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Review

Clinical value of crystalluria and quantitative morphoconstitutional analysis of urinary calculi

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HIGHLIGHTS

- The investigation of crystalluria is a tool for the detection and the monitoring of inherited and acquired diseases.
- Morphoconstitutional analysis of calculi is often very useful for identifying the cause of urolithiasis.
- Stone morphology may be of a valuable help for the early detection of severe lithogenic conditions.

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ABSTRACT

Crystalluria is a marker of urine supersaturation with substances deriving from metabolic disorders, inherited diseases or drugs. The investigation of crystalluria must be done according to a protocol which includes the delivery to the laboratory of a proper urine sample, the use of a microscope equipped with polarized light, the accurate knowledge of urine pH, and a comprehensive examination of the crystals, which is based on their identification, quantification and size measurement. For unusual crystals, infrared spectroscopy may also be needed.

If the formation of stones is always preceded by crystalluria, the reverse is not true. In addition to the crystalline composition, stone morphology provides valuable information on stone activity and, for some crystalline species, major information regarding the underlying pathology. Fourier transform infrared spectroscopy (FTIR) reliably identify specific forms of nephrolithiasis, as common-type stones made of calcium oxalate (CaOx) and/or calcium phosphate that is combined with morphology classification; using this method, stones may be classified into 6 types subdivided in 22 subtypes.

The investigation of crystalluria is an inexpensive and valuable tool for the detection and the monitoring of inherited and acquired diseases associated with urinary stone formation or acute or chronic renal function impairment from intrarenal crystal precipitation.

Selective FTIR identification of the composition of core (or the umbilication), middle part, and surface of every stone allows identification of the initiating lithogenic process (in the nucleus or in the Randall's plaque) and the factors which subsequently contributed to stone growth. In conclusion, the proposed morpho-constitutional method of urinary stone analysis, which moreover is rapid and low cost, provides clinically relevant orientations for targeted etiologic evaluation.

1. Introduction

Urine is an environment with constant supersaturation of one or more crystalline species; so it is not unusual to find crystals. Consequently, some authors consider this an unnecessary examination [1]. Crystalluria is an imbalance between promoters and inhibitors; the first are the engine of the crystal formation and the latter (made up of substances of low or high molecular weight) have physicochemical properties opposed to one or more steps of the crystallization process. The rupture of equilibrium may be due to an excessive concentration of the promoters or a defect of inhibitor concentration or change in their molecular structure [2]. A change in the ionization state of promoters or inhibitors, especially under the influence of urine pH, plays an essential role both in creating this disequilibrium, and also in its restoration by medical

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treatment. The characteristics of crystalluria may be different in subjects at risk of renal stones compared to controls [3,4]. Urinary calculi result from a long-term exposure to supersaturated urine. Because the urine composition is constantly varying according to dietary and drinking habits, the stones may contain several components, some of them being often found in very low proportion. In other cases, the pathological conditions responsible for stone formation may induce urine supersaturation regarding several chemical compounds that may crystallize simultaneously in urine and that will be present together in the stone. Thus, it is clinically relevant to identify stone constituents and provide semi quantitative evaluation of their respective proportions within a stone. Among physical methods, X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) are now universally used for stone analysis. However, when XRD or FTIR analysis is performed on a powdered sample of the whole stone, no indication is given as to the respective location (core or peripheral layers) of the diverse constituents in the case of mixed stones, even if the stone is large enough to allow a separate analysis of its different parts. Today, chemical methods are yet extensively used for stone analysis. Indeed, chemical methods fail to identify rare purine stones resulting from genetic disorders such as 2,8-dihydroxyadenine [5,6] or drug-induced calculi [7,8]. Moreover, they are unable to provide reliable information on crystalline phases present in calcium stones. Only physical methods are able to identify such a diversity of components [9,10].

If the formation of stones is always preceded by crystalluria, the reverse is not true and crystalluria may occur without resulting in stone formation. Therefore, the clinical relevance of crystalluria in kidney stone formers remains largely debated. Although some authors proposed crystalluria as an index of stone disease activity in the early seventies [3,11], routine search for crystalluria has not gained widespread popularity in clinical practice and is not currently recommended in the evaluation of stone formers. Indeed, because crystalluria is occasionally found, in 15%–20% of healthy subjects, presence of crystals in a single urine specimen was regarded as not discriminating between stone formers and non-stone formers [1,12].

