Table 1 Baseline characteristics

	Losartan group	*LS/HCTZ group*	P-*value*
Number (*n*)	48	51	
Age (year)	58±12	58±11	0.90
Sex (male/female)	26/22	30/21	0.61
Underlying kidney disease, n *(%)*			
Glomerulonephritis	39 (81)	41 (80)	0.82
Diabetic nephropathy	4 (8)	6 (12)	
Hypertensive nephrosclerosis	5 (11)	5 (8)	
Systolic blood pressure (mm Hg)	132±14	128±13	0.16
Diastolic blood pressure (mm Hg)	77±9	77±12	0.84
Pulse rate (beats min^{-1})	73±10	73±11	0.82
Total protein ($g dl^{-1}$)	6.9±0.6	6.9±0.5	0.55
Albumin ($g dl^{-1}$)	4.0±0.4	3.9±0.4	0.10
HDL-cholesterol ($mg dl^{-1}$)	65±35	61±26	0.73
LDL-cholesterol ($mg dl^{-1}$)	119±35	112±27	0.42
Triglycerides ($mg dl^{-1}$)	157±83	154±76	0.83
Blood urea nitrogen ($mg dl^{-1}$)	24±11	25±11	0.74
Creatinine ($mg dl^{-1}$)	1.4±0.7	1.5±0.7	0.68
Uric acid ($mg dl^{-1}$)	6.6±1.4	6.6±1.3	0.89
Sodium ($mEq l^{-1}$)	138±2	141±2	0.18
Potassium ($mEq l^{-1}$)	4.6±0.5	4.6±0.5	0.82
Chloride ($mEq l^{-1}$)	106±2.6	107±2.7	0.34
Calcium ($mg dl^{-1}$)	9.2±0.4	9.2±0.5	0.70
Phosphate ($mg dl^{-1}$)	3.4±0.6	3.3±0.6	0.72
Aspartate aminotransferase ($U l^{-1}$)	22±6.1	21±5.2	0.89
Alanine aminotransferase ($U l^{-1}$)	20±9.3	17±8.9	0.14
Alkaline phosphatase ($U l^{-1}$)	237±79	225±65	0.65
γ-Glutamyl transpeptidase ($U l^{-1}$)	43±29	38±37	0.14
eGFR ($ml min^{-1}$ per $1.73 m^2$)	45.9±25.1	43.8±21.9	0.67
Urinary protein/creatinine ratio ($g g^{-1}$ Cr)	1.80±1.63	1.74±1.40	0.52
Treatment during pretreatment period, n *(%)*			
Ca channel blockers	24 (50)	22 (43)	0.42
β-Blockers	3 (6)	7 (14)	0.43
α-Blockers	2 (4)	1 (2)	0.51
Statins	21 (44)	17 (33)	0.96

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II type 1 receptor blocker; Cr, creatinine; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LS, losartan; HCTZ, hydrochlorothiazide.
Data are presented as mean ± s.d.

Table 2 Medications used during the follow-up period, *n* (%)

	LS group	*LS/HCTZ group*	P-*value*
Ca channel blocker	34 (68)	25 (49)	<0.05
β-Blocker	9 (18)	7 (14)	NS
α-Blocker	3 (6)	1 (2)	NS
Statin	26 (52)	23 (45)	NS

Abbreviations: HCTZ, hydrochlorothiazide; LS, losartan; NS, nonsignificant.

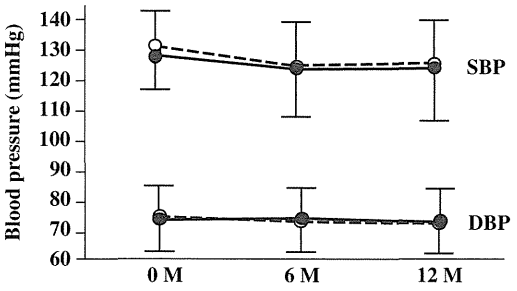

Figure 3 Changes in blood pressure during the study period. Systolic (SBP) and diastolic blood pressure (DBP) levels (mean ± s.d.) at 0, 6 and 12 months after treatment are shown. There were no differences in blood pressure between the losartan (LS) group (open circle and dash line) and the LS/hydrochlorothiazide (HCTZ) group (closed circle and solid line).

respect to nocturnal hypertension. It is thought that diuretics and salt restriction normalizes the circadian rhythm of blood pressure from non-dipper to dipper, thereby reducing the load on the circulatory system and further inhibiting cardiac events by concomitant use with RAS-inhibiting drugs.[15,16] Buter *et al.*[17] reported that the urinary protein reducing the effect of RAS inhibitors was stronger during the day than during the night. It was reported that diuretics and salt restrictions normalize the circadian rhythm of blood pressure from non-dipper to dipper type,[18,19] and the decline in proteinuria by administering diuretics is largely dependent on the decline in nocturnal blood pressure.[12]

The second point is the corrective effect from a state of excessive salt. It has been verified that the salt load promotes local tissue RAS activation in the organ, leading to the development of organ dysfunction.

The third point is that diuretics are reported to have an antioxidant effect, and there is a possibility that renal injury may be improved via a decline in oxidative stress. Skalska *et al.*[20] reported that patients taking diuretics had significantly better antioxidative protection expressed by higher levels of the ferric-reducing ability of plasma.

To date, it has not been suggested that diuretics have an effect on renal protection. Rather, as serum creatinine increases with the administering of diuretics, it was once believed that it is a drug contributing to renal impairment. However, the results from this study suggest the possibility that an increase in serum creatinine and a decline in urinary protein content indicates a decline in intraglomerular pressure owing to diuretics, and that it has an effect on renal protection in the same manner as RAS inhibitors.

According to reports in the GUARD study[8] and the ACCOMPLISH study,[9,10] although HCTZ reduced albuminuria, the decline in eGFR was also very large. Unlike the subjects of the GUARD study and the ACCOMPLISH study, those of the current study were CKD patients showing overt proteinuria in which the eGFR advanced to approximately 40–45 $ml min^{-1}$ per $1.73 m^2$. That is, the subjects were cases with a decreased functioning glomerulus count and increased intraglomerular pressure for each nephron unit. In such cases, diuretics used for a relatively long period of time such as HCTZ are believed to be effective for depression management, including management of the quantity of renal protecting body fluid as well as renal protection. It is possible that the result was affected because of Japanese people having a higher salt intake compared with Europeans and Americans. Excessive intake of salt causes excessive body fluid volume, attenuating the effect of ARB. It was hypothesized that excess extracellular fluid was discharged and a synergic effect due to concomitant use of ARB was induced by administering low-dose HCTZ to these patients.

From multiple clinical studies, proteinuria has been proven to be a predictive factor for the advancement of subsequent renal disease. According to a study by Lea *et al.*,[21] baseline proteinuria is independently related to the subsequent decline in GFR. Also, in recent years, albuminuria has been determined as being a risk factor to the cardiovascular system. In the Framingham study, proteinuria increased mortality threefold and was strongly related to other risk

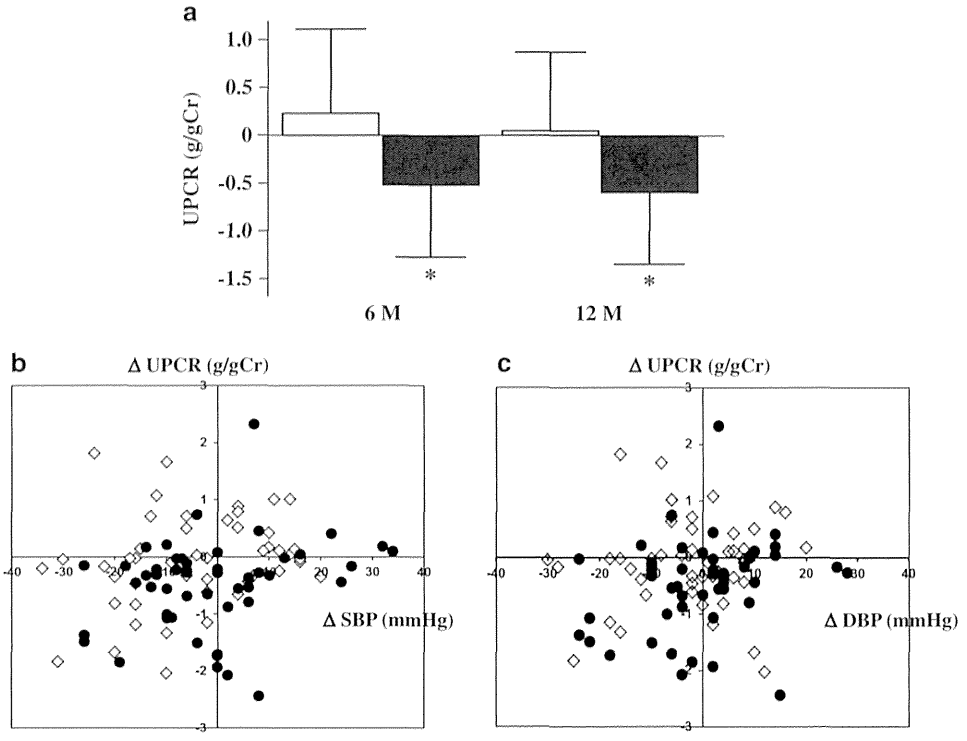

Figure 4 Changes in urinary protein-to-creatinine ratio (UPCR) and relationship between blood pressure (BP) reduction and the reduction in UPCR. (**a**) Mean changes in the UPCR ($g g^{-1}$ Cr) from baseline to 6 and 12 months in the losartan (LS) group (open column) and the LS/hydrochlorothiazide (HCTZ) group (closed column) are shown. $^*P<0.05$ *vs.* the LS group. (**b**) The relationship between systolic blood pressure (SBP) reduction (ΔSBP) and the reduction in UPCR (ΔUPCR) from baseline to 12 months was not significant. (**c**) The relationship between diastolic blood pressure (DBP) reduction (ΔDBP) and the reduction in UPCR (ΔUPCR) from baseline to 12 months was not significant. Open square, the LS group; closed circle, the LS/HCTZ group.

