:1–5.

52. Msango L, Downs JA, Kalluvya SE, Kidenya BR, Kabangila R, Johnson WD, et al. Renal dysfunction among HIV-infected patients starting antiretroviral therapy. AIDS 2011; 25:1421–5. https://doi.org/10.1097/QAD.0b013e328348a4b1 PMID: 21572304

53. Sarfo FS, Keegan R, Appiah L, Shakoor S, Phillips R, Norman B, et al. High prevalence of renal dysfunction and association with risk of death amongst HIV-infected Ghanaians. J Infect 2013; 67:43–50. https://doi.org/10.1016/j.jinf.2013.03.008 PMID: 23542785

54. Post FA, Moyle G, Stellbrink HJ, Domingo P, Podzamczzer D, Fisher M, et al. Randomized comparison of renal effects, efficacy, and safety with once-daily abacavir/lamivudine versus tenofovir/emtricitabine, administered with efavirenz, in antiretroviral-naive, HIV-1-infected adults: 48-week results from the ASSERT study. J Acquir Immune Defic Syndr 2010; 55:49–57. https://doi.org/10.1097/QAI.0b013e3181dd911e PMID: 20515994

55. Crum-Cianflone N, Ganesan A, Teneza-Mora N, Riddle M, Medina S, Barahona I. Prevalence and factors associated with renal dysfunction among HIV-infected patients. AIDS Patient Care STDs 2010; 24:353–60. https://doi.org/10.1089/apc.2009.0326 PMID: 20515419

56. Organisation WH. Paediatric ARV drug optimization 3 review [Teleconference—12 December 2017,]. 2017 [Summary report]. Available from: https://www.who.int/hiv/pub/meetingreports/pado3-review/en/index4.html.

57. Highleyman L. HIV & AIDS Information: Switching from tenofovir DF to TAF improves bone and kidney safety. Accessed 15 June 2017. Available from: http://www.aidsmap.com/Switching-from-tenofovir-DF-to-TAF-improves-bone-and-kidney-safety/page/3070140.

58. Novick TK, Choi MJ, Rosenberg AZ, McMahon BA, Fine D, Atta MG. Tenofovir alafenamide nephrotoxicity in an HIV-positive patient: A case report. Medicine (Baltimore). 2017; 96(36):e8046–e. https://doi.org/10.1097/MD.0000000000008046 PMID: 28885375