URINARY SATURATION AND RISK FACTORS FOR CALCIUM OXALATE STONE DISEASE BASED ON SPOT AND 24-HOUR URINE SPECIMENS

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1. ABSTRACT

In 222 random spot urine specimens, the calcium concentration and calcium oxalate saturation \( \text{DG(CaOx)} \) were significantly higher among stone formers than among non-stone formers, while the citrate and creatinine-corrected citrate concentrations were lower. In 188 24-hour urine specimens, magnesium excretion was lower among stone formers than non-stone formers, while the creatinine-corrected calcium concentration and \( \text{DG(CaOx)} \) were higher. Among stone formers, there was no gender difference in the urinary concentrations of calcium, oxalate, citrate, magnesium, and \( \text{DG(CaOx)} \), but the creatinine-corrected calcium, citrate, and magnesium concentrations were higher in women, as well as 24-hour citrate excretion. The levels of calcium and oxalate have a major influence on \( \text{DG(CaOx)} \), while citrate and magnesium levels have a minor influence. \( \text{DG(CaOx)} \) was correlated with calcium and oxalate excretion, as well as with the creatinine-corrected calcium and oxalate concentrations. Approximately 5% of 24-hour urine specimens showed critical supersaturation, 80% showed metastable supersaturation, and 15% were unsaturated. Hypercalciuria or hyperoxaluria was fairly common (30% and 40%) in critically supersaturated urine, while it was less common (22.4% and 8.6%) in metastably supersaturated urine and was not detected in unsaturated urine. Hypocitraturia and/or hypomagnesuria was more common (63.8-80%) at any saturation. The urinary calcium, oxalate, and citrate concentrations, as well as the creatinine-corrected calcium, oxalate, citrate, and magnesium concentrations and \( \text{DG(CaOx)} \), showed a significant correlation between 57 paired early morning spot urine and 24-hour urine specimens. The creatinine-corrected calcium and citrate concentrations of the early morning urine specimens were significantly correlated with the levels of calcium and citrate excretion in the paired 24-hour urine specimens. In conclusion, no parameter other than urinary saturation gives more than a vague indication of the risk of lithogenesis, so \( \text{DG(CaOx)} \) in either early morning urine or 24-hour urine specimens appears to be the best predictor of stone risk. Finally, the creatinine-corrected calcium and citrate concentrations in early morning urine can be used as a substitute for measuring 24-hour excretion.

2. INTRODUCTION

Urinary stones can be considered as fossils representing long-standing specific conditions of urinary supersaturation in the collecting system. The diurnal circadian rhythm, seasonal variation, heterogeneity of nephrons, and variations of pathological conditions all influence the saturation of urine (1-3). Urinary supersaturation is a prerequisite for stone formation and it should be documented during the period of stone formation and growth. With respect to lithogenesis, the circadian rhythm of stone parameters (calcium, oxalate, citrate, and magnesium) was investigated and discussed by Marshall, Vahlensieck, and Ogawa (4-6). However, it is not easy to measure all of these parameters in every patient during various situations in a day or year and individual parameters do not reflect the lithogenic potential or supersaturation of urine. Despite this, 24-hour urine collection to measure stone parameters remains the gold standard for finding the lithogenic etiology and potential (7-9).

Historically, urinary supersaturation has been used as scientific evidence of the potential for stone formation (10, 11), but complicated mathematical methods of calculating urinary saturation have made the practical use of such parameters difficult. Although a simplified method for estimating saturation was introduced by Marshall and Robertson (12), urinary saturation does not closely reflect clinical stone episodes, so various other parameters have been tested (13, 14). As a result, measurement of the risk of crystallization or saturation has subsequently been modified by various researchers (15-23). We previously used the simplified method of Marshall and Robertson (12) clinically and concluded that late night and early morning was the high-risk period for calcium oxalate crystallization (24), and similar findings were subsequently confirmed by Ahlstrand, Ogawa, and Robert (25-27).

The risk of calcium oxalate stone formation is generally discussed on the basis of several parameters (hypercalciuria, hyperoxaluria, hypocitraturia, and hypomagnesuria), but the lithogenic potential has been discussed in terms of urinary calcium oxalate saturation
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(24, 26, 28-33). There is no clear-cut relationship between the risk indices and the rate of stone formation or between stone formers and non-stone formers (34-37), but there is a tendency for a higher risk of stone formation in supersaturated urine (38-40). We also reported that the CaOx-risk index of Tiselius (16) was higher in 9 children with calcium oxalate stones than in 92 non-stone forming children (41), followed by a similar report (42) but the lithogenic potential in children still tends to be discussed only on the basis of citrate and magnesium (43). The efficacy of drugs can be evaluated in terms of changes in saturation (32, 44-57), but it is often interpreted only on the basis of calcium, oxalate, or citrate excretion (58, 59).

