Tenofovir disoproxil fumarate (TDF) is a prodrug of tenofovir diphosphate, a structural analog of deoxyadenosine triphosphate, which is the natural substrate for the viral enzyme reverse transcriptase. By competing with the natural substrate, TDF diphosphate inhibits the synthesis of viral DNA from its RNA.

Clinically important toxicities were rarely observed in phase III clinical registration trials; hence, TDF was considered to have a favorable safety profile. It was first approved by the Food and Drug Administration (FDA) for the treatment of HIV in combination with other antiretroviral drugs in 2001, and with good efficacy and safety profiles, TDF was recommended as a first-line treatment of HIV infection in both high-income and low-to-middle income countries.

In 2002, the first case of tenofovir-induced acute tubular toxicity due to TDF was reported. It consisted of both a proximal tubular injury with the combination of Fanconi syndrome and acute renal failure and a distal tubular injury in the form of nephrogenic diabetes insipidus. Since then, multiple case reports and studies have linked TDF use with various renal dysfunction, decreased bone density, and increased mortality.

A number of factors have been identified as adding risk to the development of TDF-induced nephrotoxicity including advanced age, low body mass index (BMI), diabetes mellitus (DM), hypertension (HTN), co-use of other nephrotoxic...
drugs such as protease inhibitors (PI) and didanosine, treatment experience, and genetic polymorphism in transporters involved in regulating TDF intracellular concentration.16–22

The FDA approved a new formulation of tenofovir, tenofovir alafenamide (TAF) in 2015 for the treatment of HIV. It has been reported to maintain the efficacy of TDF with less nephrotoxicity by virtue of its concentration into effector cells (smaller therapeutic dose).23

Given the multiple reports regarding the TDF nephrotoxicity with some countries already switching to TAF, we decided to investigate any toxic effects of TDF in our cohort of Omani patients. We had been following our patients by checking their electrolytes and estimated glomerular function rate (eGFR) every six to 12 months per the Infectious Diseases Society America guidelines and were satisfied with the results.24 However, we decided to add other parameters to look specifically for any tubular dysfunction including the fractional excretion of phosphate (FEPi) and urinary protein creatinine ratio (uPCR).

Our study aimed to determine the prevalence of TDF-induced nephrotoxicity in our cohort of Omani patients with HIV. We also investigated additional nephrotoxic effects of other parameters like duration of TDF treatment, age and BMI of patients at the time of the study, initial CD4 count, initial viral load (VL), concomitant use of PI, and comorbidities like DM and HTN. Our aim was to determine if we need to switch to TAF or other non-tenofovir regimens.

### METHODS

We conducted a single-center observational study on a cohort of 83 Omani patients with HIV currently on TDF-containing antiretroviral therapy. Our center is one of three main centers in the capital area. Data were collected on visits, and other related data were extracted from the electronic system in the hospital. All Omani patients currently on TDF (except three who refused) were included in the study. All non-Omani patients were excluded. We used several parameters to assess the renal function including the eGFR, serum creatinine, FEPi, and uPCR.

We used MediCal® to calculate the fractional excretion of phosphate by applying the formula:

\[
\text{FEPi} = \frac{\text{phosphate (urine)} \times \text{creatinine (serum)}}{\text{phosphate (serum)} \times \text{creatinine (urine)}}
\]

FEPi and uPCR were stratified as low, moderate, and high using MediCal® [Table 1].25

| Grade   | FEPi, % | uPCR, mg/mmol |
|---------|---------|---------------|
| Low     | < 10    | < 15          |
| Intermediate | 10–20 | 15–50         |
| High    | > 20    | > 50          |

FEPi: fractional excretion of phosphate; uPCR: urinary protein creatinine ratio.

For patients with abnormal values dictating cessation of TDF, we continued measuring these parameters to determine any potential improvement in their renal function.

