Mineralogical and morphological investigation of kidney stones of a Mediterranean region (Basilicata, Italy)

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Abstract. Kidney stones are a very frequent finding in southern Italy, but stone analysis is not routinely performed. However, it is an important requirement not only for a successful treatment of this disease, but also for the prophylaxis to prevent any recurrence.

We therefore set out to analyze 80 kidney stones collected from Basilicata Region (Southern, Italy). X-ray powder diffraction as well as optical and scanning electron microscopy and petrographic thin section analysis have been applied in order to determine the mineralogical and morphological compositions.

The internal structure and the relation between major and minor components have facilitated the classification of the kidney stones according to a detailed scheme.

As it is known from other country, calcium oxalate stones were the most frequent (59%) [39% mainly composed of whewellite, CaC\textsubscript{2}O\textsubscript{4}•H\textsubscript{2}O and 29% is mainly made of weddellite, CaC\textsubscript{2}O\textsubscript{4}•2H\textsubscript{2}O]. Uric acid stones were abundant too (18%). Calcium phosphate and cystine stone were less.

The results of the observations of kidney stones in thin petrographic sections led to the identification of more cores in the same whewellite kidney stones. In some kidney stones the core is not situated in the central zone, which represents the point of attachment on kidney wall.

Basilicata kidney stone prevalence is different from the average prevalence determined in other Mediterranean areas. The comparison showed that calcium oxalate stones seem to be more frequent, and there is a high prevalence of uric acid kidney stones and a lower prevalence of phosphate kidney stones, especially hydroxyapatite.

The relative increase frequency of uric acid stones in the northern part of the Region may be due to high-purine diets and softness water consumption.

Keywords: Kidney stone, mineralogical composition, type of stones, medical geology, geographical distribution

1. Introduction

The mineralogical approaches can give significant contributions to the study of pathological mineral deposits in the human body. Nephrolithiasis benefits greatly from this investigation because kidney stones are mineral concretions containing one or more different crystalline components. The compositional...
analysis is an important requirement not only for a successful treatment of this disease, but also for the prophylaxis to prevent any recurrence.

There are many useful techniques for gathering information on major and minor kidney stone components such as infrared spectroscopy [1,2], chemical analysis [3,4], X-ray powder diffraction [5,6] and electron microscopy [7]. Despite the many results achieved with using all these techniques, little attention was paid to the relation between the components and the crystalline structure of the stone. In fact, all the previous classifications were based only on chemical data (oxalate, phosphate, urate and cystine) [5,8–10], and can hardly be compared with modern morpho-chemical approaches [11,12].

The lack of a regional and national epidemiological research activity has made it necessary to extend our knowledge of the diagnosis and the treatment for kidney stones since the social costs to cure nephrolithiasis are progressively increasing. According to the Europe statistics, mainly taken from the National Health Care Reimbursement Fare, showed that the costs for shock-wave lithotripsy ranged from €360 to €2,740 [13]. In 2004 the Basilicata residents hospitalized for nephrolithiasis were 1,349 and the hospital cost was estimated to be approximately €1,789,730,00 [14].

Unfortunately, very few studies dealing with stone composition have been published in Italy [15–18]. Stone analysis is not routinely performed in Basilicata, although stone composition itself could give fundamental information.

All this has encouraged a regional study in order to determine the type of kidney stones affecting the Basilicata inhabitants. This study has been conducted on a qualitative level establishing (i) a relative occurrence, (ii) phase compositions and (iii) the fine inner structure of different types of kidney stones.

2. Materials and methods

All the patients hospitalized for nephrolithiasis at the San Carlo Hospital (the main medical facility in Basilicata) from January 2007 to January 2008 were selected as donors and involved in this study.

A variety of surrogate markers are used to estimate stone prevalence, including hospital discharges, physician office visits, emergency room visits, or procedures related to a primary diagnosis of stone disease, or a self-reported history of stones [19,20]. All of these surrogates are compromised in that they likely underestimate the prevalence of disease either because a patient may pass a stone without requiring any health care resources or because a patient may report a history of stones based on unsubstantiated symptoms or not report a history of stones because a stone was never collected despite classic symptoms of renal colic.

The prompt character of this study underestimates the real kidney disease prevalence because only the hospitalized cases of kidney stones were included, but this does not influence the distribution prevalence, goal of our study.

We collected more than 100 kidney stones either expelled or extracted surgically from 80 patients. In order to avoid a selection bias, more than one stone from the same patient was studied and an average composition was assigned. The stones were first fragmented and then observed to establish their original form and internal structure.

