

CLINICAL NEPHROLOGY – EPIDEMIOLOGY – CLINICAL TRIALS

Serum levels of 1,25-dihydroxyvitamin D, 24,25-dihydroxyvitamin D, and 25-hydroxyvitamin D in nondialyzed patients with chronic renal failure

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Serum levels of 1,25-dihydroxyvitamin D, 24,25-dihydroxyvitamin D, and 25-hydroxyvitamin D in nondialyzed patients with chronic renal failure.

Background. In patients with chronic renal failure (CRF), abnormalities in vitamin D metabolism are known to be present, and several factors could contribute to the abnormalities.

Methods. We measured serum levels of three vitamin D metabolites, 1,25(OH)₂D, 24,25(OH)₂D and 25(OH)D, and analyzed factors affecting their levels in 76 nondialyzed patients with CRF (serum creatinine > 1.6 and < 9.0 mg/dl), 37 of whom had diabetes mellitus (DM-CRF) and 39 of whom were nondiabetic (nonDM-CRF).

Results. Serum levels of 1,25(OH)₂D were positively correlated with estimated creatinine clearance (C_{cr} ; $r = 0.429$; $P < 0.0001$), and levels of 24,25(OH)₂D were weakly correlated with C_{cr} ($r = 0.252$, $P < 0.05$); no correlation was noted for 25(OH)D. Serum levels of all three vitamin D metabolites were significantly and positively correlated with serum albumin. Although there were no significant differences in age, sex, estimated C_{cr} , calcium and phosphate between DM-CRF and nonDM-CRF, all three vitamin D metabolites were significantly lower in DM-CRF than in nonDM-CRF. To analyze factors influencing vitamin D metabolite levels, we performed multiple regression analyses. Serum 25(OH)D levels were significantly and independently associated with serum albumin, presence of DM and serum phosphate ($R^2 = 0.599$; $P < 0.0001$). 24,25(OH)₂D levels were significantly and strongly associated with 25(OH)D ($\beta = 0.772$; $R^2 = 0.446$; $P < 0.0001$). Serum 1,25(OH)₂D levels were significantly associated only with estimated C_{cr} ($R^2 = 0.409$; $P < 0.0001$).

Conclusions. These results suggest that hypoalbuminemia and the presence of DM independently affect serum 25(OH)D levels, probably via diabetic nephropathy and poor nutritional status associated with diabetes, and that 25(OH)D is actively

catalyzed to 24,25(OH)₂D in CRF, probably largely via extrarenal 24-hydroxylase. Serum levels of 1,25(OH)₂D were significantly affected by the degree of renal failure. Thus, this study indicates that patients with CRF, particularly those with DM, should receive supplements containing the active form of vitamin D prior to dialysis.

In chronic renal failure (CRF), one of the most marked abnormalities in electrolyte metabolism is an imbalance of calcium and phosphate, the metabolism of which is strongly affected by vitamin D and parathyroid hormone (PTH). Calcium and phosphate metabolism is closely related to bone metabolism, and their metabolic abnormalities in CRF are associated with the emergence of renal osteodystrophy. This abnormality occurs as early as the decline of glomerular filtration rate to 70 ml/min and progresses as renal function deteriorates [1–4]. Serum levels of vitamin D metabolites are affected by several factors, such as calcium, phosphate, PTH, aging, seasons, renal function and intestinal absorption [1–3, 5]. In CRF, these factors are interrelated. Although serum levels of the active form of vitamin D are known to be decreased in CRF [1–4, 6–17], there are relatively few studies in which serum levels of the vitamin D metabolites have been examined in a large number of nondialyzed patients with CRF [18–21]. Recently, in both developed and developing countries, nephropathy caused by diabetes mellitus (DM) has become one of the most frequent causes of CRF. In diabetes, abnormalities in vitamin D metabolism have been reported [22–29]. Under the complicated conditions of CRF, several factors could contribute to abnormalities in vitamin D metabolism.

In this study, we measured serum levels of three vitamin D metabolites, 25-hydroxyvitamin D [25(OH)D], 24,25-dihydroxyvitamin D [24,25(OH)₂D] and 1,25-dihy-

Key words: vitamin D, hypoalbuminemia, diabetes mellitus, electrolyte metabolism, renal osteodystrophy.

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droxyvitamin D [1,25 (OH)₂D], in nondialyzed patients with CRF and analyzed factors affecting their serum levels, particularly the presence of DM.

