

Persistent proteinuria as an indicator of renal disease in HIV-infected children

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Abstract

Background Persistent proteinuria (microalbuminuria) has been reported to be a precursor of HIV-related renal disease. Screening allows for early management in order to prevent the progression of renal disease and decrease morbidity and mortality associated with chronic kidney disease in HIV. Several studies have been done on renal manifestation in HIV-infected children from American and African regions, but similar studies from Asia are lacking.

Objective To determine the prevalence of persistent proteinuria in HIV-positive children on antiretroviral therapy (ARV) in Dr. Soetomo Hospital, Surabaya.

Methods A cross-sectional study on children with HIV and treated with highly active antiretroviral therapy (HARRT) was done from August 2014 to February 2015. Microalbuminuria was measured by the ratio of urine albumin to creatinine (ACR), while proteinuria was measured by dipstick. Measurements were performed 3 times in 4-8 weeks. All subjects underwent complete evaluation of blood tests, serum creatinine, blood urea nitrogen (BUN), CD4 counts, and urinalysis. Data were analyzed using Chi-square and logistic regression tests.

Results Of 38 children on HARRT enrolled in this study, 2 subjects developed acute kidney injury (AKI), 4 subjects were suspected to have urinary tract infection (UTI), and 1 subject was suspected to have urinary tract stones. The prevalence of persistent microalbuminuria was 2.6%. There was no correlation between immunological status, WHO clinical stage, or duration of ARV and the incidence of persistent proteinuria ($P > 0.05$).

Conclusion The prevalence of persistent proteinuria is lower in younger HIV-infected children at a non-advanced stage and HIV-infected children with normal immunological status who are on HAART. We provide baseline data on the renal conditions of HIV-infected children in the era of HAART, before tenofovir is increasingly used as an antiretroviral therapy regimen in

Indonesia. [Paediatr Indones. 2016;56:343-9. doi: 10.14238/pi56.4.2016.343-9].

Keywords: HIV-infected children, proteinuria, CD4, HIV-associated chronic nephropathy

Highly active antiretroviral therapy (HAART) has been an effective means of suppressing viral replication. Thus, the number of HIV patients who are able to survive is increasing. Nevertheless, patients with human immunodeficiency virus (HIV) have an increased risk of diseases that affect multi-organ systems, including kidney disease.^{1,2} Renal abnormalities in HIV patients consist of chronic HIV-associated nephropathy (HIV-associated chronic nephropathy), including HIV-associated nephropathy (HIVAN), HIV-related immune complex disease

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(HIVICK), as well as thrombotic microangiopathy (TMA-HIV) and diseases related to opportunistic infections. HAART has been beneficial in slowing renal involvement and the rapid progression to end-stage renal disease. However, long-term exposure to HAART, in particular tenofovir disoproxil fumarate (TDF), is potentially nephrotoxic, and has been associated with nephropathy secondary to antiretroviral therapy (ARV).³⁻⁵ Several studies regarding renal manifestation in children from American and African regions have reported HIVAN to be more common in Black races and less common among other races, including Asians.^{3,6} A study in India reported that 10.7% of HIV-infected, HAART-naive children had transient proteinuria.⁷

Proteinuria is an indicator of the earliest type of kidney diseases, and reflects the level of damage to the permeability of the glomerular basement membrane or tubule reabsorption.⁸ HIV patients with persistent proteinuria often have glomerular abnormalities on renal biopsy.⁹ HIV-related kidney disease may be asymptomatic in its early stages. Hence, it is recommended to assess for the presence of renal involvement at the time of initial diagnosis.¹⁰ To date, there has been no published data on the prevalence of persistent proteinuria as a simple screening tool to detect renal involvement in children with HIV in Indonesia.

The purpose of this study was to determine the prevalence of persistent proteinuria in children with HIV infection and to analyze for possible relationships between CD4 cell count, *World Health Organization* (WHO) clinical stage of HIV, and persistent proteinuria in HIV-infected children who are on ARV therapy.

