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Poster #791

Determinants of Parathyroid Hormone Levels in HIV-positive Tenofovir-treated Patients with Normal Renal Function

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Original Abstract

Background: Secondary hyperparathyroidism may develop in HIV-positive patients in case of vitamin D deficiency and calcium or phosphorus metabolism disorders. Traditional (age, gender, BMI) and therapeutic determinants [tenofovir (TDF) exposure] have been described but the role of TDF exposure as well as of genetic polymorphisms in key genes is currently unknown.
Methods: Adult HIV-positive patients on TDF-containing HAARTs since at least six months, presenting estimated creatinine clearance (eCRCL) above 60 ml/min, with no significant comorbidity (hypertension, diabetes, urinary tract abnormalities), signing an informed consent were included. Twelve-hours TDF plasma (pC_{12}) and urinary concentration (uC_{12}) were measured through validated HPLC/MS-MS method. Results are expressed as medians (IQR); non parametric tests were used for all analysis. SNPs in the following genes were obtained through real-time PCR: *ABCB1, ABCC2, ABCC4, ABCC10, SLC22A6, SLC28A2, CYP27B1, CYP24A1, VDR.*
Results: 294 patients (75.2% male, 84.4% Caucasian) were enrolled. Age, BMI and eCRCL were 46 years (39.7-52), 23.7 kg/m^2 (22-26.5) and 91.6 ml/min (79.5-107) respectively. Median CD4 were 552 cell/uL (409-713) and 278 patients (94.6%) presented a plasma viral load <50 copies/mL after 55.2 months (27.8-88) of TDF intake. Vitamin D, parathyroid hormone (PTH) and phosphorus were 21.2 ng/dL (13.8-31.3), 52.2 pg/mL (40-66.4) and 3 mg/dL (2.7-3.4) with respectively 72.3%, 15% and 16.8% of patients presenting vitamin D deficiency (<30 ng/mL), secondary hyperparathyroidism (>79.6 pg/mL) and hypophosphoremia (<2.6 mg/dL). At multivariate linear regression (including also age, gender, BMI and time on TDF) vitamin D levels (p<0.001), cirrhosis (p=0.04) and the vitamin D receptor uncommon variant Cdx2 (rs11568820, p=0.025) were independent predictors of PTH. At multivariate linear regression PTH levels (p=0.019), HAART class (NNRTIs associated with the lowest levels, p=0.006), SLC28A2 124 (rs11854484, p=0.047) SNP and tenofovir (TFV) urinary output (urinary TFV/plasma TFV, p=0.032) were independent predictors of phosphorus levels.
Conclusions: A significant proportion of patients on long-term TDF-based treatment showed vitamin D deficiency and secondary hyperparathyroidism: the latter is more common in cirrhotic patients and in patients with non-functional vitamin D receptor. Genetic and pharmacokinetic markers can be used to identify patients at higher risk of hypophosphatemia and bone metabolism dysfunction.

Introduction

- Tenofovir (TFV) use has been associated with renal tubular dysfunction and eGFR decline over time[1]. Suggested mechanism is driven by a reduced TFV reduced urinary excretion (entrapment in tubular cells and mitochondrial toxicity). Hypophosphatemia could be the result of decreased tubular phosphates reabsorption and vitamin D (vit D) activation. Secondary hyperparatiroidism, increased bone turnover and loss of mineral density could be a consequence of phosphates loss [2-4].
- Several factors have been associated with TFV-related tubular toxicity:
  - ✧ low BMI, older age, female gender [5,6];
  - ✧ high TFV exposure [7-10];
  - ✧ single nucleotide polymorphisms in genes encoding for TFV transporters such as *ABCC2* (MRP2), *ABCC4 (MRP4)* and *ABCC10* (MRP7) [11-14];
  - ✧ boosted PIs use (associated with higher TFV exposure trough intestinal p-glycoprotein and MRP4 inhibition) [15-17];
- However, the interplay between TFV exposure, polymorphisms in genes encoding for TFV transporters or involved in vit D metabolism and phosphates/parathyroid hormone levels has not been so far investigated.

