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Dedicated to the memory of Prof. dr. Ioan Silaghi-Dumitrescu marking 60 years from his birth

PURINE METABOLISM DYSHOMEOSTASIS AND THE HETEROGENOUS NUCLEATION OF UROCONCREMENTS NOTE I. ALKALINE AND ALKALINE-EARTH METALS IN PURINE UROLITHIASIS

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ABSTRACT. Biogenesis of lithiasic concrements constitutes a special domain when taking into account the location of lithiasis (currently named calculi or stones) in the organism, e.g.: sialolithiasis, rhinolithiasis, urolithiasis, flebolithiasis a.o. as well as the type of lithiasis.

In case of purine metabolites of endogenous (strictly metabolic) and exogenous (foods) origin the concrements formation in urine implies a heterogenous nucleation mechanism where metallic ions participate, too.

Problems concerning the purine uroconcrements (mainly with uric acid) and their content in alkaline and alkaline-earth metals were approached by investigations based on physico-chemical methods. Thus, by Fourier Transform - Infrared Spectroscopy the types of urolithiasis and by atomic absorption spectroscopy the metallogram (Na, K, Ca and Mg) of purine urolithiasis were established.

Keywords: *purine metabolism and uroconcrements; alkaline and alkaline-earth metals in purine uroconcrements*

INTRODUCTION

The problem of purine uroconcrements biogenesis is integrated in the modern approach of the complex aspects of proteomics and metallomics with application in the study of urolithiasis. Under the incidence of proteomics

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falls the nucleic acids (i.e. deoxyribonucleic acid - DNA and ribonucleic acid - RNA) biodegradation, resulting pyrimidine and purine derivatives Under the incidence of metallomics falls the metallic ions presence in blood and tissues as well as their capacity to generate in urine crystallization nuclei called „primers" or „starters" [1-4].

Commonly in the biogenesis of uroconcrements take part various metabolites like: purine derivatives (mostly uric acid), oxalic acid and oxalates, cystine, phosphates a.o. beside metallic ions ([5].

Nowadays the urolithiasis (kidney stones or urinary stones) represent about 1-2% of all diseases, about 12-40% among kidney diseases and affects about 3% of the acive people.

In the biochemical pathology of urolithiasis problems concerning the etiology, pathogenesis and composition of uroconcrements implies a complex inter- and multidisciplinary approach [6]. Efficient metaphylaxy and prophylaxy in case of urolithiasis can be achieved only by the exact knowledge of stone components [7].

The aim of the study was the determination of the qualitative composition of uroconcrements by Fourier Transform - Infrared Spectroscopy (abbreviated as FT-IR or FTIR) and thus the identification of the urolithiasis type as well as the determination, by atomic absorption spectroscopy (AAS), the concentration of alkaline and alkaline-earth metals in the purine urolithiasis.

RESULTS AND DISCUSSION

Free purines in the blood flow have the following sources: tissular purines - as a results of nucleic acid degradation; exogenous nucleoproteins - brought by food intake; newly synthetized purines in the organism - de novo biosynthesis [2, 8, 9].

Issues related to purine precursors of uroconcrements are integrated in the vast domain of proteomics. In this background there are discussed metabolic aspects, homeostasis and pathobiochemistry of purine metabolites.

In figure 1 there is presented the schematic degradation of nucleic acids with purine metabolites formation. Some of the resulted metabolites are prescursor of purine uroconcrements.

As metal ions play an important role in the biogenesis of uroconcrements [3,10] the discussed subject of this study can be integrated also in metallomics, a consequential domain for environment, medicine and biology [11, 12]

It is known that between some purine derivatives and metallic ions occur the process named „heterogenous nucleation" where the starters become precursors of uroconcrements with a continuous growth. The presence of purine metabolites, of metallic ions as well as a protein and glycoprotein or mucoprotein "matrix" in the urine allow the "starters" or "primers" formation [10, 13-15].

These urolithiasis appear as the consequence of disturbances in purine nucleotides metabolism, homeostasis and excretion of the biodegraded end products, i.e. purine derivatives (uric acid, xanthine, 2,8-dihydroxyadenine).

