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Original Research Article

Evidence of Subclinical Proximal Tubular Dysfunction in HIV-Infected Patients on Tenofovir Antiretroviral Therapy

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ABSTRACT

Background: Tenofovir disoproxil fumarate (TDF) is a first line antiretroviral therapy and the most widely prescribed drug in the treatment of HIV-infection but it has been found to be potentially nephrotoxic.
Methods: In order to better evaluate the effect of tenofovir on the renal tubules, we designed a cross sectional study of 254 HIV-infected patients and groups as follows: TDF group (100 patients), Non-TDF group (102 patients) and Naïve group (52 patients). Markers of tubular toxicity together with common indicators of renal damage were assessed.
Results: There was a significant difference in estimated glomerular filtration rate (eGFR) (p=0.000), proteinuria (p=0.004), glycosuria (P=0.002) and uricosuria (p=0.000) among the different regimen groups. There was also an increase in the fractional excretion of uric acid, phosphate, glucose in the TDF regimen group compared to treatment Naïve group and Non-TDF group.
Conclusion: From our result we concluded that proximal tubular toxicity is associated with HIV-infected patients exposed to TDF regimen.

Keywords: TDF, Tubular dysfunction, HIV, Nephrotoxicity.

INTRODUCTION

Tenofovir disoproxil fumarate (TDF) is an effective and widely used nucleoside reverse transcriptase inhibitor used in the treatment of both human immunodeficiency virus (HIV) and hepatitis B virus infection. Although several researchers have suggested that TDF has a low overall toxicity profile and exhibit only a moderate effect on estimated glomerular filtration rate [1,2] several studies have since appeared in the literature describing TDF-associated renal tubular dysfunction. The main effect of TDF toxicity appears to be on the proximal tubule, and in several cases, patients can develop renal Fanconi syndrome characterized by tubular proteinuria, phosphaturia.

(Evidence by proteinuria, uricosuria, phosphaturia, glycosuria, and bicarborbonate) which is usually characterized by tubular wasting or acute kidney injury.

It have been shown that TDF-associated nephrotoxicity is the most common single diagnosed reason for HIV-related referral to tertiary health services,

accounting for about 20% of consultations. [3] Tenofovir is taken up by the kidney from the blood by the organic anion transporter (OAT), located on the surface of proximal tubules cells. [4] After two additional phosphorylations, the drug is extruded by the apical multidrug space, in which urine is formed. In agreement with this metabolism, most studies have emphasized that although patients treated with TDF may rarely experience any impairment in their glomerular function, as checked by measuring creatinine plasma concentrations or creatinine clearance, [5] tubular damage may be common. However given that HIV itself [6] and other antiretroviral therapy (e.g. indinavir) show nephrotoxic potentials, there is a need to evaluate in detail renal tubular parameters in patients treated with TDF, trying to exclude the influence of confounders.

MATERIALS AND METHODS

Study population

A cross sectional study of 254 HIV-infected patients was started in August at the HIV clinic of the University of port Harcourt teaching hospital, a reference hospital for tropical infection and disease located in Port Harcourt, Nigeria. Patients under regular follow up for at least 3 months were invited to participate in the study so far they can be allocated in to any of the three groups; patients on TDF regimen, patients on Non-TDF regimen and HIV-infected patients never exposed to antiretroviral therapy.

From every patient's social-demographics details of age, sex, occupation, education, income, and HIV status (CD4 T-lymphocyte count) were recorded at the time of recruitment in to the study. Total prior exposure to antiretroviral drugs was considered as the number of months of uninterrupted anti-HIV treatment up to inclusion. Periods of drug interruption for any reason, if occurring, were discounted. This information was obtained from the patient's treatment file and pharmacy records, as all patients recruited in to the studies had to obtain antiretroviral drugs on a monthly basis from the hospital pharmacy. Treatment was provided without any cost to patients.

Renal function tests

Blood samples for serum chemistry were put in chemistry bottles while those for hematological analyses were collected using the EDTA bottles in order to avoid hemolytic reactions. The blood samples were then taken to the laboratory for various analyses.

