TENOFOVIR-INDUCED KIDNEY AND BONE TOXICITY: CASE REPORTS AND LITERATURE REVIEW

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Abstract

Tenofovir Disoproxil Fumarate (TDF) is one of the drugs that composes the preferred initial antiretroviral regimen for the treatment of hepatitis B and HIV infections. Despite its effectiveness and few metabolic effects, it is related to renal and bone toxicity. We described the cases of two HIV-positive middle-aged women who had been using TDF for 2 and 4 years (cases 1 and 2, respectively), admitted to the emergency room. Case 1 presented with metabolic ileum and diffuse bone pain. Case 2, on the other hand, presented with bilateral coxofemoral pain after a fall from standing height. Both cases had similar laboratory tests: hyperchloremic metabolic acidosis, hypophosphatemia, hypokalemia, hypouricemia and elevated plasma creatinine. In the urinary exams, there was evidence of renal loss of electrolytes, justifying the serum alterations, in addition to glucosuria and proteinuria. Bone pain investigation identified bone fractures and reduced bone mineral density, together with increased levels of parathyroid hormone and alkaline phosphatase and vitamin D deficiency. These cases illustrate the spectrum of adverse renal and bone effects associated with TDF. TDF was discontinued and treatment was aimed at correcting electrolyte disturbances and acidosis, in addition to controlling bone disease with vitamin D and calcium supplementation. Renal changes characterize the so-called Fanconi syndrome, and occur due to mitochondrial toxicity of cells in the proximal tubule by TDF. Bone toxicity occurs due to direct interference of TDF in bone homeostasis, in addition to vitamin D deficiency and phosphaturia resulting from tubulopathy. During follow-up, both evolved with chronic kidney disease and in one of them the Fanconi syndrome did not revert. We emphasize the need for monitoring markers of bone metabolism and glomerular and tubular functions in patients using TDF.

Keywords: Tenofovir Disoproxil Fumarate; Anti-Retroviral Agents; HIV; Kidney; Nephrotoxicity; Bone.

Introduction

Tenofovir Disoproxil Fumarate (TDF) is one of the drugs that composes the preferred initial antiretroviral regimen for the treatment of hepatitis B and HIV infections. It belongs to the class of nucleotide reverse transcriptase inhibitors and despite significant efficacy, good tolerability and low incidence of metabolic adverse effects, TDF is related to more than half of the cases of tubulopathies caused by antiretroviral therapy in HIV-infected patients. In addition to nephrotoxicity, another common adverse effect is bone toxicity, caused by direct and indirect effects of the drug and characterized by osteopenia/osteoporosis, osteomalacia and fractures.[1] Below, we use two clinical cases in HIV-infected patients to illustrate the broad spectrum of clinical and laboratory manifestations caused by tenofovir toxicity in kidney and bone, emphasizing the pathophysiology of these alterations.

Case reports

Case 1: A 55-year-old woman, HIV positive for 13 years, using zidovudine, lamivudine and TDF, was admitted to the emergency room because of a 1-week progressive abdominal pain and distension, associated with interruption of flatus and feces elimination. In addition, she reported diffuse bone pain, more intense in the hip, that had started one year ago but worsened in the last month. Acute abdominal pain protocol ruled out obstructive pathologies, and the analysis of laboratory tests suggested the diagnosis of metabolic ileus (Table 1). Further investigation of the patient's bone-articular

system indicated fractures of the right ischiopubic ramus and of the left second and fifth costal arches, and osteonecrosis of the right femoral head (Figure 1-A and B).

Case 2: A 62-year-old woman, HIV-positive for 4 years, using lamivudine, efavirenz and TDF, was admitted to the emergency room due to bilateral coxofemoral pain after a fall from standing height. The investigation revealed fractures with partial consolidation of the right femoral neck and left femoral subtrochanteric region and old fractures in the ischiopubic ramus and right pubis (Figure 1-C and D). She underwent surgical correction with subsequent hospital discharge. However, after one month, she was readmitted with progressive muscle weakness, vomiting and diffuse paresthesia.

