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Measurement of the Risk of Calcium Oxalate Crystallization in Urine

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Summary. The risk of calcium crystallization (CaOx-CR) in urine was analyzed by means of crystal counting following standardized addition of oxalate. CaOx-CR was determined in 24 h urine samples from 21 stone formers and 26 normal subjects following dilution of urine to a creatinine concentration of 5 μmol per ml. The mean (± SD) CaOx-CR was in stone formers 1.42 ± 0.57 and in normal subjects 1.29 ± 0.40. CaOx-CR was also analyzed in 16 fresh urine samples diluted to 80 per cent of the original concentration whereby values between 0.36 and 3.6 were recorded. There was a good correlation between CaOx-CR and estimates of the ion-activity product of CaOx, both in urine diluted to 5 μmol of creatinine per ml and in 80 per cent diluted urine. It ist suggested that the method described is of value for evaluation and follow up of patients with CaOx urolithiasis.

Key words: Calcium oxalate, Crystallization, Urine, Urolithiasis.

Introduction

Different mechanisms are thought to contribute to the formation of renal calcium oxalate (CaOx) stones: i.e. supersaturation with respect to CaOx, decreased activity of inhibitors of crystal growth and crystal aggregation, crystallization promoters, and crystal retention within the collecting system [1–3].

A number of biochemical risk factors have been identified, most of which are determinants for the level of CaOx supersaturation [4, 5]. However, the inhibiting and promoting properties in urine are much more difficult to measure and no simple and accurate methods are available.

For the medical treatment of patients with recurrent renal stone disease it is desirable to get as much information as possible about the crystallization potential of urine; different methods have been described [6–12], but they are either complicated or insufficiently standardized.

In this paper a method for determination of the CaOx crystallization potential was evaluated by comparison with other risk indices which we used routinely in the evaluation and follow up of our stone formers.

Methods

Urine from 21 CaOx-stone formers and 26 normal subjects was collected during 24 h periods in plastic bottles containing 90 mmol of hydrochloric acid. The reason for acidification of the urine was to avoid percipitation of CaOx and calcium phosphate (CaP) during storage and to dissolve already formed crystals.

All samples were stored −20 °C until analysis, when the urine was thawed at 37 °C and carefully mixed. Adjustment to pH 5.8 was performed by adding sodium hydroxide, after which water was added to give a final creatinine concentration of 5 μmol per ml. Before the crystallization experiment, each sample was passed through a Millipore filter (pore size 0.22 μm).

To each 200 ml of urine prepared in this way, were added 100 μl-aliquots of an 0.04 mol/l Millipore-filtered sodium oxalate solution. After each addition the number of crystals in the size range 3.5 to 5 μm was determined in a Coulter Counter with Channelyzer (Model ZBI). All experiments were undertaken at room-temperature.

A computerized program was used to determine the increment in oxalate concentration (ΔOx) required for formation of 100 crystals with a diameter between 3.5 and 5 μm. The CaOx crystallization risk (CaOx-CR) was defined as the inverted ΔOx-value.

Light microscopy was performed to verify that the measured particles were CaOx-crystals.

Urine composition with respect to calcium (Ca), oxalate (Ox), magnesium (Mg), citrate (Cit), and creatinine (Cr) was routinely analyzed as previously described in detail [13, 14]. The level of supersaturation was expressed by an AP(CaOx)-index [15]:

$$\frac{k \times 3.8 \times Ca^{0.71} \times Ox}{Mg^{0.14} \times Cit^{0.10} \times V^{1.2}}$$

Where the factor k is a correction factor introduced to compensate for the dilution of urine constituents other than those included in the formula. Urine volume (V) is expressed in litres.

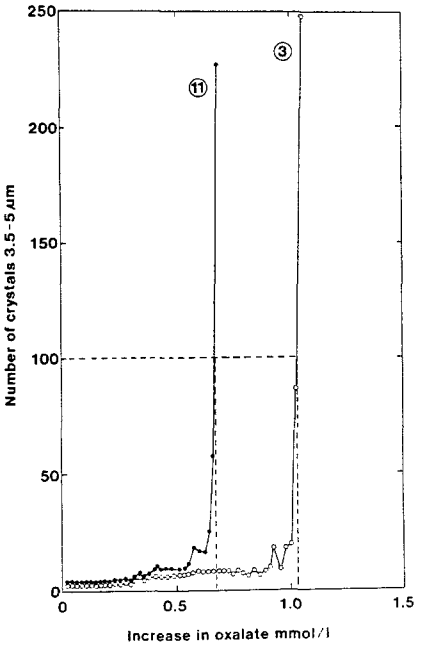

Fig. 1. The effect of increased oxalate concentration on the formation of CaOx crystals in two urine samples

