Clinica Chimica Acta 492 (2019) 23–25

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Letter to the editor

A virtuous diagnostic and therapeutic roadmap triggered by a motivated and skilful urinary sediment examination

ARTICLE INFO

Keywords:
Urinalysis
Urinary sediment
Urinary crystals
Crystalluria
2,8- dihydroxyadenine
Adenine phosphoribosyltransferase deficiency

ABSTRACT

In this paper we describe how an accurate urinary sediment examination, which revealed the presence of a severe 2,8-dihydroxyadenine crystalluria, opened the way to an articulate and successful diagnostic and therapeutic roadmap for a rare and potentially severe renal disease.

Urinary sediment (U-sed) examination is considered worldwide as an important tool for the diagnosis and the follow up of the diseases of the kidneys and of the urinary tract [1].

In this paper we describe how an accurate U-sed examination opened the way to an articulate and successful diagnostic and therapeutic roadmap for a rare and potentially severe renal disease.

Day 1 (08.02.2017). Many unusual crystals, without other formed elements, are found in a centrifuged urine sample examined in the Clinical and Research Laboratory on Urinary Sediment of Ospedale Maggiore Policlinico, Milano, Italy. By bright field microscopy at 400× magnification, the crystals appear as spherical colourless structures of different diameter (50 of which are measured: 4.3 to 11.7 μm, average ± SD: 6.3 ± 1.7, median 5.9) and with an apparent central depression (Fig. 1A). By polarized light, the crystals display a strongly birefringent "Maltese cross" appearance (Fig. 1B). By dipstick (LabStrip U11Plus, 77 Elektronika KFT, Budapest, Hungary) urine pH is 6.5, and specific gravity 1.018; haemoglobin, albumin, leukocyte esterase, nitrites, and bilirubine: negative.

Based on crystal features, two possible identification hypotheses are considered: leucine or 2,8-dihydroxyadenine (2,8-DHA) [1,2].

Day 2 (09.02.2017). Through hospital intranet we find that: 1. The urine sample belongs to an 11-year-old boy who is under observation in the Pediatric Emergency Unit, where i.v. analgesic treatment is given for a colicky pain due to stones in the right kidney; 2. Laboratory tests show normal renal function (serum creatinine 0.55 mg/dL, CKD-EPIeGFR: > 90 mL/min/1.73 m^2) and normal liver enzymes. The latter finding weakens the hypothesis of leucine crystals [1,2].

Thus, based on the combined pieces of information reported above i.e., unusual crystalluria associated with kidney stones in a boy without signs of liver disease, it is hypothesized that the patient may suffer from a congenital deficiency of adenine phosphoribosyltransferase (APRT), which leads to 2,8-DHA crystalluria.

This information is immediately forwarded to the doctor in charge of the patient in the Emergency room and to one of us (G.M.), who includes the boy in the "Urolithiasis Clinic" of the Pediatric Nephrology Institute of our hospital for further investigation and follow-up.

Day 19 (27.02.2017). A new U-sed examination is performed, which confirms the presence of a high number of crystals (without other elements), most of which are identical with those seen previously (Fig. 1C-D), while others have a brownish colour and, although still birefringent, do not show the "Maltese cross" pattern (Fig. 1E-F).

An aliquot of urine is filtered, dried, and shipped to a specialized "Crystal Laboratory" in Paris, for infrared spectroscopy (IRS) investigation.

Day 20 (28.02.2017). The measurement of APRT is performed by one of us (V. R.) using HPLC with UV detection, which demonstrates a severe enzyme deficiency: 1.1 U (= μg of AMP/min; normal value: 6.5–44.6).

Day 43 (23.03.2017). IRS confirms that urinary crystals are made of 2,8-DHA (Supplementary material, Fig. 2).

Day 48 (28.03.2017). The boy is submitted to a partial surgical removal of the renal stones.

Day 49 (29.03.2017). IRS investigation of the surgically removed stones, performed in the Clinical laboratory of our institution, confirms the presence of 2,8-DHA.

Therefore, a treatment with allopurinol 100 mg/day is started, which is increased to 200 mg/day in May.

Follow-up. Two other U-sed are examined, the first of which (on 07 August 2017) still shows the presence of a moderate amount of 2,8-DHA crystals. On December 06, 2017, after allopurinol has been increased to 300 mg/day, no more crystals are found.