METHODS

Patients

All 76 nondialyzed patients with CRF (serum creatinine > 1.6 and < 9.0 mg/dl) examined in this study visited the outpatient clinics of either Osaka City University Hospital or Shirasagi Hospital Kidney Center once or twice a month. CRF in 37 patients (21 males and 16 females) was due to long-term type II DM (DM-CRF; mean duration ± SD 17 ± 6 years, range 9 to 28 years). Patients with diabetes were treated with oral hypoglycemic agents (*N* = 18), insulin (*N* = 7), or diet therapy only (*N* = 12). All DM-CRF patients were instructed to have a food intake of 30 to 35 kcal/ideal body weight/day. CRF in the remaining 39 patients (29 males and 10 females) was due to other causes (nonDM-CRF), including chronic glomerulonephritis (*N* = 22), hypertension (*N* = 7), polycystic kidney disease (*N* = 2) and renal disease of unknown cause but not associated with DM (*N* = 8), according to medical records. None of the patients was taking vitamin D, phosphate binders, or medications that affect vitamin D metabolism, such as barbiturates or lithium. Patients with liver diseases were excluded from the study. During regular medical check-ups performed in the morning around 9:00 to 10:00 a.m. between October 1996 and January 1997, urine was collected and blood was drawn. Levels of urinary protein were estimated by a dye-impregnated paper strip method, in which +, ++, and +++ levels represented urinary protein levels of approximately 30 to 100 mg/dl, 100 to 300 mg/dl, and >300 mg/dl, respectively. After serum was separated, 2 ml was frozen and stored at -40°C, and levels of 25(OH)D, 24,25(OH)₂D and 1,25(OH)₂D were measured. The rest of the serum was used to measure levels of creatinine, albumin, calcium, phosphate and parathyroid hormone (PTH). Serum creatinine, albumin, calcium and phosphate were measured by an autoanalyzer. Serum calcium concentrations were corrected to a serum albumin level of 4.0 g/dl according to the following formula [10, 22, 30]:

$$\begin{aligned} \text{corrected Ca [mg/dl]} \\ = (4.0 - \text{albumin [g/dl]}) + \text{Ca [mg/dl]} \end{aligned}$$

Serum concentrations of PTH were measured with a kit (Allegro intact PTH kit; Japan MediPhysics Inc., Tokyo, Japan) that recognizes an intact molecule of PTH [31]. The reference range of PTH in healthy subjects was 10 to 65 pg/ml. Hemoglobin A1C of diabetic patients was measured by high performance liquid chromatography. In men, creatinine clearance (C_{Cr}) was estimated ac-

Table 1. Clinical characteristics and serum levels of three vitamin D metabolites of patients with renal failure due to nondiabetic renal diseases (NonDM-CRF) and due to diabetic nephropathy (DM-CRF)

	NonDM-CRF	DM-CRF	<i>P</i>
Age years	58 ± 12	58 ± 11	NS
Male/female	29/10	21/16	NS
Body weight kg	61.4 ± 11.6	57.6 ± 8.9	NS
Body mass index kg/m ²	24.3 ± 4.5	23.0 ± 3.0	NS
Serum creatinine mg/dl	3.6 ± 1.7	3.6 ± 1.8	NS
Estimated C _{Cr} ml/min	21.9 ± 9.1	21.4 ± 11.0	NS
Blood urea nitrogen mg/dl	50 ± 20	45 ± 15	NS
Corrected calcium mg/dl	9.0 ± 0.4	9.1 ± 0.6	NS
Phosphate mg/dl	4.2 ± 1.0	4.2 ± 0.8	NS
Serum albumin g/dl	3.9 ± 0.5	3.2 ± 0.6	<0.005
PTH pg/ml	125 ± 89	122 ± 78	NS
Fasting blood glucose mg/dl	93 ± 13	149 ± 65	<0.001
Hemoglobin A1C %	—	7.4 ± 1.9	
Urinary protein +/+/+/++	17/13/9	2/8/27	<0.001
25(OH)D ng/ml	22.3 ± 9.4	11.4 ± 5.6	<0.0001
24,25(OH) ₂ D ng/ml	0.74 ± 0.46	0.44 ± 0.20	<0.001
1,25(OH) ₂ D pg/ml	21.9 ± 10.3	15.5 ± 6.4	<0.002

