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"Daisy-like" crystals: A rare and unknown type of urinary crystal

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ABSTRACT

Background: Crystals are well known structures of urinary sediment, most of which are identified by the combined knowledge of crystal morphology, birefringence features at polarized light, and urine pH. In this paper, we report on a cohort of subjects whose urine contained a very rare type of crystal, which we first described in 2004 and which, based on its peculiar morphology, we define as "daisy-like crystal" (DLcr).
Methods: Reports on DLcr were spontaneously sent to our laboratory over a 10.5-year period by different laboratory professionals and by one veterinary clinician who, in their everyday work, had come across DLcr. After the examination of DLcr images submitted, a number of other information were requested and partly obtained.
Results: DLcr were found in 9 human beings in 7 different laboratories, located in 4 countries (Italy, Belgium, Croatia, France). DLcr were found mostly in female (8/9), at all ages (3.5 to 93 years), mostly in alkaline urine (pH 6.0 to 7.5), at variable specific gravity values (1.010 to 1.030), either as isolated particles (2/8) or in association with other crystals (5/8) and/or leucocytes or bacteria (3/8). In addition, DLcr were found in the urine of a 1-year-old dog, examined in a veterinary clinic of Czech Republic. In 3 cases, DLcr were identified by manual microscopy, while in 7 cases by automated urine sediment analyzers.
Conclusions: This paper confirms the possible presence in the urine of DLcr. However, further cases are needed to clarify their frequency, clinical meaning, and composition.

1. Introduction

Crystals are well known structures of the urinary sediment, most of which can be identified by the combined knowledge of crystal morphology, crystal birefringence features under polarized light and urinary pH [1–3]. For doubtful cases - due to unusual morphology and/or birefringence features - infrared spectroscopy investigation is also needed [4–5].

In 2004 we reported on an unusual type of crystal in the urine of a pregnant woman, with a "daisy-like" appearance and negative birefringence which, in a review of more than 1530 images contained in 58 Atlases and monographs on urinalysis published between 1844 and 1999, we could find only in two works [6]. In one Atlas, they were described as calcium carbonate crystals observed in the saliva of a dog parotid gland [7], while in the other they were identified as a combination of "creatine and zinc chloride", found in a urine sample [8]. By infrared spectroscopy investigation, we found out that our "daisy-like" crystals (DLcr) contained carbonate, even though the spectrum differed from carbonate salts usually found in urine and other biological fluids [6].

The aims of this paper are to describe a cohort of ten new cases (nine humans, one dog), whose urine contained DLcr, which were

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brought to our attention by seven laboratory professionals and one veterinary, from five European countries, who either had read the paper we had published in 2004 [6] or had contacted our Clinical and Research Laboratory on Urinary Sediment (CRLUs) at Ospedale Maggiore Policlinico, Milano, Italy, for consultation.

2. Materials and methods

The new cases have been collected in the CRLUs after receiving spontaneous reports with images by professionals from different institutions, who had come across, in their everyday practice, crystals similar to those described in 2004 (= roundish structures with a diameter of about 15 to 38 μm, made up of a combination of a variable number of triangles of different width, separated by well defined edges, whose apexes converged towards a central roundish structure containing a central dot, which could be either clear or dark) [6]. After the evaluation of the images, for each case it was asked to supply the following information: gender and age of the subject, reason for urinalysis, urine dipstick findings, the method by which DLcr were first seen, possible associated particles, and clinical information if available.

3. Results

From August 2006 to January 2017, nine human subjects, whose urine contained DLcr, were brought to the attention of CRLUs by seven different laboratory professionals from four different countries (Italy: 6; Belgium, Croatia and France: 1 each) (Table 1). Eight subjects were female and 1 male, their age ranging from to 3 to 93 years (mean ± SD: 53 ± 29.3, median 47). In all subjects but one (subject 6) the results of dipstick evaluation were available. These showed: pH 6.0 to 7.5 (mean ± SD: 6.7 ± 0.5, median 7.0) and specific gravity 1.010 to 1.030 (mean 1.017 ± 0.005, median 1.017). No dipstick abnormalities were found in five subjects, while subject 4 was found to have albumin 1 +; subject 5, nitrites 1 +; subject 7, nitrites 1 +, leukocyte 500/μL, haemoglobin 0.03 mg/dL (normal value for both analytes: absent).