The 12-to-79-year-old patients were of both genders, but 56% of stones developed in male patients. Further information was gathered from questionnaires submitted to patients asking for personal details (such as gender, age, marital status, address and educational level), socio-economic (kind of job, etc.) and medical history data (weight, height, personal and family anamnesis, dietary habits, etc. . . ).

All the kidney stones collected were dried in a dissector at room temperature for 2 weeks; then the dry samples were photographed.
Optical observations were carried out by a stereomicroscope to determine colour, shape, overall appearance, surface features and any possible occurrence of crystalline layer and/or organic matter on the surface. Special attention was paid to identify the site of stone attachment to kidney wall.

Thin sections of kidney stones embedded in a resin, which consisted of 30 μm-thick sections mounted on glass slides, were used following the typical methods adopted for a petrographic study of rocks and minerals [21]. Such petrographic observations also enabled an evaluation of the geometric arrangement and optical continuity of crystals.

During stereomicroscopy observations a plane passing as close as possible to the geometric centre of a kidney stone was recognized in order to cleave the stone into two fragments. Then one or more fragments which better displayed the structural features were observed under a high-magnifying Leica 440 Stereoscan Scanning Electron Microscope (SEM). Some fragments were mounted on an aluminium stub using a double sticky tape, and then coated with gold with a sputtering technique. Thin sections were also mounted on the stub and coated with carbon. A prolonged use of SEM to study the details of calcium oxalate kidney stones caused the desiccation of the area under study. Therefore many observations on the texture details of thin sections were made by a polarized light microscope. Half of the sample was used for XRD analysis. Kidney stones were crushed in an agate mortar in order to obtain a fine mesh powder for X-ray diffraction, so as to obtain the crystalline phase composition of the kidney stones.

The XRD measurements were performed with a microdiffractometer D/max RAPID Rigaku, using a CuKα tube working at 40 kV, 30 mA. The qualitative interpretation of the diffractometric profiles was achieved by comparing the peaks of reflection with those in the PDF card (Powder Diffraction File) [22]. Cell parameters were determined using an open-source software Unitcell [23], which makes it possible to refine the mineral cell parameters from the diffractometric profile, based on the method of nonlinear least squares.

3. Results and discussion

3.1. Mineralogical stone analysis

On the basis of the search match analysis performed on the Powder X-ray diffraction data using PDF card, constituents of all the stones have been determined.
Figure 1 shows diffractograms of 7 stones from different patients. It may be noted that all of the 7 diffractograms are similar and consist of sharp peaks. These peaks, labelled as 1 and 2 in the Fig. 1, match well with the diffractogram of the standard sample of whewellite and weddellite phase, respectively, suggesting that constituent of all of the 7 samples correspond to mixed stone with whewellite and weddellite phase. It may be noted that the positions of the peaks ($^\circ 2\theta$) corresponding to CaOx phases in all of the 7 diffractograms match very well with each other, however, intensity ratios of these peaks vary from sample to sample, indicating variation in quantity of the two phases.

They clearly show the presence of mixed constituents containing well-organized phases.

The results show that the most kidney stones studied had calcium oxalates as major or minor component, with a prevalence of whewellite (Fig. 2).

Although oxalates are the most abundant phases, are more often associated with other phases. They are, however, more frequent stones formed entirely of uric acid or cystine.

18% of stones were found to be multi-composed: weddellite plus hydroxyapatite, weddellite plus struvite and whewellite plus uric acid.

During the mineralogical analysis we recognized in 6% of the total stones analyzed the presence of a new mineralogical assemblage consisting of weddellite plus struvite [24] not included in the classification of Grases et al. [12,25].

The cell parameters calculated for the detected mineral phases were compared with the theoretical parameters, and some slight differences were found at a significant level of 95% (Student t-test).

3.2. Stereomicroscopy and SEM observations of the kidney stones

The size of the stones varied from a few millimetres to a few centimetres. The biggest stone (size, 2.0 × 1.0 × 1.0 cm) was developed by a female patient (58 years old) as a mixed calcium oxalate/uric acid stone.

Stereomicroscopy analyses showed that all samples varied from a subspherical to an irregular shape and had superficial deposits mainly composed of organic matter which is recognizable for its dark colour.

Calcium oxalate stones were found to be the most frequent, both whewellite and weddellite.

Weddellite crystals are easily identified from their dipyramidal shape (Fig. 3a) and typical yellow/white colour. We found that some of the stones analyzed were made of a single large weddellite crystals.
Fig. 3. Weddellite stones: a) dipyramidal crystals; b) one single weddellite crystal; c) weddellite plus hydroxyapatite; d) particular view of weddellite crystal dissolution and its mark on hydroxyapatite, in petrographic thin section.

