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Cystatin C: A Marker for Inflammation and Renal Function Among HIV-Infected Children and Adolescents

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Abstract

Background. Renal disease is a leading cause of morbidity in HIV-infected adults in the HAART era. Cystatin C has been proposed as a more sensitive marker of renal function, but it may be affected by ongoing inflammation. We aimed to study cystatin C levels in a cohort of HIV-infected pediatric patients at 3 Spanish centers.

Methods. Multicenter cross-sectional observational study. Renal function was assessed by means of first morning urine protein/creatinine and albumin/creatinine ratios and creatinine-estimated glomerular filtration rates (GFR), together with the following inflammation markers: cystatin C, reactive C protein, beta-2-microglobulin and 25(OH)-vitamin D levels. A control group of healthy children and adolescents was used.

Results. Eighty-three patients (51 females, median age 13.3 years; 32 males, 13.6 years) and 44 controls (24 females, median age 12.2 years; 20 males, 10.9 years) were included. Among the former, mean CD4 cell count was 860/mm^3, 29(35%) patients had a previous AIDS diagnosis, 73(88%) were on HAART and HIV viremia was undetectable in 61(73%). No differences in cystatin C levels were observed between the two groups.

In HIV-infected patients, cystatin C levels correlated with GFR (r=-0.27; p=0.01), age at first HAART (r=-0.21; p=0.05), and beta-2-microglobulin (r=0.569; p<0.01). In multivariate analysis, lower GFR (p=0.014) and higher beta-2-microglobulin levels (p=0.001) remained as independent risk factors for higher cystatin C values.

Conclusions. Cystatin C values were associated with GFR and beta-2-microglobulin. Cystatin C may be useful as a marker of renal function in HIV-infected pediatric patients, independently of ongoing inflammation or viremia.

Highly active antiretroviral therapy (HAART) has changed the natural history of pediatric HIV infection, with AIDS-related morbidity and mortality rates decreasing over time among perinatally-infected children and adolescents [1-3]. Optimizing transition pathways into adult care and long-term follow-up of metabolic and other effects of long-term exposure to HIV and HAART have now become a priority, as these vulnerable populations are at high risk of early non-AIDS defining comorbidities, including renal conditions, in their 3rd or 4th decades of life. Several factors contribute to excess kidney disease in the HIV-infected patient, including HIV itself, immune activation, drug toxicity, hepatitis C virus co-infection and traditional renal disease risk factors; some of these factors have very limited impact in the pediatric age. In the HAART era, the incidence of renal disorders in perinatal HIV infection has remained stable around 0.26 episodes per 100 patient-years [4] but it has changed from predominant classical HIV-associated nephropathy (HIVAN) to tenofovir-related toxicity [5,6]. However, persistent renal function abnormalities occur in one fifth of HIV-infected children and are associated with improved survival, black race and Hispanic ethnicity, and exposure to nephrotoxic drugs [7,8]. Current recommendations in children and adolescents with HIV and no previous evidence of kidney disease suggest screening for renal dysfunction with estimated glomerular filtration rate (GFR) at HAART initiation or change and at least twice yearly, and for kidney damage (urinalysis or quantitative proteinuria) at HAART initiation or change and at least yearly [5,9] or, if the patient is receiving tenofovir, at shorter intervals (3-6 months)[6].

The best method for estimating GFR in children remains uncertain. Creatinine (Cr)-based equations are widely used but show several limitations: muscle mass dependency, variable Cr production and Cr tubular secretion. Small molecular mass proteins have been proposed as better markers of GFR because they are freely filtered in the glomeruli: cystatin C is the most

promising of these and cystatin C-based equations have given more accurate measurements of GFR, especially in patients with GFR below 60 mL/min/1.73m^2; they have been successfully used in children with kidney dysfunction caused by several conditions [10-13]. In HIV-infected adults, the usefulness of cystatin C as a GFR marker is limited because of its strong correlation with use of HAART, HIV RNA suppression, and markers of T-cell activation [14,15]. Data on cystatin C in HIV-infected children are very limited [16,17].

