

Change in ratio levels of KIM-1 / urine creatinine and increase serum creatinine levels in human immunodeficiency virus (HIV) patients receiving tenofovir-based antiretroviral (ARV) combination therapy



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ABSTRACT

Background: Human immunodeficiency virus (HIV)-associated nephropathy refers to developing kidney disease associated with HIV infection. Tenofovir is associated with an increased risk of acute kidney injury (AKI). Kidney injury molecule-1 (KIM-1) in urine is very specific for kidney injury. This study aimed to determine the changes in the ratio between the levels of KIM-1/urine creatinine and the increase in serum creatinine levels with the incidence of decreased kidney function in people with HIV.

Method: This was an observational-analytic study. Subjects were taken using consecutive sampling at Sanglah Hospital Denpasar in 2018. KIM-1 levels were measured by the ELISA method.

Results: This study involved 35 patients (21 males and 14 females) with a mean age of 34.89 ± 10.05 years old. The average levels of KIM-1/urine creatinine at 0 hours was 11.039 ± 12.175 ng/ml, KIM-1/urine creatinine at 12 was 12.382 ± 14.671 ng/ml, KIM-1/urine creatinine at 72 was 10.272 ± 11.843 ng/ml). There were no statistically significant differences in the ratio of hours 0 to 12 hours ($p = 0.295$), and between hours 0-72 ($p = 0.413$). But there was a significant difference in creatinine levels for 3 months with the initial mean serum (0.848 ± 0.201) and 3 months of evaluation (1.002 ± 0.198) ($p = <0.001$).

Conclusion: There was no difference in the ratio of changes in serum KIM-1/creatinine levels during the evaluation of 0, 12, and 72 hours. However, there was an increase in serum creatinine levels between the beginning and 3 months of evaluation.

Keywords: HIV/AIDS, KIM-1/urine creatinine, serum creatinine, Tenofovir, AKI.

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INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS) is a disease caused by Human Immunodeficiency Virus (HIV) retrovirus, characterized by an immunosuppressive condition that triggers opportunistic infections, secondary neoplasms, and neurological manifestations.¹ Epidemiologically, HIV prevalence continues to increase. Sub-Saharan Africa, especially South Africa, has the highest incidence, accounting for 70.8% of the global burden of HIV infection. The global prevalence of HIV/AIDS in 2002 and 2012 was 31.0 million and 35.3 million, respectively.²

HIV-associated nephropathy (HIVAN) defined as kidney disease which occurs related to the infection by HIV. The direct infection of HIV to cells in the kidney resulting in HIVAN, leads to kidney damage through the viral gene products.³ Creatinine is a very useful measurement and plasma creatinine level is better than plasma urea level for assessing kidney function. An increase in plasma creatinine 1-2 mg/dl from normal level suggests a $\pm 50\%$ decline in GFR.⁴

Tenofovir Disoproxil Fumarate (TDF) is an acyclic nucleotide analog reverse transcriptase inhibitor (NtRTI). Previous studies indicated that the increase in likelihood for kidney damage, e.g., acute

tubular dysfunction or chronic decrease in GFR, related with the TDF levels. The association between TDF peak levels and toxicity for the kidney still need more exploration, since this study might benefit for the formulation of TDF nephrotoxicity management recommendation.⁵ A retrospective study in Japan which involved 493 patients with TDF showed high incidence of decreased chronic renal function, which is 10.5 per 100 patient-years.⁶

Kidney Injury Molecule-1 (KIM-1) is a transmembrane protein with immunoglobulin and mucin domains. The expression of KIM-1 is unable to be measured in normal proximal

tubular cells. However, its concentration significantly increased with cell injury or differentiation.⁷ It has been postulated that the presence of KIM-1 in urine is highly specific for injury in the kidney. It also may serve as a biomarker for injury in the renal proximal tubular, which benefits for early diagnosis of the disease.⁸ In healthy humans without any disease, the concentration of urine KIM-1 is less than 1 ng/ml. This result will be different in the patients with ischemic kidney injury, in which its concentration reached up to 3-7 ng/ml. In the six hours following the ischemic, KIM-1 levels begin to increase. It will remain elevated for 48 hours after the injury.⁹

In the presence of kidney damage in HIV patients, the use of TDF and the important role of KIM-1 in early detection of kidney damage, it is important to know about their relationship in HIV patients. This study aimed to determine whether there is a change in the ratio of urinary KIM-1/creatinine levels and an increase in serum creatinine levels in HIV patients who were receiving tenofovir-based ARV therapy.

