



PROTEINURIA AND ATYPICAL URINE CYTOLOGICAL FINDINGS OF CHILDREN ON ANTIRETROVIRAL THERAPY ATTENDING HIV CLINIC IN CALABAR, NIGERIA

NSE UMOH, MFONISO UDONKANG, GODWIN ATAGADA, IMEOBONG INYANG,
VALARIE NJAR AND RAYMOND EWORO

Email: nsirumoh1611@gmail.com

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ABSTRACT

Human immunodeficiency virus (HIV) among children has been on the rise due to increased vertical transmission and socioeconomic factors. Antiretroviral therapy (ART) has been effectively used in treatment among adults and children living with HIV (CLHIV), and urinalysis is used for routine screening for kidney health. However, there is limited study on urine cytological findings among HIV-seropositive children on ART in sub-Saharan Africa. The objectives of this study were to investigate the effects of ART on the urinary system of HIV-seropositive children by screening their urine for abnormal chemical constituents and atypical cytological epithelial cell changes. Following administration of questionnaire, freshly voided urine samples were collected from 88 participants on ART attending the Paediatric HIV Clinic of General Hospital, Calabar, Nigeria. Urinalysis was carried out using Meditest Combi-9 strips. For microscopic cytological evaluation, urine smears made from centrifuged sediments were fixed in 95% ethanol and stained with Papanicolaou technique. Age distribution showed 30 (34.1%), 44 (50%), and 14 (16.9%) of the participants were of the age range of 0-4 years, 5-9 years, and 10-14 years respectively. Based on the duration of ART administration, <1 month had 20 (22.7%), 1-6 months had 44 (50%), and >6 months had 24 (27.3%) participants. Proteinuria was reported in 36 (40.9%) of the participants. The cytological findings revealed that 46 (52.3%) had normal epithelial cells, 20 (22.7%) showed degenerative epithelial changes and 22 (25%) had squamous metaplasia. A total of 18 (20.5%) participants had crystals in their urine. In overall, the duration of use of ART was associated with proteinuria ($p < 0.001$), increased degenerative and metaplastic epithelial changes ($p < 0.001$), and crystalluria ($p = 0.002$). In conclusion, long-term ART use may predispose to atypical cytological changes associated with degeneration, squamous metaplasia, and presence of crystalluria in HIV-seropositive children. There is need to include urine cytology examination as routine screening test for CLHIV on antiretroviral therapy.

KEYWORDS: Human Immunodeficiency Virus, Urine Cytology, Children, Antiretroviral therapy, Proteinuria, Calabar

INTRODUCTION

HIV in children has been on the rise due to increased vertical transmission and socioeconomic factors.

In Nigeria, HIV is a major public health challenge (Inyang-Etoh *et al.*, 2018).

Nse Umoh, Department of Medical Bacteriology, Virology, and Mycology, Faculty of Medical Laboratory Science, University of Calabar, Calabar, Nigeria

Mfoniso Udonkang, Department of Histopathology and Cytology, Faculty of Medical Laboratory Science, University of Calabar, Calabar, Nigeria

Godwin Atagada, Department of Histopathology and Cytology, Faculty of Medical Laboratory Science, University of Calabar, Calabar, Nigeria

Imeobong Inyang, Department of Histopathology and Cytology, Faculty of Medical Laboratory Science, University of Calabar, Calabar, Nigeria

Valarie Njar, Department of Haematology and Blood Transfusion Science, Faculty of Medical Laboratory Science, University of Calabar, Calabar, Nigeria

Raymond Eworo, Department of Clinical Chemistry and Immunology, Faculty of Medical Laboratory Science, University of Calabar, Calabar, Nigeria

In 2021, the total number of people living with HIV among children aged 0-14 years was 130,000 in Nigeria (NACA, 2022). The countries effort to increase access to ARTs among children from 53,529 in 2019 to 59,423 in 2021 led to decrease in prevalence of new infections from 105,404 in 2019 to 92,323 in 2021 (NACA, 2022).

The benefits of ART have been enormous and have been shown to suppress viral load and decrease disease progression (Inyang-Etoh *et al.*, 2015a). Nigeria is among the countries that have implemented the recommendation to start ART at diagnosis. With this implementation, children are at risk of experiencing long-term antiretroviral therapy reactions as they are to adhere to the treatment for life (Dash and Rose, 2023).