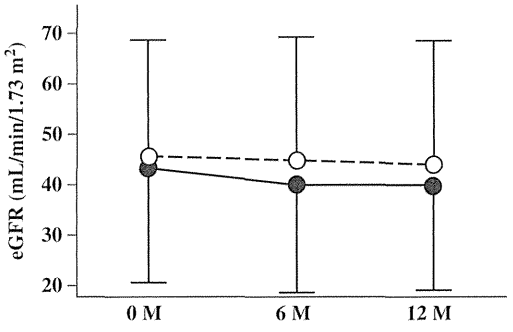

Figure 5 Changes in estimated glomerular filtration rate (eGFR) during the study period. The estimated GFR (mean ± s.d.) values at 0, 6 and 12 months after the start of treatment are shown. There were no differences in eGFR between the losartan (LS) group (open circle and dash line) and the LS/hydrochlorothiazide (HCTZ) group (closed circle and solid line).

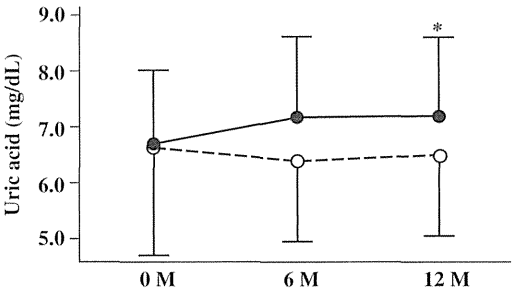

Figure 6 Changes in the uric acid levels during the study period. The uric acid (mean ± s.d.) levels at 0, 6 and 12 months after the start of treatment are shown. There was a significant difference in the uric acid levels at 12 months between the losartan (LS) group (open circle and dash line) and the LS/hydrochlorothiazide (HCTZ) group (closed circle and solid line). $^*P<0.05$ *vs.* the LS group.

factors for cardiovascular diseases.[22] In the subanalysis for the Systolic Hypertension in Europe study, proteinuria was the predictive factor for the all-cause mortality and cardiovascular events.[23] Data for basic and clinical studies to date have exhibited that renal failure and heart failure are suppressed once the proteinuria of CKD patients declines.[24]

In this study, 24-h urine collection was not carried out because it was too inconvenient and troublesome for outpatients. Twenty-four hour urine collection is the gold standard for urinary protein measurement,[25] but it has been reported by several researchers that the UPCR of occasional urine exhibits a strong correlation with 1-day urinary protein excretion.[26,27] The working group for the renal disease prognostic indicator of the National Kidney Foundation also reported that the UPCR of first morning urine or spot urine is a test value suitable at clinical sites when evaluating the proteinuria in patients with renal disease.[28]

In this study, LS/HCTZ was discontinued in three patients in the LS/HCTZ group because of aggravation of renal function. Diuretics have a danger of aggravating renal function in patients on whom sodium restriction is being carried out or in patients with declined body fluid, so sufficient attention is required.

Our study has several limitations. Blood pressure was measured at an outpatient clinic, and no investigation into home blood pressure and 24-h ambulatory blood pressure monitoring was carried out. Therefore, the improvement effect of LS/HCTZ against nocturnal hypertension on the subjects of this study has not been evaluated and

proved. Also, the sample size was 85% of the initially planned number of cases. However, we could find a significant difference in the primary outcome with the number of cases used in this study, because the difference in the amount of urinary protein was more than expected. Finally, the rate of use of CCB was significantly higher in the LS group than in the LS/HCTZ group. However, this bias did not appear to affect this result because the difference in the amount of urinary protein remained significant, even after adjustment for use of CCB.

In conclusion, in CKD patients with hypertension and overt proteinuria, the effect of reducing urinary protein was higher in the LS/HCTZ group than that in the LS group even when blood pressure was equivalently controlled. We believe that the concomitant use of ARB and thiazide diuretics should be considered for CKD patients with hypertension and overt proteinuria.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Ankle-brachial blood pressure index predicts cardiovascular events and mortality in Japanese patients with chronic kidney disease not on dialysis

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The ankle-brachial blood pressure index (ABPI) has been recognized to have a predictive value for cardiovascular (CV) events and mortality in general or dialysis populations. However, the associations between ABPI and those outcomes have not been fully investigated in predialysis patients. The present study aimed to clarify the relationships between ABPI and both CV events and mortality in Japanese chronic kidney disease (CKD) patients not on dialysis. In this prospective observational study, we enrolled 320 patients with CKD stages 3–5 who were not on dialysis. At baseline, ABPI was examined and a low ABPI was defined as <0.9. CV events and all-cause deaths were examined in each patient. A Cox proportional hazards model was applied to determine the risk factors for CV events, as well as for mortality from CV and all causes. The median follow-up period was 30 months. CV events occurred in 56 patients and all-cause deaths occurred in 48, including 20 CV deaths. Multivariate analysis showed that age and low ABPI were risk factors for CV events. It was demonstrated that age, a history of cerebrovascular disease and low ABPI were determined as independent risk factors for CV mortality. In addition, age, body mass index and low ABPI were independently associated with all-cause mortality. In patients with CKD, low ABPI during the predialysis period is independently associated with poor survival and CV events, suggesting the usefulness of measuring ABPI for predicting CV events and patient survival in CKD.
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Keywords: ankle-brachial blood pressure index; cardiovascular events; chronic kidney disease; mortality

INTRODUCTION

Patients with chronic kidney disease (CKD) have high cardiovascular (CV) morbidity and mortality.[1] Manifestations of CKD, such as hyperphosphatemia, hyperparathyroidism and chronic inflammation, are implicated in atherosclerosis in addition to traditional risk factors, such as smoking, diabetes, dyslipidemia and hypertension.[2] CKD patients had more advanced arterial wall stiffness compared with healthy subjects,[3] which is strongly associated with atherosclerosis.[4] Therefore, the presence of CKD worsens CV disease outcomes because of advanced atherosclerosis in CKD patients.

The ankle-brachial blood pressure index (ABPI) is a simple, noninvasive and reliable method of evaluating systemic atherosclerosis and peripheral artery disease.[5,6] It was demonstrated that low ABPI levels, particularly those <0.90, are indicative of generalized atherosclerosis.[7] An ABPI <0.9 is also associated with increased CV and all-cause mortality in non-CKD populations.[8–13] Several studies have also shown that a low ABPI monitored during a maintenance dialysis period is associated with the outcome of CV morbidity and mortality.[14–19] However, very few studies have documented the relationship between ABPI during the predialysis period and either CV events or mortality in predialysis patients.[20,21] Moreover, there have been very few studies investigating the relationship between low ABPI and CV events or mortality in Japanese CKD patients not on dialysis.[20] The present study aimed to determine whether ABPI during the predialysis period is associated with CV events and with both CV and all-cause mortality in Japanese CKD patients.

MATERIALS AND METHODS

In this prospective observational study, we enrolled 320 consecutive Japanese patients with CKD stages 3–5 not on dialysis, who were admitted to our hospital for evaluation of and education about CKD between January 2005 and September 2012. Patients with any malignancy or history of treatment for peripheral artery disease were excluded from this study. All patients provided written informed consent to the protocol, which was approved by the Ethics Committee of the National Kyushu Medical Center Hospital.

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Blood samples (serum creatinine (SCr), C-reactive protein, hemoglobin, serum albumin and serum phosphorus levels) were obtained in the early morning after an overnight fast. Daily proteinuria was also measured. The estimated glomerular filtration rate (eGFR; $ml\,min^{-1}$ per $1.73\,m^2$) was calculated using the following new Japanese equation: $194 \times SCr^{-1.094} \times age^{-0.287} \times 0.739$ (if female).[22]

All enrolled patients were interviewed and clinically examined at presentation. Their medical histories and outpatient records were also evaluated in detail. Demographic information (age and sex), medication history and atherosclerotic risk factors (hypertension, history of smoking, dyslipidemia and diabetes mellitus) at presentation were recorded for each patient. Hypertension was defined as systolic blood pressure $\geqslant 140$ mm Hg or diastolic blood pressure $\geqslant 90$ mm Hg, or the current use of antihypertensive drugs. Dyslipidemia was defined as plasma triglycerides $\geqslant 150\,mg\,dl^{-1}$, plasma low-density lipoprotein cholesterol $\geqslant 140\,mg\,dl^{-1}$, plasma high-density lipoprotein cholesterol $<40\,mg\,dl^{-1}$ or the use of lipid-lowering drugs based on a history of dyslipidemia. Diabetes mellitus was defined as previous or current plasma fasting glucose $\geqslant 126\,mg\,dl^{-1}$ or the use of hypoglycemic agents. Present or past cigarette smoking was distinguished. Body mass index was calculated as weight in kg divided by height in m^2. Blood pressure was measured at three separate times in a sitting position on the second day of hospitalization; the average of the three readings was recorded. Pulse pressures were calculated as the difference between systolic and diastolic blood pressures.

