

UROLITHIASIS: PECULIARITIES OF THE ETIOPATOGENY AND THE COMPOSITION OF UROCONCREMENTS

Georgeta Sofia Popescu¹, Rodica Grecu²

¹Department of Biochemistry, Faculty of Food Processing Technology, Banat's
University of Agricultural Science and Veterinary Medicine, Calea Aradului 119, RO-
300645, Timisoara, Romania, E-mail: sofiapopescu@yahoo.com;

²Reserche Centre "Raluca Ripan" Cluj-Napoca;

Abstract

The urolithiasis occurs as a result of metabolic and urodynamics disturbances in the metastable medium of the renal excretion of various organic and inorganic compounds. As results of the growth of microcrystalline formation, the process evolves towards uroconcrements, varying as a matter of shape, size, composition and clinical evolution. The analysis of urinary calculi by chemical methods is rather unsatisfactory. Physical, chemical and mineralogical tools are among the methods that are use more and more to establish the composition of urinary calculi. Infrared (IR) spectroscopy investigation of surgically extracted and spontaneously eliminated calculi permitted to find out their qualitative composition. The interpretation of spectra by means of implementing a program on computer, allowed for defining thereby the types of Urolithiasis. Method of IR spectroscopy is a physico-chemical analysis which had as aim to define the types of lithiasis.

Keywords: urolithiasis, calculi, FT-IR spectroscopy, stone chemical composition.

Introduction

Urinary calculi (nephrolithiasis) are a coccretion composed of crystalline components and organic matrix (Pak, 1992). Although the symptomatic presentations may be similary, the disorder is heterogeneous as to to composition and aetiology.

About 0.1 – 0.4 % of the population is believed to have kidney stones every year in USA and Europe. About 2–5% of population in Asia, 8 – 15% in Europe and North America and 20% in Saudi Arabia

develop kidney stones in their lifetime (Pak, 1998; Balla, 1998; Bennani, 2000; Decoster, 2002; Gault, 2000). Renal stone tend to recur, and the rate of recurrence is about 75% during 20 years (Gault, 2000).

One of most important elements which elucidate the stone formation mechanism is stone chemical composition analyses.

This paper reveals aspects regarding oxalic urolithiasis etiopatogeny and provides occasion for presenting results of research undertaken, based on results of uroconcrements analysis using especially Fourir Transform infrared (FTIR) and atomic spectroscopy methods.

Experimental

The researches have been performed on surgically removed and spontaneously eliminated calculi. There were studied 61 calculi in all. The cases studied belonged to the Urological Clinic of the Medical School, Timisoara.

First, the calculi were submitted to repeted washings with distilled water, and then they are transformed in powder wich was study. The stone was cut into two equal parts using a fine saw and was examined for presence of a nucleus. If the section shows a nucleus or other distinctive areas then their parts should be analysed separately. If the stone has homogeneous appearance then the powder from the saw cut is often sufficiente for the subsequent analyses.

A preliminary examination can give us less information about the nature of urinary calculi (see Fig. 1). Nevertheless calculi with same aspect have different compositions.

Infrared (IR) spectra of all urinary calculi were recorded as KBr pellets using a Jasco FT-IR 610 Spectrometer (Fourir-Transform Infrared Spectrometer). Metalic ions were detected by atomic absorbtion using a Varian spectrometer Spectr AA-110 (with 4 hollow cathode laps controlled by PC). Presence of phosphate was determined by using a UV-Visible spectrophotometer (Analytikjena Specord 205).

The study of metal ions and phosphate was possible after a standard digestion process using concentrate nitric acid (and hydrochloric acid) at 250°C for at least 30 min. It was necessary to neutralise digested solutions using 1M sodium hydroxide in order to determine phosphate presence, In the next step it was added 1.5 mL

ascorbic acid solution (7% w/v) followed by 1.5 mL of mixed reagent (45 mL of molybdate dissolved in 200 mL of concentrated sulphuric acid followed by 5 mL of tartrate solution) was added for colour development. Absorbance of the resulting coloured solution was read at wave length of 882 nm.