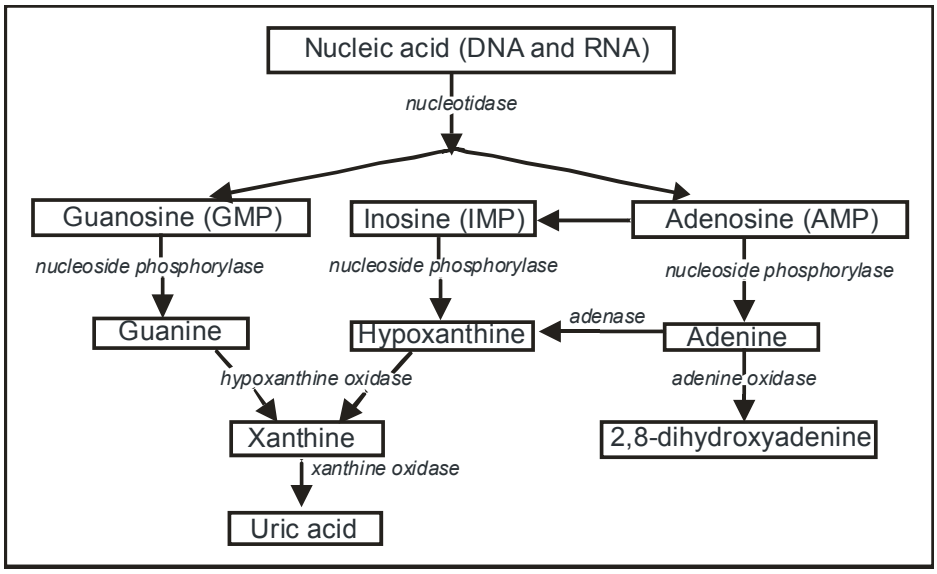

Figure 1. Main purine derivatives resulted from the biodegradation of nucleic acids

Uric acid is the end product of purine metabolism in man and is poorly soluble in biological fluids. It is excreted partly (2/3) by urinary tract and partly (1/3) eliminated by the gastrointestinal tract. Uric acid is filtered by the glomerulus and the filtered uric acid is almost completely reabsorbed in the proximal tubule. Further uric acid is secreted in to the lumen in the distal part of the proximal tubule and the daily urinary output of uric acid in a normal male on a purine-free diet is 1.6 - 3.6 mmoles (270 - 600 mg). The normal blood uric acid level is 40$\pm$70 mg/L in man and 35$\pm$60 mg/L in woman. Men tend to have higher values than women. Changes in the dietary intake of purines make relatively small differences to the blood plasma level of uric acid.

The in excess accumulation of uric acid in the urinary tract, alongside with other organic and inorganic substances (mainly metals) may lead - through coprecipitative processes - to starters formation in lithogenesis. This process is favorized by a lower urine pH and by the low urine flow rate.

Uric lithiasis has a relatively low frequency among urolithiasis but the most increased among purine lithiasis. Most uric acid stones result by the precipitation of uric acid from supersaturated urine. Uric acid urolithiasis is often accompanied by uric acid crystals in the urine sediment. Excessive urinary uric acid excretion may result from increased filtration of uric acid (from excessive dietary purine intake, metabolic errors, myeloproliferative disorders, or hemolysis), from tubular effects such as an isolated defect in renal tubular ureic reabsorption, or from generalized tubular disfunction. Uric acid excretion is increased in as many as 10% of pacients with hypercalciuria and urolithiasis. In endemic areas such as Southeast Asia and the Mediterranean region, uric acid stones are frequently not associated with hyperuricemia.

Xanthine lithiasis, rarely occured, is difficult to be found. Sometimes is necessary to make use of differential spectrophotometry, X ray diffraction or chromatography in order to diagnose it. It appears as a consequence of a metabolic disturbances in which xanthine oxidase is implicated. This enzyme catalyses the hypoxanthine oxidation to xanthine and then of the xanthine to uric acid [16,17]. Often this dysmetabolism could be observed in childhood and is the consequence of an inhereted xanthine oxidase deficiency.

