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Urine Liver-Type Fatty Acid-Binding Protein and Kidney Injury Molecule-1 in HIV-Infected Patients Receiving Combined Antiretroviral Treatment Based on Tenofovir

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Abstract

The aim of this study was to determine the presence of kidney tubular damage in the absence of overt evidence of glomerular dysfunction (GFR > 60 ml/min without proteinuria) in HIV-infected patients receiving antiretroviral therapy. Urine kidney injury molecule-1 (KIM-1) and liver-type fatty acid-binding protein (L-FABP) levels were measured by ELISA and expressed as a ratio to creatinine. Sixty-six patients (median age 38 years) and 10 healthy controls (median age 35.5 years) were included in the study. Patients with chronic diseases such as diabetes, hypertension, heart disease, or kidney disease were excluded from the study. All patients received tenofovir/emtricitabine combined with one of three other components, namely efavirenz, atazanavir/norvir, or lopinavir/norvir. A lower concentration of L-FABP/creatinine was observed in HIV-infected as compared to healthy individuals ($p = 0.0353$); KIM-1/creatinine was also lower in comparison with healthy controls but not statistically significantly. Patients receiving efavirenz had higher levels of L-FABP/creatinine in comparison to healthy controls ($p = 0.0039$). Patients with anti-HCV had higher concentrations of L-FABP/creatinine as compared to the HIV-monoinfected individuals (not statistically significant) and to healthy subjects ($p = 0.0356$). All four patients with L-FABP > 17.5 μg/g creatinine were HIV/HCV coinfected. On multivariate logistic regression urine L-FABP above 5.5 μg/g creatinine was independently associated with body weight (OR = 0.93 $p = 0.039$). This study suggests that HIV/HCV-coinfected patients with lower body weight treated with tenofovir may be at an increased risk of tubular dysfunction and should be monitored more closely. The use of protease inhibitors was not associated with an increased risk of tubular disorders.

Introduction

Combined antiretroviral therapy (cART) made it possible to extend the lifespan of human immunodeficiency virus (HIV)-infected patients. However, the decrease in the number of deaths caused by acquired immunodeficiency syndrome (AIDS) coincides with an increasing frequency of aging-associated diseases.[1,2] Therefore, quick detection and treatment of diseases that are not associated with AIDS are a matter of increasing importance.

Kidney disease often occurs in patients infected with HIV. According to the EuroSIDA cohort study, it is the fourth most common cause of death of HIV-infected patients for non-AIDS-related diseases, after malignancies, cardiovascular diseases, and liver disease.[1] Introduction of antiretroviral treatment has significantly improved the prognosis of many kidney diseases associated both directly with HIV and with the influence of the virus on the immune system.[3–5] Antiretroviral therapy may, however, also lead to a number of side effects, including nephrotoxicity. Of course, different drugs can influence kidney function in various ways. They may, for instance, contribute to the formation of kidney stones (indinavir, atazanavir), cause interstitial nephritis (indinavir), or lead to Fanconi syndrome (tenofovir).[6,7] Therefore, it is essential to quickly identify kidney injury and proceed with renoprotective intervention. There are a number of biomarkers that may be important in detecting subclinical kidney injury.

Liver-type fatty acid-binding protein (L-FABP) is a protein located in proximal renal tubules that plays an important role in the metabolism of free fatty acids.[8] An increased excretion of this protein in patients with acute renal failure was

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observed.[9] The prognostic significance of its evaluation in patients with chronic kidney disease was also confirmed.[10]

Kidney injury molecule-1 (KIM-1) is a sensitive and specific biomarker for acute kidney injury.[11] This protein is expressed in damaged tubular epithelial cells. It possesses a single transmembrane domain and undergoes membrane-proximal cleavage, which causes the release of soluble KIM-1 ectodomain into the urine.[12] The urinary KIM-1 level is closely related to tissue KIM-1 and correlates with the severity of renal damage, hence the probability that quantitation of urinary KIM-1 will be a noninvasive and sensitive means of kidney injury evaluation and even of monitoring the therapeutic effects of kidney injury.[12–14]

The aim of our study was to assess the urinary concentration of L-FABP and KIM-1 in HIV-infected patients based on selected clinical parameters and an antiretroviral treatment regimen.