25(OH)D, 25-hydroxyvitamin D; 24,25(OH)₂D, 24,25-dihydroxyvitamin D; 1,25(OH)₂D, 1,25-dihydroxyvitamin D; estimated C_{Cr} (creatinine clearance) was calculated according to Cockcroft and Gault, relying on serum creatinine, body weight, age and sex; corrected calcium is calculated by a formula considering the concentration of serum albumin as described in the text; PTH, parathyroid hormone; NS, not significant. Data are expressed as mean ± SD.

cording to Cockcroft and Gault, factoring in serum creatinine, body weight, and age [32–35] in the following formula:

$$\begin{aligned} \text{estimated C}_{\text{Cr}} [\text{ml/min}] \\ = \{(140 - \text{age})/\text{serum creatinine [mg/dl]}\} \\ \times \{\text{body weight [kg]}/72 \end{aligned}$$

For women, 15% of the value was subtracted [32, 33]. The clinical characteristics of nonDM-CRF and DM-CRF patients are summarized in Table 1.

Extraction and assay of the three vitamin D metabolites

The three vitamin D metabolites were extracted and assayed for their serum levels by Mitsubishi Kagaku Bio-Chemical Laboratory (Tokyo, Japan). The methods are described in detail in a previous report [36]. In brief, 2000 dpm of 1,25(OH)₂ [³H]D₃, 24,25(OH)₂ [³H]D₃, and 25(OH) [³H]D₃ in 20 ml of ethanol was added to 1.0 ml of serum. The samples were then mixed with 1.0 ml of 0.1 N HCl and applied to a Sep Pak tC18 cartridge (Millipore, Milford, MA, USA). Lipid extracts containing vitamin D metabolites were automatically eluted with 6 ml of acetonitrile. After the elutes evaporated, the samples were resuspended in toluene/ethanol (liter/liter) and stored at -80°C until assayed.

Samples were dried under nitrogen and resuspended in 300 ml of methanol/isopropanol/*N*-hexane (1/6/93). Vitamin D metabolites were separated by high pressure liquid chromatography (HPLC) [37] with a model 0.46 ×

30.0 cm ASP-Hypersil NH₂ column (Shandon, Cheshire, UK). The column was eluted and 4-ml fractions were collected. Absorbance was measured at a wavelength of 264 nm. After each vitamin D metabolite was separated, levels of 25(OH)D and 24,25(OH)₂D were determined by an established competitive protein binding assay using vitamin D-deficient rat serum [38]. 1,25(OH)₂D levels were determined by radioreceptor assay [39], using vitamin D receptor extract from bovine thymus (Yamasa Shoyu, Choushi, Japan). According to the company's documents, serum levels of 25(OH)D, 24,25(OH)₂D and 1,25(OH)₂D in healthy controls are 15.8 ± 6.0 ng/ml, 1.03 ± 0.56 ng/ml, and 29.0 ± 10.0 pg/ml, respectively.

Statistical analysis

Statistical analysis was performed with the Stat View IV system designed for the Macintosh computer. All data are expressed as mean ± SD. Student's *t*-test and chi-square test were performed for comparison of DM-CRF and nonDM-CRF. Correlation and linear regression analyzes were performed to examine the relationship between each vitamin D metabolite and clinical parameters, including age, body weight, estimated C_{Cr}, corrected calcium, phosphate, serum albumin, and serum PTH. Multiple regression analyzes were performed to assess the combined influence of clinical variables on serum levels of each vitamin D metabolite. Presence of DM was represented by dummy variables (0, absence; 1, presence) in multiple regression analyzes.

RESULTS

The mean serum level of 25(OH)D in the entire cohort was 16.9 ± 9.4 ng/ml, which was not significantly different from the reference range for healthy controls. The mean serum level of 24,25(OH)₂D was 0.59 ± 0.38 ng/ml, which was significantly lower than the reference range for healthy controls (*P* < 0.01). The mean serum level of 1,25(OH)₂D was 18.8 ± 9.2 pg/ml, which was significantly lower than the reference range for healthy controls (*P* < 0.0001). Serum levels of the three vitamin D metabolites in nonDM-CRF and DM-CRF are shown in Table 1. Serum levels of 25(OH)D, 24,25(OH)₂D and 1,25(OH)₂D in DM-CRF were significantly lower than those in nonDM-CRF (*P* < 0.0001, *P* < 0.001 and *P* < 0.002, respectively). There were no significant between-group differences in age, sex, body weight, body mass index, serum creatinine, estimated C_{Cr}, BUN, corrected calcium, phosphate, and serum intact PTH, although serum albumin and fasting blood glucose were significantly lower in DM-CRF than nonDM-CRF (*P* < 0.005 and *P* < 0.001, respectively). Semiquantitative assessment of urinary protein revealed significantly higher levels in DM-CRF patients than in nonDM-CRF patients (*P* < 0.001).

