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Tenofovir Associated Proximal Renal Tubular Dysfunction in HIV infected Children and Adolescents

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An abstract of a thesis submitted to the Faculty of the James T. Laney School of Graduate Studies of Emory University in partial fulfillment of the requirements for the degree of Master of Science in Clinical Research
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Abstract

Tenofovir Associated Proximal Renal Tubular Dysfunction in HIV infected Children and Adolescents
By Andres F. Camacho-Gonzalez, MD

Background: Tenofovir disoproxil fumarate (TDF) may be associated with proximal tubular dysfunction (PTD), but limited information is available in children. We investigated the association of TDF with the development of PTD in a pediatric cohort, the predictive value of β2-microglobinuria as marker of early detection of PTD, and the phosphorus and creatinine difference between TDF exposed and unexposed individuals.

Methods: Prospective cohort study measuring prevalence of PTD in HIV-infected children and adolescents receiving TDF versus non-TDF-based treatments. Subjects were categorized based on their TDF exposure: No exposure, exposure <1 or >1 year. Generalized linear mixed models were used to detect the association between TDF and PTD in both groups, and the predictive ability of β2-microglobinuria among TDF users. Tukey-Kramer procedure was used to make pairwise comparisons between the mean phosphorus and creatinine levels in each visit.

Results: 110 subjects on TDF and 68 on non-TDF regimens were followed for 12 months. 57% were males, 88% African-American, with a mean age of 15.7 years. 62% had undetectable viral load at enrollment with a mean CD4 count of 550 cells/mm^3. 13 subjects had PTD (7%). Univariate analysis identified gender and duration of TDF exposure as significant variables in predicting PTD. Generalized linear mixed models showed that women had a slight increase in the odds of developing PTD when exposed to TDF for more than 1 year (OR: 1.09 (C.I. 1.03-1.14), OR: 1.03 (0.97-1.10). Lower levels of phosphorus (p-value=<0.0001) and creatinine clearance (p-value=0.03) were seen in subjects with longer TDF exposure. β2-microglobinuria did not predict PTD among TDF users (p-value 0.21).

Conclusions: PTD was uncommon among children with HIV, but women had a slightly increased risk with TDF-exposures of more than one year. β2-microglobulin was not a useful marker for early detection of PTD. Lower levels of serum phosphorus and creatinine clearance were noted in subjects receiving TDF, but its clinical implications still need to be elucidated.

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INTRODUCTION

The aging of HIV-infected children to adolescence and beyond reflects the efficacy of antiretroviral therapy (ART). This success has been countered by an urgent need to broaden existing knowledge of the effects of a chronic disease with the long-term administration of potentially toxic medications. The kidneys are one of the primary organs affected by both the infection and drug toxicity. Prior to the advent of ART, renal disease was common and associated with increased mortality (1), most often secondary to HIV associated nephropathy (HIVAN) (2). After the introduction of ART, the incidence of HIVAN decreased and its effective implementation reversed renal dysfunction in many affected patients. However, the effect of ART on other types of renal disease (RD) has not been as apparent. Furthermore, studies from the ART era have demonstrated that the incidence, prevalence and mortality due to RD remain increased in HIV-infected adults compared to the general population (3-6). Improvements in survival coinciding with higher rates of hypertension, diabetes, atherosclerosis and other cardiovascular comorbidities, and metabolic derangements have contributed to an increased prevalence of RD in this population (7-18), although such complications are infrequently described in HIV-infected children and adolescents.

Initial studies that led to FDA approval of Tenofovir disoproxil fumarate (TDF) in adults showed that patients who were treated with this medication were not at an increased risk of developing renal dysfunction. However, post-approval studies identified potential associations between TDF usage with variable and occasionally non-reversible proximal tubular disease (PTD). Follow-up studies suggest abnormalities caused by TDF may not initially affect overall renal function (13), limiting the use of early routine testing although specific investigations of tubular function may be indicated.

In children, there is limited information on the potential association of TDF usage and RD. Here, we designed a prospective cohort study to document the prevalence of PTD and its association with chronic administration of TDF in a cohort of HIV-infected children and adolescents. In addition we evaluated the application of β2-microglobulin in urine as a screening biomarker for early detection of renal tubular disease.

BACKGROUND

HIV and Renal Disease

RD is a common complication noted in HIV-infected patients; it is estimated that approximately 30% of HIV-infected adults have abnormal kidney function (19). Nephropathy can be secondary to HIV-related and non-HIV related etiologies. The direct effect of HIV on renal parenchyma may be secondary to the host immune response, opportunistic infections (OIs) and vasculitis, causing a heterogeneous spectrum of glomerular and tubular diseases. HIVAN is most often documented and is the third leading cause of end stage renal disease in African Americans (20).

Antiretroviral Therapy and Renal Tubular Toxicity

Antiretrovirals [ARVs], such as zidovudine, stavudine lamivudine, tenofovir, ritonavir and atazanavir, have been associated with the development of renal toxicity either directly or indirectly (14). There are at least three known mechanisms of renal tubular injury secondary to antiretroviral therapy (ART): mitochondrial injury, crystal deposition and transporter defects (21). Nucleoside/Nucleotide analogues (NRTIs) are transported from the interstitium to the renal tubular cell by the human organic anion transporter (hOAT) system and are removed from the cell to the lumen by the multidrug resistant associated protein (MRP) and P-glycoprotein transporter mechanism. Increased uptake (hOAT) or decreased removal (MRP) of the NRTI may cause PTD (figure 1) (21). NRTIs may also cause mitochondrial toxicity in proximal tubular cells. Protease inhibitors (PIs) such as ritonavir and saquinavir can inhibit the hOAT and MRP mechanism, favoring accumulation of NRTIs and increasing the risk of toxicity (22). Crystal nephropathy has been widely described with indinavir. Injury occurs by deposition of crystals in the distal tubules causing obstruction,

and has been detected in 20% of individuals receiving this drug (12). The inter-individual phenotypic variability of these mechanisms result in the excretion or accumulation of ARVs (21, 23).

