Crystalluria in HIV/AIDS patients on highly active anti-retroviral therapy in the Kumasi metropolis; a cross sectional study

Richard K. D. Ephraim, Ruth C. Brenyah1, Richmond Osei2, Bright D. Bossipe, Prince Adoba, Derick N. M. Osakunor3, Hope Agbodzakey

ABSTRACT

Background: Crystalluria is associated with some highly active anti-retroviral therapies (HAART’s) used in the management of HIV/AIDS. Aims: This study used light microscopy to establish the prevalence of crystalluria among HIV/AIDS patients on HAART and identified the routine crystals present in their urine. Materials and Methods: In this simple randomised cross-sectional study, 200 HIV/AIDS participants, comprising 150 on HAART and 50 HAART-naïve were recruited from the HIV clinic at the Komfo Anokye Teaching Hospital (KATH). Urine and blood samples were collected, for urinalysis and the determination of the CD4 count, respectively. A well-structured pre-tested questionnaire was used to obtain socio-demographic data and clinical history of the participants. Results: The prevalence of crystalluria was higher among HIV-infected persons on HAART than those not on HAART (6.7% vs 4%; P = 0.733). Calcium oxalate and triple phosphate crystals were the crystal types present in their urine (3.5% and 2.5%, respectively) and was present only in HIV subjects on first line of treatment (without protease inhibitors). Participants aged between 40-50 years and those with hypersthenuria and acidic urine had the highest amount of crystalluria (41.6%, 83.3%, and 58.3%, respectively). Conclusion: HAART is associated with crystalluria in HIV patients. Light microscopy will be of diagnostic value in resource limited settings.

Key words: Crystalluria, highly active antiretroviral therapy, human immunodeficiency virus, light microscopy, urinalysis

INTRODUCTION

Crystalluria refers to crystals in urine and is a marker of urine super-saturation in both normal and pathological conditions. Crystals are frequent findings in the routine examination of urine sediment. In most instances, the precipitation of crystals is caused by transient super-saturation of the urine, ingestion of foods, or by changes of urine temperature and pH, which occurs upon standing after micturition. Formation of these crystals in urine is the necessary initial step of urolithogenesis in every type of urinary stone disease. The clinical features can either be due to renal failure from crystal deposition or nephrolithiasis that result in urinary obstruction. Highly active antiretroviral therapy (HAART) is a preferred treatment for people infected with human immunodeficiency virus (HIV) and may predispose the patient to lithiasis, which may precipitate within renal tubules either as crystals or stones and cause renal obstruction.

Studies have reported crystalluria in HIV patients on HAART using techniques like infrared spectrophotometry, mass spectrometry, high-performance liquid chromatography (HPLC), and phase-contrast microscopy. However, in a resource-limited country like Ghana, diagnosis during routine visits to healthcare facilities is made using light microscopy. Therefore, this study sought to use light microscopy to establish the prevalence of crystalluria among HIV/AIDS patients on HAART and identified the routine crystals present in their urine.
microscopy to determine the prevalence of crystalluria and identify the crystals in the urine of HIV/AIDS patients on HAART at the KATH, Kumasi, Ghana.

MATERIALS AND METHODS

This was a simple randomised cross-sectional study conducted at the Komfo Anokye Teaching Hospital (KATH), Kumasi in the Ashanti Region of Ghana from January to April, 2013. A total of 200 HIV positive participants, comprising 150 on HAART and 50 HAART-naïve were recruited from the HIV clinic. A well-structured pretested questionnaire was used to obtain personal information and clinical history of subjects after written informed consent had been obtained. Ethical approval was obtained from The Committee on Human Research, Publication and Ethics, School of Medical Sciences, Kwame Nkrumah University of Science and Technology (CHRPE/SM/KNUST) prior to the commencement of the study.

Persons with any acute or chronic infection (renal or hepatic diseases) or gouty arthritis, family history of recurrent kidney stones as well as those on drugs that can precipitate crystalluria (sulphonamides) were excluded from the study. Patients on HAART but defaulting treatment as prescribed were also excluded.

Venous blood (3 ml) was collected into ethylenediaminetetraacetic acid (EDTA) tubes for the estimation of the CD4+ count. Early morning urine (8-10 ml) was collected into transparent, clean, dry, leak-proof and wide mouth screw-capped plastic containers. The samples were then sent to the parasitology laboratory for routine urinalysis.

Each specimen was examined macroscopically and its appearance and colour recorded as either clear, hazy, turbid, straw to amber, milky, bloody, or colourless.