Using 390 urine specimens from healthy volunteers, we previously showed that hypocitraturia, hypomagnesiuria, and a high Ca/Mg ratio were poor indicators of calcium oxalate supersaturation (DG value > 0) and confirmed that critical calcium oxalate supersaturation of urine occurs late at night and early in the morning (60). Citrate and magnesium are known as important inhibitors of crystallization (61-63), and in a larger series than we reported previously (64) we studied how the levels of these parameters contribute to supersaturation and whether isolated hypocitraturia or hypomagnesiuria is clinically important. In addition, we compared the creatinine-corrected levels of calcium, oxalate, citrate, and magnesium in early morning spot urine with their respective 24-hour urinary excretion values.

3. SUBJECTS AND METHODS

A total of 222 random spot urine specimens were obtained in the morning at the outpatient clinic from 110 non-stone formers (37 women aged 46.4±22.8 years and 73 men aged 47.6±21.4 years) and 112 idiopathic calcium oxalate stone formers (37 women aged 55.3±16.4 years and 75 men aged 47.3±17.8 years), and 188 24-hour urine specimens were obtained in the ward from 22 non-stone formers (6 women aged 53.8±8.8 years and 16 men aged 54.2±18.6 years) and 166 inpatients with idiopathic calcium oxalate stones (49 women aged 56.6±13.1 years and 117 men aged 49.2±14.6 years). All of the patients had normal renal function and no serious diseases. Both 24-hour urine and early morning spot urine specimens were obtained from 57 inpatients with idiopathic calcium oxalate stones (who were aged 53±15 years) and were used for comparison of various urinary parameters. Urinary calcium (Ca) and magnesium (Mg) were measured by ICP spectrophotometry, oxalate (Ox) and citrate (Cit) were measured by capillary electrophoresis (65, 66), and urinary creatinine (Cre) was determined by the enzymatic method. The DG(CaOx) values were calculated with Finlayson's Equil2 program. The critical level for calcium oxalate supersaturation was defined as a DG value > 2.8

The DG(CaOx) values of the solubility product and formation product were reported by Ahlstrand to be -0.15 and 3.3, respectively (25) and by Robertson -0.36 and 2.8, respectively (38). The DG(CaOx) value and the values of [Ca], Ca/Cr (mg/mg), [Ox], Ox/Cr, [Cit], Cit/Cr, [Mg], and Mg/Cr were compared by Student's t-test, the Mann-Whitney test, and Scheffe's test. Correlations between parameters or groups (stone formers vs. non-stone formers) were determined by simple regression analysis and multiple regression analysis. Statistical significance was set at p<0.05 for all comparisons.

4. RESULTS

In random spot urine specimens, the mean calcium concentration was 2.329±1.614 and 2.879±1.751 mmol/l in non-stone formers and stone formers, respectively, with the latter value being significantly higher (p<0.01), as shown in Table 1. The oxalate and magnesium concentrations were not significantly different between the groups, as shown in Tables 1 and 2. The citrate concentration was 2.257±2.038 and 1.498±1.279 mmol/l in non-stone formers and stone formers, respectively, with the former value being significantly higher (p<0.01), as shown in Table 2. The Ca/Cr (mg/mg), Ox/Cr, and Mg/Cr ratios did not differ between the groups. The Cit/Cr ratio was 0.480±0.324 and 0.342±0.267 in non-stone formers and stone formers, respectively, with the former value being significantly higher (p<0.01). DG(CaOx) was 1.074±1.289 and 1.393±1.132 in non-stone formers and stone formers, respectively, being significantly higher (p<0.05) in the latter, as shown in Table 3.

In 24-hour urine specimens, the mean calcium, oxalate, citrate, and magnesium concentrations did not differ between the groups (Tables 1 and 2). Urinary calcium, oxalate, citrate, and excretion were not different between the groups, while magnesium excretion was 3.41±1.078 and 2.755±1.361 mmol/day in non-stone formers and stone formers, respectively, being significantly higher in the former group (p<0.05). The Ca/Cr (mg/mg) ratio was 0.116±0.051 and 0.148±0.075 in non-stone formers and stone formers, respectively, with the latter value being significantly higher (p<0.05), as shown in Table 3. The Ox/Cr, Cit/Cr, and Mg/Cr ratios did not differ between the groups. The mean DG(CaOx) value was 0.651±1.238 and 1.209±1.090 in non-stone formers and stone formers, respectively, with the latter value being significantly higher (p<0.05). Among stone formers, urinary calcium, oxalate, and magnesium excretion were not different between men and women, while 24-hour citrate excretion was significantly higher in women than in men (p<0.05). This gender difference was not present for the 24-hour concentrations of urinary calcium, oxalate, citrate, and magnesium for the DG(CaOx) values, while the 24-hour calcium/creatinine, citrate/creatinine, and magnesium/creatinine ratios were significantly higher in women (p<0.05).

