Microalbuminuria in Perinatally HIV-Infected Children and Adolescents in the United States

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Background. The kidney is a common target for human immunodeficiency virus (HIV), making renal disease a common noninfectious complication of HIV. Microalbuminuria is an important marker that can detect early renal damage. Timely detection of microalbuminuria is important to initiate renal management and stop the progression of renal dysfunction in people with HIV. Limited data are available about renal abnormalities in people with perinatal HIV infection. The objective of this study was to determine the prevalence of microalbuminuria in a cohort of perinatally HIV-infected children and young adults receiving combination antiretroviral therapy and investigate correlations between microalbuminuria and clinical and laboratory findings.

Methods. This was a retrospective study of 71 patients with HIV followed in an urban pediatric HIV clinic in Houston, Texas, between October 2007 and August 2016. Demographic, clinical, and laboratory data were compared between subjects with persistent microalbuminuria (PM) and those without. PM is defined as a microalbumin-to-creatinine ratio ≥30 mg/g on at least 2 occasions separated by at least 1 month.

Results. Sixteen of 71 patients (23%) met the definition of PM. In univariate analysis, patients with PM had significantly higher CD8$^+$ T-cell activation and lower CD4$^+$ T-cell nadir. Multivariate analysis demonstrated increased microalbuminuria to be independently associated with older age and CD8$^+$ T-cell activation measured as CD8$^+$HLA-DR$^+$ T-cell percentage.

Conclusions. Older age and increased activation of CD8$^+$HLA-DR$^+$ on T cells correlate with presence of microalbuminuria in this cohort of HIV-infected patients.

Keywords. HIV; immune activation; kidney; microalbuminuria; perinatal.

The rate of perinatal human immunodeficiency virus (HIV) disease has significantly declined in the United States (US) due to the implementation of preventive strategies that decreased the risk of mother-to-child transmission to approximately 1% [1]. With the introduction of combination antiretroviral therapy (cART) in the late 1990s, perinatal HIV became a manageable chronic disease, and patient management evolved from targeting opportunistic infections to management of HIV-related morbidities. Among these, renal dysfunction is one of the most important ones [2]. Known mechanisms of renal dysfunction in HIV patients are diverse, including HIV-associated nephropathy that is related to active replication of the virus itself within renal cells, immune-complex kidney disease, dysfunction secondary to prolonged exposure to antiretroviral medications such as tenofovir disoproxil fumarate (TDF), and other comorbidities including hypertension, diabetes, and hepatitis C coinfection. Apolipoprotein-L1 (*APOL1*) risk variants G1 and G2 increase the risk of chronic kidney disease (CKD), including HIV-related CKD, among African Americans. Studies investigating renal dysfunction in perinatally HIV-infected patients have used different renal parameters. Screening for microalbuminuria, a more sensitive marker of renal dysfunction than proteinuria, is encouraged in the context of HIV to detect early kidney diseases in asymptomatic patients and improve renal outcomes [3, 4].

There are few studies addressing epidemiology and risk factors for microalbuminuria in adolescents and children living with HIV in the US [5]. One demonstrated persistent renal abnormalities in one-fifth of a pediatric US cohort [3]. Worsening of glomerular filtration rate (GFR) in Ethiopian children with HIV while receiving cART has been described [6]. Prevalence of microalbuminuria in developing countries has been reported to be at similar rates or higher than in the US [7–9]. Mortality from renal failure in children living with HIV increased in the mid-2000s [10].

Microalbuminuria is strongly associated with HIV disease progression and adult HIV mortality but also with increased risk of cardiovascular disease and mortality in the general population [11–13]. Increased markers of immune activation are associated with advanced HIV disease [14, 15].

We present the prevalence of microalbuminuria in a cohort of patients with perinatally acquired HIV on cART. We investigated associations of microalbuminuria with demographic and clinical and laboratory outcomes including markers of immune activation, CD4 counts and viral loads.

