HIV-Associated CKDs in Children and Adolescents

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Introduction: Limited information is available describing the current prevalence of proteinuria and HIV-associated CKDs (HIV-CKDs) in children and adolescents living with HIV and receiving antiretroviral therapy in the United States.

Methods: To address this issue, we performed a retrospective study of children and adolescents living with HIV who received medical care at Children's National Hospital in Washington, DC, between January 2012 and July 2019. Demographic data, clinical parameters (mode of HIV transmission, viral loads, CD4 cell counts, serum creatinine, glomerular filtration rate [GFR], plasma lipid levels, proteinuria, blood pressure, renal biopsies), and medical treatments, all done as a standard of clinical care, were collected and analyzed.

Results: The majority of the 192 patients enrolled were of African descent (88%) and acquired HIV through vertical transmission (97%). The prevalence of all HIV-CKDs was 6%. Of these patients, 39% had intermittent or persistent proteinuria, and 7% percent had proteinuria with a mild decline in GFR (60–80 ml/min per 1.73 m^2), and 6% had a mild decline in GFR without proteinuria. Documented hypertension was present in 6% of the patients, mainly in association with HIV-CKD. Patients with persistent proteinuria (3%) and biopsy-proven HIV-CKD had a slow but constant progression of their renal diseases.

Conclusions: The prevalence of persistent proteinuria and HIV-CKD was lower than that reported in previous studies conducted in the United States. However, intermittent proteinuria, mild reductions in GFR, and progression of established HIV-CKD were common findings in this group of patients with predominantly vertically acquired HIV who were receiving antiretroviral therapy.

Children with vertically acquired HIV are at high risk of developing kidney diseases.[1,2] Before protease inhibitors became available in the United States in 1996, 10% to 15% of HIV-infected children developed HIV-associated nephropathy (HIVAN) and other HIV-CKDs.[1,2] Childhood HIVAN is defined by the presence of proteinuria, often nephrotic syndrome, associated with mesangial hyperplasia, global or focal segmental glomerulosclerosis, and microcystic tubular dilatation leading to kidney enlargement and failure.[1,2] However, the exact prevalence of childhood HIVAN remains unclear because this diagnosis needs to be confirmed with a kidney biopsy, and not all children living with HIV and proteinuria undergo this procedure. During the early years of the HIV/AIDS epidemic, persistent proteinuria developed in approximately 40% of all children living with HIV; often proteinuria was the only symptom preceding the diagnosis of HIVAN.[2] Almost all children with HIVAN died or progressed to end-stage kidney disease (ESKD) within 2 to 3 years of the detection of proteinuria.[1,2] Hypertension and generalized edema were usually seen during the late stages of HIVAN.[1,2] Since 1996, newly introduced antiretroviral drugs have significantly improved the clinical outcomes among children and adolescents living with HIV in the United States.[3–14]

A limited number of studies focused on the renal outcomes of children with vertically acquired HIV

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Table 1. Comparison between children with and without proteinuria living with HIV while undergoing ART