DG(CaOx) was significantly correlated with urinary calcium and oxalate concentrations (p<0.01), calcium and oxalate excretion (p<0.01), and the calcium/creatinine and oxalate/creatinine ratios (p<0.01), as shown in Table 4. DG(CaOx) was also positively correlated with the urinary citrate and magnesium concentrations (p<0.01) and magnesium excretion (p<0.05), but it was not correlated with urinary citrate excretion or with the citrate/creatinine and magnesium/creatinine ratios. Therefore, urinary citrate
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Table 1. Urinary concentrations and excretion of Ca and Ox in stone formers and non-stone formers

<table>
<thead>
<tr>
<th>Age year</th>
<th>Spot urine Stone ( - )</th>
<th>47.3±22.0</th>
<th>2.32±1.614</th>
<th>0.18±0.140</th>
<th>Ox mmol/day 0.16±0.093</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stone ( + )</td>
<td>50.±17.6</td>
<td>2.87±1.75 1</td>
<td>2.94±1.373</td>
<td>0.11±0.076</td>
<td>0.196±0.105</td>
</tr>
<tr>
<td>24-hour urine Stone ( - )</td>
<td>51.±16.2</td>
<td>2.12±1.274</td>
<td>3.48±2.011</td>
<td>0.13±0.078</td>
<td>0.206±0.116</td>
</tr>
<tr>
<td>Stone ( + )</td>
<td>49.2±14.6</td>
<td>2.11±1.304</td>
<td>3.42±2.146</td>
<td>0.137±0.078</td>
<td>0.209±0.110</td>
</tr>
<tr>
<td>F ( + )</td>
<td>56.6±13.1</td>
<td>2.135±1.217</td>
<td>3.62±1.609</td>
<td>0.117±0.075</td>
<td>0.196±0.133</td>
</tr>
</tbody>
</table>

Urinary calcium & oxalate concentrations ([Ca] and [Ox]) and 24-hour urinary calcium & oxalate excretion (Ca) and (Ox) in 222 random spot urine specimens and 188 24-hour urine specimens are compared between stone formers and non-stone formers, as well as between male (M (+)) and female stone formers (F (+)). Data are shown as mean±SD. 1: p< 0.01.

Table 2. Urinary concentrations and excretion of Cit and Mg in stone formers and non-stone formers

<table>
<thead>
<tr>
<th>[Cit] mM</th>
<th>Cit mmol/day</th>
<th>[Mg] mM</th>
<th>Mg mmol/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spot urine Stone ( - )</td>
<td>2.257±2.038</td>
<td>2.509±1.841</td>
<td></td>
</tr>
<tr>
<td>Stone ( + )</td>
<td>1.498±1.279 1</td>
<td>2.168±1.257</td>
<td></td>
</tr>
<tr>
<td>24-hour urine Stone ( - )</td>
<td>1.206±0.656</td>
<td>2.205±1.133</td>
<td>1.922±0.678</td>
</tr>
<tr>
<td>Stone ( + )</td>
<td>0.821±0.781</td>
<td>1.335±1.260</td>
<td>1.774±1.085</td>
</tr>
<tr>
<td>F ( + )</td>
<td>1.073±0.818</td>
<td>1.818±1.260</td>
<td>1.537±0.907</td>
</tr>
</tbody>
</table>

Urinary citrate & magnesium concentrations ([Cit] and [Mg]) and 24-hour urinary citrate & magnesium excretion ([Cit] and [Mg]) in 222 random spot urine specimens and 188 24-hour urine specimens are compared between stone formers and non-stone formers, as well as between male (M (+)) and female stone formers (F (+)). Data are shown as mean±SD. 1: p< 0.01, comparison with non-stone formers; 2: p< 0.05, comparison with non-stone formers; 3: p<0.05, comparison with male stone formers (M (+)).

