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TRACE ELEMENT STUDIES IN UROLITHIASIS: PRELIMINARY INVESTIGATION ON CALCIUM OXALATE AND MIXED CALCIUM OXALATE-HYDROXYAPATITE URINARY CALCULI

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In this preliminary investigation, the levels of the trace elements copper, zinc, magnesium, lead, iron, aluminium, nickel, chromium, sodium and potassium were estimated in seven calcium oxalate (CaOx) and six mixed calcium oxalate-hydroxyapatite (CaOx/APA) urinary calculi. The results were compared with those from South Africa, Turkey and Austria. Our mean results for the CaOx and CaOx/APA calculi were respectively, copper 4.3, 7.15 ng/mg; zinc 141.8, 191.1; magnesium 18520, 7203; lead 21.7, 15.5; iron 24.7, 78.35; aluminium 14.3, 42.7; nickel 0.39, 0.85; chromium 1.52, 1.88; sodium 4057, 3260; potassium 200, 150ng/mg, Only for aluminium was the difference significant: 0.05>p>0.02.

Key words: Trace elements, Urolithiasis, Urinary calculi (CaOx and CaOx/APA).

Introduction

There are over 40 elements in the human body of which about 14 are known to be essential trace elements, the amount of each of these in the whole body is less than 50 mg. They are required in the diet for well being and health and play vital role in the metabolic processes of the body. The interest in the role of trace elements in almost all fields of biomedical and environmental research has increased dra-matically in recent years.

In cases of gout, cystinuria, hyperparathyroidism, bacterial infection and in the excretion of perpetually concentrated urine, urinary stones may form [1]. On the other hand, calculi may form in the absence of any of these conditions or when the urine is rarely concentrated. In general, little is known about the reasons for stone formation or about the mechanisms of nucleation and initial growth. Although some attention has been focused on the possible role trace elements may play in urolithiasis and *in vitro* experiments have been carried out. The only reports concerning the analysis of trace elements in urinary calculi themselves are from Cape Town (South Africa) [2], Ankara (Turkey) [3], Vienna, (Austria) [4], Udiapur (India) [5], U.S.A. [6] and Japan [7], to our knowledge. Only the first three of these have given results for CaOx and CaOx/APA calculi, the calculi were not classified in the others.

In this paper we have reported our results for the trace element analysis of urinary CaOx and CaOx/APA calculi which were removed from the patients surgically in one of the Karachi hospitals and we have made comparisons with the results reported from Cape Town, Ankara and Vienna.

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Materials and Methods

Water. All water used was doubly distilled and deionised water stored in a previously acid soaked and well washed polythene container. It was tested for zero response by atomic absorption spectroscopy for each element under investigation.

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

Chemicals. 'Spectrosol' grade reagents from B.D.H., Poole, Dorset, U.K. were used throughout.

Analysis. After careful cleaning and drying, 20 urinary calculi were selected and ground to fine powders using an Agate mortar and pestle. Initial analysis of each for calcium, magnesium, ammonium, uric acid, phosphate and oxalate by a kit method classified it. Seven were found to be CaOx and six were mixed CaOx/APA calculi and were used in this preliminary study.

250 mg of each powdered stone were 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. After cooling and dilution to 250 ml in a graduate flask with pure water, analysis for copper, zinc, magnesium, lead, aluminium and iron was carried out using a Hitachi Z-8000 atomic absorption spectrometer by flame aspiration and for nickel and chromium by graphite furnace. Attempts at the analysis of manganese, cobalt and cadmium were also made, but these were not estimatable in any of our stones. The instrument was calibrated for each element using our own prepared standard stone solution and controls of spectrosol

grade in 1% solution. Sodium and potassium were estimated by Flame Photometer, Corning, Model 410.

Results and Discussion

Results for the CaOx urinary calculi are given in Table 1 and those for CaOx/APA in Table 2, together with those from Cape Town, Ankara and Vienna [2-4]. Of note are the wide variation of results for a given type of stone between localities and the wide range within the same locality.

As a preliminary investigation, we analysed seven CaOx and six CaOx/APA mixed urinary calculi obtained surgically in Karachi for the trace elements shown (Tables 1 and 2). It was also attempted to analyse manganese, cobalt and cadmium, but levels were below the lower limit of detection of the instrument in all the calculi. The group from South Africa [2] were also unable to obtain results for manganese, but those from Turkey [3] found mean levels of 1.92 and 3.13 ng/mg for CaOx and CaOx/APA calculi respectively. In general, trace element levels were very much higher in the Viennese stones [4] and results were obtained for manganese, cadmium and cobalt and CaOx stones: 0.23-29.5, 0-9.1 and 0-27.3 ng/mg respectively and for CaOx/APA: 0-15-0.59, 0.09-0.63 and 0.10-0.71. It should be noted that there were only three specimens of the second type of stone. Of interest are the easily estimatable quantities of chromium in all our calculi and of nickel in nearly all though in most cases far lower than those found in Austria.

