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Calcium oxalate saturation in dialysis patients with and without primary hyperoxaluria

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Abstract Calcium oxalate supersaturation of the blood is associated with deposition of crystals in various tissues. We measured the serum levels of oxalate, citrate, calcium, and magnesium to estimate their saturation in 112 hemodialysis patients without primary hyperoxaluria and two boys with primary hyperoxaluria. Serum levels of oxalate and citrate were determined by high-performance capillary electrophoresis, while calcium and magnesium were measured by ICP spectroscopy. The serum levels of oxalate, citrate, calcium, and magnesium were 44.9 ± 16.5, 138.1 ± 54.9 μmol/l, 2.30 ± 0.28, and 1.07 ± 0.18 mmol/l, respectively, while the levels in patients with primary hyperoxaluria were 83.9 ± 34.3, 197.9 ± 63.5 μmol/l, 2.53 ± 0.15, and 1.14 ± 0.34 mmol/l, respectively. Serum calcium oxalate saturation (SS), as calculated by the Equil program, was significantly correlated with the serum oxalate level. Most patients showed metastable supersaturation ($1 < SS < 8.9$), which was associated with a serum oxalate level of more than 30 μmol/l. Serum saturation exceeded the formation product ($SS = 8.9$) in some specimens from patients with type 1 primary hyperoxaluria. The serum calcium oxalate saturation [SS(CaOx)] showed a significant positive correlation with the levels of oxalate [Ox], calcium [Ca], and citrate [Cit]: $[SS(CaOx)] = -0.3562 + 34.634[Ox] + 0.394[Ca] - 0.483[Mg] + 0.101[Cit]$, (all mmol/l, $r = 0.9848$, $P < 0.01$). This formula is useful for estimating the saturation. In conclusion, the serum oxalate level is a good indicator of calcium oxalate saturation and should be monitored accurately while keeping it lower in dialysis patients.

Keywords Calcium oxalate saturation · Hyperoxaluria · Hyperoxalemia · Hemodialysis

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Introduction

Calcium oxalate deposition in various tissues has been reported in association with renal failure [1–4]. The plasma oxalate level rises as renal insufficiency or failure develops since renal excretion is the only outlet for oxalate produced in humans [5]. Hyperoxalemia may occur as a result of the ingestion of oxalate and its precursors, vitamin B6 deficiency, or decreased renal function [1]. Crystallization often occurs in blood supersaturated with respect to calcium oxalate, and renal deposition of calcium oxalate crystals is an inevitable complication of hyperoxalemic syndromes. Hyperoxalemia may result in the diffuse deposition of calcium oxalate crystals in multiple organs [6–18]. Oxalate crystal deposition disease, commonly known as systemic oxalosis, is a life-threatening disorder that complicates primary hyperoxaluria types 1 and 2 (PH1 and 2) or end-stage renal disease managed with long-term dialysis. Oxalate crystal deposits are mainly found in the kidneys, thyroid, myocardium, bone, skin, and vessels, and less often in the joints, spleen, and lungs [8]. An essential condition for the formation of such crystals to form is supersaturation with respect to calcium oxalate [19]. Marangella et al. [20–22] previously described a method of calculating serum saturation that represents a noninvasive method for assessing the risk of systemic oxalosis. It is strongly recommended to measure the saturation during long-term ascorbate therapy [23]. In this report, we included serum citrate to calculate the saturation in a similar manner to the Tiselius index [24], and investigate its applicability to patients on regular hemodialysis and patients with hyperoxaluria.