Table 3. Urinary Cre-corrected Ca, Ox, Cit, and Mg concentrations and DG(CaOx) in stone formers and non-stone formers

<table>
<thead>
<tr>
<th>Ca/Cre mg/mg</th>
<th>Ox/Cre mg/mg</th>
<th>Cit/Cre mg/mg</th>
<th>Mg/Cre mg/mg</th>
<th>DG(CaOx)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spot urine Stone ( - )</td>
<td>0.116±0.078</td>
<td>0.020±0.015</td>
<td>0.480±0.324</td>
<td>0.074±0.043</td>
</tr>
<tr>
<td>Stone ( + )</td>
<td>0.140±0.103</td>
<td>0.018±0.011</td>
<td>0.342±0.267 1</td>
<td>0.063±0.041</td>
</tr>
<tr>
<td>24-hour urine Stone ( - )</td>
<td>0.116±0.051</td>
<td>0.017±0.007</td>
<td>0.431±0.250</td>
<td>0.084±0.031</td>
</tr>
<tr>
<td>Stone ( + )</td>
<td>0.148±0.075 2</td>
<td>0.021±0.012</td>
<td>0.313±0.274</td>
<td>0.071±0.034</td>
</tr>
<tr>
<td>F ( + )</td>
<td>0.125±0.063</td>
<td>0.019±0.009</td>
<td>0.237±0.180</td>
<td>0.063±0.028</td>
</tr>
</tbody>
</table>

Urinary Ca/Cr, Ox/Cr, Cit/Cr, and Mg/Cr ratios and the DG(CaOx) value in 222 random spot urine specimens and 188 24-hour urine specimens are compared between stone formers and non-stone formers, as well as between male (M (+)) and female stone formers (F (+)). Data are shown as mean±SD. 1: p< 0.01, comparison with non-stone formers; 2: p< 0.05, comparison with non-stone formers; 3: p<0.01, comparison with male stone formers (M (+)); 4: p<0.05, comparison with male stone formers (M (+)).

excretion is independent of its urinary saturation. As expected, there was a significant positive correlation between the concentrations of calcium, oxalate, citrate, magnesium, and creatinine (p<0.01), except in the case of calcium and oxalate (p>0.05). DG(CaOx) could be expressed as follows on the basis of multiple regression analysis of 408 spot and 24-hour urine specimens (r = 0.9779, p <0.01). DG(CaOx) = 3.0070 + 2.6485xlog([Ca]) + 2.6353xlog([Ox]) - 0.6292xlog([Cit]) - 0.6122xlog([Mg]). This formula combined multiple variables and showed that DG(CaOx) was positively correlated with [Ca] and [Ox], while it was negatively correlated with [Cit] and [Mg], findings that were theoretically reasonable.

For a total of 146 24-hour urine specimens, hypercalciuria (> 200 mg/day), hyperoxaluria (> 30 mg/day), hypocitraturia (< 320 mg/day), and hypomagnesiuria (< 75 mg/day) were stratified into three saturation levels [critical supersaturation (DG>2.8) 6.8%, metastable supersaturation (2.8>DG>0) 79.5%, and unsaturated (0>DG) 13.7%] according to our previous report (60), as shown in Figures 1-3. Hypercalciuria or hyperoxaluria was fairly common (30% + 40%) in critically supersaturated urine (10 specimens), less common (22.4% + 8.6%) in metastably supersaturated urine (116 specimens), and was not detected in unsaturated urine (20 specimens). In contrast, hypocitraturia and/or hypomagnesiuria, either isolated or in combination with other abnormalities, was common (63.8-80%) at any saturation level. Therefore, hypocitraturia and hypomagnesiuria were nonspecific changes that could be present irrespective of the calcium oxalate saturation. However, there was a small subset of patients with isolated hypocitraturia (approximately 10%) or isolated hypomagnesiuria (approximately 10%) that was associated with critical supersaturation.

In 402 spot and 24-hour urine specimens, the creatinine-corrected values for hypercalciuria (Ca/Cr > 0.200), hyperoxaluria (Ox/Cr > 0.300), hypocitraturia (Cit/Cr < 0.320), and hypomagnesiuria (Mg/Cr < 0.075)
Table 4. Relationship between DG(CaOx) and various urinary risk factors

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Regression function</th>
<th>Regression Coefficient (r)</th>
<th>Probability p</th>
</tr>
</thead>
<tbody>
<tr>
<td>log[Ca]</td>
<td>Y = 2.96993X + 0.36858</td>
<td>0.78440</td>
<td>0.0000</td>
</tr>
<tr>
<td>log(Ca)</td>
<td>Y = 2.02111X + 0.19658</td>
<td>0.48183</td>
<td>0.0000</td>
</tr>
<tr>
<td>log(Ca/Cr)</td>
<td>Y = 1.45260X + 2.56207</td>
<td>0.34707</td>
<td>0.0000</td>
</tr>
<tr>
<td>log[Ox]</td>
<td>Y = 3.06383X – 5.19166</td>
<td>0.81512</td>
<td>0.0000</td>
</tr>
<tr>
<td>log(Ox)</td>
<td>Y = 2.73124X – 4.97490</td>
<td>0.66648</td>
<td>0.0000</td>
</tr>
<tr>
<td>log(Ox/Cr)</td>
<td>Y = 1.95392X + 4.8557</td>
<td>0.42523</td>
<td>0.0000</td>
</tr>
<tr>
<td>log[Cit]</td>
<td>Y = 3.06383X – 5.19166</td>
<td>0.81512</td>
<td>0.0000</td>
</tr>
<tr>
<td>log(Cit)</td>
<td>Y = 2.02111X + 0.19658</td>
<td>0.48183</td>
<td>0.0000</td>
</tr>
<tr>
<td>log(Cit/Cr)</td>
<td>Y = 1.45260X + 2.56207</td>
<td>0.34707</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