In this study, we determined cystatin C plasma levels in a large cohort of HIV-infected children and adolescents, and how these levels correlated with markers of renal function and inflammation.

MATERIALS AND METHODS

We performed a multicenter cross-sectional observational study in a cohort of 84 HIV-infected children (52 females) and adolescents up to age 18 years, followed up in three tertiary hospitals in Spain (Hospital Sant Joan de Déu and Hospital Vall d'Hebron, Barcelona; and Hospital Virgen del Rocío, Seville).

Epidemiologic data and medical history, including CDC clinical category [6], are routinely obtained for all patients at enrollment. The complete history of past and current use of antiretroviral drugs is collected and each quarterly visit which includes a clinical interview with assessment of adherence to HAART, complete physical examination, body mass index (BMI) calculation (expressed in Z-score), adjusted for age and sex [18], as well as blood count, plasma Cr, CD4 cell count (flow cytometry, FACSCalibur; BD Biosciences, San Jose, CA, USA) and determination of quantitative plasma HIV RNA level (in Barcelona: NucliSENS EasyQ HIV-1 assay; BioMérieux Laboratories, France; in Seville: COBAS AmpliPrep/COBAS TaqMan HIV-1

Test, version 2.0, Roche Diagnostic System, Branchburg, NJ, USA; lower limit of detection: <20 HIV RNA copies/mL for both tests).

To assess renal function, a first-morning urine sample was collected and the protein/creatinine ratio (Pr/Cr; normal ≤200 mg/g) and the albumin/creatinine ratio (Alb/Cr; normal ≤30 mg/g) were calculated. Glomerular filtration rate was estimated from Cr levels according to Schwartz formula in patients aged up to 11 years and according to the Cockroft–Gault formula in patients ≥12 years [19]. The cut-off for decreased GFR was <90 mL/min/1.73m^2 for age, sex, and height-adjusted values from the Spanish healthy population [19,20].

The inflammation status was evaluated by measuring C-reactive protein (CRP; in mg/L); beta-2-microglobulin, analyzed through a latex particle-enhanced immunoturbidimetric assay (ARCHITECT c-system, Quantia b2-microglobulin assay; Biokit SA, Barcelona, Spain), normal ≤2.5 mg/L [21] and 25(OH)-vitamin D levels (ARCHITECT 25-OH Vitamin D assay; Abbott Diagnostics, Wiesbaden, Germany), normal values > 30 ng/mL. Plasma cystatin C was measured using a turbidimetric immunoassay (Multigent cystatin C assay; Abbott Diagnostics, Wiesbaden, Germany); normal ≤1.38 mg/L [22].

Patients with previously diagnosed active nephropathy, thyroid disease or corticosteroid therapy at assessment were excluded, as well as urine samples obtained after vigorous exercise or during active infection. A cohort of 44 healthy children (24 females) referred to Hospital Sant Joan de Déu for non-nephro-urological minor surgery was used as a control population; they provided a first morning urine sample and a blood sample to determine Cr and cystatin C. Informed consent and assent at inclusion from parents or legal guardians and patients aged 12 or older, respectively, were obtained, as was local ethics committee approval at each Centre.

Statistical analysis

Categorical and continuous variables were described as percentages and median values and interquartile ranges, respectively. The parametric unrelated samples Student's t test was used for normally distributed data; the Mann-Whitney test was applied to non-normally distributed data. Pearson's and Spearman test were used to identify correlations between quantitative variables. A multivariate analysis to identify factors related to cystatin C levels was performed; factors examined included those showing a significant association in the bivariate analysis and/or having clinical importance. The analysis was carried out using SPSS 15.0 Software, and statistical significance was set at $p \leq 0.05$.

