MAJOR FACTORS MODULATING THE SERUM OXALIC ACID LEVEL IN HEMODIALYSIS PATIENTS

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1. ABSTRACT

Ascorbic acid overload and vitamin B6 deficiency have been implicated in the development of hyperoxalemia in dialysis patients, but there is still disagreement about this. Hemodialysis patients who are exposed long-term hyperoxalemia may develop secondary oxalosis with an increased risk of cardiac, vascular, and bone disease, and thus may benefit from maintaining a low serum oxalic acid level. In 452 hemodialysis patients, the serum level of oxalic acid was 47.2±22.9 µmol/l before and 16.9±10.5 µmol/l after a 4-hour dialysis session, while the ascorbic acid levels were 39.0±92.7 µmol/l and 6.5±18.6 µmol/l, the glycolic acid levels were 7.3±10.1 µmol/l and 0.6±2.3 µmol/l, and the citric acid levels were 141.3±54.7 µmol/l and 117.6±37.2 µmol/l, respectively. Most patients (65.3%) had low serum ascorbic acid levels (<10 µmol/l) before hemodialysis. The serum level of oxalic acid [Ox] showed a significant positive correlation with the levels of ascorbic acid [AA], glycolic acid [Gly], and creatinine [Cre]: $[Ox] = 21.711 + 0.181 \times [AA] + 0.174 \times [Gly] + 0.171 \times [Cre]$, (all µmol/l, p<0.05). In 124 dialysis patients, the 4-pyridoxic acid level was 8.9±19.6 µmol/l before and 3.9±8.8 µmol/l after dialysis, and it was not correlated with oxalic acid or glycolic acid. Most dialysis patients（65.3%）had low serum levels of ascorbic acid, but a subgroup of patients (12%) had high serum ascorbic acid levels （>100 µmol/l) associated with hyperoxalemia (88.2±24.5 µmol/l). High-dose vitamin C supplementation may aggravate hyperoxalemia in hemodialysis patients, so attention should be paid to avoiding this risk.

2. INTRODUCTION

In patients with renal insufficiency or failure, the plasma oxalic acid level increases due to accumulation of oxalic acid and additional metabolic factors that promote endogenous synthesis of oxalic acid.[1] Ascorbic acid, glycolic acid, and glyoxylic acid are important metabolic precursors of oxalic acid, and a high intake of any of these precursors may cause hyperoxalemia in patients with chronic renal failure.[1-7] Ascorbic acid is generally considered to be a key antioxidant, and its deficiency may contribute to oxidative stress in dialysis patients.[8] To restrict potassium intake, most dialysis patients only eat a limited amount of fruit and vegetables, so their dietary intake of ascorbic acid is poor, often causing asymptomatic scurvy.[8-12] Increased lipid peroxidation and depletion of chain-breaking antioxidants due to subclinical scurvy may contribute to an increased risk of atherosclerosis in these patients. Cardiovascular disease is the major cause of mortality in patients on hemodialysis for chronic renal failure.[13] In addition, inadequate iron mobilization and defective iron utilization may cause hyporesponsiveness to recombinant erythropoietin in hemodialysis patients with iron overload.[14] Intravenous administration of ascorbic acid has been shown to effectively control erythropoietin hyporesponsive anemia associated with iron overload.[14-17] Vitamin B6 deficiency has also been implicated as another cause of secondary hyperoxalemia, which is often associated with hyperglycolic acidemia.[18-26] Ascorbic acid and vitamin B6 are small water-soluble molecules (with a molecular weight of 176.13 and 205.64, respectively), which are removed from the blood during dialysis.[27] During hemodialysis, oxalic acid shows similar behavior to that of creatinine (molecular weight: 113). An increased plasma oxalic acid level seems to be an important factor promoting the deposition of calcium oxalate in patients with uremia.[1] Therefore, we studied the relative contribution of these substances to modulation of the serum oxalic acid level in patients on hemodialysis.

