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Comparison of Levels of Nephropathy Biomarkers in HIV-infected Individuals and Healthy Volunteers

HIV ile Enfekte Bireyler ile Sağlıklı Gönüllülerde Nefropati Biyobelirteçlerinin Düzeylerinin Kıyaslanması

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Abstract

Introduction: The number of human immunodeficiency virus (HIV)-infected individuals (HIIs) is increasing day by day and life expectancy is prolonged with the use of highly active antiretroviral therapy (HAART). With the advancing age, chronic diseases that may cause renal dysfunction will also begin to appear. In addition, HIV-associated nephropathy due to inflammation caused by HIV itself and renal dysfunction due to HAART use have been reported in the literature. Considering the social and medical problems caused by HIV, nephropathy as an additional disease makes patient management difficult. Therefore, it is important to detect nephropathy in the early period and take precautions. Creatinine is not always sufficient in the evaluation of nephropathy. New searches are needed in demonstrating nephropathy in HIIs compared to healthy individuals. It was aimed to evaluate cystatin C and NGAL levels in serum, and KIM1 and NGAL levels in urine to determine whether nephropathy developed in HIIs.

Materials and Methods: Eighty-eight HIIs in whom renal dysfunction was not detected before and 81 healthy individuals were prospectively evaluated. Cystatin C and NGAL levels were studied in serum samples, while KIM1 and NGAL were studied in urine samples. Serum creatinine, spot urine protein and creatinine levels were recorded in these patients.

Results: Of all participants, 114 (67.5%) were male and 55 (32.5%) were female. It was observed that the estimated-glomerular filtration rate (eGFR) was higher, cystatin C level in serum, and NGAL level in serum and urine were higher in the control group than the patient group. Serum creatinine, urinary protein and urinary creatinine levels were higher in the HIV-infected patient group compared to the healthy control group ($p<0.05$). The estimated-glomerular filtration rate was lower in patients using tenofovir disoproxil fumarate (TDF) than in patients not using TDF ($p=0.017$). When all participants were evaluated, urinary NGAL level was higher in women ($p<0.001$).

Conclusion: In our study, HIIs had higher creatinine levels and lower eGFR compared to the control group. In the control group, cystatin C in serum and NGAL in urine and serum were higher. These parameters did not correlate with creatinine level and creatinine-based eGFR in serum, which were standardized to assess renal function. Our study was a cross-sectional study and only one measurement was made. In our study, the NGAL level was found to be higher in women, especially in the urine, and it was necessary to pay attention to the gender factor when using nephropathy markers. Low eGFR was noteworthy in patients using TDF.

Keywords: HIV, nephropathy, cystatin C, KIM1, NGAL

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Öz

Giriş: İnsan immün yetmezlik virüsü (HIV) ile enfekte bireylerimizin (HEB) sayısı her geçen gün artmakta ve yüksek etkili antiretroviral tedavi (HAART) kullanımı ile yaşam süreleri uzamaktadır. Yaşın ilerlemesi ile renal fonksiyon bozukluğu yapabilecek kronik hastalıklar da görülmeye başlayacaktır. Ayrıca HIV'in kendisinin neden olduğu enflamasyona bağlı HIV ilişkili nefropati ve HAART kullanımına bağlı renal fonksiyon bozuklukları literatürde bildirilmektedir. İnsan immün yetmezlik virüsünün sosyal ve medikal problemleri düşünüldüğünde ek bir hastalık olarak nefropatinin yol açtığı komplikasyonlar hasta yönetimini güçleştirmektedir. Bu yüzden erken dönemde nefropatinin tespit edilip önlemlerin alınması önemlidir. Kreatinin nefropatinin değerlendirilmesinde her zaman yeterli gelmemektedir. Sağlıklı bireylere kıyasla HEB'lerde nefropatiyi göstermede yeni arayışlara ihtiyaç duyulmaktadır. HIV ile enfekte bireylerde nefropati gelişip gelişmediğini belirlemede, sistatin C, idrarda KIM1, serum ve idrarda NGAL düzeylerinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Daha önce renal fonksiyon bozukluğu tespit edilmeyen 88 HEB ve 81 sağlıklı birey prospektif olarak değerlendirildi. Serum örneğinde sistatin C ve NGAL düzeyleri çalışılırken, idrar örneklerinde ise KIM1 ve NGAL düzeyleri çalışıldı. Bu hastalarda serum kreatinin, spot idrarda protein ve kreatinin düzeyleri kaydedildi.