Subjects and methods

After oral informed consent was obtained, blood samples were collected before hemodialysis from 112 patients (72 men and 40 women) aged 58 ± 13 years on chronic hemodialysis, as well as two boys with primary hyperoxaluria (aged 6 months and 9 years at diagnosis, respectively) from whom blood samples were obtained on 17 and 13 occasions over several years (3 years and 1 year, respectively). The patients had been on dialysis for 12.1 ± 7.6 years (mean ± SD) because of end-stage renal disease including chronic glomerulonephritis, diabetic nephropathy, and SLE. They were being dialyzed for 4 h three times a week using dialyzers with an effective surface area of 0.8–2.1 m^2, which were either the AM-BC-11–20P, APS-13–21S (Asahi Medical Co.), FB-150–210U (Nipro Co.), BG-1.0–1.6U (Toray Medical Co.), or FDX-12GW (Nikkiso Co.). The two boys were diagnosed as having type 1 primary hyperoxaluria from biochemical data (hyperglycolic academia) and the results of liver biopsy. They were maintained on peritoneal dialysis and hemodiafiltration (FB70F Nipro Co.) for several years because of chronic renal failure (serum creatinine 4 ± 1 and 6 ± 2 mg/dl, respectively) and subsequently underwent liver–kidney transplantation (the details will be reported elsewhere). This study has been approved by the medical ethics committee of the Faculty of Medicine, University of the Ryukyus. Serum was deproteinized using an Ultrafree C3 THK filter and diluted threefold with water. The serum levels of oxalate, ascorbate, glycolate, and citrate were determined by high-performance capillary electrophoresis (HPCE: Hewlett-Packard ^{3D}CE, Germany) using a pH 7.7 buffer solution for HPCE (Fluka), 20 mM tetraborate/30 mM SDS buffer, and organic acids buffer for HPCE (pH 5.6, Agilent Technologies) [25, 26]. The serum levels of calcium and magnesium were measured by ICP spectroscopy, and serum creatinine was determined by the enzymatic method. Calcium oxalate saturation [SS(CaOx)] was calculated using Finlayson's Equil program for Windows™ (SS stands for the supersaturation ratio) [27, 28]. The Equil program originally requires 14 variables and was modified to calculate the SS(CaOx) of serum calcium oxalate employing four variables as similar to that reported before [29]. A new index [SS(CaOx)] was created using four parameters and multiple regression analysis in the same way as reported elsewhere [29]. Results are reported as the mean ± standard deviation (SD). Correlations between parameters were investigated by univariate and multivariate regression analysis. Statistical significance was set at $P < 0.05$ for all comparisons.

Results

The serum levels of oxalate, citrate, calcium, and magnesium in the hemodialysis patients were 44.9 ± 16.5, 138.1 ± 54.9 μmol/l, 2.30 ± 0.28, and 1.07 ± 0.18 mmol/l, respectively, while the levels of the patients with primary hyperoxaluria were 83.9 ± 34.3, 197.9 ± 63.5 μmol/l, 2.53 ± 0.15, and 1.14 ± 0.34 mmol/l, respectively (Table 1). Serum calcium oxalate saturation [SS(CaOx)] was significantly correlated with the serum oxalate level. Most patients showed metastable supersaturation [1 < SS(CaOx) < 8.9], [20, 28, 29], which was associated with a serum oxalate level of more than 30 μmol/l (Fig. 1). Calcium oxalate saturation exceeded the formation product [SS(CaOx) = 8.9] in some serum specimens from the PH1 patients (Fig. 2). Serum calcium oxalate saturation [SS(CaOx)] showed a significant positive correlation with the serum levels of oxalate [Ox], calcium [Ca], and citrate [Cit] and a negative correlation with the magnesium level [Mg]: $[SS(CaOx)] = -0.3562 + 34.634[Ox] + 0.394[Ca] - 0.483[Mg] + 0.101[Cit]$, (all mmol/l, $r = 0.9848$, $P < 0.01$). This new simplified method of calculating [SS(CaOx)] provided a better approximation to the Equil method ($r^2 = 0.9365$, $P < 0.01$) (Fig. 3).

Discussion

Calculation of serum supersaturation with respect to calcium oxalate monohydrate using an iterative computer model of polyelectrolyte equilibrium was first introduced by Worcester as RSR = 1.8[Ox] + 0.11, but it seems likely that it should be RSR = 0.018[Ox μmol/l] + 0.011 (calculated from the data in their Fig. 4). Close agreement was reported between the computer-based model and direct measurement procedures [19]. A similar index was proposed by Marangella to estimate the saturation of calcium oxalate monohydrate as βCaOx = 0.02[Ox μmol/l] + 0.03, in which βCaOx equals to RSR or SS(CaOx) (supersaturation ratio or relative supersaturation) in the Equil program [20]. Another simplified method for estimating serum calcium oxalate saturation was proposed by Marangella, which was β CaOx − 2 = 0.018[Ox mmol/l] + 1.16[Ca mmol/l] –0.17[Mg mmol/l] − 1.16. The calcium concentration

Table 1 Serum levels of calcium, magnesium, oxalate, and citrate, and serum calcium oxalate saturation, in 112 hemodialysis patients and two boys with primary hyperoxaluria