Tenofovir and Renal Tubular Dysfunction

Tenofovir disoproxil fumarate (TDF) is a nucleotide analogue that inhibits HIV reverse transcriptase, thereby terminating DNA synthesis. TDF has activity against HIV-1 and 2 as well as hepatitis B virus and is excreted unchanged by the kidneys through a combination of glomerular filtration and tubular secretion (24). 20-30% of the drug is actively transported to the proximal tubule by the hOAT mechanism in the basolateral membrane (especially hOAT1 and hOAT 3) and secreted into the tubular lumen by the apical membrane transporter MRP-4 and MRP-2 (see figure 1). It is considered one of the preferred ARVs in naïve and experienced patients above 12 years of age, because of its proven antiviral efficacy as well as its bioavailability as a single tablet combination with emtricitabine and efavirenz administered once daily (25). Initial clinical trials suggested that renal toxicity was rare and reversible in subjects receiving TDF (26-28). However, multiple case reports and studies in HIV-infected adults now suggest the converse (10, 11, 15, 17). A prospective study undertaken by Labarga and colleagues specifically examining renal tubular damage with TDF usage documented that 22% of subjects receiving this drug had tubular dysfunction compared to 6% of subjects treated with other ART regimens. None had abnormal plasma creatinine or creatinine clearance (13). TDF-induced proximal tubular dysfunction (PTD) can present clinically with a preserved or decreased renal function. The latter will manifest as acute renal failure (ARF), chronic renal failure (CRF) or with a declining glomerular filtration rate (GFR) in the initial phases of the disease. Clinically, patients can present either with a partial or complete Fanconi syndrome (renal tubular acidosis, glycosuria with

normoglycemia, aminoaciduria, hypophosphatemia, hypouricemia and tubular proteinuria (29, 30)), as well as with other manifestations such as osteomalacia, decreased bone mass (due to phosphate wasting) and vitamin D deficiency (31-34).

Risk factors for the development of TDF-associated PTD include: pre-existing renal disease, longer exposure to ART, older age, black race, CD4 nadir less than 200 cells/mm^3, poorly controlled HIV, and concomitant use of other nephrotoxic medications including ARVs such as ritonavir (35, 36).

Tenofovir Toxicity in the Pediatric Population

In HIV-infected children there are few studies documenting the incidence of PTD and its association with TDF usage (8, 9, 18). A retrospective study from the United Kingdom and Ireland among a cohort of HIV-infected children and adolescents documented that 7.5% of subjects receiving TDF had serious adverse events, half of these due to renal toxicity (16). A prospective study from the Pediatric AIDS Clinical Trial Group (PACTG) undertaken in 2100 HIV-infected children documented a 22% incidence of renal dysfunction. Those who received TDF were twice as likely to develop renal laboratory abnormalities compared to those who did not receive this drug (7). Recently, Soler-Palacin and colleagues followed 40 patients on TDF-based regimens over a 2 year period. The investigators noted a decrease in total phosphorus reabsorption in 74% of the individuals and the presence of proteinuria in 89% of subjects (27% with nephrotic range proteinuria). These findings suggest that TDF usage is an important risk factor in the development of renal abnormalities. The impact of TDF renal toxicity in pediatric subjects should also be considered on its consequences on bone metabolism. Children and adolescents have a higher rate of bone turnover so that the potential effects of TDF usage may be greater than that among HIV-infected adults. Studies

undertaken in macaque models have shown that exposure to TDF at doses of 30/mg/kg/day for more than 8 months were associated with a rachitic-type picture (widened growth plates, bony deformities, growth restriction, increase alkaline phosphatase and hypophosphatemia). Lower doses showed no difference in bone density and growth after 5 yers of follow-up(33). Another study by Gafni and colleagues in a small number of pediatric patients, suggested that TDF use was associated with decreased bone mineral density after 24 weeks of follow-up (37).

Biomarkers and early detection of Renal Tubular Dysfunction

The earlier diagnosis of PTD could be important in minimizing the potential of disease progression related to TDF toxicity. Renal tubular defects may not manifest with significant changes in serum creatinine or creatinine clearance and could be missed by routine screening (13, 21). Pediatric subjects may have increased morbidity related to chronic phosphorus wasting with premature osteopenia and osteoporosis due to bone mineral loss. (25, 38, 39). This emphasizes the critical need to identify earlier markers of tubular injury. The net transport of amino acids by the proximal tubular epithelium is very efficient and approximately 99% of free amino acids filtered in the glomerulus are reabsorbed in the proximal tubules and retained in the body. Spilling of amino acids into the urine may be secondary to PTD (40). β2-microglobulin is freely filtered at the glomerulus and reabsorbed and catabolized by the proximal renal tubules. Higher concentrations of β2-microglobulin in urine have been associated with PTD (41). Gatanaga and colleagues, in a cross sectional study of HIV-infected adults receiving ART, found that 43% of individuals exposed to TDF-containing regimens had a significant elevation of β2-microglobinuria compared to 7% of individuals on a different ART regimen. However, the majority of subjects in both groups

had normal serum creatinine and creatinine clearance (42). Similar results were noted in a cross-sectional study among HIV-infected children and adolescents: 12 out of 44 receiving TDF had increased β2-microglobinuria versus 2 out of 48 in the non-TDF group (43). Based on the above information, some experts recommend a comprehensive evaluation of renal tubular function in individuals receiving TDF (13, 44, 45).

Methods

Hypothesis:

1. Treatment with TDF is associated with the development of PTD among HIV-infected children and adolescents on antiretroviral therapy.
2. Urinary β2-microglobulin is a predictive marker of PTD in HIV-infected individuals treated with TDF as part of their ART.

Study Design. This was an observational prospective cohort study.

Study Area. This study was conducted at the Ponce Family and Youth Clinic, which is considered one of the largest and most comprehensive outpatient treatment facilities in the country for HIV-infected infants, children and adolescents. The clinic is nested within the larger Grady Infectious Disease Program, which is federally funded through the Ryan White care act and provides care annually for approximately 5,500 patients (including 400 HIV-infected children and adolescents) in 20 county eligible metropolitan areas that include Atlanta. The Grady Infectious Disease Program is the largest free-standing facility offering care to HIV infected patients in the US. The center provides multiple services for patients in one location, including a primary care clinic, family clinic, dental clinic, mental health clinic, clinical research area, pharmacy, and subspecialty clinics, including nephrology and gastroenterology.

Participants. The population of interest consisted of HIV-infected children and adolescents between 0-24 years of age without previous renal disease and who were already receiving ART or were due to commence ART at the time of enrolment. Patients with previous renal disease, diabetes or diarrhea (temporary criteria) were excluded from the study.

Procedures. Upon agreement to participate in the study, basic demographic, clinical and laboratory information about the patient was abstracted. At the enrollment visit, patients had the following study tests: baseline urine amino acids and urine β2-microglobulin, a serum chemistry panel that included sodium, potassium, chloride, magnesium, phosphorus, renal function tests (BUN and creatinine), liver function tests, hemoglobin, hematocrit and urinalysis.