Bulgular: Tüm katılımcıların 114'ü erkek (%67,5), 55'i kadındı (%32,5). Kontrol grubunda tahmini-glomerüler filtrasyon hızı (eGFR), serumda sistatin C, serumda ve idrarda NGAL düzeylerinin hasta grubundan daha yüksek olduğu gözlendi. Serum kreatinin, idrar protein ve idrar kreatinin değerleri hasta grubunda, sağlıklı kontrol grubu ile karşılaştırıldığında daha yüksek bulundu ($p<0,05$). Tenofovir disoproksil fumarat kullanan hastalarda eGFR, kullanmayan hastalara göre daha düşüktü ($p=0,017$). Tüm katılımcılar değerlendirildiğinde kadınlarda idrarda NGAL düzeyi daha yüksekti ($p<0,001$).

Sonuç: Çalışmamızda HEB'lerde, kontrol grubuna göre kreatinin yüksekliği ve eGFR düşüklüğü mevcuttu. Kontrol grubunda ise serumda sistatin C, idrarda ve serumda NGAL düzeyleri daha yüksekti. Bu parametreler; renal foksiyonları değerlendirmede standartlaşmış olan serumda kreatinin ve kreatinin bazlı eGFR ile korelasyon göstermemiştir. Çalışmamız kesitsel bir çalışma olup tek ölçüm yapılmıştır. Çalışmamızda özellikle idrarda NGAL düzeyi kadınlarda daha yüksek saptanmış olup, nefropati belirteçlerini kullanırken cinsiyet faktörüne dikkat etmek gereklidir. Tenofovir disoproksil fumarat kullanımı olan hastalarda eGFR düşüklüğü dikkati çekmektedir.

Anahtar Kelimeler: HIV, nefropati, sistatin C, KIM1, NGAL

Introduction

Human immunodeficiency virus (HIV) is a retrovirus that basically infects immune system cells and disrupts their functions, affects all systems in the body, and can cause local and systemic complications. While the number of HIV-infected individuals (HIIs) is increasing day by day, the diagnosed patients also age over time. It is expected that the rate of development of kidney disease in these patients will increase as a result of the increase in the frequency of comorbidities such as hypertension, diabetes mellitus, lipometabolic disorder and hepatitis in HIV-positive patients, all of which now affect younger individuals. While HIV itself can lead to renal disorders called HIV-associated nephropathy (HIVAN) or HIV-associated immune complex disease due to inflammation, renal impairment can also occur as a result of the highly active antiretroviral therapy (HAART) regimen we use in the treatment.

Renal insufficiency causes health problems such as the need for dialysis, anemia treatment, osteoporosis, and an increase in the risk of cardiovascular disease, in addition to the deterioration of patients' quality of life. Studies have shown that proteinuria and estimated glomerular filtration rate (eGFR), which are traditional methods of indicating kidney disease, may not be sufficient for early diagnosis. Biomarkers of cystatin C, kidney injury molucule-1 (KIM1) and neutrophil gelatinase-associated lipocalin (NGAL) have been studied in different patient populations before, and some studies have shown significant results in demonstrating early nephropathy. There are studies in the literature showing that high serum cystatin C levels are also associated with renal and cardiovascular complications, and that high serum KIM1 levels help predict the incidence of chronic kidney disease (CKD) in HIIs[1,2]. Also KIM1 has also been used to predict mortality, decrease in eGFR and tenofovir disoproxil fumarate (TDF) toxicity[3-5]. It has been observed in studies that NGAL has a 10-fold increase in serum levels and a 100-fold increase in urine levels in intensive care patients who develop acute renal failure secondary to sepsis, ischemia or nephrotoxins[6]. However, there is still not enough proven data on these biomarkers. In this study, it was planned to investigate the contribution of cystatin C and NGAL levels in serum, and KIM1 and NGAL levels in urine in early diagnosis of renal dysfunction, which may be one of the complications in HIIs.