	Ca (mmol/l)	Mg (mmol/l)	Oxalate (mmol/l)	Citrate (mmol/l)	SS value
Non-PH HD patients	2.315 ± 0.201	1.076 ± 0.161	0.045 ± 0.016	0.139 ± 0.055	1.610 ± 0.601
PH patients	2.535 ± 0.145	1.136 ± 0.335	0.084 ± 0.034	0.198 ± 0.064	6.384 ± 2.543

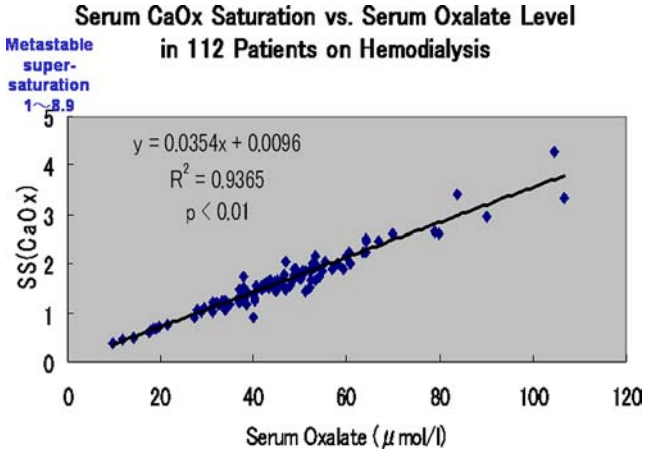

Fig. 1 Serum calcium oxalate saturation [SS(CaOx)] was significantly correlated with the serum oxalate level in the hemodialysis patients. Most patients showed metastable supersaturation [1 < SS(CaOx) < 8.9], which was associated with a serum oxalate level of more than 30 μmol/l

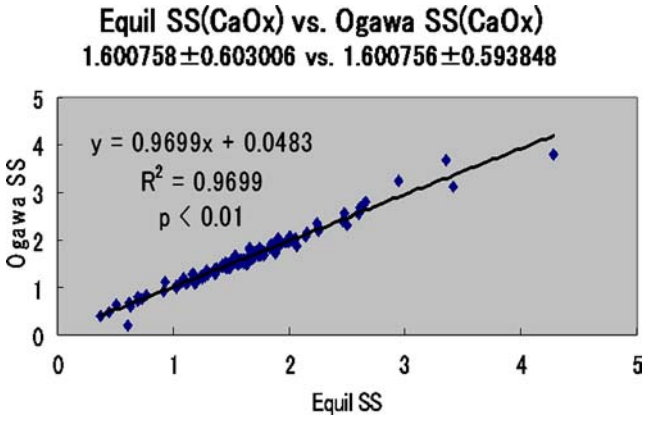

Fig. 3 There is a significant correlation between values obtained with the new simplified method [SS(CaOx)] and the Equil method ($r^2 = 0.9365$, $P < 0.01$)

[Ca] in this index was serum ultrafiltrable calcium, so the level was 1.0–1.4 mmol/l [22]. Our proposed index $\{[SS(CaOx)] = -0.3562 + 34.634[Ox\,mmol/l] + 0.394[Ca\,mmol/l] - 0.483[Mg\,mmol/l] + 0.101[Cit\,mmol/l]\}$ is similar to βCaOx−2, except for two points. These are used of the serum calcium level (2.3 ± 0.2 mmol/l) and employing units of mmol/l for data in square brackets, which make our index simpler and more practical.

Accordingly, the serum saturation of calcium oxalate monohydrate mainly depends on the serum oxalate concentration. The threshold of serum calcium oxalate supersaturation or solubility product may be associated with the serum oxalate level over the range of 55–90 μmol/l (Worcester) [19], 44–46 μmol/l (Marangella) [21], and 30 μmol/l (Hoppe [30] and our data). Hyperoxalemia is defined as a serum oxalate level > 3.2 mg/dl (= 32 mg/l = 355 μmol/l) according to the Roche Lexikon Medizin, but this is abnormally high even if reduced by tenfold (35.5 μmol/l). The fasting plasma oxalate concentration of healthy subjects is reported to be 3.8 ± 1.5 μmol/l (mean ± SD) [20]. In dialysis patients, we previously found that the serum oxalate level was 47.2 ± 22.9 μmol/l before and 16.9 ± 10.5 μmol/l after a 4-h dialysis session and that most dialysis patients had low serum levels of ascorbate. But a subgroup of patients (12%) had high serum ascorbate levels that were significantly associated with hyperoxalemia [31]. We also confirmed that ascorbate overload, vitamin B6 deficiency, and inadequate dialysis are the major factors increasing the serum oxalate level in dialysis patients [31]. These findings are also compatible with the classical notion that hyperoxalemia may occur as a result of the ingestion of oxalate and its precursors, vitamin B6 deficiency, and/or inadequate dialysis in dialysis patients [1]. We did not measure the dietary content of oxalate and its precursors, but such measurement of the dietary content may clarify the contribution of exogenous oxalate to hyperoxalemia. The safety concerns related to hyperoxalemia are still poorly understood [32], but it may be reasonable for hyperoxalemia to be defined as > 30 μmol/l (near the solubility product), while critical hyperoxalemia can be roughly defined as > 120 μmol/l (since the formation product was 120–250 μmol/l according to our data).