Fig. 4. Texture of columnar whewellite crystals growth (polarized light). b) Detail of columnar whewellite crystals growth on different cores made of organic matter and small whewellite crystals. Whewellite kidney stone with a decentrate core, optical microscopy observation (c) and thin petrographic sections (d).

(Fig. 3b), in other cases entire weddellite crystals dissolved either losing their mark on the stone structure (Fig. 3c-d) or transforming into whewellite the most thermodynamically stable form [12]. This pseudomorphism begins at the centre of weddellite crystal and works outward. Well-formed subhedral to euhedral whewellite crystals were observed in the interior portions of large weddellite crystals [24]. The surfaces of weddellite crystals are rarely replaced, probably because the contact with the aqueous environment of the urinary system maintains the stability of weddellite [5]. Whewellite pseudomorphosis was observed in weddellite crystals with bigger dimensions with respect to pure weddellite crystals ($p < 0.005$).

Kidney stones made of weddellite crystals are arranged in a rosette-like form. The surface crystals were generally found with a well-developed crystal faces. In these stones, within coarse surface crystals, a moderately developed porosity was observed, often filled with fine hydroxyapatite spherules or struvite.

Kidney stones composed of whewellite form small spheroids, with minor surface irregularities. The interior appeared homogenous and consisted of closely packed fine whewellite crystals creating concentric laminations to form a rhythmic structure (Fig. 4a-b).

The kidney stone cores appeared to consist of fine whewellite crystals and organic matter. The position
of the cores can vary and is synonymous of a different growth mechanism. In some kidney stones the core is not situated in the central zone and it is made of an organic matrix and small whewellite crystals [26]. A peripheral core involves the development of a stone with a hemispherical concave surface which represents the point of attachment on kidney wall (Fig. 4c–d). In others cases it is located at the centre, which means that kidney stones have grown up in the kidney cavities.

In both cases the external morphology of stones reflects the internal structure. This is more evident for calcium oxalate monohydrate stones; whereas uric acid stones may have the same external shape but can have compact or disordered internal structures (Fig. 5).

The results of the observations of kidney stones in thin petrographic sections led to the identification of more cores in the same whewellite kidney stones (Fig. 6).

The spherulitic aggregates are widely present in minerals and have been widely studied in the past [27–29]. Spherulites are the best crystals form adapted to non-equilibrium growth conditions such as in a urinary solution. Growth conditions include a viscous crystallization medium, a low crystallization temperature, a high degree of supersaturation promoting and a high rate of crystal growth which, in turn, cause both the mechanical capture of inclusions and the development of structural impurities [30]. For calcium oxalates, high supersaturation favour weddellite crystallization first which becomes whewellite during aggregation process. These growth steps enable the fusion of the aggregates (Fig. 6A1).
Kidney stones are likely to be the result of multiple simultaneous processes of nucleation and growth. The chromatic differences observed correspond to the microrytms of growth where small whewellite crystals and organic matter become the core of a new growth (Fig. 3). This type of structure gives the whewellite stones a hardness that makes them more difficult to be fragmented. In fact we identified a high percentage of recurrence among people who developed this type of stones treated by Shock Wave Lithotripsy. The serious consequence of fragmentation not performed correctly is the inability to spontaneously expel fragments (as too large) which remain inside the cavity and may function as substrate for a new crystallization [31,32].

Structural analyses make it possible to appreciate the porosity of different types of kidney stones. There is the misconception that porous structures correspond to softer kidney stones, blurring the hardness of each mineral with the hardness of its aggregates. In the case of most calcium phosphates, despite a hardness of 5 on the Mohs scale, the phosphate kidney stones are among the least hard to be destroyed by lithotripsy techniques. Macroporosity was not usually observed in stones, but small pores were present in some mixed stones where different mineral phases were in contact. The type of the mineral affects the porosity. Stones that contain weddellite are porous as well as those made of struvite.

Struvite, another mineral entirely constituting some of the stones analyzed, belongs to the group of phosphate so it was difficult to recognize the single struvite crystals at the stereo-microscope, because they appear white and look similar to the other minerals of the same group. Struvite stones are recognizable thanks to their large size and coral form. Despite their porosity, these stones were among the heaviest samples (average weight 155 mg) also because struvite stones use to have a bigger size than others.

Cystine stones are easily recognizable for their appearance: round form and yellow wax colour. The crystals are recognizable by SEM and in thin section by their hexagonal shape. For this type of samples a compact internal structure without porosity was recognized.

3.3. Etiological factors

56% of the patients involved in the study developed a single intact or fragmented kidney stone, while the remaining 44% expelled several stones simultaneously.