3. SUBJECTS AND METHODS

After oral informed consent was obtained, blood was collected before and after a 4-hour dialysis session from 452 patients (271 men and 181 women) aged 63±13 years who were on chronic hemodialysis. The dialyzers used had an effective surface area of 0.8～2.1 m^2 and included the following: AM-BC-11～20P and APS-13～21S (Asahi Medical Co.), FB-150～210U and PES-150～210D (Nipro Co.), CL-EE-15～18N (Terumo Co.), BG-1.0～1.6U and BS-1.3U～1.6UL (Toray Medical Co.), FLX-12～18GW and FDX-12GW (Nikkiso Co.), PS-1.6MW and PS-1.9UW (Kawasumi Lab. Inc.), and KF-m08～m15 (Kuraray Med. Inc.). Serum was deproteinized using an Ultrafree C3 THK filter and diluted 3-fold with water. The serum levels of oxalic acid, ascorbic acid, glycolic acid, 4-pyridoxic acid,

Table 1. Serum parameters before and after a 4-hour dialysis session. The percent reduction of oxalic acid was similar to that of creatinine, while the decrease of ascorbic acid or glycolic acid was greater than that of creatinine and the decrease of citric acid was smaller.

	Before HD (μmol/l)	After HD (μmol/l)	% reduction
Oxalic acid	47.2±22.9	16.9±10.5	64.2
Ascorbic acid	39.0±92.7	6.5±18.6	83.4
Glycolic acid	7.3±10.1	0.6±2.3	92.1
Citric acid	141.3±54.7	117.6±37.2	16.8
Creatinine	102.5±30.1	40.3±14.5	60.6

and citric acid 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 an organic acids buffer for HPCE (pH 5.6, Agilent Technologies).[2,28,29] The detection limits of serum oxalic acid, ascorbic acid, glycolic acid, 4-pyridoxic acid, and citric acid were 1 μmol/l, 1 μmol/l, 5 μmol/l, 4 μmol/l, and 5 μmol/l, respectively, while the coefficient of variation was 5-16% (unpublished data), which was comparable to other reported values.[28,29] We have found that the serum oxalic acid level is significantly correlated with and almost equal to the plasma oxalic acid level (using heparin or EDTA) ($p<0.01$)(unpublished data). There were 15 dialysis patients taking vitamin C (120-600 mg/day) and their serum levels of ascorbic acid and oxalic acid were measured. Results are reported as the mean ± standard deviation (SD). Correlations between parameters were determined by both univariate and multivariate regression analysis. Statistical significance was set at $p<0.05$ for all comparisons.