A CaOx-risk index [15]:

$$\frac{(Ca/Cr)^{0.71} \times (Ox/Cr)}{(Mg/Cr)^{0.14} \times (Cit/Cr)^{0.10}}$$

was also used to summarize the metabolic risk situation in the individual urine relative to the creatinine excretion.

Freshly voided urine was analyzed from 16 patients. The original pH was measured, after which the samples were acidified to pH 3.0. Following re-adjustment to the original pH, water was added to give a final urine concentration of 80 per cent. The urine was then Millipore-filtered and subjected to CaOx-CR determination as described above.

Results

Figure 1 shows a typical result of two crystallization measurements, in which increments in oxalate concentration of 0.67 and 1.06 mmol/l were required to obtain 100 crystals in the size range 3.5 to 5 µm. Light microscopy of all samples at the end point clearly demonstrated crystals of CaOx-dihydrate.

The relationship between CaOx-CR and AP(CaOx)-index in stone formers and normal subjects is shown in Fig. 2. There was a good correlation in both groups (r = 0.73 and 0.76 respectively).

When CaOx-CR was compared with the CaOx-risk index (Fig. 3), a good correlation was also obtained in both stone-formers (r = 0.74) and normal subjects (r = 0.72).

The mean (± SD) CaOx-CR was in stone formers 1.42 ± 0.57 and in normal subjects 1.29 ± 0.40. The cumulative frequency distributions are shown in Fig. 4. Despite a signi-

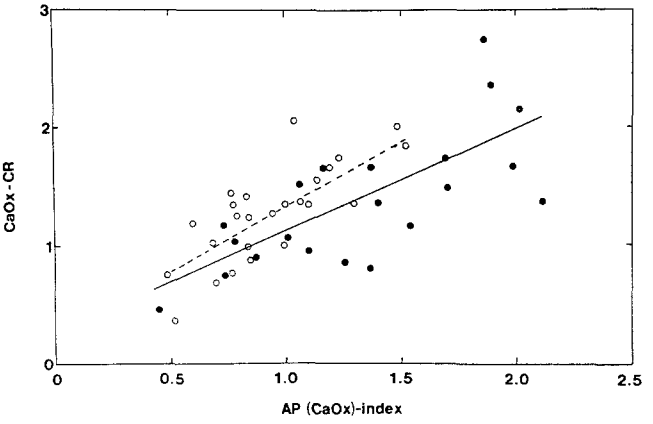

Fig. 2. Relationship between AP(CaOx)-index and CaOx-CR in urine from normal subjects (○) and stone formers (●). Urine was diluted to a creatinine concentration of 5 µmol/ml

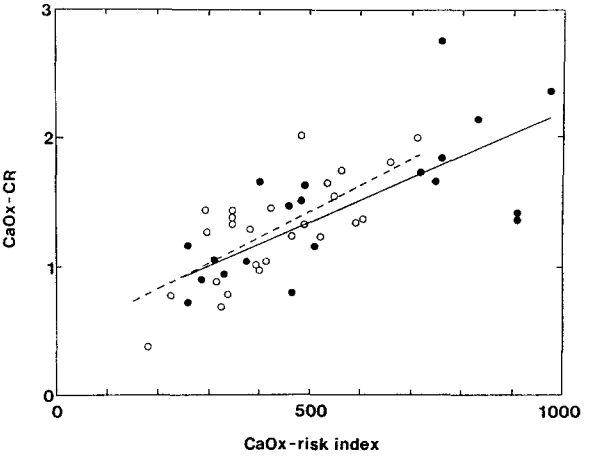

Fig. 3. Relationship between CaOx-risk index and CaOx-CR in urine from normal subjects (○) and stone formers (●). Urine was diluted to a creatinine concentration of 5 µmol/ml