CaOx urinary calculi (Table 1). In general, the concentration of copper, lead, iron and aluminium in the calculi from Austria [4] were much greater than those in our calculi and in those from other areas, where reported. The levels of nickel and chromium were also greater than in ours, no other groups analysed these elements. However, our levels for zinc and magnesium were the highest, those for magnesium being extremely high. The Austrians did not estimate the later element. The South African group [2] was the only other which estimated sodium and potassium, ours were higher for the former and lower for the later.

TABLE 1. TRACE ELEMENT CONCENTRATION IN CaOx URINARY CALCULI.

Element		Concentration (ng/mg)			
		Present study n =7	Cape Town[2] n=33	Ankara[3] n =14	Vienna[4] n=13
Cu	Range	2 - 8	0.5-5.9	-	3.9-122
	Mean	4.3	1.2	16.4	26.3
Zn	Range	15.5-384	2.1-1316	-	0-598.7
	Mean	141.8	98.3	73.9	75
Pb	Range	3 - 63	7-131	-	0-868
	Mean	21.7	15.7	49.0	138
Mg	Range	342-122400	100-8700	-	-
	Mean	18520	554	570	-
Fe	Range	17.1-39.1	0.5-79	-	26.4-1388
	Mean	24.7	23.1	78.9	286
Al	Range	6- 35	5 - 89	-	0-179
	Mean	14.3	15.6	-	54.5
Ni	Range	0-1.48	-	-	0.12-126.3
	Mean	0.39	-	-	18.8
Cr	Range	0.94 - 2.10	-	-	0-191
	Mean	1.52	-	-	28.1
Na	Range	800 - 16000	129-10636	-	-
	Mean	4057	1553	-	-
K	Range	50-800	90-4059	-	-
	Mean	200	664	-	-

TABLE 2. TRACE ELEMENT CONCENTRATION IN CaOx/APA URINARY CALCULI.

Element		Concentration (ng/mg)			
		Present study n=6	Cape Town[2] n=18	Ankara[3] n= 5	Vienna[4] n= 3
Cu	Range	5-11	23.3-29.6	-	0.9-11.7
	Mean	7.15	26.6	37.75	5.6
Zn	Range	29.8-547	113-1381	-	4.4-770
	Mean	191.1	483	475	384
Pb	Range	11-19	7-119	-	3.2-154
	Mean	15.5	47	35.9	61.0
Mg	Range	560-36300	300-18500	-	-
	Mean	7203	3000	1557	-
Fe	Range	23.9-146.7	9-129	-	18.3-49.4
	Mean	78.35	48	161.3	31.3
Al	Range	22-103	12-72	-	5.7-32.5
	Mean	42.7	30	-	18.4
Ni	Range	0-3.50	-	-	0.18-1.79
	Mean	0.85	-	-	0.96
Cr	Range	1.46-2.36	-	-	0.43-1.65
	Mean	1.88	-	-	1.01
Na	Range	1000-9100	1428-15212	-	-
	Mean	3260	3884	-	-
K	Range	100-350	350-3270	-	-
	Mean	150	1063	-	-

Our levels for copper and lead were relatively low, the later being rather surprising in view of the high blood lead levels in the population reported by one of us [8].

South African researchers in a later paper [9] pointed out that the combined mean trace element levels of all their calculi were in the same order as the mean of the normal concentrations in urine namely Na > K > Zn > Fe > Pb > Cu. We also found this sequence in the calculi of this group but in addition in the stones Cu > Cr > Ni > whereas normally in urine it is Cu > Ni > Cr. The Austrian group also found the same sequence (except for sodium and potassium) as in our calculi, but in those from Turkey it was Fe > Zn > Pb > Cu.

CaOx /APA urinary calculi (Table 2). The Viennese group [4] reported the highest levels for lead, but the lowest for copper, iron and aluminium. Our levels together with those from Vienna for copper, were relatively low. Our levels for lead and zinc were the lowest and for magnesium the highest. Our nickel levels were similar to those from Vienna, but chromium was higher while potassium was lower than those levels found in South Africa [2].

Although the groups from South Africa [9] and Turkey [3] (where applicable) found that Na > K > Zn > Fe >Pb >Cu as in urine, we found that Na > Zn > K > Fe > Pb> Cu > Cr > Ni in this group of stones, and the Austrians found Zn > Pb > Fe > Cu > Cr > Ni but for only a small number of samples (n = 3).

In comparing our own results for the two groups of stones (CaOx and CaOx/APA), only statistically significant difference was in the aluminium levels, being higher for the CaOx/APA calculi, 0.05>P>0.02 (Wilcoxon Rand Sum Test).

Of note are the levels of aluminium and the very high levels of magnesium which we found in both types of calculus. The relatively low lead levels are surprising in view of the high degree of lead pollution in the atmosphere of Karachi and the high blood lead levels of the population [8]. The trace element levels in the CaOx calculi were much higher of lead as those in both types of stone, from environmentally conscious Vienna. The results from individual calculi were astonishingly high.

These results require further investigations and should include analyses of the blood and urine of the patient and of the drinking water in his/her place of abode. The notable aluminium concentrations in the calculi also need further investigation, as the normal range in urine is extremely low.

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