4. RESULTS

The serum level of oxalic acid was 47.2±22.9 μmol/l before hemodialysis and 16.9±10.5 μmol/l after hemodialysis, while the ascorbic acid levels were 39.0±92.7 μmol/l and 6.5±18.6 μmol/l, the glycolic acid levels were 7.3±10.1 μmol/l and 0.6±2.3 μmol/l, the 4-pyridoxic acid levels were 8.9±19.6 μmol/l and 3.9±8.8 μmol/l, and the citric acid levels were 141.3±54.7 μmol/l and 117.6±37.2 μmol/l, respectively. In addition, serum creatinine was 102.5±30.1 μmol/l before dialysis and 40.3±14.5 μmol/l after dialysis. All of these substances were dialyzable, with the percent reduction of oxalic acid, ascorbic acid, glycolic acid, 4-pyridoxic acid, citric acid, and creatinine being respectively 64.2%, 83.4%, 92.1%, 56.1%, 16.8%, and 60.6% after the 4-hour dialysis session (Table 1). The majority of the patients (65.3%) had low levels of ascorbic acid (<10 μmol/l) before dialysis. Some patients had high levels of 4-pyridoxic acid prior to hemodialysis, but most (78.6%) had undetectable levels (<5 μmol/l). The serum glycolic acid level was very low in comparison with that found in patients with primary hyperoxaluria (unpublished data: 572.2±221.2 μmol/l). By univariate regression analysis, the serum oxalic acid [Ox] level showed a significant correlation with the levels of ascorbic acid and creatinine, but not with the levels of glycolic acid and citric acid (Figures 1-4). By univariate analysis, the serum glycolic acid level was not correlated with the 4-pyridoxic acid level (p=0.193) (Figure 5), suggesting that the latter does not have an important influence on glycolic acid. On multivariate analysis, the
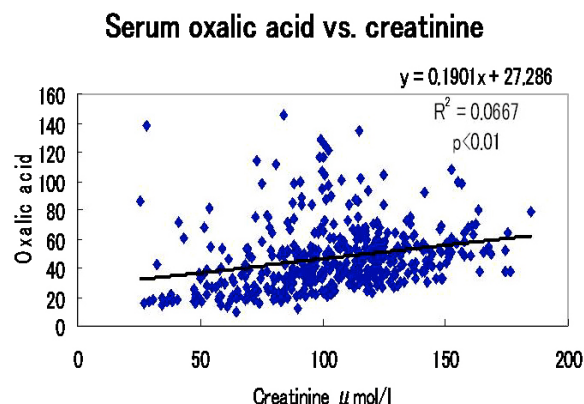
serum oxalic acid level [Ox] showed a significant positive correlation with the levels of ascorbic acid [AA], glycolic acid [Gly], and creatinine [Cre]: $[Ox] = 21.711 + 0.181$ x $[AA] + 0.174$ x $[Gly] + 0.171$ x $[Cre]$ (all μmol/l, $p<0.05$). However, the serum oxalic acid level was not correlated with the citric acid level (p=0.912). An ascorbic acid level > 100 μmol/l was seen in 54 out of 452 patients (12%). Their ascorbic acid level was 227.7±141.8 μmol/l, and it was associated with a high oxalic acid level (88.2±24.5 μmol/l). An oxalic acid level > 100 μmol/l was seen in 12 of 54 patients with high ascorbic acid levels (22.2%). Oral intake of vitamin C at a dose of 120-600 mg/day caused an increase of the serum oxalic acid level in 15 patients. The mean dose of vitamin C (371±197 mg/day) was not correlated with either the mean serum ascorbic acid level (239.9±199.9 μmol/l) or the oxalic acid level (79.8±23.8 μmol/l). However, the mean serum levels of ascorbic acid and oxalic acid were significantly correlated in this subgroup (p < 0.05) (Figure 6).

5. DISCUSSION

To minimize potassium intake and fluid intake, most dialysis patients only eat a limited amount of fruit and vegetables, as a consequence of which they have a low dietary intake of vitamin C, folic acid, and vitamin B6. Because their dietary intake of water-soluble vitamins is marginal, these should be supplemented at doses as close as possible to the recommended dietary intake.[27] Ascorbic acid is generally considered to be a key antioxidant, and its deficiency may contribute to oxidative stress in dialysis patients. The antioxidant capacity of serum can be increased by multivitamin supplementation to help prevent radical-induced damage in patients with chronic renal failure.[30] Ascorbic acid deficiency is often seen in dialysis patients due to its removal during dialysis (the percent reduction was 83.4% in the present study after a 4-hour dialysis session) combined with a low dietary intake.[31] Our results are compatible with those reported by Tsapas *et al.*[12], while Wang *et al.* reported that the median plasma ascorbic acid concentration was reduced by 33% and that patients on chronic hemodialysis have very low plasma ascorbic acid levels unless they receive supplementation.[32] The serum ascorbic acid level was reportedly 2.3-9.0 mg/l without supplementation and rose to 3.2-39.0 or 15.3-37.7 mg/l after supplementation with vitamin C at 100 mg/day or 500 mg/day, respectively.[31] In the present study, approximately 65% of our patients had low ascorbic acid levels (< 10 μmol/l) prior to hemodialysis, so they needed appropriate supplementation. Jejunal absorption of L-ascorbic acid is impaired in rats