Methods

This study was approved by the Ethics Committee of Dr. Soetomo Hospital, Surabaya Indonesia. A cross-sectional study was carried out in the *Unit Perawatan Intermediet Penyakit Infeksi/UPIPI* (Intermediate Care Unit of Infectious Diseases) of Dr. Soetomo Hospital from August 2014 to February 2015. Subjects were taken by consecutive sampling from all HIV-infected children who did not suffer from acute illness, urinary tract infection (UTI), glucosuria, uncontrolled

hypertension, heart failure, and history of chronic kidney disease (CKD) related to other diseases. The independent variables were CD4, clinical stage, and duration of ARV, while persistent proteinuria was the dependent variable.

Serial observations were done during three visits at intervals of 4-8 weeks. On the first visit, subjects had their blood drawn for a complete blood count, renal function (BUN, serum creatinine), and CD4 cell count. They also underwent urine collection for urinalysis and albumin-creatinine ratio (ACR). On the second and third visits, only urinalysis and urine ACR were performed. Persistent proteinuria was defined as a positive test result in at least 2 out of 3 times for both the quantitative test and urinalysis dipstick qualitative method. The minimum required sample size for this study was 28 subjects.

Results

During the study period, 42 children were enrolled in the study, but 4 children dropped out during the observation period. Hence the total sample size was 38 children. The characteristics of subjects are shown in **Table 1**.

Two subjects had AKI with eGFR values of 28.4 and 47.55 mL/min/1.73 m², respectively. The first subject who suffered from AKI was a 61 month old child, with normal immunological status, and had been on first-line ARV regimen for 25 months duration. The second subject was younger (29 months of age), with severe immunological status, and had been on first-line regimen therapy for 2 months. **Table 2** shows the relationships between persistent proteinuria and immunological classification, duration of ARV, adherence to ARV therapy, as well as age. Urinalysis revealed that 4 subjects had suspected UTI, based on leukocytes >5 cells/HPF, and 1 subject had suspected urinary stones (urolithiasis), based on proteinuria and microscopic hematuria with oxalate crystals.

A quantitative test for proteinuria showed 7 subjects with microalbuminuria. Positive proteinuria results on the second and third examinations were found in 5 out of 38 subjects. The prevalence of persistent proteinuria in this study was 2.6% (1/38 subject).

Table 1. Characteristics of subjects

| Characteristics | Frequency (n=38) |
|---|------------------|
| Gender, n | |
| Male | 24 |
| Female | 14 |
| Mean age, years (SD) | 6.8 (3.4) |
| Age groups, n | |
| <5 years | 10 |
| 5-10 years | 22 |
| >10 years | 6 |
| Nutritional status, n | |
| Well-nourished | 22 |
| Moderate malnutrition | 16 |
| Severe malnutrition | - |
| Short stature, n | |
| Yes | 9 |
| No | 29 |
| WHO clinical stage pre-therapy, n | |
| Stage I | 2 |
| Stage II | - |
| Stage III | 25 |
| Stage IV | 11 |
| WHO clinical stage during study, n | |
| Stage I | 32 |
| Stage II | - |
| Stage III | 6 |
| Stage IV | - |
| WHO immunological classification, n | |
| None or insignificant | 25 |
| Mild | 5 |
| Advanced | 3 |
| Severe | 5 |
| Mean absolute CD4, cells/mm ³ (SD) | 757.8 (346.4) |
| Regimens of ARV | |
| First-line, n | |
| AZT/3TC/NVP | 23 |
| AZT/3TC/EFV | 8 |
| D4T/3TC/NVP | 2 |
| Second-line, n | |
| DDI/3TC/Aluvia | 3 |
| ABC/3TC/Aluvia | 1 |
| D4T/3TC/Aluvia | 1 |
| Mean duration of ARV, years (SD) | 4.1 (3.2) |
| Duration of ARV therapy, n | |
| 0 - 3 months | 3 |
| 3 - 12 months | 3 |
| 12 - 60 months | 17 |
| > 60 months | 15 |
| Adherence to ARV, n | |
| Yes | 35 |
| No | 3 |