Table 1 Baseline characteristics of study participants grouped by ABPI

	All (n = 320)	*ABPI < 0.9 (n = 42)*	*ABPI ⩾ 0.9 (n = 278)*	P-*value*
Age (years)	72 (62–78)	77 (70–82)	71 (61–77)	<0.01
Male	218 (68)	32 (76)	186 (67)	0.22
Smoking	174 (54)	29 (69)	145 (52)	0.04
Hypertension	300 (94)	40 (95)	260 (94)	0.66
SBP (mm Hg)	137 ± 18	146 ± 20	136 ± 18	<0.01
DBP (mm Hg)	72 ± 11	68 ± 10	73 ± 11	0.01
Pulse pressure (mm Hg)	65 ± 15	78 ± 16	63 ± 14	<0.01
Diabetes mellitus	162 (51)	30 (71)	132 (47)	<0.01
Dyslipidemia	232 (73)	33 (79)	199 (72)	0.33
History of IHD	60 (19)	15 (36)	45 (16)	<0.01
History of CVD	60 (19)	15 (36)	45 (16)	<0.01
Body mass index ($kg\,m^{-2}$)	22 (20.2–24.9)	21.3 (19.4–22.7)	22.1 (20.4–25.3)	<0.01
Serum albumin ($g\,dl^{-1}$)	3.5 (3.0–3.8)	3.3 (3.0–3.8)	3.5 (3.0–3.8)	0.49
C-reactive protein ($mg\,dl^{-1}$)	0.09 (0.04–0.21)	0.11 (0.04–0.23)	0.09 (0.04–0.21)	0.75
Hemoglobin ($g\,dl^{-1}$)	10.3 (8.7–11.7)	9.8 (8.6–11.3)	10.4 (8.8–11.7)	0.51
Serum phosphorus ($mg\,dl^{-1}$)	3.8 (3.3–4.3)	3.7 (3.2–4.3)	3.8 (3.3–4.3)	0.73
Proteinuria (g per day)	1.5 (0.4–3.5)	1.4 (0.4–3.2)	1.5 (0.4–3.6)	0.95
eGFR ($ml\,min^{-1}$ per $1.73\,m^2$)	18.4 (12.6–32.2)	21.3 (12.0–28.9)	18.1 (12.7–32.4)	0.85
ABPI	1.09 (0.98–1.16)	0.75 (0.66–0.82)	1.11 (1.04–1.17)	<0.01
Follow-up period (months)	30 (19–46)	27 (13–38)	30 (19–46)	0.12

Abbreviations: ABPI, ankle-brachial blood pressure index; CVD, cerebrovascular disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; IHD, ischemic heart disease; SBP, systolic blood pressure.
Values are expressed as the mean plus/minus s.d., number (percent) or median (interquartile range).

Table 2 Logistic regression analysis of determinant factors for low ABPI

	Univariate		*Multivariate*	
Variables	*OR (95% CI)*	P-*value*	*OR (95% CI)*	P-*value*
Age (years)	1.07 (1.03–1.11)	<0.01	1.06 (1.02–1.12)	<0.01
Male *vs.* female	1.58 (0.77–3.52)	0.22		
Smoking	2.05 (1.04–4.22)	0.04	2.09 (0.95–4.83)	0.07
Pulse pressure (mm Hg)	1.06 (1.04–1.08)	<0.01	1.05 (1.03–1.08)	<0.01
Diabetes mellitus	2.77 (1.39–5.82)	<0.01	1.65 (0.70–3.99)	0.25
Dyslipidemia	1.46 (0.69–3.36)	0.33		
History of IHD	2.88 (1.39–5.78)	<0.01	2.10 (0.92–4.74)	0.08
History of CVD	2.88 (1.39–5.78)	<0.01	2.54 (1.11–5.74)	0.03
Body mass index ($kg\,m^{-2}$)	0.87 (0.79–0.96)	<0.01	0.17 (0.01–3.21)	0.24
Serum albumin ($g\,dl^{-1}$)	0.84 (0.52–1.39)	0.49		
C-reactive protein ($mg\,dl^{-1}$)	1.09 (0.57–1.75)	0.76		
Hemoglobin ($g\,dl^{-1}$)	0.95 (0.80–1.11)	0.50		
Serum phosphorus ($mg\,dl^{-1}$)	0.93 (0.61–1.37)	0.73		
Proteinuria (g per day)	1.00 (0.87–1.15)	0.95		
eGFR ($ml\,min^{-1}$ per $1.73\,m^2$)	1.00 (0.98–1.03)	0.85		

Abbreviations: CI, confidence interval; CVD, cerebrovascular disease; eGFR, estimated glomerular filtration rate; IHD, ischemic heart disease; OR, odds ratio.

Table 3 Cox hazards analysis for CV events and mortality from both CV and all causes

	CV events				*CV mortality*				*All-cause mortality*			
	Univariate		*Multivariate*		*Univariate*		*Multivariate*		*Univariate*		*Multivariate*	
Variable	*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Age (years)	1.05 (1.02–1.08)	<0.01	1.03 (1.00–1.07)	0.02	1.08 (1.03–1.15)	<0.01	1.07 (1.01–1.13)	0.01	1.12 (1.08–1.16)	<0.01	1.10 (1.05–1.14)	<0.01
Male	1.64 (0.91–3.17)	0.10			2.82 (0.95–12.09)	0.06			1.48 (0.79–2.98)	0.22		
Smoking	1.26 (0.74–2.19)	0.40			1.02 (0.42–2.54)	0.96			0.81 (0.46–1.43)	0.47		
Pulse pressure (mm Hg)	1.02 (1.01–1.04)	<0.01	1.01 (0.99–1.03)	0.52	1.01 (0.99–1.04)	0.33			1.03 (1.01–1.04)	<0.01	1.00 (0.98–1.02)	0.84
Diabetes mellitus	1.68 (0.99–2.94)	0.06			1.80 (0.74–4.80)	0.20			1.22 (0.69–2.18)	0.50		
Dyslipidemia	1.28 (0.71–2.48)	0.42			0.69 (0.28–1.85)	0.45			0.87 (0.48–1.69)	0.68		
History of IHD	2.76 (1.50–4.88)	<0.01	1.82 (0.95–3.34)	0.07	2.44 (0.86–6.14)	0.09			2.60 (1.37–4.72)	<0.01	1.43 (0.73–2.72)	0.29
History of CVD	2.04 (1.09–3.63)	0.03	1.23 (0.63–2.33)	0.53	5.92 (2.45–14.70)	<0.01	4.32 (1.77–10.87)	<0.01	2.01 (1.05–3.69)	0.04	1.32 (0.66–2.55)	0.42
Body mass index ($kg\,m^{-2}$)	0.93 (0.86–0.99)	0.03	0.98 (0.90–1.06)	0.64	0.90 (0.79–1.01)	0.08			0.85 (0.77–0.92)	<0.01	0.89 (0.79–0.99)	0.03
Serum albumin ($g\,dl^{-1}$)	0.75 (0.52–1.10)	0.14			0.76 (0.41–1.44)	0.39			0.63 (0.43–0.94)	0.03	0.69 (0.40–1.20)	0.18
C-reactive protein ($mg\,dl^{-1}$)	1.15 (0.79–1.49)	0.41			1.18 (0.57–1.76)	0.58			1.23 (0.85–1.58)	0.23		
Hemoglobin ($g\,dl^{-1}$)	0.86 (0.75–0.98)	0.02	0.96 (0.81–1.13)	0.60	0.82 (0.65–1.02)	0.07			0.79 (0.68–0.91)	<0.01	0.92 (0.77–1.10)	0.39
Serum phosphorus ($mg\,dl^{-1}$)	1.19 (0.88–1.59)	0.24			1.15 (0.70–1.82)	0.56			1.00 (0.71–1.37)	1.00		
Proteinuria (g per day)	1.06 (0.95–1.17)	0.29			0.89 (0.70–1.08)	0.25			0.91 (0.79–1.03)	0.15		
eGFR ($ml\,min^{-1}$ per $1.73\,m^2$)	0.98 (0.96–1.00)	0.03	0.98 (0.96–1.01)	0.16	0.98 (0.94–1.01)	0.26			0.98 (0.96–1.00)	0.12		
ABPI <0.9	3.71 (2.04–6.46)	<0.01	2.47 (1.24–4.81)	0.01	5.40 (2.11–13.11)	<0.01	3.15 (1.21–7.84)	0.02	4.34 (2.34–7.77)	<0.01	2.16 (1.06–4.30)	0.03

Abbreviations: ABPI, ankle-brachial blood pressure index; CI, confidence interval; CV, cardiovascular; CVD, cerebrovascular disease; eGFR, estimated glomerular filtration rate; HR, hazard ratio; IHD, ischemic heart disease.

For the measurement of ABPI, bilateral arm systolic blood pressure and bilateral ankle systolic blood pressure (posterior tibial artery) were taken with the subject in a supine position, and the measurement for each leg and ipsilateral arm was used to calculate ABPI. Patients who had ABPI <0.9 in either leg were categorized as having low ABPI.[23]

CV events were defined as follows: procedures of percutaneous coronary intervention or coronary artery bypass grafting for ischemic heart disease, congestive heart failure or cerebrovascular disease (such as brain infarction and hemorrhage); procedures of carotid endarterectomy for internal carotid artery stenosis; and procedures of percutaneous transcatheter angioplasty, lower-limb amputation or bypass surgery for peripheral artery disease; dissecting aneurysm of the thoracic and/or abdominal aorta, rupture of thoracic and/or abdominal aortic aneurysm, pulmonary embolism or sudden death.

Statistical analysis

Continuous data are expressed as either the mean ± s.d. or the median (interquartile range), depending on their distribution. Categorical data are expressed as numbers (with %). The significance of differences between ABPI <0.9 and ⩾0.9 was examined using the χ^2 test for categorical data, the Wilcoxon's rank-sum test for nonparametric data and the unpaired Student's *t*-test for parametric data. A logistic regression analysis was performed to elucidate the associations between low ABPI and traditional and nontraditional CV risk factors. Covariates associated with low ABPI in univariate analysis were analyzed by multivariate analysis to determine the independent risk factors for low ABPI. A Cox proportional hazards model was also applied to elucidate the traditional and nontraditional CV risk factors associated with CV events and both mortality and all-cause mortality. Covariates associated with these outcomes that were significant in univariate analysis were selected as risk factors in multivariate analysis. The odds and hazard ratios and the 95% confidence interval were calculated for each variable. Survival curves were estimated by the Kaplan–Meier method and evaluated by the log-rank test. Data were analyzed using the JMP10 statistics package (SAS Institute, Cary, NC, USA). A *P*-value below 0.05 indicated a significant difference.