Antiretroviral therapies have been reported to cause several toxicities including renal toxicity (Barata *et al.*, 2023; Spach, 2025). Documented reports have indicated that ARTs such as Tenofovir can cause tubular and interstitial nephritis. Protease inhibitor like Lopinavir and Indinavir can cause kidney stone formation (Barata *et al.*, 2023). Short and long-term monitoring of these side effects have been suggested by some authors to ensure better quality of life of affected persons (Dash and Rose, 2023; Spach, 2025).

One of the routine screening methods to assess kidney health is urinalysis. Urine protein was included as important test to assess the state of the kidneys before initiation and for monitoring of antiretroviral drugs (Spach, 2025). A study by Ikobo *et al.* (2020) showed that proteinuria was associated with ART use among children living with HIV (CLHIV). Apart from the kidneys, other parts of the urinary system such as the ureter, bladder, and urethra can be screened for the effects of ART.

Cytological examination is another test that can be useful in routine screening for urinary tract pathologies. Previous study on adults living with HIV reported abnormal urine cytological findings in HIV-seropositive persons receiving ART (Udonkang *et al.*, 2018). However, there is limited data on urine cytological findings among HIV-seropositive children on ART in Sub-Saharan Africa. This study investigated the effects of ART on the urinary system of HIV-seropositive children by screening their urine for abnormal chemical constituents and atypical cytological epithelial cell changes.

MATERIALS AND METHODS

Study area

The study was carried out in the Paediatric HIV Clinic of the General Hospital in Calabar, Cross River State, Nigeria. The General hospital is a secondary health facility located within Calabar Metropolis and a site for treatment and care for persons living with HIV.

Study design, subjects and data collection

The study utilized cross-sectional design and simple random sampling to select 88 children within the ages of 0-14 years in both males and females on ART. An interview-administered questionnaire was used to obtain age and duration of ART use from the study participants.

Sample size calculation

The sample size was calculated based on HIV prevalence of 2.8% (NACA, 2022) with 5% precision at 95% confidence interval using the sample size formula for Cross sectional study of Sadiq *et al.* (2025). Forty-four subjects were gotten but 88 were recruited to increase participant variability.

Inclusion/Exclusion criteria: The study subjects were HIV-seropositive children who were on ART for at least two weeks whose parents gave consent to participate. The children whose parents did not give consent were excluded from the study.

Ethical approval

Ethical approval was obtained from the Ethics and Research Committee of Cross River State Ministry of Health, Calabar with approval number: CRSMOH/RP/REC/2021/106.

Sample collection and preparation

Each participant gave fresh voided urine sample collected into sterile universal container. The macroscopic appearances of the urine samples were first assessed. Each sample was properly mixed and divided into two parts. Part A was used for urinalysis and part B was used for Papanicolaou staining technique.

Urinalysis

Urinalysis was carried out using Medi-Test Combi-9 dipstick strip (Macherey-Nagel Duren, Germany) based on manufacturer's instruction. Each apportioned sample was mixed while a urinalysis strip was dipped in completely and removed immediately. The colour changes on each strip were read after comparing with the colour chart on the urinalysis strip container.

Cytological procedure/Papanicolaou Staining Technique

Before staining, each urine sample was spun in a centrifuge for 5 minutes. The supernatant was discarded while the sediment was used to make a smear on an albumenized glass slide. The smears were fixed in 95% ethanol for 15 minutes. The smears were stained with Papanicolaou method according to method of Cibas and Ducatman (2014). The stained slides were examined microscopically with a light microscope using x40 and x100 objectives.

Statistical analysis:

The results were analyzed using Statistical Package for Social Sciences (SPSS) version 27 (Armonk, New York: IBM Corporation). All results were expressed as percentages. Chi-square was used to test the association between duration of ART use with the urinalysis and cytological findings.

Results were statistically significant at probability level $P \leq 0.05$.

