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Analysis of calcium, iron, copper and zinc contents of nucleus and crust parts of urinary calculi

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Summary. In this study we compared the concentrations of calcium, iron, copper and zinc in the nucleus and the crust parts of various kinds of urinary calculi. We found that the mean concentrations of iron, zinc and copper in the nucleus parts of the stones were statistically higher than those in the crust parts. We attempted to explain this finding by two different approaches. Inter- and intracorrelations were also sought between the elements in the nucleus and crust parts. These correlations suggested a complex relationship between the elements of the urinary calculi.

Key words: Urinary calculi - Calcium - Iron - Copper - Zinc

Although many theories have been put forward to explain the stone-formation process [1, 17], none gives a satisfactory explanation and several questions still remain to be clarified [18]. Therefore, it seemed reasonable to investigate various aspects of the stone-formation process in more detail. In this regard, we have previosly carried out several studies to obtain more insight into the possible functions of some of the major and minor elements in the stone-formation and growth processes in the urinary tract [4-8]. In some of our previous work [4-6], however, we studied whole stone, without separating the inner nucleus and outer crust parts, which were different in chemical structure and composition. Knowledge of the trace element status of both the inner nucleus and outer crust parts may provide useful information about the stone precipitation in the urinary tract.

Several reports have described the possible functions of iron, copper, zinc, calcium and other elements in stone-formation disease [4, 5, 8-10, 15, 16]. Indeed, some major and minor elements may play a role in influencing, causing, accelerating or inhibiting the precipitation of the urinary tract calculi. Furthermore, it has been shown that some trace elements tend to enhance the growth rate of deposits of crystalline calcium compounds [2]. Similar observations were also demonstrated in previous reports [20, 22]. All these observations need to be investigated in more detail before reaching a definite explanation on stone formation.

Materials and methods

Of 37 urinary tract calculi used in this study, 34 had nucleus and crust parts with the same chemical composition. Ten calculi contained pure calcium oxalate (group A); 8 had calcium oxalate and calcium phosphate (group B); 3 were pure uric acid (group C); 3 had calcium oxalate and uric acid (group D); 3 had calcium oxalate, calcium phosphate, calcium carbonate and magnesium ammonium phosphate (group E); 7 had calcium oxalate and magnesium ammonium phosphate (group G). The 3 calculi in group F, however, had calcium oxalate type nuclei and magnesium ammonium phosphate, and calcium oxalate mixed-type crust.

Complete urinary tract calculi were obtained from the Urology Department, ibn-i Sina Hospital, Ankara. After washing with deionized water, the calculi were dried at 40°C for 24 h and then kept in a dessicator for about 2 months, until analysis.

Calculi were gently crushed in an agate mortar by using an agate mortar pestle and then the inner nucleus parts were removed with the help of a small ceramic spatule, when necessary. Usually, however, there was no need to use a ceramic spatule to take out the nucleus because the nucleus and crust parts of many calculi could be easily separated after crushing. In fact, as a rule, we only used the stones whose nucleus and crust parts were clearly distinguishable and easily separated. The remaining outer parts of the stones were used as the crust fraction.

After the nuclei and crusts had been separated, each part was ground and the structural compositions were analyzed by infrared spectroscopy. In the qualitative analysis of the calculi, a Hitachi model 215 IR grating spectrophotometer was used. From ground samples, a small amount was taken and mixed with potassium bromide. A pellet was made by using the last mixture containing stone sample (approx. 20%) and potassium bromide (approx. 80%) as described by Chiara et al. [3]. The infrared spectra of the pellet were then taken from 4000 to 659 nm. By using the spectra of the pellet and previously identified peaks for specific groups, qualitative structural compositions of the stones were analyzed [11, 12].