This may indicate that the process of biomineralisation is not unique or exclusive, but can occur simultaneously to other processes on the same site and from the same urinary solution. Even assuming that the different samples have been generated at different times, their genesis does not interfere or influence the other stones already formed.

56% of the analyzed stones were developed by men, thus confirming their increased susceptibility. The 41-to-60-year-old group was found to be the most affected. By analyzing the questionnaire the physical predisposition to nephrolithiasis could also be assessed, and it was seen that both obese people and those who are overweight (based on the value of body mass index) are more affected by nephrolithiasis.

The most significant socio-economic information given by the patients (type of work, sport activity) and their medical histories (personal and family medical history, dietary habits) showed no clear evidence of the heredity of the disease or a clear link with other diseases. An excessive sweating and insufficient physical activity are common features among patients, and most patients had already suffered from stones (high percentage of recidivism). This is statistically significant (99% confidence).

Struvite stones were expelled exclusively by females (age > 55 years), who are historically speaking more susceptible to this type of stones. The only cystine stone found belongs to a 47-year-old woman. The development of these stones is mainly related to hypercistinuria which is due to genetic features, as in this case, which shows a genetic predisposition to the disease.
An abundance of uric acid stones could be observed only in male patients (86% of cases). The most determinant risk factor for the crystallization of uric acid is the presence of a strongly acidic environment (pH < 5.5) in which uric acid crystals cannot dissolve [33]. In fact, acidic urinary pH decreases uric acid solubility and consequently favours crystallization of uric acid.

All this together with hyperuricosuria is strictly connected to dietary habits and the type of water ingested. An excessive intake of animal proteins [34–37] was found in overweight patients who also showed high alcohol consumption [38]. The lack of soft drink intake as well as the use of weakly bicarbonate water (bottled or not) do not promote urine alkalisation that is necessary to dissolve uric acid crystals, that are the easiest type of stones to prevent and treat because are easily soluble in vivo.

For calcium oxalate stones, the focus has been placed on the potential sources of oxalate. Some of these are produced directly by the human body and the amount produced is directly proportional to body weight [39]. A direct relation between diets rich in oxalates and hyperoxaluria is well-known [40,41].

According to the questionnaire submitted the consumption of foods containing oxalates (e.g. spinach and beets), was low. Unfortunately, the number of vegetable containing oxalates was very large, so this result may depend on a lack of information in the questionnaire.

The lower amount of oxalates ingested may however be offset by a reduced calcium intake among patients, confirming the hypothesis made by some authors about the antagonism between calcium and oxalate. The urinary excretion of the latter could increase only when the calcium is present in a lower quantity [43–45].

An increased intake of mineral waters causing a pH growth seems to be associated with oxalate stones mixed with percentages of phosphates. For all the cases reported there is a low consumption of liquid, which does not reach 2 L/a day, to ensure a high urinary volume [46,47]. Among people with calcium oxalate stones, there is an average excess body mass especially in cases of whewellite stones formed in kidney cavities.

3.4. Classification of Basilicata stones and geographical distribution

The internal structure and the relation between the major and minor components provided information to classify the studied kidney stones according to the latest classification proposed by Grases et al. [12, 25]. This classification was modified, as indicated in Table 1, lacking a suitable group for the new type of stone composed of weddellite and struvite. We added a new group labelled as 4*, seeing the similarity with the already existing group 4. This group will be subdivided into two subtypes depending on the amount of struvite [24].

We decide to assume that hospitalization rates tally with stone prevalence rates. So, the prevalence percentage of Basilicata stones has been calculated (Fig. 7).

As is known from other country, calcium oxalate stones were the most frequent (59%) [39% mainly composed of whewellite, CaC$_2$O$_4$*H$_2$O and 29% is mainly made of weddellite, CaC$_2$O$_4$*2H$_2$O]. Uric acid stones were abundant too (18%). Calcium phosphate and cystine stone were less.

We found difficulty in comparing our results with the previous studies since they used old classifications [11,12] to distinguish only three categories: oxalate, phosphate and urate [5,8–10] instead of seven types of stone using morphoconstitutional information, as in this study. Therefore, our results were compared with the prevalence found following the studies performed only by two authors in other communities such as the Balearic Islands [25] and France [48], in order to identify similarities and differences correlated with different food habits and various environmental influences.

Basilicata kidney stone prevalence is different from the average prevalence determined in other areas [25,48]. The comparison showed that in Basilicata calcium oxalate stones seem to be more frequent,
and there is a high prevalence of uric acid kidney stones and a lower prevalence of phosphate kidney stones, especially hydroxyapatite (Fig. 7). This abundance is not unusual for Italy [17,18].