We classified FEPi as low (< 10%), moderate (10–20%), and high (> 20%) in the presence of low serum phosphate (sPO4)(< 0.8).26

We classified the severity of uPCR as that for chronic kidney disease with low (< 15 mg/mmol), moderate (15–50 mg/mmol), and severe (> 50 mg/mmol) with normal levels < 5 mg/mmol in a healthy adult.27

eGFR was obtained from the laboratory values calculated using the Modification of Diet in Renal Disease formula. A drop > 25% was considered significant.28

Increased serum creatinine was defined as a ≥ 1.5-fold increase in baseline creatinine per the acute kidney injury definition in kidney disease improving global guidelines.27

We studied the effects of duration of TDF use, age and BMI of the patients at the time of the study, gender, initial CD4 count, and initial VL. We also investigated any impact of DM and HTN and concomitant use of PI on the TDF-associated nephrotoxicity [Table 2]. We could not study the effect of BMI at the start of TDF as some old data on weights were not recorded in the system.

VL used to be measured with less sensitive assays with < 400 copies/mL being the minimal cutoff detected while the current assays detect < 20 copies/
To overcome this problem, we considered all values < 400 copies/mL as undetectable. We divided the patients into groups of low (L), intermediate (IM), and high (H) according to their FEPi and uPCR values. We used medians to describe the clinical parameters of the groups since the distribution of data was not normal. To investigate the impact of cofactors, we used Fisher’s exact test as more than 80% of cells had values < 5.

We used an alpha threshold of 0.05 for statistical significance. We used the SPSS Statistics (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.) program.

**RESULTS**

From 7 June 2016 to 9 August 2017, we collected the blood and urine samples of 83 patients currently on TDF. Fifty-one (61.4%) patients were males and 32 (38.6%) were females. The median age and BMI at the time of the study, duration of TDF use, initial CD4 count, and VL are given in Table 3.

Twenty-one (25.3%) patients were concomitantly on PI in the form of darunavir/ritonavir or lopinavir/ritonavir (Kaletra, AbbVie).

Fifteen patients (18.1%) had DM (eight males and seven females), 14 (16.9%) had HTN (seven males and seven females), and five (6.0%) patients had both DM and HTN.

FEPi was high in two (2.4%) patients, moderate in 26 (31.3%), and low in 55 (66.3%) patients. Two patients had FEPi > 10% with hypophosphatemia, and three patients had FEPi > 20%, two with normal sPO4 and one with hypophosphatemia. Among these five patients, two had moderate uPCR, and none had severe uPCR. One patient had HTN and two had DM. No patients had Fanconi syndrome.

uPCR was low, moderate, and high in 45 (54.2%), 28 (33.7%), and 10 (12.0%) patients, respectively. Seventeen (20.5%) and eight (9.6%) patients had uPCR of 15–20 mg/mmol and 20–50 mg/mmol, respectively. The median uPCR was 14.6 (range = 5.7–435.0). We obtained urine samples of 25 patients not on TDF (total number of patients not on TDF = 34). Their median uPCR was 14.2 (range = 7.4–286.0), and six (17.6%) had uPCR of 20–50 mg/mmol.

Serum creatinine increased in 44 (53.0%) patients with a mean of 1.3 (range = 1.0–2.2) and median of 1.2, decreased in 20 (24.1%) patients with a mean of 0.8 (range = 0.1–0.9) and median of 0.9, and did not change in the remaining 19 (22.9%) patients. Six patients had an increase in the serum creatinine of 1.5-fold the baseline.