We assumed that the study of these biomarkers may help in the diagnosis of kidney injury on the subclinical stage, when it is not yet recognizable by the parameters commonly used and recommended for monitoring the safety of antiretroviral treatment such as glomerular filtration rate (GFR) or urinalysis.

We intended to find a group of patients with an increased excretion of these proteins who might require closer monitoring and initiation of renoprotective intervention.

Materials and Methods

This cross-sectional study was designed to determine the presence of kidney tubular damage in the absence of overt evidence of glomerular dysfunction (GFR > 60 ml/min without proteinuria) in HIV-infected patients receiving antiretroviral therapy. The study was approved by the ethics committee.

Inclusion criteria involved patients with an at least 1 year of antiretroviral therapy containing tenofovir/emtricitabine (TDV) and one of three other components, namely lopinavir/ritonavir (LPV/r), atazanavir/ritonavir (ATV/r), or efavirenz (EFV). All patients had eGFR > 60 ml/min and normal urinalysis. Written informed consent was obtained from all participants (of the study).

Exclusion criteria included chronic diseases such as diabetes, hypertension, diagnosed heart disease, kidney disease as well as active infection, antibacterial or antiviral therapy, or use of nonsteroidal antiinflammatory drugs and other potentially nephrotoxic agents within 6 months before the study. HIV/HBV coinfection (HBsAg positive) and proteinuria defined as below were also exclusion criteria.

Urine analysis

Random urine samples were obtained during routine clinical visits. Urinalysis, the presence of proteinuria, and creatinine concentration in the samples were evaluated.

Urine protein excretion was measured using the turbidimetric method. Proteinuria was defined by the presence of above 200 mg protein/g creatinine in the urine sample. After centrifugation a portion of the urine was frozen within 3 h of collection and stored at −80°C. This material was later used to assess the concentration of L-FABP and KIM-1 by the immunoenzymatic method.

A similar procedure was applied to 10 healthy controls, not subjected to any medication, in whom hepatitis C virus (HCV), hepatitis B virus (HBV), and HIV infection were excluded before the study (anti-HCV negative, HBsAg negative, anti-HIV negative). The control group was matched to the study group in terms of age and gender.

Enzyme-linked immunosorbent assay (ELISA) for measurement of urinary protein level

For detection of KIM-1 and L-FABP levels in the urine of patients and controls specific commercially available ELISA kits were used.

Collected spot urine samples were centrifuged at $10{,}000 \times g$ for 2 min at 4°C to remove particulate matter. Aliquoted supernatants were immediately frozen at −80°C until analysis. Prior to the quantitation the frozen aliquot (1–1.5 ml) of each urine sample was stabilized at room temperature and vortexed gently. Each urine sample and standards were tested in duplicate. For the washing steps Stat-Matic Plate Washer II (Sigma-Aldrich) was used. The absorbance was read by a Multifunctional Microplate Reader VICTOR X4 (Perkin Elmer, USA). All ELISA results were analyzed with WorkOut 2.5 software and expressed as the mean concentration in nanograms protein/ml.

KIM-1 determination. ELISA analysis with the human type KIM-1 antigen ELISA reagent kit (R&D, Minneapolis, MN) was performed to detect the urinary levels of KIM-1. The detection range of the kit was 0–10 ng protein/ml and the sensitivity was 0.009 ng/ml. The coefficients of variations (CVs) values for intraassay and interassay precision were no more than 7.8%. The assay, which recognizes recombinant and natural human KIM-1, was used according to the manufacturer's directions. Briefly, standards and samples were pipetted into the wells of the microtiter plate precoated with a monoclonal antibody specific to KIM-1. Any antigen present was bound by the immobilized antibody and any unbound substances were removed by washing. Then, an enzyme-linked polyclonal antibody specific for KIM-1 was added to the wells. After washing to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color was developed in proportion to the amount of KIM-1 bounded in the initial step. The color development was stopped with sulfuric acid and the optical density of each well was determined within 30 min, using a microplate reader set to 450 nm and 570 nm. Optical imperfections in the plate were corrected by subtraction readings at 570 nm from those at 450 nm. The urinary KIM-1 concentration was determined by referring to the four parameter logistic (4-PL) curve generated by software used for analysis.