Correlation of DG(CaOx) with the calcium concentration ([Ca] mM), 24-h urinary calcium excretion ([Ca] mmol/day), and calcium/creatinine ratio (Ca/Cr mg/mg); the oxalate concentration ([Ox] mM), 24-h urinary oxalate excretion ([Ox] mmol/day), and oxalate/creatinine ratio (Ox/Cr mg/mg); the citrate concentration ([Cit] mM), 24-h urinary citrate excretion ([Cit] mmol/day), and citrate/creatinine ratio (Cit/Cr mg/mg); the magnesium concentration ([Mg] mM), 24-h urinary magnesium excretion ([Mg] mmol/day), and magnesium/creatinine ratio (Mg/Cr mg/mg).

Table 5. Correlations of urinary parameters between early spot urine and 24-hour urine specimens

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Spot urine</th>
<th>24-h urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ca] mM</td>
<td>2.900±1.620</td>
<td>2.120±1.363</td>
</tr>
<tr>
<td>[Ox] mM</td>
<td>0.167±0.096</td>
<td>0.119±0.070</td>
</tr>
<tr>
<td>[Cit] mM</td>
<td>1.492±1.515</td>
<td>0.813±0.651</td>
</tr>
<tr>
<td>[Mg] mM</td>
<td>2.211±1.340</td>
<td>1.627±1.100</td>
</tr>
<tr>
<td>[Cre] mg/dl</td>
<td>98.26±59.68</td>
<td>58.24±28.17</td>
</tr>
<tr>
<td>Mg/Cr mg/mg</td>
<td>0.147±0.121</td>
<td>0.157±0.093</td>
</tr>
<tr>
<td>Ox/Cr mg/mg</td>
<td>0.0175±0.0096</td>
<td>0.020±0.013</td>
</tr>
<tr>
<td>Cit/Cr mg/mg</td>
<td>0.306±0.228</td>
<td>0.287±0.200</td>
</tr>
<tr>
<td>DG(CaOx)</td>
<td>1.471±1.107</td>
<td>1.041±1.274</td>
</tr>
</tbody>
</table>

Correlations between early spot urine specimens (Y) and 24-hour urine specimens (X) obtained from 57 inpatients with idiopathic calcium oxalate stones for urinary calcium, oxalate, citrate, magnesium, creatinine, DG(CaOx) value, calcium/creatinine, oxalate/creatinine, citrate/creatinine, and magnesium/creatinine ratios. The following correlations were obtained. [Ca]: Y = 0.492X + 1.856 (r = 0.41, p = 0.001); [Ox]: Y = 0.435X + 0.115 (r = 0.32, p = 0.016); [Cit]: Y = 1.069X + 0.623 (r = 0.46, p = 0.0003); [Mg]: No correlation; [Cre]: Y = 0.908X + 45.364 (r = 0.43, p = 0.001); Ca/Cr: Y = 0.671X + 0.042 (r = 0.52, p = 0.0000); Ox/Cr: Y = 0.259X + 0.012 (r = 0.35, p = 0.008); Cit/Cr: Y = 0.839X + 0.066 (r = 0.74, p = 0.0000); Mg/Cr: Y = 0.475X + 0.030 (r = 0.46, p = 0.0003); DG(CaOx): Y = 0.277X + 1.1831 (r = 0.32, p = 0.016). Data are shown as mean±SD. 1: p < 0.01, comparison with spot urine; 2: p < 0.05, comparison with spot urine.
Table 6. Correlations between 24-hour excretion and Cre-corrected concentrations of urinary parameters