RESULTS

Among 84 HIV-infected patients at study entry, three children had previous renal diagnosis, including recurrent urinary tract infections, trauma-related hematuria, and diabetic nephropathy; only the patient with diabetic nephropathy was excluded from the analysis. Eighty-three (51 females, median age 13.3 years; 32 males, median age 13.6 years) HIV-infected children and adolescents and 44 (24 females, median age 12.2 years; 20 males, median age 10.9 years) healthy controls were included in the study; baseline characteristics are summarized in ***Table 1.*** Among the former, mean CD4 cell count was 860/mm^3, 29 (35%) patients had a previous AIDS diagnosis, 73 (88%) were on HAART and plasma HIV RNA was undetectable in 61 (73%). HAART regimens included tenofovir, a protease inhibitor and a non-nucleoside in 28 (34%), 49 (59%) and 31 (37%) patients, respectively. No child was receiving indinavir.

At assessment, no patient had symptoms consistent with urinary protein loss or kidney damage; Cr levels also remained within normal range in all cases. Healthy children were younger than HIV-infected patients (median 11.5 vs 13.4 years; p=0.018), had slightly higher BMI values

(0.04 vs -0.2; p=0.03) and lower, albeit not abnormal, estimated GFR levels (125 vs 137 mL/min/1.73m^2; p=0.036). No other significant differences were observed in epidemiological data, renal function variables or cystatin C levels between HIV-infected children and the healthy population. Overall, cystatin C levels were not associated with age, gender, ethnicity or BMI (data not shown).

In our series, proteinuria was detected in one fifth of HIV-infected patients, and two 17-year-old girls showed estimated GFR <90 ml/min/1.73m^2; both had normal CD4 counts and undetectable viremia, were receiving a tenofovir-based HAART regimen and had no other known risk factors for renal dysfunction.

Cystatin C remained within normal limits in all cases, and vitamin D values were insufficient in 59 (71%) patients. Among HIV infection-related variables, higher values of cystatin C were observed in treatment-naïve children (0.95 vs 0.87 mg/L; p= 0.019) and in patients receiving protease inhibitors (0.88 vs 0.82; p= 0.06). Cystatin C values inversely correlated with age at first HAART (r= -0.21; p=0.059). Plasma HIV viral load at assessment, HIV infection transmission route, previous AIDS diagnosis, nadir or current CD4 cell counts, hepatitis B or C virus co-infection, cumulative time on HAART, prior or current use of tenofovir, and current use of non-nucleosides were not associated with cystatin C levels.

With regard to renal function, cystatin C negatively correlated with estimated GFR (r=-0.272; p=0.013), but showed no association with proteinuria or albuminuria. Finally, a strong correlation between cystatin C and beta-2-microglobulin levels was observed (r= 0.569; p<0.001), but not with vitamin D or CRP. No other associations were found in bivariate analysis. In adjusted multivariate analysis, lower Cr-estimated GFR values (p=0.014) and beta-2-

microglobulin levels (p=0.001) remained as independent risk factors for higher cystatin C values (***Table 2***).

DISCUSSION

Since 1985, cystatin C has increasingly been used as a marker of renal function. Cystatin C is a proteinase inhibitor with low molecular weight produced by all nucleated cells. It protects the connective tissue from the injury of intracellular enzymes released after cellular death or secreted by malignant cells. A possible immune function in chronic viral infections has been described as well [23-26]. Cystatin C is freely filtered and completely reabsorbed and catabolized in the proximal renal tubules. Therefore, its urine concentration is minimal and its blood concentration is constant. Unlike Cr levels, cystatin C values remain constant after the first year of life and are not dependent on muscle mass or anthropometric data, which are major advantages in children. Consistent with these properties, in our results, age, gender and BMI did not affect cystatin C levels, as previously described [16,17,26,27].

When a renal injury occurs, cystatin C plasma levels increase [23-25,27]. Other conditions that may influence cystatin C values are thyroid illness, corticosteroid therapy, and chronic viral infections, such as HIV infection [23]. Studies conducted in adults with kidney transplant, chronic kidney disease, and diabetes mellitus have shown that cystatin C detects renal injury earlier than Cr [28-31]. Similar findings have been described in children, although data are still scarce [10-12,32,33].