Two females and two males had subnormal initial creatinine levels when they presented with emaciation and normal levels at the time of the study. They all had normal phosphate excretion. One patient had both DM and HTN, and two had DM alone.

eGFR remained > 90 mmol/L in 50 patients (60.1%), decreased in 12 (14.5%) patients, increased in four (4.8%) patients, and was undetermined in 17 (20.5%) patients. The undetermined values were due to data unavailability in the record systems. Among those in whom the eGFR dropped, only one patient had a drop > 25%. This patient also had a 1.5-times increase in serum creatinine, a uPCR of 32 and normal FEPi. He had neither DM nor HTN. In the

| Characteristics | Median | Min  | Max  |
|-----------------|--------|------|------|
| Duration, weeks | 178    | 3    | 554  |
| Age, years      | 42     | 21   | 80   |
| Body mass index | 27     | 17.4 | 42.7 |
| CD4             | 205    | 3    | 1745 |
| Viral load, copies/L | 37250  | 0    | 9,523,428 |

**Table 2: Categories of parameters used in the study.**

| Category | Duration, weeks | Age, years | Body mass index | CD4 | Viral load, copies/L |
|----------|-----------------|------------|-----------------|-----|---------------------|
| 1        | 0–99            | 21–30      | Underweight: < 18.5 kg | 0–49 | 0–999 |
| 2        | 100–199         | 31–40      | Normal weight: 18.5–24.9 kg | 50–199 | 1000–9999 |
| 3        | 200–299         | 41–50      | Overweight: 25.0–29.9 kg | 200–349 | 10000–99999 |
| 4        | 300–399         | 51–60      | Obese: ≥ 30.0 kg | > 350 | 100000–999999 |
| 5        | 400–499         | 61–70      |                 | 1000000 |
| 6        | 500–599         | 71–80      |                 |       |

**Table 3: Median with maximal and minimal values for 83 patients with HIV taking TDF treatment.**

TDF: tenofovir disoproxil fumarate.
remaining patients, the drop ranged from 1.1–24.4 with a mean of 8.2%.

No significant impact was found on the severity of FEPi and uPCR by the duration of TDF use, BMI, DM, gender, initial VL, or concomitant use of PI (p-values were all > 0.050) [Table 4].

**DISCUSSION**

The reported prevalence of TDF renal effects in HIV cohorts is widely variable ranging from 2.4% occurring after six to nine months to 20%. The prevalence is higher in Asian cohorts from Japan and India.²⁹⁻³¹

Various parameters have been used to investigate the nephrotoxic effects of TDF including eGFR, serum creatinine, FEPi, and proteinuria in the form of uPCR or urinary albumin-creatinine ratio. Some studies, however, used more specific markers for tubular injury as TDF was shown to cause proximal tubular injury due to mitochondrial toxicity. Such markers include β2 microglobulin, retinol binding protein/creatinine ratio, and α-1 microglobulin.⁹,³²,³³

Renal tubular dysfunction was reported in 10–22% of HIV-positive patients receiving TDF using variable parameters and definitions.⁹,³⁴,³⁵

Due to unavailability of tubular specific markers in our setting, we opted to measure FEPi, uPCR, serum creatinine, and eGFR. Interpretation of the results, however, proved to be difficult due to lack of consensus regarding clear definitions for TDF-induced toxicity.

Regarding FEPi, five (6.0%) patients had FEPi suggestive of tubular dysfunction (using the Waheed et al,³⁶ definition) with tubular FEPi being > 20% and normal or low sPO₄ or FEPi > 10% with low sPO₄ of < 0.8 mmol/L. On the other hand, using the definition of tubular dysfunction by Hamzah et al,⁶ we had only one patient satisfying tubular FEPi (FEPi of > 20% with serum hypophosphatemia). Moreover, changes in FEPi were seen to occur early post-TDF introduction by some groups and as late as 64 months by others often in the presence of normal sPO₄ and the absence of other features of Fanconi.³⁷,³⁸

For eGFR and serum creatinine, TDF-associated dysfunction was defined as a 25% drop in the eGFR or a 1.5-fold increase in serum creatinine while others did not define any cutoff values for either when comparing TDF versus TAF for renal toxicity but found the TAF group had a lower reduction in eGFR.³⁹,⁴⁰

Six of our patients (7.2%) had a 1.5-fold increase in serum creatinine from baseline, one of which also had a 25% drop in their eGFR. None had abnormal FEPi. However, four of the six patients had subnormal initial creatinine levels due to their low weight, which rapidly picked up after treatment. Therefore, it could be considered that only two patients has serum creatinine 1.5 above baseline.