From the thoroughly ground sample, approximately 200-500 mg was placed in a ceramic pot and ashed in an oven at about 600°C until a slightly greyish-white ash was obtained. The time the samples were left in the oven ranged from 30 min (e.g. for uric acid calculi)

Table 1. Mean ± SD values for calcium, iron, zinc and copper contents of nucleus and crust parts of urinary calculi

Groups	Nucleus, ppm				Crust, ppm			
	Ca, %	Fe	Zn	Cu	Ca, %	Fe	Zn	Cu
A	21.0	236.5	289.2	108.3	21.8	53.2	45.9	46.5
$n=10$	± 3.3	± 225.3	± 164.9	± 84.3	± 3.5	± 46.8	± 21.7	± 24.4
B	22.5	113.7	195.0	152.2	21.7	32.3	72.5	65.0
$n=8$	± 4.0	± 134.3	± 151.1	± 107.0	± 1.5	± 18.3	± 70.0	± 44.8
C	10.0	20.0	17.0	38.3	0.3	9.3	9.7	13.4
$n=3$	± 12.7	± 5.6	± 5.7	± 25.0	± 0.4	± 9.1	± 14.2	± 12.0
D	16.5	26.5	42.3	49.5	8.5	16.5	10.0	19.5
$n=3$	± 4.9	± 2.1	± 1.4	± 2.1	± 4.9	± 9.2	± 2.8	± 0.7
E	23.5	107.5	178.5	112.5	22.0	25.5	133.0	163.0
$n=3$	± 0.7	± 33.2	± 98.2	± 7.0	± 1.4	± 16.3	± 53.7	±182.4
F	19.2	30.5	85.4	62.0	16.0	29.0	136.2	46.5
$n=3$	± 8.5	± 7.8	± 29.7	± 2.1	± 2.8	± 4.2	± 43.8	± 6.4
G	19.4	102.5	219.2	93.0	22.5	42.5	76.8	59.8
$n=7$	± 17.0	± 62.9	114.0	± 50.0	± 2.4	± 12.5	± 29.1	± 21.8

Table 2. Intra-correlation coefficients among nucleus and crust elements

Group		Ca-Fe	Ca-Zn	Ca-Cu	Fe-Zn	Fe-Cu	Zn-Cu
A	Nucleus	0.28	No	0.22	0.44	0.62	0.34
$n=10$	Crust	No	−0.66	0.26	−0.38	0.33	−0.44
B	Nucleus	0.75	0.69	0.72	0.88	0.80	0.98
$n=8$	Crust	−0.43	−0.93	−0.34	0.56	0.30	0.24
G	Nucleus	No	−0.45	0.55	0.80	No	−0.45
$n=7$	Crust	−0.46	−0.36	0.34	0.87	−0.90	−0.57

Table 3. Inter-correlation coefficients *(r)* between nucleus and crust elements and student's *t*-test results *(P)*

Group		Ca-Ca	Cu-Cu	Zn-Zn	Fe-Fe
A	*r*	0.47	No	0.61	− 0.29
$n=10$	*P*	NS	< 0.025	< 0.0005	< 0.025
B	*r*	− 0.33	0.23	0.71	0.91
$n=8$	*P*	NS	< 0.05	< 0.05	< 0.025
G	*r*	0.94	0.58	0.48	0.33
$n=7$	*P*	NS	NS	< 0.005	< 0.025

Group A: Pure calcium oxalate calculi group (nucleus and crust)
Group B: Calcium oxlate + calcium phosphate mixed calculi group (nucleus and crust)
Group C: Pure uric acid calculi group (nucleus and crust)
Group D: Calcium oxalate + uric acid calculi group (nucleus and crust)
Group E: Calcium oxalate + calcium phosphate + calcium carbonate + magnesium ammonium phosphate mixed calculi group (nucleus and crust)
Group F: Calcium oxalate (nucleus) and magnesium ammonium phosphate + calcium oxalate (crust) calculi group
Group G: Calcium oxalate + magnesium ammonium phosphate (nucleus and crust) mixed calculi group
NS, Not significant; No, correlation

to 4 h (e.g. for calcium phosphate calculi) depending on the type of calculi. After dry-ashing, about 3 ml acid solution, composed of a concentrated nitric acid/concentrated perchloric acid mixture (6/1, v/v), was added to the ashes and they were heated moderately until all perchloric acid fumes disappeared and a homogenized solution was obtained. The final solution was prepared by diluting this solution with deionized water to a defined volume. Analyses were carried out on this solution.

Element concentrations were determined by atomic absorption spectrophotometry (Hitachi Polarized Zeeman Effect, 180/80 AAS), using a standard addition technique and a graphite furnace system [14]. Throughout the experiments, the necessary precautions were taken to avoid element contamination.

The statistical significance of the differences between the mean element concentrations of the groups were assessed using student's *t*-test. A *P* value of less than 0.05 was considered significant.

Results

Results are given in Tables 1-3. From the tables, the following conclusions were made.