Serum oxalic acid vs. ascorbic acid
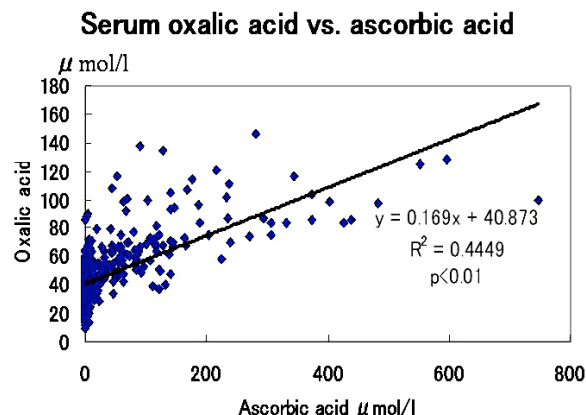
Figure 1. Relationship between serum oxalic acid and ascorbic acid levels. Univariate analysis showed a strong correlation between the levels of oxalic acid and ascorbic acid. The majority of patients (65.3%) had low ascorbic acid levels (<10 μmol/l) before hemodialysis. An ascorbic acid level > 100 μmol/l was often associated with an oxalic acid level > 100 μmol/l.

Serum oxalic acid vs. creatinine

Figure 2. Relationship between serum oxalic acid and creatinine levels. Univariate analysis showed a significant correlation between serum oxalic acid and creatinine levels.
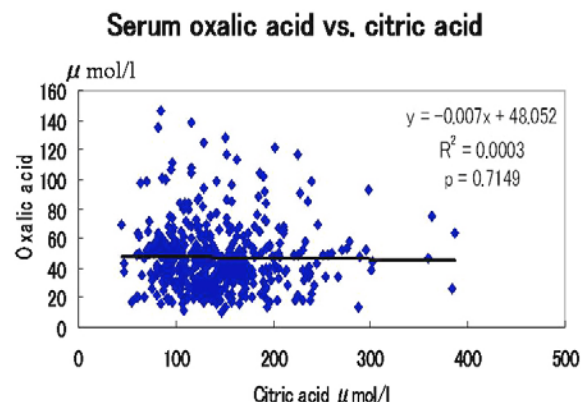
Serum oxalic acid vs. glycolic acid
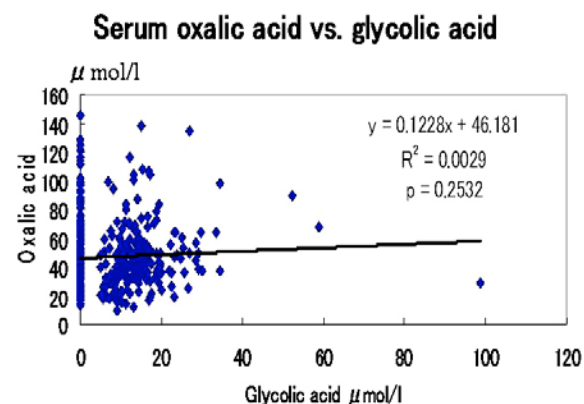
Figure 3. Relationship between serum oxalic acid and glycolic acid levels. Univariate analysis showed no correlation between serum oxalic acid and glycolic acid levels.

with renal failure, despite in vitro evidence of increased intestinal permeability, and this change appears to be

mediated by reduced nutrient intake and weight loss.33) A high peritoneal clearance of ascorbic acid leads to significant loss into the dialysate in patients on peritoneal dialysis and ascorbic acid lost during peritoneal dialysis is not adequately replaced by dietary intake of vitamin C.12) The total ascorbic acid content of the body, when fully saturated, is estimated to be about 4-6 g, with approximately 200 mg in the plasma and extracellular fluid, while the rest is intracellular. Plasma ascorbate is in equilibrium with tissue ascorbic acid, and as the vitamin is removed from the plasma it is replaced by release from various tissue stores, so it may also be lost during dialysis in this way.12)