Spot Urine samples were collected from patient's using urine sample bottle while 24hours urine was collected using urine bag. Urine samples were aliquoted within 30minutes of collection and sent to the chemistry laboratory for analyses. Urine analyses were performed within 4hours of collection to avoid denaturation of some parameters. Venous blood biochemistry included creatinine, phosphorus, total calcium, uric acid, and glucose. In parallel, creatinine, phosphorus, calcium, uric acid, and glucose, were also measured in 24 hours urine.

Proximal tubular dysfunction was diagnosed with two or more of the following abnormalities: proteinuria (defined as positive protein on urine dipstick), glycosuria (defined as ≥0.1 mg/dl), Uricosuria (≥0.05mg/dL), and phosphaturia (≥20.0 mg/dL), if they were repeatedly present.

Statistical analysis

Continuous values are given as mean and standard deviation while categorical data are given as percentages. The three groups of patients analyzed were first compared using chi-squared for categorical data and parametric tests for continuous variables. The level of significance was 0.05. All statistical analyses were performed using SPSS v20.0 software package (SPSS inc., Chicago, Illinois, USA).

RESULTS

A total of 254 HIV-infected patients were recruited in the study. At recruitment, 100 patients were under TDF (group 1), 102 patients under Non-TDF (group 2) and 52 patients were antiretroviral naive.

Characteristic of patients

The results of the educational backgrounds of the study population were as follows: 6 (2.4%) patients had no formal education, 27 (10.6%) patients had primary education, 150 (59.1%) patients had secondary education, 68 (26.8%) patients had university education and 3(1.2%) patients (p>0.272) which was not statistically significant.

Among the study population, 53 (20.9%) patients were jobless at present, 107 (42.1%) were business men and women, 54 (21.3%) were students, 26 (10.2%) were artisans and only 14 (5.5%) were civil servants. The difference among the groups was statistically significant (P >0.000).

The means age of the distinct patients' population; group 1, group 2 and group 3 are 35.23, 35.49 and 32.50 years respectively, but without a significant difference (p=0.069), although the gender distribution among the patients study population was statistically significant (p=0.002) and were observed to be; 85 (33.46%) in males and 169 (66.54%) in females as shown in table 1.

The stages of renal disorder are mainly based on measured or estimated glomerular filtration rate (eGFR). There are five stages but kidney function is normal in stage 1, minimally reduced in stage 2, and moderately reduced in stage 3. Our results show that 90 (35.43%) patients have values greater than 90 (eGFR stage 1), 142 (55.9%) have values within 90-60 (eGFR stage 2) and only 22 (8.7%) have values within 59-30 (eGFR stage 3). Table 2 shows the eGFR stage in the distinct patient populations to be as follows; Stage 1(>90) [TDF 32 (12.60%), Non-TDF 49(19.30%) and Drug-Naïve 9 (3.50%)], stage 2 (60-90) [TDF 59 (23.20%), Non-TDF 50(19.70%) and Drug-Naïve 33 (13%)], and stage 3(30-59) [TDF 9 (3.50%), Non-TDF 3(1.20%) and Drug-Naïve 10 (3.90%)]. The difference among the eGFR stages was statistically significant (P=0.000). Table 3 shows that a significant percentage of tenofovir-exposed group meet the criteria for proximal tubular dysfunction (tubulopathy) compared with Non-TDF group [13 (72.2%), 5 (27.8%) respectively and this difference was significant (P = 0.043). Table 4 shows no significant difference between tubulopathy in the TDF group and Naïve group but there was a greater incidence of tubulopathy in the TDF group compared to the treatment Naïve group (2.60 and 7.90% respectively).

Table 5 shows the fractional excretion of the different biomarkers to be as follows:

Fractional excretion of uric acid was higher in the tenofovir (TDF) group as compared with those not exposed to TDF, (TDF 0.78mg/dl, Non-TDF 0.57mg/dl and Drug-Naïve 0.36mg/dl) the difference between the regimen groups was statistically significant (P = 0.000).