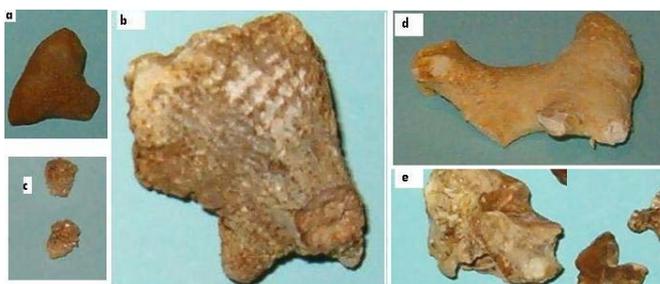


Fig. 1. a) uric acid; b) oxalate; c) oxalate; d) carbapatite e) phosphate

Qualitative wet chemical analysis for cations and anions in the urinary calculi was carried out as per routine technique commonly using in Biochemistry laboratories (Popescu, 2002, 2006).

Results and Discussions

The chemical composition of urinary calculi were determined by FTIR spectroscopic method. An amount of 1-2 mg is generally sufficient to obtain a good spectrum. This quantity can be reduced. IR spectroscopy is a physical method based on the property of atom groups to absorb infrared radiations of different wavelengths, according with their nature and their environment. The molecular composition and the crystal lattice influence the absorption and lead to a characteristic spectrum. IR spectroscopy is a very convenient for analysis of renal stone because the time required is short, a small amount of sample is required, and many components can be determined by a single analyse (in a single spectrum) for a sample.

Epidemiological studies concerning urolithiasis in Romania showed that the highest frequency is in the South West of Romania, i.e. 186.8 cases at 1000.000 habitants, and about 31% of the patients admitted in the Urological Clinic of Timisoara are suffering with this

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disease. That is the reason why we started studied on the qualitative and quantitative composition of urolithiasis.

The results obtained by FT-IR spectroscopy on 61 uroconcrements are presented in table 1. It can observe that among all urolothiasis analysed by us (61) a number of 13 were simple urolithiasis and 48 mixed (binary and ternary).

Table 1. Types of oxalate urolithiasis (simple and mixed)

Urolithiasis type	Calculi Composition		No. of investigated cases		
			Total	Men	Women
Simple	Oxalates (O)		6	4	2
	Urate (U)		5	3	2
	Phosphates (P)		2	-	2
Total simple urolithiasis			13	7	6
mixed (binary and ternary).Mixed	binary	O-P	5	3	2
		P-O	2	1	1
		U-O	8	6	2
		O-Carb	13	6	7
	ternary	O-P-Carb	16	7	8
		O-U-P	2	2	-
		Carb-P-U	2	1	1
Total mixed urolithiasis			48	26	22
T o t a l			61	33	28

O – Oxalates; P –Phosphates; U–Urates (urate or uric acid); CA – Carbapatit;

One can observe that among all the urolithiasis analysed by us a number of 52 were with oxalates: 6 simple and 46 mixed (binary and ternary).

The toxic nature of oxalic acid and its nephrotoxicity was well recognized in the nineteenth century (Hodgkinson, 1977). Its injurious effects are considered to be a result of the physical properties of its calcium salts, the calcium oxalates, which are insoluble at physiological pH.

In the etiopatogeny of urolithiasis the metals play an important role. Boshamer (1961) was the first who observed that metals intervene in the urinary crystal formation. The human urine, considered as a metastable solution, contains beside nitrogenous compounds, protein, polysaccharides, organic acids also anions and inorganic cations (Na, K, Ca, Mg, Zn, Cu, Mn, Fe). Between the organic and inorganic

compounds precipitative and/or co-precipitative processes might occur (Thomas, 1995; Popescu, 2005).

Metals intervene to a large extent in their evolution and in the development of calculous formations. Thus, while the sodium salt is readily soluble, the calcium salt – prevailing in oxalic urolithiasis - is scarcely soluble. In our research involving FTIR spectroscopy, we recorded the IR spectra of the chemically pure compounds. Thus, we recorded the spectrum for oxalic acid the underlying compound of oxalic lithogenesis. We proceeded similarly, with the other compounds too, present in the composition of calculi. Metals may intervene either indirectly as effectors (inhibitors-activators) of metabolic processes, or directly as substituents engaged in competing interactions owing to the difference in the solubility products of oxalic, phosphatic salts etc. From among metals Zn^{2+} has a dissolving effect for Ca^{2+} and is considered as litholytic substance; Al^{3+} favours lithogenesis - being antidissolving; Fe^{3+} favours lithogenesis by blocking pyrophosphates considered and considered as lithogenic substance (Chisholm, 1984; Coe, 1988; Daranyi, 2000, Popescu, 2005)

In this study the concentration of the main alkaline and alkaline-earth metals and trace metals in simple (6 cases) and mixed (46 cases) oxalic uroconcrements was determined by means of AAS. The analytical results are presented in table 2 and 3.