Parameters	HIV-CKD ($n = 12$)	Intermittent proteinuria ($n = 63$)	No proteinuria ($n = 117$)	P value
Mean age in 2012, yr	13.25 ± 3.6	12.3 ± 4.1	10.8 ± 5.0	Kruskal-Wallis
Median IQR, 25%–75%	13 (12–16.5)	12 (10–16)	11 (7–16)	0.096
Mean age in 2019, yr	18.25 ± 3.9	17.63 ± 3.4	15.85 ± 4.9	Kruskal-Wallis
Median IQR, 25%–75%	18.5 (18–20)	18 (16–21)	17 (13–20)[a]	0.036
Ethnicity, %				
African descent	12 (100%)	59 (93.6%)	100 (85.4%)	χ^2 0.66
Hispanic	0 (0.0%)	2 (3.1%)	8 (6.8%)	χ^2 0.40
Caucasian	0 (0.0%)	1 (1.6%)	6 (5.1%)	χ^2 0.37
Asian	0 (0.0%)	1 (1.6%)	3 (2.5%)	χ^2 0.79
Sex, % male	5 (42%)	23 (36.5%)	53 (45%)	χ^2 0.42
Hypertension, %	3 (25%)[a]	5 (8%)	4 (3.5%)	χ^2 0.006[a]
ACEI/ARB use, %	7 (58%)[a]	2 (3%)	0 (0%)	χ^2 0.0001[a]
Mean SCr in 2012, mg/dl	0.81 ± 0.3	0.60 ± 0.18	0.57 ± 0.2	ND
Median SCr in 2012, IQR	0.70 (0.5–1.0)[a]	0.60 (0.5–0.7)	0.50 (0.4–0.7)	Kruskal-Wallis 0.0078[a]
Mean SCr in 2019, mg/dl	2.2 ± 3.6	0.66 ± 0.2	0.65 ± 0.21	ND
Median SCr in 2019, IQR	0.85 (0.75–1.0)[a]	0.6 (0.5–0.8)	0.68 (0.5–0.8)	Kruskal-Wallis 0.0031[a]
Mean eGFR in 2012, ml/min per 1.73 m^2	94 ± 27	124 ± 30	126 ± 28	ND
Median eGFR in 2012, IQR	100 (76–119)[a]	120 (96–129)	120 ± (100–135)	Kruskal-Wallis 0.20
Mean eGFR in 2019, ml/min per 1.73 m^2	81 ± 43	118 ± 31	109.5 ± 30	ND
Median eGFR in 2019 (IQR)	72 (67–114)[a]	121 (87–141)	103 (87–123)	Kruskal-Wallis 0.0053[a]
Viral load copies/ml, n, %[b]				
<50	2 (16%)	21 (33 %)	41 (35%)	χ^2 0.43[a]
50–1000	(8.3%)	13 (23%)	26 (22%)	χ^2 0.52
1001–10,000	3 (25%)	8 (12%)	20 (17%)	χ^2 0.51
10,000	6 (50 %)	21(33%)	30 (25%)	χ^2 0.35
CD4 cells/mm^3, n, %[b]				
<200	2 (16%)	4 (7 %)	3 (2.5%)	ND
200–499	4 (33%)	11 (18%)	22 (18 %)	χ^2 0.43
500–1000	4 (33%)	33 (53 %)	61 (52%)	χ^2 0.44
>1000	2 (16%)	14 (22%)	32 (27%)	0.54

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; ART, antiretroviral therapy; CD4, CD4 cell count; eGFR. estimated glomerular filtration rate; IQR, interquartile range; ND, not done; SCr, serum creatinine.
[a]$P < 0.05$ was considered statistically significant.
[b]Number of patients (%) showing viral load and CD4 cell count values within the corresponding range at the end of the study period. Comparisons between proportions in each group were done using χ^2 tests.
Data are expressed as the mean ± SD of the mean. Viral loads and CD4 cell counts were obtained within 3 months of the end of the study period and reported grouping patients in different categories of HIV-RNA values, and different ranges of CD4 cell counts.

have been conducted since more effective combination ART (cART) became a standard of care treatment for children and adolescents in the United States.[3,4] These studies often included children with short follow-up times, children in whom HIV-CKD developed during the pre-ART era before 1996, or both. In this study, we aimed to evaluate the more recent prevalence of proteinuria and HIV-CKD among children and adolescents living with HIV being treated with cART in the Washington, DC, area.

METHODS

Study Design and Population

We performed a retrospective study of children and adolescents (from birth to 24 years of age) living with HIV who received medical care at Children's National Hospital in Washington, DC, between January 2012 and July 2019, and did not show clinical evidence of kidney disease during the pre-cART era before 1996.

Children's National Hospital Special Immunology Services program has provided testing, care, and treatment for the majority of children and adolescents living with HIV in the Washington, DC, metropolitan area since 1991. Patients were seen in clinic every 3 months as standard of care, and all treatments were provided according to the national Health and Human Services pediatric and adolescent guidelines.[15] The Special Immunology Services program maintains a de-identified clinical database for research purposes. The study was approved by the institutional review board at Children's National Hospital.