METHODS

This is a 3-months observational-analytical cohort study to determine the comparison of increased urinary KIM-1/Creatinine ratio and serum creatinine level for early detection of renal impairment in HIV patients who were receiving a combination of Tenofovir-based ARV. End of study was the emergence of impaired renal function for 3 months. Design of the study is described in Figure 1. The study was conducted in a VCT outpatient ward, from September 2018 to December 2018.

The study sample was all of outpatients aged > 18 years, with HIV infection, who had not received any ARV therapy and would be treated with tenofovir-based ARV therapy at VCT clinic. Inclusion criteria were HIV patients who were willing to be treated by tenofovir-based ARV combination therapy, aged \geq 18 years, serum creatinine levels <1.2 g/dL before therapy, taking tenofovir-based ARVs, willing to be included in this study with a written informed consent. The exclusion criteria were currently on acute infection, had renal function impairment

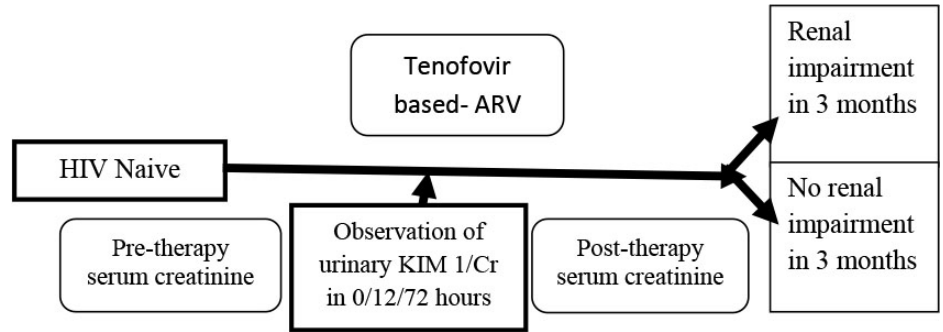


Figure 1. Design of the study.

in the last 6 months, had a history of hospitalization more than once in the last 3 months, and patient died within 3 months of observation. The sample size calculated with sample size formula ($Z\alpha = 1.96$, $Z\beta = 1.28$, mean difference effect size of 20, and standard deviation of 40), obtained a minimum total sample of 35. The samples were taken using consecutive sampling.

Dependent variables were urinary KIM-1/Creatinine and serum creatinine levels, while the confounding factors were age, sex, CD4, BMI, and blood pressure. The KIM-1 level tested was the urinary KIM-1/creatinine level using Quantikine Human TIM-1/KIM-1/HAVCR Immunoassay reagent measured by the Enzyme-linked immunosorbent assay (ELISA) method in pg/mL units. A high KIM-1 was defined when the urinary KIM-1 was above the median value (0.156-10 pg/mL). BMI was defined as a person's weight (measured in kilograms) divided by the square of the person's height (measured in meters) (kg/m^2). The BMI Classified into <18.5 (underweight), 18.5-24.9 (normal/ideal), 25.0-29.9 (overweight), 30.0 or more (obese). CD4 levels were measured by immunofluorescence flow cytometry method with the results in numbers with a range of 404-1612/mL. CD4 <200/mL is considered to be low.

The data obtained were analyzed descriptively to describe the general characteristics. For independent and dependent variables, the median and standard deviation values were used. Kolmogorov-Smirnov normality test is used to analyze normality between data. Comparative test using paired t-test to analyze the increase in urinary KIM-1/Creatinine levels as an early predictor of renal impairment in HIV patients before

and the increase of serum creatinine 3 months after taking Tenofovir. If the data were not normally distributed, a Wilcoxon test was performed. Multivariate analysis by ANCOVA was done to control the confounding variables (age, gender, comorbid disease) which could affect the changes in urinary KIM-1/creatinine and serum creatinine for 3 months. Kruskal-Wallis test was used to determine the difference between 2 or more groups of independent variables on the dependent variables. Analysis was performed using statistical software SPSS for windows version 23.0. P-value <0.05 was considered statistically significant.

RESULTS

Baseline characteristics of subjects can be seen in Table 1. Out of a total 35 subjects, mean age of patients was 34.89 ± 10.05 years old, with male predominance. Mean of CD4 level among samples was 151.20 ± 96.07 mL.

Comparative tests were performed on urinary KIM-1/creatinine at 0, 12, and 72 hours as well as baseline and final creatinine serum (Table 2). No significant difference was found between urinary KIM-1/Creatinine at 0 and 12 hours ($p = 0.295$). Urinary KIM-1/Creatinine at 0 and 72 hours was also not significantly different ($p = 0.413$). However, there was a significant difference between the baseline vs. final creatinine serum. The final creatinine serum creatinine was significantly higher than baseline level ($p < 0.001$). The comparison chart of urinary KIM-1/creatinine and creatinine serum can be seen in Figure 2 and Figure 3.