RESULTS

The median age of the 320 patients (218 men and 102 women) in this study was 72 years (range, 30–92 years). The primary causes of renal disease were diabetic nephropathy (35%, 113 patients), hypertensive nephrosclerosis (33%, 104 patients), chronic glomerulonephritis (21%, 67 patients), other defined causes (8%, 27 patients) and unknown (3%, 9 patients). The median follow-up period was 30 months (range, 2–104 months). At the end of follow-up, 48 all-cause deaths were recorded. The causes of death were as follows: CV deaths in 20 patients, infection in 13, malignancy in 5, other defined causes in 8 and unknown in 2. In addition, CV events occurred in 56 patients.

The clinical characteristics of the patients with and without low ABPI are summarized in Table 1. Low ABPI was found in 42 patients (13%). The median age of patients with low ABPI was significantly higher than that of patients without low ABPI. The prevalences of smoking, diabetes mellitus and a history of ischemic heart disease or cerebrovascular disease were significantly higher in patients with low ABPI. Systolic, diastolic and pulse pressures were also significantly higher in patients with low ABPI, whereas body mass index was significantly lower. There were no significant differences in the values of serum albumin, C-reactive protein, hemoglobin, serum phosphorus, proteinuria and eGFR between the two groups.

Table 2 shows the results of logistic regression analysis of determinant factors for low ABPI. Univariate analysis demonstrated that age, smoking, pulse pressure, diabetes mellitus, history of ischemic heart disease and of cerebrovascular disease, and body mass index were significantly associated with low ABPI. Multivariate

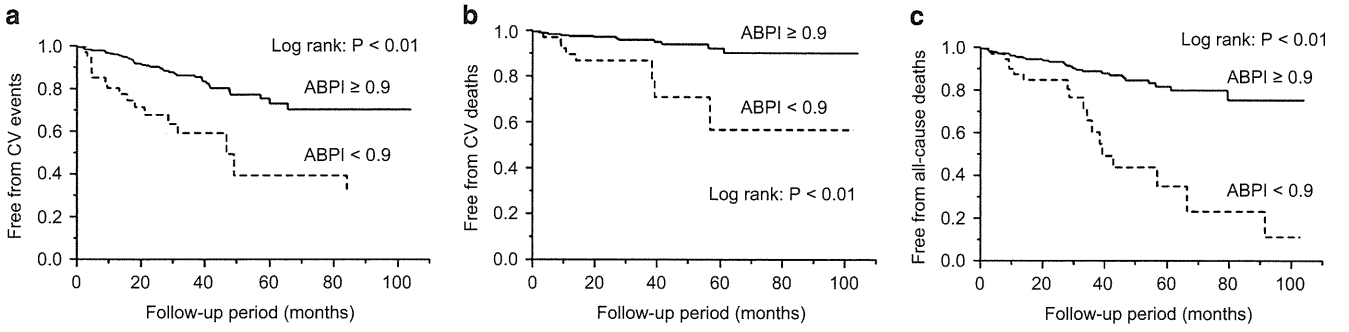

Figure 1 The associations between ankle-brachial blood pressure index (ABPI) and cardiovascular (CV) events, as well as with mortality from CV and all causes. Kaplan–Meier curves with log-rank tests of freedom from CV events (**a**) and deaths (**b**), and all-cause deaths (**c**) according to groups with ABPI <0.9 and $\geqslant 0.9$.

analysis showed that age, pulse pressure and history of cerebrovascular disease remained as independent determinant factors for low ABPI.

We analyzed the risk factors for CV events and both mortality and all-cause mortality using a Cox hazards analysis, as shown in Table 3. In multivariate analysis, age and low ABPI were independently associated with CV events. Age, a history of cerebrovascular disease and low ABPI were identified as independent risk factors for CV mortality. In addition, age, body mass index and low ABPI were independently associated with all-cause mortality. Figure 1 shows Kaplan–Meier curves of freedom from CV events (Figure 1a) and mortality (Figure 1b), as well as all-cause mortality (Figure 1c) in subjects with ABPI <0.9 and $\geqslant 0.9$. Patients with low ABPI experienced more CV events as well as both CV deaths and all-cause deaths.

DISCUSSION

Previous studies demonstrated the relationships between low ABPI and both CV events and mortality in predialysis patients. One report addressed the association of low ABPI with CV and all-cause mortality, but not with CV events.[21] In another report, clinical end points were defined as composite events of all-cause deaths or CV events.[20] On the other hand, the present study investigated separately the effects of low ABPI on CV events as well as on CV and all-cause mortality; it was demonstrated that low ABPI is independently associated with all these clinical end points. Additionally, although there have been limited data regarding the risk factors for having low ABPI in CKD patients,[24] our study simultaneously investigated those factors using a multivariate analysis to further explore the relationship between low ABPI and outcomes. In addition, given that very few studies regarding the association of low ABPI with outcomes have been conducted in Japanese predialysis patients,[20] the results of the present study may contribute to the clarification of the relationship between low ABPI and outcomes in this population.

In previous studies, old age, diabetes mellitus, a history of ischemic heart disease or cerebrovascular disease, increased pulse pressure, low serum albumin and low eGFR levels were identified as risk factors for atherosclerosis in patients with CKD.[25–27] In the present study, multivariate logistic regression analysis showed that pulse pressure, old age and a history of cerebrovascular disease were independent determinant factors for low ABPI. A previous study demonstrated that pulse pressure was a determinant factor for low ABPI in non-CKD patients.[28,29] On the other hand, patients with CKD exhibit vascular abnormalities, including arterial stiffness and early wave reflection, that contribute to elevated pulse pressure.[30,31] Pulse pressure was also positively and significantly associated with low ABPI in CKD patients.[32] In addition, higher levels of pulse pressure have been associated with carotid stenosis,[33] left ventricular hypertrophy,[34] myocardial infarction,[35] CV death[36] and congestive heart failure[37] in both normotensive and hypertensive populations. However, in the present study multivariate analysis showed that low ABPI, but not pulse pressure, was an independent risk factor for CV events and mortality. Taken together, these previous and present findings suggested that ABPI, rather than pulse pressure, was a useful method to predict CV events and mortality in CKD patients.

The present study also showed that low ABPI was an independent risk factor for all-cause mortality. No report thus far has explained clearly the association between low ABPI and all-cause mortality. Results of the National Health and Nutrition Examination Survey demonstrated that there are high prevalences of both traditional and nontraditional CV risk factors among persons with peripheral artery disease.[38] In addition, the Atherosclerosis Risk In Communities (ARIC) study reported that more patients with peripheral artery disease had hypertension, diabetes and a smoking habit.[39] In the present study, patients with low ABPI also had significantly higher prevalences of hypertension, diabetes, smoking, high pulse pressure and a history of ischemic heart and cerebrovascular disease compared with patients having normal ABPI. Previous studies demonstrated that hypertension,[40] diabetes[41] and smoking[42] are independent risk factors for all-cause mortality in general populations. Other studies also showed that pulse pressure was an independent risk factor for all-cause mortality in patients with CKD stages 4 and 5,[43] peritoneal dialysis[44] and hemodialysis.[45] All these clinical features might explain why patients with low ABPI had a higher mortality rate from CKD. However, risk factors such as pulse pressure and a history of cerebrovascular disease independently related to low ABPI were not associated with all-cause mortality in multivariate analysis. Therefore, the precise reason for the association between low ABPI and all-cause mortality has remained uncertain.

The present study has some limitations. First, the study subjects were in only one regional hospital; thus, the selection of patients was limited and the sample size was relatively small. Second, our study had an imbalanced gender ratio. Our study recruited the consecutive patients who were admitted to our hospital, and the number of male patients was two times the number of female patients. In general, male-predominant study groups tend to have a high risk of CV outcomes[46,47] and all-cause mortality. In patients on dialysis, previous studies with male predominance ($>60\%$) have addressed the association between male gender and mortality; one report showed a significant association between male gender and all-cause mortality

in a multivariate analysis,[15] but another study did not observe such a relationship.[16] In our present study, the univariate analysis showed that male gender was not associated with CV outcomes or all-cause mortality. Third, it has been reported that abnormally high ABPI ($\geqslant$1.3) predicts both CV mortality and all-cause mortality in CKD and hemodialysis patients.[18,19] However, we could not find an association between an abnormally high ABPI and CV events or death from CV or all causes, because only 18 patients had ABPI $\geqslant$1.3 in the present study. A larger cohort study will be needed to avoid study bias and to document more precisely the association between low ABPI and CV events, as well as mortality from CV and all causes.

In conclusion, the present study demonstrated that low ABPI was independently associated with CV events as well as with mortality from CV and all causes. This finding suggests that ABPI measurement could have a predictive value for CV disease outcome and patient survival.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Relationship Between Residual Renal Function and Serum Fibroblast Growth Factor 23 in Patients on Peritoneal Dialysis

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Abstract: Fibroblast growth factor 23 (FGF23) levels in dialysis patients are influenced by various factors, including phosphorus load. However, the clinical parameters that determine serum FGF23 levels in patients on peritoneal dialysis (PD) remain unclear. The aim of the present study was to examine the effects of clinical factors, on serum FGF23 levels, with an emphasis on residual renal function (RRF). This cross-sectional study included 56 outpatients undergoing PD therapy. Urine volume ≥100 mL/day or renal creatinine (Cr) clearance was used as a surrogate marker for RRF. Clinical characteristics were compared between patients with and without RRF. Linear regression analysis was conducted with serum FGF23 level as the dependent variable and renal Cr clearance as the main independent variable. The median and interquartile range of serum FGF23 levels were 5970 (1451–11 688) pg/mL. Patients with RRF showed higher urinary and total phosphate eliminations, and lower serum FGF23 and phosphate levels than patients without RRF. Multivariate linear regression analysis showed that the renal Cr clearance and serum phosphate and dialysis history were negatively associated with serum FGF23 levels, even after adjusting for potential confounders including peritoneal Cr clearance. Further, the predictabilities of serum FGF23 were comparable among renal Cr clearance, Kt/V for urea, and renal phosphate clearance. RRF determined by renal Cr clearance or residual urine volume is an independent negative determinant of serum FGF23 levels in PD patients. **Key Words:** Creatinine clearance, Fibroblast growth factor 23, Peritoneal dialysis, Residual renal function, Serum phosphate.

