SEVERE IMPAIRMENT OF SOLUTE-FREE WATER CLEARANCE IN PATIENTS WITH HIV INFECTION

Running head: HIV-associated damage at the loop of Henle

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Abstract

Renal disease is a well recognized complication among individuals with HIV infection. Either viral infection itself and the use of some antiretroviral drugs contribute to this major cause of serious non AIDS-defining condition that may affect both the glomeruli and the renal tubules. The thick ascending loop of Henle constitutes the main location for free-water clearance determining kidney´s ability to concentrate and dilute urine in a direct and indirect fashion, respectively. We compared this renal capacity in patients with HIV infection (with or without tenofovir-based antiretroviral therapy) with matched seronegative controls by means of an acute overload of hypotonic sodium chloride solution (Chaimovitz´ test).
Regardless of the presence of antiretroviral treatment, HIV-infected individuals showed a markedly impaired renal capacity to handle water excess under challenge conditions.
This finding may have clinical significance in patients at risk for decreases in serum osmolality such as the elderly, neurocognitive impaired and those with hyponatremia due to concomitant medications.

(Abstract word count: 155)

Key words: HIV infection, renal disease, tubular damage, free-water clearance, osmolality, hyponatremia

Introduction

Renal disease is increasingly recognized as a cause of morbidity and mortality among patients with HIV infection. Among the several conditions contributing with this fact are the longer survival of patients on antiretroviral treatment, specific effects of some antiretroviral drugs and viral-related factors [1,2].

Traditional risk factors for renal disease such as hypertension, diabetes and hyperlipidemia are more prevalent in seropositive patients and are also associated with the advancing age of the HIV-positive population [3]. In addition, these variables can be associated with exposure to combination antiretroviral therapy (cART) which may produce direct toxic effects on the nephron. Although the underlying mechanism is still unclear, tenofovir may produce proximal tubule damage characterized by proteinuria, normoglycemic glycosuria, hypokalemic renal tubular acidosis and phosphaturia [4]. These relatively infrequent findings may appear in the context of preserved renal function, although in some studies a decrease in the estimated Glomerular Filtration Rate (eGFR) has been reported [5,6,7]. Both decreased eGRF and proximal tubule damage –through diminished activation of Vitamin D- may contribute with overall cardiovascular risk in patients with HIV infection [8,9].

Atazanavir has also been reported as associated with an increased risk of reduction in eGFR or Chronic Kidney Disease (CKD) when used in combination with tenofovir [3, 10].

Proteinuria is an important finding in patients with HIV infection. Its prevalence in patients under cART ranges between 10 and 30%, and constitutes an independent risk factor for progression of CKD and all-cause mortality [11, 12, 13]. Urine protein-creatinin ratio (UPCR) or urine albumin-creatinin ratio (UACR) are commonly accepted measures of quantitative daily proteinuria [14].

Albuminuria indicates glomerular damage, while proteinuria includes albumin as well as other proteins that may reflect either renal tubular or glomerular damage. UACR or UPCR are less susceptible to the variability that may result from fluctuations in intravascular volume [15, 16]. An increased UACR is suggestive of glomerular injury, while a high UPCR with low UACR may identify patients with renal tubular disorders [17].

Usually renal compromise assessment have restricted to eGFR, but not tubular compromise. While eGFR could be ultimately affected by tubular disease, usual evaluations performed in patients with HIV infection –including proteinuria- are oriented towards glomerular health. Nevertheless, both drugs and HIV “per se” may produce tubular damage [18, 19, 20]. Among the main function of the renal tubules is to produce reabsorption of substances and to determine the amount of solute-compromised and solute-free water that will be excreted in urine. The evaluation of tubular function is somewhat more complex since requires the analysis of fractional excretion of solutes and/or determination of specific proteins that are normally reabsorbed at the proximal tubule such as retinol-binding protein (RBP) or Beta-2-microglobulin [21, 22].