Proteinuria precedes changes in eGFR and represents an early marker of renal damage, but we found uPCR the most difficult to interpret. Although studies suggest that the proteinuria caused by TDF is of tubular origin, there is no consensus regarding the numerical value definition of tubular proteinuria.⁴¹

Peyriere et al defined tubular proteinuria as uPCR > 200 mg/g (20 mg/mmol) with a urinary albumin-creatinine ratio of < 0.4 and found it to occur in 20% of patients on TDF.⁴² Proteinuria of 30 mg/dL was used by Huang et al but was seen to occur with equal prevalence in TDF and non-TDF exposed Taiwanese patients.⁴³

Moreover, it was shown when ritonavir or cobicistat were not used, there was only marginal difference in safety between TDF and TAF.⁴⁴ Additionally, pathological proteinuria, defined as uPCR of 150 mg/g, was also found to be 20% in HIV patients, but with no difference observed between the groups receiving highly active anti-retroviral therapy (HAART) and not receiving HAART.⁴⁵

| Characteristics          | FEPi, p-value | uPCR, p-value |
|--------------------------|---------------|---------------|
| Duration                 | 0.149         | 0.489         |
| Age                      | 0.292         | 0.019         |
| BMI                      | 0.468         | 0.289         |
| Diabetes mellitus        | 0.224         | 0.714         |
| Hypertension             | 0.042         | 0.131         |
| CD4                      | 0.050         | 0.664         |
| Viral load               | 0.651         | 0.355         |
| Gender                   | 0.377         | 0.279         |
| Protease inhibitor use   | 0.883         | 1.000         |

Hypertension and initial CD4 had an impact on the severity of FEPi (p < 0.042 and p < 0.050, respectively), but not on uPCR. FEPi: fractional excretion of phosphate; uPCR: urinary protein creatinine ratio; BMI: body mass index.
We had eight (9.6%) patients with tubular proteinuria (considering the level of uPCR to be 20–50 mg/mmol), the rest had either mild or severe uPCR. One had HTN, one had DM, and one had both HTN and DM. One of the patients also had an increase in eGFR of > 25%. However, in the non-TDF group, 17.6% of patients had this range of uPCR. This indicates that moderate uPCR could not be taken as an indicator for TDF-associated nephrotoxicity. Moreover, there was no difference in uPCR between the TDF group and the non-TDF group (median 14.2 vs. 14.6). Proteinuria of any level, therefore, could be multifactorial with DM, HTN, and HIV contributing factors.

Three patients who had very high uPCR (> 150 mg/mmol) had no difference in their uPCR after six months or more post-cessation of TDF. One patient had both DM and HTN, and the high uPCR level could be attributed to that. Another cause could be HIV-associated nephropathy (HIVAN), especially since this patient was of African origin and 90% of patients with HIVAN are of black origin. A young patient with vertical transmission also had persistent uPCR of nephrotic range. We are planning a renal biopsy.

Collectively, we found one patient with high FEPi and two patients with abnormal eGFR/serum creatinine making it 3.6%. Considering high FEPi as a marker of tubular dysfunction, its prevalence in patients on TDF is only 1.2%, which is much lower than the reported prevalence. This does not prove the toxicity is due to the use of TDF as we did not compare this with patients not on TDF nor did we perform a renal biopsy. We cannot use uPCR as indicative of TDF-associated nephrotoxicity since it occurred equally in TDF and non-TDF groups.

Currently, we are measuring FEPi, eGFR, serum creatinine of the patients not on TDF to compare them with the TDF group. Furthermore, we are planning to get renal tubular specific markers to investigate TDF effects on renal tubules further.

The major limiting factor of our study was its small sample size. However, it can be thought of as a basis for any planned studies in other hospitals in the country and indeed in the Gulf region. In addition, our study population come from all parts of Oman and so represent all ethnic backgrounds and regions.