Fibroblast growth factor 23 (FGF23) is a phosphaturic hormone that is excreted by osteocytes in the bone in response to various stimuli, including phosphate (Pi), calcitriol, and parathyroid hormone (PTH) (1). FGF23 increases urinary Pi excretion by regulating the sodium-Pi co-transporter in the renal proximal tubules, providing a negative Pi balance in response to Pi loading (2). Several lines of evidence have revealed that serum FGF23 levels show a compensatory increase as early as chronic kidney disease (CKD) stage 2, and also play a pivotal role in maintaining serum Pi levels within the physiological range (3). In addition, a recent clinical study showed that the within-subject variability in serum FGF23 levels was lower than that of intact PTH and Pi, indicating the greater stability of serum FGF23 levels (4). Although there is little evidence regarding the clinical significance of measuring serum FGF23 levels in patients with CKD (5), serum FGF23 is nonetheless a promising tool for the assessment of Pi balance.

Clinical studies have shown that serum FGF23 levels are associated with high cardiovascular morbidity and mortality rates (6–8). However, whether serum FGF23 is simply a surrogate marker or a player that directly affects the cardiovascular system remains debatable. Faul et al. recently demonstrated

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direct effects of FGF23 on cardiac hypertrophy in an in vivo model (9), and a more recent clinical study showed that FGF23 inhibited calcitriol production in human monocytes (10). Given the possible role of FGF23, it is important to identify the clinical factors affecting serum FGF23 levels and to control these levels within the appropriate range. Because FGF23 production is stimulated in response to Pi loading, serum FGF23 is thought to be closely associated with Pi elimination, both renal and peritoneal, in peritoneal dialysis (PD) patients. However, few reports have identified the clinical factors associated with serum FGF23 levels in these patients (4,11).

The main aim of the present cross-sectional study was to determine the clinical factors associated with serum FGF23 levels in PD patients, with particular emphasis on the relationship between residual renal function (RRF) and serum FGF23 levels. We also investigated which RRF-related indicators (Kt/V for urea, renal creatinine [Cr] clearance, and renal Pi clearance) provided the most accurate predictions of serum FGF23 levels in PD patients.

MATERIALS AND METHODS

Study design and participants

This cross-sectional study included 60 outpatients who received PD therapy at Kyushu University Hospital between September 2010 and April 2012. Patients who did not undergo measurement of serum intact FGF23 levels were excluded ($N = 4$). The remaining 56 patients were enrolled in the analysis. The study protocol was approved by the local ethics committee of Kyushu University Hospital (No. 24–56) and was registered in the clinical trial registry (UMIN000009315). The study was performed according to the Ethics of Clinical Research (Declaration of Helsinki). Written informed consent was obtained from each patient prior to study participation.

Data collection

Peritoneal dialysis patients at Kyushu University Hospital received regular follow-up examinations once or twice per month. Blood samples were collected after overnight fasting, and 24-h urine and peritoneal dialysate were collected in the morning at each 6-month examination. Demographic and clinical data at the time of each regular visit were also recorded. Blood, urine and peritoneal dialysate samples were centrifuged at room temperature at 700 *g* for 10 min, and stored at –80°C until analysis, except in the case of standard biochemical analyses. Renal Cr clearance was used as the surrogate marker for RRF (12). Peritoneal membrane transport category was determined by a peritoneal equilibration test performed during the same month the data were gathered, according to the standard method. Estimated Pi intake was calculated based on the normalized protein catabolic rate: estimated Pi intake (mg/day) = normalized protein catabolic rate (g/kg per day) × body weight (kg) × 15 (mg/g) (12).

Measurement of biochemical mineral-related parameters

The levels of urea nitrogen, Cr, albumin, calcium (Ca) and Pi in the serum, urine, and peritoneal dialysate were measured using an auto-analyzer with standard procedures (Hitachi 911 Auto Analyzer, Hitachi, Japan). The serum-corrected Ca level was adjusted to the serum albumin level, according to Payne's formula, which is commonly used in patients with hypoalbuminemia (13). Serum FGF23 levels were measured using a two-site enzyme-linked immunosorbent assay (ELISA) kit (Kainos Laboratories, Tokyo, Japan) with an intra-assay coefficient of variation (CV) of <5% and inter-assay CV of <5% (14). Whole PTH was measured using an ELISA kit (Scantibodies Laboratory, Santee, CA, USA) with an intra-assay CV of 3.2–4.8% and inter-assay CV of 3.6–6.8% (15).

Calculations of Kt/V for urea, weekly Cr clearance and Pi clearance

Determinations of the Kt/V for urea and Cr clearance were performed as described previously (12). Renal and peritoneal Pi clearances (L/week per 1.73 m^2) were calculated as follows: urine (or dialysate) Pi (mmol/L)/plasma Pi (mmol/L) × 24-h urine (or dialysate) volume (L/day) × 7 (corrected for 1.73 m^2 body surface area). The total Pi clearance was equal to the sum of the renal and peritoneal Pi clearances. Normalized protein catabolic ratio was calculated from the Randerson equation divided by the patient's body weight.

Statistical analysis

All statistical analyses were conducted using the JMP 10.0 software program (SAS Institute, Tokyo, Japan). The results were expressed as the means (SD) for variables with a normal distribution, medians (interquartile range) for variables with a skewed distribution, and frequencies (percentage) for categorical variables, as appropriate. Serum FGF23 levels were transformed into logarithms to improve the skewed distribution. To clarify the association between RRF and serum FGF23 level, we determined the association using two types of RRF indicator in the following analyses: continuous variable

determined by renal Cr clearance, and categorical variable determined by urine volume. RRF as a categorical variable was defined as a urine volume ≥100 mL/day. To determine the dose-response association between renal Pi clearance and serum FGF23 level, we also divided the patients into tertiles according to renal Pi clearance. Differences between two groups were compared by unpaired *t*-tests for parametric variables, Wilcoxon's signed rank tests for skewed distributions, or χ^2 tests for categorical variables. Linear regression was also used to analyze the determinants of serum FGF23 level. In multivariate linear regression analysis, age, sex, underlying kidney disease, dialysis history, serum Ca, serum Pi, use of vitamin D receptor activator, use of Pi-binder and estimated Pi intake were used as covariates. The fit of the models for log serum FGF23 level was compared using the coefficient of determination (R^2), adjusted R^2, Akaike's information criterion, and Bayesian information criterion. A two-tailed *P*-value <0.05 was considered to be statistically significant in all analyses.

RESULTS

Patient characteristics

A total of 56 PD patients were included in the present analysis. A summary of their baseline characteristics is shown in Table 1. The mean age was 54 ± 14 years, 34 patients (61%) were male and 17 patients (30%) were diabetic. The median and interquartile range of dialysis history were 557 (182 1079) days, serum Cr was 893 ± 292 μmol/L, serum-corrected Ca was 2.40 ± 0.12 mmol/L, and the serum Pi level was 1.7 ± 0.4 mmol/L. Serum FGF23 was 5970 (1451, 11 688) pg/mL (range, 49–25 240 pg/mL). When patients were stratified by age or sex, there were no significant differences in serum FGF23 levels between the groups. Renal Cr clearance was 30.2 (7.5, 45.9) L/week, peritoneal Cr clearance was 32.1 (25.8, 41.1) L/week, and total Cr clearance was 65.1 (50.4, 76.1) L/week. Urine volume was 0.615 (0.263, 1.238) L/day. The median dialysate volume was 6 L/day, and 46% of patients used automated PD. Pi-binders were used by 84% of patients, and vitamin D receptor activator by 46%.

TABLE 1. *Patient characteristics* (N = 56)

Variable	Value
Demographics and basic data	
Age, years	54 ± 14
Sex, male	34 (61)
Underlying kidney disease	
diabetes mellitus	17 (30)
Dialysis history, days	557 (183, 1078)
Body mass index, kg/m^2	22.6 ± 3.1
Serum biochemistry	
Albumin, g/L	34 ± 4.1
Urea nitrogen, mmol/L	10.5 ± 2.3
Cr, μmol/L	892 ± 292
Corrected calcium, mmol/L	2.40 ± 0.12
Pi, mmol/L	1.7 ± 0.4
Whole PTH, nmol/L	7.9 (4.8, 21.3)
FGF23, pg/mL	5970 (1451, 11688)
Parameters related to dialysis and RRF	
Total Kt/V for urea	1.66 (1.45, 1.90)
Renal Kt/V for urea	0.47 (0.16, 0.73)
Total Cr clearance, L/week	65.1 (50.4, 76.1)
Renal Cr clearance, L/week	30.2 (7.5, 45.9)
Total Pi clearance, L/week	66.7 (51.8, 86.7)
Renal Pi clearance, L/week	23.4 (5.7, 38.7)
Urine volume, L/day	0.62 (0.26, 1.24)
Dialysis-related parameters	
Dialysate volume, L/day	6.0 (4.8, 8.5)
Use of automated peritoneal dialysis	26 (46)
Use of icodextrin	19 (34)
Use of 2.5% glucose dialysate	12 (21)
Pharmacotherapy	
Use of Pi-binder	47 (84)
Use of VDRA	26 (46)

Data are given as mean ± SD, median (25th percentile, 75th percentile), or number (percentage). Cr; creatinine, Kt/V; urea clearance, Pi; phosphate, PTH; parathyroid hormone, RRF; residual renal function, SD; standard deviation, VDRA; vitamin D receptor activator.