Patients 1 and 2 had been using an antiretroviral therapy (ART) regimen containing TDF for 2 and 4 years, respectively. Although the patients were admitted with different clinical conditions (acute abdomen vs. femur fracture), both had the same pattern of laboratory alterations as shown in Table 1: hyperchloremic metabolic acidosis, hypophosphatemia, hypokalemia, hypouricemia and elevation of plasma creatinine. Urine analysis demonstrated an "alkaline" urinary pH, glycosuria and proteinuria. The increase of the fractional excretion (FE) of phosphate and potassium and the reduction in the maximum phosphorus transport/glomerular filtration rate (TmP/GFR) ratio indicated renal loss of these electrolytes. In addition, parathyroid hormone (PTH) and alkaline phosphatase were increased, while 25-hydroxyvitamin D (calcidiol) was reduced. Dual-energy x-ray absorptiometry (DXA) T-score of the femoral neck was -2.7 in case 1 and -4.5 of the lumbar spine in case 2.

Diagnosis

Tenofovir-related renal and bone toxicity: Acute Kidney Injury (AKI), Fanconi syndrome (FS), vitamin D deficiency, secondary hyperparathyroidism (SHPT), bone mineral density (BMD) reduction and bone fractures.

Follow up

TDF was discontinued and the ART regimen was switched, in case 1 to abacavir, lamivudine and fosamprenavir/ritonavir, and in case 2 to abacavir, lamivudine, darunavir/ritonavir. In both cases, the initial treatment was based on oral replacements of potassium, bicarbonate, vitamin D (cholecalciferol) and calcium. Later, a bisphosphonate (70 mg/week of alendronate sodium) was introduced for osteoporosis. In case 1, the metabolic ileum improved after correction of the electrolyte and acid-base disturbances. During the outpatient follow-up, complete resolution of hypokalemia,

hypophosphatemia, hypouricemia and acidosis, as well as SHPT and urinary alterations (proteinuria and glycosuria) were observed, allowing the suspension of oral replacements after 6 months. In addition, DXA control showed a T-score of -0.9 in the femoral neck after 18 months. However, GFR only partially improved, evolving with chronic kidney disease (CKD) stage 3A (CKD-EPI 56 mL/min/1.73m^2) after 32 months (Table 1). In case 2, during the first 12 months of treatment, it was necessary to maintain the oral replacements initially introduced. Then, the patient lost follow-up, returning 24 months later. New tests showed persistence of the findings of proteinuria, glycosuria, metabolic acidosis, SHPT and CKD stage 3B (CKD-EPI 42 mL/min/1.73 m^2), suggesting irreversibility of the FS (Table 1).

Discussion

These clinical cases illustrate the broad spectrum of adverse effects of TDF involving the kidney and bone, as we will review below. Unlike the kidney damage associated with the HIV, which has a predilection for the glomerulus, the main site of renal aggression by antiretroviral drugs is the tubulointerstitial compartment. In two large retrospective studies involving kidney biopsies from HIV-positive patients, one containing 222 and the other 437 patients, TDF nephrotoxicity accounted for about 13-16% of all histological findings, and for 49-59% of cases with tubulointerstitial diseases.[2,3] Among drug-related tubulointerstitial nephrotoxicity, TDF accounted for the main offending agent, about 70%.[2] In general, TDF renal toxicity increases over time of drug use, becoming more evident after the first year. Additional risk factor include age (>50 years), low body weight (<60 kg), use of other nephrotoxic drugs, male gender, reduced glomerular filtration rate (GFR), comorbidities (HIV-Hepatitis C coinfection, diabetes, hypertension), advanced HIV infection (low CD4 lymphocyte counts, AIDS) and vitamin D deficiency.[1,4,5]