L-FABP determination. The level of L-FABP in spot urine samples was determined by means of the Human L-FABP ELISA Kit (CMIC Holdings Co., Tokyo, Japan) with a specific cross-reactivity of 100% with human L-FABP. The sensitivity of the assay was 3 ng/ml and the CV value was no more than 10% in the case of eight times the simultaneous measurement of the same specimen. The assay, which employs the quantitative sandwich enzyme immunoassay technique with a monoclonal antibody for L-FABP precoated onto a microplate, was used according to the manufacturer's directions. Briefly, after incubation with pretreatment solution, standards and samples were added to microplate wells filled with

assay buffer. Any antigen present was bound by the immobilized antibody and any unbound substances were removed by washing and then the second antibody conjugate was added. After incubation time and washing the plate, an enzyme reaction process was initiated by adding the substrate and was terminated by the stop solution. The colorimetric signal produced with the substrate in proportion to the amount of bounded L-FABP was detected at 490 nm. Urinary L-FABP concentrations were determined by comparing the OD of the samples to the five parameter logistic (5-PL) curve generated by software used for analysis.

Urine concentration of KIM-1 and L-FABP was expressed as a ratio to creatinine in order to account for variations in urine concentrations among individuals.

Patients were also subjected to blood tests, which are routinely performed in the course of antiretroviral therapy such as ALT (enzymatic method without the addition of pyridoxal-5′-phosphate), $CD4^+$ T lymphocytes (flow cytometry), creatinine concentration (kinetic compensated Jaffe method), and HIV viremia (RT-PCR method; Cobas AmpliPrep/Cobas TaqMan HIV-1 Test, Roche Diagnostics). The GFR was estimated using the Modification of Diet in Renal Disease Study formula.

Test results for the presence of anti-HCV (HCV EIA II COBAS CORE test) and HCV-RNA (RT-PCR method, Cobas AmpliPrep/Cobas TaqMan HCV TERst, Roche Diagnostics) as well as the duration of antiretroviral therapy and the time of following the present treatment regimen were obtained from the medical record.

Statistical methods

The average values and standard deviation of quantitative traits were calculated for parameters with normal distribution. Variables that were not normally distributed were expressed as median (lower–upper quartiles). These variables were compared according to the Mann–Whitney test. To compare categorical variables between groups Chi-square distribution, Yates' correction for continuity or Fisher's exact test (according to the size of the studied group) was used. Univariate regression and multivariate logistic regression were applied in order to determine whether higher urine L-FABP was related to selected predictors.

Results

Out of 290 patients receiving antiretroviral therapy the inclusion criteria were met by 66 patients, including 24 women and 42 men.

The study group consisted of 20 patients treated with tenofovir disoproxil fumarate (TDF)/EFV, 26 treated with TDF/LPV/r, and 20 treated with TDF/ATV/r.

Analysis of KIM-1 and L-FABP: comparison with the control group

Comparison between the study group and the control group is presented in Table 1. A statistically higher concentration of L-FABP/creatinine was observed in HIV-infected patients receiving antiretroviral therapy as compared to healthy individuals. Levels of KIM-1/creatinine were also lower in healthy controls, although this difference was not statistically significant.