Table 1: Age and Sex Characteristics of the study population

	Mean Age	No. of patient	Std. Deviation	P-Value
SEX				
Male	36.92	85 (33.46%)	7.69	
Female	33.70	169 (66.54%)	7.97	(Z-test) p = 0.002
Total	34.77	254 (100%)	8.01	
ART Regimen group				
TDF	35.23	100 (39.37%)	7.68	
N-TDF	35.49	102 (40.16%)	8.68	(ANOVA) p = 0.069
D-NAIVE	32.50	52 (20.47%)	6.93	
Total	34.77	254 (100%)	8.02	

Values in parenthesis showed the percentage of patients in the study population

Table 2: Prevalence of Renal disease as defined by eGFR

			ART Regimen class			Total
			ART-Naive	Non-TDF	TDF group	
eGFR Stages	>90 (stage 1)	Count	9	49	32	90 (35.43)%
		% within eGFR class	10.00%	54.40%	35.60%	
		% of Total	3.50%	19.30%	12.60%	
	90-60 (stage 2)	Count	33	50	59	142 (55.9%)
		% within eGFR class	23.20%	35.20%	41.50%	
		% of Total	13.00%	19.70%	23.20%	
	59-30 (stage 3)	Count	10	3	9	22 (8.7%)
		% within eGFR class	45.50%	13.60%	40.90%	
		% of Total	3.90%	1.20%	3.50%	

X^2 Value = 21.869 P=0.000 (Significant)

Table3: Treatment experience groups and proximal tubular dysfunction

			TDF STATUS		Total
			NON TDF	ON TDF	
Tubulopathy status	**Absent**	Count	97	87	184
		% within Tubulopathy STATUS	52.7%	47.3%	100.0%
		% within TDF STATUS	95.1%	87.0%	91.1%
	Present	Count	5	13	18
		% within Tubulopathy STATUS	27.8%	72.2%	100.0%
		% within TDF STATUS	4.9%	13.0%	8.9%
Total		Count	102	100	202
		% within Tubulopathy STATUS	50.5%	49.5%	100.0%
		% within TDF STATUS	100.0%	100.0%	100.0%

X^2 = 4.833 P = 0.043 (Significant)

Table 4: Difference between patients on TDF, ART-Naïve and Tubulopathy

			Tubulopathy		Total
			Absent	Present	
ART Regimen	**ART-Naive**	**Count**	48	**4**	52
		% within ART class	92.30%	**7.70%**	100.00%
		% within Tubulopathy	35.30%	**25.00%**	34.20%
		% of Total	31.60%	**2.60%**	34.20%
	TDF	**Count**	88	**12**	100
		% within ART class	88.00%	**12.00%**	100.00%
		% within Tubulopathy	64.70%	**75.00%**	65.80%
		% of Total	57.90%	**7.90%**	65.80%
Total		**Count**	136	**16**	152
		% within ART class	89.50%	**10.50%**	100.00%
		% within Tubulopathy	100.00%	**100.00%**	100.00%
		% of Total	89.50%	**10.50%**	100.00%

X^2 =0.674 P =0.300 (Not Significant)

Table 5: Fractional Excretion Parameters in the distinct patient populations

	HAART with TDF	**HAART, Non-TDF**	**Drug-naïve**	**Difference among groups**	**Z-Test Value**
Fractional excretion of Uric acid (mg/dl)	**Mean (S.E)**	**Mean (S.E)**	**Mean (S.E)**	**(ANOVA) P-value**	**(Z-Test) P-value**
	0.78 (0.06)	0.57 (0.04)	0.36 (0.05)	P= 0.000	**1 versus 2 p=0.008, 1 versus 3 p= 0.000, 2 versus 3 p= 0.003**
Fractional excretion of Phosphate (mg/dl)	10.55 (0.59)	10.34 (1.32)	8.49 (0.77)	P= 0.416	**1 versus 2 p=0.998, 1 versus 3 p= 0.102, 2 versus 3 p= 0.538**
Fractional excretion of Glucose (mg/dl)	0.18 (0.13)	0.04 (0.01)	0.04 (0.01)	P= 0.431	**1 versus 2 p=0.648, 1 versus 3 p= 0.660, 2 versus 3 p= 0.997**
Estimated glomerular filtration rate (mg/dl)	80.65 (2.21)	92.90 (2.39)	75.63 (3.19)	P= 0.000	**1 versus 2 p=0.001, 1 versus 3 p= 0.475, 2 versus 3 p= 0.000**

