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Early Diagnosis of Renal Tubular Dysfunction in HIV-Infected Patients; a Case of Interleukin (IL)-18 and other Common Indicators of Renal Toxicity

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Abstract

Background: At present renal dysfunction in clinical practice is measured using serum creatinine values to calculate eGFR (estimated glomerular filtration rate) but creatinine is a late marker of renal dysfunction and is only raised when up to 30-50% of renal function is lost.

Methods: Serial urine samples were analyzed by enzyme-linked immunosorbent assay for IL-18. Urinary IL-18 together with other common indicators of renal damage was assessed in 325 HIV patients; of which 66 developed renal dysfunction after 12 weeks of follow-up.

Result: Marked increase in IL-18 (p=0.000) was observed at an earlier stage in the renal disease group compared to a delayed elevation of eGFR, serum creatinine, fractional excretion of phosphate and fractional excretion of uric acid which was evident only after 4 weeks .

Conclusion: This finding seems to suggest that IL-18 can be used as an early marker of subclinical renal tubular dysfunction in HIV-infected patients, owing to the fact that IL-18 increases in urine only under conditions of marked tubular damage, apoptotic tubular cell shedding, and cell necrosis, associated with deterioration of renal function.

Keywords: Urinary IL-18; HIV; Tubular dysfunction; Serum creatinine

Introduction

Interleukin-18(IL-18) is a proinflammatory cytokine produced by leukocytes, blood vessels and kidney tubules [1]. IL-18 production is also observed in the villi and at the top of crypts, in the most differentiated cells facing an antigen-rich environment. A substantial increase in IL-18 levels is known to occur during acute tubular necrosis and elevated urine levels are a relatively specific and sensitive marker of tubular injury [1,2]. Originally identified as the gamma interferon-inducing factor, interleukin-18 (IL-18) was rediscovered as a pro-inflammatory cytokine related to the IL-1 family of cytokines that play an important role in both innate and adaptive immune responses against viruses and intracellular pathogens. The proinflammatory activity of IL-18 is mediated by the production of inflammatory cytokines, chemokines, nitric oxide, and prostaglandins and is amplified by IL-18-induced IFN-gamma production which in turn activates macrophages.

However, a major challenge in the prevention and management of kidney disease in HIV infection is the reliance on serum creatinine to detect impaired kidney function [3]. Serum creatinine is of limited value and any change in its concentration is a delayed response with

levels rising significantly above baseline only when 25-50% of renal function has been lost [3]. Secondly, it is a marker of glomerular filtration and therefore, not specific to damage at other sites of the nephron. Early diagnosis and management of kidney dysfunction in the primary care setting are essential to maintain quality of life and improve outcome in patients with renal diseases [4]. Therefore, urine testing may identify HIV infected individuals at increased risk for mortality even when a significant number of them may be asymptomatic. Indeed, many studies have emphasized that although patients on antiretroviral therapy including TDF may not experience noticeable renal impairment as measured by creatinine clearance, tubular damage may be common [5].

Material and Methods

Study population

A total of 325 HIV-infected patients treated with various antiretroviral therapies such as tenoforvir, efavirenz, lamivudine, ritonavir nivirapine, zidovudine etc were enrolled in this study from April to September 2015. Out of these 123 patients were on tenoforvir (TDF) based regimen while 202 patients were on non-TDF based regimen. Patients with severe comorbidities and/or organ failures, hepatitis B&C, pregnancy and metabolic disorders were excluded from the study. Also, patients with moderate to severe renal failure or

nephrotic syndrome were not enrolled in the study. Clinical data of each patient were collected from the medical record. Immunovirologic status and CD4 cell count at baseline was also recorded. All patients gave informed consent to participate in the study.

Study protocol and specimen collection

The study period was 12 weeks. Serum and urine samples were obtained at different time points and specifically at commencement (visit 1), and after 4 weeks (visit 2), and 12 (visit 3) weeks of starting the antiretroviral therapy. A fasting blood sample was obtained during the protocol visits; the patients also provided a sample of the 24-h urine collection and a morning spot urine sample. Serum and blood samples were analyzed for routine chemistry and a complete blood count. The 24-h urine samples were centrifuged and sent to the central laboratory for evaluation of routine parameters (protein, albumin, electrolytes, urate, creatinine). In order to eliminate the effect of incorrect urine collection, all assayed parameters were expressed as ratio versus urinary creatinine. The spot urine sample was centrifuged and stored at -70°C until being tested for urinary markers. Serum and urinary IL-18 were analyzed using enzyme-linked immunosorbent assay (ELISA), while estimated glomerular filtration rate (eGFR) was calculated using the modification of diet in renal disease (MDRD) formula. Fractional excretion of phosphate and uric acid was also calculated from urinary and serum phosphate and uric acid.