After enrollment, subjects were evaluated every 4 months with sodium, potassium, chloride, magnesium, phosphorus, renal function tests (BUN and creatinine), liver function tests, hemoglobin, hematocrit, urinalysis and urinary β2-microglobulin. If previous laboratory tests suggested proximal renal tubular dysfunction, urinary phosphorus and creatinine and serum uric acid were obtained in addition to the baseline tests (see figure 2). This information was used to calculate tubular reabsorption of phosphorus (TRP) and confirm renal phosphorus wasting (see formula below). Patient glomerular filtration rate (GFR) was calculated at each visit using either the Schwartz equation (if age <18 years) or the Modification of Diet in Renal Disease (MDRD) equation (if age ≥ 18 years).

1. $TRP = 1 - \frac{\text{Urine Phosphorus} * \text{Serum Creatinine}}{\text{Serum Phosphorus} * \text{Urine Creatinine}}$

2. Schwartz Equation (Eq): $GFR = \frac{k * \text{height (cm)}}{\text{Serum Creatinine}}$

Where k is a constant as follows:

-0.33 for infants with birth weight <2.5 kg

-0.45 for infants born full-term

-0.55 for children and adolescent girls

-0.7 for adolescent boys

3.MDRD

GFR=170* Serum Creatinine$^{-0.99}$ * age$^{-0.176}$ * 1.18 (if Black) * 0.762 (if female) * BUN$^{-0.17}$ * Albumin$^{0.318}$

Enrollment Schema: See Figure 2

Outcome Variable: The outcome variable was the development of PTD. Patients were considered to have PTD if they had low bicarbonate level <21 mEQ/L or renal phosphate wasting (phosphorus <2.5 and/or TRP: <86%), or glycosuria (1+ or more in urine dipstick) in a non-diabetic patient.

Exposure Variable: TDF exposure was determined based on the initiation of TDF-based regimen (date 1) and the follow-up date (date-2) as follows: If a subject was on TDF at a given visit (date 2) duration on TDF was calculated by the difference in dates (i.e., exposure = date 2 – date 1). If a subject was on TDF at a given visit but not on TDF at the following visit (date 2), then duration of TDF was calculated using the midpoint between visits (i.e., exposure = ½ (date2 - date1). For patients who had only one visit and were lost to follow-up, exposure was determined by calculating the midpoint between the enrollment and the time they started their TDF-based regimen. Exposure was then classified initially into 3 TDF duration groups: **0**: never on TDF; **1**: < 12 months on TDF and **2**: ≥ 12 months on TDF.

Other covariates:

Other variables that where considered included demographic information (age in years, gender and race (black/non-black)), family history of kidney disease, perinatal HIV infection (yes/no), AIDS diagnosis (yes/no), urine amino acids (abnormal, normal), length of disease (date of HIV diagnosis until enrollment in days), poor HIV control (CD4 count less than 200 cells/mm^3 and viral load >1000 viral copies), utilization of other nephrotoxic medications such as: acyclovir, trimethroprim-sulfamethoxazole, dapsone, and pentamidine;

utilization of other nephrotoxic ARVs such as: ritonavir, atazanavir, lopinavir, didanosine and stavudine; CD4 count (categorized as < 200 cells/mm^3 or > 200 cells/mm^3), serum creatinine (abnormalities based on age), serum phosphorus (hypophosphatemia considered if <2.5 mg/dl), serum uric acid, and creatinine clearance (calculated with either the Schwartz or the MDRD formula depending on the age) classified according to guidelines of the National Kidney Foundation as follows: Mild reduction: 60-89 ml/min per 1.73 m^2; moderate reduction: 30-59 ml/min per 1.73 m^2; severe reduction 15-29 ml/min per 1.73 m^2; kidney failure <15 ml/min per 1.73 m^2. Urine test variables included glucose (abnormal if 1+ or more in urine dipstick, proteinuria (abnormal if ≥1+ in two consecutive tests), urine creatinine, urine phosphorus and urinary β2-microglobulin. Variables included in the physical exam were: height, weight and blood pressure (classified according to age, sex and height for children less than 18 years of age based on standardized tables).

Sample Size Calculation:

Confidence Level	Size (N)	Target Width	Actual Width	Proportion (P)	Lower Limit	Upper Limit	Width if P = 0.5
0.95	356	0.09	0.09	0.25	0.205	0.295	0.104
0.95	399	0.09	0.09	0.3	0.255	0.345	0.098
0.95	139	0.1	0.1	0.1	0.05	0.15	0.166
0.95	**196**	**0.1**	**0.1**	**0.15**	**0.1**	**0.2**	**0.14**
0.95	246	0.1	0.1	0.2	0.15	0.25	0.125
0.95	289	0.1	0.1	0.25	0.2	0.3	0.115

A priori the expected proportion of cases of renal tubular acidosis was 0.15 (46); therefore, a sample size of 196 was required to estimate a two-sided 95% confidence interval with a width of 0.10. To compare the proportion of patients with PTD among those subjects taking TDF and those subjects taking a non-TDF containing ART regimen, a sample size of

101 subjects in the TDF arm and 75 subjects in the non TDF arm was calculated in order to achieve 80% power to detect an OR of 3 with a significance level of 0.05. The actual number of enrollees were 110 subjects on a TDF-regimen and 68 on a non-TDF-regimen.

Statistical Methods.

All statistical analyses were performed using SAS 9.2 (Cary, NC). Statistical significance was assessed using a significance level of 0.05. Appropriate descriptive statistics (e.g., frequencies and percentages, means and standard deviations, or medians and interquartile ranges) were calculated for all variables of interest. Time of initiation of their latest antiretroviral therapy was documented even if it was before the time of enrollment in the study.

Baseline risk factors were tested individually against the probability of developing PTD. Since the longitudinal analysis included multiple observations on each subject over time, we performed generalized linear mixed model logistic regression with a subject specific random effect to account for correlated outcomes. Factors that had a p value of less than 0.1 where use to construct the logistic regression model and included the terms gender, and TDF exposure.

For the second hypothesis we looked at β2-Microglobulin and β2-Microglobulin/creatinine ratio as potential markers of PTD among TDF users. Our decision for this was based on the following causal pathway: TDF causes PTD and PTD causes elevation of urinary b2-microglobulin. Generalized linear mixed model logistic regression was also used to determine this association. Sensitivity and specificity were not measured since no association was found.

