

TRACE ELEMENT STUDIES IN UROLITHIASIS; PRELIMINARY INVESTIGATION ON MIXED CALCIUM OXALATE-STRUVITE URINARY CALCULI

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In this study, the levels of the trace elements copper, zinc, lead, iron, aluminium, nickel, chromium along with magnesium, sodium and potassium were estimated in fifteen mixed calcium oxalate-struvite (CaOx/STR) urinary stones. The mean values of the combined results were, copper 4.24, zinc 1302.10, lead 23.25, iron 36.48, aluminium 28.83, nickel 0.69, chromium 1.93, magnesium 4530441, sodium 54.13 and potassium 5.93 ng mg⁻¹. It was observed that zinc, aluminium and potassium levels were higher than in calcium oxalate (CaOx) calculi $0.05 > P > 0.02$ and potassium levels were higher than in mixed calcium oxalate-hydroxy apatite (CaOx/APA) calculi, $P < 0.01$. A combination of all the results was also compared with similar data from South Africa, Turkey, Austria, India, U.S.A. and Japan.

Key words: Trace elements, Urinary calculi, Urolithiasis.

Introduction

More than 40 elements have been detected in various parts of human body out of which 14 are termed as essential trace elements, as their concentration is less than 50 mg kg⁻¹ body weight (Heydorn 1983) These elements play important role in the metabolic processes of the body.

To investigate the role played by trace elements in urolithiasis, it is necessary to estimate their concentrations in a number of calculi of different types.

The stone formation has been observed in cases of gout, cystinuria, hyperparathyroidism, bacterial infection and in the excretion of perpetually concentrated urine. On the other hand, calculi may form in the absence of any of these conditions or when the urine is rarely concentrated. Very little is known about the aetiology and initial mechanisms of the formation of urinary calculi except in certain cases of clearly defined disease states. There are no reports on the analysis of mixed calcium oxalate-struvite (CaOx/STR) stones, however, a few papers dealt with other types of urinary calculi or those without discerning the type (Levinson *et al* 1987; Wandt and Pougnet 1986; Aziza Khanam and Aatur Rehman 1978; Joost and Tessadri 1987; Komleh *et al* 1989 and Hofbauer 1991).

In this work analytical findings on fifteen CaOx/STR urinary calculi which were removed by surgery at a hospital have been reported. We have also tried to make a compari-

son between our total results with those from six other countries.

Materials and Methods

Double distilled deionised water used in all studies which was tested for zero response by atomic absorption spectroscopy for each element under investigation.

All glassware and disposable pipette tips were soaked for at least 24 h. in 20% nitric acid to ensure metal free surfaces. They were then washed at least six times in pure water before oven drying.

'Spectrosol' grade reagents from B.D.H., Poole (Dorset, U.K.) were used throughout the course of investigation.

Analysis by kit method. Stones were carefully cleaned, dried and then ground to fine powder using agate mortar and pestle. Initial analysis for calcium, magnesium, ammonium, uric acid, phosphate and oxalate was carried out by a kit method for classification.

Sample preparation. Sample of each powdered stone was digested with a mixture of 2 ml of conc nitric acid and 1 ml of perchloric acid, both of spectroscopically pure grade, until a clear solution was obtained (Manser *et al* 1995). It was cooled and diluted to 250 ml.

Preparation of standard urinary stone solution. Trace element analysis was carried out on a Hitachi Z-8000 atomic absorption spectrometer equipped with Zeeman background correction and a data processor. Flame atomization was used

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for iron, copper, zinc, magnesium and aluminium and graphite furnace atomization for lead, nickel, chromium. Sodium and potassium were estimated by flame photometer (Corning Model 410).

The instrument was calibrated for each element using standard urinary stone solution.

Several urinary stones were carefully cleaned with water and oven dried at 110°C before grinding to powder in an agate mortar and pestle. About 10g of the combined powders was dissolved in 80 ml of concentrated nitric acid ('Spectrosol' grade, BDH, Poole, U.K.) and heated until all was in solution and any chemical reaction had ceased. The resulting solution was diluted to one litre with pure water.