Chemistry analysis was performed on the urine sample for the pH, specific gravity (SG), blood and protein using a dipstick (DIRUI A, China).

The urine was centrifuged at 500-1000 g for 3-5 minutes and a wet preparation of urine deposits examined using the ×10 and ×40 objectives of a light microscope (Olympus, Olympus Corporation of the Americas, USA) for the presence and identification of crystals.

The CD4+ T-lymphocyte count was estimated using the BD FACS Count system (FACS Count, California, USA).

Data was analysed using Microsoft excel 2010 and GraphPad Prism (version 5) (www.graphpad.com). Data were summarised using frequency tables and bar charts. For all statistical comparisons, a P-value < 0.05 was considered as statistically significant.

RESULTS

Table 1 shows the general characteristics of the study participants. Majority of the participants were females in their third decade of life. Most of the females (61.9%) had basic education with only a few (2.6%) having tertiary education. There was a significantly higher SG among the females compared to the males (\(P = 0.040\)), and the urine pH was also higher among the females than the males but the difference was not significant (\(P = 0.570\)). Eight (5.1%) and 15 (9.5%) females had proteinuria and haematuria, respectively, whereas none of the males had proteinuria and haematuria. Majority of the females had crystalluria compared to the males. The females had an insignificantly higher mean CD4+ count compared to the males. Majority of the participants were on medication with greater than 2 years duration of HIV/AIDS.

The prevalence of crystalluria among the HIV/AIDS subjects is shown in Table 2. The prevalence of crystalluria was higher among HIV subjects on HAART (6.7%) than those who were HAART-naïve (4%) (\(P = 0.730\)).

| Parameter                  | Total n (200) | Male n (45) | Female n (155) | P-value |
|----------------------------|---------------|-------------|----------------|---------|
| Sex                        |               |             |                |         |
| Male                       | 45 (22.5)     |             | 155 (77.5)     | —       |
| Female                     |               | 15 (41)     |                | 0.260   |
| Age (years)                | 39.36±0.51    | 40.47±0.76  | 39.03±0.62     | 0.240   |
| Educational background     |               |             |                |         |
| Illiterate                 | 25 (20.0)     | 4 (8.9)     | 21 (13.5)      | 0.020   |
| Basic                      | 128 (64)      | 32 (71.1)   | 96 (61.9)      | 0.290   |
| Secondary                  | 14 (7.0)      | 5 (11.1)    | 9 (5.8)        | 0.310   |
| Tertiary                   | 8 (4.0)       | 4 (8.9)     | 4 (2.6)        | 0.080   |
| Biochemical assay          |               |             |                |         |
| SG                         | 0.01±0.01     | 0.01±0.00   | 0.01±0.01      | 0.040   |
| pH                         | 6.60±0.27     | 6.31±0.33   | 6.69±0.35      | 0.570   |
| Protein                    |               |             |                |         |
| Negative                   | 172 (86.00)   | 40 (88.99)  | 132 (85.20)    | 0.630   |
| Trace                      | 15 (7.50)     |             | 15 (9.70)      | 0.020   |
| 1+                         | 7 (3.50)      |             | 7 (4.50)       | 0.350   |
| 2+                         | 1 (0.50)      |             | 1 (0.60)       | 1       |
| Blood                      |               |             |                |         |
| Negative                   | 162 (81.0)    | 43 (95.6)   | 119 (76.8)     | 0.004   |
| Trace                      | 21 (10.5)     |             | 21 (13.5)      | 0.056   |
| 1+                         | 6 (3.0)       |             | 6 (3.8)        | 0.340   |
| 2+                         | 5 (2.5)       |             | 5 (3.2)        | 0.600   |
| 3+                         | 4 (2.0)       |             | 4 (2.5)        | 0.580   |
| Crystalluria               | 12 (6.0)      | 3 (6.7)     | 9 (5.8)        | 0.730   |
| Medical History            |               |             |                |         |
| CD4 count (cells/UL)       | 4572±1173.32  | 4035±321.58 | 4722±202.27    | 0.090   |
| Medication                 |               |             |                |         |
| Yes                        | 150 (75.00)   | 36 (80.0)   | 114 (73.50)    | 0.440   |
| No                         | 50 (25.00)    | 9 (20)      | 42 (26.50)     | 0.440   |
| Duration on medication     |               |             |                |         |
| <1 year                    | 42 (21.0)     | 10 (22.9)   | 32 (20.9)      | 0.840   |
| 1-2 yrs                    | 44 (22.0)     | 10 (22.9)   | 34 (21.9)      | 1       |
| >2 years                   | 54 (27.0)     | 16 (35.6)   | 38 (23.0)      | 0.590   |

SG – Specific gravity

Table 1: General characteristics of study participants
oxalate (3.5%) and triple phosphate (2.5%) crystals were the only crystals detected in the urine of the participants.