Table 2. Correlations between clinical parameters and each vitamin D metabolite

	25(OH)D		24,25(OH) ₂ D		1,25(OH) ₂ D	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Age		NS		NS		NS
Body weight		NS		NS		NS
Estimated C _{Cr}		NS	0.252	0.0391	0.429	0.0001
Corrected calcium		NS		NS		NS
Phosphate	-0.296	0.0105	-0.260	0.0252	-0.406	0.0003
Serum albumin	0.605	0.0001	0.229	0.0477	0.440	0.0001
PTH		NS	-0.237	0.0463		NS
24,25(OH) ₂ D	0.694	0.0001	—			NS
1,25(OH) ₂ D	0.468	0.0001	NS			—

Abbreviations are: C_{Cr}, creatinine clearance; 25(OH)D, 25-hydroxyvitamin D; 24,25(OH)₂D, 24,25-dihydroxyvitamin D; 1,25(OH)₂D, 1,25-dihydroxyvitamin D; *r*, correlation coefficient; NS, not significant; corrected calcium is calculated by a formula considering the concentration of serum albumin as described in the text; PTH, parathyroid hormone.

Correlations between each vitamin D metabolite and clinical parameters were examined (Table 2). The degree of renal failure was assessed by estimated C_{Cr} rather than serum creatinine, since estimated C_{Cr} values are more closely related to glomerular filtration rate than are serum creatinine levels. Effects of age, body weight and gender on serum creatinine are minimized in the formula used to calculate estimated C_{Cr} [32–35]. Although there was no significant correlation between serum 25(OH)D and estimated C_{Cr}, there was a significant, positive association between serum 1,25(OH)₂D and estimated C_{Cr} (*r* = 0.429, *P* < 0.0001). There was also a significant, positive correlation between serum 24,25(OH)₂D and estimated C_{Cr} (*r* = 0.252, *P* < 0.05), although the relationship was far weaker than that between serum 1,25(OH)₂D and estimated C_{Cr}. Correlations between each vitamin D metabolite and estimated C_{Cr} are shown in Figure 1. Although there were no significant associations between serum corrected calcium levels and each vitamin D metabolite, there were significant, negative correlations between serum phosphate levels and each vitamin D metabolite. There were also significant, positive correlations between serum albumin and each vitamin D metabolite. With respect to interrelationships among the three vitamin D metabolites, there were strong, significant, positive relationships between 25(OH)D and 24,25(OH)₂D and between 25(OH)D and 1,25(OH)₂D. There was no significant correlation between age and each vitamin D metabolite.

Because the clinical parameters that correlated with vitamin D metabolites were also interrelated, multiple regression analyzes were performed to assess the combined influence of these factors on serum levels of the three vitamin D metabolites. A model including serum albumin, presence of DM, phosphate, estimated C_{Cr} and serum PTH as independent variables was used to analyze the combined influence of these factors on serum