The objective of the present study was to analyze the overall tubular function, and in particular that from the proximal tubule and the thick ascending loop of Henle (TALH) in patients with HIV infection receiving or not tenofovir-containing antiretroviral treatment in comparison with seronegative controls, by applying an hyposaline fluid acute overload (Chaimovitz` test)

Materials and Methods

Patients with confirmed HIV infection were selected in accordance with pre-specified criteria. Inclusion criteria were as follows: adult patients with confirmed HIV-1 infection who agreed to provide written informed consent. Patients under antiretroviral treatment must had a stable regimen for over six months and undetectable (<50 copies/ml) viral load for at least three months.

Exclusion criteria included patients with HIV primary infection, personal history of nephropathy, history or current evidence of acute or chronic renal failure, plasma creatinin >1.3 mg/dl, creatinin clearance <60ml/min, presence of glucosuria/proteinuria, prior heart failure, concurrent opportunistic infection, current use of tenofovir plus didanosine, chronic active hepatitis B or C, and use of potentially nephrotoxic agents in the prior week before the test (e.g diuretics, angiotensin converting enzyme antagonists, AT1 angiotensin II receptor antagonists or non-steroidal anti-inflammatory agents).

A group of seronegative patients were also selected for comparisons

Chaimovitz test

The functional evaluation of the proximal tubule and the TALH can be carried out using the Chaimovitz' test, which is based on the exploration of the tubular response to an acute fluid overload [23, 24, 25]. This salt and water overload -twenty cc/Kg of mineralized water PO and two liters of IV hypotonic solution (0.45%) intravenously infused in two hours- suppresses the activity of anti-diuretic hormone (ADH) and aldosterone and thus “defunctionalizes” the distal tubule. Sodium is the major solute reabsorpted at the proximal tubule, and thus the function of this segment can be analyzed through its local sodium clearance. In addition, the analysis of the fractional excretion (FE) of phosphorus and uric acid -mainly reabsorpted at the same segment- may constitute an alternative way of assessing proximal tubule functioning. In contrast, water

handling may account for the evaluation of the TALH, since this segment is responsible for the generation of free water clearance or local sodium reabsorption which in turn contribute to medullar hypertonicity that determine consequent water reabsorption. As previously shown that the presence of renal incompetence for sodium reabsorption can be detected by the Chaimovitz' test [26].

This study was approved by the Institutional Review Board and all participants provided written informed consent prior to the performance of all the study evaluations.

Comparisons between two groups were performed with Mann-Whitney Wilcoxon Rank Sum Test and between three groups with Kruskal-Wallis Test. Comparisons within groups pre and post dilution were performed with Wilcoxon Signed Rank test, with $p<0.05$ as the level of significance

Results

A total of 31 patients were included in the analysis. Among patients with HIV infection, ten individuals (1 female) were currently receiving stable antiretroviral treatment regimen including tenofovir (Tenofovir Group), while eleven patients (1 female) were not receiving tenofovir (Non-tenofovir Group) - in fact three patients were not receiving antiretroviral treatment-. Median CD4+ T-cell count was 464 cells/mm^3 (r280-869) and 475 cells/mm^3 (r293-1876) in the Tenofovir and Non-tenofovir groups, respectively. Ten seronegative patients (2 females) were selected as control. Median age was 48 and 51 years in both HIV positive groups, and 24,5 years in the control group.

All baseline values in both HIV+ groups were within physiological range, except for urinary osmolarity (UOsm) that was lower than expected for a fasting state.

Baseline serum values and fractional excretion of solutes were similar between HIV+ groups with the exception of plasma magnesium that was lower in the Tenofovir Group (data not shown).

Serum sodium and plasma osmolarity were significantly lower and urine osmolarity was significantly higher than normally expected values in both HIV+ groups either at baseline and at maximal dilution status.

At dilutional status, most serum and fractional excretion values were similar between HIV+ groups, with the exception of plasma magnesium that was higher and fractional excretion of potassium and calcium that were lower in the tenofovir group.

Serum urea, calcium, magnesium were lower and FE of calcium was higher in the dilutional status in both HIV+ groups in comparison with basal values (Table 1).

