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SYMPOSIUM PAPER

Ultra structural study of laminated urinary stone

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Abstract Several modalities of stone analysis are utilised in different laboratories. However, the treating clinician finds it hard to assess the initiation and progression of stone formation. The pathogenesis of calculogenesis still remains a mystery. The purpose of this paper is to assess the pathological mechanisms of stone nucleation and growth by observing the ultra microscopic morphology of the different layers of laminated stones; 130 fragments from 28 randomly selected laminated stones of more than 10-mm diameter were analysed. Wet chemical analysis of the stones was performed. Surface and cross-sectional morphology of the entire stones and the individual fragments was assessed using optical microscopy and images were recorded using ordinary camera. They were further analysed using FTIR for confirmation. By morphological analysis, whewellite, weddellite, uric acid, and phosphate were the main minerals identified. Mixtures of these minerals were also found. Concentric lamination, radial striation, frond formation, and amorphous pattern were the main cross-sectional morphologies obtained. The calculi analysed had differences in their outer and inner portions. This was more pronounced in stones containing predominantly whewellite and uric acid. Whewellite was the outer component in most mixed stones. Uric acid was more in the inner layers of mixed stones than the surface.

Keywords Urinary stone · Morphology · Wet chemistry · Optical microscopy · FTIR · Whewellite · Weddellite · Uric acid · Phosphate

Introduction

Several methods of investigation are available for analysis of urinary stones. Ordinary chemical analysis still remains the common procedure. Higher analytical procedures like Raman spectroscopy [1], X-ray diffraction [2], Fourier transform infrared (FTIR) spectroscopy [3, 4], and scanning electron microscopy (SEM) [5–7], still remain investigative domains of research establishments. However in-depth study of the genesis of each stone formed in the urinary tract requires recognition of the composition during the different phases of development of the stone [8]. This is possible only by ultra structural morphological and chemical analysis. Surface morphology and cross-sectional morphology of urinary stones can be well studied using optical microscope [9]. The pathological mechanism of stone nucleation and growth can also be elucidated only by observing ultra structural morphology. This work was performed using optical microscopy and FTIR to study the variations in composition of different layers of human urinary tract stones.

Materials and methods

Twenty-eight randomly selected laminated stones of more than 10-mm diameter were analysed. Wet chemical

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analysis was performed using standard biochemical procedures [10].

Subsequently 130 fragments from different areas of the stones namely central core and outer zones of up to five were analysed separately. Surface morphology and cross-sectional morphology were assessed using ordinary camera images in close up mode.

Zoom stereo microscope with magnification of 125 was used to get the optical microscopic images. These were recorded with an 8 mega pixel camera. Morphology of the entire stones and the individual fragments was assessed. Each fragment was then analysed using FTIR [11]. These results were compared with those of optical microscopy for confirmation.

Results and discussion

The results were recorded as general morphology, qualitative wet chemical analysis, optical microscopic study and FTIR of the whole stones and then compared with the similar observations of the surface and interior layers. An average of 4.6 fragments was assessed for each stone. Types of calculi with respect to minerals present were identified as whewellite (COM), weddellite (COD), uric acid (UA), phosphate, and mixed.

Of the 28 stones studied, 15 were single stones and the rest constituted part of multiple stones retrieved from the patients. All the stones had been removed using open surgery from the kidney. The weight varied between 5.1 and 6.7 g. Depending on the mineral composition, the stones showed variation in the formation pattern. The variations occurred in the surface and sectional morphology as colour, friability, concentric laminations, radial striations, frond formation, amorphous pattern, and presence of cavities.

The surface was black (Fig. 1a) in 5, yellow (Fig. 1b) in 6, brown (Fig. 1c) in 10, and 8 had multiple patches (Fig. 1d). The cross-section showed the concentric laminations (Fig. 2a) in 17, radial striations (Fig. 2b) in 9, frond formation (Fig. 2c) in 3, amorphous pattern (Fig. 2d) in 5, and cavities in 4. The colour of the cross section was not uniform in any stone. Black, brown, yellow, and dirty white were intermixed in all stones analysed. Cavities were noted in seven stones. Fifteen were friable and thirteen were not.

On qualitative wet chemical analysis, all the stones contained more than one chemical component namely calcium (100%), phosphate (78%), oxalate (63%), magnesium (32%), and uric acid (23%). Further detailed assessment was not possible.