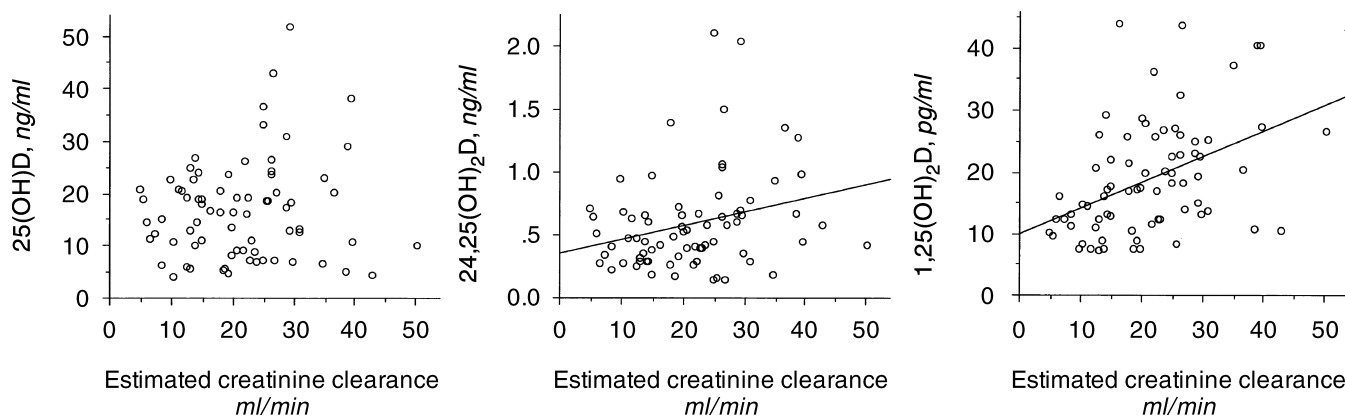


Fig. 1. Relationship between vitamin D metabolites and estimated creatinine clearance (C_{Cr}). There was a significant, positive correlation between 1,25(OH)₂D and estimated C_{Cr} ($r = 0.429$, $P < 0.0001$). There was also a significant correlation between 24,25(OH)₂D and estimated C_{Cr} ($r = 0.252$, $P < 0.05$), although the relationship was weaker than that between 1,25(OH)₂D and estimated C_{Cr} . There was no significant correlation between 25(OH)D and estimated C_{Cr} .

25(OH)D levels. Each of these independent variables had a significant correlation with at least one of the vitamin D metabolites. In multiple regression analysis using serum 24,25(OH)₂D and 1,25(OH)₂D levels as dependent variables, serum level of 25(OH)D was added as an independent variable, since 25(OH)D is a substrate for 24,25(OH)₂D and 1,25(OH)₂D (Table 3). Serum levels of 25(OH)D were significantly associated with serum albumin, presence of DM and phosphate as independent variables in order of importance ($R^2 = 0.599$, $P < 0.0001$), although estimated C_{Cr} and PTH were not significantly associated with 25(OH)D levels. Serum levels of 24,25(OH)₂D were significantly, strongly, and positively associated with serum 25(OH)D ($\beta = 0.752$, $P < 0.0001$) and serum albumin ($R^2 = 0.446$, $P < 0.0001$), although not with PTH, estimated C_{Cr} , phosphate or presence of DM. Serum levels of 1,25(OH)₂D correlated significantly only with estimated C_{Cr} ($R^2 = 0.409$, $P < 0.0001$) and not with serum albumin, 25(OH)D, phosphate, presence of DM, or PTH.

DISCUSSION

In this study, we measured serum levels of three vitamin D metabolites, 25(OH)D, 24,25(OH)₂D and 1,25(OH)₂D, in nondialyzed CRF patients and analyzed factors associated with their serum levels. As previously reported, we found normal serum levels of 25(OH)D in these patients and reduced serum levels of 1,25(OH)₂D [1–3, 10, 15, 18, 40]. There was a significant, positive correlation between serum 1,25(OH)₂D levels and estimated C_{Cr} . These results are consistent with previous reports in which serum levels of 1,25(OH)₂D decreased as renal mass declined in patients with CRF [1–3, 15, 18, 40]. Decreased renal mass and uremic factors are believed to reduce the activity of 25(OH)D 1 α -hydroxy-

lase, which is mostly located in the mitochondria of the proximal tubules [1–3, 15, 41]. There have been relatively few reports on serum 24,25(OH)₂D levels in patients with CRF. Koenig et al reported that in nondialyzed patients with CRF, levels of 24,25(OH)₂D were significantly lower than those of healthy subjects [18]. In the present study, serum levels of 24,25(OH)₂D in nondialyzed patients with CRF were significantly lower than those of healthy controls, and there was a weak relationship between serum 24,25(OH)₂D and estimated C_{Cr} , which is consistent with the report by Koenig et al. However, in contrast to the significant, relatively strong, positive correlation seen between serum 1,25(OH)₂D levels and estimated GFR, the correlation between serum 24,25(OH)₂D levels and estimated GFR was far weaker. Compared to 25(OH)D 1 α -hydroxylase, which is mostly localized in the kidney, 25(OH)D 24-hydroxylase is localized not only in the kidney but also to a large extent in other parts of the body such as the intestines and bones [5, 7, 42–45]. In CRF, extrarenal metabolism of vitamin D is reportedly increased by extrarenal 25(OH)D 1 α -hydroxylase and 25(OH)D 24-hydroxylase activity [5, 7, 8, 12, 19, 20, 40, 42, 46–48], which is known to be present in macrophages, skin, bones, and other tissues [8, 12, 47, 48]. Considering the far weaker correlation between 24,25(OH)₂D levels and estimated C_{Cr} despite the presence of a relatively stronger significant correlation between 1,25(OH)₂D levels and estimated GFR in the present study, it is suggested that a relatively larger amount of extrarenal 25(OH)D 24-hydroxylase is present or activated than that of extrarenal 25(OH)D 1 α -hydroxylase under conditions of renal mass reduction in CRF.