Materials and Methods

Among the newly diagnosed patients or patients receiving treatment who were admitted to the Çukurova University Faculty of Medicine Infectious Diseases and Clinical Microbiology outpatient clinic between May 2018 and May 2019, those who wanted to participate in the study were included. Patients with CKD and those with known hypertension and the use of anti-hypertensive drugs were excluded in the study. The study included 88 patients with a sampling error of 0.10 and $\alpha=0.05$. As the control group, 81 healthy volunteers who were admitted to the infectious diseases outpatient clinic without underlying hypertension, diabetes mellitus or kidney disease were included. Written informed consent was obtained from all patients in the

study. Approval was obtained from Çukurova University Faculty of Medicine Ethics Committee (no: 33, date: 13.04.2018).

Demographic data, disease characteristics, comorbidities, CD4 cell count, HIV RNA values, and previous antiretroviral treatments (ART), if any, of HIIs included in the study were recorded. Detailed physical examination of each patient was performed and blood pressure arterial (mmHg) values from the brachial artery were recorded with a single measurement.

Serum creatinine, sodium, potassium, calcium and phosphorus levels, protein and creatinine levels in spot urine, venous blood gas, complete blood count of the patients included in the study were recorded, and eGFR value was calculated by taking the serum creatinine level using the modification of diet in renal disease method[7].

Blood (in a tube with EDTA) and urine samples were collected from the patients to measure KIM1, NGAL and cystatin C levels. Cystatin C and NGAL levels were measured in the serum sample taken at the first admission during the start of the study, while KIM1 and NGAL levels were measured in the urine samples. The samples were centrifuged at 2000-3000 rpm for 20 minutes, and the supernatant parts were transferred to screw cap cryo tubes and stored at -80 °C until they were measured.

Human cystatin C was measured with Cys-C ELISA Kit (96 Tests) (YLbiont, Shanghai, China), human NGAL with ELISA Kit (96 tests) (YLbiont, Shanghai, China), and human KIM1 with ELISA Kit (96 tests) (YLbiont, Shanghai, China). Biomarkers were studied with the BiotekELX800 Elisa device. Cystatin C, NGAL, and urine and serum KIM1 levels were studied in accordance with the specified kit protocol.

Statistical Analysis

The Statistical Package for the Social Sciences (IBM statistics 21) package program was used for statistical analysis of the data. Categorical measurements were summarized as numbers and percentages, and continuous measurements as mean, deviation, and minimum-maximum. In comparison of continuous measurements between groups, distributions were controlled and independent Student's t-test was used for two-group variables and ANOVA analysis was used for more than two variables. Correlation (Spearman's rho) analysis was used to determine the level of correlation between the patients' current eGFR, cystatin C level in serum, NGAL level in serum, NGAL level in urine and KIM1 level in urine. The relationship between eGFR and nephropathy biomarkers and risk factors in HIIs was evaluated by Spearman's correlation test and scatter plots were made. The extent to which variables with a significant linear relationship predicted the eGFR level was analyzed by multivariate linear regression analysis. Statistical significance level was accepted as less than 0.05 in all tests.

Results

A total of 169 subjects over the age of 18 were included in the study, including 88 patients (52.1%) as the patient group and 81 healthy volunteers (47.9%) as the control group. While 76 (86.4%) of 88 HIIs in the patient group were men, 38 (46.9%) of 81 healthy volunteers in the control group were men. The proportion of male patients in the patient group was higher than the control group. When the mean age of the patients was evaluated, the mean age of 88 patients in the patient group was 38.53±12.32 years, and the mean age of 81 healthy volunteers in the control group was 38.56±13.05 years. The mean systolic and diastolic blood pressures of the patient and control groups are shown in the table (Table 1). Of HIV-infected patients 75% (85.23%) were using ART, and 57 of patients using ART were receiving TDF treatment. The mean absolute CD4 count of the patients was 716 (±294).