As for eGFR, it was found that there was a decrease in eGFR from the analysis of mean difference of the baseline and

3 month eGFR ($p < 0.001$) (Figure 4). Based on the analysis of the incidence of CKD assessed from the initial eGFR and 3 months of evaluation after taking tenofovir, it was found that 2 subjects had CKD (5.7%) after 3 months observation, although there was no significant difference (Table 3).

Table 1. Characteristics of Subjects.

Characteristic	n=35
Age (years), mean \pm SD	34.89 \pm 10.05
Sex, n (%)	
Male	21 (60%)
Female	14 (40%)
Body Mass Index (kg/m ²), median (IQR)	19.53 (15.94-25.01)
CD4 (mL), mean \pm SD	151.20 \pm 96.07
Systolic pressure (mmHg), mean \pm SD	108.88 \pm 9.63
Diastolic pressure (mmHg), mean \pm SD	66.86 \pm 7.18

CD4: Cluster of differentiation 4; SD: standard deviation; IQR: Interquartile range

Table 2. Comparison of urinary KIM-1/creatinine and baseline vs. final creatinine serum.

Variable	Mean \pm SD	p
Urinary KIM-1/Creatinine-0	11.039 \pm 12.175	0.295
Urinary KIM-1/Creatinine-12	12.382 \pm 14.671	
Urinary KIM-1/Creatinine-0	11.039 \pm 12.175	0.413
Urinary KIM-1/Creatinine-72	10.272 \pm 11.843	
Baseline serum creatinine	0.848 \pm 0.201	<0.001
Final serum creatinine	1.002 \pm 0.198	

KIM-1: Kidney Injury Molecule-1

Table 3. Comparison of the occurrence of CKD (eGFR < 60) at baseline and end of study.

Variable	Baseline	End of study	Increase in incidence	p-value
eGFR < 60	0 (0%)	2 (5.7%)	2 (5.7%)	0.157

eGFR: estimated Glomerular Filtration rate

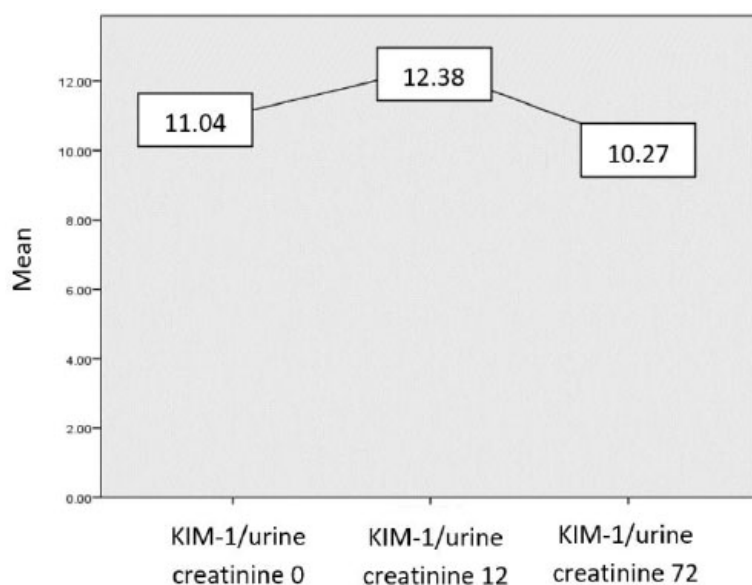


Figure 2. Mean of urinary KIM-1/creatinine at 0, 12 and 72 hours.

DISCUSSION

This current study is a 3-month cohort study to determine the comparison of increased levels of urinary KIM-1/creatinine and serum creatinine for early detection of kidney function disorders in HIV patients who were receiving Tenofovir-based ARV combination therapy.