Free water clearance (sodium reabsorption at TALH) was significantly lower, while urine osmolarity was higher in the dilutional state in HIV+ patients than in seronegative controls (Table 2).

Discussion

Chronic kidney disease associated with HIV-1 infection can affect not only the glomerular portion of the nephron but also the different portions of the renal tubules. CKD may persist clinically silent for many years, supporting the current recommendation to include laboratory determinations in addition to clinical evaluation in the follow up of patients with HIV infection [27].

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The restriction of routine analysis to a periodical determination of BUN and serum creatinine may result insufficient to detect early tubular damage. While HIV-related kidney disease has been usually regarded as glomerular damage, tubular dysfunction -mainly associated with drug toxicity- is an important and frequently overlooked component of renal pathology.
Different approaches have been previously published aiming to detect tubular damage in patients with HIV [21]. Tenofovir-related tubular damage has been associated with subclinical mitochondrial damage in patients switching to tenofovir-based regimens from thymidine analogs [28]

In our study, most basal (fasting) serum and fractional excretion values of electrolytes were similar between HIV+ groups, and -as expected- were within normal range (Table 1). However natremia and serum osmolarity values were lower and urine osmolarity values were somewhat higher than expected. This findings suggest the presence of an impairment in urine concentration in both HIV+ groups.
Analyzing the values obtained with acute volume overload there were no significant differences in serum or in FE values between both HIV+ groups, except for magnesemia -which was lower in the Non-tenofovir group, and FE of potassium and calcium which were significantly higher in the Non-tenofovir group (Table 1). While volume expansion usually increases electrolyte excretion, the differences found in both HIV+ groups may warrant further confirmation.

Urine osmolarity, plasma sodium clearance and free water clearance showed no significant difference between HIV+ groups, but were significantly different from seronegative controls (Table 2). Urine osmolarity difference suggests that kidney`s dilutional effort was insufficient to protect plasma sodium and plasma osmolality in HIV-positive patients under these conditions.

Although Chaimovitz´ test has been reported as an approach to evaluate tubular function in the young and elderly population, to our knowledge this is the first report of the use of this test in patients with HIV-1 infection. Although this test may not be feasible for routine screening of seropositive patients under follow up, an approach towards evaluating concentration/dilution capacity may be warranted at least in some selected groups, such as the elderly, patients with neurocognitive impairment, prior kidney disease or those with concomitant use of potentially interacting medication

In this selected group of patients with no evidence of baseline renal compromise, we found no significant abnormalities in proximal sodium handling nor in the FE of solutes transported mainly by the proximal tubule, such as phosphate and uric acid. However free-water clearance was significantly lower in patients with HIV infection than in seronegative controls, which may reflect an attempt of the kidney to preserve sodium that is being lost at the TALH in these patients. Although extremely uniform in our study, this finding may require further confirmation given the reduced number of patients evaluated.

We consistently found a significant functional reduction in the free-water clearance in all HIV+ individuals evaluated regardless of the presence of tenofovir.
These findings suggest that kidney`s ability to either concentrate and dilute urine is severely impaired in patients with HIV infection, with the potential risk to develop osmolality abnormalities under contraction or water overload scenarios.

In the clinical setting, these findings indicate an increased risk for dehydration or hyponatremia in HIV-infected patients undergoing water deprivation or water overload respectively, as well as in the context of receiving drugs that could cause hyponatremia such as diuretics or psychoactive drugs.