Discussion

Initially, 42 children enrolled in the study, 4 children dropped out (9.5%). Subjects were predominantly male, similar to several other studies.^{11,12} The mean age of the study subjects was 6.8 (SD 3.4) years. Age

is an important factor as 95% of HIV cases in children occur through vertical transmission from infected mothers. As such, patient age is reflective of disease duration.¹³

With regards to nutritional status, 42.1% of subjects were malnourished, consistent with another study.¹⁴ Malnutrition in HIV patients has been correlated with advanced WHO clinical stage and decreased % of CD4.¹⁵ Growth hormone deficiency and insensitivity are found in children with HIV and are suspected to be the cause of growth failure in HIV patients.¹⁶ In our study, 9 subjects had short stature.

Most of our subjects met the WHO classification of clinical stage I, and 15.8% had clinical stage III. Another study had a majority of subjects in WHO clinical stages I and II, and a zero prevalence of microalbuminuria.¹⁷ A retrospective cohort in 286 children with HIV showed that persistent proteinuria was more common in clinical stages III and IV.¹⁸ Our subjects' mean CD4 count was 757.8 (SD 346) cells/mm³. Regarding WHO immunological classification, 25 out of 38 subjects had no immunosuppression. A study in which the majority of subjects had normal immunological status showed zero prevalence of microalbuminuria.¹⁷

All subjects in our study were on HAART, for a mean duration of 4.1 (SD 3.2) years. In other studies where most subjects were on HAART for <5 years duration, the prevalence of microalbuminuria was 11.7%.¹⁹ Uzoma *et al.* reported that in 77.3% of children with HIV who took HAART, none had microalbuminuria.¹⁷

Two subjects in this study had AKI. AKI is quite common in children with HIV.^{7,14} In the era before HAART, AKI was associated with younger age, severe immunosuppression, sepsis, and opportunistic infections. In the era of HAART, acute tubular necrosis, hemolytic uremic syndrome, HIVAN, and drug-induced microtubular obstruction are common causes of AKI. Around 14% of AKI in HIV patients is caused by nephrotoxic effects of ARV, occurring after 3 months of HAART treatment.²⁰ Both subjects with AKI in our study received AZT/3TC/NVP. One case report showed a correlation between lamivudine (3TC) and renal tubular acidosis (RTA).²¹ Two other drugs, zidovudine (AZT) and nevirapine (NVP) have not been reported for nephrotoxic effects.²²

Table 2. The relationships between persistent proteinuria and immunological classification, duration of ARV, adherence to ARV therapy, as well as age (N=38)

| Characteristics | Persistent proteinuria (+) (n=1) | Persistent proteinuria (-) (n=37) | P value |
|---------------------------------|-------------------------------------|--------------------------------------|---------|
| Immunological classification, n | | | |
| None or insignificant | - | 25 | 0.342 |
| Mild | - | 5 | |
| Advanced | - | 3 | |
| Severe | 1 | 4 | |
| Clinical stage, n | | | |
| Stage I | - | 32 | 0.19 |
| Stage II | - | - | |
| Stage III | 1 | 5 | |
| Stage IV | - | - | |
| Duration of ARV therapy, n | | | |
| 0-3 months | 1 | 2 | 0.158 |
| 4-12 months | - | 3 | |
| 13-60 months | - | 17 | |
| >60 months | - | 15 | |
| Adherence to ARV therapy, n | | | |
| Yes | 1 | 34 | 1.000 |
| No | - | 3 | |
| Age groups, n | | | |
| <5 years | 1 | 9 | 0.237 |
| 5-10 years | - | 22 | |
| >10 years | - | 6 | |

No subjects in our study received tenofovir disoproxil fumarate (TDF). TDF is approved by the Food and Drug Administration for use in children aged 2 years and older and is recommended by the WHO for use as a preferred first-line nucleotide reverse transcriptase inhibitor in adults and adolescents aged 10 years and older. The simplicity of its once daily dosing, few metabolic side effects, and efficacy against hepatitis B virus, make TDF suitable for use in a large-scale program.²³ A number of observational studies have documented TDF-associated nephrotoxicity following its widespread use in patients.²⁴⁻²⁶