The *2,8-dihydroxyadenine lithiasis* is very rare and in numerous cases may be confounded with uric lithiasis. At the origin is a defect in purine metabolism consisting in the deficiency of the adenine phosphoribosyl-transferase enzyme [18, 19]

Literature data concerning the purine derivatives concentration in urine, compiled by Altman and Dittmer [20] are presented in Table 1.

Table 1. Concentrations of purine nucleobases and other purine derivatives in urine

Nr.	Purine metabolites	Values (mg/kg body)	
		Mean	Range
1.	Adenine	0.020	0.016 – 0.240
2.	Guanine	0.006	0.003 – 0.009
	8-hydroxy-7-methyl-guanine	0.020	0.016 – 0.030
	7-methyl-guanine	0.090	0.080 – 0.110
	N^2-methyl-guanine	0.007	0.006 – 0.009
3.	Hypoxanthine	0.140	0.080 – 0.190
	1-methyl-hipoxanthine	0.006	0.003 – 0.010
4.	Xanthine	0.090	0.070 – 0.120
5.	Uric acid	2.000	0.800 – 3.000
	1,3-dimethyl-uric acid	traces	
6.	Purine bases - total		0.200 – 1.000

The presence of oxypurines, end products of purine catabolism, is conditioned by pH level. Data presented by Klinenberg et al., cited by Watts [21] reveal the solubility of the purinic metabolites: hypoxanthine, xanthine and uric acid (Table 2). It is noticed that solubility differs in serum and urine conditioned by the purine metabolites and the pH of the medium.

In order to identify the chemical substances present in urolithiasis "standard spectra" of the appropriate chemically pure compounds were recorded and the specific FT-IR absorption bands for every compound have been identified, confirmed by the available literature data [22-25].

Table 2. Data concerning pH-solubility relationship of purine metabolites

Medium	pH	Solubility (mg/ 100 mL)		
		Uric acid	Xanthine	Hypoxanthine
Serum	7.4	7	10	115
Urine	5.0	15	5	140
	7.0	200	13	150

Thus, there were recorded standard spectra for uric acid, xanthine, 2,8-dihydroxy-adenine, oxalates, phosphates, cystine, carbonates. Such a standard FT-IR spectrum for uric acid is given in figure 2.