In normal condition APRT, a purine salvage enzyme, catalyzes the transformation of adenine into adenosine monophosphate. In APRT deficiency, adenine is oxidized by xanthine dehydrogenase to 2,8-DHA, which is highly insoluble at any body pH with a consequent precipitation of crystals in the urine [3](Supplementary material, Fig. 2).

Two types of APRT deficiency are recognized, based on residual APRT activity on erythrocyte lysate: type I which is found mainly in hemizygous or compound heterozygous Caucasians, and is characterized by the total absence of APRT activity; type II, which is present mostly in hemizygous Japanese population, and is characterized by a variable residual APRT activity [3].

APRT deficiency has been described in all ethnic groups, although the majority of reported cases come from France, Iceland and Japan [4]. According to Edvarsson et al. [3], who described a cohort of 23 patients from Iceland, the condition can be seen both in male and

https://doi.org/10.1016/j.cca.2019.01.026
Received 28 January 2019; Received in revised form 29 January 2019; Accepted 29 January 2019
Available online 30 January 2019
0009-8981/

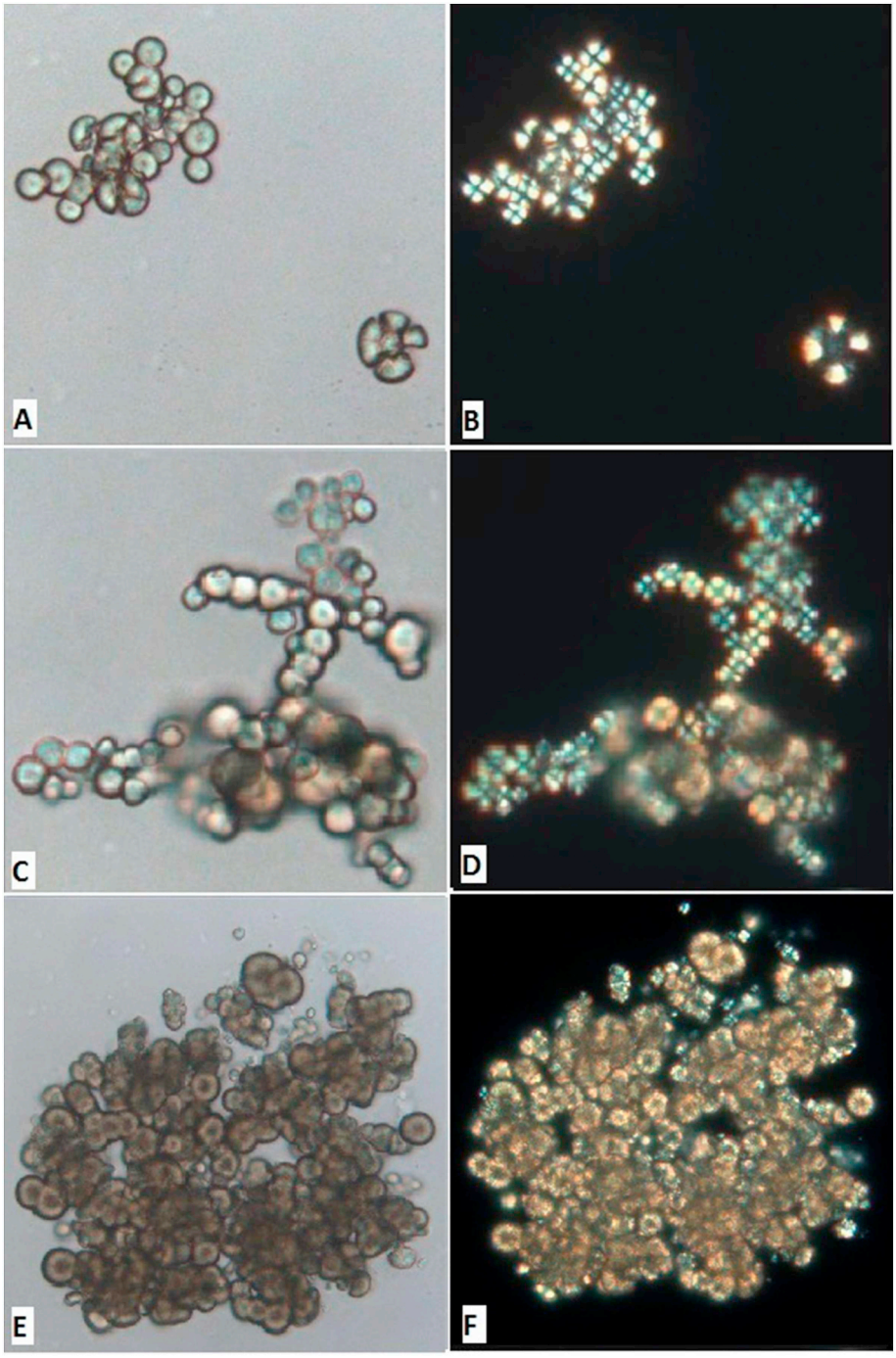

Fig. 1. A. Day 1. A cluster of typical spherical 2,8-DHA crystals as seen by bright field microscopy. At the bottom right, a large crystal with the same colourless appearance but a different "flower-like" morphology. **B.** The same crystals as seen by polarized light. **C.** Day 19. Crystals similar to those seen at day 1, arranged as a nice female ballet dancer. **D.** The same crystals as seen by polarized light. **E-F.** A cluster of crystals seen in the same urine sample. Why they have a brownish colour and a birefringence without the "Maltese cross" appearance is not clear at the moment. Original magnification for all images: 400x.

female of any age (from 0.5–62 yrs) in whom it can cause recurrent radiolucent stone disease (65% of cases), acute kidney injury due to precipitation of 2,8-DHA crystals within the renal tubules and interstitium (26%), and/or chronic kidney disease, probably due to chronic interstitial nephritis (17%).

APRT deficiency can first be suspected after the finding of urinary crystals, which are seen in virtually all untreated patients [4] and/or recurrent radiolucent stone disease in the absence of uric acid metabolic imbalance; in both cases, IRS investigation is mandatory [4,5]. The definite diagnosis is obtained with the measurement of APRT enzyme activity in erythrocyte lysate [4] and can be completed by the measurement of 2.8-DHA in the urine [4,6], and genetic molecular analysis [6,7].