It remains controversial whether oral vitamin C supplementation is indicated in patients on hemodialysis, but the recommended daily allowance (RDA) of 60 mg/day should generally be given.34) Intravenous ascorbic acid is a possible adjuvant therapy in patients with "iron-overload" anemia who are hyporesponsive to erythropoietin.35) Although the long-term effect of intravenous ascorbic acid on hemodialysis patients is unknown, the potential risk of secondary oxalosis should be considered.36,37) All of our dialysis patients had markedly elevated oxalic acid levels before and after dialysis. The predialysis ascorbic acid level showed an extremely good correlation with the serum oxalic acid level. Excessive intake of vitamin C in the diet or as a supplement may lead to high serum ascorbic acid levels that provoke hyperoxalemia, which may then contribute to vascular disease in patients on chronic hemodialysis.37)
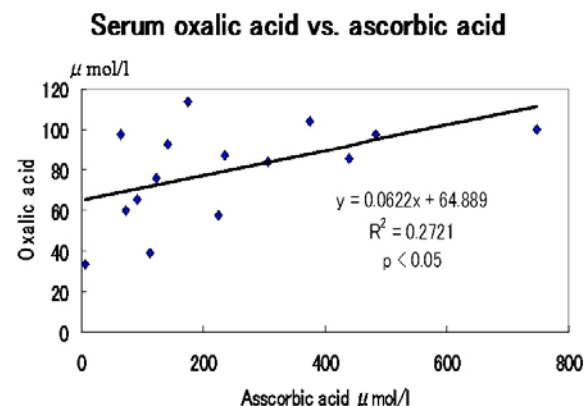
The relationship between ascorbic acid (vitamin C) and oxalic acid has been discussed for around 40 years, but remains to be clarified, with some studies showing no effect of ascorbic acid on oxalic acid levels and others showing extreme elevation of oxalate in response to an ascorbate load. Three potential degradation pathways for ascorbic acid can be postulated, which are bacterial and nonbacterial breakdown in the bowel, endogenous metabolism (oxidative and nonoxidative), and alkaline degradation in the urine (*in vivo* and *in vitro*).38) High-dose vitamin C supplementation is reported to cause saturation of the body pool of ascorbic acid, particularly in patients with renal failure, and may potentially lead to hyperoxalemia.6,37,39,40) Both pre-dialysis and post-dialysis oxalic acid levels were reported to be significantly increased after an increase of the vitamin C dose, but there was no significant correlation between plasma oxalic acid and ascorbic acid levels,41) and no significant changes of plasma oxalic acid were observed at the vitamin C doses tested (25-50 mg).8) There was also no change of oxalic acid during treatment with vitamin C at 300 mg intravenously three times per week for eight weeks.14) The mean (±SEM) plasma oxalic acid level rose from 30.3±3.5 μmol/1 to 48.4±6.1 μmol/1 in patients given oral vitamin C supplementation at 500 mg/day for 3 weeks.42) Daily oral supplementation with vitamin C at a dose of 0.57 mmol (100 mg) resulted in a 19% increase of the plasma oxalic acid level to 57.8±6.1 μmol/l (p < 0.03), with a concomitant 60% increase of the plasma ascorbic acid level to 91±6 μmol/l.43) The plasma oxalic acid level rose to

Figure 4. Relationship between serum oxalic acid and citric acid levels. Univariate analysis showed no correlation between serum oxalic acid and citric acid levels.
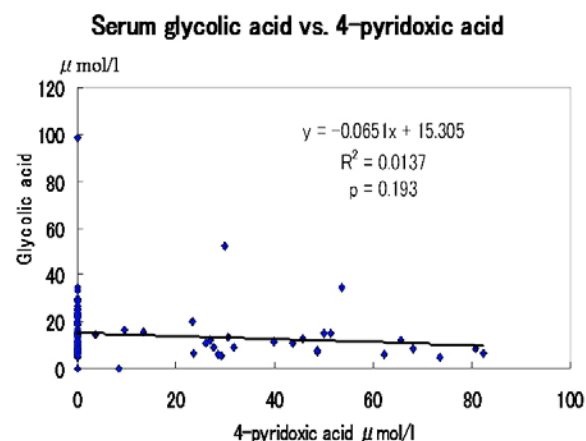
Figure 5. Relationship between serum glycolic acid and 4-pyridoxic acid levels. Univariate analysis showed no correlation between serum glycolic acid and 4-pyridoxic acid levels.

Figure 6. Relationship between serum oxalic acid and ascorbic acid levels before hemodialysis in 15 patients given oral vitamin C at a dose of 120-600 mg/day. Univariate analysis showed a significant correlation between serum oxalic acid and ascorbic acid levels.