Optical microscopic study revealed the surface morphology and cross-sectional morphology in greater detail. Compositional analysis was also possible. COM was identified by the bosselated, mulberry appearance on the surface and by the concentric laminations and radial striations on section, COD by the spiculated surface and dipyramidal crystals (sugar candy appearance), uric acid by the smooth reddish appearance with fibrous strands (cotton candy

Fig. 1 Stone surface colour. **a** Black, **b** yellow, **c** brown, **d** multiple patches

Fig. 2 Cross-sectional morphology. **a** Concentric lamination, **b** radial striation, **c** frond formation, **d** amorphous pattern

appearance), phosphates by the white amorphous appearance, struvite by coffin lid appearance, and newberyite by parchment appearance. By observing the surface and cross sections by optical microscopy, it was possible to recognise the ultra structural composition of stones. Stones were predominantly COM in 8, predominantly COD in 3, predominantly UA in 4, and mixed in 13. FTIR, however, showed predominant COM in 15, predominant COD in 6, predominant uric acid in 2, phosphate in 1, and mixed composition in 4. It is interesting to note that mixed stones were reported more by optical microscopy than by FTIR analysis. This is probably because optical microscopy studies various layers of the stone, whereas FTIR usually takes up only one fragment from any one region of the whole stone and thus may not be representative of the whole sample. It is thus felt that in order to understand the stone morphology and composition as a whole, we need to do segmental analysis of larger stones.

On performing the morphological study, optical microscopy and FTIR of the fragments of the stones, the findings were not totally tallying with the results of the analysis of full stones. It is seen from Table 1 that white colour was seen in the central core more often. Black and mixed colours were more in the outer fragments. Concentric laminations were more in the peripheral part of the stones. Amorphous pattern was more in the centre. COM component was more in the centre and outer areas of the stones. FTIR of the fragments showed that COM was the most common component (73%), followed by UA—36%, COD—27%, ammonium urate—12%, brushite—9%, struvite—5%, apatite—5%, and newberyite—5%. Components in mixed stones were COM (88%), COD (45%), UA (43%), and phosphates (27%). Mixtures were mostly COM + UA and COM + COD. Radial striations were seen mostly along with concentric lamination in COM stones. Frond formation was characteristic in phosphate stones. Since optical microscopy is easier to perform, it is advisable to scan through the cross sections of large stones in order to understand the total morphology and composition.

It is thus seen from the data that urinary stone formation occurs in different phases, which are separated by periods of inactivity. If a complete picture of the genesis of the stone is to be obtained, more detailed assessment of the interior is needed. Analysis of stone fragments gave an idea about the particular segment of the stone analysed. Presence of different compositions in the different layers indicates a conglomeration of metabolic effects in different times of evolution of the stones. The presence of protein in the intermediate brown coloured areas indicates either superimposition of protein matrix in between layers of stone or blood components. It might indicate that the stones in the urinary tract might have formed over a period of time with intervening periods of inactivity and in some cases collection of blood on the surface produced by the spiky crystal protrusions. Mixture of uric acid and calcium oxalate is an indicator of the close relation between the oxalate and uric acid metabolisms. This will help in deciding more appropriate prophylaxis for the patients.

Table 1 Characteristics of stone as a whole and fragments from inside stones

Characteristic	Type	Full stone* 28	Fragments (inner to outer)					
			1	2	3	4	5	Total
			28	23	27	24	28	130
Colour	White	7	10	3	2	2	4	21
	Brown	10	6	2	6	7	5	26
	Yellow	6	4	5	5	6	4	24
	Black	5	5	8	6	4	7	30
	Mixed	8	3	5	8	5	8	29
Friability	Friable	15	14	12	8	16	15	70
	Non-friable	13	14	10	15	8	13	60
Cross-sectional morphology	Concentric lamination	17	5	5	10	16	12	48
	Radial striation	9	4	11	7	9	9	40
	Frond formation	3	0	0	6	7	7	20
	Amorphous pattern	5	17	7	12	6	7	49
	Cavities	7	2	1	4	5	6	18
Optical microscopy	COM	8	13	12	9	8	8	50
	COD	3	2	4	5	6	7	24
	UA	4	4	3	2	3	2	14
	Phosphate	0	2	1	1	3	3	10
	Mixed	13	7	6	7	7	6	33
FTIR	COM	15	12	6	4	9	5	36
	COD	6	3	4	4	5	5	21
	UA	2	8	5	2	7	4	27
	Phosphate	1	2	2	2	2	4	12
	Mixed	4	7	4	7	5	7	30
	Protein	0	2	0	3	4	0	9

**The full stone analysis was any part of the stone fragmented from the full stone*

Conclusion

The surface and the interior were different in those stones containing predominantly COM and UA. The analytical findings differed in the different layers of the stone. Uric acid component was more in the inner layers of mixed stones than the surface. COM was the outer component in most mixed stones.

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