Effect of RRF on serum FGF23 level

The clinical parameters stratified with or without RRF are shown in Table 2. Serum FGF23 levels in patients with RRF were significantly lower than those in patients without RRF (Fig. 1a). Further, renal and total Pi excretion were significantly higher in patients with RRF than in patients without RRF (Fig. 1b,c), though peritoneal Pi clearance was significantly lower in patients with RRF than in those without RRF (Fig. 1d). In contrast, the estimated daily Pi intake was comparable between the two groups (Fig. 1e), indicating that serum FGF increased in response to decreased Pi elimination from the kidney.

Simple linear regression analysis of serum FGF23 levels and clinical factors

To identify potential confounders affecting the association between serum FGF23 levels and RRF, we performed linear regression analyses between serum FGF23 and other clinical parameters. Univariate analysis revealed that log serum FGF23 level was significantly associated with dialysis history (standardized $\beta = 0.46$, $R^2 = 0.212$, $P < 0.001$), serum albumin (standardized $\beta = 0.43$, $R^2 = 0.185$, $P = 0.001$), blood urea nitrogen (standardized $\beta = 0.35$, $R^2 = 0.122$, $P = 0.009$), serum Cr (standardized $\beta = 0.54$,

TABLE 2. *Clinical characteristics according to presence or absence of residual renal function (RRF)*

Variable	Without RRF ($N = 16$)	With RRF ($N = 40$)	P-value
Age, years	50.6 ± 13.7	55.4 ± 13.4	0.558
Sex, male	8 (50)	27 (68)	0.361
Dialysis history, days	828 (356, 1282)	427 (20.5, 923)	**0.047**
Presence of diabetes mellitus	6 (38)	11 (28)	0.331
Serum Cr, μmol/L	1096 ± 203	804 ± 283	**0.002**
Serum Pi, mmol/L	1.94 ± 0.48	1.61 ± 0.35	**<0.001**
Serum corrected Ca, mmol/L	2.48 ± 0.15	2.40 ± 0.13	0.155
Serum whole PTH, pmol/L	5.8 (2.7, 23.0)	9.0 (5.8, 21.3)	0.126
Use of automated peritoneal dialysis	12 (75)	17 (43)	0.057
Use of icodextrin	14 (88)	22 (55)	**<0.001**
Use of 2.5% glucose dialysate	9 (56)	3 (8)	**<0.001**
Urine volume, L/day	0.05 (0, 0.08)	0.90 (0.59, 1.31)	**<0.001**
Dialysate volume, L/day	8.4 (6.4, 10.4)	6.0 (4.5, 8.0)	**0.002**
D/P Cr 4 h	0.76 (0.67, 0.90)	0.68 (0.58, 0.78)	**0.021**
Use of a Pi-binder	15 (94)	32 (80)	0.310

Data are expressed as mean ± standard deviation (SD) or median (25th percentile, 75th percentile) for continuous variables, and as number (percentage) for categorical variables. Patients with RRF were defined as having urine volume ≥100 mL/day. Ca, calcium; Cr, creatinine; Pi, phosphate; PTH, parathyroid hormone; RRF, residual renal function. A P-value <0.05 was considered to be statistically significant.

$R^2 = 0.289$, $P < 0.001$), serum Ca (standardized $\beta = 0.30$, $R2 = 0.087$, $P = 0.028$), serum Pi (standardized $\beta = 0.52$, $R^2 = 0.271$, $P < 0.001$), dialysate volume (standardized $\beta = 0.42$, $R^2 = 0.175$, $P = 0.002$), renal Cr clearance (standardized $\beta = -0.50$, $R^2 = 0.271$, $P < 0.001$), peritoneal Cr clearance (standardized $\beta = 0.30$, $R^2 = 0.089$, $P = 0.027$), use of Pi-binder ($P = 0.011$) and use of automated peritoneal dialysis

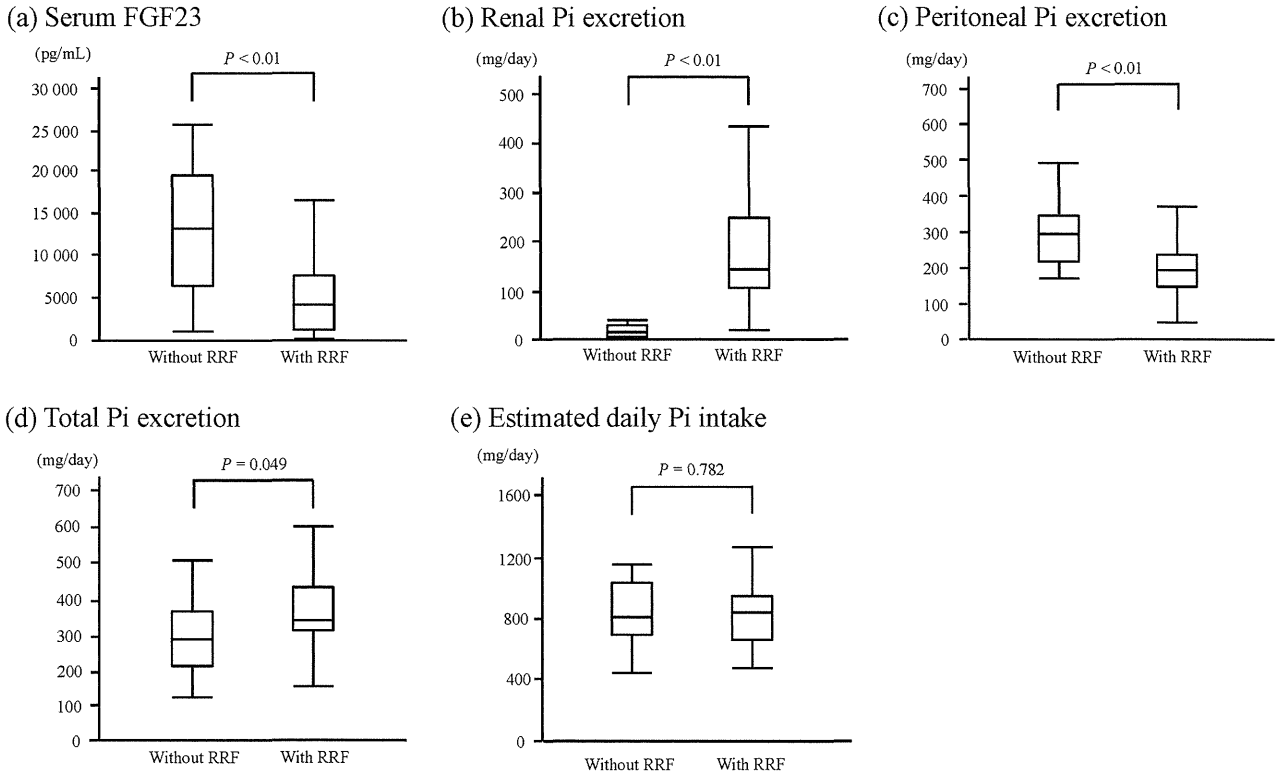

FIG. 1. Effects of residual renal function (RRF) on phosphate (Pi) homeostasis. Serum fibroblast growth factor 23 (FGF23) level (a), renal Pi excretion (b), peritoneal Pi excretion (c), total Pi excretion (d), and estimated daily Pi intake (e) are shown. Patients with RRF were defined as having urine volume ≥100 mL/day. Boxes represent the interquartile range, with the upper and lower edges representing the 75th and 25th percentiles, respectively. The central horizontal lines represent the median levels. The vertical whiskers above and below the boxes represent the ranges of 5–95% percentiles. Patients with RRF were defined as having urine volume ≥100 mL/day. Cr; creatinine, A P-value <0.05 was considered to be statistically significant.

TABLE 3. *Multivariate linear regression model for log serum fibroblast growth factor 23 (FGF23) level* (N = 56)

Variable	β-coefficient	95%CI	*P*-value
Age, per 10 years	−0.032	−0.236, 0.210	0.792
Sex (male)	−0.232	−0.615, 0.152	0.229
Presence of diabetes mellitus	0.358	−0.021, 0.737	0.064
Dialysis history, per 100 days	0.072	0.006, 0.138	**0.034**
Serum Ca, per 1 mmol/L	0.033	−0.129, 0.195	0.683
Serum Pi, per 1 mmol/L	0.149	0.059, 0.240	**0.002**
Use of VDRA	−0.269	−0.607, 0.068	0.114
Peritoneal Cr clearance, per 10 L/week	0.018	−0.248, 0.285	0.892
Renal Cr clearance, per 10 L/week	−0.191	−0.352, −0.035	**0.018**
Estimated dietary Pi intake, per 100 mg/day	0.065	−0.162, 0.292	0.564

Ca, calcium; CI, confidence interval; Cr, creatinine; Pi, phosphate; VDRA, vitamin D receptor activator. A *P*-value <0.05 was considered to be statistically significant.

($P = 0.020$). However, log serum FGF23 level was not significantly associated with peritoneal transporter category ($P = 0.396$) or D/P Cr 4 h ($P = 0.482$).

Multivariate analysis to determine the effect of renal Cr clearance on log serum FGF23

We performed multivariate linear regression analysis to determine if there was an independent association between renal Cr clearance and log serum FGF23 level. Based on the results of univariate analysis and previous reports, age, sex, dialysis history, presence of diabetes mellitus, serum Ca and Pi levels, use of Pi-binders, use of vitamin D receptor activators, and estimated daily Pi intake were included as covariates. Renal Cr clearance showed a significant ($P < 0.05$) and inverse dose-response relationship with log serum FGF23, while peritoneal Pi clearance did not (Table 3).