In summary, based on FEPi we had only one patient (1.2%) satisfying tubular dysfunction. Taking other parameters (apart from uPCR), we had a total of three patients (3.6%) who had some renal dysfunction. uPCR cannot be used to indicate TDF-associated dysfunction.

**CONCLUSION**

Based on FEPi values only for tubular dysfunction, the prevalence of TDF-associated nephrotoxicity in our cohort was very low. However, a larger sample size and better agreed upon definition for TDF-induced nephropathy are required to confirm this.

**Disclosure**

The authors declared no conflicts of interest. No funding was received for this study.

**REFERENCES**

1. Viread (tenofovir disoproxil fumarate) tablet product labelling. Foster City, Gilead Sciences, Inc. 2003 Oct., CA, 2010 [cited June 5 2018]. Available from: www.viread.com, ac.

2. Sax PE, Gallant JE, Klotman PE. Renal safety of tenofovir disoproxil fumarate. AIDS Read 2007 Feb;17(2):90-92, 99-104, C3.

3. US Food and Drug Administration. FDA report: background pack- age for NDA 21-356: VIREAD (tenofovir disoproxil fumarate) 2001. [cited June 5 2018 ]. Available from: http://www.fda.gov/cder/approval/v.htm.

4. Gallant JE, Staszewski S, Pozniak AL, DeJesus E, Suleiman JM, Miller MD, et al; 903 Study Group. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients; a 3-year randomized trial. JAMA 2004 Jul;292(2):191-201.

5. Izzedine H, Hulot JS, Vittecoq D, Gallant JE, Staszewski S, Launay-Vacher V, et al; Study 903 Team. 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 Apr;20(4):743-746.

6. Clumee N, Pozniak A, Raffi F; EACS Executive Committee, European AIDS Clinical Society (EACS) guidelines for the clinical management and treatment of HIV-infected adults. HIV Med 2008 Feb;9(2):65-71.

7. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Recommendations for a public health approach. World Health Organization 2013 [cited June 5 2018]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/24716260.

8. Verhelst D, Monge M, Meynard JL, Fouqueray B, Mougenot B, Girard PM, et al. Fanconi syndrome and renal failure induced by tenofovir: a first case report. Am J Kidney Dis 2002 Dec;40(6):1331-1333.

9. 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 Mar;23(6):689-696.

10. Karras A, Lafaurie M, Furco A, Bourgarit A, Droz D, Sereni D, et al. Tenofovir-related nephrotoxicity in human immunodeficiency virus-infected patients: three cases of renal failure, Fanconi syndrome, and nephrogenic diabetes insipidus. Clin Infect Dis 2003 Apr;36(8):1070-1073.

11. Créput C, Gonzalez-Canali G, Hill G, Piketty C, Kazatchkine M, Nochy D. Renal lesions in HIV-1-positive patient treated with tenofovir. AIDS 2003 Apr;17(6): 935-937.
27. Khwaja A. KDIGO clinical practice guidelines for acute kidney injury. Nephron Clin Pract 2012;120(4):c179-c184. doi:10.1159/000339789.

28. Lee JE, Lee S, Song SH, Kwak IS, Lee SH. Incidence and risk factors for tenofovir-associated nephrotoxicity among human immunodeficiency-virus-infected patients in Korea. Korean J Intern Med 2017 Oct 12. [cited 2018 April 2]. Available from: https://doi.org/10.3904/kjim.2016.418.

29. Antoniou T, Raboud J, Chirhin S, Youn D, Govan Y, Gough K, et al. Incidence of and risk factors for tenofovir-induced nephrotoxicity: a retrospective cohort study. HIV Med 2005 Jul;6(4):284-290. doi:10.1111/j.1468-1299.2005.

30. Ryom L, Mocroft A, Kirk O, Werm SW, Kamara DA, Reiss P, et al; D:A:D Study Group. Association between antiretroviral exposure and renal impairment among HIV-positive persons with normal baseline renal function: the D:A:D study. J Infect Dis 2013 May;207(9):1359-1369.