Data Collection

Demographic data (race, age, sex, weight, and height) and clinical parameters (mode of HIV transmission, HIV RNA viral loads, CD4 cell counts, serum and urine creatinine, plasma lipid levels, urine analysis, proteinuria, renal biopsies) obtained as a standard of clinical care were collected from the Special Immunology

Services clinical database and included diagnostic and treatment data generated by the nephrology division. We recorded all viral load and CD4 count data throughout the study period, and reported the viral load (number of copies of HIV RNA/ml) and CD4 cell count (expressed as cells per cubic millimeter) obtained within 3 months of the end of the study period. Patients were grouped in different categories of HIV-RNA values, as well as by different ranges of CD4 cell counts, as shown in Table 1. The serum creatinine (SCr) value was measured at Children's National Hospital central laboratory using either the Jaffe method until 2014 or an enzymatic method until the end of the study in 2019. The reference SCr values for both assays in healthy children and adolescents are provided in Supplementary Table S1. Median SCr values were reported in milligrams per deciliter for the first year of study entry in 2012 and at the end of the study in 2019 (Table 1). For patients with proven HIV-CKD, we collected all available measurements of SCr values over the study period. Lipid data included total cholesterol, high-density lipoprotein, low-density lipoprotein, and triglyceride levels, all measured as a standard of care, were obtained within 3 months of the end of the study period. Results were reported in milligrams per deciliter (mg/dl), divided into 3 groups: normal, borderline, and abnormal values (Supplementary Table S2). Abnormal values for total cholesterol, high-density lipoprotein, low-density lipoprotein, and triglycerides were defined as greater than 200 mg/dl, less than 40 mg/dl, greater than 130 mg/dl, and >130 mg/dl, respectively.

Data on the standard of care casual blood pressure measurements; use of angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs), and the results of the renal biopsies were collected. Hypertension was defined as systolic and diastolic blood pressure levels greater than the 95th percentile for height, age, and sex, documented at 3 different consecutive visits at any time point in the study.

All patients were screened for proteinuria by urine dipstick annually according to standard of care. A single urine protein dipstick value of 1+ or greater (>30 mg/dl) was considered abnormal. Proteinuria was quantitated by the random urine protein-to-creatinine ratio (Upr/cr) in mg/mg. The normal Upr/cr ratio in children older than 2 years was considered less than 0.2, whereas the nephrotic range value was greater than 2.0. To calculate the estimated GFR (eGFR), we used either the original Schwartz equation [eGFR = k (0.55 female or 0.7 male) × height (cm)/plasma creatinine (mg/dl)], or the updated Schwartz bedside equation [eGFR = k = 0.413* × height (cm)/plasma creatinine (mg/dl)] in patients 18 years old and younger. The original equation was used whenever the SCr was measured with the Jaffe method, and the updated equation when SCr was measured with the enzymatic method.[16] The Chronic Kidney Disease Epidemiology Collaboration 2009 eGFR formula was used to calculate the GFR in patients older than 18 years.[17]

Statistical Analysis

For the overall data analysis, patients were divided into 3 groups. The control group (no proteinuria group) included patients whose testing showed either trace or negative proteinuria by dipstick throughout the entire study period. The intermittent proteinuria group included all patients whose dipstick testing was abnormal urinary dipsticks (≥1+) or Upr/cr ratio values at any point during the study period but became proteinuria-free in all subsequent measurements. The HIV-CKD group included all patients with persistent proteinuria and/or biopsy proven HIV-CKD and eGFR less than or equal to 90 ml/min per 1.73 m^2.

All datasets were tested for Gaussian distribution with the D'Agostino and Pearson omnibus test for normality. Continuous variables were expressed as means with standard error, and categorical variables were expressed as proportions. Results were expressed as mean (standard error) when normally distributed. The medians with 25th to 75th interquartile ranges (IQR) were used for non-normally distributed variables. Intergroup comparisons were analyzed by 1-way analysis of variance with the Bonferroni multiple-comparison procedures for pairwise comparisons. For nonparametric data, post-test comparisons were performed with the Kruskal-Wallis test. Variables expressed as proportions were analyzed using the χ^2 test whenever appropriate. Correlations were estimated using the Pearson correlation coefficient, r. P values less than 0.05 were considered significant. Calculations of the area under the curve for viral loads were made using the trapezoidal rule, as previously described.[18,19] The statistical analysis was done using the Prism 6 software program (GraphPad Software Inc., La Jolla, CA).