We used linear associations between phosphorus level and TDF exposure as well as between creatinine clearance and TDF exposure at each visit in a 2- factor repeated measures analysis of variance model. If significant interactions were detected then simple effects were

reported; otherwise, we reported the analysis of the main effects. The Tukey-Kramer multiple comparison procedure was used to make pairwise comparisons between groups while controlling the overall type-I error rate.

Ethics. Both verbal and written informed consent was obtained from parents/ guardians in at the time of screening. The study was approved by the Emory, Grady and Children's Healthcare of Atlanta Institutional Review Boards.

RESULTS

Descriptive analysis

178 patients were enrolled in the study: 110 on a TDF-based regimen and 68 on a non TDF-based regimen. Table 1 and 2 provide a summary of patient characteristics throughout the study. The majority of the patients were male (57%), African-American (88%) and with a mean age of 15.7 years. 62% of patients had an undetectable viral load at enrollment with a mean CD4 count of 550 cells/mm^3. Patients on a TDF-based regimen were older, horizontally infected and had lower CD4 counts at enrollment than those on non TDF-based regimens. The majority of patients completed 3 visits (75%); there was a decrease in the number of patients following-up at the final visit with only 59% of patients completing the study at the time of this analysis. Since patients were allowed to change their ARVs throughout the study based on the discretion of their treating physician or because of poor compliance, the TDF variable in Table 1 shows exposure to TDF based on three categories: no exposure, exposure less than 12 months and exposure longer than 12 months. Compliance never dropped below 78% and only a very small percentage of patients stopped their medication.

Primary Outcome

Throughout the study duration, there were 13 patients with PTD occurrences (Figure 3). Of those patients with PTD, 1 was never on a TDF-based regimen during the study (male), 3 were on TDF for less than a year (1 male, 2 females) and 9 were on TDF for 1 year or more (2 males, 7 females). 1 patient on a TDF-based regimen developed Fanconi syndrome and required switching of his ARVs with subsequent resolution of PTD.

Univariate and Multivariate models

We examined the association of risk factors with the development of PTD first by univariate analysis (Table 3 and 4). Gender (p=0.06) and TDF group (p=0.05) were the only two

variables considered as having a p-value significant enough to include in the multivariate model. Multivariate analysis of TDF exposure, gender and their interaction term was found to be significant.

The results of the logistic regression showed that women exposed to TDF for more than one year had a slight increase in the odds of developing PTD (p-value: 0.0016) (Table 5).

A second model looking at TDF exposure only with two levels (TDF exposure less than 1 year or no exposure VS TDF exposure for more than 1 year), as well as gender showed also than females on TDF for more than one year had an slight increase in the odds of developing PTD (p-value=0.003) (Table 6).

Analysis of Phosphorus levels, Creatinine clearance and TDF Exposure

We examined the linear association between serum phosphorus levels and TDF exposure as well as creatinine clearance and TDF exposure. Phosphorus mean value was obtained per visit number and risk category. After multiple comparisons adjustment there was a significant difference between phosphorus levels in subjects who were on TDF for less than a year or no TDF and those with TDF exposure of more than 1 year (p-value = <0.0001) (Table 7, Figure 4). The estimate of the effect of TDF on phosphorus level was -0.478 mg/dl. In a similar fashion we examined the linear association between creatinine clearance and both TDF groups and noted a significant difference between both risk groups (p-value=0.03), with an estimate of -9.75 ml/min per 1.73 m^2 (Table 8, Figure 5).

β2-Microglobulin and β2-microglobulin/creatinine ratio as predictors of PTD

In order to look for β2-microglobulin and β2-microglobulin/creatinine ratio as potential markers for the development of PTD we selected only those subjects who were on a TDF based regimens (n=110). Univariate analysis showed no association between PTD and β2-Microglobulin (p-value=0.21) or β2-microglobulin/creatinine ratio (p-value=0.1)

DISCUSSION

The prevalence of PTD in our cohort was 7% (13/178) and there was a slight increase in the odds of developing PTD in female patients who were exposed to TDF for more than 1 year (OR: 1.09 p-value=0.0016). No association was found between PTD and severity or duration of disease as well as the use of other potentially nephrotoxic ARV or non-ARV agents as noted in other studies (47). One patient who developed PTD was not on a TDF-containing regimen and the majority where exposed to TDF for more than one year. One patient on a TDF based regimen developed Fanconi syndrome that resolved after switching to a non-TDF antiretroviral regimen.

The prevalence of PTD in our study was not insignificant given that this condition is uncommon to the general population. In adult studies, prevalence reports have been variable. Labarga and colleagues, in a prospective observational study of 284 patients documented an overall prevalence of PTD in 22% of patients on TDF-based regimens compared to 6% in those on non-TDF based regimens (13). In another cross-sectional study that included 399 adults, Dauchy and colleagues reported a PTD prevalence of 6.5% (C.I: 4.2-9.5%) (48). This is the first pediatric study to report on the prevalence of PTD in HIV-infected children and adolescents. As in this study, both of the adult reports showed an association between the risk of PTD and the duration of exposure to TDF. In Labarga's study, estimates for tubular dysfunction at four years were 25% and in Dauchy's the odds of PTD were 5 times higher after five years of TDF exposure(13, 48). The higher risks reported in the adult studies may be related to their longer follow-up time when compared to ours.

Lower phosphorus levels can occur as a consequence of PTD exposure and although some authors have suggested that serum phosphorus levels have great variability and that

hypophosphatemia may be a late finding of PTD due to compensation by bone turnover, others have suggested using serum phosphorus as a potential marker for early detection of PTD (49). In our cohort, a clear decline of serum phosphorus levels was noted in subjects who received TDF for more than 1 year-compared to those receiving TDF for less than 1 year or no TDF at all (P-vale=0.0001). The clinical significance of this trend is unclear since despite the difference all subjects had mean values of phosphorus that were in the normal range for age and only seven patients (5 on TDF and 2 on non-TDF regimens) had a transient hypophosphatemia grade 2 or above based on the Division of AIDS (DAIDS) Adverse event Grading Table. Recently, Soler-Palacin and colleagues followed 40 pediatric patients on TDF-based regimens over a 2 year period. The investigators noted a decrease in total phosphorus reabsorption in 74% of individuals, suggesting a potential role for TDF as a risk factor in the development of proximal tubular abnormalities (50). Contrary to our results, in a prospective study of 63 HIV-infected patients followed over a three-year period, Kinai et al noted no difference in the serum phosphorus level of patients on TDF and non-TDF based regimens (45). These differences may be due to the higher rate of bone turnover in the pediatric population compared to adults. In addition, the impact of lower serum phosphorus levels in children may reflect in poor growth and development. Related studies in children are conflicting with two reports noting a potential reduction of bone mass on patient on TDF-based regimen (25, 38) and two reports reporting the opposite (51, 52). The four studies are not comparable since the characteristics of the subjects were different, with patients with advanced immunosuppression in those studies showing an association. Further studies are required to determine the effects of lower phosphorus levels in the pediatric population.