<table>
<thead>
<tr>
<th>Risk factor (f)</th>
<th>Mean±SD</th>
<th>Y: log(f) X: log(f/Cre)</th>
<th>Regression function</th>
<th>Coefficient (r)</th>
<th>Probability p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca (mg/day)</td>
<td>137.6±90.8</td>
<td>0.3732X + 2.3942</td>
<td></td>
<td>0.3835</td>
<td>0.0032</td>
</tr>
<tr>
<td>Ca/Cre (mg/mg)</td>
<td>0.1474±0.1206</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ox (mg/day)</td>
<td>17.3±12.1</td>
<td>0.0313X + 1.1931</td>
<td></td>
<td>0.0246</td>
<td>0.8558</td>
</tr>
<tr>
<td>Ox/Cre (mg/mg)</td>
<td>0.0175±0.0096</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cit (mg/day)</td>
<td>240.7±165.9</td>
<td>0.7074X + 2.7104</td>
<td></td>
<td>0.6757</td>
<td>0.0000</td>
</tr>
<tr>
<td>Cit/Cre (mg/mg)</td>
<td>0.306±0.2283</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg (mg/day)</td>
<td>59.9±30.1</td>
<td>0.0394X + 1.7729</td>
<td></td>
<td>0.0439</td>
<td>0.7455</td>
</tr>
<tr>
<td>Mg/Cre (mg/mg)</td>
<td>0.0636±0.0368</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparison of the 24-hour urinary calcium (Ca mg/day), oxalate (Ox mg/day), citrate (Cit mg/day), and magnesium (Mg mg/day) excretion with the Ca/Cre, Ox/Cre, Cit/Cre, and Mg/Cre ratio (mg/mg), respectively, of early spot urine specimens obtained from 57 inpatients with idiopathic calcium oxalate stones. Data are shown as mean±SD.

**Figure 1.** The prevalence of hypercalciuria (> 200 mg/day), hyperoxaluria (> 30 mg/day), hypocitraturia (< 320 mg/day), and hypomagnesuria (< 75 mg/day) in 10 critically supersaturated 24-hour urine specimens.

**Figure 2.** The prevalence of hypercalciuria (> 200 mg/day), hyperoxaluria (> 30 mg/day), hypocitraturia (< 320 mg/day), and hypomagnesuria (< 75 mg/day) in 116 metastably supersaturated urine specimens.

**Figure 3.** The prevalence of hypercalciuria (> 200 mg/day), hyperoxaluria (> 30 mg/day), hypocitraturia (< 320 mg/day), and hypomagnesuria (< 75 mg/day) in 20 unsaturated urine specimens.

(p<0.01). The mean 24-hour urinary magnesium excretion was 59.9±30.1 mg/day and the magnesium/creatinine ratio (mg/mg) was 0.0636±0.0368 in spot urine specimens, again with no significant correlation between the two parameters (p>0.10), as shown in Table 6. The calcium/creatinine, oxalate/creatinine, citrate/creatinine, and magnesium/creatinine ratios were significantly correlated between early morning spot urine and 24-hour urine specimens, with the regression coefficients (r values) being 0.5162, 0.3489, 0.7360, and 0.4646 (p<0.01), respectively. The mean±SD was 0.147±±0.1206 and 0.156±0.0927, 0.0175±0.0096 and 0.019±0.0129, 0.306±0.2283 and 0.286±0.2002, and 0.063±0.0368 and 0.070±0.0360, respectively. The former set of mean ratios showed slightly lower values than the latter set except for the mean citrate/creatinine ratio.

5. DISCUSSION

In this study, the measurement of 24-hour urinary calcium, oxalate, and citrate excretion could not discriminate stone formers from non-stone formers, while the mean 24-hour urinary magnesium excretion was significantly higher in non-stone formers than in stone formers. The creatinine-corrected urinary calcium, oxalate, and magnesium levels in spot urine were also unable to discriminate stone formers from non-stone formers, but the mean creatinine-corrected citrate level was higher in non-stone formers than in stone formers. Because nephrolithiasis is a heterogenous group of disorders, stones develop due to a wide variety of metabolic or environmental disturbances (1-7). Therefore, determination of calcium oxalate saturation is necessary to gain an understanding the physicochemical events involved in renal stone formation (38, 67). We found that the DG(CaOx) value for either 24-hour urine or spot urine could discriminate stone formers from non-stone formers despite the heterogenous composition of these groups of patients. Finlayson’s Equil program seems to be useful for discriminating stone formers from non-stone formers. The differential Gibbs’ free energy (DG) value of calcium oxalate represents the chemical potential of the calcium oxalate system in states from a solution to a precipitate, thus reflecting the extent of calcium oxalate saturation (17). We have reported previously that all of the methods for estimating the ion-activity product of calcium oxalate,
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Critical Supersaturation (DG>2.8) and Cre-corrected Risk Factors

Figure 4. The prevalence of creatinine-corrected hypercalciuria (Ca/Cre > 0.200), hyperoxaluria (Ox/Cre > 0.300), hypocitraturia (Cit/Cre < 0.320), and hypomagnesiuria (Mg/Cre < 0.075) in 31 critically supersaturated urine specimens.

Metastable Supersaturation (2.8>DG>0) and Cre-corrected Risk Factors

Figure 5. The prevalence of creatinine-corrected hypercalciuria (Ca/Cre > 0.200), hyperoxaluria (Ox/Cre > 0.300), hypocitraturia (Cit/Cre < 0.320), and hypomagnesiuria (Mg/Cre < 0.075) in 311 metastably supersaturated urine specimens.