RESULTS

The study analyzed data from 192 children and adolescents from the database. The majority of these patients were of African descent (89%) and acquired HIV-1 through vertical transmission (~97%) (Table 1). The remaining patients acquired HIV mainly through sexual transmission. Eighty-four percent of the patients were born in or after 1996, and 32 patients (16%) were born before 1996.
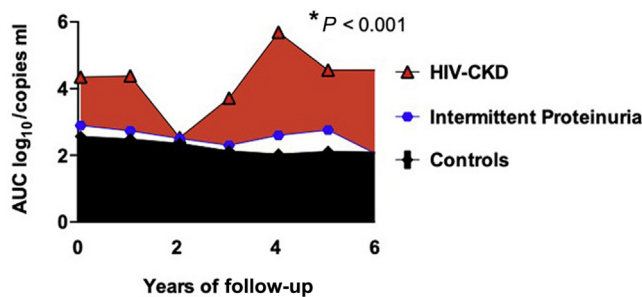
Figure 1. HIV RNA viral load and CD4 cell counts in children and adolescents living with HIV. The study controls were patients with trace or no proteinuria. Patients with intermittent proteinuria (≥1+ by dipstick) are described in the Methods section. HIV–chronic kidney disease (CKD) indicates patients with persistent proteinuria, those with biopsy-proven HIV-CKD, or both, and estimated glomerular filtration rates less than 90 ml/min per 1.73 m^2. The red area shows the HIV RNA viral load values expressed as the area under the curve (AUC) for the corresponding years of follow-up. The black area shows representative CD4 cell counts obtained within 3 months of the end study period. *$P < 0.05$ was considered statistically significant by 1-way analysis of variance (Kruskal-Wallis test).

Figure 2. Estimated glomerular filtration rate (eGFR) decline over-time and viral load in children living with HIV-1. The study controls were patients with trace or no proteinuria; Patients with intermittent proteinuria (≥1+ by dipstick) are described in the Methods section. HIV–chronic kidney disease (CKD) indicates patients with persistent proteinuria, those with biopsy-proven HIV-CKD, or both and eGFR values less than 90 ml/min per 1.73 m^2. The graph shows the median eGFR decline in values between January 2012 and July 2019. The dashed lines indicate the percent number of patients in each group with mean viral load (VL) values less than 10,000 viral copies/ml.

One hundred and seventeen patients (61%) had either trace or negative proteinuria by dipstick testing throughout the study period; 63 patients (33%) had intermittent proteinuria, and 12 patients (6%) had HIV-CKD, defined by the presence of persistent proteinuria without biopsy, and biopsy-proven HIVAN or other HIV-CKD (Table 1). There were no children with clinical evidence of renal disease before the use of cART in 1996. Patients allocated to the control, intermittent proteinuria, and HIV-CKD groups had annual urine testing for proteinuria for an average period of 5.56 ± 0.16 years ($P = 0.028$, Kruskal-Wallis); 6.25 ± 0.17 years; and 6.0 ± 0.5 years (mean ± SD), respectively. Eighty-four percent of the patients had annual urine testing for proteinuria for a period ranging from 5 to 7 years, and the remaining patients for periods of 4 years (4.6%), 3 years (3%), and 2 years (8%), respectively. We did not estimate the prevalence of hematuria in the cohort because not all patients had complete urinalysis records reporting the number of red blood cells in the urine.

A single patient with horizontally acquired HIV had intermittent proteinuria with a mild reduction in eGFR level (<80 ml/min per 1.73 m^2). None of the patients who acquired HIV through horizontal transmission (3%) developed persistent proteinuria or biopsy-proven HIV-CKD. Most patients with documented intermittent proteinuria experienced resolution of proteinuria after 1 or 2 years, and their eGFR increased to normal levels with the resolution of proteinuria in approximately 7% of the cases. The proteinuria decreased in 6 patients (3%) with persistent proteinuria treated with ART, ACEIs, or ARBs; however, it did not resolve completely after 4 or more years of
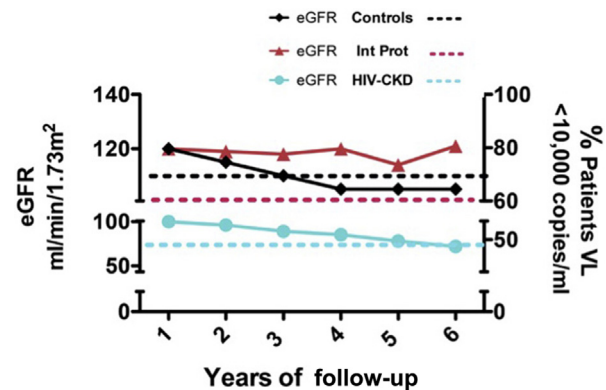
treatment, and the eGFR levels remained slightly reduced (60–80 ml/min per 1.73 m^2). Ten percent of the children (12 of 117) included in the no proteinuria group had a mild reduction in eGFR (60–80 ml/min per 1.73 m^2) over the study period. As expected, the magnitude of proteinuria in the HIV-CKD group was inversely correlated with the eGFR values ($r = 0.35$), and more patients in the HIV-CKD and intermittent proteinuria groups were treated with ACEIs or ARBs relative to the control group (Table1).

