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A Comparative Study of Two Renal Stone Analysis Methods

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Abstract

Background and Aims: The scanning electron microscopy (SEM) and Fourier transform infrared spectrophotometry (FTIR) methods are particularly useful for the analysis of renal calculi due to their simplicity and the information they provide, and the results can guide therapeutic approaches and prevention of recurrence. The aim of this study was to compare SEM and FTIR as methods for renal calculus analysis.

Methods: Analysis of renal calculi from the Tadla-Azilal region (Morocco) were performed by optical stereomicroscopy, followed by FTIR or SEM. The results obtained by the two methods were compared.

Results: Both methods clearly showed a predominance of calcium oxalate monohydrate (COM; 54%) in renal calculi from the study region, while calcium oxalate dihydrate calculi represented only 13.5%. Purine calculi were almost as frequent as phosphate calculi (24.3%), and struvite calculi comprised 8.1%. The high prevalence of COM calculi could be the result of several factors including sedentary lifestyles and eating habits, the latter involving a major contribution to oxalates from frequent consumption of tea.

Conclusions: The results showed good agreement between the two methods, which provided appropriate information on the composition and crystal structure of renal calculi.

Keywords: Renal Stone Analysis, Scanning Electron Microscopy, Fourier Transform Infrared Spectrophotometry, Etiology, Composition Prevalence

Introduction

Recurrence of renal calculi appears to have increased in recent years despite the development of more effective surgical techniques (1, 2), emphasizing the importance of laboratory analyses of urinary stones. Investigation of urinary calculi has been an important part of clinical chemistry for more than a century (3). The great majority of routine clinical laboratories only perform a qualitative analysis of the calculus by means of wet chemistry kits or infrared spectroscopy of the whole pulverized calculus, omitting details concerning minoritary components,

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percentages of different compounds or internal structure. The information obtained with wet methods or infrared spectroscopy is valuable on setting up therapeutic advice but is not valid for guidance on the concrete etiological causes of the lithiasis (4). At present it is accepted that no single method provides total information on the structure and composition of the stone, and at least two different methods have to be combined for accurate study of calculi. We report here the results of an initial study of 37 cases of renal calculi in the Tadla-Azilal region, Morocco, based on analyses using stereomicroscopy and Fourier transform infrared spectrophotometry (FTIR), or scanning electron microscopy (SEM) coupled to X-ray micro-analysis.

The objective of this study was to compare SEM and FTIR as methods for renal calculus analysis, and to study the etiological profile of urolithiasis in the Tadla-Azilal region, Morocco, through analysis of 37 cases of renal calculi.

Materials and Methods

Sample Preparation

Sample collection was conducted through the Urology Department of the Beni Mellal regional hospital and private clinics during the period October 2006-June 2007. Renal calculi were extracted surgically or obtained following spontaneous expulsion. As a consequence of the limited number of samples, we only consider two patient age groups: 20-50 years of age and >50 years of age.

The samples were cleaned, dried and stored under ambient conditions until analyzed. Initial observations were made using optical stereomicroscopy to assess the presence of papillary umbilication, indicating the presence of Randall's plaque. The surface characteristics were evaluated for color, texture, crystallinity, size, presence of layers, homogeneity, presence of foreign bodies, and any other notable features. The calculus was then cut longitudinally through the core to yield two symmetrical sections for examination by optical stereomicroscopy, FTIR and SEM.

Analysis of Samples

Analysis by FTIR (Bruker Vector 22, Bruker Optics, Ettlingen, Germany) was based on a previously published technique (5). Briefly, this involved examination of the surface and internal structures, collection of material representing each part using a needle. The collected material was mixed with potassium bromide and transformed into a transparent pellet using a mold and a special press, in preparation for measurement by FTIR.

SEM analysis involved gold-coating the calculus section prior to examination with a scanning electron microscope (Hitachi S-3400, Hitachi, Tokyo, Japan) coupled to X-ray micro-analysis (XFlash Detector 4010, Bruker AXS, Berlin, Germany), as described previously (4).

Results

The ratio of occurrence of calculus in men and women was 2.7:1. The age of patients with lithiasis in the study ranged from 24 to 70 years, with an average of 47.2 years (49.1 years for men, 42.1 years for women).

The results of application of FTIR and SEM coupled to X-ray micro-analysis for the analysis of 37 urinary calculi appear in Table 1. As can be seen, there was a good correlation between the results obtained by the two methodologies. In Table 2 the frequency of different subtypes of renal calculi found in the studied calculi are indicated. As can be seen, calcium oxalate monohydrate (COM) calculi were the most frequently found in the studied collection. Finally, in Table 3 the overall frequencies considering a single main component are indicated.