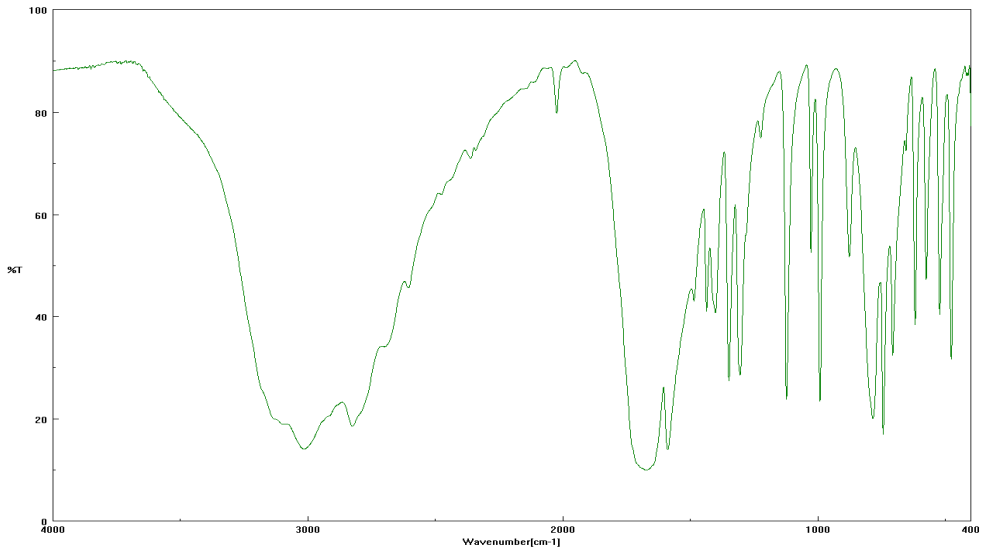

Figure 2. Standard spectrum of uric acid obtained by FT-IR

In the standard FT-IR spectrum of uric acid the significant bands were found: in the 2800-3300 cm^{-1} range for lactame and lactime O-H group streching vibrations (with peaks at 2825 cm^{-1} and 3014 cm^{-1}); in the 990-1150 cm^{-1} range for the purine skeleton ring vibrations (with peaks at 992 cm^{-1} and 1122 cm^{-1}); in the 700-800 cm^{-1} range for the purine skeleton ring vibrations (with peaks at 706 cm^{-1}, 743 cm^{-1} and 783 cm^{-1}). Also, regarding the uric acid spectrum, the characteristic vibrations for C-N, N-H and C=O peaks will be presented below.

Spectra for two uroconcrements, one with simple and other with mixed composition, are given in figure 3. In the case of the simple urolithiasis the recorded spectrum presents specific IR bands corres-ponding to certain wave numbers which are characteristic for urates (U) - fig.3a. The bands presented peaks at 1036 cm^{-1} - for the vibration type C-N stretching; 3014 cm^{-1} - for the vibration type N-H stretching and 1672 cm^{-1} - for the vibration type C=O stretching.

In the case of the mixed urolithiasis, containing uric acid and oxalates (U-O) - fig.3b, there were found not only the bands with characteristic peaks and vibrations types specific for uric acid (mentioned above) but also specific bands with characteristic peaks for oxalates. Specific bands and types of vibrations for oxalates are: 1620 cm^{-1} and 1319 cm^{-1} for the C=O asymmetric and symmetric streching, 781 cm^{-1} for the COO deformation and 518 cm^{-1} for the COO rocking (out-of-plane) vibrations.

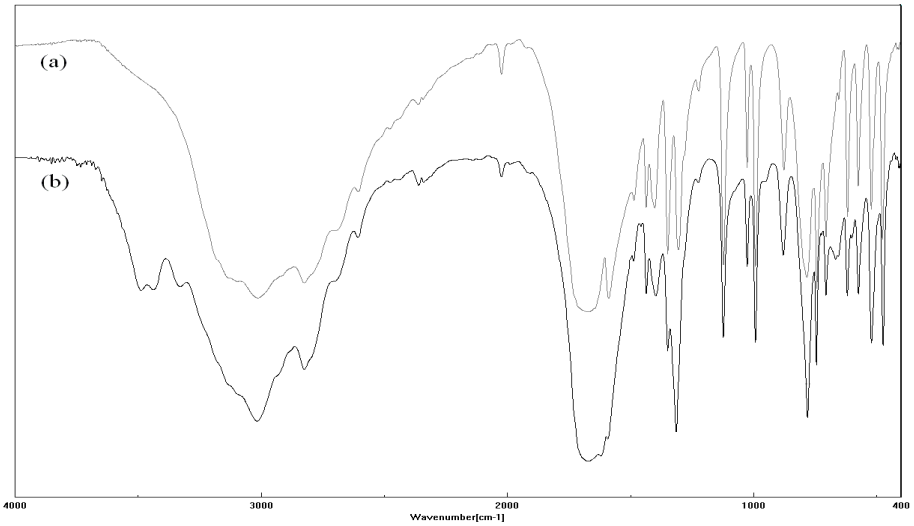

Figure 3. Spectra of some uroconcrements obtained by FT-IR : (a) simple calculus with uric acid – urates (U); (b) mixed calculus with urates and oxalates (O-U)

In case of calculi with mixed composition there are selected the expressive peaks of the IR spectra of the uroconcrements. For the identification of substances present in such a mixture, e.g. uroconcrements with urates and oxalates, Estepa and Daudon [26] proposed the building of „calibration curves" used in the evaluation of peaks and the estimation of the prevailing component. Appliance of this method will permit the evaluation of mixed uroconcrements. In the present paper three characteristic peaks were taken into consideration in the evaluation of uroconcrements with mixed composition.