References

1. Phair J, Pallela F. Renal disease in HIV-infected individuals. Curr Opin HIV AIDS 2011; 6:285-9
2. Estrella MM, Fine DM, Atta MG. Recent developments in HIV-related kidney disease. HIV Ther 2010 September 4(5):589-603
3. Mocroft A, Kirk O, Reiss P et al. Estimated glomerular filtration rate, chronic kidney disease and antiretroviral drug use in HIV-positive patients. AIDS 2010; 24(11):1667-87.
4. Mathew G, Knaus SJ. Acquired Fanconi´s syndrome associated with tenofovir therapy. J Gen Intern Med 2006; 21(11):C3-C5.
5. Fernandez-Fernandez B, Montoya-Ferrer A, Sanz AB, Sánchez-Niño MD, Izquierdo MC, Poveda J, Sainz-Prestel V, Ortiz-Martin N, Parra-Rodriguez A, Selgas R, Ruiz-Ortega M, Egido J, Ortiz A. Tenofovir nephrotoxicity: 2011 Update. AIDS Res Treat 2011; 1-11.
6. Rodriguez-Novoa S, Alvarez E, Labarga P, Soriano V. Renal toxicity associated with tenofovir use. Exp Opin Drug Safety 2010; 9(4);545-99
7. Cooper RD, Wiebe N, Smith N, Keiser P, Naicker S, Tonelli M. Systematic review and meta-analysis: renal safety of tenofovir disoproxil fumarate in HIV-infected patients. Clin Infect Dis 2010; 51(%):496-505.
8. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events and hospitalization. N Engl J Med 2004; 351(13):1296-370.
9. Choi AI, Li Y, Deeks SG, Grunfeld C, Volberding PA, Schlipak MG. Association between kidney function and albuminuria with cardiovascular events in HIV-infected persons. Circulation 2010; 121:651-8.
10. Dazo C, Fahey P, Puls RL, Winston A, Boesecke C, Avhingsanon A, Amin J, Rooney JF, Belloso WH, Cooper DA, Emery S for the Altair Study Group. Small and significant and non-progressive decline in glomerular filtration rate is observed in therapy-naive

HIV-positive subjects commencing ritonavir-boosted atazanavir compared to either efavirenz or zidovudine/abacavir, all with tenofovir/emtricitabine after 48 weeks, a randomized controlled study. Presented at the 18th Conference on Retroviruses and Opportunistic infections (CROI 2011), Boston, MA, March 2011.

11. Wyatt CM, Hoover DR, Shi Q, Seaberg E, Wei C, Tien PC, Karim R, Lazar J, Young MA, Cohen MH, Klotman PE, Anastos K. Microalbuminuria is associated with all-cause and AIDS mortality in women with HIV infection. J Acquir Immune Defic Syndr. 2010 Sep 1;55(1):73-7.
12. Gardner LI, Holmberg SD, Williamson JM, Szczech LA, Carpenter CC, Rompalo AM, Schuman P, Klein RS. Development of proteinuria or elevated serum creatinine and mortality in HIV-infected women. HIV Epidemiology Research Study Group.J Acquir Immune Defic Syndr. 2003 Feb 1;32(2):203-9.
13. Choi AI, Scherzer R, Bacchetti P, Tien PC, Saag MS, Gibert CL, Szczech LA, Grunfeld C, Shlipak MG.. Cystatin C, albuminuria, and 5-year all-cause mortality in HIV-infected persons. Source University of California, San Francisco, CA, USA. Am J Kidney Dis. 010 Nov;56(5):872-82. Epub 2010 Aug
14. Gupta SK, Smurzynski M, Franceschini N, Bosch RJ, Szczech LA, Kalayjian RC; AIDS Clinical Trials Group Longitudinal Linked Randomized Trials Study Team The effects of HIV type-1 viral suppression and non-viral factors on quantitative proteinuria in the highly active antiretroviral therapy era. Antivir Ther. 2009;14(4):543-9.
15. Kalayjian R, Renal Issues in HIV infection, Curr HIV/AIDS Rep 2011; 8: 164-17
16. Kalayjian R, Kidney disease in HIV-infected persons. Curr Infect Dis Rep 2012; 14:83–90
17. Samarawickrama A, Nambiar K, Gilleece Y, Fisher M, Host S. Value of Urine Protein/Creatinine and Albumin/Creatinine Ratios in Assessing Renal Disease in HIV

infection. 17th Conference on Retroviruses and Opportunistic Infections, San Francisco, CA. Abstract 737. 2010.