Unsaturation (DG<0) and Cre-corrected Risk Factors

Figure 6. The prevalence of creatinine-corrected hypercalciuria (Ca/Cre > 0.200), hyperoxaluria (Ox/Cre > 0.300), hypocitraturia (Cit/Cre < 0.320), and hypomagnesiuria (Mg/Cre < 0.075) in 60 unsaturated urine specimens.

which also reflects the saturation, were correlated well with each other (68), so that any indices accounting for urinary calcium oxalate saturation could discriminate the two groups better than any other single parameters. Similarly several risk formulas (AP(CaOx) index, calcium/magnesium, calcium/citrate, calciumxoxalate/magnesium/citrate, and Parks and Coe score) were confirmed by Tiselius to be equally useful in terms of the predictive power of stone risk and in discriminating between stone formers and non-stone formers (23). The risk indices are governed by the strong contribution of the calcium and oxalate concentrations as well as the weak inhibitory effects of citrate and magnesium. However, the calcium, oxalate, citrate, and magnesium concentrations were positively associated with DG(CaOx) if compared individually. Citrate inhibits urinary stone formation by making a complex with calcium, while magnesium exerts a solubilizing effect because magnesium oxalate has a slightly greater stability constant than calcium oxalate (69). These two inhibitors apparently have an important influence on stone formation and their inhibitory effect can be easily calculated and expressed as the urinary saturation (e.g., the DG(CaOx) value). Although most researchers believed that urinary citrate and magnesium levels should be lower in the supersaturated urine of stone formers because these are inhibitory factors, it was actually the other way around because the DG(CaOx) value increased along with increasing citrate and magnesium levels (68). This paradoxical and puzzling phenomenon may be accounted for by the fact that the urinary levels of all these substances are closely associated with each other depending on the extent of concentration or dilution of the urine, and that their relative ratios influenced by urinary concentration and dilution may play an important role in determining the DG(CaOx) value or the extent of saturation. Therefore, the Ca/Mg and Ca/Cit ratios have been used as better indices of stone risk by some researchers (23, 70-72). According to the formula for calculation of the DG(CaOx) value \( \text{DG(CaOx)} = 3.0070 + 2.6455 \log([\text{Ca}]) + 2.6353 \log([\text{Ox}]) - 0.6292 \log([\text{Cit}]) - 0.6122 \log([\text{Mg}]) \), DG(CaOx) is negatively associated with the citrate and magnesium concentrations. Interpreting this formula suggests that the calcium and oxalate concentrations make an almost equally important contribution to the DG(CaOx) value, while citrate and magnesium make an almost equal minor contribution. The former vs. the latter shows a ratio of approximately 2.6:0.6, so calcium and oxalate concentrations exert 4-fold more influence on the saturation than citrate and magnesium concentrations. Finlayson reported that urinary oxalate is about 15 times more important than urinary calcium for stone formation (73). However, our findings do not support Finlayson because the DG(CaOx) formula shows that calcium and oxalate have an equal influence on stone formation.

In this study, the 24-hour urinary calcium, oxalate, and magnesium excretion were shown to be positively correlated with the DG(CaOx) value, but 24-hour urinary citrate excretion was not. This may suggest that 24-hour urinary citrate excretion is not an important risk factor for stone formation. Hypercalciuria and hyperoxaluria were mostly observed in CaOx-supersaturated urine and their prevalence was almost equal. Mild hyperoxaluria was emphasized to be the main clinical cause of idiopathic calcium stone disease by Robertson and Hughes (74), who claimed that urinary oxalate is the most important risk factor for calcium oxalate stone formation. We do not deny the concept of mild hyperoxaluria, but the clinical prevalence of hypercalciumia and hyperoxaluria appears to be equal, while hypocitraturia and hypomagnesiuria were even more common, but were not associated with any particular level of urinary saturation. Because hypocitraturia tended to be a non-specific finding, both
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hypocitraturia and hypomagnesiuria are not good predictors of urinary calcium oxalate saturation, but may be associated with the underlying etiologic conditions. However, there was a small group of patients who had isolated hypocitraturia with critical calcium oxalate supersaturation, where both the calcium and oxalate concentrations were borderline. When we introduced “isolated hypocitraturia” as a concept, we intended it to mean CaOx-supersaturated urine that showed hypocitraturia without obvious hypercalciuria, hyperoxaluria, or hypomagnesiuria, excluding unsaturated urine, while “isolated hypomagnesiuria” meant CaOx-supersaturated urine that showed hypomagnesiuria without hypercalciuria, hyperoxaluria, or hypocitraturia (again excluding unsaturated urine) (60). Hypomagnesiuric hypocitraturia was introduced as a new entity by Preminger (62), but we would like to define it to mean CaOx-supersaturated urine that showed hypomagnesiuria without hypercalciuria, hyperoxaluria, or hypocitraturia (60). Hypomagnesiuric hypocitraturia was introduced as a new entity by Preminger (62), but we would like to define this as calcium oxalate supersaturation associated with hypomagnesiuria and hypocitraturia. In the present study, 10% of critically supersaturated urine filled this definition. Although citrate and magnesium are important inhibitors of stone formation, isolated hypomagnesiuria or hypocitraturia is often an inadequate explanation for stone formation/non formation. Assessment of the urinary saturation of stone components should be included in metabolic screening and is mandatory for clinical evaluation of drug efficacy, however, the magnitude of the drug effect was minor in terms of saturation (75). Hypocitraturia or hypomagnesiuria is a poor indicator of urinary saturation as such, but to compare the frequency of these risk factors may be a good idea (76).