The reason why urinalysis had been performed was available for all subjects but two (subject 4 and 6) (Table 1). In addition, it was known that at the time of urine sediment examination, all seven subjects were taking one or more drugs, two of which (sulfamethoxazole for urinary tract infection, subject 2; sulfasalazine for spondyloarthritis, subject 8) under certain conditions, may induce the precipitation in the urine of crystals made up of the drug itself. However, the morphology of such crystals, as described in the literature, was not at all resembling that of DLcr [9,10]. Finally, it was reported that subject 1 was at the 15th week of pregnancy and the day before urinalysis she had eaten a "large amount" of spinach and other vegetables; subject 3 was at the 20th week of pregnancy and used to follow a diet rich in fruits and vegetables; subject 9 was a regular consumer of "large quantities" of vegetables.

DLcr had been seen by phase contrast manual microscopy (without polarized light) in subject 2 (Fig. 1, left), while in all the other subjects they had been seen first by automated instruments (Table 1): iQ200 (Beckman Coulter Inc., Brea, USA): 4 subjects (Fig. 1, center); sediMAX (A. Menarini Diagnostics, Firenze, Italy): 3 (Fig. 1, right); UF-1000i (Sismex Corporation, Kobe, Japan): 1. Indeed, in this last case the instrument had just reported the presence of "crystals", which were subsequently examined by phase contrast manual microscopy (without polarized light) and found to have a daisy-like appearance. After examination of the urine by the automated instrument, for subjects 5 and 8 manual microscopy was also performed, which confirmed the presence of DLcr for both subjects. The DLcr observed in subject 5 were measured and found to have a diameter of 25 to 30 μm and to be non-birefringent at polarized light, both features being in agreement with our previous observation [6].

In 4 cases (subjects 2,7,8,9) DLcr were associated with calcium phosphate crystals, while in subject 4 they were found together with amorphous granules of unclear nature and bi-hydrate calcium oxalate. Other particles included leukocytes (subjects 4 and 8) and bacteria (subject 5) (Table 1).

For subjects 1 and 3, a new urine sample was examined 10 and 15 days respectively after the finding of DLcr and, in both cases, no crystals were found.

The 10th case was a 1-year-old male crossbred dog, whose diet was very poor in vegetables, and whose urine was examined in November 2015 by one of us (JK, a veterinary clinician practicing at Brno, Czech Republic) for a suspicion of urinary tract infection. Manual urine dipstick (Decaphan Leuco, Erba Lachema, Brno, Czech Republic) performed on fresh and spontaneously emitted urine, showed a pH of 7.0 and a specific gravity of 1.018, without abnormalities. After centrifugation (at 6000 rpm for 3 min), removal of the supernatant urine,

Table 1
Main data of the nine new subjects with urinary DLcr.

Subject	Date/country	Subject		Reason for urinalysis	Urine dipstick	Daisy-like crystal identification	Associated particles
		Gender	Age (years)				
1	August 2006/ Croatia	Female	40	Pregnancy	pH 7.0, SG 1.020 No abnormalities	Automated analyzer (iQ200)	No
2	April 2003/ Italy	Female	3	UTI	pH 7.0, SG 1.020 No abnormalities	Manual microscopy	Starlike calcium phosphate (+ +)
3	October 2011/ Italy	Female	38	Pregnancy	pH 6.5, SG 1.018 No abnormalities	Automated analyzer (iQ200)	No
4	March 2014/ Belgium	Male	19	NA	pH 6.0, SG 1.030 Albumin +	Automated analyzer (sediMAX)	WBC: 2/HPF, amorphous granules (+), bi-hydrate calcium oxalate crystals (+)
5	February 2015/ Italy	Female	77	Endometrial carcinoma	pH 7.5, SG 1.010 Nitrites +	Automated analyzer (iQ200)	Bacteria (+ +)
6	September 2015/ France	Female	78	NA	pH 7.0, SG NA	Automated analyzer (sediMAX)	NA
7	May 2016/Italy	Female	93	Cardiopathy	pH 6.0, SG 1.011 Nitrites +, Leukocytes 500/μL	Automated analyzer (iQ200)	Calcium phosphate crystals (+)
8	December 2016/ Italy	Female	47	Spondylo-arthritis	pH 6.5, SG 1.017 No abnormalities	Automated analyzer (sediMAX)	WBC 5–10/HPF, needle-like calcium phosphate crystals
9	January 2017/ Italy	Female	82	High blood pressure	pH 7.5, SG 1.017 No abnormalities	Automated analyzer (UF1000i) + manual microscopy	Calcium phosphate crystals (+)