Since the implementation of HAART in the late 1990s, a substantial decrease in death and AIDS-related conditions has been observed. On the other hand, non-AIDS diseases - including renal disorders - have emerged and are now leading causes of morbidity and mortality in this

population. Ongoing inflammation plays a central role in the development of non-AIDS events, and higher levels of inflammatory markers such as cystatin C, CPR, interleukin 6, D-dimer, beta-2-microglobulin, vitamin D and A amiloid have been described in the HIV-infected population as compared to healthy controls [34,35].

The interaction between cystatin C and HIV infection has been widely studied. In one of the SMART Trial sub-studies [15] patients randomized to interrupt HAART showed an increase in cystatin C values that positively correlated with plasma HIV RNA levels and which returned to previous levels upon treatment resumption. Moreover, cystatin C values correlated with inflammatory markers, whilst no changes in other renal function parameters were observed. Similar findings were subsequently confirmed by other authors [5,36,37]. The latter would potentially invalidate the use of cystatin C as a marker of renal function in treatment-naïve HIV-infected patients and those on virologic failure.

To date, data on cystatin C in HIV-infected children and adolescents are limited to two cross-sectional case-control studies conducted in Nigeria among naïve patients with advanced HIV disease [16,17]. In both cases, consistently higher cystatin C levels and lower cystatin-calculated GFR were reported in HIV-infected children as compared to healthy controls. The authors attribute these findings to a high rate of HIVAN and chronic kidney disease in their cohorts, but HIV viral load or markers of inflammation were not performed; therefore, the influence of the inflammatory status on cystatin C levels (and cystatin-calculated GFR) could not be taken into account in these studies. The very different clinical and immunological situation in the two Nigerian cohorts (low CD4 counts, naïve status, black ethnicity, malnutrition and advanced HIV disease) as compared to our cohort makes comparisons difficult. Mean cystatin C levels (0.87 mg/L) in our study are lower than those reported by other studies in pediatric (1.01 mg/L) [16]

and adult HIV cohorts (1.03 mg/L) [38]; most importantly, no differences in cystatin C values were found with healthy controls, as opposed to previous studies [16,17,39,40]. The optimal immunologic and clinical situation in our cohort, including renal function, probably explains these findings.

Only 2 out of 83 patients showed estimated GFR <90 and none <60 ml/min/1.73m^2 in our study; of note, both girls were receiving HAART regimens including tenofovir. Albuminuria or proteinuria rates were similar between HIV-infected patients and healthy controls and did not correlate with inflammatory markers. In HIV-infected adults, inflammatory activity has been implicated as a cause in the development of microalbuminuria [41] which, in turn, is a well-known risk factor for kidney and cardiovascular disease in both HIV-infected [42,43] and uninfected individuals [44]. Again, low numbers and lack of significant renal disease in our study probably prevented us from observing these associations.

In this study, uncontrolled viremia and lower CD4 counts were not associated with higher cystatin C levels, as previously described [16,37,41]. Instead, cystatin C remained significantly associated with beta-2-microglobulin and Cr-estimated GFR in multivariate analysis, suggesting that, in well-controlled HIV-infected cohorts, cystatin C is associated with inflammation but also remains a useful marker of renal function.

Our study has several limitations, including small numbers, its cross-sectional design and the lack of data on other comorbidities that may influence renal function, such as diabetes or hypertension. Secondly, the HIV-infected cohort and the healthy children were significantly different in terms of age and BMI, although we think these findings had no clinical relevance and we believe cystatin C levels to be independent of age and anthropometric data. Differences in estimated GFR are probably explained by younger age among controls. Thirdly, most of the

patients in our study were receiving HAART at assessment, including tenofovir (36%) and protease inhibitors (64%), which are known to be nephrotoxic; only the use of protease inhibitors was associated with higher cystatin C levels in bivariate analysis, but this relationship disappeared in multivariate analysis. Finally, our findings in a well-controlled group of HIV-infected children and adolescents with highly preserved renal function [8] may not be applicable in the setting of uncontrolled HIV infection and/or renal impairment.