Results and Discussion

Trace elements were estimated in fifteen mixed CaOx/STR urinary calculi, given in Table 3 Table 4 and 5 give the ranges and mean values respectively, of the combined results for all calculi analysed so far, i.e. CaOx/STR together with those calcium oxalate (CaOx) and mixed calcium oxalate into calcium oxalate-hydroxyapatite (CaOx/APA) calculi. Also listed are the combined results of work reported from South Africa (Wandt and Pougnet 1986) Turkey (Durak *et al* 1988) and Austria (Hofbauer *et al* 1991) as well as those from India (Komleh *et al* 1989) U.S.A. (Levinson *et al* 1978).

The proportion of different types of calculi varies greatly depending on regions. All of ours contained calcium oxalate except three, but only 51/102 (50%) from South Africa (Wandt and Pougnet 1986) 19/20 (65.5%) from Turkey (Durak *et al* 1988) and 16/25 (64%) from Austria (Hofbauer *et al* 1991) contained calcium oxalate. Of these 14 (35%)

of our, 33 (64.7%) from South Africa (Wandt and Pougnet 1986) and 14 (73.7%) from Turkey and 13 (81.2%) from Austria were basically CaOx; 4 (35%) of ours, 18 (35.3%) from South Africa, 5(26.3%) from Turkey and 3 (18.8%) from Austria were CaOx/APA and 9(22.5%) of ours.

Table 1A
Instrumental conditions for ASS

Graphite furnace	Elements		
	Pb	Cr	Ni
Lamp current	7.5 mA	7.5 mA	10.0 mA
Wavelength	283.3 nm	359.3nm	232.0 nm
Slit width	1.3nm	1.3 nm	0.2 nm
Cuvette	Graphite tube		Graphite
Tube	Graphite tube		
Carrier gas(Ar)	200 ml min ⁻¹	200 ml min ⁻¹	200 mlmin ⁻¹
Interrupted gas	30 ml min ⁻¹	30 ml min ⁻¹	30 ml min ⁻¹
Sample volume	20 ul	20 ul	20ul
Calculation mode	Integration	Integration	Integration
Calculation	10.0 sec	10.0 sec	10.0 sec

Table 2

Temperature programme for lead, chromium and nickel

No.Stage	Pb		Cr		Ni		Time Sec.
	Temp°C		Temp°C		Temp°C		
	Star	End	Start	End	Start	End	
1.Dry	80	120	80	120	80	120	30
2.Ash	400	400	700	700	700	700	30
3.Atcmisation	2000	2000	2900	2900	2700	2700	10
4.Cleaning	2400	2400	3000	3000	2800	2800	3

Table 1

Instrumental conditions for AAS

Flame atomisation	Elements				
	Fe	Cu	Zn	Mg	Al
Lamp current (mA)	10.0	7.5	10.0	7.5	10.0
Wavelength (nm)	248.3	324.8	213.8	285.2	309.3
Slit width (nm)	0.2	1.3	1.3	1.3	1.3
Oxidant	air	air	air	air	N ₂ O
Oxidant Pressure (Kg cm ⁻²)	1.60	1.60	1.60	1.60	1.40
Fuel	C ₂ H ₂	C ₂ H ₂	C ₂ H ₂	C ₂ H ₂	C ₂ H ₂
Fuel Pressure (kgcm ⁻²)	0.30	0.30	0.20	0.20	0.40
Burner height (mm)	5.5	7.5	7.5	7.5	10.0
Calculation mode	Integration				
Calculation time (sec)	1.0	1.0	1.0	1.0	

Table 3

Trace elements concentration in CaOx/STR urinary calculi.

Element	Concentration (ng mg ⁻¹), n=15	
	Range	Mean
Copper	1-7.0	4.24
Zinc	23.5-7146	1302.10
Lead	3.95-50.20	23.25
Magnesium	71.71-122407	45304.41
Iron	12.0-70.21	36.48
Aluminium	6.0-53.0	28.83
Nickel	0.00 -1.050	0.69
Chromium	1.294-2.72	1.93
Sodium	21.00-160	54.13
Potassium	2.00 - 13.00	5.93

were CaOx/STR. South Africa, Turkey and Austria had none of this type.