Estimated-GFR, cystatin C level in serum, NGAL level in serum, NGAL level in urine and KIM1 level in urine were evaluated in the patient and control groups (Table 2). It was observed that the mean values of eGFR, cystatin C level in serum, NGAL level in serum, NGAL and KIM1 levels in urine in the control group were

Table 1. Characteristics of the patient and control groups

	Patient group (n=88)		**Control group (n=81)**	
Age (year)	38.53±12.32		38.56±13.05	
Gender	Female n=12	Male n=76	Male n=38	Female n=43
Mean systolic blood pressure (mmHg)	120.92±11.70		115.3±9.23	
Mean diastolic blood pressure (mmHg)	76.36±6.64		72.1±7.19	
HAART* use TDF use**	75 (85.23%) 57 (64%)			
Mean CD4 count Min-max	716±294 Min-max (31-1603)			

**Use of TDF for more than 6 months.

*HAART: Highly active antiretroviral treatment, Min-max: Minimum-maximum, TDF: Tenofovir disoproxil fumarate

higher than the patient group (p<0.05). Although the mean of KIM1 level in the urine in the patient group was higher than the control group, it was not statistically significant (p>0.05). Serum creatinine levels and urine protein and creatinine levels were higher in the patient group than in the control group (p<0.05) (Table 2).

In the patient group, there were 57 (64%) patients who had been using TDF for more than six months. The nephrological parameters of patients with and without TDF use were compared. Estimated-GFR was found to be lower in patients with TDF use (p=0.017). There was no statistical difference between the patients with and without TDF use in terms of creatinine level in serum, urinary protein/creatinine, protein level in urine, serum cystatin C level, urine and serum NGAL levels, and KIM1 level in urine (p>0.05) (Table 3).

There were no statistically significant differences between the male and female groups in terms of cystatin C level in serum, NGAL level in serum, NGAL level in urine, KIM1 level in urine and current eGFR values (p>0.05). Urinary NGAL level in the female group was found to be statistically significantly higher than the male group (p<0.001) (Table 4).

The relationship between eGFR level and nephropathy biomarkers and risk factors in HIIs was analyzed by the Spearman correlation test. A statistically significant correlation was found between age, serum cystatin C level, duration of TDF use, and urine KIM1 level and eGFR (rho=-0.594, p<0.001; rho=0.218, p=0.041; rho=-0.337, p=0.001; and rho=0.213, p=0.046, respectively) (Table 5). Scatter plots for variables with significant correlations are shown in Figure 1.

When multivariate linear regression analysis of factors associated with eGFR level in HIIs was performed, age (B=-0.990, p<0.001) and duration of TDF use (B=-0.200, p=0.013) were found to be statistically significant (F=15.114; p<0.001) (Table 6).

Table 2. Nephrological parameters in patient and control groups

	Patient group (n=88)	Control group (n=81)	p
	Mean±SD	Mean±SD	
Age (years)	38.53±12.32	38.56±13.05	0.991
Serum creatinine level (mg/dl)	0.84±0.13	0.68±0.14	**<0.001**
Urine protein level (mg/dl)	18.38±20.69	10.62±8.10	**0.002**
Urine creatinine level (mg/dl)	155.23±86.82	118.67±68.89	**0.003**
Urine protein/creatinine	125.34±147.69	111.82±182.30	0.596
Current eGFR (ml/min/1.7)	107.90±23.48	122.98±24.39	**<0.001**
Serum cystatin C level	36.53±52.03	54.23±52.17	**0.029**
Serum NGAL level	835.24±1525.30	1300.08±1455.31	**0.045**
Urine NGAL level	111.02±72.59	242.95±62.28	**<0.001**
Urine KIM1 level	0.58±0.28	0.55±0.20	0.467