A difference in estimated glomerular filtration rates (eGFR) between patients receiving TDF and non-TDF based regimens, was noted. Although this observation may not be clinically significant (all of the subjects had normal eGFR), longer follow-up will be required to assess if the rate of decline persists. Studies in adults have shown significant differences in GFR in patients on TDF and non-TDF groups (45). A recent study by Vrouenraets et al in which both eGFR and measured GFR (mGFR) were calculated found that eGFR, but not mGFR was significantly decreased in patients on TDF-based regimens after 48 weeks of follow-up. The authors attributed this difference to the effect of TDF in the inhibition of tubular creatinine excretion (53).

The earlier detection of proximal tubular defects may be important and routine kidney function tests may not suffice for this purpose since they primarily follow glomerular function (31). Multiple studies have shown that creatinine levels and glomerular filtration rates are reported as normal despite abnormalities in proximal tubular function, and may not be the appropriate tests to follow subjects receiving TDF-based regimens. We studied the potential use of β2 microglobulin or β2-microglobulin/creatine ratio as markers for early detection. We were unable to prove the utility of these markers as significant predictors of PTD. Previous studies in both children and adults have shown that urinary excretion of β2 microglobulin is increased in patients receiving TDF-based regimens and could potentially be used as a predictor of disease (42, 43). In our patient population we did see an increase in the mean level of β2-microglobulin in those subjects with longer TDF exposures, but it did not reach statistical significance (p-value=0.08). Other markers of PTD such as N-Acetyl-B-D-glucosaminidase (NAG) and Retinol Binding Protein (RBP) (54) have been studied but

not in the pediatric population. Further studies are needed to establish a good marker for early detection of PTD in HIV-infected children.

Our study had several limitations: there were few cases of PTD in the follow-up period limiting our power. Nevertheless, this is the largest pediatric and adolescent study to date specifically examining the development of PTD in patients exposed to TDF. We also included the time of initiation of therapy even if this was prior to enrollment in order to compensate for the shorter follow-up period. Second, the follow-up of adolescent patients was complicated and there was some variability in the study visits throughout the study. Therefore, retention rates in the first three visits were relatively good, but there was a larger decline in the fourth visit. To compensate for this we will continue following those patients beyond the thesis period to have a completed study for peer-reviewed publication. Third, there is no consensus in the definition of PTD in the literature and this may have resulted in an overestimation of prevalence. Finally, although we found statistically significant differences in the development of PTD among females exposed to TDF for more than one year as well as lower levels of serum phosphorus in this same population, they may not be clinically significant. Further studies with longer follow-up times are required to fully answer these questions.

In conclusion, there are few cases of PTD in HIV children and adolescents exposed to TDF. There is a slight increase in the risk of developing PTD in women who have been exposed to TDF for a period longer than one year. Lower levels of phosphorus and creatinine clearance were noted in subjects taking TDF for prolonged periods of time, but follow-up studies are required to determine the clinical significance of these findings. β2-microglobulin and β2-Microglobulin/creatinine ratio were not useful markers for early detection of PTD.

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Table 1. Characteristics of the Outcome Variable and other risk Factors over the 1 year study period

Variable	Level	Visit #1 (N = 178) (%)	Visit #2 (N = 166) (%)	Visit # 3 (N = 134) (%)	Visit # 4 (N = 104) (%)
PTD	Yes	3 (1.7)	2 (1.2)	4 (3.0)	4 (3.8)
TDF	No Exposure	67 (37.6)	58 (34.9)	37 (27.6)	26 (25.0)
	Less than 1 year of exposure	62 (34.8)	46 (27.7)	31 (23.1)	11 (10.6)
	More than 1 year of exposure	49 (27.5)	62 (37.3)	66 (49.3)	67 (64.4)
Poor HIV Control[1]	Yes	10 (6.3)	12 (7.7)	9 (7.5)	5 (5.4)
	No	149 (93.7)	143 (92.3)	111 (92.5)	88 (94.6)
Use of other non HIV nephrotoxic Medication	Yes	34 (19.1)	6 (3.6)	5 (3.7)	11 (10.6)
	No	144 (80.9)	160 (96.4)	129 (96.3)	93 (89.4)
Use other nephrotoxic HIV Medication	Yes	137 (77)	127 (76.5)	100 (74.6)	87 (83.7)
	No	41 (23)	39 (23.5)	34 (25.4)	17 (16.3)
CD4 count	<200 cells/mm	24 (13.5)	25 (15.1)	21 (15.7)	17 (16.3)
	>200 cells/mm	154 (86.5)	141(84.9)	113 (84.3)	87 (83.7)
β2 Microglobulin	>160	75 (42.1)	68 (41.0)	48 (35.8)	44 (42.3)
	<160	103 (57.9)	98 (59.0)	86 (64.2)	60 (57.7)
β2 Microglobulin/Creatinine	>300	23 (12.9)	20 (12.0)	11 (82)	10 (9.6)
	<300	155 (87.1)	146 (88.0)	123 (91.8)	94 (90.4)
Blood Pressure	Normal	8 (4.5)	11 (6.6)	3 (2.2)	3 (2.9)
	Abnormal	170 (95.5)	155 (93.4)	131 (97.8)	101 (97.1)

PTD= Proximal Tubular Dysfunction
TDF=Tenofovir
1. Missing 19 on Visit 1, 11 on visit 2, 14 on visit 3 and 11 on visit 4.