Abbreviations. UTI: urinary tract infection. NA: not available. SG: specific gravity; WBC: white blood cells; HPF: high power field.

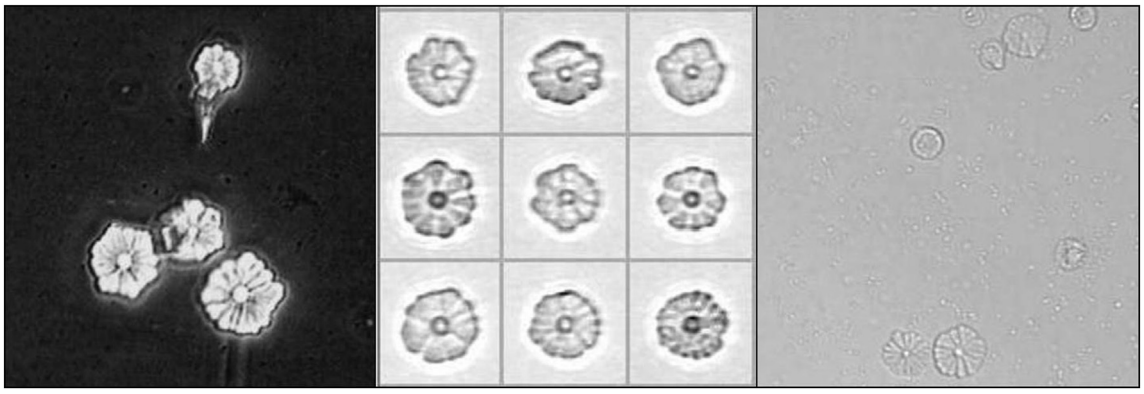

Fig. 1. Left. Subject 2: DLcr by phase contrast microscopy (original magnification 400 ×). Center. Subject 3: DLcr by iQU200. Right. Subject 6: DLcr by sediMAX.

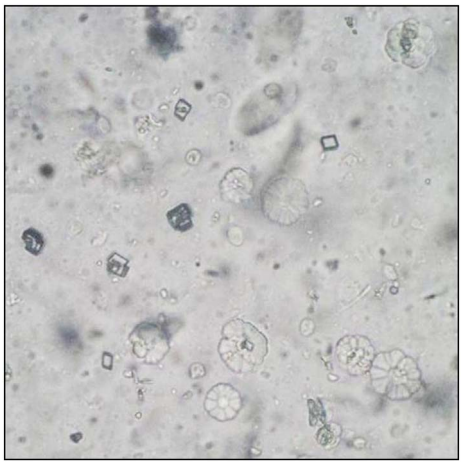

Fig. 2. DLcr, intermingled with other plate-like crystals found in the urine of the dog (bright field microscopy, original magnification 400 ×).

and sediment resuspension, the sample was analyzed by an Olympus CX31 microscope (Shinjuku, Tokyo, Japan) equipped with bright field light but without device for polarized light. The sample examination revealed the presence of 5 to 15 DLcr/high power field (400 ×) intermingled with tiny rhombohedral thin plates, either individual or heaped one upon another (Fig. 2). A new urine sample, examined 5 days later, confirmed the presence of DLcr in the same amount.