Although the pathological mechanisms are not fully elucidated, the renal damage generated by TDF seems to be related to the mitochondrial DNA toxicity of proximal tubular cells through inhibition of the gamma polymerase enzyme.[4,6] Consequently, there will be dysfunction in the mitochondrial oxidative phosphorylation process with generation of oxidative stress and low levels of ATP. These changes will interfere with the expression of transport proteins on the luminal surface of tubular cells and their production of calcitriol. Furthermore, mitochondrial damage can also stimulate apoptosis of tubular cells by activating the caspase pathway (Figure 2-Ia). Additionally, in rats, TDF nephrotoxicity was accompanied by downregulation of endothelial nitric oxide synthase protein abundance and severe renal vasoconstriction (renal blood flow and increased renal vascular resistance).[7] Histological findings in renal biopsy are characterized by

acute tubular necrosis associated with eosinophilic intracytoplasmic inclusions, which correspond to dysmorphic and giant mitochondria.[6]

About 76-90% of cases of TDF-induced kidney damage displayed proximal tubular dysfunction, clinically manifested by AKI, proteinuria (predominantly tubular proteinuria) and urinary active sediment. On the other hand, CKD was among the indications for kidney biopsy in about 24% of cases.[2,3] Usually, there is an impairment in the reabsorption of glucose, phosphate, uric acid, amino acids, tubular proteins (as β2-microglobulin) or bicarbonate in the proximal tubule. Renal loss and increased urinary excretion of all these substances simultaneously won't always be present, but when it does (27-40% of cases), it characterizes FS.[2,3,4] The main transport affected is involved in phosphorus reabsorption, and hypophosphatemia is a frequent finding. Another manifestation described in case series by Zaidan et al was nephrogenic diabetes insipidus, present in approximately 7% of patients with tubulointerstitial damage attributed to TDF.[2] An experimental study demonstrated that TDF reduces the expression of aquaporin-2 channels in collecting duct cells (Figure 2-Ib).[7]

Clearance of TDF occurs through a combination of glomerular filtration and tubular secretion. About 20-30% of the drug is actively transported across the basolateral membrane of proximal tubule cells by organic anion transporters, in particular OAT-1 and to a lesser extent OAT-3. Then, the secretion into the tubular lumen occurs via energy-dependent pumps, the so-called multidrug resistance proteins (MRP-2 and MRP-4) (Figure 2-Ia).[4] In this scenario, the increase in the intracellular concentration of TDF, aggravating the tubular lesion, can occur in the following situations: drop in GFR, resulting in increased drug elimination by the secretory pathway; or by the use of drugs that increase the activity of OAT-1 and OAT-2 transports and/or reduce MRP-2 and MRP-4. As an example, ritonavir-booster protease inhibitors (PI/RTV) compete with TDF for MRP-2 transport, increasing the risk of kidney injury from TDF by 3.7 times.[8] Studies of pharmacokinetic have shown that boosted protease inhibitors regimens (bPIs) significantly increase the area under de curve of TDF plasma concentrations by 25–37%.[9] Non-steroidal anti-inflammatory drugs, acyclovir and ganciclovir may also increase TDF nephrotoxicity by inhibiting MRP-4. Furthermore, a simple polymorphism in the ABCC2 gene (1249 G → A), responsible for encoding MRP-2, is associated with a 5-6 times greater risk of tubular toxicity and progression to FS.[6] In contrast, drugs such as probenecid, an uricosuric that inhibits OAT-1, has been shown to be potentially effective in reducing TDF nephrotoxicity.[4,10]

Tenofovir Alafenamide (TAF), a new prodrug of tenofovir, has recently emerged as an alternative to TDF. After oral administration, whereas TDF is hydrolyzed by intestinal and plasma esterases to tenofovir, TAF is predominantly metabolized intracellularly by cathepsin A to tenofovir. Thus, the pharmacokinetics of TAF allowed a reduction of about 91% in plasma concentrations of the active metabolite of tenofovir when compared to TDF, reducing the exposure of the kidney and bone to the drug. On the other hand, it increased intracellular concentrations by 6.5 times, allowing the ingestion of low doses of the medication (25mg of TAF is bioequivalent to 300 mg of TDF in terms of plasma levels of tenofovir).[9,11] In phase 3 studies, patients with HIV and chronic hepatitis B who started treatment with TAF-containing regimens had significantly lower decrease in GFR, less proteinuria, and less reduction in BMD compared with those given TDF-containing regimens. In addition, patients who were using TDF and migrated to TAF had increased BMD.[11] These findings were also reinforced by a meta-analysis of randomized controlled trials conducted by Tao et al.[12]