Table 2 Descriptive Statistics of other Covariates of Interest

Variables of Interest	Level	N (%) N=178
Gender	Male Female	101 (57) 77 (43)
Race	Black Non-Black	157 (88) 21 (12)
Age in years (Mean ± SD)		15.7 (±6)
Family History of Renal Disease	Yes No	13 (7) 165 (93)
Perinatal HIV Infection	Yes No	125 (70 53 (30)
AIDS Diagnosis	Yes No	127 (71) 51 (29)
Urine Amino Acids	Abnormal Normal	19 (11) 159 (89)
Days Since HIV Diagnosis (Mean ± SD)		3961.9 (±4527.7)

Table 3 Univariate Analysis of Time Varying Risk Factors and the Development of PTD

Variable	Odds Ratio	Confidence Interval	p-value
TDF 0 (no exposure)			0.05
TDF1 (less than 1 year of exposure)	1.01	0.98-1.05	
TDF2 (more than 1 year of exposure)	1.03	1.01-1.07	
Poorly Controlled HIV Infection?	0.97	0.92-1.03	0.35
Use of other non HIV nephrotoxic Medication	0.99	0.95-1.03	0.67
Use of other nephrotoxic HIV Medication	0.99	0.96-1.03	0.72
CD4 Count (<200 or >200 cells/mm^3)	1.02	0.98-1.06	0.40
β2 Microglobulin	0.97	0.95-1.01	0.09
β2 Microglobulin/creatinine	0.96	0.92-1.01	0.09
Blood Pressure	1.02	0.96-1.09	0.485

TDF= Tenofovir

Table 4 Univariate Analysis of Constant Risk Factors on the Development of PTD

Variable	Level	PTD (N=13)	No PTD (N=167)	Odds Ratio	Confidence Interval	p-value
		N (%)	N (%)			
Gender	Male vs. Female	4 (4) 9 (12))	97 (96) 68 (88)	3.21	0.95-10.85	0.06
Race	Black vs. Non-Black	12 (8) 1 (5)	145 (92) 20 (95)	0.60	0.07-4.90	0.64
Age	Per year			1.12	0.98-1.28	0.10
Family History of Kidney Disease	Yes No	1 (8) 12 (7)	12 (92) 153 (92)	0.94	0.11-7.86	0.96
Perinatal	Yes No	8 (6) 5 (9)	117 (94) 48 (91)	1.52	0.47-4.89	0.48
AIDS	Yes No	9 (7) 4 (8)	118 (93) 47 (92)	1.12	0.33-3.8	0.86
Urine Amino Acids	Yes No	2 (11) 11 (7)	17 (90) 148 (93)	0.63	0.129-3.09	0.57

Table 5 Association of TDF Exposure with PTD adjusted for Gender: 3 Levels of TDF[1]

Variable	Odds Ratio	Confidence Interval	P-value
TDF among Males			
TDF1Vs No TDF	**1.00**	**0.95-1.04**	**0.97**
TDF2 Vs No TDF	**1.01**	**0.96-1.05**	**0.8**
TDF among Females			
TDF1Vs No TDF	**1.03**	**0.97-1.10**	**0.24**
TDF2 Vs No TDF	**1.09**	**1.03-1.14**	**0.0016**

1. **TDF was categorized in 3 levels:**
    a. **TDF0: No Exposure**
    b. **TDF1= Exposure to TDF for less than 1 year**
    c. **TDF2= Exposure to TDF for more than 1 year**

Table 6 Association of TDF Exposure with PTD adjusted for Gender: 2 Levels of TDF[1]

Variable	Odds Ratio	Confidence Interval	P-value
TDF 2 vs 1among Males	1.01	0.97-1.04	0.77
TDF 2 vs 1 among Females	1.07	1.02-1.11	0.003

TDF: 1=Less than 1 year or no exposure
2=More than 1 year

Table7 : Difference in Phosphorus mean Level at Enrollment/Follow-up Visit and TDF Exposure

	Visit Number			
TDF	**Enrollment**	**Follow-up Visit 1**	**Follow-up Visit 2**	**Follow-up Visit 3**
1	**4.33 (0.75)**	**4.42 (0.85)**	**4.59 (0.78)**	**4.28 (0.92)**
2	**4.08 (0.77)**	**3.93 (0.84)**	**3.89 (0.72)**	**3.83 (0.74)**

TDF: 1=Less than 1 year or no exposure
2=More than 1 year

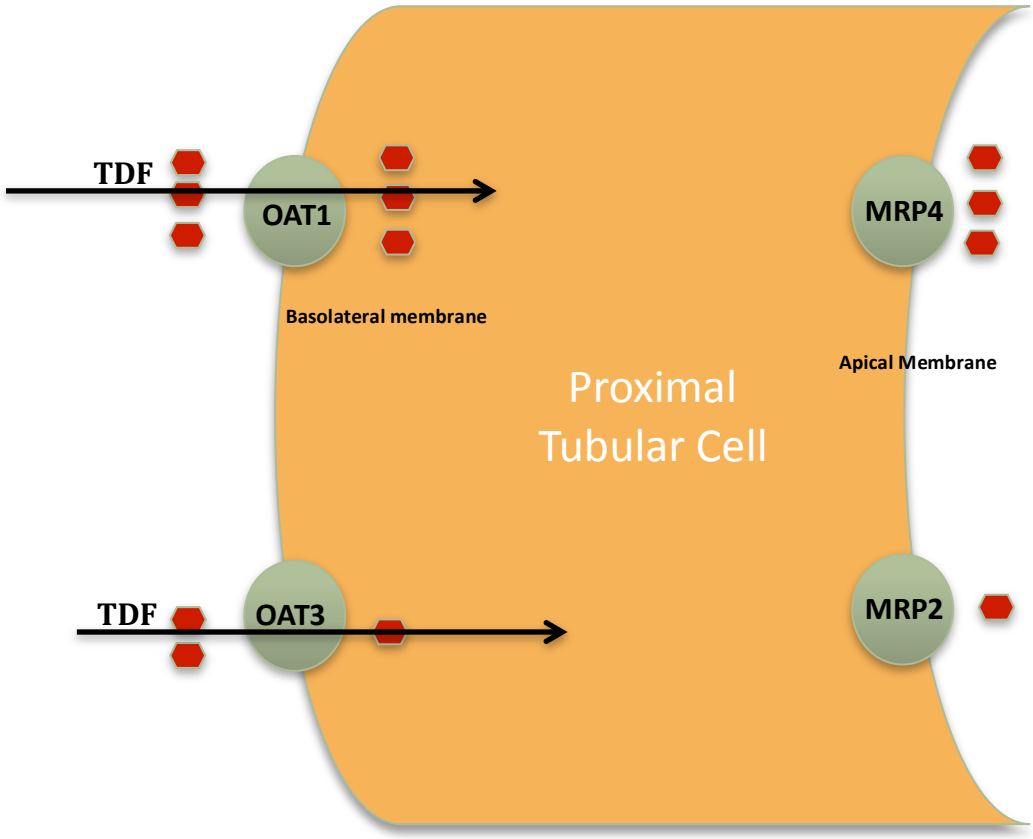
Table 8: Difference in Creatinine Clearance mean Level at Enrollment/Follow-up Visit and TDF Exposure