Comparisons of different Pi-elimination-related indicators for estimating log serum FGF23

To obtain a better model for predicting log serum FGF23 levels, we compared the goodness-of-fit for each model using Kt/V for urea, renal Cr clearance, and renal Pi clearance as RRF indicators related to renal Pi elimination. We confirmed the association among renal Pi clearance, renal Kt/V for urea, and renal Cr clearance. Simple linear regression analysis revealed that renal Pi clearance, a direct indicator of the renal Pi elimination, was significantly associated with Kt/V for urea ($R^2 = 0.89$, $P < 0.001$) and renal Cr clearance ($R^2 = 0.91$, $P < 0.001$).

To determine which indicator was most suitable for predicting log serum intact FGF23 level, we calculated R^2, adjusted R^2, Akaike's information criterion, and Bayesian information criterion, and compared the fits of the multivariate linear regression models for log serum FGF23 level. This multiple linear regression model used the parameters shown in Table 3 as covariates. According to the three indices for goodness-of-fit, the predictabilities of renal Kt/V for urea and renal Cr clearance were similar to that of renal Pi clearance, indicating that both renal Kt/V for urea and renal Cr clearance were as useful as renal Pi clearance for estimating serum FGF23 levels in clinical settings (Table 4).

DISCUSSION

The present study demonstrated that renal Cr clearance was negatively associated with log serum FGF23 level, even after adjusting for other confounding

TABLE 4. *Comparison of models for estimating log serum fibroblast growth factor 23 (FGF23) level using three phosphate (Pi)-elimination-related indicators*

Pi-elimination-related indicator	R^2	Adjusted R^2	AIC	BIC	*P*-value for model
Renal Kt/V for urea	0.56	0.45	184	200	**<0.001**
Renal Cr clearance, L/week	0.57	0.46	183	199	**<0.001**
Renal Pi clearance, L/week	0.57	0.47	182	198	**<0.001**

Multivariate linear regression analysis was used to estimate serum FGF23 levels. Each model included one Pi-elimination-related indicator (Kt/V for urea, renal Cr clearance, or renal Pi clearance); age, sex, with or without diabetes, dialysis history, serum Ca and Pi, peritoneal Pi clearance, use of vitamin D receptor activator, and estimated Pi intake were used as covariates. AIC, Akaike's information criterion; BIC, Bayesian information criterion; Ca, calcium; Cr, creatinine; R^2, coefficient of determination. A *P* value <0.05 was considered to be statistically significant.

factors, including age, sex, diabetes, dialysis history, serum Ca and Pi, estimated dietary Pi intake, use of vitamin D receptor activator, and peritoneal Cr clearance. Furthermore, the accuracies of renal Kt/V for urea, renal Cr clearance, and renal Pi clearance for predicting serum intact FGF23 levels in PD patients were similar.

Recent clinical studies showed that serum FGF23 levels were an independent risk factor for end-stage renal disease, vascular calcification, cardiovascular events and overall and cardiovascular mortality in CKD patients (6–8,16). However, whether FGF23 is only a marker for cardiovascular events, or acts as a player in the pathogenesis of cardiovascular diseases remains unclear. A recent experimental study showed that FGF23 itself induced cardiac hypertrophy in a mouse model (9), suggesting that controlling serum FGF23 levels could have a therapeutic effect in maintaining appropriate mineral and bone statuses. In this context, the current study revealed that several clinical factors were associated with serum FGF23 levels, which could help improve the management of these levels in PD patients. However, vitamin D receptor activators, which are known to have beneficial effects on mortality based on observational studies (17), increase serum FGF23 levels. There is currently no satisfactory explanation for the conflicting results regarding the relationship between FGF23 and vitamin D. Further studies are therefore needed to determine if serum FGF23 levels can be used as a therapeutic target, and if actively maintaining lower serum FGF23 levels could decrease cardiovascular and non-cardiovascular mortalities in CKD patients.

Mounting evidence has shown that RRF has beneficial effects on the control of volume, solute clearance and mortality in PD patients (18,19). Patients with RRF also have advantages in the management of mineral and bone metabolisms (20). Epidemiological studies have revealed that patients with RRF have a greater renal Pi excretion and lower serum Pi level, enabling appropriate Pi homeostasis (20,21). Because renal Pi clearance is closely associated with RRF and renal Cr clearance (22), the present study highlighted the clinical importance of preserving RRF, determined by renal Cr clearance, independent of serum Pi level, in the management of serum FGF23 levels among patients undergoing PD.

Serum Pi level and dialysis history were independently associated with serum FGF23 levels in the present study, in accordance with a recent observational study in PD patients (4). Although serum FGF23 levels increased in response to Pi load (23), the precise mechanisms underlying the Pi-load-induced activation of FGF23 production in osteocytes remains unclear. Several factors, including vitamin D derivatives, Ca, estrogen and PTH have been shown to stimulate FGF23 production by osteoblasts and osteocytes directly (24–26). However, direct exposure of osteocytes to Pi did not stimulate FGF23 production in an in vitro study (27). In addition, although the clinical significance of the association between dialysis history and serum FGF23 levels remains unknown, it is reasonable to conclude that a long dialysis history may indicate a greater accumulation of Pi in the body. Hence, further studies are needed to determine the clinical significance of the association between higher serum Pi levels and higher serum FGF23 levels.

It is important to determine which indicators of RRF predict serum FGF23 levels most accurately in PD patients. Sedlacek et al. reported that renal Pi clearance was more closely associated with renal Cr clearance than with renal Kt/V for urea (22). This indicates that the behavior of Pi in terms of elimination from the kidneys is more similar to that of Cr than of urea nitrogen (28). In the present case, all three indicators were similarly effective based on several statistical indices for the goodness-of-fit for the model. In addition, the present study also confirmed that renal Cr clearance could be used as a substitute measurement for renal Pi clearance and as a surrogate marker for serum FGF23 levels. Considering that renal Cr clearance and Kt/V for urea are more routinely evaluated than renal Pi clearance, measuring these parameters represents a valid option for assessing serum FGF23 levels in PD patients.

The peritoneal transporter category (or D/P Cr 4 h) was not associated with serum FGF23 levels in the present study, and previous studies have shown conflicting results regarding the effect of peritoneal transporter category on Pi elimination (29,30). Several clinical studies showed that peritoneal transporter category was associated with Pi clearance; patients with a high D/P creatinine value had greater Pi elimination through the dialysate (31). However, no previous studies have examined the effect of peritoneal transporter type on serum FGF23 levels (4), highlighting the importance of the present study. Theoretically, because peritoneal transporter category is highly associated with peritoneal and total Pi elimination (32), patients with a high D/P Cr 4 h value should have higher transperitoneal Pi elimination. Moreover, transperitoneal Pi elimination also depends on the total volume of peritoneal fluid, and the statistical analysis of the relationship between serum FGF23 and transporter category should thus be adjusted by the peritoneal dialysis volume. Hence, further large-scale studies are required to allow the

accurate determination of the effect of peritoneal transporter category on serum FGF23 levels in PD patients.

This study had some limitations. First, the cross-sectional study design limits the interpretation of causality between renal Cr clearance and serum FGF23 levels. In a physiological state, serum FGF23 increases urinary Pi excretion, working to lower both the total Pi burden and serum FGF23 level. However, it is reasonable to conclude that serum FGF23 levels increase in response to reduced urinary Pi excretion, because serum FGF23 increases as CKD stage progresses in CKD patients, justifying the negative association between renal Cr clearance and serum FGF23 levels. Second, we did not gather data on dietary Pi intake by medical interviews, but instead used estimated Pi intake to take account of the effect of dietary Pi intake.

CONCLUSION

Renal creatinine clearance is negatively associated with serum FGF23 levels in peritoneal dialysis patients, even after adjusting for potential confounders. Because FGF23 has recently been shown to have deleterious effects on cardiovascular and non-cardiovascular systems, controlling serum FGF23 levels may represent a potential therapeutic strategy for peritoneal dialysis patients in the near future. In this regard, maintenance of renal phosphate elimination by preserving residual renal function can retard the progressive increase in the serum FGF23 levels, thus helping to prevent cardiovascular events in peritoneal dialysis patients.

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Effects of Sleepiness on Survival in Japanese Hemodialysis Patients: J-DOPPS Study

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Key Words
Hemodialysis · Sleep disorder · Sleepiness · Survival

Abstract
Sleep disorder and poor sleep quality are common in chronic hemodialysis (HD) patients. They have been claimed as a cause of morbidity and mortality. The relationship between the degree of sleepiness and survival has not been studied. We studied the degree of sleepiness in 1,252 adult HD patients (age ≥20 years) recruited into the Dialysis Outcomes Practice Pattern Study in Japan (J-DOPPS III), using the Japanese version of the Epworth Sleepiness Scale (JESS) questionnaire. Demographic data were presented for three subgroups: low, intermediate, and high JESS score. Cox proportional hazard regression analysis was performed to estimate the independent effect of several variables on survival. The hazard ratio for mortality was 2.312 (95% CI 1.267–4.220; p = 0.006) for those with a high JESS score (vs. those with a low JESS score) after adjusting for age, vintage (length of time on HD), sex, diabetes, body mass index, cardiovascular disease, HD treatment regimen (time, frequency, and single-pool Kt/V), laboratory data (serum albumin, creatinine, and total cholesterol), and medication (antihypertensive drugs, erythropoietin, vitamin D, and phosphate binders). Patients ≥70 years of age with comorbid conditions (congestive heart failure, stroke, and diabetes) showed a significantly higher JESS score (≥16). The JESS score did not show interaction by age. Results showed that the degree of sleepiness is related to survival in Japanese HD patients, particularly in elderly patients.