Values in parenthesis showed the standard error of mean

Fractional excretion of phosphate (TDF 10.55mg/dl, Non-TDF 10.34mg/dl and Drug-Naïve 8.49mg/dl), Fractional excretion of phosphate was higher in the TDF group compared to other groups but the difference was not statistically significant (P = 0.416).

Fractional excretion of glucose (TDF 0.18mg/dl, Non-TDF 0.04mg/dl and Drug-Naïve 0.04mg/dl) values was also

higher in the tenofovir group but the difference was not statistically significant (P = 0.431).

Estimated glomerular filtration rate (eGFR), as measured by serum creatinine levels was overall within normal limits and comparable among study groups, TDF, Non-TDF and Drug-Naïve, (80.65, 92.90,75.63 units, respectively). The difference between the regimen group was statistically significant (P = 0.000). EGFR value was higher in the TDF group compared to other regimen groups.

DISCUSSION

In this cross sectional study we found that HIV-infected patients on TDF have a higher risk to develop renal tubular dysfunction compared to their counterpart on other antiretroviral therapy. A growing number of existing and updated literature have described an association between treatment with tenofovir and renal tubulopathy or impaired glomerular filtration rate (GFR) in patients with human immunodeficiency virus (HIV) infection. [7, 8] Our results show an increase in proximal tubular dysfunction in the TDF regimen group compare with the other two groups.

The relatively low prevalence of eGFR<60ml/min/1.73m2 in our results suggests that HIV-infected individuals may experience a significant kidney injury prior to the clinician's ability to detect due to non-appreciable loss of filtration function. The measurement of markers of tubulopathy, in addition to eGFR, could constitute a novel and more comprehensive method of screening for and quantifying kidney injury in HIV-infected individuals. The ability to detect early kidney damage is particularly important in the HIV- infected population because HIV-related muscle loss makes creatinine-based eGFR less accurate and HIV-related therapies can be nephrotoxic.

There has been several report of evidence of mitochondrial toxicity in HIV-infected patients exposed to TDF antiretroviral therapy. Such reports come from studies performed on kidney tissue of patients treated with TDF. These patients, showed significantly reduced levels of mt DNA under certain conditions, like the coadministration of didanosine compared to other patients never treated by TDF or HIV-negative patients. [9] However, a recent report on the morphologic aspects of renal toxicity of TDF emphasized a specific finding represented by the presence of eosinophilic intracytoplasmic inclusions compared to other forms of toxic acute tubular necrosis. The researchers were able to identify giant mitochondria, once again as underlining the specific damage produced to these organelles in cases of renal toxicity of TDF. [10] However, other experimental studies have confirmed the role of TDF in inducing significant mitochondrial toxicity targeted to proximal renal tubular cells, and sparing other metabolically active cells like hepatocytes. [11]

This result strongly agree with other workers that Tenofovir secretion by proximal renal tubule may lead directly to a greater drug accumulation in the renal tubular cells and, consequently, to tubular damage and renal toxicity. [12] Therefore, we hypothesize that a subclinical tubular dysfunction not detected by creatinine (eGFR) occurs after accumulation of TDF in proximal tubular cells. Finally this study has shown that TDF is not toxic to the glomerulus but show specificity of tubular toxicity at the proximal tubules.

CONCLUSION

In conclusion, the present review shows that TDF-associated toxicity is specifically to the proximal tubules and its effect on the tubules may be progressive as its long term use can lead to end stage renal disease. Thus, a periodic monitoring of renal function parameters in all patients taking tenofovir, part of antiretroviral therapy is strongly advised.

Competing Interest: The authors declared that no competing interests exist.

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