METHODS

Patients and Data Collection

A retrospective study of a cohort of HIV-positive pediatric and adolescent subjects spanning October 2007 to August 2016 is reported. Subjects received medical care from the pediatric HIV clinic at the University of Texas McGovern Medical School (Houston, Texas). Medical care of patients was independent of this study. Institutional review board approval was obtained. Inclusion criteria comprised perinatal transmission of HIV and having at least 2 urine microalbumin-to-creatinine ratio (MC) measurements during the study period. As a standard of care, patients had serum creatinine and MC checked annually unless clinical status required more frequent measurements, as well as HIV viral load (VL) and lymphocyte subsets every 3–6 months. The data were incomplete due to patients' lack of adherence with clinic visits. According to the corresponding laboratory reference, MC was abnormal if it was ≥30 mg/g. Data were extracted from electronic medical records. When serum creatinine was available, we calculated GFR using the creatinine-based "bedside Schwartz" equation for subjects 1–18 years old and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation (2009) for participants aged >18 years [16].

Analyses

Data were first analyzed cross-sectionally. Subjects were divided into 2 groups based on the results of the MC measurements (Figure 1). Patients with abnormally elevated MC values (≥30 µg/mg) on at least 2 occasions separated by at least 1 month were classified as the persistent microalbuminuria (PM) group [17]. Patients with no or only 1 abnormal MC value were placed in the nonpersistent microalbuminuria (NPM) group. Demographic, laboratory, and clinical parameters were compared between the 2 groups using univariate analysis (Table 1). Due to deviation from normality, most variables are presented as median with interquartile range (IQR) and compared using Mann-Whitney U tests for continuous variables. Categorical variables were tested using the Pearson χ^2 test or Fisher exact test. Using a multivariate analysis, we calculated incidence rate ratios across 3 age groups: <11, 11–17, and ≥18 years. A P value <.05 was considered statistically significant. Analyses were done using Stata version 15 software (StataCorp, College Station, Texas).

RESULTS

Of the 149 patients with HIV seen in our clinic, 71 met the eligibility criteria and are included in this analysis (Figure 1). The 71 patients had a total of 331 MC results. The mean, median, and standard deviation of number of MC results per patient was 5.4, 5, and 2.8, respectively. Sixteen of the 71 patients (23%) met our definition of persistent microalbuminuria (PM group), while 55 of the 71 patients (77%) fit the under the NPM group (Figure 1). The median of number of MC results per patient in the PM group and the NPM group was 8 (IQR, 5–10) and 4 (IQR, 2–6), respectively. Demographic, clinical, and laboratory data are presented in Table 1. The 71 patients were followed in our clinic for a median of 55 months (IQR, 23–75 months). Age in this cohort ranged between 14 months and 25 years. The median age of subjects for both groups was comparable (12.2 and 12.7 years in NPM and PM respectively). There were more females in the cohort (57%), and non-Hispanic Black race was the most common in both groups. The median of VL for the PM group was higher than for the NPM group; however, this was not statistically significant. Median CD4$^+$ T-cell percentage was comparable in
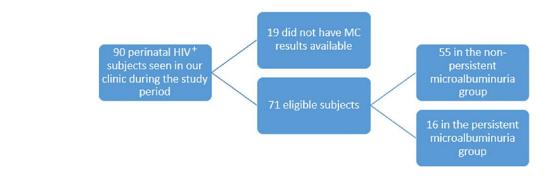

Figure 1. Study flowchart. Abbreviations: HIV$^+$, human immunodeficiency virus positive; MC, microalbumin-to-creatinine ratio.