SD: Standard deviation, eGFR: Estimated-glomerular filtration rate

Table 3. Nephrological parameters by TDF use

	TDF use*		p
	Yes (n=57)	No (n=31)	
	Mean±SD	Mean±SD	
Age (years)	39.9±11.09	35.8±14.1	0.136
Serum creatinine level (mg/dl)	0.856±0.133	0.834±0.15	0.49
Urine protein level (mg/dl)	17.2±12.39	20.53±30.79	0.567
Urine protein/creatinine (mg/dl)	0.116±0.079	0.142±0.225	0.535
Current eGFR (ml/min/1.7)	103.55±20.92	115.91±26.07	**0.017**
Serum cystatin C level	29.43±44.71	49.56±62.04	0.117
Serum NGAL level	629.59±1227.7	1213.36±1924.89	0.134
Urine NGAL level	111.6±85.6	109.91±39.94	0.889
Urine KIM1 level	0.59±0.34	0.57±0.125	0.708

*Use for more than 6 months.

SD: Standard deviation, TDF: Tenofovir disoproxil fumarate

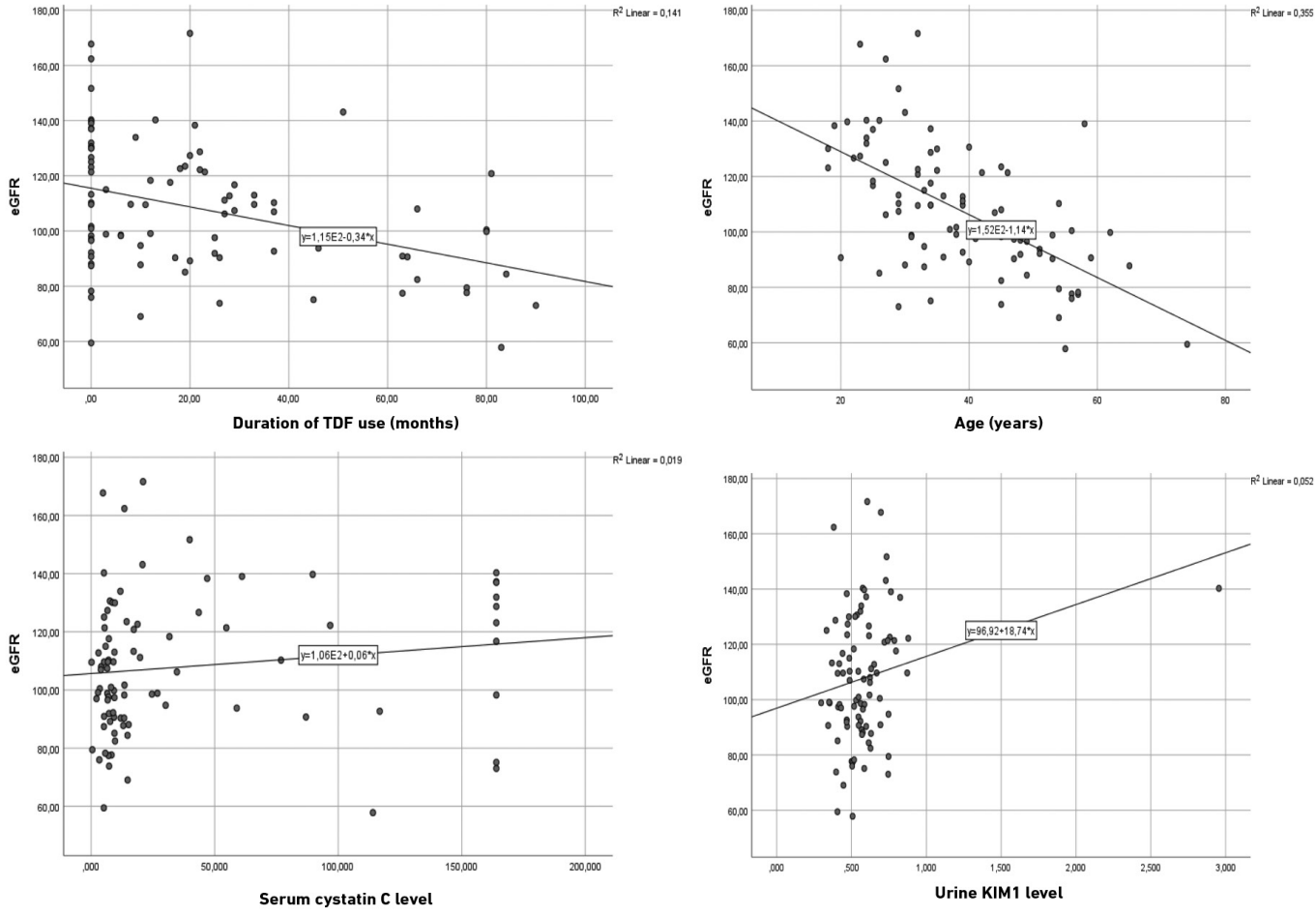

Figure 1. Scatter plot of variables correlated with eGFR level

TDF: Tenofovir disoproxil fumarate, eGFR: Estimated-glomerular filtration rat

Table 4. Comparison of nephrological parameters according to gender in the whole population

Parameters	Gender			p
	Male (n=114)	Female (n=55)	Total (n=169)	
	Mean±SD Median (min-max)	Mean±SD Median (min-max)	Mean±SD Median (min-max)	
Serum cystatin C level	39.82±51.23 13.39 (0-164)	55.78±54.51 24.6 (0-164)	45.01±52.69 16.39 (0-164)	0.065
Serum NGAL level	941.96±1508.35 257.61 (1.107-7194.6)	1298.62±1485.42 610.11 (8.61-5171.05)	1058.03±1505.85 308.19 (1.107-7194.6)	0.150
Urine NGAL level	154.26±93.71 118.73 (53-720)	215.69±82.84 234.87 (69-380)	174.25±94.58 147.51 (53-720)	**<0.001**
Urine KIM1 level	0.56±0.26 0.54 (0.191-2.955)	0.59±0.21 0.58 (0.150-1.003)	0.57±0.24 0.54 (0.150-2.955)	0.440
eGFR	113.21±23.73 110.26 (59.4-173.1)	119.11±27.30 117.96 (57.83-197.95)	115.13±25.02 114.12 (57.83-197.95)	0.151

Min-max: Minimum-maximum, SD: Standard deviation, eGFR: Estimated-glomerular filtration rate