However, the supposed security benefits of TAF over TDF seem to be overestimated, since the adverse effects are more apparent when TDF is administrated as part of bPIs. In a meta-analysis of randomised controlled trials conducted by Hill et al., recently updated, TAF was compared with TDF in boosted or unboosted subgroups for treatment of HIV and chronic hepatitis B.[9,13] This study demonstrated that, compared with TAF, TDF was associated with higher risks of bone and renal adverse events only when boosted with RTV or cobicistat (COBI). By contrast, when RTV and COBI were not used (unboosted subgroups), there were no differences between TAF and TDF for HIV RNA suppression, clinical adverse events, discontinuation for renal adverse events, bone fractures or discontinuation for bone-related adverse events. In addition, a recent systematic review described by Fraga et al. also emphasized that renal and bone toxicity of TDF appears to be a potential problem in HBV/HIV co-infected patients, not being clinically relevant in monoinfected HBV treated with single drug therapy.[14] It is speculated that the absence of TDF dose adjustment when combined with bPIs (TAF is reduced from 25 mg to 10 mg per day, but the TDF dose is maintained at 300 mg per day) results in lower tolerability of TDF compared to TAF regimens.[13]

HIV infection is an isolated risk factor for reduced BMD in children and adults. Osteopenia and osteoporosis rates in the HIV-positive population range from 42-67% and 12-23%, respectively, with a prevalence of about 6.7 and 3.7 times higher when compared to the uninfected population. The result is a risk of fracture 60% higher than the general population. In this scenario, the vast majority of ART regimens contribute to a reduction in BMD. Studies have shown a 2-6%

reduction in BMD in the first 2 years of ART initiation, and regimens containing TDF cause greatest reductions in BMD when compared to others.[15,16]

Experimental studies have shown that TDF directly interferes with bone homeostasis, through the reduction of extracellular adenosine levels, mediated by inhibition of ATP release from cells.[17] As a result, there will be stimulation for osteoclast differentiation and osteoblast inhibition, with increased bone resorption. In addition, TDF interferes with the binding of calcidiol with its carrier protein (DBP, vitamin D binding protein) reducing its availability for production of 1.25-dihydroxyvitamin D (calcitriol) in the kidney, the biologically active form.[18] The summary of TDF-induced bone toxicity mechanisms is illustrated in Figure 2-II.

The indirect effects of TDF on bone are related to mitochondrial toxicity of the renal cells of the proximal convoluted tubule, which may trigger the following changes: 1) urinary calcidiol loss due to the inability to reabsorb DBP and reduction of the mitochondrial 1-alpha-hydroxylase enzyme-mediated conversion of calcidiol to calcitriol; 2) phosphaturia: consequent to reduced expression of the NaPi-IIa cotransport; 3) metabolic acidosis: secondary to bicarbonate reabsorption deficit (Figure 2-Ia).[4,7,19] Reducing calcitriol will reduce the absorption of calcium and phosphorus in the intestine and stimulate the development of SHPT. Acidemia and SHPT stimulated the increase in osteoclastic activity/bone turnover induced by TDF itself. Furthermore, phosphate wasting may be associated with osteomalacia, characterized by impaired mineralization of bone. As DXA scanning may not differentiate between osteoporosis and osteomalacia, the presence of osteomalacia may not be recognized. However, clinical (bone pain and fractures) and laboratory (hypophosphatemia, vitamin D deficiency and elevated serum alkaline phosphatase level - a compensatory increase in osteoblast activity) findings may suggest the presence of this entity, as observed in our patients.[19,20]

