ORIGINAL ARTICLE

Renal Adverse Effects of Tenofovir Containing Regimens in HIV-Infected Children and Adolescents in North India

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ABSTRACT

Objective: To study the prevalence of abnormal renal functions among children living with HIV (CLHIV) receiving tenofovir disoproxil fumarate (TDF) containing antiretroviral therapy (ART).

Methods: A prospective, observational study was conducted among CLHIV aged 10 years to 21 years attending the pediatric HIV clinic. We included CLHIV weighing ≥ 30 kg who had been receiving TDF-containing regimens for at least 6 months, with estimated glomerular filtration rate (eGFR) > 60 ml/min/m^2 at enrolment and for whom baseline laboratory parameters were available before starting ART. Clinical and laboratory parameters like serum creatinine, serum phosphate, urinary protein and glucose estimation, CD4 count and viral load were noted from records. The mean change in serum creatinine, estimated glomerular filtration rate (eGFR), creatinine clearance, serum phosphate, and presence of urinary glucose and protein by dipstick were assessed at 3- and 12-months follow-up.

Results: We enrolled 70 patients with mean (SD) age 14.99 (2.45) years who had been receiving TDF-based ART for a mean (SD) duration of 14.60 (12.80) months. At 3-months and 12-months follow-up, 32.85% and 41.42% patients, respectively, had eGFR below 90 mL/min/1.73m^2, while 4.2% and 2.8% patients, respectively, had eGFR between 50-60 mL/min/1.73m^2. One patient had creatinine clearance below 50 mL/min/1.73m^2. Four patients had hypophosphatemia at the first and last follow-up respectively, and five patients had proteinuria. There was no statistically significant change in CD4 counts, serum potassium, or serum uric acid during study duration.

Conclusion: TDF-containing ART regimen is associated with decreased eGFR, creatinine clearance and proteinuria.

Keywords: *Antiretroviral therapy, Creatinine clearance, eGFR*

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INTRODUCTION

According to the India HIV Estimates Report 2020, the national adult (15-49 years) human immunodeficiency virus (HIV) prevalence was estimated at 0.22% in 2020; 0.23% among males and 0.20% among females. The national adult prevalence continued to decline from an estimated peak level of 0.54% in 2000-2001 through 0.33% in 2010 to 0.22% in 2020 corresponding to a 33.3% decline over the last 10 years [1]. The decline in HIV prevalence has been attributed to the use of robust antiretroviral therapy (ART) regimens and better screening and testing services. The U.S. Food and Drug Administration (FDA) has approved the use of tenofovir disoproxil fumarate (TDF) in children aged ≥ 2 years and weighing ≥ 10 kg when used as a component of ART [2]. TDF is an acyclic nucleotide analogue reverse transcriptase inhibitor (NtRTI) which is structurally similar to adefovir and cidofovir [3]. TDF is eliminated by the kidney through glomerular filtration and tubular secretion, and its clearance is in the proximal tubule of the nephrons and is controlled by active transport [4]. A high plasma concentration of TDF causes intracellular accumulation in renal tubular cells which increases the risk of renal toxicity.

Due to a long intracellular half-life, TDF allows for once-daily dosing and fosters treatment adherence with a better bioavailability [5]. TDF is recommended as the first line therapy in children living with HIV (CLHIV) [6]. The most common adverse effects of TDF are gastrointestinal symptoms and others include adverse effect on blood lipids, fat accumulation, and mitochondrial toxicity [7]. Systematic review and meta-analyses of randomized controlled trials and various observational studies have

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suggested that TDF-containing ART regimens are linked to a considerable loss of renal function [8]. Interactions between TDF and other anti-retroviral agents have been implicated in the development of renal toxicity [9]. Renal toxicity can manifest in the form of proximal or glomerular dysfunction, acute kidney injury, chronic kidney disease and end stage renal disease [10]. TDF-containing ART has shown to lead to a modest decline in renal function, more so in the initial 180 days of therapy, although the effect on estimated glomerular filtration rate (eGFR) was shown to stabilize between 180 and 720 days [11]. A decline in eGFR greater than 25% relative to baseline has been reported as ranging from 6 to 40.8% across different geographical locations of the world [12].