The HIV RNA viral load was significantly higher in the HIV-CKD group compared with all other groups (Figure 1). Lower CD4 cell counts were also found in the HIV-CKD group, but this difference was not statistically significant compared with the other groups (Supplementary Figure S1). A secondary analysis comparing the eGFR decline over time with the mean viral load is shown in Figure 2. Briefly, the HIV-CKD group had a clinically significant decline in eGFR and in more patients with a high viral load (Figure 2).

Hypertension developed in 12 children (6%). The prevalence of hypertension was higher in children with HIV-CKD (25%) and intermittent proteinuria (8%) compared with the control group (3.5%; Table1). Children in the HIV-CKD group had higher total cholesterol and low-density lipoprotein levels, as well as hypertriglyceridemia; however, these changes were not statistically significant compared with all other groups (Supplementary Table S2).

The 6 patients with biopsy proven HIV-CKD were of African descent and acquired HIV through vertical transmission. HIVAN developed in 3 patients (Figure 3a–
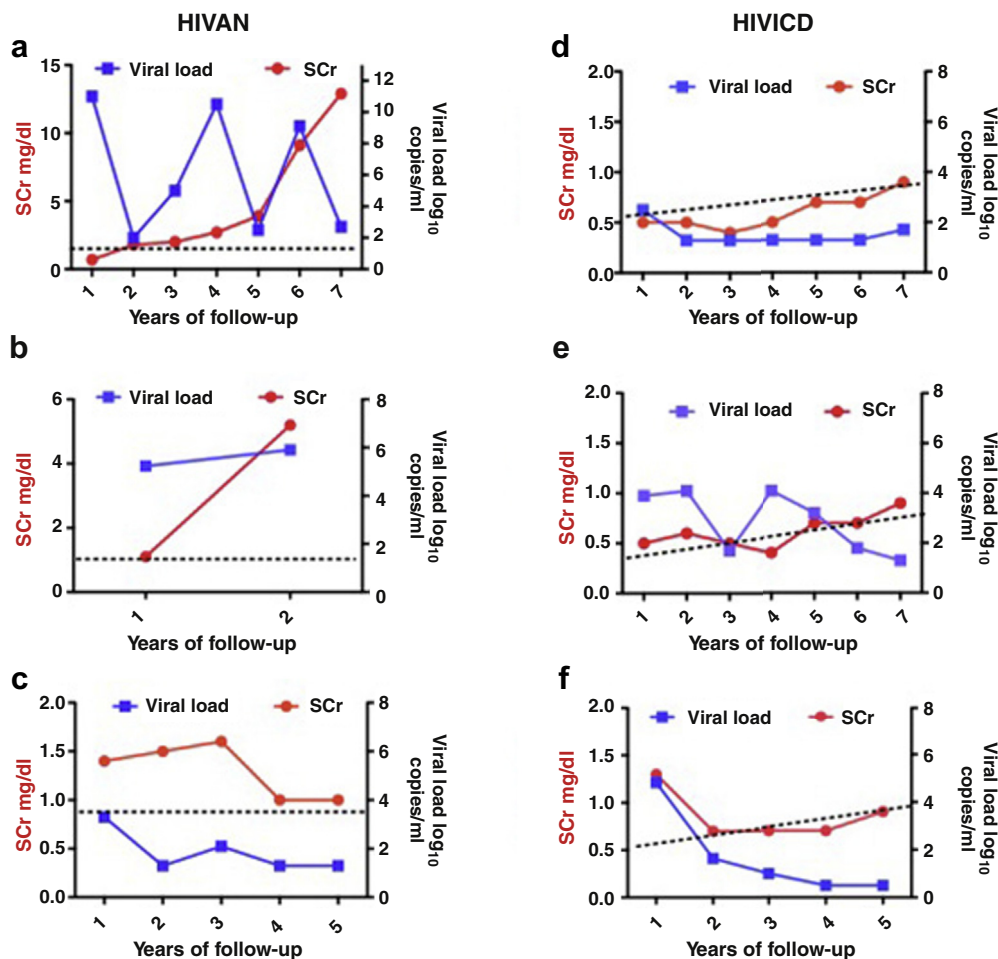
Figure 3. Longitudinal follow-up of children and adolescents with biopsy-proven HIV–chronic kidney disease (HIV-CKD); patients with persistent proteinuria, biopsy-proven HIV-CKD, or both; and estimated glomerular filtration rate less than 90 ml/min per 1.73 m^2. The panels show the relationship between the serum creatinine (SCr) and HIV RNA viral load in patients with HIV-associated nephropathy (HIVAN) (a–c), and those with HIV-associated immune complex kidney diseases (HIVICD) (d–f). Each panel shows 1 representative SCr value and HIV RNA values per year of follow-up. The dashed lines indicate the cutoff normal SCr values adjusted to the age of each patient.