AIM OF THE STUDY
To study the demographic, therapeutic and genetic determinants of parathyroid hormon (PTH) and phosphate levels in HIV-positive TFV-administerd patients with normal renal function.

Material & Methods

Cross-sectional, observational study
Main inclusion criteria:
- ▪Adult HIV-positive patients
- ▪estimated creatinine clearance ≥60 mL/min (Cokroft-Gault equation)
- ▪on TDF-containing regimens for ≥6 months
- ▪intake of TDF 12±2 hours before sampling

Paired twelve-hour plasma and urinary concentration were measured by validated HPLC/MS-MS

Single Nucleotide Polymorphisms by real-time PCR
- ◆*ABCB1* (P-glycoprotein): rs1045642 (3435C>T)
- ◆*ABCC2* (MRP2): rs717620 (24 G>A) and rs2273697 (1249 G>A)
- ◆*ABCC4* (MRP4): rs1059751 (*879 T>C), rs1751034 (3348 T>C)
- ◆*ABCC10* (MRP7): rs2125739 (2759 C>T) and rs9349256 (1791+526 G>A)
- ◆*SLC22A6* (OAT1): rs4149170 (453 G>A)
- ◆*SLC28A2* (CNT2): rs11854484 (124 C>T)
- ◆*CYP27B1: rs4646536 (+2838* C>T)
- ◆*CYP24A1:* rs2248359 (T>C)
- ◆ vit D receptor, *VDR*: rs11568820 (Cdx2 A>G)

Data are expressed as medians (interquartile ranges)

Results

Table 1. Patients' Characteristics (n=294)

gender Male (n, %)	221	75.2%	HCV-positive n, %	46	23.7%
ethnicity Caucasian (n, %)	248	84.4%	Cirrhosis n ,%	13	4.4%
age Years (med, IQR)	46	40-52	TDF duration months (med, IQR)	55.2	28-88
BMI Kg/m^2 (med, IQR)	23.7	22- 26.5	vit D ng/mL	21.2	14-31.3
estimated CR CL ml/min (med, IQR)	91.6	79.5- 107	PTH pg/mL	52	40-66.4
CD4 n/uL (med, IQR)	552	435- 702	Third drug: PI	132	45 %
			NNRTI	143	49 %
			Raltegravir	12	4%
HIV RNA <50 cps/mL n (%)	278	94.6%	Other	7	2.4%

Vit D deficiency (<30 ng/mL) %	**72.3**	**PTH >79.6 pg/mL %**	**15**	**Hypophosphoremia (<2.6 mg/dL) %**	**16.8**

12-hour TFV concentrations (ng/mL), (med, IQR)

plasma (pTFV)	66	47-90
urinary (uTFV)	23383	13512-36908
urinary-to-plasma ratio (u/pTFV)	390.7	224.6-559.6

Table 2. Variables Associated with Parathyroid Hormon Levels

	Univariate		Multivariate linear regression		
	ρ	p	β	p	95 CI
Age	0.16	0.005	-0.071	0.274	-0.1;0.6
Gender	-	0.122	-0.048	0.449	-11.9;5.3
BMI	0.07	0.188	-0.039	0.530	-1.2;0.6
duration of TDF exposure	0.09	0.105	0.117	**0.063**	-0.0;0.2
Vit D levels	-0.21	**<0.001**	-0.278	**<0.001**	-0.9;-0.3
Cirrhosis	-	**0.023**	-0.125	**0.042**	-38.8;-0.6
VDR Cdx2 A>G	-	0.160	-0.139	**0.025**	-16.2;-1

At multivariate linear regression analysis
- *vitamin D levels* (p=0.001, 95CI -0.9;-0.3), Fig. 1
- *cirrhosis* (p=0.042, 95CI -38.8;-0.6),
- *vitamin D receptor Cdx2 (* p=0.025, 95CI -16.2;-1), Fig.2

were independent predictors of PTH levels.