The treatment of toxicity induced by TDF should be directed to the changes found in the clinical presentation. The focus will be on the correction of electrolyte disturbances and metabolic acidosis associated with tubulopathy, in addition to the control of SHPT and reduced bone mass with vitamin D and calcium supplementation. Replacement of TDF for a non-tenofovir regimen or TAF is suggested by expert opinion if: GFR is ≤ 60 mL/min, urine protein/creatinine ration (UP/C) > 50 mg/mmol, glucosuria in non-diabetics, confirmed hypophosphatemia of renal origin and osteopenia/osteoporosis in the presence of increased urine phosphate leak. In addition, replacement should also be considered in the following

situations: GFR > 60 mL/min, but decrease in eGFR by 5 mL/min per year for at least 3 consecutive years or confirmed 25% GFR decline from baseline, UP/C 15-50 mg/mmol, co-morbidities with a high risk of CKD (i.e. diabetes and hypertension), body weight < 60 kg or use of a PI/RTV as a third agent.[1] After drug withdrawal, there is a tendency for the recovery of renal functions, which can vary from days to months, a period in which oral replacements of potassium, sodium bicarbonate, phosphate and cholecalciferol may be necessary. However, progression to CKD and tubular alterations may remain in up to 60-70% of patients.[2,6] In cases where the FS becomes persistent, calcitriol supplementation may be necessary due to the kidney's inability to produce active vitamin D. Treatment of osteoporosis, when present, may include antiresorptive drugs such as bisphosphonates or denosumab. However, it is important to emphasize that the use of these drugs can aggravate hypophosphatemia and induce fractures in patients with FS/osteomalacia and, therefore, should only be considered when these conditions are stabilized.[20]

In conclusion, we would like to emphasize that monitoring glomerular and tubular function along with mineral and bone metabolism markers is crucial in HIV-positive patients using TDF. Serum dosages of creatinine, phosphorus, potassium, bicarbonate, PTH, 25-hydroxyvitamin D and alkaline phosphatase, as well as a urine sample (first morning) for evaluation of UP/C, phosphaturia (quantified as FE of phosphate) and urinalysis are recommended annually/biannually in the outpatient follow-up, in addition to DXA every 2 years.[1] This monitoring should be more rigorous in patients who have risk factors for nephrotoxicity and in those who have a history of fragility fracture, osteopenia/osteoporosis or a high FRAX score. In addition, it is important to pay attention to the association of other drugs that may aggravate TDF toxicity, especially when TDF is used in combination with bPIs.

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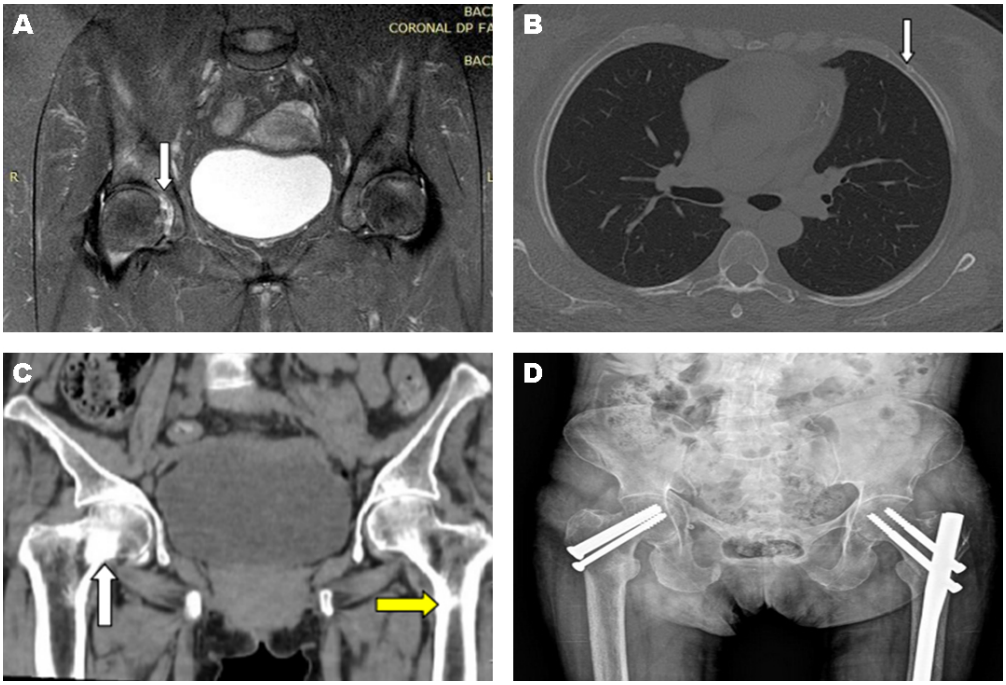
Table 1 - Laboratory tests referring to clinical cases 1 and 2