Table 1. Demographic and Laboratory Characteristics of Patients

Characteristics	NPM Group	PM Group	P Value
No. of patients (%)	55 (77)	16 (23)	
Age at first albumin-creatinine ratio, y	12.2 (7.6–15.2)	12.7 (8.2–14.5)	.945
Female sex, %	49	81	.025
Black race, %	67	75	.76
Albumin-creatinine ratio, mg/g	4.6 (3.2–7.7)	35.6 (10.99–77.2)	<.01
Viral load, RNA copies/mL	84 (34–1600)	382 (48–8945)	.154
CD4$^+$ %	32 (28–40)	32 (23–38)	.393
CD8$^+$ %	38 (28–48)	42 (37–59)	.048
CD8$^+$CD38$^+$ T-cell %	12 (9–18)	18 (14–30)	.024
CD8$^+$HLA-DR$^+$ T-cell %	7 (3–12)	14 (6–23)	.011
CD4$^+$ T-cell % nadir	22 (16–29)	18 (11–22)	.016
Cumulative viral load^a, log$_{10}$	7.6 (7.3–8.1)	8 (7.4–8.3)	.065
% of patients who received TDF	38	38	.757
Lowest eGFR^b, mL/min/1.73 m^2	90 (83–106)	82 (78–101)	.118
Undetectable viral load, %	46	25	.163

Data are presented as median (interquartile range) unless otherwise indicated. Shown are the demographic, immunologic, viral, and renal function data for this cohort, comparing subjects with PM and NPM. For outcomes where individual patients had repeated measures, the median of all measurements was calculated and the median of these medians is presented for each group. Lowest level of detection for VL is 50 RNA copies/mL.
Abbreviations: eGFR, estimated glomerular filtration rate; NPM, nonpersistent microalbuminuria; PM, persistent microalbuminuria; TDF, tenofovir disoproxil fumarate.
^{a}Cumulative viral load is the total amount of viral replication measured over the study period [18].
^{b}Lowest eGFR indicates the lowest eGFR the patient had during the study period.

both groups, but median of the nadir CD4$^+$ T-cell percentage was significantly lower in the PM group in the univariate analysis. Twenty-seven patients (38%) in the cohort received TDF before the first MC measurement. The duration of TDF exposure ranged between 19 and 3157 days. There were more undetectable VL measurements among patients in the NPM group.

Our multivariate analysis showed that the incidence rate ratio of microalbuminuria was significantly higher in patients aged ≥18 years compared with those <11 years of age (1.98 vs 1.17, respectively; P = .027). The same pattern of age discrimination applied for patients with higher CD8$^+$HLA-DR$^+$ T cells (0.21/1000 patient-days at CD8$^+$HLA-DR$^+$ T-cell percentage of 40 vs 0.07 at CD8$^+$HLA-DR$^+$ T-cell percentage of 1; P = .022). There was overall a trend toward an increase of microalbuminuria's incidence rate ratio in female patients (Table 2).

DISCUSSION

We demonstrate that approximately one-quarter of a population of participants with perinatally acquired HIV cared for by an HIV specialty clinic and receiving cART had compromised renal function. Compromised renal function was associated with age and poorer HIV clinical status and was strongly associated with increased activation of HLA-DR$^+$CD8$^+$ lymphocytes.

Table 2. Multivariate Analysis

Characteristic	Incidence Rate Ratio (95% CI)	P Value
Age, y		
<11	1.17 (.73–1.88)	.514
11–17	Ref	Ref
≥18	1.98 (1.08–3.61)	**.027**
Race		
Black	0.92 (.42–2.01)	.835
Not Black	Ref	Ref
Male sex	0.43 (.18–1.03)	.058
CD4 T-cell %	1.0 (.97–1.03)	.882
CD8 T-cell %	0.97 (.93–1.01)	.140
CD8$^+$CD38$^+$ T-cell %	1.0 (.97–1.03)	.992
CD8$^+$HLA-DR$^+$ T-cell %	1.03 (1.0–1.05)	**.022**
Viral load	1.0 (1.0–1.0)	.159
Days of TDF intake	1.0 (1.0–1.0)	.111
eGFR	0.99 (.98–1.0)	.013

P value in bold represent signficant correlation of microalbuminuria with specific variables.
Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; TDF, tenofovir disoproxil fumarate.