Treatment with xanthine dehydrogenase inhibitors, allopurinol or febuxostat, prevent the production of 2,8-DHA and hence the formation of crystals and stones [4].

In conclusion, this paper demonstrates the utility and importance of a skilful U-sed examination in the early diagnosis of a potentially severe renal disease, which in the long-term can lead to dialysis or kidney transplant, with the possibility, in the latter case, of a recurrence of

crystalluria and renal function impairment, as different authors have reported [8–10].

A. Day 1. A cluster of typical spherical 2,8-DHA crystals as seen by bright field microscopy. At the bottom right, a large crystal with the same colourless appearance but a different “flower-like” morphology. B. The same crystals as seen by polarized light. C. Day 19. Crystals similar to those seen at day 1, arranged as a nice female ballet dancer. D. The same crystals as seen by polarized light. E–F. A cluster of crystals seen in the same urine sample. Why they have a brownish colour and a birefringence without the “Maltese cross” appearance is not clear at the moment. Original magnification for all images: 400×.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2019.01.026.

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G. Garigali[a], G. Marra[b], V. Rizzo[c], F. de Liso[d], A. Berrettini[e], M. Daudon[f], G.B. Fogazzi[a,*]

[a] *Laboratorio Clinico e di Ricerca sul Sedimento Urinario, U.O.C. di Nefrologia, Dialisi e Trapianto di Rene, Fondazione, IRCCS, Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy*
[b] *U.O.C. di Nefrologia, Dialisi e Trapianto Pediatrico, Fondazione, IRCCS, Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy*
[c] *Dipartimento di Medicina Diagnostica, U.O.C. di Servizio Analisi Chimico-Cliniche, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy*
[d] *Laboratorio di Analisi Chimico-Cliniche e Microbiologia, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy*
[e] *U.O.C. di Urologia Pediatrica, Fondazione, IRCCS, Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy*
[f] *Laboratoire des Lithiases, Service des Explorations Fonctionnelles APHP, Hôpital Tenon, Paris, France*
E-mail address: giovanni.fogazzi@policlinico.mi.it (G.B. Fogazzi).

[*] Corresponding author at: Clinical and Research Laboratory on Urinary Sediment, U.O. di Nefrologia, Dialisi e Trapianto di rene, Fondazione, IRCCS, Ca' Granda Ospedale Maggiore Policlinico, Via della Commenda, 15, 20122 Milano, Italy.