Impairment of vitamin D metabolism has been reported to be present in diabetic animal models such as rats with streptozotocin-induced diabetes, BB rats and

Table 3a. Factors affecting serum levels of 25-hydroxyvitamin D (25(OH)D) (Multiple regression analysis)

Variables				
Dependent	Independent	β	<i>P</i>	
25(OH)D	Serum albumin	0.468	< 0.0001	$R^2 = 0.599$ ($P < 0.0001$)
	Presence of DM	-0.369	0.0002	
	Phosphate	-0.201	0.0397	
	Estimated C_{Cr}	-0.035	0.7232	
	PTH	0.020	0.8318	

Table 3b. Factors affecting serum levels of 24,25-dihydroxyvitamin D [24,25(OH)₂D] (Multiple regression analysis)

Variables				
Dependent	Independent	β	<i>P</i>	
24,25(OH) ₂ D	25(OH)D	0.772	< 0.0001	$R^2 = 0.466$ ($P < 0.0001$)
	Serum albumin	0.322	0.0185	
	PTH	-0.170	0.1257	
	Estimated C_{Cr}	0.157	0.1770	
	Phosphate	0.079	0.5030	
	Presence of DM	-0.055	0.6567	

Table 3c. Factors affecting serum levels of 1,25-dihydroxyvitamin D [1,25(OH)₂D] (Multiple regression analysis)

Variables				
Dependent	Independent	β	<i>P</i>	
1,25(OH) ₂ D	Estimated C_{Cr}	0.332	0.0031	$R^2 = 0.409$ ($P < 0.0001$)
	Serum albumin	0.248	0.0868	
	25(OH)D	-0.192	0.2173	
	Phosphate	-0.143	0.2414	
	Presence of DM	-0.096	0.4494	
	PTH	0.026	0.8191	

Abbreviations are: C_{Cr} , creatinine clearance; DM, diabetes mellitus; PTH, parathyroid hormone; β , standard regression; R^2 , multiple coefficient of determination.

GK rats [23, 24, 26, 28, 49]. In these animal models, 25(OH)D 1 α -hydroxylase and 24-hydroxylase activities appear to be impaired [23, 24, 50]. In human studies, however, different results with respect to serum levels of 25(OH)D, 24,25(OH)₂D and 1,25(OH)₂D have been reported; specifically, serum levels of vitamin D metabolites differ between diabetics and control subjects [25, 27–29, 51]. Reduced serum levels of 1,25(OH)₂D in diabetics were reported in some studies but not in others. While these studies examined subjects with normal renal function, there have been few reports in which vitamin D metabolite levels were examined in patients with DM-CRF [13, 52]. In our study, all three vitamin D metabolite levels were significantly lower in DM-CRF than in nonDM-CRF, despite the fact that there were no significant differences in age, sex, body weight, corrected calcium, phosphate or PTH levels. Our results contradict those of Lu et al [13], which showed no significant difference in serum 25(OH)D and 1,25(OH)₂D between DM-CRF and nonDM-CRF, although 25(OH)D levels had a tendency to be lower in DM-CRF. In our study, serum albumin levels were significantly lower in DM-CRF than