RESULTS

Age, clinical data, urinalysis, and cytological findings

In this study, majority 44(50%) of the 88 children on ART were within the age group of 5-9 years and ART duration of 1-6 months (Table1). Proteinuria was observed in 36(40.9%) of the participants, while 18(20.5%) were positive for ascorbic acid. All other chemical constituents of urine were reported negative for all the urine specimens. Degenerative changes

20(22.7%), squamous metaplasia 20(22.7%), and crystalluria 18(20.5%) were seen in the urine of the children. In Table 2, the ART group of >6 months had 16 (18.2) cases of proteinuria. There was a significant association between duration of ART use and proteinuria among the subjects ($\chi^2=20.809$, $p<0.001$). Cytological findings showed more degenerative changes of 16(18.2%) within ART group of 1-6 months and more squamous metaplasia in 16(18.2%) within ART group of >6 months. The association between duration of ART use and the abnormal cytological findings was statistically significant ($\chi^2=47.052$, $p<0.001$). Crystalluria was found in 8(9.1%) of the 1-6 months and >6 months ART groups and there was a statistical significantly associated ($\chi^2=17.753$, $p=0.002$).

Table 1: Demographic and clinical data, urinalysis, and cytological findings of the subjects

Demographic and clinical data					
Age (years)	n (%)	ART Duration (months)	n (%)		
0-4	30 (34.1)	<1	20 (22.7)		
5-9	44 (50.0)	1-6	44 (50.0)		
10-14	14(16.9)	>6	24 (27.3)		
Total	88 (100)	Total	88 (100)		
Urinalysis					
Proteinuria	n (%)	Ascorbic acid		Other Chemical Constituents	n (%)
Positive	36 (40.9)	Positive	18 (20.5)	Positive	0 (0)
Negative	52 (59.1)	Negative	70 (79.5)	Negative	88 (100)
Total	88 (100)	Total	88 (100)	Total	88 (100)
$\chi^2=23.511$	$p=0.001$				
Papanicolaou staining					
Cytological findings	n (%)			Crystal	n (%)
Normal	46 (52.3)			Positive	18 (20.5)
Degenerative changes	20 (22.7)			Negative	70 (79.5)
Squamous metaplasia	22 (25)			Total	88 (100)
Total	88 (100)				

Table 2: Associations between duration of ART use with proteinuria, cytological findings, and crystalluria

ART duration (months)	Proteinuria		
	Negative n(%)	Positive n(%)	Total n(%)
<1	20 (22.7)	0 (0)	20 (22.7)
1-6	24 (27.3)	20 (22.7)	44 (50.0)
>6	8 (9.1)	16 (18.2)	24 (27.3)
Total	52 (59.1)	36 (40.9)	88 (100)
Statistics	$\chi^2=20.809$	$p=<0.001$	

ART duration (months)	Cytological findings			
	Negative n(%)	Degenerative changes n(%)	Squamous metaplasia n(%)	Total n(%)
<1	20 (22.7)	0 (0)	0 (0)	20 (22.7)
1-6	22 (25.0)	16 (18.2)	6 (6.8)	44 (50.0)
>6	4 (4.5)	4 (4.5)	16 (18.2)	24 (27.3)
Total	46 (52.3)	20 (22.7)	22 (25.0)	88 (100)
Statistics	$\chi^2=47.052$	$p=<0.001$		

ART duration (months)	Presence of crystal		
	Negative n(%)	Positive n(%)	Total n(%)
<1	40 (45.5)	2 (2.3)	42 (47.7)
1-6	18 (20.5)	8 (9.1)	26 (29.5)
>6	12 (13.6)	8 (9.1)	20 (22.7)
Total	70 (79.5)	18 (20.5)	88 (100)
Statistics	$\chi^2=17.753$	$p=0.002$	

Photomicrographs

The photomicrographs are shown in Plate 1A-F. Normal superficial and transitional epithelial cells are shown in plate 1A and 1B respectively. Plate 1C is a degenerating superficial epithelial cell with

hyperchromatic cytoplasm and pyknotic nucleus. Plate 1D is a metaplastic cell with double nuclei and 1E is a clump of keratinized anucleated epithelial cells. Plate 1F shows crystals in urine