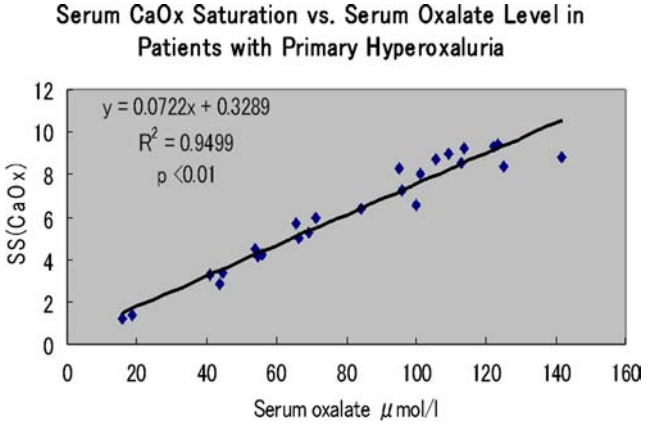

Fig. 2 Serum calcium oxalate saturation [SS(CaOx)] was significantly correlated with the serum oxalate level in the patients with primary hyperoxaluria. Serum saturation exceeded the formation product [SS(CaOx) = 8.9] in some specimens from these patients

In addition to calcium oxalate deposition in the tissues and organs caused by hyperoxalemia, the following other possible toxicities of oxalate and calcium oxalate should also be kept in mind. Elevated levels of oxalate and calcium oxalate crystals provoke increased synthesis of inflammatory mediators and extracellular matrix by renal cells. The renal epithelial cells are often injured and undergo apoptosis and/or necrosis, while reactive oxygen species are produced during interactions between oxalate/oxalate crystals and renal cells [33, 34]. Calcium oxalate monohydrate crystal stimulation of renal epithelial cells results in rapid and robust activation of p38 MAP kinase, as well as a program of events that

includes alterations of gene expression, initiation of DNA synthesis, cell growth, and apoptosis [35, 36]. The cause of uremic atherogenesis is still unknown. However, Recht reported that uremic levels of oxalate severely inhibit the proliferation and migration of human endothelial cells without affecting other cell types. Since the physical, cellular, and molecular events of endothelial injury are clearly established as key factors in the development of atheromatous plaque, and since inhibition of cell proliferation and migration would enhance endothelial injury, oxalate has been proposed as an atherogenic uremic toxin [37]. Plasma oxalate exerts an atherogenic effect by elevating intracellular calcium in endothelial cells and preventing re-endothelialization, so care must be taken to keep the serum oxalate level low in dialysis patients. Routine hemodialysis usually maintains a blood oxalate level below that associated with a risk of calcium oxalate crystallization, except in the presence of ascorbic acid overload and primary hyperoxaluria, but hyperoxalemia may still exert an atherogenic effect on endothelial cells.

In conclusion, the serum oxalate level is a good indicator of calcium oxalate saturation, which can be calculated by Ogawa's formula using four parameters ([Ox], [Ca], [Mg], and [Cit]). Serum calcium oxalate levels remain within the metastable supersaturation range in hemodialysis patients without PH (90%), while occasionally exceeding metastable supersaturation to enter the labile supersaturation range in PH patients, although the number studied was so small that further investigation is required. Therefore, we need to study more cases of primary hyperoxaluria in the future. The serum oxalate level should be monitored accurately and should be kept below 100 μmol/l to prevent vascular damage due to oxalate and to avoid crystal deposition.

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