Fig.1

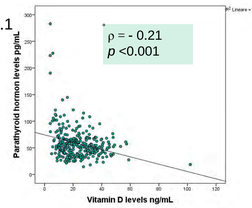


Fig.2

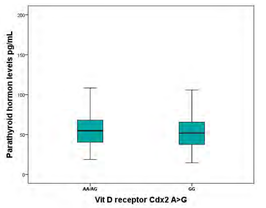


Table 3. Variables Associated with Phosphorus levels

	Univariate		Multivariate linear regression		
	ρ	p	β	p	95 CI
Age	-0,192	0.001	-0.091	0.150	-0.011;0.002
Gender	-	**<0.001**	-0.249	**<0.001**	-0.441;-0.151
BMI	-0.022	0.706	0.005	0.938	-0.016;0.017
duration of TDF exposure	-0.153	**0.011**	-0.069	0.269	-0.031;0.001
PTH	-0.176	**0.003**	-0.144	**0.019**	-0.005;-0.001
HAART associated class	-	**0.001**	-0.169	**0.006**	-0.204;-0.035
SLC28A2 124 C>T	-	**0.002**	-0.124	**0.047**	-0.175;-0.001
urinary/plasmaTFV	0.081	0.195	0.133	**0.032**	0.000;0.000

At multivariate linear regression analysis
- *gender* (p<0.001, 95CI -0.4;-0.1)
- *PTH levels* (p=0.019, 95CI -0.005;-0.001), Fig. 3
- HAART type [third drugs, with NNRTI associated with lower levels (p=0.006,95 CI -0.204;-0.035], Fig. 4
- urinary tenofovir output (u/p TFV) (p=0.032, 95 CI 0.000-0.000). Fig. 5
- *SLC28A2 124* (p=0.047, 95CI -16.2;-1), Fig. 6

were independent predictors of phosphorus levels.

Fig.3

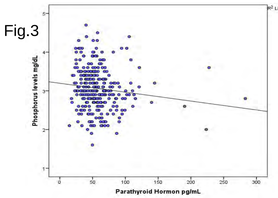


Fig.4

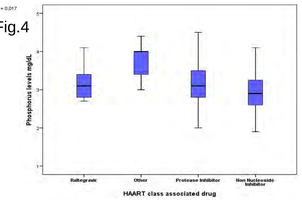


Fig.5

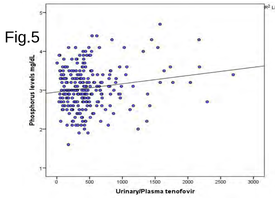
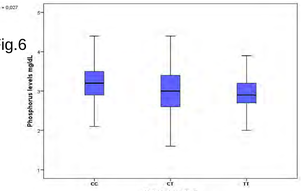


Fig.6



Conclusions

- This is the first study evaluating the interplay between pharmacokinetics and pharmacogenetics of TFV, vit D, phosphates and PTH levels in patients.
- Lower phosphoremia was associated with reduced tenofovir urinary excretion together with female gender and TFV efflux transporter gene *SLC28A2* 124 TT genotype. This finding confirmed the contribution of both genetic factors and tenofovir entrapment to proximal tubular dysfunction. NNRTIs use was also associated with decreased phosphoremia. A possible explanation is induction of cytochrome P450 enzymes, such as 24-hydroxylase, responsible of vit D metabolism. The result could be a decreased reabsorption of phosphates and consequently lower phosphoremia.
- Higher PTH levels could be driven by low phosphoremia but also a role of lower vit D levels and single nucleotide polymorphism in vit D receptor gene (CDx2 GG genotype) was observed. These findings provide a possible explanation to reported variable impact of vit D supplementation on PTH levels in TFV-intakers[18].
- Genetics (*SLC28A2,* vitamin D receptor) and pharmacokinetics (urinary TFV) deserve further evaluation to identify TFV-intakers at higher risk of secondary hyperparathyroidism.

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