Since 1984, we performed routinely a search for crystalluria, simultaneously with full blood and 24-h urine biochemistry, at each visit in all patients referred to our stone clinic. We subsequently recorded all laboratory data, together with stone episodes, in our stone formers patients over the past two decades. We were thus able to analyze the relationships between serially determined crystalluria and laboratory parameters and recurrence of stone episodes in the cohort of consecutive patients who were referred to us after they had formed one or several stones.

2. Crystalluria

2.1. Analytical aspect

Many techniques have been published sometimes with the use of inaccessible design in clinical practice: electronic scanning microscope [13], particle counter [3], urine filtration [14], evaporation [3,11] or centrifugation [15].

As part of clinical research, these techniques have provided useful information for the understanding of the phenomena involved in the crystal formation and the physicochemical characteristics of the crystals, and the difference between normal subjects and stone formers. However, the majority of these methods are not applicable in clinical practice. Optical microscopy examination with polarized light is the most suitable and most informative technique. It is usable by all laboratories that perform microscopic examination of urine [15,16].

2.2. Laboratory procedures

Based on the common observation that urine produced during the night is usually the most concentrated, therefore carries the highest risk of supersaturation and crystal formation, we performed all crystalluria studies in fresh first-voided morning urine samples, according to a uniform protocol described elsewhere [15]. In short, urine samples brought to the laboratory within 2 h of voiding were kept at room temperature and were rapidly processed. Urine-specific gravity and pH were measured. Undiluted urine was then homogenized by gentle shaking and turning over (neither centrifuged nor filtered) and immediately placed in a Malassez cell (CML, Nemours, France) containing 10 mm^3, then examined by light microscopy using a polarizing microscope (Optiphot-2) (Nikon, Champigny-sur-Marne, France). The entire cell was examined at $\times$200 magnification to localize crystals and aggregates, then at $\times$400 magnification (high power field). All crystals and aggregates were counted on the entire cell and their size determined using the included micrometric scale. The results were expressed as number of crystals per mm^3. Only one Malassez cell was examined in each instance. Crystalluria examinations were performed in a “blind” manner (i.e., without knowledge of the clinical status of the patients and of the results of laboratory determinations). The mean number of crystalluria studies beyond the baseline one was 6.8 $\pm$ 5.3 per patient, with a median of five determinations (range 3–33). We defined as “crystalluria index” the ratio of the number of urine samples with crystalluria to the total number of examined urine samples. This marker was proven to be clinically relevant for assessing the risk of stone recurrence in calcium stone formers [17]. Of note, in this study, we did not consider small calcium phosphate grains less than 2 μm in diameter. The average value of all 24-h urine biochemistry parameters was used in the follow-up period.

In the case of crystalluria studies performed in order to identify genetic diseases or drug crystallization explaining an acute renal failure, we can spin the urine as we seek to detect and identify specific crystals, particle counting being less relevant in such conditions.

2.3. Clinical aspects

Clinical interpretation of crystalluria must integrate different criteria that may apply to certain species. These criteria are: chemical nature of the crystals, crystalline nature, crystal form, aggregation rate, crystal size, crystalluria frequency. Using these criteria involved recognition of crystals observed by their morphological characteristics and appearance in polarized light, and considering the pH of urine. The crystals attached boards can help the identification.

2.3.1. Importance of chemical nature for unusual crystals

For diagnostic, some crystals are significant because of their presence alone:

- cystine, which reveals a congenital cystinuria;
- dihydroxyadenine resulting from a defect of adenine phosphoribosyltransferase;
- Orotic acid salt as a marker of uridine monophosphate synthetase deficiency;
- xanthine which is observed in homozygous xanthine deshydrogenase deficiency or in the treatment of Lesh-Nyhan syndrome by high doses of allopurinol;
- leucine met in leucinosis or Hartnup's disease;
- tyrosine met in tyrosinosis or some serious liver diseases;