According to the Turkish Society of Nephrology and European AIDS Clinical Society guidelines, the evaluation of nephropathy begins with a decrease in eGFR level below 90 ml/min/1.7. In this case, when HIIs were divided into groups according to the presence of nephropathy (eGFR <90); there was no significant difference between the groups except for age (p<0.001) (Table 7).

Discussion

Since the introduction of HAART in the late 1990s, a significant reduction in mortality and AIDS-related clinical manifestations has been observed. However, internal problems such as kidney diseases and non-AIDS diseases seen in non-HIV-infected populations started to appear in HIIs and became one of the leading causes of morbidity and mortality in this population. Creatinine is the most commonly used marker to evaluate renal function. However, creatinine levels may be misleading or cause delays in demonstrating nephropathy due to the lower muscle mass of elderly and female patients[8]. A normal creatinine level in patients with nephropathy may not mean that eGFR is also normal[9]. In addition, the presence of proteinuria may not indicate tubular damage[10]. This situation led to the search for

Table 5. The relationship between eGFR level and nephropathy biomarkers and risk factors in HIV-infected individuals

	Spearman's rho coefficient	p value
Age (years)	-0.594	**<0.001**
Duration of TDF use (months)	-0.337	**0.001**
Serum cystatin C level	0.218	**0.041**
Serum NGAL level	0.203	0.057
Urine NGAL level	0.074	0.492
Urine KIM1 level	0.213	**0.046**

HIV: Human immunodeficiency virus, TDF: Tenofovir disoproxil fumarate

Table 6. Multivariate linear regression analysis of factors associated with eGFR level in HIV-infected individuals

	B	SE	t value	p value
Constant	144.022	8.965	16.065	**<0.001**
Age (years)	-0.990	0.178	-5.557	**<0.001**
Duration of TDF use (months)	-0.200	0.079	-2.537	**0.013**
Urine KIM1 level	12.249	6.945	1.764	0.081
Serum cystatin C level	-0.018	0.040	-0.455	0.650

Regression coefficient: B; SE: Standard error; R^2=0.421; corrected R^2=0.394; F=15.114; p<0.001.