14 times normal in a hemodialysis patient who received a total dose of 91.0 g of ascorbic acid intravenously over several months. Increased oxalic acid synthesis from ascorbic acid may be responsible for hyperoxalemia, leading to a high oxalic acid content in the myocardium, aorta and lungs, and to deposition of calcium oxalate in the soft tissues. Accordingly, high doses of vitamin C should be avoided in hemodialysis patients with chronic renal failure.5) Ono *et al.* could not find any beneficial effect of vitamin C supplementation on morbidity or mortality in regular hemodialysis patients but secondary hyperoxalemia was aggravated by supplementation. These observations suggest that vitamin C supplementation may be both harmful and unnecessary in dialysis patients provided they have an adequate diet.44,45) The plasma ascorbic acid level and vitamin B6 status were reported to show no correlation with the plasma oxalic acid level, while it was correlated with the duration of dialysis (p = 0.02).36)

Vitamin B6 deficiency increases the urinary excretion of oxalic acid in rats, due to impairment of glyoxylate transamination by peroxisomal alanine:glyoxylate aminotransferase (AGT).25) Endogenous synthesis of oxalic acid has been reported to increase in the presence of vitamin B6 deficiency, probably due to defective transamination of glyoxylate (the direct source of oxalic acid) to glycine. Hepatic AGT has the major role in glyoxylate transamination in mammals.46) Low serum AST and ALT levels in hemodialysis patients are partly due to deficiency of pyridoxal-5'-phosphate (the active form of vitamin B6), which acts as a coenzyme for these transaminases.20) Vitamin B6 deficiency not only decreases alanine:glyoxylate aminotransferase activity, but also down-regulates alanine-glyoxylate aminotransferase gene expression by hepatocytes, and thus leads to hyperoxaluria and hyperglycolic aciduria secondary to impaired metabolism of oxalic acid precursors. Hyperoxaluria combined with hypocitruria may contribute to calcium oxalate stone formation in patients with vitamin B6 deficiency.26) Administration of pyridoxine was reported to decrease the mean plasma oxalic acid level by 46% (32.0 to 56.1%) in 6 out of 8 chronic hemodialysis patients,1) but high-dose vitamin B6 supplementation did not decrease plasma oxalic acid levels in dialysis patients.21) Conversion of glycolic acid to glycine is probably increased in uremia.1) When the plasma glycolic acid and oxalic acid levels were measured in 20 patients on chronic hemodialysis, the mean values were 173.7±52.9 μmol/l and 128.7±25.6 μmol/l, respectively, versus 145.8±37.8 μmol/l and 16.8±6.0 μmol/l in healthy volunteers.47) However, the plasma glycolic acid level was 7.8±1.7 μmol/l in 12 healthy controls versus 6.1±0.8 μmol/l in 9 dialysis patients without oxalosis.48) Our present data on glycolic acid are compatible with this report and indicate that there is no direct association between the serum oxalic acid and glycolic acid levels. A high plasma concentration of 4-pyridoxic acid (> 10,000 nmol/l) which is the end product of vitamin B6 was observed that might have had a negative effect on vitamin B6 metabolism, causing the plasma level of pyridoxal (active form) to decrease. In reality, however, pyridoxal was elevated along with a high 4-pyridoxic acid level, presumably as a result of vitamin B6 supplementation.49) Our data of glycolic acid and 4-pyridoxic acid suggest that the serum 4-

pyridoxic acid level is not associated with hyperglycolic acidemia and does not necessarily imply the vitamin B6 status. The vitamin B6 status of dialysis patients should be monitored by using the serum pyridoxal-5'-phosphate level, which was reported to be 57.8±13.2 nmol/l by Mydlik and 26.8±3.8 nmol/l by Okada.50,51) They did not assess the correlation between serum pyridoxal-5'-phosphate and glycolic acid levels. However, vitamin B6 deficiency seems to increase the serum glycolic acid level in a subgroup of dialysis patients, although uremic plasma may potentially inhibit a wide range of enzymes that metabolize precursors of glycolic acid and glyoxylic acid.52) Others have also found that vitamin B6 does not contribute to hyperoxalemia,43) while a pharmacological dose of pyridoxine (800 mg/day) reduces the plasma oxalic acid level.53) Dialysis itself may stimulate the formation of oxalic acid by removing inhibitors,54) but this possibility requires further study.