4. Discussion

Crystals were the first particles seen by microscopy in the urine [11], and today a wide spectrum of crystals is recognized and described in the Atlases on urinary sediment and in the monographs on urinalysis [1,2,12–18]. The most frequent and known crystals include calcium oxalate (both mono- and bi-hydrated), uric acid, calcium phosphate, triple phosphate, amorphous urates and phosphates. Rare, and therefore less known crystals, include: ammonium biurate, calcium carbonate and hippuric acid, which are considered as devoid of clinical implications; cholesterol, cystine, leucine, tyrosine, and 2,8-dihydroxyadenine which, instead, always indicate either organic or congenital diseases; and crystals due to drugs [1,2,12–18]. The knowledge of the morphological spectrum of each type of crystal, combined with the knowledge of its birefringence features at polarized light and of urinary pH, are clue to the correct identification of crystals. With this approach, the majority of crystals met in the everyday practice can be identified, while for a minority of them some clinical information about the patient and/or infrared spectroscopy are also needed [3–5,9,10].

In this paper we describe a rare type of crystal which we saw for the first time in March 2003 in the urine of a pregnant woman with normal renal function and urinalysis, and a urine pH of 7.5. The crystals had a “daisy-like” appearance and were non birefringent at polarized light. Infrared spectroscopy demonstrated that the DLcr were of unclear nature, containing carbonate ions with a spectrum which differed from carbonate salts usually found in urine and other biological fluids [6].

After the report of that case in 2004 [6], we have been contacted by other laboratory professionals who also have found DLcr in the urine of some subjects, whose urine had been examined for various clinical reasons. Thus we have been able to collect a cohort of nine subjects, which shows that DLcr are found: almost exclusively in female, in subjects with a wide spectrum of ages, mostly in alkaline urine, at different specific gravity values, either as isolated particles or in association with other crystals (calcium phosphate or calcium oxalate) and/or leukocytes or bacteria. In addition, DLcr were also found in the urine of a dog, a fact which demonstrates that they are not confined to human beings. Also of note is the rarity of these crystals, as confirmed by the observation that subjects 3, 5 and 7 were brought to our attention over a 55-month period (from October 2011 to May 2016) by one of us (AR), who works in an Italian laboratory in which about 327,000 urine sediments are analyzed each year (i.e., 1 sample with DLcr/500,000 samples). Finally and interestingly, DLcr could be seen by both by manual microscopy, as it was the case for subjects 2, 9 (Table 1) and the dog, and by two different types of automated urine microscopy analyzers, each one being based on an original system of image capturing [19,20]. This demonstrates that also this approach to urine sediment analysis, when used by motivated persons, can stimulate investigation.

Other points of potential interest in the present cohort, which however do not enable sound conclusions due to fragmentary or missing information are: the possible association of DLcr with a diet rich in vegetables, observed in at least three subjects and in the first case we reported [6]; the possible persistence of DLcr in subsequent samples, which was noticed in the dog (after a 5 day interval) but not in two humans at an interval of 10 and 15 days respectively.

An important aspect which cannot be evaluated with this cohort is the relationship between DLcr and renal function, serum creatinine being available only for one subject out of nine (subject 8: 0.72 mg/dL, 63 μmol/L).

Finally, a crucial point deals with DLcr composition which, based on infrared spectroscopy investigation we performed on our previous case [6], includes a carbonate salt, which differs from that of calcium carbonate (calcite), as shown in Fig. 3, and from other forms of calcium carbonate (i.e., aragonite and vaterite), which may be observed in biological fluids such as pancreatic juice or bile. X-rays microanalysis might be useful to clarify this important aspect, but unfortunately, we have not had the possibility to perform this investigation for any subject of the present cohort.

In conclusion, this paper confirms the possible presence in the urine of human beings and dogs of DLcr, which were first reported 13 years