	Visit Number			
TDF	**Enrollment**	**Follow-up Visit 1**	**Follow-up Visit 2**	**Follow-up Visit 3**
1	**149.1 (34.7)**	**153.6 (39.9)**	**157 (34.7)**	**150 (36.9)**
2	**146.5 (31.6)**	**138.5 (32.0)**	**147 (38.5)**	**138.3 (28.6)**

TDF: 1=Less than 1 year or no exposure
2=More than 1 year

FIGURES

Figure 1: Transport Mechanism of Tenofovir in the proximal Tubular Cell.



TDF= Tenofovir
OAT: Organic anion transporter
MRP: multidrug resistant associated protein

Figure 2 Enrollment Schema

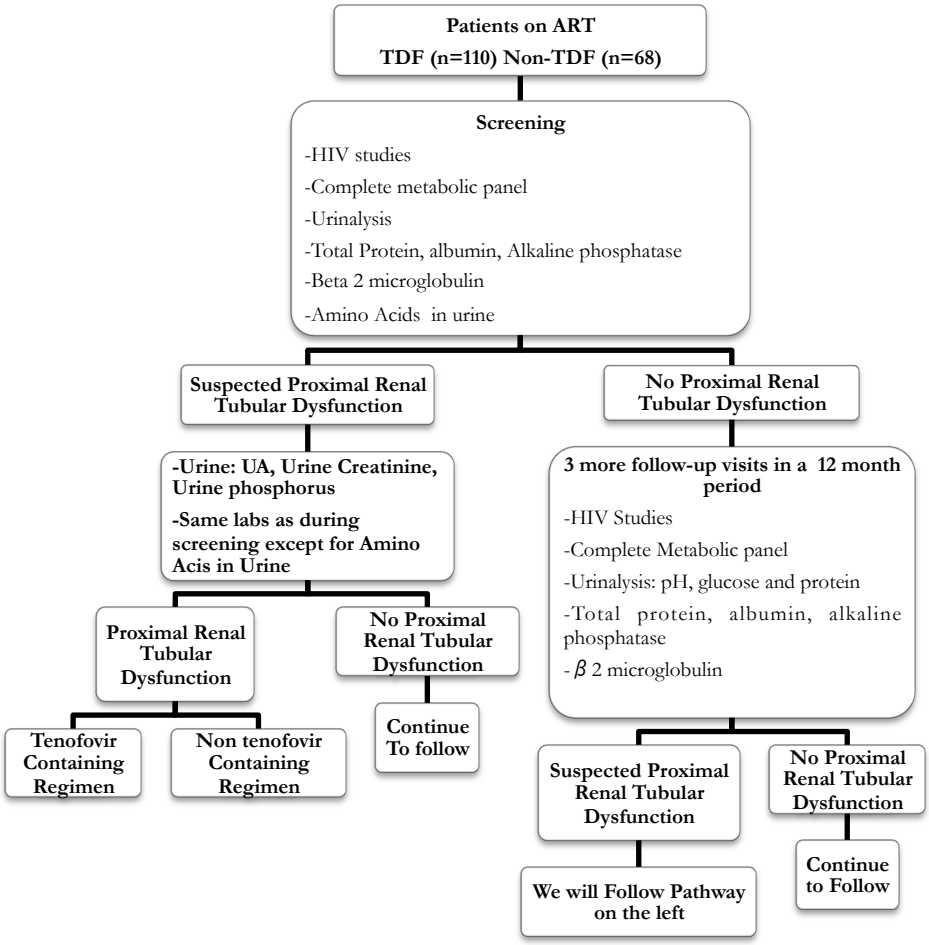


Figure 3 Number of PTD Occurrences per visit