Statistical analysis

Continuous variables were expressed as mean and standard deviation while categorical data were given as percentages. The three groups of patients analyzed were first compared using chi-squared for categorical data and parametric tests for continuous variables. The level of significance was considered as $p \leq 0.05$. All statistical analyses were performed using SPSS version 20.0 software package (SPSS inc., Chicago, Illinois, USA). Carley et al., [6], simple normograms for the calculation of sample size for clinical diagnostic study were used for the calculation of sample size.

Result

A longitudinal study was started in April 2015 at the HIV clinic of the University of Port Harcourt Teaching hospital, a reference Hospital for tropical infectious diseases located in Port Harcourt Rivers State, Nigeria. The patients were divided in to two (2) groups according to incidence of renal damage. The first group comprised patients who developed renal damage (66 patients of which 38 patients were on tenofovir based regimen). The second group had normal renal function.

Characteristic of patients

The gender distribution among the patients study population was statistically significant (p=0.002) and was observed to be; 102 (31.38%) in males and 223 (86.62%) in females as shown in Table 1. As seen in Table 1, no differences were noted between the two groups with respect to renal disease, gender, ethnic origin, or urine output while the mean age of the renal disease group and the non-renal disease group was 36.44 and 34.57 but without a significant difference (p=0.069). Table 2 shows the CD4 counts and viral loads of the two groups to be as follows: renal disease group [visit 1; 244.43/<1000 copies/ml, visit 2; 341.45/<100 copies/ml, visit 3; 579.12/<50 copies/ml], Non-renal disease group [visit 1; 248.45/<1000 copies/ml, visit 2; 269.78/<1000 copies/ml, visit 3; 343.24/<50 copies/ 6.62%) in females as shown in tsml].

	Mean Age	No. of patients	Std. Deviation
SEX			
Male	36.22	102 (31.38%)	7.75
Female	34.8	223 (68.62%)	7.98
Total	35.51	325 (100%)	8.03
Study group			
Renal disease group	36.44	66 (20.31%)	7.68
Non renal disease group	34.57	259 (79.69%)	8.68
Total	35.51	325 (100%)	8.02
Values in parenthesis show the percentage of patients in the study population			

Table 1: Baseline Characteristics of the study population.

CD4 (ul/cells)	Non-renal disease group	Renal disease group	Differences among groups
	Means (SD)/VL	Means (SD)/VL	(z-test, p-value)
visit 1	248.45 (59.8)/<1000 copies/ml	244.43 (86.5)/<1000 copies/ml	0.583
visit 2	269.78 (51.8)/<1000 copies/ml	341.45 (131.2)/<100 copies/ml	0
visit 3	343.24 (64.9)/<50 copies/ml	579.12 (94.8)/<50 copies/ml	0

Table 2: Mean CD4 of the study population within 12 weeks.

Estimated glomerular filtration rate (eGFR) shows a slight increase in trend only after 4 weeks in the group of patients with renal damage (Figure 1).
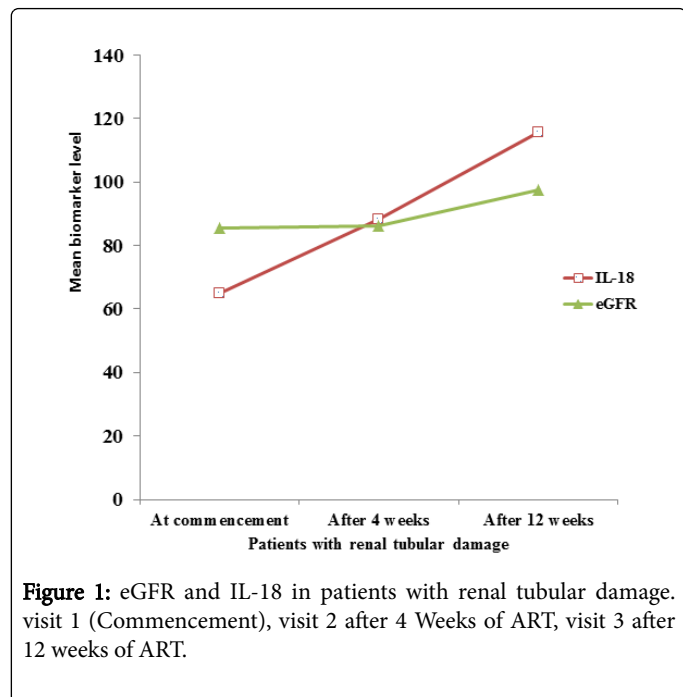
Figure 1: eGFR and IL-18 in patients with renal tubular damage. visit 1 (Commencement), visit 2 after 4 Weeks of ART, visit 3 after 12 weeks of ART.