Limited studies are available showing TDF-related nephrotoxicity in the pediatric age group. A randomized trial in children receiving TDF as part of their highly active antiretroviral therapy (HAART) regimens reported a favorable safety profile [13]. Whereas, another pediatric cohort study from UK revealed renal toxicity especially with concurrent use of didanosine and lopinavir-ritonavir [14]. A case control study, in which serum renal function markers were determined in patients receiving TDF suggested a possible association between its use and development of hypophosphatemia [15]. A study from Africa reported a reduction in eGFR < 90ml/min/1.73m^2 in 35.9% of children receiving tenofovir disoproxil fumarate [16].

National AIDS Control Organization (NACO) guidelines recommend TDF based antiretroviral regimens as the preferred first line treatment of HIV in adults, adolescents, pregnant women and children in India [17]. To the best of our knowledge, there were no large observational studies in India assessing renal adverse effects of TDF containing regimens in CLHIV in India. So, this study was planned to assess the prevalence of renal dysfunction and its risk factors in CLHIV receiving TDF-containing regimens.

METHODS

A single-centre, prospective, observational study was conducted between August 01, 2021 to July 31, 2022, among CLHIV attending the pediatric HIV clinic of a tertiary hospital in North India to evaluate the renal adverse effects of TDF-containing regimens. All eligible CLHIV aged 10 years to 21 years or weighing ≥ 30 kg who had been receiving TDF-based ART for at least 6 months, with eGFR more than 60 ml/min/m^2 and whose baseline parameters were available before starting ART treatment were enrolled. Children with history of hypertension, diabetes mellitus, congenital heart disease, chronic liver failure, cardio vascular disease, nephrotic syndrome or hepatitis B and C infection or those whose care was transferred to another medical facility for logistic reasons were excluded. A written, signed and informed consent was obtained from the adult participants/parents/ caretakers of all participants before enrolment. Oral assent was taken from children between 10-12 years in presence of parents/guardians and a written assent was obtained from children and adolescents aged 12-18 years. Approval was granted by institutional ethics committee for human studies. This research was registered with Clinical Trial Registry of India (CTRI).

There are no studies assessing the prevalence of renal dysfunction in children receiving TDF-based regimens from India. A case series from India found no renal adverse effects with the use of TDF in children [18]. Based on estimates provided by NACO [17], a prevalence of TDF-associated renal dysfunction was assumed as 5% [17]; a sample size of 73 children is needed at 80% power, α error of 0.05 and 95% confidence interval to ascertain the prevalence of renal dysfunction in CLHIV receiving TDF-based ART.

Baseline serum creatinine, CD4 count and viral load with clinical characteristics including age, and weight, height and body mass index (BMI) of selected patients were noted from hospital records. All participants were followed-up regularly at the HIV clinic every month for 12 months after enrolment. Serum creatinine, CD4 count, viral load, serum phosphate and urinary glucose and proteins were estimated at 3- and 12-months follow-up during the study period.

Blood glucose was measured by glucometer (my life Pura X) with measurement range from 10 to 600 mg/dL (0.6 to 33.3 mmol/L). Urinary protein and glucose was assessed by standard dip stick method (Siemens Uristix). Siemens Uristix has a urinary detection threshold of 15-30 mg/dL for albumin and 75-125 mg/dL for glucose.

CD4+ cell count was analysed with Sysmex Partec's CyFlow Counter System flow cytometer. CD4 count was assessed at 6 monthly intervals. Viral load testing for HIV-1 viral load was done by Taqman Plasma (Quantitative) using test principle of real time PCR.

Calculation of eGFR was done by simple height independent equation of 'Pottel' where eGFR = 107.3/ (Scr/Q), Q = 0.0270 × age + 0.2329 (age in years) and Scr is serum creatinine (mg/dL). Creatinine clearance was calculated by the 'Cockcroft-Gault equation' where creatinine clearance (CrCl) = {(140-age) × weight/ (serum creatinine × 72)} × 0.85 (if female), CrCl (creatinine clearance) in mL/minute, age in years, weight in kg and serum creatinine in mg/dL [19-21]. Serum phosphate was

graded from 1 to 4 according to the Division of AIDS (DAIDS) adverse events table [22]. TDF-associated renal alteration was defined by decrease of eGFR by > 25% from baseline [12]. Proximal tubular dysfunction was defined by hypophosphatemia as < 2.7 mg/dL, proteinuria as 1+ or 30 mg% on urine dipstick, glycosuria as at least 1+ or 30 mg% (in the presence of normal serum glucose), hypouricemia as < 2 mg/dL and hypokalemia as < 3.56 mEq/L [22,23].