The alkaline and alkaline-earth metals concentration in simple oxalate urolithiasis decreased as follows: $Ca > Na > Mg > K$. In case of binary (O-P and P-O) urolithiasis Ca had the highest concentration being followed by Mg and Na. Potassium had the lowest concentration in O-P and P-O urolithiasis while magnesium in U-O and U-Chol urolithiasis. In ternary O-U-P urolithiasis the metals concentration decreased as follows: $Ca > Na > K > Mg$.

The trace metals concentration in simple oxalate urolithiasis decreased as follows: $Fe > Zn > Pb > Mn > Cu$.

In case of binary O-P and P-O urolithiasis Zn had the highest concentration being followed by Fe and Pb. Copper had the lowest concentration in O-P urolithiasis while manganese in P-O urolithiasis. In the binary U-O urolithiasis Fe had the highest concentration and Mn the lowest. Lead was in the highest concentration in oxalate-phosphate. Lead, a toxic metal for the organism, has a rather high concentration in all the studied oxalates urolithiasis. This fact might be explained by the

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nature of cholesterol and the valence configuration of lead divalent character of Pb Pb(II) and Pb(IV).

Table 2. Concentration of the main trace metals in oxalic urolithiasis

Urolithiasis Type		No.	Metallic concentration in various urolithiasis (µg/g)			
			Na	K	Ca	Mg
		n	X±SD	X±SD	X±SD	X±SD
<i>Simple</i>	O	6	2 420 ± 737	786.5 ± 162	231756 ± 24401	1653 ± 494
<i>Mixed binary</i>	O-P	5	4 489 ± 285	272.0 ± 162	187 292 ± 58126	5 219 ± 1464
	P-O	2	9 101 ± 2610	1 973 ± 681	149 140 ± 50310	24360 ± 7107
	U-O	7	1 810 ± 577	473.5 ± 79.8	191 092 ± 63112	407.3 ± 189
	O-CA	13	4 592 ± 286	271.1 ± 160	197 514 ± 58158	5 785 ± 1486
<i>Mixed ternary</i>	OPCA	15	7 813 ± 2111	1 973 ± 685	138 132 ± 49311	22561 ± 6986
	O-U-P	2	2 191 ± 526	982.8 ± 76.9	152 469 ± 49820	831.9 ± 319

In ternary O-U-P urolithiasis the trace metals concentration decreased as follows: Zn > Fe > Pb > Cu > Mn. The concentration of the studied trace metals revealed a difference function of uroconcrement kinds. This fact proves that in the composition of the simple oxalate and mixed oxalate-phosphate urolithiasis the concentration of the trace metals is higher than in the mixed urates-oxalates excepting copper. It is known that copper has two possible valence configurations: Cu (I) or cuprous ions and Cu (II) or cupric ions. While divalent copper ion has affinity for ionic bonds, monovalent copper ions are involved in coordinate covalent bonds. Between metal ions and other compounds of uroconcrements there are two kinds of bindings: ionic (e.g. OOC-COO^- , PO_4^{3-} or PO_3^{2-}) and polar (e.g. uric acid, cholesterol) ones which could explain the higher concentration of trace metals in oxalate and oxalate-phosphate concrements.

Table 3. Concentration of the main trace metals in oxalic urolithiasis

Urolithiasis Type		No.	Metallic concentration in various urolithiasis ($\mu\text{g/g}$)				
			Zn	Fe	Cu	Mn	Pb
		n	X \pm SD	X \pm SD	X \pm SD	X \pm SD	X \pm SD
<i>Simple</i>	O	6	370.9 \pm 101	465.0 \pm 149	19.49 \pm 6.29	32.87 \pm 6.23	85.31 \pm 21.64
<i>Mixed Binary</i>	O-P	5	587.0 \pm 140	242.7 \pm 61.7	17.32 \pm 4.76	18.26 \pm 4.02	203.1 \pm 56.4
	P-O	2	164.9 \pm 52.0	237.9 \pm 70.2	21.86 \pm 9.12	17.05 \pm 5.81	69.80 \pm 19.68
	U-O	7	51.43 \pm 10.3	45.19 \pm 17.0	18.03 \pm 5.81	8.93 \pm 3.24	58.04 \pm 17.11
	O-CA	13	584.2 \pm 139	242.7 \pm 61.7	17.32 \pm 4.76	18.26 \pm 4.02	203.1 \pm 56.4
<i>Mixed Ternary</i>	OPCA	15	164.8 \pm 51.6	237.3 \pm 70.1	21.86 \pm 9.03	16.77 \pm 5.79	69.80 \pm 19.68
	O-U-P	2	216.9 \pm 88.1	159.3 \pm 79.5	20.51 \pm 5.47	12.86 \pm 4.07	137.7 \pm 42.7