Table 1. Study Group and Healthy Controls

	Patients median (LQ-UQ)	*Healthy controls median (LQ-UQ)*	p
Age (years)	38 (33–45)	35.5 (29–48)	>0.05
Weight (kg)	71 (72–83)	77,5 (61–83)	>0.05
KIM-1 (ng/ml)	0.94 (0.49–2.00)	1.42 (0.84–3.01)	>0.05
KIM-1 (μg/g creatinine)	0.93 (0.56–1.80)	0.79 (0.38–1.38)	>0.05
L-FABP (ng/ml)	0.96 (0–4.13)	0 (0–0.49)	=0.0662
L-FABP (μg/g creatinine)	0.77 (0–5.14)	0 (0–0.24)	=0.0353
L-FABP/KIM-1	0.60 (0–2.74)	0 (0–0.16)	=0.0728
Women[a]	24	4	>0.05
Men[a]	42	6	

[a]Number of subjects.

KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid-binding protein.

L-FABP and KIM-1 depending on the treatment regimen (Table 2)

Patients receiving the regimen based on EFV had higher levels of L-FABP/creatinine compared to individuals treated with other regimens (not statistically significant) and to healthy controls ($p=0.0039$).

However, it should be noted that patients treated with EFV were significantly older than patients treated with protease inhibitors. Furthermore, there were significantly fewer individuals with HIV/HCV coinfection in this group.

In comparison with the control group, patients receiving EFV were older ($p=0.024$) and had a higher level of L-FABP/creatinine ($p=0.039$).

No statistically significant differences of the analyzed parameters were observed between the control group and patients treated with protease inhibitors (PIs).

L-FABP and KIM-1 depending on the presence of anti-HCV (Table 3)

Patients with anti-HCV were significantly younger in comparison to both the control group ($p=0.004$) and the treated patients in whom no anti-HCV was observed ($p=0.0016$). Patients with anti-HCV had higher concentrations of L-FABP/creatinine as compared to the HIV-monoinfected individuals (not statistically significant) and to healthy subjects ($p=0.0356$)

The chance of L-FABP ≥5.14 μg/g creatinine (Table 4 and 5)

First, a univariate logistic regression was performed. It demonstrated a statistical significance of the influence of weight on the occurrence of L-FABP ≥5.14 μg/g creatinine (Table 4). Subsequently, a forward stepwise logistic regression was carried out. Addition of particular variables considered in the analysis to the only statistically significant variable identified in the univariate regression, namely weight, did not change the outcome; its influence on the occurrence of L-FABP ≥5.14 μg/g creatinine was still found to be statistically significant, with an insignificant impact of other variables (Table 5).

Table 2. Estimated Parameters According to Treatment Scheme

	PIs N *(%)*	*EFV* N *(%)*	p
Women	18 (75)	6 (25)	>0.05
Men	28 (66,7)	14 (33,3)	
Intravenous drug usage	28 (77,8)	8 (22,2)	>0.05
Sexual route	18 (60,0)	12 (40,0)	
L-FABP ≥5.14 (μg/g creatinine)	9 (60,0)	6 (40,0)	>0.05
L-FABP <5.14 (μg/g creatinine)	37 (72,5)	14 (27,5)	
eGFR >90 (ml/min)	36 (75,0)	12 (25,0)	>0.05
eGFR ≤90 (ml/min)	10 (55,6)	8 (44,4)	
HIV viremia <50 (copies/ml)	40 (67,8)	19 (32,2)	>0.05
HIV viremia ≥50 (copies/ml)	6 (85,7)	1 (14,3)	
Anti-HCV (+)	33 (82,5)	7 (17,5)	=0.005
Anti-HCV (−)	13 (50,0)	13 (50,0)	
HCV-RNA (+)	24 (82,8)	5 (17,2)	=0.0409
HCV-RNA (−)	22 (59,5)	15 (40,5)	
	Median (LQ-UQ)	**Median (LQ-UQ)**	
Age (years)	35 (32–40)	44 (39–49.5)	=0.0022
Years on present treatment scheme	3 (2–4)	2.5 (1–5)	>0.05
Years on cART	4.5 (4–8)	4.5 (1.5–8.5)	>0.05
CD4 lymphocytes (cells/μl)	454.5 (288–613	435.5 (316–638.5)	>0.05
ALT (U/liter)	28.5 (20–58)	30.5 (18.5–43)	>0.05
Creatinine (mg/dl)	0.84 (0.7–0.93)	0.84 (0.78–0.96)	>0.05
Weight (kg)	74,5 (72–51)	64 (87–62)	=0.0302
eGFR (ml/min)	100.01 (91.98–117.25)	97.70 (83.42–110.7)	>0.05
KIM-1 (μg/g creatinine)	0.94 (0.54–1.80)	0.93 (0.58–1.41)	>0.05
L-FABP (μg/g creatinine)	0.77 (0–3.80)	1.01 (0–6.21)	>0.05
L-FABP/KIM-1	0.45 (0–2.04)	0.91 (0–5.1)	>0.05