In conclusion, in this study cystatin C levels in HIV-infected children were similar to those of healthy children, and correlated both with beta-2-microglobulin and with Cr-estimated GFR after adjustment for viremia. Further longitudinal studies including larger number of patients are needed to identify how useful cystatin C may be in the follow-up of renal function and inflammation activity in HIV-infected children and adolescents.

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Legends.

Table 1. Epidemiological data, renal function and inflammatory markers from both HIV-infected patients and healthy controls; results expressed as n (%), unless stated otherwise.

Table 2. Risk factors (multivariate analysis) for cystatin C levels.

(Table 1)

Patient characteristics	**HIV-infected patients N=83**	**Controls N=49**	**p**
Female gender	51 (61.4)	24 (54.5)	0.452
Black ethnicity	11 (13.4)	2 (4.5)	0.125
Age (years); median (IQR)	13.4 (10.1-16.4)	11.5 (7.3-14.5)	0.015
BMI, Z-score; median (IQR)	-0.2 (-0.8 to 0.3)	0.04 (-0.5 to 1.2)	0.03
Vertically-transmitted HIV infection	73 (87.9)	NA	
Age at HIV infection diagnosis (years); median (IQR)	0.7 (0.2-2.6)	NA	
Nadir CD4 cells/mm^3; median (IQR)	423 (254-636)	NA	
AIDS diagnosis	29 (34.9)	NA	
Hepatitis B virus co-infection	1 (1.2)	NA	
Hepatitis C virus co-infection	1 (1.2)	NA	
Details on previous ARV therapies			
Age at 1st HAART (years); median (IQR)	1.7 (0.4-3.8)	NA	
Prior tenofovir use	22 (26.5)	NA	
Prior indinavir use	2 (2.4)	NA	
Cumulative time on HAART (months); median (IQR)	113 (73-168)	NA	
At assessment:			
Naïve	5 (6.0)	NA	

Tenofovir use	28 (36.4)	NA	
HAART regimen including an NNRTI	31 (40.2)	NA	
HAART regimen including a PI	49 (63.6)	NA	
CD4 cells/mm^3; median (IQR)	860 (610-1200)	NA	
Log_{10} RNA-HIV copies/mL; median (IQR)	1 (1-2)	NA	
Undetectable HIV viremia	61 (73.5)	NA	
Renal function and inflammatory variables			
Abnormal albumin/creatinine ratio	7 (10.8)	5 (11.3)	0.787
Abnormal protein/creatinine ratio	13 (20.3)	4 (9.1)	0.207
GFR; median (IQR)	137 (124-150)	125 (118-145)	0.036
GFR < 90 mL/min/1.73m^2	2 (2.4)	0 (0)	0.296
Cystatin C, mg/L; mean (SD)	0.87 (0.15)	0.88 (0.12)	0.881
Cystatin C >1 mg/L	15 (18.4)	9 (18.1)	1
Abnormal beta-2-microglobulin	9 (10.8)	ND	
Abnormal vitamin D	59 (71.1)	ND	
CRP, mg/L; median (IQR)	1.1 (0.6-2.4)	ND	

IQR, interquartile range; BMI, body mass index; ARV, antiretroviral; HAART, highly active antiretroviral therapy; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; SD, standard deviation; GFR, glomerular filtration rate; CRP, C-reactive protein; NA, not applicable; ND, not determined.

(Table 2)

	Beta coefficient	95% CI	p
HAART regimen including a PI	0.032	-0.032 to 0.097	0.317
Age at first HAART (years)	0.002	-0.009 to 0.009	0.995
Undetectable HIV viremia	-0.012	-0.089 to 0.065	0.752
Estimated glomerular filtration rate	-0.001	-0.002 to 0.000	0.027
Beta-2-microglobulin values	0.105	0.044 to 0.165	0.001

HAART, highly active antiretroviral therapy; PI, protease inhibitor; CI, confidence interval