In the FT-IR spectra of uroconcrements one can mark out various non-specific peaks for the type of urolithiasis. The presence of those peaks can be explained by the heterogenous nucleation process where the different characteristic lithogenetic compounds (e.g. urates, oxalates, phosphates etc.) and metallic ions precipitate on a „matrix" support. The existence of the preliminary matrix in the biogenesis of calculi is known and accepted long time ago [13]. Recent data revealed that renal stones are concretions containing 97.5% polycrystalline aggregate and 2.5% glycoprotein or mucoprotein matrix [27].

Based on the „standard spectra" and the recorded spectra for each uroconcrements there were possible to establish the qualitative chemical composition of the uroconcrements and to classify them into simple and mixed (binary and ternary) ones. In table 3 there are presented the distribution of urolithiasis types according to their chemical composition and gender of the patients.

Table 3. Synopsis on the composition of the uroconcrements investigated by FT-IR

Type of urolithiasis	Composition		Number of cases		
			Total	Men	Women
Simple	Purine derivatives	Urates (U)	34	14	20
		Xanthine (X)	--	--	--
		2,8-dihyroxyadenine (2,8-DHA)	--	--	--
	Oxalates (O)		38	23	15
	Phosphates (P)		19	6	13
	Cystine (C)		6	2	4
Simple uroconcrements - total			97	45	52
Mixed	Binary	Oxalates-urates (O-U)	11	3	8
		Urates-oxalates (U-O)	6	2	4
		Oxalates-phosphates (O-P)	31	13	18
		Oxalates-cholesterol (O-COL)	3	2	1
		Phosphates-oxalates (P-O)	15	3	12
		Phosphates-carbonates (P-CARB)	2	1	1
	Temary	Oxalates-urates-phosphates (O-U-P)	5	2	3
		Phosphates-oxalates-carbonates (P-O-CARB)	2	2	--
Mixed uroconcrements – total			75	27	50
Total uroconcrements			172	72	102

Metals are important components of human organism but they are not produced or destroyed by the body. Being present in our environment, i.e. food, water, air, soil they are introduced in the organism mainly by food and water.

The occurrence of the metals in uroconcrements is an outcome of their presence in urine where they initiate the co-precipitative processes. It was observed in the urine of the patients with calculosis an increase of Ca and Mg concentration, while the concentrations of Na and K displayed minor variations.

By means of AAS, we determined the concentration of alkaline metals in the simple and mixed purine urolithiasis. The results are presented in Table 4.

Table 4. Quantity of alkaline metals in the purine urolithiasis

Type of urolithiasis		**Metals concentration (μg/g calculus)**			
		Sodium		**Potassium**	
		n	X ± SD	n	X ± SD
Simple	Urates (U)	34	586.12 ± 251.64	34	193.81 ± 125.18
Mixed	O-U	11	1722.31 ± 503.17	11	497.72 ± 184.16
	U-O	6	1364.05 ± 414.17	6	322.57 ± 156.74
	O-U-P	5	2249.33 ± 516.64	5	912.43 ± 206.17

n – number of cases; X- mean value; SD – standard deviation

Higher sodium concentration was found in case of mixed ternary and binary urolithiasis, more exactly in O-U-P and O-U lithiasis. Also, one can observe the indirect role of oxalates in the urolithiasis formation.

Regarding the alkaline-earth metals Ca and Mg their concentration was determined also by AAS. The results presented in table 5 revealed augmented quantities for Ca and Mg in the mixed purine urolithiasis O-U and O-U-P. Their quantity in the urolithiasis and especially that of Mg alkaline metals by the presence of phosphates, cases when result ammonio - magnesian phosphates.

Table 5. Quantity of alkaline-earth metals in the purine urolithiasis

Type of urolithiasis		**Metals concentration (μg/g calculus)**			
		Calcium		**Magnesium**	
		n	X ± SD	n	X ± SD
Simple	Urates (U)	34	611.43 ± 270.33	34	137.61 ± 91.27
Mixed	O-U	11	187 342.00 ± 52 814.00	11	412.93 ± 127.31
	U-O	6	121 416.00 ± 47 200.00	6	376.14 ± 102.19
	O-U-P	5	179 614.00 ± 50 139.00	5	969.56 ± 257.08

The analytical data of metallograms obtained by our investigations are similar with those in the literature [10, 28–31]. But, one must note that the composition of the uroconcrements and especially in metals is different from one patient to another and from one region to another. From these observations result that the difference is dependent not only on the purine metabolites (resulting from nucleoproteins) but also by the specific metallic composition of food nutrients and of the water. That is why metallomics - applied in the domain of uroconcrements biogenesis – integrate not only physiological and pathobiochemical aspects but also those related to the habitual environment (conditioned by geochemical peculiarities).