Element concentrations in the nucleus parts of the calculi showed differences for each group. For example, iron, copper and zinc concentrations in the nucleus parts of groups A, B and G were significantly different.

Regarding the crust elements, there were also differences between the groups. However, no great differences were found between crust and nucleus calcium concentrations for groups A, B and G. In general, iron, copper and zinc concentrations in the nucleus parts of the calculi were higher when compared with their crust counterparts (Table 1).

Both positive and negative intra-correlations were found between the elements in the nucleus and crust parts. For example, there were only positive correlations between calcium and other elements in the nucleus of group B, while there were both positive and negative correlations in the crust parts. In this regard, positive correlations were found between the iron and zinc levels in both nucleus and crust parts in groups A, B and G, with the exception of crust parts in group A. Similar positive correlations were also found between iron and copper in all groups except group G (Table 2).

On the other hand, in the inter-correlation analysis made between nucleus and crust elements, positive correlations were found in all groups except for iron in group A and calcium in group B (Table 3).

Discussion

It has long been suggested that some major and minor elements together with other unclarified factors may play a role in the formation of urinary calculi. The absence of detailed studies on the possible functions of various elements in the formation of urinary calculi has however resulted in different approaches in studying the mechanisms of stone formation in the urinary tract [1, 5, 10, 13, 15, 16, 20, 21]. It seems that the major problem leading to faculty evaluations is that the contents of the nucleus and crust parts of the urinary calculi have not identified separately.

Since the initiation phase of the stone-formation process is as important as the promotion and growth phases, the element status in the local environment where the stone forms during the first initiation period may be of particular significance. In this regard, nucleus element status may give more useful information about the precipitation and initiation phases and, similarly, crust element status may give more information about the promotion and growth phases.

Our findings that different kinds of calculi contain different amounts of elements reflects the variations in the mechanisms of their formation in the urinary tract. This is seen clearly in the mean concentrations of iron, copper and zinc in the nucleus and crust parts of groups A, B and G.

The nucleus and crust concentrations of iron, zinc and copper were quite different (Table 1). In general, nucleus element concentrations were significantly higher than those in the crust parts. Therefore, it is not possible to compare the results of this present study with our previous reports [4, 5] or with those of other workers, which were established in whole stone without separation into inner and outer parts [13, 19, 21]. In general, our nucleus element concentrations were higher than those obtained in other studies but crust element levels were lower [4, 5, 13, 15, 19, 21]. In fact, large differences in element concentrations in various kinds of urinary calculi have been reported previously [4, 5, 13, 15, 19, 21]. Therefore, it is difficult to make comparisons with previous reports.

The results of the correlation analyses suggested that the elements of the calculi occurred in complex reciprocal relationships. Therefore, any change in the metabolism of a particular element may also influence the metabolism of some other elements. This interaction may result in abnormal body element metabolism, which may lead to the formation of calculi in the urinary tract.

In view of these results, how can one explain high iron, zinc and copper concentrations in the nucleus parts of the urinary calculi? The following two hypotheses can be postulated.

First, the presence of high concentrations of one or more elements in a local environment within the urinary tract, due to various causes, may initiate precipitation of stone nuclei at a local focus. That event may eventually lead to the precipitation of other elements, which may cause high levels of some elements, including iron, zinc and copper, in the nucleus. Therefore, high element concentrations in the nucleus may be one of the factors leading to stone formation in the urinary tract. These precipitation and initiation phases must then be followed by the stone growth and promotion phases.

Second, rapid precipitation of a stone at a local focus may lead to concomitant precipitation of other elements, causing high concentrations of some elements, including iron, zinc, and copper, in the nucleus. We therefore suggest that the presence of high levels of elements in the nucleus is a result of the precipitation process, rather than its cause.

At present, it is difficult to ascertain which of the explanations is correct. It is also possible that both mechanisms may play part in the event, resulting in a vicious circle. On the one hand, the presence of excess amounts of elements such as iron, zinc and copper in a local environment may become an initiating factor in the process of rapid stone precipitation; on the other, rapid stone precipitation of various aetiologies may also accelerate the precipitation of some major and minor elements as well in the environment where stone precipitation occurs. However, this explanation does not necessarily exclude the possibility that one of them may have a role in the stone formation and growth process in the urinary tract. This subject requires further investigation to reach a more definitive explanation of the problem.

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