Statistical Analysis: The data was tabulated and entered in Microsoft Excel and analyzed using SPSS version 23. The change in serum creatinine, eGFR, creatinine clearance, CD4 counts, and serum phosphate data were analyzed using Wilcoxon signed rank test. $P < 0.05$ was considered statistically significant.

RESULTS

A total of 70 patients (41 boys, 29 girls) aged 10 years to 19.5 years were included in the study. The mean (SD) age of participants was 14.99 (2.45) years. The body mass index (BMI) ranged from 15.06 kg/m^2 to 23.28 kg/m^2 with mean (SD) BMI of 18.00 (1.82) kg/m^2. The characteristics of the study participants at the time of enrolment are shown in **Table I**. The study selection process is illustrated in **Fig. 1**.

All patients were on TDF-based regimens at the time of enrolment. Most of the participants (48/70) had been receiving TDF-based ART for the past 6-12 months. Of the remaining, 11, 7, and 4 participants had received TDF-based ART for 12-24 months, 24-36 months, 36-72 months, respectively. The mean (SD) eGFR (ml/min/1.73 m^2) at enrolment was 106.71 (6.52). This decreased significantly by 13.1% to 92.75 (14.87) at 3 months follow-up ($P < 0.001$) and by 14.71% to 91.01 (14.77) at 12 months follow-up ($P < 0.001$) (**Fig. 2a**).

Table I Characteristics of HIV-Infected Children and Adolescents on Tenofovir (TDF) Containing Regimens at enrolment (*N*=70)

Parameter	*Values*
Male	41 (58.6%)
Female	29 (41.4%)
Age (y)[a]	14.99 (2.45)
Weight (kg)[a]	33.98 (3.68)
Height (cm)[a]	146.03 (10.58)
BMI (kg/m^2)[a]	18.00 (1.82)
Duration of TDF containing regimens (months)[a]	14.6 (12.80)
CD4 count (cell/mm^3)[a]	622.11 (304.90)
Serum creatinine (mg/dL)[a]	0.59 (0.16)
eGFR (mL/min/1.73m^2)[a]	106.71 (6.52)
Creatinine clearance (mL/min)[a]	93.94 (12.61)

Values are in *n* (%), or [a]mean (SD)
ART Antiretroviral therapy, eGFR Estimated glomerular filtration rate, TDF Tenofovir disoproxil fumarate