Introduction

The prevalence of sleep disorder increases with declining kidney function and is very common among chronic hemodialysis (HD) patients [1–3]. Survival of HD patients is poor compared with the non-HD population [4]. The degree of sleep disorder or sleep quality (SQ) predicts the quality of life and mortality in HD patients [5]. The Dialysis Outcomes and Practice Patterns Study (DOPPS) is a prospective, observational study designed to examine the relationship between HD practices and patient outcomes and, as such, provides an opportunity to study mul-

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tiple topics in large numbers of HD patients around the world including Japan [6, 7]. Among the DOPPS countries, the mean SQ score was highest and the death risk was lowest in Japan [8]. Data on SQ were collected from 11,351 patients in 308 dialysis units in 7 countries in the DOPPS between 1996 and 2001 through a patient self-reported SQ scale, ranging from 0 (worst) to 10 (best). The main variable of interest was patient self-reported SQ as derived from the Kidney Disease Quality of Life Short Form (KDQOL-SF-36™). Patients were asked, 'On a scale of 0–10 (where 0 represents 'very bad' and 10 represents 'very good'), how would you rate the quality of your sleep overall?' However, the questionnaire-based surveys have several problems as they have not been formally validated for Japanese, and in particular dialysis, patients. Dialysis patients have multiple confounding variables such as volume expansion, malnutrition, and chronic inflammation.

We used the Japanese version of the Epworth Sleepiness Scale (JESS) questionnaire among the participants of the Japanese DOPPS I (1996–2001). It was developed to apply to Japanese subjects, as the original version was not appropriate for Japan [9]. The ESS comprises questions about subjective sleepiness in eight circumstances [10, 11]. In the present study, we examined the association between the JESS score and survival rate. Results were adjusted for baseline characteristics and possible confounders related to the JESS score. In addition, we investigated the variables related to sleepiness in order to gain a clinical strategy to improve SQ [8] and life expectancy in chronic HD patients. We used JESS as a proxy of SQ.

Methods

Data Source and Study Patients

The DOPPS is a prospective cohort study on HD practices and patient outcomes. The study sampling plan and methods have been described previously [6, 7]. Data for the present study were from the Japanese DOPPS III (J-DOPPS III; 2005–2008; n = 2,293). The JESS questionnaire was used only in Japan for J-DOPPS III participants. The minimal number of registered patients for DOPPS was 20 in each unit (61 units). Among these units, the number of patients sampled proportional to the unit size ranged from 2 to 36 (5.1–76.9%). We excluded patients who did not respond (n = 954) or provided inadequate responses (n = 87). Therefore, we studied 1,252 adult HD patients (57.6% of total HD patients) aged ≥20 years who responded to the JESS [8] questionnaire in 2006–2007. Two questions were replaced from the original ESS. Each question was graded from 0 to 3. Based on the JESS scores, which consists of eight questions, patients were categorized into three subgroups: low (JESS score 0–10), intermediate (JESS score 11–15), and high (JESS score 16–24). High JESS scores denote high daytime sleepiness.

Outcomes and Exposures

The primary outcome was mortality. In Cox regression models, the duration of observation was from the date the JESS questionnaire was applied until the earliest of the following events: death, kidney transplantation, transfer to another dialysis unit, or the end of the observation period of J-DOPPS III.

Statistical Analysis

Demographic data were presented as mean (standard deviation, median, 25th percentile, 75th percentile, or percentage) for three subgroups (low, intermediate, and high JESS score). We defined comorbidities for cardiovascular disease (CVD), a composite of coronary artery disease, cerebrovascular disease, peripheral vascular disease, and other CVD. The Kruskal-Wallis test and analyses of covariance were performed to compare the significance of continuous variables, and the χ^2 test or Fisher's exact test were used for categorical variables. We evaluated the association of the JESS-based subgroups with the cumulative incidence of all-cause mortality during follow-up using the Kaplan-Meier method. Differences between the groups were assessed with the log-rank test. Cox proportional hazards models were used to estimate the hazard ratios (HRs) for mortality associated with the JESS subgroups. Laboratory and clinical variables were included as baseline covariates. The continuous variables such as body mass index (BMI), single-pool Kt/V, serum albumin, total cholesterol, creatinine, hemoglobin, phosphate, adjusted calcium, and intact parathyroid hormone were categorized before inclusion in the models. The effect modification of the JESS subgroups and age group was evaluated by adding an interaction term (JESS subgroups × age group) to the multivariate analysis model. A p value of <0.10 was considered as statistical significance level for interactions.

Demographics and baseline clinical characteristics associated with JESS scores were assessed in multivariate logistic regression analyses. All significant univariate variables ($p < 0.20$) and clinically established factors were entered into a multivariate logistic model predicting the JESS subgroup. The fitting of multivariate logistic regression models was evaluated by the Hosmer-Lemeshow test, C statistics, and Akaike's information criterion. Missing values were imputed multiply using the chained equation method by PROC MI [12]. Results from 20 such imputed datasets were combined for the final analysis using Rubin's formula [13] which was implemented in PROC MIANALYZE in SAS. The proportion of missing data was below 10% for all imputed covariates, with the exception of Kt/V (16%), total cholesterol (18%), and intact parathyroid hormone (50%). A p value of <0.05 was considered statistically significant in survival analyses. Data were analyzed with SAS statistical software (version 9.2, SAS Institute Inc., Cary, N.C., USA).

Results

Study Subjects

Baseline characteristics are summarized in table 1. A high JESS score was observed among those with a higher mean age, a high prevalence of diabetes mellitus, and a shorter HD vintage (length of time on HD). Laboratory

Table 1. Patient background

Variables	Low score group (0–10)	Intermediate score group (11–15)	High score group (16–24)	p value
Patients, n (% of total)	971 (77.6%)	191 (15.3%)	90 (7.2%)	
Age, years	60.9±11.7	62.3±12.5	67.3±11.9	<0.001†
Vintage, years	5.9 [2.0, 11.7]	4.8 [1.1, 10.4]	3.7 [1.3, 8.4]	0.005§
Male gender	581 (59.8%)	133 (69.6%)	55 (61.1%)	0.039#
Primary disease (DM), n (%)	253 (26.1%)	58 (30.4%)	37 (41.1%)	0.007#
Comorbidities, n (%)				
Coronary artery disease	217 (22.4%)	36 (18.9%)	24 (26.7%)	0.317#
Cancer (other than skin)	76 (7.8%)	13 (6.8%)	10 (11.1%)	0.450#
Other CVD	224 (23.1%)	44 (23.0%)	26 (28.9%)	0.454#
Cerebrovascular disease	86 (8.9%)	14 (7.3%)	17 (18.9%)	0.004#
Congestive heart failure	153 (15.8%)	40 (20.9%)	23 (25.6%)	0.021#
Diabetes mellitus	286 (29.5%)	66 (34.6%)	40 (44.4%)	0.008#
Gastrointestinal bleeding	23 (2.4%)	5 (2.6%)	3 (3.3%)	0.845#
HIV	5 (0.5%)	1 (0.5%)	0 (0%)	0.792‡
Hypertension	677 (69.7%)	136 (71.2%)	69 (76.7%)	0.373#
Lung disease	19 (2.0%)	3 (1.6%)	4 (4.4%)	0.248‡
Neurologic disorder	86 (8.9%)	28 (14.7%)	15 (16.7%)	0.007#
Psychological disorder	25 (2.6%)	7 (3.7%)	4 (4.4%)	0.464#
Peripheral vascular disease	93 (9.6%)	26 (13.6%)	14 (15.6%)	0.074#
Recurrent cellulitis	19 (2.0%)	1 (0.5%)	3 (3.3%)	0.221‡
BMI	20.7 [18.9, 23.1]	20.6 [18.6, 22.6]	21.1 [18.7, 23.1]	0.227§
Single pool Kt/V	1.37±0.26	1.31±0.26	1.30±0.28	0.003†
Dialysis frequency (3 times/week), n (%)	934 (96.2%)	176 (92.2%)	85 (94.4%)	0.005‡
Dialysis time, min/session	238.7±30.8	236.0±28.9	230.9±29.2	0.163†
Albumin, g/dl	3.82±0.39	3.75±0.40	3.70±0.48	0.010†
Total cholesterol, mg/dl	153.7±35.8	149.4±32.0	144.3±32.4	0.389†
Creatinine, mg/dl	11.28±2.77	11.01±3.01	9.62±2.96	<0.001†
Hb, g/dl	10.48±1.24	10.40±1.20	10.19±1.39	0.105†
Adjusted calcium, mg/dl	9.35±0.86	9.32±0.83	9.52±0.90	0.289†
Serum phosphorus, mg/dl	5.44±1.33	5.35±1.38	5.15±1.42	0.138†
Intact parathyroid hormone, pg/ml	173.0 [94.0, 283.0]	155.0 [78.0, 317.0]	157.5 [63.5, 226.5]	0.255§
Erythropoiesis-stimulating agent, n (%)	792 (81.6%)	165 (86.4%)	82 (91.1%)	0.028#
Antihypertensive drug, n (%)				0.467#
Calcium channel blocker	202 (20.8%)	35 (18.3%)	20 (22.2%)	
ACEI or ARB	104 (10.7%)	15 (7.9%)	7 (7.8%)	
Combined	227 (23.4%)	54 (28.3%)	27 (30.0%)	
Vitamin D, n (%)				0.039#
Oral	384 (39.6%)	65 (34.0%)	26 (28.9%)	
Injection	204 (21.0%)	32 (16.8%)	19 (21.1%)	
Phosphate binder, n (%)				0.030#
Calcium based	531 (54.7%)	100 (52.4%)	46 (51.1%)	
Non-calcium based	149 (15.4%)	28 (14.7%)	13 (14.4%)	
Combined	149 (15.4%)	31 (16.2%)	6 (6.7%)	
Hypnotic agent, n (%)	317 (32.7%)	58 (30.4%)	34 (37.8%)	0.466#

Values represent mean ± SD or median [Q1, Q3], unless otherwise specified. # χ^2 test; ‡ Fisher's exact test; † ANOVA; § Kruskal-Wallis test. DM = Diabetes mellitus.