CD4 counts contributed to the high prevalence of TDF-associated nephrotoxicity, i.e. CD4 cell counts in interquartile range of 117-324 cells/ μ L.¹⁰ Older age is also related to lower epidermal growth factor (EGF). The EGF is a marker protein for tubular regeneration. It may facilitate renal ability to recover from injury and dampen the progression of CKD.¹¹

In our study, there was an insignificant increase of urinary KIM-1/creatinine ($p=0.295$) between measurement before administration of TDF therapy (urinary KIM-1/creatinine 0) and 12 hours after administration of TDF. Mean of urinary KIM-1/creatinine at 0 was 11.039 \pm 12.175, increased after 12 hours (urinary creatinine 12) became 12,382 \pm 14,671 on average. This may be due to the absence of significant tubular ischemia after 12 hours of TDF administration. At first administration, TDF is excreted in the urine (70-80%) via filtration and tubular secretion, mainly in the form of unchanged TDF within 72 hours. After repeated oral doses, TDF will be excreted by the body after 24 hours. The mechanism of acute kidney disease (AKD) in patients in TDF therapy is thought to be associated with renal clearance. Higher plasma levels of TDF is directly increasing the accumulation of TDF in renal tubular cells which results in renal toxicity.¹²

Glomerular filtration and proximal tubular secretion involved in the elimination of tenofovir in the urine, which is unchanged.¹³ Organic anion transporters (hOAT1, and to a lesser extent, OAT3), which located at the basolateral membrane, helped transport of approximately 20-30% of the drug into proximal renal tubular cells.¹⁴ It can be a mitochondrial poison. This event supported by the finding of ultrastructural mitochondrial abnormalities, found in TDF-induced tubulopathy.¹⁵

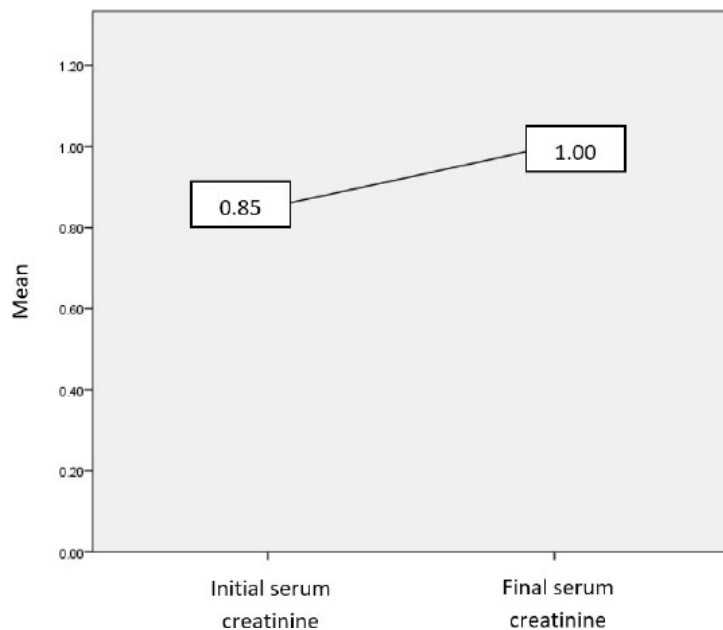


Figure 3. Mean of baseline and final serum creatinine in 3 months.

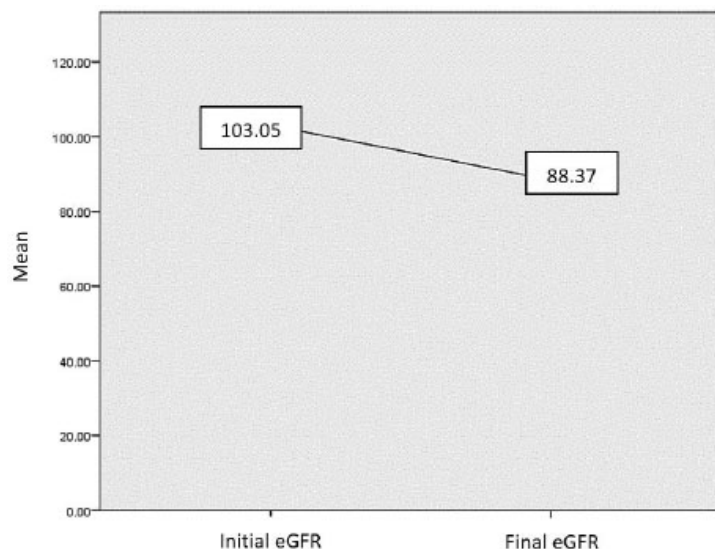


Figure 4. Mean of baseline and final eGFR in 3 months.