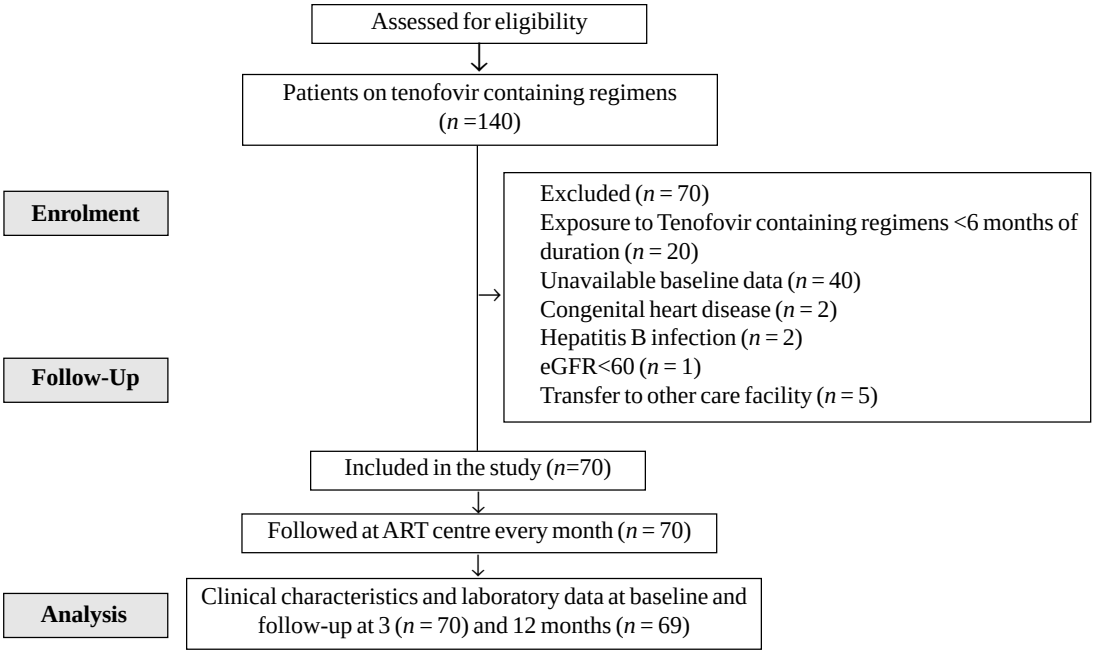


Fig. 1 Flow of participants in the study

A significant increase in serum creatinine of 26.85% was noted from enrolment; 0.5904 (0.16) to 0.7489 (0.14) at 3-months follow-up ($P < 0.001$). We found a significant increase in serum creatinine (mg/dL) of 32.91% from enrolment; 0.59 (0.16) to 0.78 (0.14) at 12 months follow-up ($P < 0.001$) (**Fig. 2b**). In our study we have found 4.28% and 7.14% patients had serum creatinine more than 1 mg/dL at 3-months follow-up and 12-months follow-up, respectively.

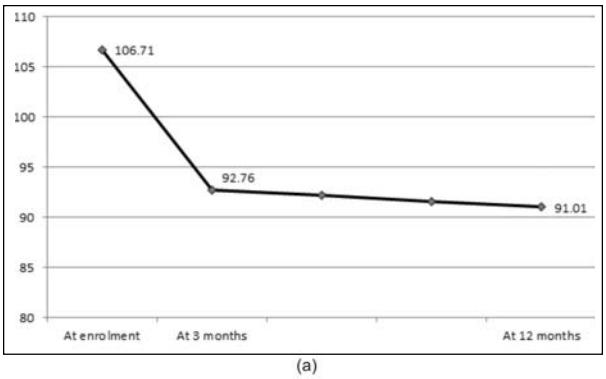
A significant decrease in mean (SD) creatinine clearance of 9.5% was seen from baseline 93.94 (12.61) to 85.04 (15.61) at 3-months follow-up ($P < 0.001$). We found a significant decrease in the mean (SD) creatinine clearance of 10.4% from baseline, 93.94 (12.61) to 84.20 (10.37) at 12 months follow-up ($P < 0.001$).

In our study creatinine clearance below 90 mL/min was seen in 60% participants ($n = 42$**)**, out of these 7.1% participants ($n = 5$) had creatinine clearance between 50 to 60 mL/min and 1.4% participants ($n = 1$) had creatinine clearance below 50 mL/min at 3-months follow-up (aged 18 years; duration of TDF containing ART: 19 months). Thus, only one patient had decreased creatinine clearance below 50 mL/min who was shifted to an alternate regimen without TDF as per the NACO Guidelines. Also, at the last follow-up at 12 months, creatinine clearance below 90 mL/min was seen in 47% participants ($n = 33$), out of these 11% participants ($n = 8$) had creatinine clearance between 50 to 60 mL/min. Our results suggest statistically significant decrease in creatinine clearance along with the duration of TDF treatment (Wilcoxon signed rank test) (**Fig. 2c**). 20% (14/70) patients had a decrease in eGFR by >25% from baseline at 12-months follow up.

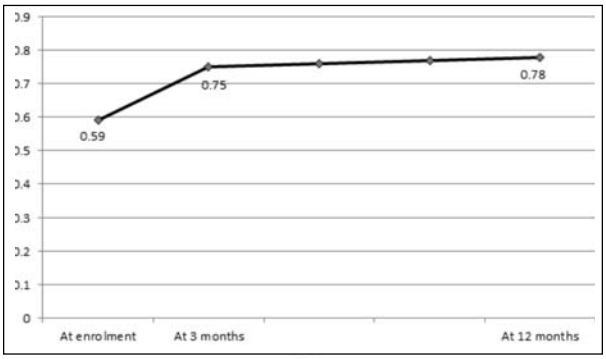
A decrease in mean (SD) serum phosphate of 5.23% was observed from the first follow-up [4.29 (3.76)] to [4.08 (0.76)] at the last follow-up ($P = 0.058$). Out of 70 patients, three patients had grade 1 and one patient had grade 2 hypophosphatemia at the first follow-up. At the last follow-up, one patient had grade 1 and three participants had grade 2 hypophosphatemia.

Proteinuria was not observed in any patient with treatment of TDF containing regimens during the study on first follow-up but at the last follow up 5 patients (7.1%) were having 1+ proteinuria. None of the patients developed glucosuria during this study on any follow-up.