Table 3 shows the prevalence of crystalluria among HIV subjects in relation to CD4+ cell counts. HIV subjects with 200-500/μl CD4+ T cell counts had the highest prevalence of crystalluria (50%) whereas those with greater than 500/μl CD4+ T cell counts had the lowest prevalence (16.7%).

Table 4 shows the prevalence of crystalluria in HIV subjects on HAART in relation to medication use. Crystalluria was only found in subjects on first-line treatment (CBV + NVP or CBV + EVP). No crystals were found in the urine of subjects on second-line treatment, who had protease inhibitors as part of their medication (ABC + TDF + EFV; TDF + ALUVIA + ABC).

Table 5 shows the prevalence of crystalluria stratified by age. HIV subjects in the age group 40-49 had the highest prevalence of crystalluria (41.6%). Both calcium oxalate and triple phosphate were present among all the age groups. However, the prevalence of calcium oxalate was highest in age groups 30-39 (66.7%), and the prevalence of triple phosphate crystals was also highest in the age groups 20-29 and 50-59.

Figure 1 shows the distribution of crystals among HIV patients on HAART stratified by SG (a) and pH (b). Majority (83%) of the crystalluric patients produced hypersthenuric urine whereas 16.7% produced isosthenuric urine. Over half of the participants (58.3%) of the crystalluric subjects produced acidic urine whereas the remaining 41.7% produced alkaline urine.

**DISCUSSION**

Crystalluria is the presence of crystals in urine and has been associated with the use of protease inhibitors class of HAART in HIV patients. Several studies conducted in advanced countries used sophisticated methods to examine crystalluria in HIV patients on HAART.\(^7,8\)

This study used light microscopy to determine the prevalence of crystalluria among HIV/AIDS persons on HAART, and also identified the routine crystals present in their urine. The prevalence of crystalluria, using light microscopy, was higher among HIV-infected persons on HAART than in HAART-naïve participants,

**Table 2: Prevalence of crystalluria among the study participants**

| Crystal          | Total n (200) | HIV individuals (On-HAART) n (150) | HIV individuals (HAART-naïve) n (50) | P-value |
|------------------|---------------|-----------------------------------|--------------------------------------|---------|
| Calcium Oxalate  |               |                                   |                                      |         |
| Triple phosphate |               |                                   |                                      |         |

**Table 3: Prevalence of crystalluria among HIV participants on HAART in relation to CD4+ counts**

| CD4 T cell count | Total (%) | Crystal type |
|------------------|-----------|--------------|
| <200/μl          | 4 (33.3)  | 3            |
| 200-500/μl       | 6 (50.0)  | 3            |
| >500/μl          | 2 (16.7)  | 1            |

**Table 4: Crystalluria prevalence in HIV subjects on HAART type**

| Medication         | Number of patients n (150) | Patients with crystalluria n (10) |
|--------------------|----------------------------|-----------------------------------|
| CBV+NVP            | 44 (29.3)                  | 3 (30)                            |
| CBV+EFV            | 64 (42.7)                  | 7 (70)                            |
| ABC+TDF+EFV        | 32 (21.3)                  | 0                                 |
| TDF+ALUVIA+ABC     | 10 (6.7)                   | 0                                 |

**Table 5: Prevalence of crystalluria stratified by age**

| Ages (years) | Total n (%) | Calcium oxalate n (%) | Triple phosphate n (%) |
|--------------|-------------|-----------------------|------------------------|
| 20-29        | 2 (16.7)    | 1 (14.3)              | 1 (20.0)               |
| 30-39        | 3 (25.0)    | 2 (18.6)              | 1 (20.0)               |
| 40-49        | 5 (41.6)    | 3 (42.8)              | 2 (40.0)               |
| 50-59        | 2 (16.7)    | 1 (14.3)              | 1 (20.0)               |
and calcium oxalate and triple phosphate crystals were present in the urine.

The calcium oxalate and triple phosphate crystals found in this study is consistent with earlier observations made in Milano Italy, which recorded a high prevalence of calcium oxalate and triple phosphate crystals in the normal population. The presence of calcium oxalate crystals corroborate the findings of, who demonstrated a high prevalence of calcium oxalate crystals in the normal population in a cross-sectional study conducted in Morocco. This is expected due to the presence of calcium oxalate crystals in normal subjects, often as a consequence of ingestion of vegetable foods, in stone formers, in patients with hyperoxaluria, or after ethylene glycol poisoning. Triple phosphate crystals are also mostly found during urinary tract infection caused by urea-splitting bacteria.