Exams	Case 1				Values of reference	Case 2			
	Admission	2 months	12 months	36 months		Admission	2 months	12 months	36 months
Blood									
Creatinine (mg/dL)	2.41	1.55	1.31	1.1	0.5-0.9	2.2	1.41	1.5	1.42
Urea (mg/dL)	25	44	45	27	16-40	53	-	78	52
pH	7.15	7.36	7.41	-	7.35-7.45	7.25	7.32	7.3	7.27
Bicarbonate (mmol/L)	11	24.5	24.2	-	22-26	10.6	22.7	19.4	15.4
Anion Gap	18	11.5	13.8	-	10-12	10.4	-	-	-
Chloride (mEq/L)	114	106	103	106	98-109	118	-	106	106
Sodium (mEq/L)	143	142	141	142	135-145	139	-	140	141
Potassium (mEq/L)	2.3	4.34	4.4	4.4	3.5-5.1	1.7	3.7	3.8	4.0
Magnesium (mEq/L)	-	2.22	2.46	2.3	1.7-2.5	1.5	1.97	-	-
Calcium (mg/dL)	-	8.9	10.4	9.5	8.6-10	8.9	9.8	9.2	9.3
Phosphorus (mg/dL)	2.1	-	3.74	4.1	2.5-4.5	1.7	2.32	2.4	-
Uric acid (mg/dL)	2	1.98	-	2.5	2.6-6.7	1.2	-	-	-
Glucose (mg/dL)	92	-	104	-	60-110	-	-	93	93
Parathormone (pg/mL)	98	35	69	67	15-68.3	606	55.7	197	156
Alkaline Phosphatase (U/L)	758	1210	154	163	68-240	1032	978	1032	-
25-OH Vitamin D (ng/mL)	17	-	32	30	>30	9	46	22	29
Urine sample									
pH	7.0	7.0	-	5.0	5.5-6.5	8.0	-	6.0	7.0
Density	1.005	1.020	-	1.009	1.015-1.025	1.010	-	1.019	1.018
Albumin (mg/dL)	++	75	absent	absent	<15	+	100	100	150
Glucose (mg/dL)	++	100	absent	absent	<30	+++	300	300	300
Anion Gap	14	-	-	-		38	-	-	-
Albumin-creatinine ratio (mg/g)	-	-	-	-	-	-	3,800	-	-
24-Hour urine									
24-hour proteinuria (mg)	1,680	74	-	46	<150	-	-	-	-
24-hour glucosuria (g)	11	absent	-	-	<0.5	-	-	-	-
FE* Phosphate (%)@	56	-	-	-	15-20	52	49	-	-
FE* Potassium (%)	35	32	-	-	4-16	51	25	-	-
FE* Uric acid (%)	31	42	-	5	<10	-	-	-	-
TmP/GFR** (mg/dL)	0.22	-	-	-	2.2-3.6	0.36	-	-	-