18. Vashistha H, Husain M, Kumar D, Singhal PC. Tubular cell HIV-1 gp120 expression induces caspase 8 activation and apoptosis. Ren Fail. 2009;31(4):303-12.
19. Szczech L. Renal dysfunction and tenofovir toxicity in HIV-infected patients. Topics HIV Med 2008; 16(4):122-6,
20. Vrouenraets SM, Fux CA, Wit FW, Garcia EF, Furrer H, Brinkman K, Hoek FJ, Abeling NG, Krediet RT, Reiss P, Prepare Study Group. Persistent decline in estimated but not measured glomerular filtration rate on tenofovir may reflect tubular rather than glomerular toxicity. AIDS 2011; 25(17):2149-55.
21. Hall AM, Edwards SG, Lapsley M, Connolly JO, Chetty K, O´Farrell S, Unwin RJ, Williams IG. Subclinical tubular injury in HIV-infected individuals on antiretroviral therapy: a cross-sectional analysis. Am J Kidney Dis 2009; 54(6):1034-42.
22. Labarga P, Barreiro P, Martin-Carbonero L, Rodriguez-Novoa S, Solera C, Medrano J, Rivas P, Albalater M, Blanco F, Moreno V, Vispo E, Soriano V. Kidney tubular abnormalities in the absence of impaired glomerular function in HIV patients treated with tenofovir. AIDS 2009; 10(6):329-36.
23. Chaimovitz C, Levi J, Better O, Oslander L, Benderli A. Studies on the site of renal salt loss in a patient with Bartter´s syndrome. Pediatr Res 1973;7;89.
24. Macías-Nuñez JF, García Iglesias C, Bondía Roman A, Rodriguez Commes L, Corbacho Becerra L, Tabernero Romo JM, De Castro del Pozo S. Renal handling of sodium in old people: a functional study. Age and ageing 1978; 7:178-81.
25. Musso CG, Fainstein I, Kaplan R, Macías Nuñez JF. Función tubular renal en el muy anciano. Rev Esp Geriatr Gerontol 2004; 39(5):314-9.

26. Musso CG, Macías-Nuñez JF. Dysfunction of the thick loop of Henle and senescence: from molecular biology to clinical geriatrics. Int Urol Nephrol. 2011 Mar;43(1):249-52. Epub 2010 Nov 12.
27. Gupta SK, Eustace JA, Winston JA, Boydstun II, Ahuja TS, Rodriguez RA, Tashima KT, Roland M, Franceschini N, Palella FJ, Lennox JL, Klotman PE, Nachman SA, Hall SD, Szczech LA. Guidelines for the management of chronic kidney disease in HIV-infected patients: recommendations of the HIV Medicine Association of the Infectious Diseases Society of America. Clin Infect Dis 2005; 40:1559-85.
28. Maggi P, Montinaro V, Bellacosa C, Pietanza S, Volpe A, Graziano G, Strippoli GF, Angarano G. Early markers of tubular dysfunction in antiretroviral-experienced HIV-infected patients treated with tenofovir versus abacavir. AIDS Patient Care STDS. 2012 Jan;26(1):5-11. Epub 2011 Dec 2.

Tables

Table 1. Comparison between HIV+ Tenofovir Group and HIV+ Non-tenofovir group at baseline and at maximum dilution (Chaimovitz´Test). Median (range) values

	HIV+Tenofovir Baseline	Group Dilution	*p*-value	HIV+ Non-tenofovir Baseline	Group Dilution	*p*-value	Between groups *p* value
Serum Urea (mg/dl)	32.5 (14-53)	26 (14-44)	0.01	36 (15-57)	33 (12-50)	0.003	NS
Serum creatinin (mg/dl)	0.82 (0.65-1.2)	0.81 (0.65-1.1)	NS	0.85 (0.58-1.07)	0.84 (0.52-1.0)	NS	NS
Serum glucose (mg/dl)	84 (68-99)	106.5 (75-145)	0.01	81 (69-105)	156 (60-220)	NS	NS
Serum uric acid (mg/dl)	4.2 (3.9-7)	4.85 (3.9-5.8)	NS	4 (3.1-5.4)	4.95 (2.8-6.6)	NS	NS
Serum calcium (mg/dl)	9.1 (8.1-10)	8.1 (7.7-8.8)	0.01	9.05 (8.8-10.4)	8.5 (7.8-8.7)	0.01	NS
Serum sodium (mmol/l)	137.5 (136-141)	134 (131-137)	0.009	136 (135-143)	133 (129-140)	0.01	NS
Serum potassium (mmol/l)	3.95 (3.7-5.2)	3.7 (2.7-5.0)	NS	4 (3.6-5.6)	3.6 (2.9-4.3)	0.005	NS
Serum phosphate (mmol/l)	4.1 (2.3-4.8)	2.9 (2.1-3.9)	NS	3.45 (3.2-4.3)	2.85 (2.1-3.2)	0.01	NS