31. Pujari SN, Smith C, Makane A, Youle M, Johnson M, Bele V, et al. Higher risk of renal impairment associated with tenofovir use amongst people living with HIV in India: a comparative cohort analysis between Western India and United Kingdom. BMC Infect Dis 2014 Mar;14.173.

32. Woodward CL, Hall AM, Williams IG, Madge S, Copas A, Nair D, et al. Tenofovir-associated renal and bone toxicity. HIV Med 2009 Sep;10(8):482-487.

33. Kang J, Liu J, Ding H, Li X, Wang Q, Guo X, et al. Urine alpha1-microglobulin is a better marker for early tubular dysfunction than beta2-microglobulin among tenofovir-exposed human immunodeficiency virus-infected men who have sex with men. Braz J Infect Dis 2015 Jul-Aug;19(4):410-416.

34. Nishijima T, Shimbo T, Komatsu H, Takano M, Tanuma J, Tsukada K, et al. Urinary beta2-microglobulin and alpha1 microglobulin are useful screening markers for tenofovir-induced kidney tubulopathy in patients with HIV-1 infection: a diagnostic accuracy study. J Infect Chemother 2013 Oct;19(5):850-857.

35. Ezinga M, Wetzels JG, Bosch ME, van der Ven AJ, Burger DM. Long-term treatment with tenofovir: prevalence of kidney tubular dysfunction and its association with tenofovir plasma concentration. Antivir Ther 2014;19(8):765-771.

36. Waheed S, Attia D, Estrella MM, Zafar Y, Atta MG, Lucas GM, et al. Proximal tubular dysfunction and kidney injury associated with tenofovir in HIV patients: a case series. Clin Kidney J 2015 Aug;8(4):420-425.

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

38. Fischa T, Griebewald A. Urinary markers of tubular injury in HIV-infected patients. Biochem Res Int 2016;2016:1501785.

39. Jin S, Kim MH, Park JH, Jung HJ, Lee HJ, Kim SW, et al. The incidence and clinical characteristics of acute severe creatinine elevation more than 1.5mg/dl among the patients treated with tenofovir/emtricitabine-containing HAART regimens. Infect Chemother 2015 Dec;47(4):239-246.

40. Wang H, Lu X, Yang X, Xu N. The efficacy and safety of tenofovir alafenamide versus tenofovir disoproxil fumarate in antiretroviral regimens for HIV-therapy:meta-analysis. Medicine (Baltimore) 2016;95(4):e146.

41. Campbell LJ, Dew T, Salota R, Cheseem E, Hamzah L, Ibrahim F, et al. Total protein, albumin and low-molecular-weight protein excretion in HIV-positive patients. BMC Nephrol 2012 Aug;13:85.

42. Peyriere H, Couriol A, Casanova M-L, Badouin S, Cristol J-P, Reyes J. Long-term follow up of proteinuria and estimated glomerular filtration rate in HIV patients with tubular proteinuria. PLoS One 2015 Nov;10(11):e0142991.

43. Huang Y-S, Chan C-K, Tai M-S, Lee K-Y, Lin S-W, Chang SY, et al. Kidney dysfunction associated with tenofovir exposure in human immunodeficiency virus-1-infected Taiwanese patients. J Microbiol Immunol Infect 2017
44. Hill A, Hughes SL, Gotham D, Pozniak AL. Tenofovir alafenamide versus tenofovir disoproxil fumarate: is there a true difference in efficacy and safety? J Virus Erad 2018 Apr;4(2):72-79.

45. Antonello VS, Antonello IC, Herrmann S, Tovo CV. Proteinuria is common among HIV patients: what are we missing? Clinics (Sao Paulo) 2015 Oct;70(10):691-695.

46. Kopp JB, Winkler C. HIV-associated nephropathy in African Americans. Kidney Int Suppl 2003 Feb;83(83):S43–S49.