in nonDM-CRF. Considering that vitamin D metabolites are mostly bound to vitamin D-binding proteins, whose serum levels are closely associated with serum albumin levels [1–3, 53], serum albumin may be strongly associated with serum levels of vitamin D metabolites. Significant positive correlations between serum albumin and the three vitamin D metabolites in our study also support this speculation. The more exaggerated hypoalbuminemia in DM-CRF in the present study was presumably caused primarily by significantly higher excretion of urinary protein. Furthermore, other factors such as malnutrition in DM-CRF may contribute to hypoalbuminemia. Hemoglobin levels, one of the indices of nutritional status, were lower in DM-CRF than in nonDM-CRF (10.4 ± 1.6 g/dl vs. 11.2 ± 1.9 g/dl, $P < 0.05$) even though some patients with anemia in both the DM-CRF and nonDM-CRF groups were receiving erythropoietin therapy, as we recently reported [54]. Therefore, the significantly lower serum levels of all three vitamin D metabolites in DM-CRF than in nonDM-CRF in our study may have been caused by significantly decreased serum albumin

levels in DM-CRF, which is characterized by significantly higher excretion of urinary protein and malnutrition.

By linear regression analysis, we found several significant correlations between levels of each vitamin D metabolite and clinical parameters. This led us to apply multiple regression analysis to determine the combined influence of various factors on serum levels of each vitamin D metabolite. In multiple regression analysis to determine factors affecting 25(OH)D levels, which represent the nutritional status of vitamin D [1–3, 5, 15, 55], the presence of DM was significantly and independently associated with serum levels of 25(OH)D in addition to serum albumin (Table 3a). This indicates that the presence of DM is an independent risk factor for reduced 25(OH)D levels. The mean 25(OH)D level of 11.4 ± 5.6 ng/ml in DM-CRF patients was significantly lower than that of healthy subjects ($P < 0.005$), which suggests that these patients have vitamin D deficiency [5, 6, 34, 55–58]. Serum 25(OH)D levels are affected by sunlight, liver function and intestinal absorption of vitamin D [1–3, 15, 55]. In our study, serum samples were collected over a relatively short time from late autumn to winter, and patients with liver dysfunction were excluded. We speculate that one of the reasons for the independent and significant association between the presence of DM and 25(OH)D levels involved dietary habits of diabetics. These patients were instructed to avoid excessive food (30 to 35 kcal/ideal body weight/day), particularly foods containing a large amount of fat in which vitamin D can be dissolved, in order to control their blood sugar levels, a practice which may lead to decreased vitamin D intake. We speculate that another contributing factor may have been impaired intestinal absorption of vitamin D in diabetic patients, since patients with advanced diabetic nephropathy often have gastroenteropathy associated with diabetic autonomic neuropathy [59, 60]. Furthermore, diabetic patients with nephropathy may be sicker than nonDM-CRF patients in terms of hypoalbuminemia (more frequent presence of nephrotic syndrome), leading to less outside activity and sunlight exposure necessary for synthesizing vitamin D; however, outside activity was not assessed in the present study. Multiple regression analysis to evaluate serum levels of 24,25(OH)₂D demonstrated that 24,25(OH)₂D levels were strongly and positively associated with 25(OH)D. 25(OH)D 24-hydroxylase is reported to be activated by an excessive amount of 25(OH)D and represents the catabolic pathway of vitamin D [1–3, 15, 19]. Even though renal 25(OH)D 24-hydroxylase activity may be reduced in chronic renal failure [18, 61], the results of the present study suggest that 25(OH)D 24-hydroxylase can be activated as serum substrate [25(OH)D] levels increase. This speculation is consistent with the results of animal and human studies of renal failure [19, 46, 62], in which 24,25(OH)₂D levels correlated with 25(OH)D levels after 25(OH)D adminis-