eGFR (estimated glomerular filtration rate; MDRD formula); PIs, protease inhibitors; EFV, efavirenz; L-FABP, liver-type fatty acid-binding protein; HCV, hepatitis C virus; KIM-1, kidney injury molecule-1; cART, combined antiretroviral therapy; ALT, alanine aminotransferase.

Table 3. Estimated Parameters According to Anti-Hepatitis C Virus Serology

	Anti-HCV (+) N	*Anti-HCV (−)* N	p
Women	15 (62,5)	9 (37,5)	>0.05
Men	25 (59,5)	17 (40,5)	
Intravenous drug usage	35 (97,2)	1 (2,8)	<0.0001
Sexual route	5 (16,7)	25 (83,3)	
eGFR >90 (ml/min)	29 (60,4)	19 (39,6)	>0.05
eGRF ≤90 (ml/min)	11(61,1)	7 (38,9)	
HIV viremia <50 (copies/ml)	35 (59,3)	24 (40,7)	>0.05
HIV viremia ≥50 (copies/ml)	5 (71,4)	2 (28,6)	
PIs	33 (71,7)	13 (28,3)	=0.005
NNRTI	7 (35,0)	13 (65,0)	
	Median (LQ-UQ)	**Median (LQ-UQ)**	
Age (years)	34.5 (32.5–40)	45 (36–51)	=0.0016
Years on present treatment scheme	3 (2–4)	3 (1–5)	>0.05
Years on cART	5 (4–8.5)	4 (2–8)	>0.05
CD4 lymphocytes (cells/μl)	475 (315.5–646)	428 (270–512)	>0.05
ALT (U/liter)	46.5 (22.5–70)	24.5 (16–33)	=0.0012
Creatinine (mg/dl)	0.86 (0.76–0.95)	0.82 (0.68–0.93)	>0.05
Weight (kg)	71 (77–60)	75 (85–64)	>0.05
eGFR (ml/min)	97.68 (87.86–114.69)	103.41 (88.53–117.25)	>0.05
KIM-1 (μg/g creatinine)	0.93 (0.57–1.98)	1.018 (0.5–1.62)	>0.05
L-FABP (μg/g creatinine)	0.77 (0–5.60)	0.71 (0–3.67)	>0.05
L-FABP/KIM-1	0.52 (0–5.85)	0.71 (0–2.10)	>0.05

eGFR (estimated glomerular filtration rate; MDRD formula); HCV, hepatitis C virus; PIs, protease inhibitors; NNRTI, nonnucleoside reverse transcriptase inhibitor; cART, combined antiretroviral therapy; ALT, alanine aminotransferase; KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid-binding protein.

Table 4. The Chance of Liver-Type Fatty Acid-Binding Protein ≥5.14 μg/g Creatinine—Results of the Univariate Regression

	OR	*95% CI*	p
Age	1.00	0.94–1.07	>0.05
CD4 lymphocytes (cells/μl)	1.15	0.88–1.50	>0.05
HIV viremia (copies/ml)	0.71	0.12–4.21	>0.05
Anti-HCV	3.29	0.80–13.41	>0.5
Years of treatment	1.09	0.82–1.43	>0.05
Years on present treatment scheme	0.99	0.84–1.16	>0.05
PIs/NNRTI	0.57	0.17–1.93	>0.05
eGFR	0.68	0.19–2.43	>0.05
Weight (kg)	0.94	0.88–0.99	0.0247

According to this analysis weight was the only variable of statistical significance with respect to the occurrence of L-FABP≥ 5.14 μg/g creatinine.