Four (10%) subjects in this study were suspected to have UTIs. Another study in Nigeria found an increased frequency of UTI in adolescents and young adults with HIV who had advanced clinical WHO stages.²⁷ One subject in our study was suspected to have urolithiasis. This subject received an ARV regimen of AZT/3TC/EFV. There are multiple etiologies of kidney stones, which facilitate the occurrence of super saturation/crystallization of relatively insoluble substances in the urine.²⁸ Approximately 1-2% of stones in the urinary tract are caused by drug induction.²⁹ The ARV drugs indinavir

and atazanavir are lithogenic. Two cases of urinary tract stones were reported to have been related to efavirenz/EFV.³⁰

Persistent proteinuria was found in 1 of 38 subjects in this study, with a prevalence of 2.6%, derived from quantitative test with urine ACR values in the range of 30-300 mg/g (microalbuminuria). This result was lower than the prevalence in other studies, which were about 10-33% in Nigeria, Africa, India, and the United States.^{7,11,18,31} These data were higher than the findings in Enugu, Nigeria, which reported a microalbuminuria prevalence of 0%.¹⁷ One study reported AN 11.1% prevalence of microalbuminuria in subjects on HAART. However, the study only evaluated a single measurement of microalbuminuria.¹⁹ Low prevalence in our study was associated with lower risk factors observed in subjects, for example, younger age,^{3,11} non-advanced clinical staging,^{9,31} high CD4 cell count,^{9,11,18} and already having received antiretroviral therapy at the time of study.^{7,17}

We found no relationships between persistent proteinuria and age, clinical stage, immunological classification, or duration of ARV. This was due to

the low prevalence of proteinuria (only 1 subject). The characteristics of the subject with persistent proteinuria was <5 years of age, had advanced clinical staging, and had received ARV drugs for 3 months.

Several studies have shown that duration of infection (which is described by the age of the subjects in HIV vertical transmission) significantly influenced the incidence of proteinuria.^{3,11} HIV-associated nephropathy in children is different from that in adults. Children with HIVAN who had mesangial hyperplasia showed slower progression towards ESRD, so the incidence of proteinuria was more common in teenagers.² Other studies showed that proteinuria occurred in a younger age group.³¹ Age is not the sole cause of persistent proteinuria, as other factors, including the degree of immunological suppression and provision of ARV also affect proteinuria.³

Subjects with persistent proteinuria had advanced clinical classification and severe immunosuppression. The HIV virus weakens the immune system, leading to opportunistic infections. Clinical staging and immunological status proved to be risk factors for proteinuria.⁹ Children with opportunistic infections have lower CD4 levels than children without opportunistic infections.¹⁵ Anochie *et al.* showed that 83.3% of HIV-infected children with microalbuminuria presented the clinical symptoms of AIDS and CD4 count of 0 cells / L3.³ Other studies have found a correlation between CD4 cell count and levels of ACR ($r=-0.22$; $P=0.03$).³⁴ Moreover, lower CD4 level (<25%) is a risk factor for microalbuminuria.¹¹ Research on subjects who received ARV showed a significant correlation between clinical stage renal abnormalities and ARV ($P<0.049$).¹⁹ Clinical stage and degree of immunosuppression further reflect the high number of HIV virus particles in the patient's body, aggravating the risk of renal cell injury as a result of the HIV virus.³

The one subject with persistent proteinuria in our study had received ARV for 3 months. A retrospective cohort study showed that ARV provision for 5.6 (SD 0.1) years repaired proteinuria. Improvement of proteinuria by administering HAART for 5 years was associated with a decrease in viral load ($r=0.5$; $P<0.001$).¹⁸ Microalbuminuria was found in 11.1% of HIV-infected children who received ARV. In subjects with abnormalities of the kidney, 87.5% had been taking ARV < 5 years.¹⁹