TDF: Tenofovir disoproxil fumarate, eGFR: Estimated-glomerular filtration rate, HIV: Human immunodeficiency virus

Table 7. Characteristics of HIV-infected individuals according to the presence of nephropathy

	Nephropathy		
	Yes (n=18)**	**No (n=70)****	**p value**
Age (years)	51.5 (34-56)	34 (29-45)	0.001***
Gender (female/male)	5 (27.8)/13 (72.2)	7 (10)/63 (90)	0.064****
HIV-RNA (copies/ml)	20 (0-19075)	20 (0-155)	0.528***
CD4 count (/mm^3)	619.5 (509-863.25)	649 (545-949.5)	0.485***
HAART* use	14 (78)	61 (87)	0.256****
TDF use	13 (72%)	44 (63%)	0.458*****
Duration of TDF use (months)	23 (0-76)	12 (0-28.5)	0.077***
Serum cystatin C level	8.838 (5.72-14.762)	11.959 (39.872-6.68)	0.346***
Serum NGAL level	156.712 (94.10-259.39)	174.19 (86.79-727.24)	0.450***
Urine NGAL level	105.945 (91.48-129.05)	98.135 (81.92-117.84)	0.347***
Urine KIM1 level	0.542 (0.5-0.615)	0.56 (0.47-0.65)	0.698**

HIV: Human immunodeficiency virus, TDF: Tenofovir disoproxil fumarate, *HAART: Highly active antiretroviral treatment, **n (%); median (interquartile range), ***Mann-Whitney U test, ****Fisher's exact test, *****chi-square test

biomarkers to evaluate tubular damage especially in the early period.

Cystatin C has been shown as a marker of tubular damage in patients with CKD[11]. It has been shown that serum creatinine level, which is the clinical standard for assessing renal function, is affected by muscle mass, so it may be insufficient to diagnose CKD among people with HIV infection[12]. It is advantageous because it is independent of factors such as age. It is thought to be particularly good at predicting mild to moderate renal function[13,14]. Cystatin C has been shown to be a stronger predictor of mortality among HIIs compared to creatinine[15,16]. Two cross-sectional case-control studies conducted in Nigeria reported that the cystatin C level was higher and the eGFR calculated with cystatin was lower in HIV-infected children compared to healthy controls. The authors argued that it might be a marker of HIV-related nephropathy and future CKD[17,18]. However, the effects of HIV viral load and inflammatory markers such as C-reactive protein (CRP) and erythrocyte sedimentation rate on cystatin C level were not addressed in the study. In this study which we conducted on HIIs, cystatin C level was found to be low while expected to be high in the patient population, and this might be associated with the absence of nephropathy and CKD in our patient group, and it was thought that inflammation or other variables in the patient group could explain the high cystatin C levels in the control group. Chazot et al.[2] stated that serum cystatin C concentration was affected by many parameters such as CRP, HIV viral load, CD4 cell count, and it was not suitable for use in HIIs. However, in the study of Deyà-Martínez et al.[20], mean cystatin C level was lower than those reported in other studies in pediatric and adult HIV cohorts, and cystatin C level was higher in healthy controls, similar to our study[19-21].

Urine KIM1 level has been shown to be an important biomarker, especially in predicting tubular damage in the early period[22]. In a study of HIV-infected women, high levels of IL-18 and KIM1 levels were found to be independent of predicted cystatin C-based GFR (eGFRcys) or albuminuria. As a result, it was shown to be associated with decreased kidney function[5]. Another study from the United Kingdom stated that an elevated urine KIM1/creatinine ratio in HIIs using ART was an important predictor of renal dysfunction[23]. The KIM1 level was higher in the patient group compared to the control group, but no statistically significant difference was found in our study. This might be because, since the study was cross-sectional, these biomarkers were studied with a single measurement without serial measurements. Perhaps the increase in KIM1 could be statistically demonstrated in repeated measurements.