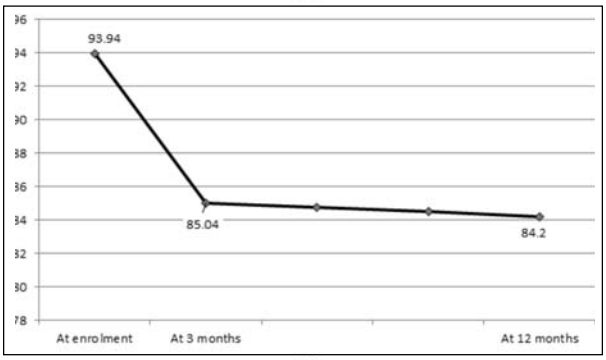
A marginal decline ($P > 0.05$) in CD4 counts was observed in CLHIV on TDF-containing regimens at 3-months and 12-months follow-up; mean (SD) CD4 count at enrolment, 3-months and 12-months was 622.11 (304.90), 610.44 (232.96) and 577.44 (230.04) respectively.



(a)



(b)



(c)

Fig. 2a Estimated eGFR (ml/min/1.73 m^2); 2b Serum creatinine (mg/dL); 2c Serum creatinine clearance (mL/min)

DISCUSSION

The WHO recommends that serum creatinine evaluation must be conducted before initiation of TDF and the patients with compromised renal function should not be started with TDF [2]. We observed that TDF containing regimens are associated with a significant decrease in eGFR, creatinine clearance and increase in serum creatinine values over follow up in North Indian HIV-infected children and adolescents.

A major limitation of our study is the heterogeneity in duration of treatment using TDF-based ART for all participants and the less duration of follow-up. Ideally, all children being started on TDF should be followed up on

WHAT THIS STUDY ADDS?

- Tenofovir-containing regimens are associated with a decreased eGFR, creatinine clearance and increase in serum creatinine values over follow up in North Indian HIV-infected children and adolescents.

same time points since commencement on TDF-based therapy. Besides, dietary factors, other medications and drug-drug interactions were not studied which may affect creatinine levels. Ours was an observational study from a single centre. The use of the Cockcroft-Gault formula is another limitation of the study. The strength of our study is that it enrolled largest number of HIV-infected children and adolescents from India.

We observed that TDF containing regimens are associated with a significant decrease in eGFR, creatinine clearance and increase in serum creatinine values over follow up in North Indian HIV-infected children and adolescents. All our patients on TDF containing regimens had normal renal function at baseline and had no pre-existing co morbidities. All study participants except one who was receiving TLD including the one who developed renal insufficiency were not previously on medications which could interfere or cause renal dysfunctions.

The mean decline in eGFR from baseline was statistically significant among our HIV-infected patients during all follow ups. This was consistent with the findings of another study among HIV-infected children [16] where in eGFR <90mL/min/1.73m^2 was noted in 35.9% of patients. In our study, eGFR below 90 mL/min/1.73m^2 was seen in 32.85% and 41.42% at the first and the last follow-up respectively. Previously, TDF has shown to impact the renal function in the initial 6 months of starting and the effect was shown to plateau thereafter [16]. Most of our participants had been on TDF-based ART for 6-12 months, implicating that continued decline in eGFR may occur even beyond 12 months of starting TDF warranting close monitoring of renal functions.

We observed that 4.2% (6/70) and 2.8% (4/70) patients had an eGFR below 60 mL/min/1.73m^2 at the first and the last follow-up respectively. A study by Vigano et al [25] observed a moderate reduction in renal function in underweight children. We observed non-significant hypophosphatemia from first to last follow-up which was similar to findings by Mashingaide-Mano et al [16]. We found urinary dip stick was positive for protein in 7.1% of participants. The prevalence of proteinuria was similarly reported in study of Mashingaide-Mano et al [17]. However, there was no occurrence of glycosuria during study period. Our study provides some evidence for TDF associated renal impairment in children and adolescents. We recommend more studies with longer follow-up to assess the same.

Ethics Approval: Institutional Ethics Committee, Medical College and associated hospitals; No.ECR/922/inst/UP/2017 I, dated July 01, 2021.

Contributors: RK, SAS, SKS: Collected the data, reviewed the literature and drafted the first version of the manuscript; MVS, SAS, MM: Conceptualized the study and revised the manuscript; NM, AS, RKY, RS, MS: Collected the data, reviewed the literature and statistical analysis; MVS, MM, SAS, AS: Critically reviewed the final version of submitted manuscript. All authors contributed to drafting of the manuscript and approved the final version of the manuscript; MVS: Shall act as guarantor of the paper.

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