Figure 2 compared increase in urinary IL-18 and serum creatinine (which is a known biomarker of renal damage) in the renal disease group. The graph shows that interleukin-18 increased exponentially with renal damage while serum creatinine increased only slightly after 4 weeks (visit 2) in the renal disease group. There was no increased in serum Interleukin-18 levels measured before and after the 12 weeks of exposure to antiretroviral therapy.
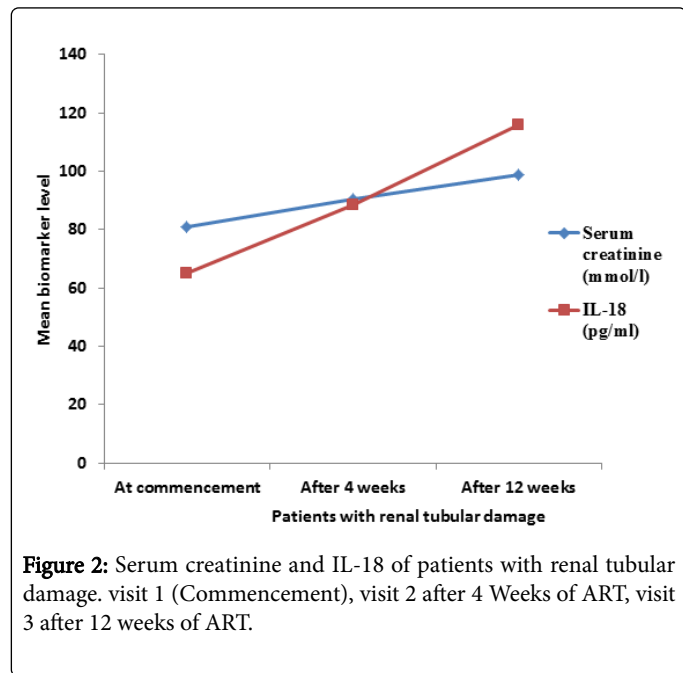

Figure 2: Serum creatinine and IL-18 of patients with renal tubular damage. visit 1 (Commencement), visit 2 after 4 Weeks of ART, visit 3 after 12 weeks of ART.

Figure 3 compared increase in urinary IL-18 and fractional excretion of phosphate (which is a known biomarker of renal damage) in the renal disease group. The graph shows that urinary interleukin-18 increased exponentially with renal damage while fractional excretion

of phosphate (FEP) only showed a trend after 4 weeks (visit 2) in the renal disease group. As shown in Figure 4, fractional excretion of uric acid also showed a minimal upward trend with increase in renal damage while urinary interleukin-18 increased progressively with renal damage at each time point in the group of patients with renal damage.
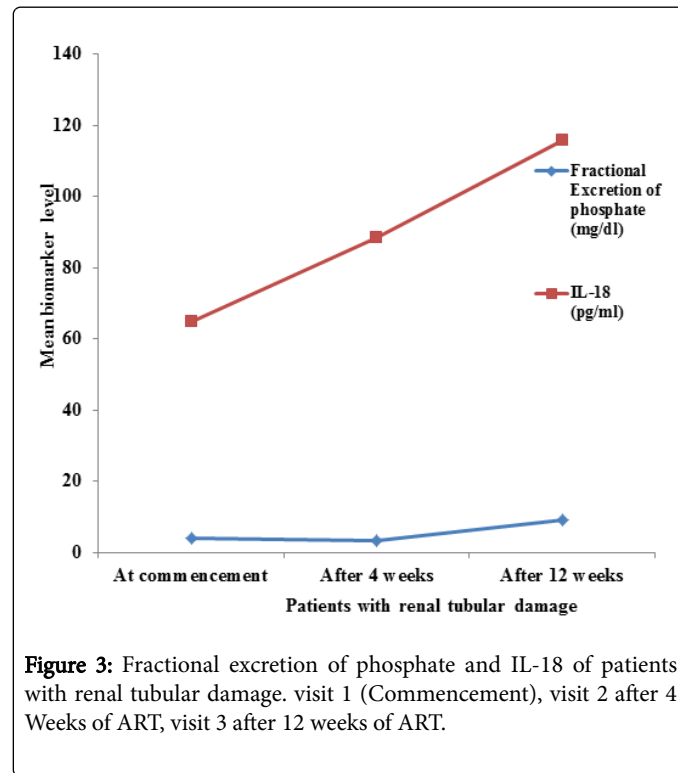