c) and HIV-immune complex kidney diseases developed in 3 additional patients (Figure 3d–f). Two patients with HIV-immune complex kidney disease had significant microscopic hematuria (>10 red blood cells per high-power microscopic field). In contrast, patients with biopsy-proven HIVAN did not have hematuria. Nephrotic-range proteinuria developed in all patients. Over time, the proteinuria decreased in 5 patients treated with ART and ACEIs or ARBs. However, none achieved a complete remission of the proteinuria by the end of the study period. One patient with biopsy-proven HIVAN had persistent nephrotic-range proteinuria for 5 years while undergoing cART and ACEI treatment, and developed ESKD requiring dialysis and a kidney transplant (Figure 3a). Another child with biopsy-proven HIVAN had a poor response or adherence to ART and died within 2 years with a GFR less than 15 ml/min per 1.73 m^2 (Figure 3b). A third patient with biopsy-proven HIVAN had persistent nephrotic range proteinuria for 6 years and completed the study with a mild reduction in

GFR (70 ml/min per 1.73 m^2) (Figure 3c). Two patients with biopsy-proven HIV-associated membranoproliferative glomerulonephritis (Figure 3d,e) had a decreased viral load in response to ART for more than 5 years; they completed the study period with proteinuria and a mild reduction in eGFR levels of 66 and 70 ml/min per 1.73 m^2, respectively. Finally, another patient with biopsy-proven HIV–immune-complex glomerulonephritis (Figure 3f) had significant improvement in proteinuria and viral load, and the eGFR increased from 44 to 84 ml/min per 1.73 m^2. However, during the second year, this patient developed nephrotic range proteinuria, and completed the study years later with a similar mild reduction in GFR and moderate proteinuria, despite the low viral load (Figure 3f).

DISCUSSION

Our study describes the prevalence and clinical outcome of proteinuria and HIV-CKD in a large group

of children and adolescents undergoing cART who received medical care in the Washington, DC, area between January 2012 and July 2019. Overall, the prevalence of persistent proteinuria and biopsy-proven HIVAN or other HIV-CKD was lower than that documented in previous observational cohort studies done in the United States.[3,4] However, periods of intermittent proteinuria or a mild reduction in GFR were still commonly observed in our cohort. Moreover, we report a slow but continuous progression of the renal diseases of children and adolescents with biopsy-proven HIV-CKD despite combination ART and overall low HIV RNA viremia.