Table 1. Components detected after the analysis of 37 urinary calculi by FTIR or SEM

Sample numbers	Results	
	FTIR, Daudon (5)	LB + SEM, Grases (4)
5, 6, 7, 8, 10, 11, 15, 26, 27, 28, 30, 31, 32, 33	COM	COM
1	COM	COM + UA
3, 12, 14, 22, 36	COM + UA	COM + UA
4, 16, 29	COM + HAP	COM + HAP
9	COM + COD + UA	COM + COD + UA
20, 25	COM + COD + HAP	COM + COD + HAP
21	COM + COD + HAP	COM + COD
24	COM + COD + UA	COD + UA
23	COM + UA + Struvite + HAP	COM + UA + Struvite + HAP
2, 17, 35	COD + HAP	COD + HAP
18	Struvite + HAP	Struvite + HAP
34	Struvite + UA	Struvite
13, 19, 37	UA	UA

FTIR, Fourier Transform Infrared Spectrophotometer; **SEM,** Scanning Electron Microscope (Hitachi S-530) coupled to a micro-analysis by X-ray; **COM,** Calcium Oxalate Monohydrate; **COD,** Calcium Oxalate Dihydrate; **HAP,** Hydroxyapatite; **UA,** Uric Acid

Table 2. Frequency of different subtypes encountered in the calculus of the adult according to the classification of Grases (4)

Type of calculus	Structure and composition	Number	%
1a	Papillary COM calculi with core of COM	5	13.5
2a	Unattached COM calculi with core of COM	11	29.7
2b	Unattached COM calculi with core of HAP	2	5.4
2c	Unattached COM calculi with core of UA	2	5.4
3aII	COD	1	2.7
3b	COD with HAP minoritary	4	10.8
6	Struvite	3	8.1
8a	UA	1	2.7
8aI	UA: structure compact and radial	1	2.7
8aII	UA: structure in layers, non radial	1	2.7
9II	UA + COM	6	16.2
Total		37	99.9

COM, Calcium Oxalate Monohydrate; **COD,** Calcium Oxalate Dihydrate; **HAP,** Hydroxyapatite; **UA,** Uric Acid

Table 3. Frequency (%) considering the single main component of the calculus

	Constituting	Frequency Overall
Calcium oxalates	COM	54%
	COD	13.5%
Phosphates	HAP	-
	Struvite	8.1%
Purines	UA (Anhydrous)	24.3%
	UA (dihydrate)	-

COM, Calcium oxalate monohydrate; **COD,** Calcium Oxalate Dihydrate; **HAP,** Hydroxyapatite; **UA,** Uric Acid

Discussion

Good agreement was found between the results of FTIR and SEM analyses of renal calculi (Table 1). Certain components of the calculi identified by SEM were not detected by FTIR, probably because in the latter technique the main absorption bands masked the signal of some minor components. Using SEM it was possible to recognize the components and their crystallization forms on the calculus surface and in the section. Consequently, to obtain maximum information it is necessary to use both analysis techniques to derive all the constitutional and structural data.

We found that the ratio of occurrence of calculus in men and women was 2.7:1. This result is consist ent with previous studies (6-8), showing that there is a slight male predominance in the formation of urinary calculi in the human population. As the num-berof patients involved in the study was limited, the role of both age and sex as factors in development of urolithiasis could not be clearly defined. However, the average age of affected individuals (47.2 years) was consistent with previous studies (9, 10).

As shown in Table 2, the calculi were classified into different types following the classification system of Grases et al (4). Type 1 (calcium oxalate monohydrate papillary calculi; COM) had a frequency of 13.5%. Type 2 (COM unattached calculi) was the most common type, representing 40.5% of the total. This type occurred as three subtypes, with subtype 2a (core COM and organic matter; OM) being dominant with a frequency of 29.7% (Figure 1).

The SEM methodology enabled differentiation of authentic COM calculi (where COM was the initially-formed crystalline phase) from COM calculi derived from the crystalline transformation of a calcium oxalate dihydrate (COD) form. SEM clearly showed that the latter exhibited a typical disorganized non-compact structure with clearly identifiable COD phantoms.

Types 1 and 2 are mainly formed in the context of hyperoxaluria. Thus, it is interesting to note that in the study area, as in most parts of Morocco, tea is consumed much more than dairy products. This could partly explain the high proportion of COM

Figure 1. Micrograph taken by Scanning Electron Microscopy, showing the calcium oxalate monohydrate "leaves" with organic matter as a minority

(i.e. hyperoxaluria) calculi found. However, high proportions of COM calculi have been reported in countries where less tea is consumed (11-13).

. Type 3 calculi (COD) are associated with hypercalciuria of absorptive, renal or resorptive origin (Figure 2). Purines occurred in type 8 (UA, uric acid composition; frequency 8.1%) or type 9 (UA + COM; frequency 16.2%) calculi. Infectious calculi (type 6) occurred at low frequency (8.1%).

UA has a tendency to act as a lithogen germ nucleus for some calculi that ultimately form as calcium oxalate (14). The purine calculi have been linked to hyperuricuria with or without hyperuricemia (15), or to low urinary pH, which is associated with a defect in renal ammoniogenesis.

Considering only the main component, the frequency of each calculus type in relation to sex, showed that COM calculi occurred most frequently in males and females, and in general we found that the occurrence of COM calculi increased with age. For phosphate and purine calculi the trend was reversed, with patients aged below 50 years being most at risk for the development of urolithiasis. Overall, regardless of age and sex factors (Table 3), calcium oxalate dominated the 37 calculi studied, followed by calcium phosphate and purine calculi.