KIM-1 is a biomarker that has the potential to predict the presence of AKD. An increase in KIM-1 can be detected within hours after kidney damage occurs. Thus, KIM-1 has a higher sensitivity than serum creatinine which increases after 24-72 hours of kidney damage. Khebra et al conducted a study in 2019 on 45 patients who underwent open heart surgery, in which 60% of the samples were diagnosed with AKD and 40% of patients were not.¹⁶ In adult patients who were undergoing cardiac surgery, urinary KIM-1 can predict the development of AKI better than NGAL.^{17,18}

Previous studies which involved kidney disease patients found that urinary KIM-1 was increased in all patients. Acute tubular necrosis (ATN) had highest levels of KIM-1.^{9,19} A study by Han et al measured KIM-1 by using Western blot and ELISA methods. Analysis with ELISA showed false positive results. Comparing to other study which explore the difference in urine KIM-1, the study also found difference in urine KIM-1, in which they compare the urine KIM-1 in patients with renal disease and controls, regardless of the kidney disease's type.²⁰ In regards to different etiologies for the kidney disease, urine KIM-1 in renal cell

carcinoma patients (normalized to urinary creatinine level) was increased (0.39 ± 0.08 ng/mg UCR; $n = 21$), compared to healthy subjects (0.05 ± 0.01 ng/mg UCR; $p < 0.005$; $n = 30$) and patients with prostate cancer (0.12 ± 0.03 ng/mg UCR; $p < 0.02$; $n = 10$).²¹

In our study, urinary KIM-1/creatinine ratio was also measured at 72 hours after TDF therapy (KIM-1/urinary creatinine 72). There was no significant difference between urinary KIM-1/creatinine 0 (11.039 ± 12.175) and KIM-1/creatinine 72 (10.272 ± 11.843) ($p=0.413$). Excretion of TDF initial dose through the kidneys is completed within 72 hours. There is accumulation of plasma TDF following repeat dosing, however it is not predicted to have a toxic effect on renal tubular. Therefore, in our study, there was no significant increase in KIM-1/urinary creatinine 72.¹²

Declining kidney function in patients who were treated with TDF has been studied by Yazie et al, 2018 by observing the decrease in eGFR and increase in serum creatinine among 63 HIV patients on TDF therapy, which were calculated in the first, second, and sixth months of therapy. A significant decrease in eGFR was only found in the sixth month of TDF administration (-8.37 ± 18.4), analyzed using one way ANOVA ($p < 0.05$) and post hoc test t-test paired with Bonferonni correction ($p < 0.05$). In that study, 25.4% (16) of patients had more than 25% reduction in eGFR and diagnosed with renal dysfunction.²²

In our study, serum creatinine (1.002 ± 0.198) was significantly increased at the end of study, compared to the baseline serum creatinine (0.848 ± 0.201 ; $p < 0.001$). Serum creatinine was measured at 3 months after administration of TDF. It is estimated that there has been an accumulation of plasma TDF levels at time after repeated doses for 3 months and then increases the accumulation levels of TDF in renal tubular.¹² It subsequently resulted in a significant increase in serum creatinine after 3 months. Likewise, eGFR measurement showed a statistically significant decrease ($p < 0.001$).

In our study, urinary KIM-1/creatinine measured before, at 12 hours, and at 72 hours after administration of TDF did

not show a significant difference after the variables controlled for age and sex. The difference in measurement time only gives rise to 0.5% variability which is not significant. This is thought to be due to inappropriate sampling time, in which significant renal tubular damage did not occur in 12 hours and 72 hours after TDF administration.¹² The process of declining kidney function on average occurs in sixth month to one year after the administration of TDF therapy with various clinical manifestations.^{22,23}

Our study has some limitations. We did not evaluate the increase of urinary KIM-1/Creatinine levels for a longer period of time, hence the changes in the kidneys can be more reflected. We also did not specifically monitor the course of treatment assessed on follow up, history of drug consumption, laboratory evaluation, and compliance.

CONCLUSION

Our study found that there was no change in the ratio of urinary KIM-1/Creatinine levels between 0, 12 hours and 72 hours in HIV patients receiving Tenofovir-based ARV therapy. However, there was an increase in serum creatinine levels during the 3 months of observation and it was supported by a decrease in eGFR levels.

CONFLICT OF INTEREST

There is no competing interest regarding the manuscript.

ETHICS CONSIDERATION

The research was approved by the Ethics Committee of the Research and Development Unit of the Faculty of Medicine, Udayana University, with the number 12/UN14.2.2.VII.14/LP/2019.

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AUTHORS CONTRIBUTION

Conceptualization and methodology, IDGTKM; design of the study and definition of intellectual content, IDGTKM and IGRW; literature search, IDGTKM; clinical studies and experimental studies,

IDGTKM and IGRW; data acquisition, IDGTKM; data analysis, IDGTKM and IGRW; statistical analysis, IGRW; manuscript preparation, IDGTKM; manuscript editing, IGRW; manuscript review and guarantor, IDGTKM and IGRW. All authors have read and agreed to publish the manuscript.

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