The use of antiretroviral drugs affected the low prevalence of proteinuria in this study because all subjects included in the final evaluation had been on ARV. ARV therapy restores renal epithelial changes characterized by a significant decrease in albuminuria.³⁵ Studies with subjects who had not received antiretroviral drugs had a higher prevalence of proteinuria than our study.^{7,9} Another study reported zero prevalence in subjects who received ARV.¹⁷ In addition, Fredrick *et al.* found no relationship between microalbuminuria and antiretroviral drugs.¹¹

Case reports of renal biopsies from HIV patients, performed before and after ARV treatment, showed that the loss of synaptopodin, which causes podocyte proliferation, is reversible. The provision of HAART improves synaptopodin expression, thereby inhibiting proliferation and decreasing the degree of podocyte interstitial infiltration. In vitro studies showed that HIV-1 RNA was present in tubular cells and glomerular epithelial cells. Viral transcription persists during therapy, but was not accompanied by clinical symptoms (i.e., patients had improvement of clinical symptoms). As such, it is thought that the small number of viral proteins produced from viral RNA is not enough to sustain the symptoms of nephropathy.³⁶ Winston *et al.* showed that HAART therapy resulted in decreased HIV-1 RNA levels in plasma and decreased viral infection in the kidney, leading to improvement of morphological and functional abnormalities of HIV-1-associated nephropathy.³⁵ Giving HAART not only serves to suppress viral replication, but is associated with a decrease in the release of cytokines from peripheral blood mononuclear cells (tumor necrosis factor- α , interleukin-2, and interferon- γ). Some of these cytokines have been associated with renal cell injury and induction of proteinuria.⁵

Earlier administration of ARV could affect the prognosis of patients with renal involvement. An adult case report showed that improvement of renal biopsy findings occurred after 3 months of ARV administration, followed by improvement of proteinuria.³⁵ In the early stages, injury of podocytes by the HIV virus is reversible, so that repair will allow the actin cytoskeleton foot branching process to recur, in order to form an interdigitating pattern. Persistent injury of podocytes causes attenuation of body cells, podocyte hypertrophy, detachment from the glomerular basement membrane (GBM), and

podocyte death, followed by the formation of podocyte synechiae via attachment of parietal epithelial cells to the GBM. Attachment causes filtration to occur in the wrong direction, toward the interstitium. Podocyte loss will eventually lead to glomerulosclerosis and renal failure.^{37,38}

The prevalence of persistent proteinuria in this study was 1/38 (2.6%) subjects, obtained from a quantitative test with urine ACR values in the range of 30-300 mg/g (microalbuminuria). There was no relationship between persistent proteinuria events in HIV-infected children and CD4 levels, clinical stage, or duration of ARV. Variations in prevalences of proteinuria were associated with varying racial backgrounds.³⁹ HIVAN was not only found to be more common in the African races, but families of HIVAN patients had higher incidence of kidney disease.⁴⁰ However, some studies did not include a family history of kidney disease as one of the variables.¹² Variations in the prevalence of proteinuria were also affected by different methodologies. Several previous studies used different methods to measure protein or different cut-off points.^{7,12,17}

To date, this was the first study of persistent proteinuria in HIV-infected children in Indonesia. Proteinuria tests were conducted by two methods (qualitative and quantitative methods, simultaneously) to improve accuracy and reduce the possibility of false positives. We provide the baseline data of kidney condition in the era of HAART, before tenofovir was increasingly used as one of the ARV regimens in Indonesian HIV pediatric patients. However, we did not explore the history of renal insufficiency in the subjects. Also, urine specimens were collected at random times instead of morning urine. Also, proteinuria was observed in a limited time range over 3 visits, therefore, all subjects with a first-time positive test on the third examination were no longer observed. Further research is needed to explore and observe subjects who showed transient proteinuria.

In conclusion, early detection plays important role in identifying persistent proteinuria. Therefore, proteinuria tests should be performed immediately at the time of diagnosis, prior to receiving ARV. Further study must extend the period of observation for subjects with transient proteinuria at the third examination, to obtain the more accurate data on renal involvement status (persistent proteinuria).

Conflict of interest

None declared.

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