Significant amounts of glycolic acid are present in vegetables, fruits, and beverages,55) so dietary intake of glycolic acid may increase the plasma oxalic acid level, but its contribution may be minimal because dialysis patients usually eat cooked vegetables and have lower serum glycolic acid levels.

During a 6-hour hemodialysis session, the plasma oxalic acid concentration was reported to decrease by 53.5%, but there was no decrement of the plasma glycolic acid level.47) When oxalic acid clearance across a 1 m^2 hollow-fiber Cuprophan dialyser (dialysate flow rate of 500 ml/min and blood flow of 175-225 ml/min) was measured in 19 patients at 1 h after the commencement of dialysis, the mean (±SD) clearance was 96.5 (±27.0) ml/min. Oxalic acid clearance is similar to that of other small molecules, such as creatinine and phosphate. Patients on routine hemodialysis have marked hyperoxalaemia, which may be exacerbated by vitamin C supplementation.56) Calcium oxalate crystals form when supersaturation of oxalate occurs. Although the mechanism of crystal deposition is not clear, saturation of plasma exceeding the solubility product may be associated with an increased risk of crystal deposition in body tissues.57) The plasma oxalic acid concentration is a crucial factor in determining the saturation of calcium oxalate.58) Chronic deposition of calcium oxalate crystals in the tissues is a life-threatening disorder that complicates the hyperoxalemic syndromes. The plasma saturation of calcium oxalate can be estimated from the serum levels of oxalic acid, calcium, and magnesium, and saturation is associated with a serum oxalic acid level of 44-46 μmol/l.59-61) Though plasma oxalic acid levels ranged between 6 and 116 μmol/l (4 to 78 times greater than the upper limit of the reference range), no extrarenal oxalate deposits were found in any of the tissues examined (inferior epigastric artery biopsy or necropsy specimens), but the renal deposition of oxalate was associated with a plasma oxalic acid concentration of more than 20 μmol/l.62) In dialysis patients, the oxalic acid level was even higher, being 137.5±56.0 μmol/l. Hemodialysis reduces the plasma oxalic level by about the same amount as the creatinine concentration. A high plasma oxalic acid level seems to be an important factor in

the occurrence of uremic calcification in various organs, with those most frequently involved being the kidneys, thyroid, and heart, while less prominent deposits are found in the spleen and the lungs. 63,64) Moderate to severe renal oxalosis is more frequently encountered in patients who have been on hemodialysis for a long period.65,66) Various other organs are also reported to be involved by oxalosis, including bone, skin, and the cardiovascular system.67-76) Hyperoxalemia is associated with an increased risk of cardiac and vascular disease even in the absence of primary hyperoxaluria. Plasma oxalic acid levels were significantly higher in dialysis patients with radiologically detectable calcification of medium-sized arteries than in those without such calcification, but the duration of dialysis was also significantly longer in the patients with calcification.56)

It may be necessary to monitor the plasma oxalic acid level during long-term therapy with intravenous ascorbic acid. In fact, monitoring of both oxalic and ascorbic acid is recommended in these patients. High-dose intravenous vitamin C therapy has been shown to be effective for erythropoietin-hyporesponsive anemia associated with iron overload. Clinical studies have shown that intravenous doses of vitamin C from 300 mg to 500 mg can be given up to three times a week for a maximum of 12 weeks without any significant deleterious effects.15-17) However, a large-scale prospective controlled trial is needed to assess the long-term safety and efficacy of intravenous vitamin C therapy in iron-overloaded hemodialysis patients receiving erythropoietin for anemia.

In conclusion, ascorbic acid overload and underdialysis appear to be the most important determinants of the serum oxalic acid level in dialysis patients, while vitamin B6 deficiency seems not so important. High doses of vitamin C tend to aggravate hyperoxalemia in hemodialysis patients, so accurate monitoring of the serum ascorbic acid and oxalic acid levels is strongly recommended.77) The absorption of ascorbic acid and oxalic acid is variable in dialysis patients, so care must also be taken to avoid vitamin deficiency.

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