About the comparison of sensitivity and specificity of the proposed methods for renal stone analysis, it should be considered that both are qualitative procedures of the identification of components of the calculus and, it is also very important to consider the overall macrostructure and microstructure of the calculus.

The quantity of sample needed for FTIR can be less than one microgram. If inspection of the superficial and cross-section of the stone reveals a homogeneous appearance, the identification of calculus composition can be performed by powdering the whole stone and taking an average sample for FTIR study. But if inspection reveals a heterogeneous appearance such as areas of differing colors or textures, lamination or

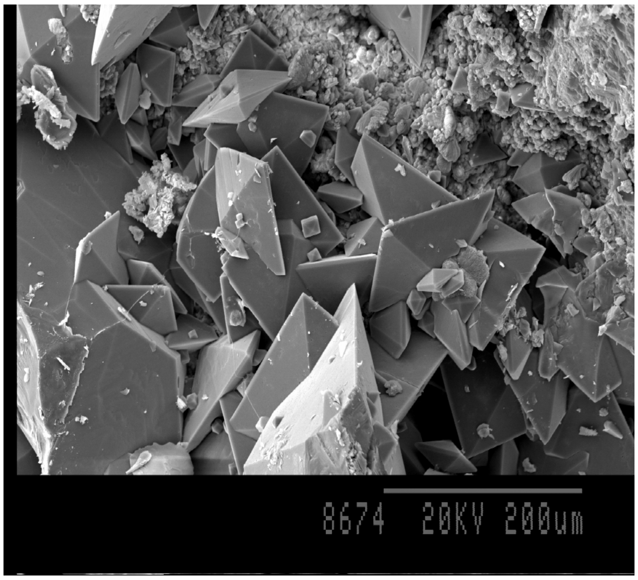

Figure 2. Micrograph taken by Scanning Electron Microscopy, showing calcium oxalate dihydrate in "pyramids", with HAP as a minority

other structural details, it may become necessary to scrape off bits of calculus material from the different regions with a scalpel and perform several identifications by FTIR. Identification is very simple if a reference spectrum that matches that of the unknown material is found. When an exact reference spectrum match cannot be found, a band by band assignment is necessary to determine the composition of the solid. In a mixture of components it is considered that a minor component is possible to be detected when it is present at an amount not inferior to 1/10 part of the major component. The main advantages of FTIR use in the identification of calculi components are the speediness, simplicity and specificity for the identification of rare components and the availability of the instrument.

The SEM provides three outstanding improvements over the optical microscope: it extends the resolution limits so that picture magnifications may be increased up to 30000x to 60000x, it improves the depth-of-field resolution more dramatically, by a factor of approximately 300, and finally, it entails the observation of several surfaces of a sample because of the possibility of rotating the sample in several directions. But in addition to image formation, this instrument may provide elemental analysis of micron-sized areas of the specimen observed when coupled with a special device for energy dispersive X ray microanalysis.

Microstructural observation of calculi emphasizes on several fine structure features. In this sense, it is possible to locate inducer components of the calculus development as apatite nests of several microns of size in calcium oxalate calculi core. SEM may emphasize crystalline conversions which sometimes are not evident under a binocular stereoscopic microscope or with FTIR. Thus, the presence of etching on the surface of COD calculi and big crystals of COM, suggest a high degree of transformation of COD into COM as a consequence of a long time of permanence of the calculus in the kidney. On the other hand, the observation of external details may be crucial in determining the history of the calculus. In this way, the observation of an external cavity coated with tubular apical cells suggests the presence of a point of attachment to the renal papilla and hence, the existence of a epithelial microlesion. Finally, SEM provides information about the nature of crystalline compounds, shape of the crystals, internal structure, location of components, crystalline conversions, crystallite size distribution, characteristics of the aggregates and some data about intimate relations between crystals and organic matrix or relationships between different crystalline species.

Conclusions

This study analyzed urinary calculi using FTIR and SEM techniques. In general there was a good correlation between the two methods, indicating that both are appropriate for renal calculi analysis. Nevertheless, the SEM methodology enabled identification of some minor components in the core, which was not possible using FTIR. Moreover, SEM enabled dif ferentiation of authentic COM calculi (COM was the initially-formed crystalline phase) and COM calculi derived from crystalline transformation of the COD form. However, the FTIR technique is less expensive than SEM, does not require highly qualified technicians, and allows the identification of rare compounds that could be difficult to identify using SEM alone. Thus, the combination of optical stereomicroscopy with FTIR, or of optical stereomicroscopy with SEM are recommended, but where possible the optimum for the study of renal calculi would be to dispose of optical stereomicroscope, FTIR and SEM.

Overall, in Morocco this pathology affects a higher proportion of males, as it does in developed countries, and oxalocalcic calculus is by far the main form among renal calculi.

The data from patients in the study area of Middle Atlas show a strong dietary contribution to the development of lithiasis, probably related to the high level of tea consumption. The slight decrease in urinary infections indicates an improvement in hygiene conditions and/or the treatment of urinary tract infections.

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Conflict of Interest

None declared.

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