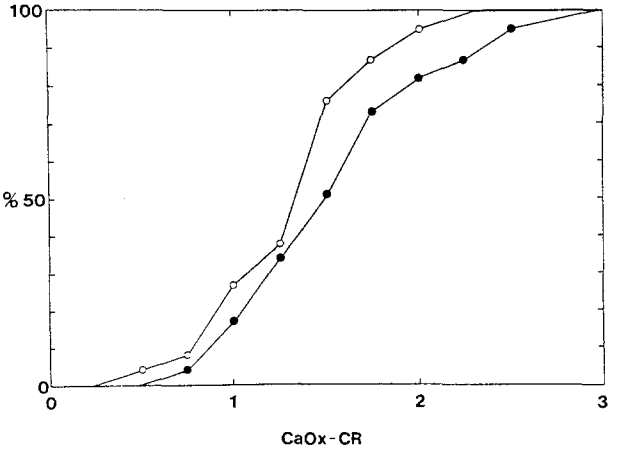

Fig. 4. Cumulative frequency distribution curves of CaOx-CR values in urine from normal subjects (○) and stone formers (●). Urine was diluted to a creatinine concentration of 5 µmol/ml

ficant difference in terms of AP(CaOx)-index: 1.35 ± 0.48 in stone formers and 0.94 ± 0.27 in normal subjects ($p < 0.001$), the CaOx-CR was not significantly different between the two groups.

The CaOx-CR was also determined in 16 fresh urine samples collected between 6.00 and 10.00 h. Values between 0.36 and 3.6 were recorded and even in these urines was there a good correlation to the AP(CaOx)-index ($r = 0.6$).

Discussion

The CaOx-CR was the net result of nucleation, crystal growth and crystallization inhibition. Whether an aggregation occurs as well was not possible to show, but in light microscopy no large aggregates were visible.

The interval between addition of sodium oxalate and crystal counting is of fundamental importance to the results and had to be standardized carefully. The crystal counting was always undertaken 2 min after the previous additive and the next addition of sodium oxalate 3 min later.

Dilution of the urine samples to a creatinine concentration of 5 μmol per ml was done in order to standardize the experimental situation and to enable comparison with our routinely used CaOx-risk index [15].

An AP(CaOx)-index was calculated for each urine sample, but because of the dilution of those urine constituents which are not included in the index formula, it was necessary to correct the index for the degree of dilution. Previous experience has shown that for clinical purposes, the AP(CaOx)-index is a fair approximation of the ion-activity product of CaOx [15].

A good correlation was obtained between CaOx-CR and both AP(CaOx)-index and CaOx-risk index. This is an important observation, because previous studies have shown significantly higher values of CaOx-risk index in stone formers compared with normal subjects [15, 16].

In the routine investigation of crystallization properties in urine, it is of less relevance to dilute urine to a fixed creatinine concentration. The measurements should be performed either in whole urine or in urine diluted to 80 per cent concentration, which enables treatment of the urine for dissolution of CaOx and CaP crystals. It would thus be possible to study the CaOx-CR in fresh urine collected during the different risk periods of the day [17].

If the level of CaOx-supersaturation only determined CaOx-CR, a similar difference as for AP(CaOx)-index between stone formers and normal subjects might be expected. Although the mean CaOx-CR was higher in stone formers the difference did not reach statistical significance. The reason for this is unclear, but can be explained by differnt levels of inhibitory activity. A rough estimate of the level of inhibiting activity might be obtained by diving the AP(CaOx)-index and the CaOx-CR. By this calculation the mean inhibition values for stone formers and normal subjects were 0.98 ± 0.28 and 0.76 ± 0.19 respectively ($p < 0.01$). A higher inhibitory potential was found among stone formers in contrast to measurements of the inhibition index in diluted urine [18, 19]. Although it is not possible to draw any definite conclusions from these very approximate calculations the results are interesting and might indicate an increased excretion of inhibitors in response to a high CaOx-supersaturation.

Such a hypothesis is supported by previous observations of a peak inhibitory activity during the morning which was simultaneous with a high CaOx-supersaturation [17].

In as much as CaOx-CR directly reflects the propensity of CaOx crystal formation in urine, its determination might be of considerable value in the biochemical work up of stone formers. A program for evaluation and follow up of these patients should therefore start with analysis of individual risk factors in one or several urine collections. These results would provide the basis for selection of appropriate therapy. The effect of treatment could then be followed by repeated determinations of CaOx-CR.

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