In the etiopathogeny of urolithiasis metals play an important role. They 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.

Urolithogenesis is a process which begins with the appearance of the so-called “primers” resulted by the heterogenous nucleation mechanism, involving the presence of specific lithogenic organic (urates, oxalates, cystine) or inorganic (phosphates, carbonates) components and metallic ions.

In the uroconcrement biogenesis can compete compounds with ion and/or non-ion structure. Beside these usually anionic structures there are ionic structures generated by some conditions: the pH , ionic strenght, osmolality a.o. – situations in which such forms may generate hydroxylated derivatives of purines (uric acid, xanthine, 2,8 –DHA).

To organic or inorganic compounds of uroconcrements may bond metallic ions, such as Na^{+}, K^{+}, $Ca^{2+,}$ Mg^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Pb^{2+} a.o. and non-metallic ions (NH_4^{+}). Though the metallic trace elements are in very small quantities they compete (obviously in a lesser degree) to the heterogenous nucleation mechanism of calculi. In the next paper (Note II) analytical data on the trace metals found in the uroconcrements studied by us will be presented.

Establishment of urolithiasis types and quantity of their metallic components are of interest not only for an accurate clinical guideline but also for the prophylaxy and metaphylaxy of urolithiasis.

CONCLUSIONS

By means of infrared spectroscopy there were established the following types of purine urolithiasis: simple - with urates (U); mixed - with oxalates-urates (O-U); urates-oxalates (U-O); oxalates-urates-phosphates (O-U-P).

The alkaline and alkaline-earth metals concentration in the simple and mixed purine urolithiasis, determined by atomic absorption spectroscopy, revealed a decrease as follows :

Ca > Na > K > Mg in case of U and O-U lithiasis
Ca > Na > Mg > K in case of U-O and O-U-P lithiasis

In mixed urolithiasis the concentration of Ca, Na, K and Mg was higher than in simple urolithiasis due to the presence of oxalates and phosphates which determine ionic bonds with the metals in uroconcrements.

EXPERIMENTAL SECTION

Samples obtainment. The study have been performed on the surgically removed or spontaneously eliminated urinary stones obtained from 172 patients admitted and treated in the Clinic of Urology Timişoara. The samples were collected during a period of 6 years.

Analytical determinations. First, in order to study the qualitative and quantitative composition of the kidney stones they were submitted to repeated washing with distilled water (to remove blood, mucous etc.), air-dried and finally powdered.

Next, the qualitative chemical composition of each urinary stone was determined by the Fourier Transform - Infrared Spectroscopy (FT-IR). For the spectra recording a JASCO FT-IR/410 spectrophotometer (Jasco, Japan), in the 400-4000 cm^{-1} wavenumber range at 4 cm^{-1} resolution, was used. The samples have been homogenized with KBr and converted in pellets using a manual 15 Ton Specac Pellet Press (Specac Ltd, U.K.). Initially there were recorded the FT-IR spectra of the chemically pure substances (presumed to be also in the composition of kidney stones) in order to create a database of „standard spectra" and afterwards the spectra of the powered kidney stones (with unknown composition). Subsequently to the obtainment of FT-IR spectra of uroconcrements the types of urolithiasis were established.

Further on by means of atomic absorption spectroscopy (AAS) the quantity of the alkaline (Na, K) and alkaline-earth (Ca, Mg) metals in the simple and mixed (binary and ternary) purine urolithiasis was determined. The concentrations of Na, K, Ca and Mg were expressed in μg/g calculus. For this investigation a PYE UNICAM apparatus Series SP 1900 was used.

Statistic evaluation. The analytical data were statistically processed by a computerized method. Mean values (X) and standard deviation (SD) of the metals concentration in the simple and mixed purine urolithiasis were determined.

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