Concentration of metals in uroconcrements is different, being dependent on the type of urolithiasis. The variations are due to the solubility differences of the compounds, the pH and the molality of the medium as well as to the morpho-functional state of the kidney and urinary tract.

Conclusions

Men develop more frequently oxalic urolithiasis than women, but women had been founded more urolithiasis than men in case of phosphate (simple and mixed) urolithiasis. Concentration of metals in uroconcrements is different, being dependent on the type of urolithiasis. The obtained analytical data may provide important information for the management of the stone-forming patients. The knowledge of the qualitative and quantitative composition of the oxalic uroconcrements is of interest for the clinical guideline in the prophylaxy, therapy and metaphylaxy of urolithiasis.

References

- * * * (1997). *Aspects sanitaires et nutritionnels des oligo-éléments et des éléments en traces*. Ed. OMS, Genève, Publ. InfoPrint Singapour
- Balla, A.A., Salah, A.M., Salah, A.M, Khattab, A.H., Khambal, A., Bongartz, D., Hoppe, B., Hesse, A. (1998). Mineral composition of renal stone from Sudan, *Urol Int*, 61(3), 154–156.
- Bennani, S., Debagg, A., Oussama, A., el Mirini, M., Benjelloun, S.(2000). Infrared spectrometry and urolithiasis, *Ann Urol*, 34(6), 376-383.
- Boshamer. K. (1961). *Die Steinerkrankungen*. Kapitel X, in "Handbuch der Urologie" (Alkn C.A., Din V.W., Wildbolz E., Eds.), Springer-Verlag, Berlin-Göttingen-Heidelberg.
- Chisholm, G.D., Williams, D.I. (1984). *Scientific Foundation of Urology*, W. Heineman Medial Books Ltd., London.
- Coe F.L., Parks J.H. (1988). *Nephrolithiasis. Pathogenesis and treatment*. 2nd ed., Year Book Medical Publisher, Inc., Chicago-London-Boca Raton.
- Daranyi, G., Bucuras, V., Dragan, P., Garban, Z., Vincu, M., Popescu, G.S. (2000). *Profile uroconcrementsmetallogram in simple lithiasis with oxalates, phosphates and urates*, pp. 1101 – 1108, in Mengen- und Spurenelemente, 20. Arbeits-tagung 1999", Friederich-Schiller-Universität Jena, (Hrsg. Anke M. et al.) Verlag Harald Schubert, Leipzig.
- Decoster, M., Bigot, J.C., Carre, J.L., Morin, J.F., Mahe, J.L., Tanquerel, Cledes J., Floch, H.H. (2002). Epidemiologic study of urinary calculi in Western France, *Press med*, 31(3), 113-118.
- Gault, M.H., Chafe, L. (2000). Relationship of frequency, age, sex, stone weight and composition in 15624 stones, *J Urol*, 64(2), 302-307.
- Hodgkinson, A. (1977). *Oxalic acid in biology and medicine*. Academic Press, London.
- Pak, C.Y.C. (1992). *Kidney stone, Textbook of Endocrinology*, Williams, pp. 1519 – 1536.
- Pak, C.Y.C. (1998). *Kidney stone, Textbook of Endocrinology*, Lancet, 351, pp. 1519 – 1536.
- Popescu, G.S. (2002). *Particularitățile ale metalogramelor în diferite tipuri de urolitiaze*, Referat Dr., doctorantură Univ. Politehnica Timișoara
- Popescu, G.S. (2006). A chemical and phisico-chemical analysis combination of urinary calculi (Manuscript - Submitted).
- Popescu, G.S., Gârban, G., Gârban, Z., Velciov, A.-B., Dragan, P. (2005). Trace metals in simple and mixed oxalate urolithiasis, in "5th International Symposium on trace elements in human: new perspectives", Athens.
- Thomas, J., Fompeydie, D., Thomas, E., Arvis, G. (1995). La lithiase renale uro-oxalique. Quelques particularites. *J Radiol.*, 76(1), 61-67.