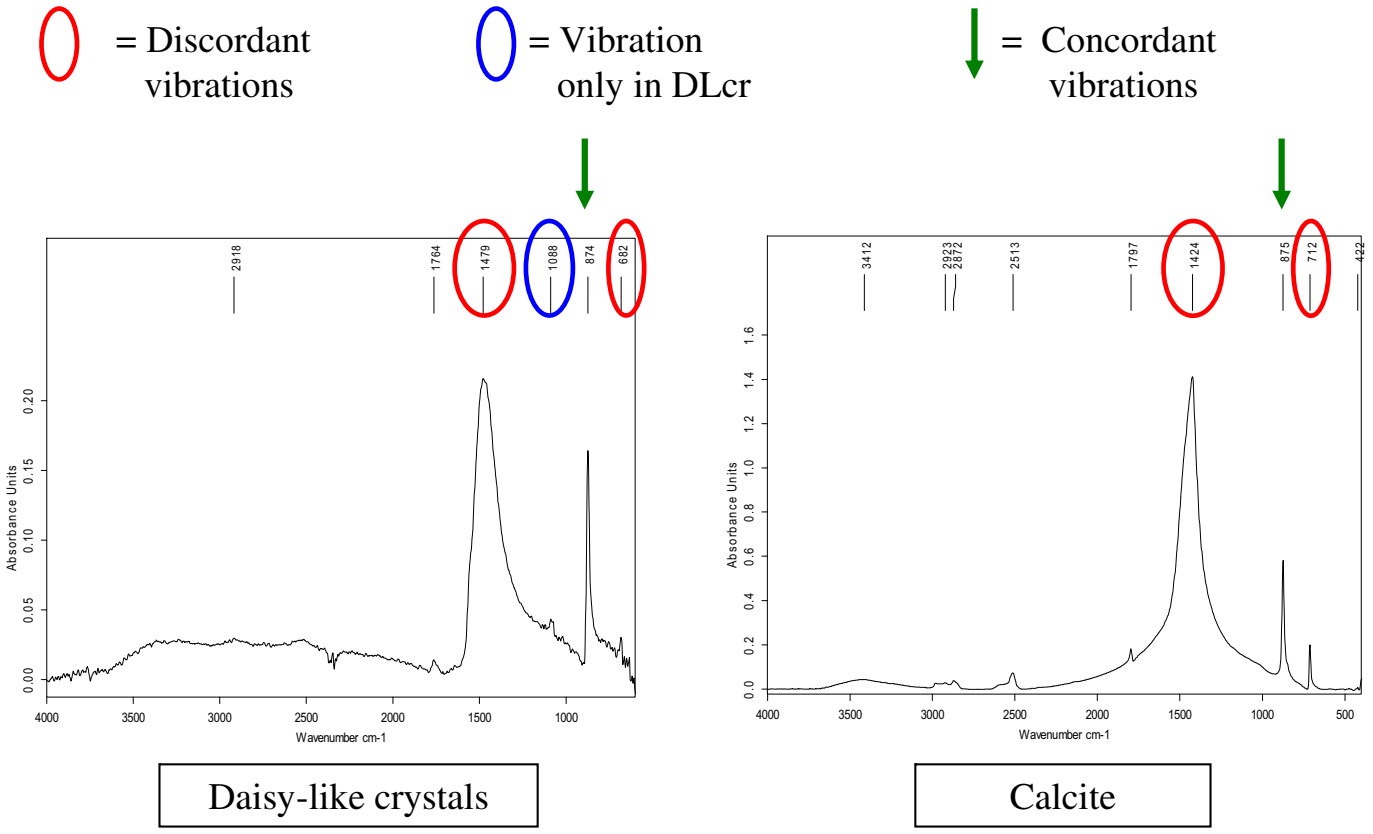

Fig. 3. Left. The infrared spectroscopy of DLcr found in the patient described in 2004 (from Fig. 2 of: Fogazzi GB, Baroni S, Garigali G, Daudon M. An unusual type of crystalluria (appearing only once every 130 years?). Nephrol Dial Tranplant 2004; 19: 1907–9) [6]. Right. The infrared spectroscopy of calcium carbonate (calcite). On the spectrum of DLcr, the $\nu 3$ and $\nu 4$ vibrations of the CO_3 group are located at 1479 and 688 cm^{-1} respectively in place of 1424 and 712 cm^{-1} for calcite. Moreover, the $\nu 1$ vibration was observed at 1088 cm^{-1} for DLcr while it is not visible in IR mode for calcite. The unique vibration shared by the two carbonates, DLcr and calcite, is the $\nu 2$ vibration of the CO_3 group, located at 874 cm^{-1} for DLcr and 875 cm^{-1} for calcite.

ago [6]. However, further cases are needed to clarify their frequency, clinical meaning and, above all, accurate composition.

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