OR, odds ratio; PIs, protease inhibitors; eGFR (estimated glomerular filtration rate; MDRD formula); HCV, hepatitis C virus; PIs, protease inhibitors; NNRTI, non nucleoside reverse transcriptase inhibitor.

According to this analysis, weight was the only variable of statistical significance with respect to the occurrence of L-FABP ≥5.14 μg/g creatinine.

Patients with the highest L-FABP/ceratinine

In four HIV-infected individuals despite high eGFR an increase of L-FABP/creatinine above 17.5 μg/g was observed. This group consisted of two persons receiving TDF/EFV, one receiving TDF/LPV, and one receiving TDF/ATV/r. In all of these subjects HCV-RNA was detected (Table 6).

Discussion

TDF is a widely used drug recommended as a first choice in the treatment of HIV-infected patients. It is well know that this drug is eliminated by active tubular secretion and may cause renal tubular damage. Therefore, during treatment with TDF it is necessary to monitor renal function regularly and perform tests such as serum creatinine, eGFR, and urine proteinuria.[15–18]

Large observational studies, including the EuroSida study, have implied that the use of tenofovir and PIs causes a greater risk of renal function impairment in comparison with treatment with TDF and a nonnucleoside reverse transcription inhibitor (NNRTI).[19]

The EuroSida investigators concluded that every additional 10 years of exposure to TDF was associated with a 16% decline in eGFR. The decreasing eGFR was reported in 8%, 22%, and 11% of patients treated with LPV/r, ATV/r, and indinavir (IDV), respectively. Adding TDF to these regimens resulted in a faster eGFR decline. For instance, the use of a combination of ATV and TDF resulted in a decrease in eGFR by 41%.[19]

However, the tests that were assessed in this study are especially suitable for recognizing glomerular disease and may not detect mild tubular disease.

The purpose of our study was to analyze the markers of subclinical renal tubular injury in patients treated with tenofovir. We demonstrated that patients treated with tenofovir and efavirenz had higher L-FABP in comparison with healthy controls. Moreover, 15 out of 66 patients had an L-FABP above 5.14 μg/g creatinine and 4 above 17.5 μg/g creatinine. Many other studies described the incidence of proximal tubular dysfunction in patients on ARV therapy.

In the study conducted by a Japanese group, 25% of HIV-infected patients on cART had subclinical tubular damage.[20] Hall *et al*. showed that the level of retinol-binding protein, which is normally reabsorbed by the proxima tubule, and *N*-acetyl-β-D-glucosaminidase (NAG; a proximal tubule lysosomal enzyme) was increased in the urine of HIV-infected patients treated with tenofovir compared to healthy controls.[21] However, in the article presented by Kinai *et al*. urine β_2-microglobulin levels (normally reabsorbed by the proximal tubule) were increased in patients after the initiation of TDF.[22]

Table 5. The Chance of Liver-Type Fatty Acid-Binding Protein ≥5.14 μg/g Creatinine—Multivariate Logistic Regression

	OR[a]	*95% CI*	p
Age	1.04	0.94–1.16	>0.05
CD4 lymphocytes (cells/μl)	1.28	0.91–1.81	>0.05
HIV viremia (copies/ml)	0.63	0.05–8.34	>0.05
Anti-HCV	5.34	0.60–47.60	>0.5
Years of treatment	0.83	0.64–1.08	>0.05
Years on present treatment scheme	1.17	0.72–1.92	>0.05
PIs/NNRTI	0.83	0.12–5.97	>0.05
eGFR	0.52	0.10–3.37	>0.05
Weight (kg)	0.93	0.86–0.99	0.039

[a]Odds ratio (OR) adjusted for all variables used in this model.

According to this analysis weight was the only variable of a statistical significance with respect to the occurrence of L-FABP≥ 5.14 μg/g creatinine.