Serum magnesium (mmol/l)	2.25 (2.0-2-4)	2.0 (1.8-2.2)	0.02	2.05 (1.9-2.2)	1.8 (1.5-2.0)	0.01	0.03
Serum chloride (mmol/l)	104 (96-107)	104.5 (96-108)	NS	103 (100-107)	105 (99-107)	NS	NS
Est.GFR (MDRD) (ml/min/1.73m^2)	103.9 (65-125)	103.9 (72-134)	NS	98 (79.4-139)	100 (79.4-141)	NS	NS
FE sodium (%)	0.59 (0.25-1.8)	0.78 (0.48-1.27)	NS	0.52 (0.4-1.0)	0.87 (0.36-1.56)	0.01	NS
FE potassium (%)	4.9 (3.3-11.1)	7.8 (1.18-19)	NS	6.4 (0.8-13)	13.3 (3.5-18)	0.005	0.04
FE chloride (%)	0.68 (0.47-3.7)	1.4 (1.1-1.7)	NS	0.58 (0.28-5.7)	1.6 (0.81-5.0)	NS	NS
FE phosphate (%)	14 (6-20)	15.6 (3.79-24)	NS	15.5 (3-23)	10.75 (0.6-19)	NS	NS
FE calcium (%)	0.68 (0.34-1.5)	1.4 (0.99-1.7)	0.02	0.58 (0.46-1.3)	2.0 (0.19-2.8)	0.02	0.04
FE magnesium (%)	2.05 (0.8-3)	2.7 (2.2-7.48)	NS	1.53 (0.44-4.9)	4.2 (0.45-5.0)	0.04	NS
FE urea (%)	0.47 (0.21-0.6)	0.46 (0.27-3.0)	NS	0.57 (0.2-4.6)	0.65 (0.44-1.1)	NS	NS
FE uric acid (%)	4.7 (4.1-7.9)	7.9 (6.0-10.0)	NS	4.4 (0.36-8.7)	10 (2.0-29)	NS	NS
Plasma osmolality (mOsm/l)	282.5 (279-292)	278.5 (270-283)	0.009	285 (277-298)	278 (273-289)	0.005	NS

Urine osmolality (mOsm/l)	418.5 (142-759)	138.5 (38-594)	0.005	659 (369-854)	92 (37-323)	0.003	NS

Table 2. Chaimovitz´ test specific results. Comparison between HIV+ groups and seronegative controls.

	HIV+ Tenofovir Group	HIV+ Non-tenofovir Group	Seronegative controls	*p*-value
Urine osmolality (mOsm/l)	138.5 (38-594)	92 (37-323)	40 (30-59)	0.001
Free-water clearance (ml/min/1.73 m^2)	3.07 (-1.36-6.9)	5.5 (-0.42-8.6)	15.2 (11.8-18.2)	<0.001
Proximal sodium clearance (ml/min/1.73 m^2)	1.15 (0.59-4.1)	1.4 (0.47-2.2)	17.8 (15.3-22.0)	0.001
Distal sodium reabsorption (Henle) (%)	71 (18-93)	91 (61-98)*	86 (81-92)	NS
Osmolar clearance (ml/min/1.73 m^2)	2.17 (1.0-6.3)	2.6 (0.78-3.39)*	3.15 (2.2-4.0)	0.005

*p<0.05 between HIV+ groups