Our finding of a 23% prevalence of renal impairment is comparable with other studies. In a US cohort of children and adolescents receiving cART, frequency of persistent proteinuria is 8% [3]. In several non-US cohorts, the prevalence of microalbuminuria ranged between 18% and 29% [7–9]. An Indian study showed an incidence of microalbuminuria at approximately 20% regardless if patients were receiving cART. More recently, lower prevalence of microalbuminuria at 8.5% was reported in perinatally infected South African subjects mostly with well-controlled HIV disease [19].

Improvement of proteinuria following initiation of cART has been reported [20]. A Nigerian study, however, did not find an association between renal disease and cART type or duration [21]. Another Indian study showed a prevalence of albuminuria at 26% in children living with HIV [22]. In an Ethiopian study, blood urea nitrogen and GFR worsened following 6 months of cART [6]. Clearly, renal dysfunction remains a consequence of pediatric HIV infection despite widespread use of cART. It can, at best, be partially ameliorated by cART. However, details of the association of renal compromise with clinical care, including cART, cannot be resolved from the available studies because of differences in methodologies.

We did not find a statistically significant association between duration of TDF exposure and microalbuminuria. The incidence of TDF-associated renal toxicity is variable across studies [23–25]. In a cohort of Italian children and adolescents with perinatally acquired HIV treated with a TDF-containing cART regimen, proteinuria was not detected [24]. A meta-analysis linked chronic nephrotoxicity with TDF use in adult patients to the duration of its use for 4 years or more [26]. In our cohort, 11 patients in our cohort received TDF for >4 years, and among these, 4 patients had PM. Larger studies and further

studies will be required to reach definitive conclusions. Furthermore, a large biopsy-documented TDF nephrotoxicity study showed that nonalbumin proteinuria is a significant feature of the disease, making MC measurement a less reliable marker in detecting early TDF renal toxicity [27]. There is more evidence supporting the substitution of TDF with tenofovir alafenamide to preserve renal function.

In our study, older patients (≥18 years) were more likely to develop PM. Black race in this study did not associate with the increased occurrence of microalbuminuria. Consistent with other studies, lower CD4+ T-cell nadir was more frequent in the PM group [28].

An increase in peripheral blood CD8+HLA-DR+ T cells associated significantly with microalbuminuria. CD8+HLA-DR+ T cells are markers of immune activation and HIV disease progression. The association between immune activation markers and microalbuminuria has not been well studied. One adult study found an association between proteinuria and increased expression of both HLA-DR and CD38 on CD8+ T cells [29]. The association between increased expression of HLA-DR on CD8+ T cells and microalbuminuria may suggest that albuminuria may be a surrogate marker of advanced HIV disease. Increased coexpression of HLA-DR and CD38 represents a well-established activation phenotype of CD8+ T cells documented in acute viral infections including HIV [30]. The CD8+ T-cell activation observed in this study differs from that commonly observed in acute viral infections in that the level and significance of HLA-DR increase is greater than that of CD38 [31–33]. In some instances, an increase in the CD8+HLA-DR+ phenotype is associated with increased T-regulatory cells [34].

Our study has several limitations. The study is retrospective. More extensive and longitudinal studies are needed to draw clinical conclusions. Many patients seen in our practice were excluded due to unavailable MC measurements. The number of available MC results available varied between the PM and NPM groups (8 vs 4), with a sizeable fraction of the NPM group having only 2 results. Thus, there was considerably less chance of picking up abnormal MC results in the NPM group. The database linked to this study lacks important variables, such as comorbidities of participants (eg, hypertension, diabetes, baseline renal disease) and nephrotoxic medication history other than TDF. MC measurement timing depended on patients' adherence to their scheduled clinic visits (every 3–4 months), making the interval between MC measurements per patient highly variable.

In summary, microalbuminuria is present in about one-quarter of a cohort of US children and adolescents with HIV. MC testing is an important screening tool to detect early renal disease in children and adolescents with perinatally acquired HIV. Increasing age, lower CD4+ nadir, and increased CD8+HLA-DR+ T cells are associated with microalbuminuria. Given its association with an immune activation maker,

microalbuminuria may have the potential to predict advanced HIV disease.

Notes

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