Crystal formation in HIV subjects on HAART has been associated with the use of protease inhibitors. On the contrary, in this study, only HIV subjects on first line of treatment (without protease inhibitors) presented with crystalluria. This might be due to the use of light microscopy in the examination of the urine samples rather than more specific methods such as infrared spectrophotometry, mass spectrometry, high-performance liquid chromatography (HPLC) and phase-contrast microscopy with polarised filters.

The highest prevalence of crystalluria was found in HIV patients aged between 40 and 50 years. This is concordant with the higher frequency of crystalluria observed in normal adults aged 40 years and above in a cross-sectional study.

In agreement with observations made in this study, low pH and increased SG (hyperstenuria) have been shown to influence crystals and stone formation in a retrospective descriptive study conducted among proven urolithiasis patients. Crystalluria leads to stone formation, which can predispose HIV patients to acute renal failure. Crystal formation depends mainly on the composition of urine, since urine is a metastable liquid containing several coexisting substances that can crystallise to generate renal calculi. The presence of crystalluria among HIV patients in our study, suggests that these patients are at risk of urolithiasis and possibly renal failure, if the necessary corrective measures are not taken.

Inability to compare our findings with that obtained from the use of more specific methods serves as a major limitation of this study.

**CONCLUSION**

HAART associated crystal formation in urine is common among HIV patients. This is linked with an increased SG and low pH. In low resource settings light microscopy is of diagnostic value in the detection of crystalluria.

**ACKNOWLEDGEMENT**

We are grateful to the participants and, staff the HIV clinic and Parasitology Laboratory, of Komfo Anokye Teaching Hospital, Kumasi.

**REFERENCES**

1. Daudon M, Jungers P, Lacour B. Clinical value of crystalluria study. Ann Biol Clin (Paris) 2004;62:379-93.
2. Ryall RL, Bagley CJ, Marshall VR. Independent assessment of the growth and aggregation of calcium oxalate crystals using the Coulter counter. Invest Urol 1981;18:401-5.
3. Fogazzi GB. Crystalluria: A neglected aspect of urinary sediment analysis. Nephrol Dial Transplant 1996;11:379-87.
4. Yarlagadda SG, Perazella MA. Drug-induced crystal nephropathy: An update. Expert Opin Drug Saf 2008;7:147-58.
5. Famularo G, Di Toro S, Moretti S, De Simone C. Symptomatic crystalluria associated with indinavir. Ann Pharmacother 2000;34:1414-8.
6. Kopp JB, Miller KD, Mican JA, Feuerstein IM, Vaughan E, Baker C, et al. Crystalluria and urinary tract abnormalities associated with indinavir. Ann Intern Med 1997;127:119-25.
7. Daugas E, Rougier JP, Hill G. HAART-related nephropathies in HIV-infected patients. Kidney Int 2005;67:393-403.
8. Izzedine H, Harris M, Perazella MA. The nephrotoxic effects of HAART. Nat Rev Nephrol 2009;5:563-73.
9. Mbarki M, Oussama A, Elbouadili A, Semmoud A, Berkani M, Touhami M, et al. Study of spontaneous crystalluria on a series of patients in the Tadla Azilal Moroccan area. Arch Esp Urol 2006;59:653-9.
10. Finch AM, Kasidas GP, Rose GA. Urine composition in normal subjects after oral ingestion of oxalate-rich foods. Clin Sci (Lond) 1981;60:411-8.
11. Ishtiyaq A, Jayadevan S, Jayakumary M, Manda V, Mohammad AHAF, Elsheba M. Effect of urinary pH and specific gravity in urolithiasis. Gulf Medical Journal, 2012;1:26-31.
12. Röling J, Schmid H, Fischereder M, Draenert R, Goebel FD. HIV-associated renal diseases and highly active antiretroviral therapy-induced nephropathy. Clin Infect Dis 2006;42:1488-95.
13. Ansari MS, Gupta NP. Impact of socioeconomic status in etiology and management of urinary stone disease. Urol Int 2003;70:255-61.

**How to cite this article:** Ephraim RK, Brenyah RC, Osei R, Bossipe BD, Adoba P, Osakunor DN, et al. Crystalluria in HIV/AIDS patients on highly active anti-retroviral therapy in the Kumasi metropolis; a cross sectional study. Niger Med J 2014;55:504-7.

**Source of Support:** Nil, **Conflict of Interest:** None declared.