tration, suggesting increased extrarenal activity of 25(OH)D 24-hydroxylase. Furthermore, our results suggest that extrarenal 25(OH)D 24-hydroxylase actively catalyzes vitamin D in CRF where both renal 25(OH)D 24-hydroxylase and 1 α -hydroxylase activities are apparently reduced [18, 19, 46, 61, 62]. In multiple regression analysis of 1,25(OH)₂D levels, as seen in linear regression analysis, 1,25(OH)₂D levels were strongly and significantly associated only with estimated C_{cr}, and not with 25(OH)D levels, phosphate, presence of DM, serum albumin and PTH (Table 3c). Although 1,25(OH)₂D levels are affected by PTH, calcium, phosphate and 25(OH)D levels in normal renal function [1–3, 15, 55], the present study showed that none of these factors had a significant influence on 1,25(OH)₂D levels in CRF. The results also suggest that renal 25(OH)D 1 α -hydroxylase activity, which is decreased due to reduced renal mass, is still a major regulatory factor that determines serum levels of 1,25(OH)₂D in CRF, even though extrarenal 25(OH)D 1 α -hydroxylase activity can be activated in this condition [8, 12, 15, 40, 47, 48]. In this study, it was unclear whether free 1,25(OH)₂D and free 25(OH)D unbound to vitamin D-binding protein were reduced. In recent studies by Boonen et al [34, 35], both total and free 25(OH)D levels were significantly lower in osteoporotic elderly patients with significantly reduced vitamin D-binding protein and without renal failure than in individuals without osteoporosis, although there was a significant decrease in total 1,25(OH)₂D but not free 1,25(OH)₂D. The results suggest that serum levels of free 25(OH)D, a metabolic precursor that has little hormonal function, are not tightly or feedback controlled, and are independent of the free/total ratio of 1,25(OH)₂D, which acts as a tightly regulated hormone. Thus, low 25(OH)D levels in patients in the present study suggest the presence of hypovitaminosis D. However, since serum concentrations of total and free 1,25(OH)₂D decrease with a high degree of correlation as renal function declines in CRF patients and free 1,25(OH)₂D falls as glomerular filtration rate decreases in nephrotic patients [18], the reduced levels of total 1,25(OH)₂D in our study may reflect reduced free 1,25(OH)₂D levels, leading to a deficiency in the active form of vitamin D in CRF.

In the present study, PTH levels in DM-CRF patients were not significantly different from those of nonDM-CRF patients. Among patients receiving hemodialysis or peritoneal dialysis, PTH levels have been reported to be lower in those with diabetes than those without; however, few studies have compared the effect of diabetes on PTH levels in predialysis patients with renal failure [63–66]. In rats with streptozotocin-induced diabetes and azotemia, PTH levels were not significantly different from those in nondiabetic azotemic rats, although indices of bone turnover rate were lower in the former than in the latter [67]. In a clinical study, Fajtova et al reported

that there were no significant differences in intact PTH levels between diabetic and nondiabetic patients with chronic renal failure not receiving dialysis [68]. Our results are consistent with the results of Fajtova et al. Considering vitamin D metabolite levels in the present study, it may be suggested that PTH secretion from parathyroid glands in secondary hyperparathyroidism is not sufficiently suppressed due to lowered $1,25(\text{OH})_2\text{D}$ levels in DM-CRF, even though PTH secretion is reportedly lower in diabetic patients [69]. Low-dose calcitriol or alphacalcidol supplementation in predialysis renal failure has been recommended in several clinical trials [70–73]. In these studies, supplementation with the active form of vitamin D safely and effectively prevented the progression of hyperparathyroidism and related bone disorders in patients with mild to moderate renal failure. In the present study, since $1,25(\text{OH})_2\text{D}$ levels were lowered in CRF and the nutritional status of vitamin D was impaired in some of the CRF patients, particularly those with DM-CRF, and furthermore since extrarenal $25(\text{OH})\text{D}$ 24-hydroxylase activity may actively catalyze $25(\text{OH})\text{D}$ into $24,25(\text{OH})_2\text{D}$ in CRF, we recommend supplementation with the active form of vitamin D, rather than the nonactive form of vitamin D, in these patients, particularly those with DM-CRF.

In summary, this study showed that nondialyzed patients with CRF have abnormal vitamin D metabolism. In particular, all of the vitamin D metabolites were significantly lower in DM-CRF than in nonDM-CRF. The reduced $25(\text{OH})\text{D}$ levels in DM-CRF are believed to be associated with hypoalbuminemia and poor nutritional status of vitamin D possibly due to impaired intake, absorption and intrinsic synthesis. $24,25(\text{OH})_2\text{D}$ levels were strongly and significantly correlated with $25(\text{OH})\text{D}$ levels, presumably largely via activation of extrarenal $25(\text{OH})\text{D}$ 24-hydroxylase whose activity is not associated with declining renal function. $1,25(\text{OH})_2\text{D}$ levels were significantly associated only with decreased renal function. Since serum levels of $1,25(\text{OH})_2\text{D}$ and/or $25(\text{OH})\text{D}$ are decreased and extrarenal $25(\text{OH})\text{D}$ 24-hydroxylase activity may actively catalyze $25(\text{OH})\text{D}$ in CRF, we feel that supplementation with the active form of vitamin D is required in nondialyzed patients with CRF, particularly those with DM-CRF.

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