Two large pediatric studies in the United States explored the prevalence and outcome of renal diseases in children living with HIV during the cART era.[3,4] These studies were published more than 10 years ago and included a significant number of patients with HIV-CKD acquired during the pre-cART era.[3,4] In our study, there were no children with clinical evidence of renal disease before the use of cART in 1996. One large multicenter US study included 3451 children and youths (<21 years of age) living with HIV who were followed up for a minimum period of 30 months before 2005.[3] This study by Andiman *et al.*[3] included a larger proportion of Caucasian (13.5%) and Hispanic children (28.4%) compared with our study and considered single trace measurements of proteinuria by urinary dipstick as an indicator of HIV-renal dysfunction.[3] Approximately 22% of patients in this large cohort had proteinuria or an elevated SCr, and elevated SCr was more common than persistent proteinuria (15% vs. 8%) in this cohort of children and youths. Given the different experimental designs, study populations, and criteria used to define renal dysfunction, it is challenging to compare the findings of this study with our data. Nonetheless, our study shows that trace proteinuria is not a reliable indicator to predict CKD, and that single measurements of proteinuria cannot be used to estimate the prevalence of CKD in children and adolescents living with HIV.

The second large US study[4] described the renal outcome of 286 children living with HIV in Miami with follow-up from 1989 to 2007. This study by Chaparro *et al.* reported a 33% prevalence of proteinuria (defined by Upr/cr ratio >0.2), including 11.2 % with nephrotic range proteinuria (Up/cr ratio ≥1).[4] The majority of children in this study were of African descent (∼85%), and the average follow up period was 5.6 ± 0.1 years. Approximately 50% of the patients with nephrotic-range proteinuria died or developed ESKD during the study period, and 4.5 % of the patients with intermediate proteinuria (Up/cr >0.2 <1.0) progressed to nephrotic-range proteinuria. The majority (63%) of children with intermediate proteinuria had resolution of proteinuria by the end of the study period. This study included a significant number of patients in whom HIV-CKD and other AIDS-related illnesses developed during the pre-cART era.[4] Our findings in a cohort of predominately children and adolescents of African descent living with HIV underscore the progress that has been made in the treatment of such patients during the past 20 years and support the notion that cART is effective in preventing renal diseases in children and adolescents with vertically acquired HIV.

Several challenges remain for the treatment of children and adolescents living with HIV, including those related to long-term adherence, persistence of viral reservoirs, and inflammation resulting in kidney damage.[20–23] Childhood HIVAN is seen predominantly in children of African descent with elevated HIV viral loads and is characterized by persistent proteinuria, which in the early stages is not associated with edema, reduced GFR or hypertension, or both.[1,2,24] These clinical changes are associated with mesangial hyperplasia, focal segmental or collapsing glomerulosclerosis, multicystic tubular dilatation, and renal enlargement.[1,2] cART has been shown to decrease the prevalence of collapsing glomerulopathy in adults.[8,9,13,25] In general, collapsing glomerulopathy has a worse outcome but is less frequently seen in children with HIVAN compared with adults.[1,2,4] Nonetheless, our findings suggest that once the typical renal histologic features of HIVAN are established in children or adolescents, ongoing cART cannot prevent its long-term progression, particularly in non-adherent children and adolescents living with HIV with persistent HIV viremia.

Polymorphisms in apolipoprotein L1 (*APOL1*) account for the majority of the excess risk for developing HIVAN in adult populations of African descent.[26–28] More specifically, those who are homozygous for the G1 or G2 renal risk alleles (or G1/G2 compound heterozygotes) have a 30-fold increased risk of HIVAN.[28,29] The *APOL1* risk alleles have also been associated with the pathogenesis of hypertension-induced CKD in African Americans.[26] In our cohort of predominately children and adolescents of African descent, lipid changes and hypertension were observed in the HIV-CKD group. This group also included the majority of patients treated with ACEIs or ARBs. Consequently, the low prevalence of hypertension observed in the control and intermittent proteinuria groups cannot be accounted for those receiving anti-proteinuric and anti-hypertensive therapy. Furthermore, the role of the *APOL1* risk alleles in young children with hypertension, HIVAN, or both is not well defined because previous studies included a limited number of young children with HIVAN, hypertension, or both. In fact, a single US study of older children and adolescents with perinatal HIV infection concluded that patients carrying the high-risk *APOL1* alleles have a 3.5-

fold increased odds of developing CKD,[27] which is less than the risk predicted for young adults. However, this study included in its definition of CKD children with proteinuria (dipstick ≥1+) for more than 6 months on 2 or more occasions; in our study, children with persistent proteinuria (dipstick ≥1+) for more than 6 months on 2 or more occasions did not necessarily develop CKD. Nonetheless, 6% of the patient in our cohort had a mild reduction in GFR despite having no significant proteinuria. Therefore, it is possible to speculate that these patients, together with those who had a slow but continuous progression of HIV-CKD while undergoing cART, may carry the *APOL1* risk alleles. Further studies are warranted to explore this possibility and to determine how the *APOL1* risk alleles may affect the renal outcome of children and adolescents born with HIV.