HCV, hepatitis C virus; PIs, protease inhibitors; NNRTI, nonnucleoside reverse transcriptase inhibitor; eGFR (estimated glomerular filtration rate; MDRD formula).

Table 6. Patients with Liver-Type Fatty Acid-Binding Protein >17.5 μg/g Creatinine

	Patients			
	1	*2*	*3*	*4*
Sex	M	M	M	F
Age (years)	38	37	45	43
Route of infection	N	N	N	N
CD4 lymphocytes (cells/μl)	437	218	536	642
HIV viremia (copies/ml)	<50	<50	<50	<50
Anti-HCV	+	+	+	+
HCV-RNA	+	+	+	+
Years of treatment	3	3	12	9
Years on present treatment scheme	3	3	2	2
Drugs	ATV/r	LPV	EFV	EFV
Creatinine (mg/dl)	0.96	1.09	1.02	0.77
eGFR (ml/min)	93.17	81.36	84.33	87.37
Weight (kg)	75	71	50	49
L-FABP (μg/g creatinine)	17.7	44.61	108.49	26.3
KIM-1 (μg/g creatinine)	3.08	2.19	2.36	0.93

N, intravenous drug usage; eGFR (estimated glomerular filtration rate; MDRD formula); HCV, hepatitis C virus; L-FABP, liver-type fatty acid-binding protein; KIM-1, kidney injury molecule-1.

Our results showed an increased urine concentration of L-FABP in HIV/HCV-coinfected patients. The coincidence of chronic hepatitis C and renal dysfunction is widely recognized.

HCV nephropathy is well-known and is not a rare phenomenon.[23,24]

Viral RNA as well as viral proteins have been found in glomerular structures and in the tubular epithelial cells of HCV-infected patients.[25,26] Regardless of the tubulointerstitial injury associated with different glomerular lesions, HCV may lead to tubular injury in its own right.[23] In the large EuroSida study, patients with HCV replication were at increased risk of developing chronic kidney disease.[27]

Our results suggested that there was no increased risk of subclinical renal tubular injury in patients treated with PIs in comparison to those on EFV-based therapy. Many clinical studies concentrate on renal tubular dysfunctions depending on the type of cART regimen. Ando *et al*. demonstrated that urine β_2-microglobulin, urine α_1-microglobulin, and γ-glutamyl transpeptidase were not statistically significant different between groups of patients treated with TDF + PIs compared to TDF + EFV.[20]

In our study we observed a higher level of L-FABP in patients treated with TDF with EFV in comparison to the healthy control group; however, no differences in L-FABP level were found between the healthy group and patients treated with TDF plus PI. We observed that patients treated with a PI-based regimen had a higher body weight and, in our opinion, the weight of patients can influence TDF safety. Moreover, in our study only low body weight was an independent risk factor for a higher L-FABP/creatinine level. Multiple studies indicated that low body weight, older age, and higher tenofovir plasma level were associated with a higher risk of tubular dysfunction during TDF-based therapy.[28,29] Thus, it could be speculated that the observed increased body mass on PI treatment may influence the redistribution of tenofovir, resulting in a decline or risk of tubular dysfunction. On the other hand, it has been suggested that ritonavir can slow TDF renal clearance and increase TDF plasma concentration,[30] which can be associated with the development of tubular toxicity.

Our results are in contrast with the large observational studies, which demonstrate that the use of tenofovir in combination with PIs has been linked to kidney toxicity. However, in these analyses the authors assessed parameters suitable for evaluation of glomerular but not tubular function.[19,31,32]

Our study was cross-sectional and it was conducted in patients without overt renal dysfunction, therefore it is difficult to determine their prognostic value in predicting the development of chronic kidney disease.

Thus, larger studies are clearly warranted to further investigate the clinical significance of these results.

In conclusion, this study suggests that patients treated with TDF who are HIV/HCV coinfected and have a lower weight may have an increased risk of tubular dysfunction and should be monitored more closely. The use of protease inhibitors was not connected with the increased risk of tubular disorders.

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