Risk factors for stone formation, including hypercalciuria and hyperoxaluria, are often observed in association with high urinary saturation. Supersaturation, as expressed by a high DG(CaOx) value caused by one or more of these risk factors, can undoubtedly lead to stone formation irrespective of the underlying etiologic condition if it persists for a long time. More than 90% of critical calcium oxalate supersaturation occurs late at night or in the early morning, so examination of early morning spot urine may generally be sufficient to predict the risk of critical supersaturation (69). In the present study, the urinary calcium, oxalate, citrate, and magnesium concentrations, as well as the DG(CaOx) value, were well correlated between early morning spot urine and 24-hour urine, although generally higher in early morning urine, so the DG(CaOx) value of early morning spot urine specimens may be a better indicator of stone risk because it could represent the highest saturation throughout the day. Therefore, the target of therapy for stone formers should be to reduce the saturation of early morning spot urine. The urinary calcium/creatinine, oxalate/creatinine, magnesium/creatinine, and citrate/creatinine ratios were well correlated between early morning and 24-hour urine, with the former three ratios being lower in spot urine. Therefore, calculation of the urinary calcium/creatinine, oxalate/creatinine, and magnesium/creatinine ratios from early morning spot urine specimens may slightly underestimate urinary excretion relative to the values obtained from 24-hour urine specimens.

Regarding the comparison of urinary data between males and females, urinary calcium and oxalate concentrations were not significantly different between men and women as well as calcium supersaturation (77,78), while urinary calcium, oxalate, and magnesium excretion were all reported to be significantly higher in stone-forming men than women (79). Our data obtained from stone-forming inpatients on a hospital diet are consistent with the former report except that urinary citrate excretion was higher in stone-forming women than in men and the creatinine-corrected urinary substances (Ca, Ox, Cit, and Mg) tended to be higher in women. Urinary excretion of various substances is influenced by the diet (80), so a prospective study is warranted on groups of stone formers with well-defined diets.

Although 24-hour urine collection remains the gold standard for metabolic evaluation and can detect a metabolic abnormality in approximately 70% of stone formers if performed properly (81), it is influenced by various factors, including the season (1, 2, 82), day-to-day variations, exercise, dietary changes (80), and activity levels. Incorrect sampling can cause significant errors in the excretion data (83), and a single 24-hour sample is not sufficient for evaluating patients before metabolic treatment (84). Supersaturation values are, however, reasonably stable in most patients during months to years required for stones to form, and urine samples collected in standard practice and sent to a central laboratory may accurately reflect the supersaturation values (85). Use of spot urine specimens has been recommended by some authors because of simplicity and the good correlation with 24-hour urine specimens for various parameters, but spot urine values have not been verified as a surrogate measure for 24-hour urinary excretion of all the substances of interest (86, 87). In the present study, urinary oxalate and magnesium excretion were not correlated with the oxalate/creatinine ratio or the magnesium/creatinine ratio, respectively. There are some substances (e.g., uric acid) for which the creatinine ratio does not accurately predict the 24-hour excretion (88). Therefore, measurement of early morning spot urine may be useful for identifying some risk factors, including hypercalciuria and hypocitraturia as well as calcium oxalate saturation, but hyperoxaluria and hypomagnesiuria in early morning spot urine do not have the same implications as these findings in 24-hour urine. However, the currently accepted definitions of normal values are not firmly supported, so the traditional definitions of normal 24-hour urine values need to be reassessed (89).

We have not mentioned macromolecular promoters and inhibitors or the concept of inhibitory macromolecule deficiency, but routine determination of such substances is impractical (90-94). In the future, macromolecular factors may eventually be incorporated in the Equil2 program.

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