The NGAL is a protein of which amount is increased in tubules and urine as a result of epithelial damage[24]. In a study with serum and urine NGAL levels, information was obtained about the progression of chronic kidney damage independent of eGFR[25,26]. Paragas et al.[27] showed that the NGAL level increased significantly in a study in which they evaluated 13 patients with biopsy-confirmed HIVAN. Contrary to expectations, in our study, NGAL level in the urine was found to be higher in the healthy control group ($p<0.001$). The reason for this may be the predominance of female individuals in the control group and male individuals in the patient group. In the examination performed in the whole population (n=169), in which the patient and control groups were evaluated together, urinary NGAL level was higher in females ($p<0.001$). A study conducted in patients with type 1 diabetes mellitus showed that urinary NGAL level was higher in women. It was stated that the difference between men and women in urine biomarkers should be taken into account[28].

Because NGAL can be produced by neutrophils and other organs in addition to the kidney, clinical conditions such as urinary tract infections or sepsis may result in NGAL levels in the urine that do not reflect the absolute severity of kidney damage[29]. Decavele et al.[30] showed increased urinary NGAL levels in urinary tract infections and pyuria. That is, NGAL can act as an inflammatory marker. In this case, as with cystatin C level, the NGAL level we found can be attributed to inflammation. In our study, the participants did not have pyuria. However, systemic inflammatory markers (sedimentation, CRP) were not evaluated.

It has been observed that kidney functions are affected by causes such as tubular necrosis, interstitial nephritis, and crystal nephropathy in the acute or chronic processes in patients receiving HAART[31]. Data are showing that TDF in particular causes tubular damage and lowers eGFR[32]. In our study, eGFR in patients using TDF in our HIV-infected patient group was found to be lower. These results may support TDF nephrotoxicity. However, the number of samples is larger and it should be supported by measurements spread over more than one time. Cystatin C level in serum, NGAL level in urine and serum, and KIM1 level in urine were not different between patients who did not use TDF and who used TDF.

Study Limitations

Our study had some limitations. The most important of these limitations was the lack of support for the effect of existing biomarkers with serial measurements. Human immunodeficiency virus-positive participants in the study were initially selected from individuals without nephropathy and other systemic diseases. However, we cannot conclude whether nephropathy will develop in the future in these patients. More powerful data can be obtained with serial measurements. However, in our study, creatinine-based eGFR and creatinine level in serum and urine gave significant results compared with the control group. Our limitations were that there were gender differences,

simultaneous inflammatory parameters were not studied, and the number of patients in a single center was small.

Conclusion

In our study, although eGFR was lower in the patient group, creatinine level in serum and urine and protein level in urine were higher, cystatin C and NGAL levels in urine and serum were lower in the control group. Although KIM1 level in the urine was higher in the patient group, the difference was not statistically significant. These parameters were not correlated with serum creatinine level and creatinine-based eGFR, which was standardized in assessing renal function. However, it would not be appropriate to evaluate it with a single measurement. Larger cohort studies with serial measurements will be more accurate. In our study, NGAL level was found to be higher in women, especially in the urine, and it was concluded that it was necessary to pay attention to the gender factor when using nephropathy markers. In addition, low eGFR draws attention in patients using TDF. Although there are studies that support cystatin C, KIM1 and NGAL as nephropathy markers, the influencing factors should be evaluated while studying these parameters.

Ethics

Ethics Committee Approval: Approval was obtained from Çukurova University Faculty of Medicine Ethics Committee (no: 33, date: 13.04.2018).

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.C., Design: A.C., Data Collection or Processing: D.E., Y.D., Ö.G.Ö., Analysis or Interpretation: A.C., O.A., Literature Search: D.E., A.C., Y.T., Writing: D.E.

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