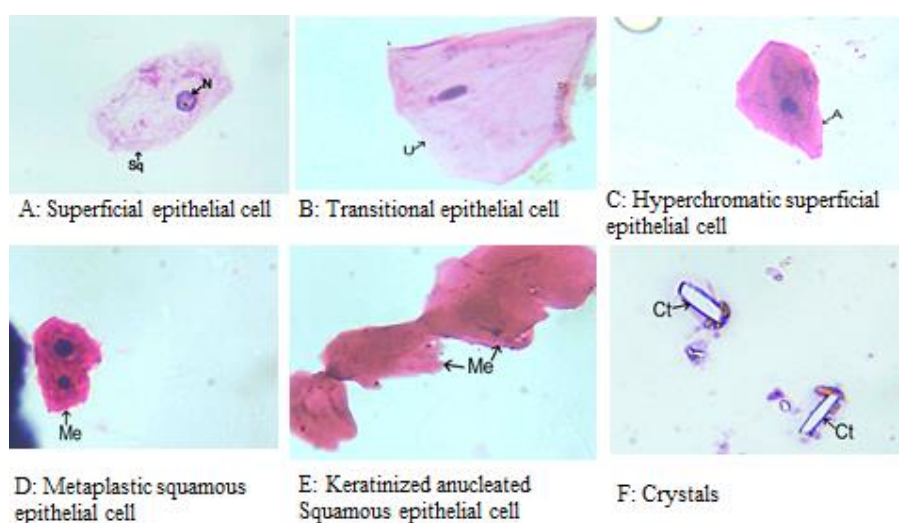


Plate 1: Epithelial cells and crystals in voided urine from HIV-seropositive children on ART. Plate 1A and 1B show normal superficial and transitional epithelial cells, 1C is degenerating epithelial cell with hyperchromasia and pyknosis, 1D shows metaplastic squamous epithelial cell with karyolysis, 1E is keratinized squamous cell, and 1F is crystal. Pap x400 magnification.

Plate 1: Epithelial cells and crystals in voided urine from HIV-seropositive children on ART.

DISCUSSION

The use of ARTs is aimed at suppressing viral load which in turn improves the quality of life for people living with HIV (Archibong *et al.*, 2022). However, strict adherence to treatment has been a major challenge due to some side effects including cases of nephrotoxicity (Ikobo *et al.*, 2020). Thus, children on ART need a well-tailored approach to monitoring ART-related nephrotoxicity.

In this study, majority of CLHIV who received ART were found to be less than 5 years of age. While this data may indicate that CLHIV have access to ARTs in this locality, it is consistent with the report of Iboko *et al.* (2020), who conducted a similar study on children receiving ART. Proteinuria was common among CLHIV in this study, with a frequency of 40.9% among the subjects. This value was higher than 21.8% occurrence of proteinuria, attributed to the effect of ART in a previous study (Iboko *et al.*, 2020). Proteinuria, which on its own triggers processes that lead to rapid progression of renal damage, has been used as a surrogate marker for nephropathy (Spach, 2025). These proteins appear in urine due to defective glomerular filtration (Longhitano *et al.*, 2024).

The cytological findings in this study revealed degenerative changes and squamous metaplasia. These changes are reversible in nature. Degenerative changes involving pyknotic nuclei and hyperchromasia are as a result of toxicity. Metaplasia on the other hand is a pre-malignant change found in severe toxicities and injuries. Similar degenerative changes and metaplasia were reported in HIV-seropositive adults on ART (Udonkang *et al.*, 2018). Other studies had reported degenerative changes and dysplasia in urine cytology of cigarette smokers (Inyang *et al.*, 2018) and degenerative changes and metaplasia in urine cytology of *Schistosoma haematobium* infections (Inyang-Etoh *et al.*, 2018b, Etuk *et al.*, 2018).

Crystal formation in urine was observed among the children. Triple phosphate crystal was seen. Crystalluria is believed to be initiated by urine supersaturation with minerals (Tamborino *et al.*, 2024). Crystals in urine have been found to predispose individuals to nephrolithiasis. Thus, their presence in urine may serve as a marker of nephrolithiasis. This finding has been confirmed following report of nephrolithiasis due to ART use (Plawecki *et al.*, 2023).

The strength of this study is anchored on the importance of incorporating urine cytological examination in routine clinical visits for CLHIV. Urine cytological examination is non-invasive, simple, and cost effective. It will help reveal the state of health of the urinary system for better outcomes and quality of life. The limitation of the study bothers on inability to access other relevant data of the subjects that would have helped in understanding the duration of HIV infection.

CONCLUSION

Children living with HIV taking ARTs have proteinuria. They are also predisposed to abnormal cytological changes involving epithelial degenerative changes and squamous cell metaplasia. The use of ART was also associated with crystalluria. This study shows that the use of ART may increase the risk of nephrotoxicity and atypical epithelial cell changes. There is need to incorporate urine cytological examination as a screening test during routine clinical visits for children living with HIV.

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