CaOx/STR urinary calculi. There is no report in the literature about the trace element levels in this type of urinary stone, hence a comparison of the results of this preliminary study with those from other parts of the world cannot be made. It was surprising to note that lead levels in these stones, were low while in blood, lead levels were estimated to be high in Karachi.

Zinc levels were very high and magnesium levels were particularly so, especially in some individual calculi even al-

lowing for the fact these calculi were partly composed of struvite ($MgNH_4PO_4$). Also of interest were the chromium and nickel results.

The Austrian group (Hofbauer *et al* 1991) could find no significant differences in level of any of the trace elements between any of their groups of calculi. We found that the levels of zinc, aluminium and potassium were higher in the CaOx/STR calculi than in the CaOx, $0.05 > P > 0.02$ and potassium levels were higher than in the CaOx/APA calculi $P < 0.01$. The Wilcoxon Rank Sum Test was applied as the distributions were non-Gaussian.

Table 4
Comparison of trace elements concentration range in urinary calculi ($ng\ mg^{-1}$)

Element	Present Study n = 40	South Africa n = 102	Turkey n = 29	Austria n = 25	India n = 60	U.S.A n = 69	Japan n = 10
Copper	1.0-11.8	1.00-10.00	-	0.59-122.19	-	-	4.80-27.00
Zinc	23.25-7146	0.5-1381	-	0.00-5987.4	-	-	239.00-913.00
Lead	3.95-65.4	14.0-139.00	-	0.00-867.97	-	-	19.00-58.00
Magnesium	71.71-122407	100.0-95000	-	-	-	-	1500.00-31400
Iron	12.0-146.70	0.5-156.00	-	11.21-1388.2	-	-	118.00-340.00
Aluminium	6.0-102.60	5.00-89.00	-	0.00-178.58	-	-	-
Nickel	0.00-3.50	-	-	0.12-126.27	-	-	-
Chromium	0.94-2.72	-	-	0.00-191.20	-	-	-
Sodium	8.00-160.00	129.00-50042.00	-	-	-	-	1690-7650
Potassium	0.50-14.00	90.00-6756.00	-	-	-	-	520.00-8140.00

Table 5
Comparison of trace elements mean concentration in urinary calculi ($ng\ mg^{-1}$)

Element	Present Study n = 40	South Africa n = 102	Turkey n = 29	Austria n = 25	India n = 60	U.S.A n = 69	Japan n = 10
Copper	5.13	1.4	18.4	17.93	314	2.5	14
Zinc	605.41	270	161.0	542	15834	420	555
Lead	27.48	22	33.5	84	-	65	34
Magnesium	30280	23800	1487	-	14415	-	11200
Iron	44.02	27.0	77.9	166.5	-	33	215
Aluminium	39.90	32	-	34.5	-	180	-
Nickel	0.74	-	-	10.3	-	-	-
Chromium	1.80	-	-	15.6	-	-	-
Sodium	44.78	4670	-	-	-	3574	4570
Potassium	4.29	1737	-	-	-	15265	2890

A comparison of combined results with those of six other countries. A combination of all the results for the three classes of urinary calculi namely CaOx, CaOx/APA and CaOx/STR calculi are given in Table 5. The results of the research groups in South Africa (Wandt and Pougnet 1986), Turkey (Durak *et al* 1988) and Austria (Hofbauer *et al* 1991) are treated similarly. Results from India (Komleh *et al* 1989) U.S.A. (Levinson *et al* 1978) are also given.

The highest mean zinc level by far was from India (Komleh *et al* 1989) where many of the patients were zinc miners. India also had the highest mean copper level. Environmentally conscious Austria and U.S.A. had the highest lead levels while ours and those from South Africa were low. Magnesium levels from Turkey (Durak *et al* 1988) were very low, while the other groups, including ours, reported very high mean levels. The mean values of sodium from U.S.A., South Africa and Japan were very high.

This study indicates that not only the proportions of the different types of urinary calculi vary from region to region, but also the levels of a given trace element, in a given type of stone. The ranges, where given, are usually very wide. Further investigations are required on a much greater number of samples, with analysis of the blood and urine of the patient and of the drinking water supply especially with regards to lead and aluminium. Analysis of the different layers of concretion in some of the larger stones would be of interest.

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