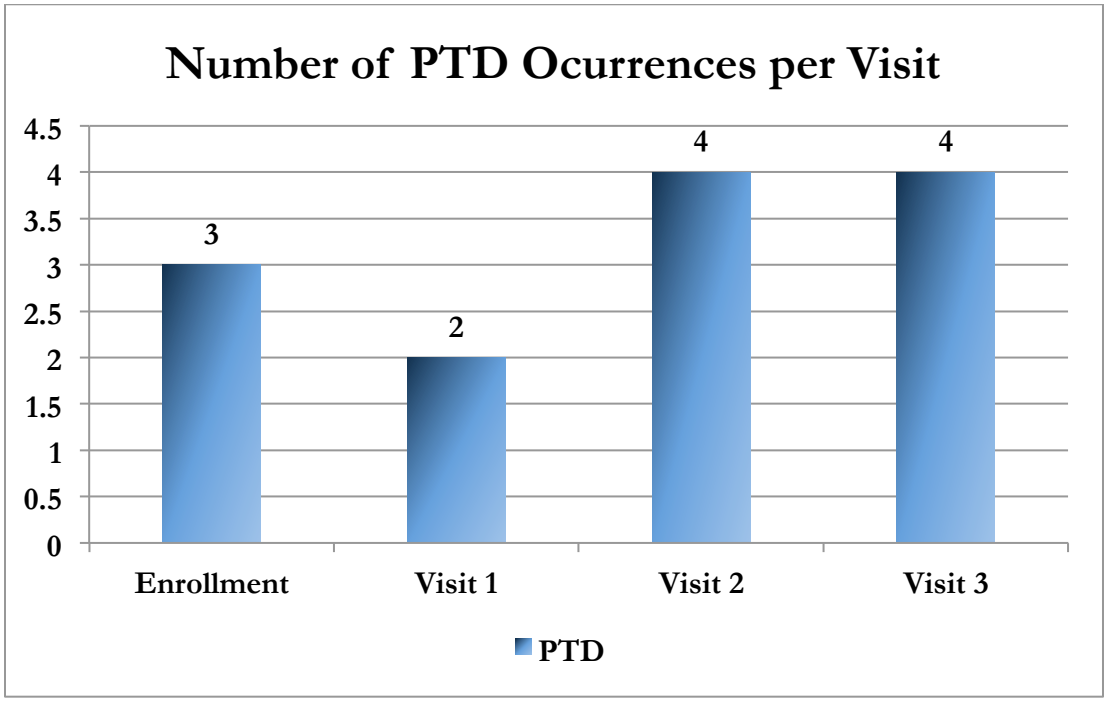
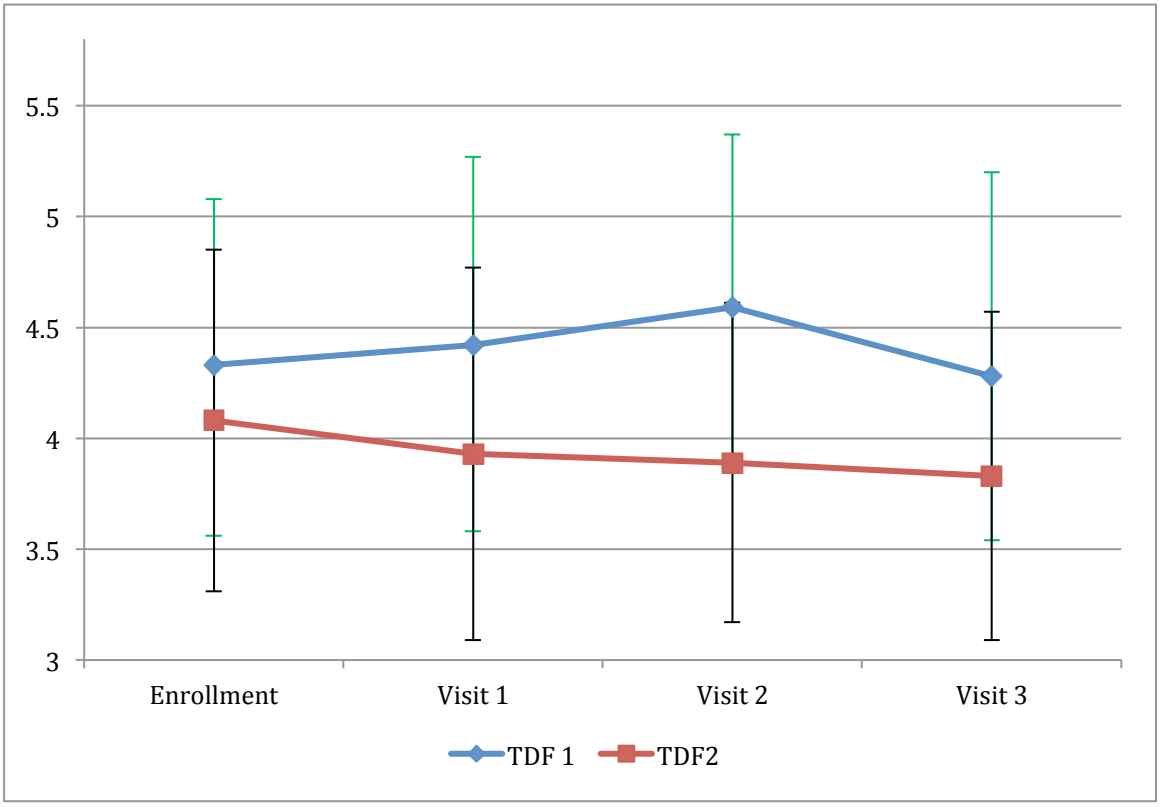
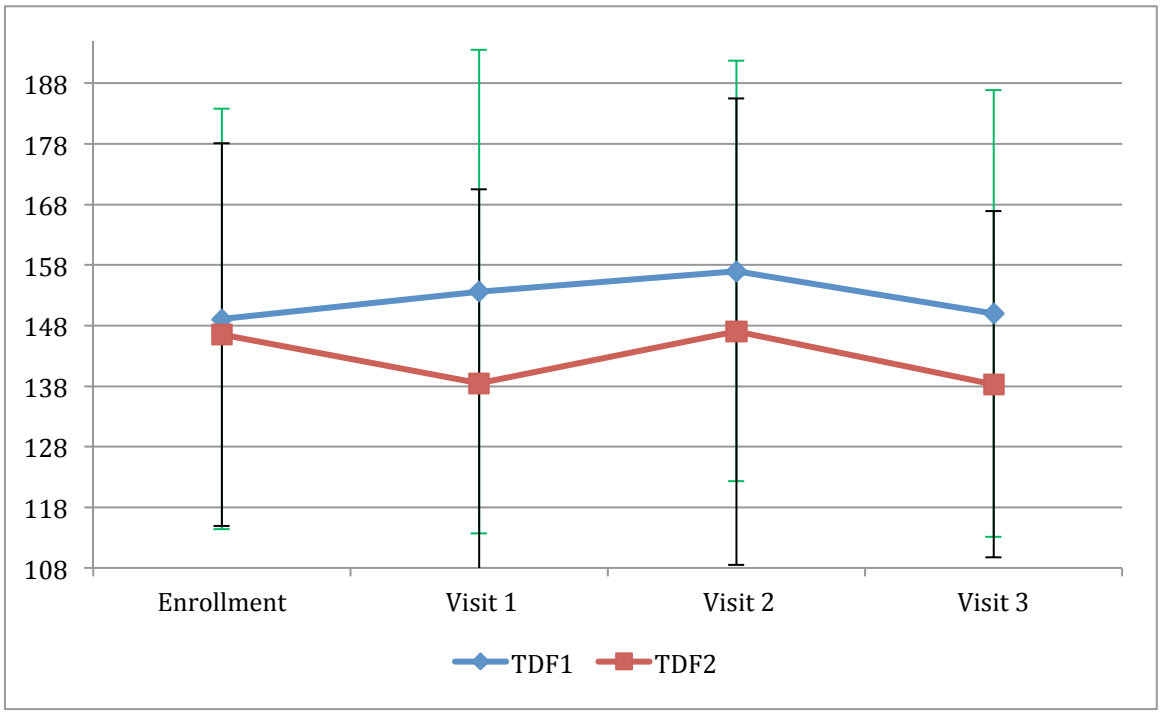


Figure 4 Mean Phosphorus level per Visit Number among TDF user for more than 1 year and TDF user for less than 1 year or no TDF use



TDF1 = TDF use for less than one year or no TDF use
TDF2= TDF use for more than one year.

Figure 5 Mean Creatinine Clearance per Visit Number among TDF user for more than year and TDF user for Less than 1 year or no TDF use



TDF 1 = TDF use for less than one year or no TDF use
TDF 2= TDF use for more than one year