Many patients in our study, as well as those monitored in Miami,[4] had improvement in proteinuria while undergoing cART over time. In adults living with HIV, the addition of ACEIs or ARBs to ongoing cART positively alters the decline in renal function.[30,31] This benefit was not so evident in our study, probably because these agents were used mainly in children and adolescents with established HIV-CKD. In adult patients with a low CD4 cell count and high viral load, cART has been shown to be often less effective in preventing the progression of HIVAN[8,9,13,25,32] compared with patients who have low HIV viral load and normal CD4 counts at the time of HIVAN diagnosis.[8,9,25,32] In our cohort of children and adolescents with vertically acquired HIV, ACEIs and ARBs were introduced relatively late in the course of the HIV-CKD and could be potentially considered at earlier stages.

Interestingly, a single adult study[32] and 1 case report in a child[33] suggest that HIVAN can develop in people living with HIV even with supressed HIV viral load. Because single viral load measurements are not representative of the long-term viral suppression, we estimated the area under the curve for the viral load of patients in our study throughout several years and compared them among patients in whom HIV-CKD developed and control patients without renal disease and proteinuria. Our findings clearly indicate a higher prevalence of persistent HIV viremia in children and adolescents with HIV-CKD and suggest that nonadherence to ART plays an important role in precipitating HIVAN and other HIV-CKDs in children and adolescents living with HIV.

Our study has several limitations, including its retrospective nature, single center, use of 2 different methods to measure SCr, and the standard of care assessment of proteinuria by urinary dipsticks, not a quantitative method to follow the progression of proteinuria. In addition, we did not rule out the presence of orthostatic proteinuria. Nonetheless, for 84% of the patients we had annual records of proteinuria for 5 to 7 years. Furthermore, in all relevant cases, proteinuria was quantified by Upr/cr ratios. The presence of proteinuria for several years associated with mild changes in eGFR is not consistent with the diagnosis of orthostatic proteinuria. We did not have systematic measurements and documentation of patients' ART adherence and did not analyze the composition of ART; therefore, we did not assess the potential renal cytotoxic effects of certain antiretroviral drugs, such as tenofovir and cobicistat, and other drugs capable of producing proteinuria and changes in GFR.[34,35] All these factors, in addition to the *APOL1* risk alleles, should be taken into consideration in planning future observational and treatment studies. Together our data suggest that children and adolescents living with HIV, who have proteinuria (≥1+ by dipstick) or a mild reduction in GFR, should be monitored more closely to assess their viral load status and initiate the corresponding treatment as soon as possible.

In conclusion, cART is associated with a low prevalence of persistent proteinuria and HIV-CKD in children and adolescents living with HIV in the Washington, DC, area compared with earlier US studies. However, a significant number of children and adolescents living with HIV on ART continue to demonstrate intermittent or persistent proteinuria and have mild reductions in GFR levels. Moreover, higher viral loads are seen in children and adolescents with biopsy-proven HIVAN or other HIV-CKDs, and these renal diseases continue to progress slowly throughout the years despite ongoing cART. In summary, our findings underscore the need to continue closely monitoring renal outcomes in children and adolescents living with HIV in the United States.

DISCLOSURE

ACKNOWLEDGMENTS

AVAILABILITY OF DATA AND MATERIAL

The Special Immunology Services program at Children's National Hospital provides testing, care and treatment for the majority of children and adolescents living with HIV in

metropolitan Washington DC since 1991, and maintains IRB approved de-identified clinical database for research purposes.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Reference serum creatinine values for the Jaffe's and enzymatic methods.

Table S2. Comparison of the lipid profile in children and adolescents living with HIV on ART with and without proteinuria or HIV-CKD.

Figure S1. CD4 cell counts in children and adolescents living with HIV.

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