The relative increase frequency of uric acid stones in the northern part of the Region may be due to high-purine diets and softness water consumption, in fact municipalities with a prevalence of uric acid stones are supplied with soft drinking water. All the patients with uric acid stones also use bottled water with low bicarbonates content (150–200 mg/L) and low hardness (2–7°F). These characteristics prevent the dissolution of uric acid because they do not favour an alkaline environment.

Another risk factor related to the development of stones, especially uric acid ones, is the temperature. A relation between low temperatures and the development of stones is possible because low temperatures, not enticing for frequent consumption of liquids, determine a lower urine volume.

The greater oversaturation due to a reduced urinary volume is also considered as a cause of uric acid stones spread in areas with low average annual temperatures. It has been observed that the rate at which stones are submitted to the laboratory varies during the year, a small number of stones are expelled in

### Table 1

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Description</th>
<th>TYPE</th>
<th>Description</th>
<th>SUBTYPE</th>
<th>Description</th>
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<tr>
<td>1</td>
<td>Calcium oxalate monohydrate (whewellite) - papillary kidney stone</td>
<td>1a</td>
<td>core constituted by whewellite</td>
<td>1al</td>
<td>core constituted by organic matter</td>
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<tr>
<td></td>
<td></td>
<td>1b</td>
<td>core constituted by hydroxyapatite/organic matter</td>
<td>1bl</td>
<td>core constituted by hydroxyapatite</td>
</tr>
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<td></td>
<td></td>
<td>2a</td>
<td>core constituted by whewellite + organic matter</td>
<td>2b</td>
<td>core constituted by hydroxyapatite + organic matter</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2c</td>
<td>core constituted by uric acid</td>
<td>3a</td>
<td>weddelite only</td>
</tr>
<tr>
<td></td>
<td>Calcium oxalate dihydrate (weddelite)</td>
<td>3a</td>
<td>without transformation in whewellite</td>
<td>3bl</td>
<td>core constituted by hydroxyapatite</td>
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<tr>
<td></td>
<td></td>
<td>3b</td>
<td>hydroxyapatite in small quantities</td>
<td>3bl</td>
<td>containing little amounts of hydroxyapatite among weddelite crystals</td>
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<tr>
<td></td>
<td></td>
<td>3c</td>
<td>papillary</td>
<td>3bl</td>
<td>containing little amounts of hydroxyapatite and organic matter among weddelite crystals</td>
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<td>4a</td>
<td>alternative weddelite/hydroxyapatite layers</td>
<td>4b</td>
<td>disordered weddelite/hydroxyapatite deposits</td>
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<tr>
<td></td>
<td>Weddelite + Struvite mixed stone</td>
<td>4b</td>
<td>alternative weddelite/struvite layers</td>
<td>4c</td>
<td>disordered weddelite/struvite deposits</td>
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<td>Hydroxyapatite</td>
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<td>5b</td>
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<td>Struvite</td>
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<td>papillary stone</td>
<td>6b</td>
<td>unattached (no papillary) stone</td>
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<td>urates</td>
<td>8aII</td>
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<td></td>
<td>8aIII</td>
<td>disordered anhydrous/dihydrate uric acid deposits</td>
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<td>Whewellite + uric acid mixed stone</td>
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<td>papillary stone</td>
<td>9b</td>
<td>unattached (no papillary) stone</td>
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<tr>
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<td>Cystine</td>
<td>11a</td>
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<td>11c</td>
<td>artefacts</td>
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</table>
the hot summer, thus suggesting that there is no link with high temperatures.

In these areas there is also a lower total annual radiation than in the rest of the region. And it is well-known that an increased exposure to sunlight stimulates the production of vitamin D which, in turn, promotes the absorption of calcium in the body. Calcium is the most abundant cation involved in the processes of crystallization of stones, especially of oxalates and phosphates.

4. Conclusions

This is the first Italian study that takes into account a very detailed classification of kidney stones introduced in 2002 by Grases et al. [25].

This study emphasizes the importance of stone analysis in patients with nephrolithiasis, in order to avoid any recurrence or a potential progression towards a chronic kidney failure. Preferably, stone analysis should have been conducted by using one or more additional methods.

In Basilicata region there is a high prevalence of oxalate stones, and an unusual higher prevalence of uric acid respect to other European communities. The geographical distribution of uric acid patients allows correlating their prevalence with some environmental and dietary factors.

The detailed compositions of stones should be kept in mind when dealing with patients affected by stones, especially for the prevention and prophylaxis of stone recurrence.

Dietary intervention on a large scale and health education in this regard may be helpful as far as prevention is concerned.

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