Figure 3: Fractional excretion of phosphate and IL-18 of patients with renal tubular damage. visit 1 (Commencement), visit 2 after 4 Weeks of ART, visit 3 after 12 weeks of ART.
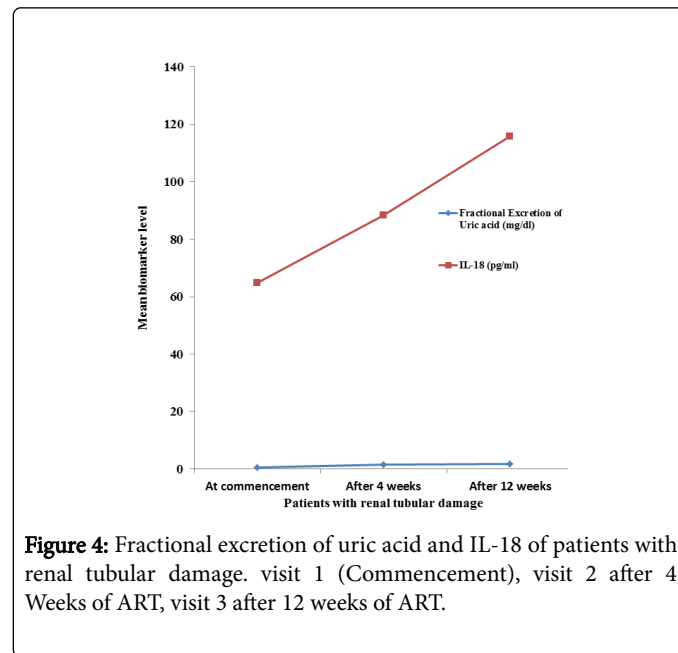

Figure 4: Fractional excretion of uric acid and IL-18 of patients with renal tubular damage. visit 1 (Commencement), visit 2 after 4 Weeks of ART, visit 3 after 12 weeks of ART.

Discussion

Renal dysfunction in clinical practice is measured using creatinine values to calculate eGFR (estimated glomerular filtration rate).

Creatinine is a late marker of renal dysfunction and is only raised when up to 30-50% of renal function is lost, [7,8]. This is because creatinine is more specific to the glomerulus and not to the tubules. Nephrotoxic drugs including antiretroviral therapy (ART), such as tenofovir have adverse effect on the proximal tubule of the kidneys. Severe renal dysfunction leading to end stage renal disease is associated with very high mortality especially in Africa where over 80% of these patients die due to insufficient facilities, poverty and late presentation of patients. Thus, there is need to detect renal dysfunction at a much earlier stage to prevent progression and so reduce possible mortality. Early detection can enable clinicians to stratify patients on the basis of drug therapy, avoiding nephrotoxic drugs and thereby reducing prevalence of overt renal dysfunction. Interleukin-18 has been described as a promising marker already in use to asses' renal function after transplantations, in metabolic diseases and heart diseases [9-12]. In this study there was a statistically significant difference in urinary IL-18 levels between each time point in the renal disease group, thus confirming earlier results from classic laboratory tests [9]. This early increase in urinary IL-18 levels compared with other common indicators of renal function with time, suggest a possible progression of renal toxicity from a subclinical stage to an end stage renal disease. From this study, it is important to note a consequential occurrence of the late increase (after 4 weeks) in eGFR, serum creatinine, fractional excretion of phosphate and fractional excretion of uric acid compared to the early increase in urinary IL-18 (at commencement). Therefore, it is hypothesized that a subclinical tubular dysfunction not detected by creatinine (eGFR) and other common renal indicators, can be detected by urinary IL-18.

The findings appear to confirm the statement that IL-18 can be used as an early marker of renal tubular dysfunction in HIV-infected patients. However, this finding is not unpredicted since IL-18 increases in urine only under condition of a marked tubular damage, apoptotic tubular cell shedding, and cell necrosis, associated with deterioration of renal function [13]. Another finding in this study is the significant difference in mean CD4 levels among the groups. It was observed that there was an increase in the mean CD4 level (<50 copies/ml) in the renal disease group compared to the non-renal disease group. This observation suggests that despite been at greater risk of developing renal dysfunction, they have a better immunological outcome.

Conclusion

Tubular toxicity has become an increasingly common comorbidity with a prevalence estimate of 7-33% among persons with HIV [14-16], and knowing that such an injury can be progressive, the aim of the study was therefore, to find early markers of the disease to help identify patients at higher risk of renal disease. The present findings appear to indicate that urinary IL-18 could be a reliable and accurate marker of subclinical tubular dysfunction [17], compared to other common indicators of renal function such as eGFR, serum creatinine etc, in HIV-infected patients. Finally, it is likely that not just one biomarker such as creatinine or IL-18 would be accurate and valuable for the investigation of renal injury in HIV-infected patients, but rather a combination of biomarkers and clinical parameters, will emerge as powerful tools for the early prediction and risk stratification of subclinical tubular dysfunction in HIV-infected patients.

Acknowledgments

Author's Contributions

TI and OC conceived and designed the experiments. DN performed the experiment and wrote the manuscript. OK and OP analysed the data and helped with the writing of the manuscript. OA contribute to data interpretation. OI contributed reagents, materials and analysis tools. All authors read and approved the final manuscript.

Conflict of interest

The authors confirm that this article content has no conflicts of interest.

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