Abbreviations: FE = Fractional Excretion; TmP/ GFR** = Transport Maximum for Phosphate reabsorption/Glomerular Filtration Rate; @FE of phosphate (%) calculator = [(PO4(urine)/ PO4(serum) / (Creatinine(urine) / Creatinine(serum)] x 100.*



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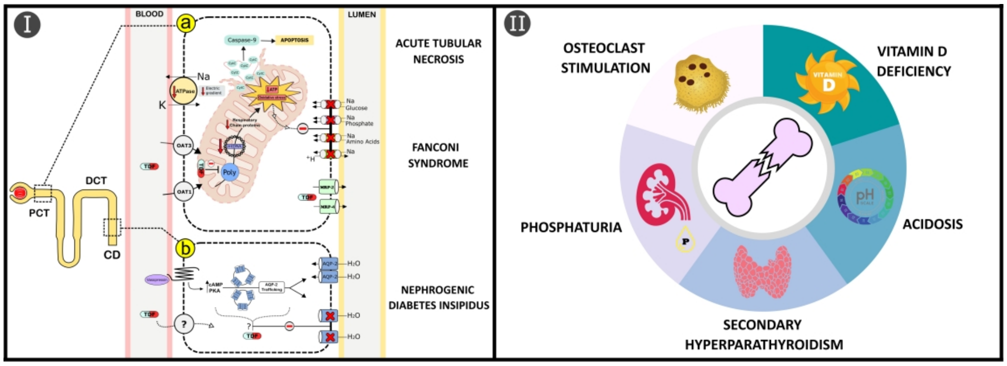
I
BLOOD
LUMEN
a
b
Caspase-9
APOPTOSIS
Na
K
PCT
DCT
CD
ACUTE TUBULAR NECROSIS
FANCONI SYNDROME
NEPHROGENIC DIABETES INSIPIDUS
II
OSTEOCLAST STIMULATION
VITAMIN D DEFICIENCY
PHOSPHATURIA
ACIDOSIS
SECONDARY HYPERPARATHYROIDISM

Legends of the Figures

Figure 1. Image exams referring to clinical cases 1 (A and B) and 2 (C and D). (A) MRI of the hip with osteonecrosis of the right femoral head (white arrow). (B) CT of chest with incomplete fracture in the fifth left costal arch (white arrow). (C) CT of the pelvis with fractures with partial consolidation of the right femoral neck (white arrow) and left subtrochanteric (yellow arrow). (D) Postoperative control pelvis x-ray.

Figure 2. Potential pathogenic mechanisms of TDF-induced bone and renal toxicity. (Ia) After entering the proximal convoluted tubule (PCT) epithelial cells by OAT1/OAT3 transporters, TDF triggers functional and structural abnormalities in the mitochondria through the inhibition of the DNA polymerase γ enzyme (Polγ), compromising mitochondrial DNA (mtDNA) synthesis and the production of respiratory chain proteins. In this scenario, there will be a reduction in the supply of ATP to the cell, in addition to the generation of oxidative stress, leading to decreased basolateral Na/K-ATPase activity, and interfering with the trafficking and endosomal recycling of apical membrane transporters in polarized epithelial cells. Furthermore, the release of proteins from mitochondria to the cytosol, including cytochrome c (CytC), will stimulate pathways of apoptosis (caspase-9) and damage to the cell's DNA. (Ib) In the distal nephron, collecting duct (CD) cells can also be targeted for damage by TDF, through reduced expression of aquaporin-2 (AQP-2) channels on the luminal surface. However, since there is no expression of OAT1/OAT3 in human collecting duct cells, the mechanisms of entry into cells, as well as the reduction in the expression of AQP-2 in the luminal membrane are still unknown. The spectrum of kidney damage includes acute tubular necrosis, Fanconi syndrome and nephrogenic diabetes insipidus. (II) TDF interferes directly with bone homeostasis, stimulating osteoclastic differentiation, and indirectly through PCT epithelial cells damage, reducing the production of calcitriol, responsible for the development of secondary hyperparathyroidism, and inducing phosphaturia and systemic acidosis by bicarbonaturia.