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Association between proteinuria and mineral metabolism disorders in chronic kidney disease: the Japan chronic kidney disease database extension (J-CKD-DB-Ex)

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Chronic kidney disease-mineral and bone disorder (CKD-MBD) are recognized as a systemic disease affecting the prognosis of patients with CKD. Proper management of CKD-MBD is important to improve the prognosis of patients with CKD. Although proteinuria is recognized as a poor prognostic factor in these patients, few reports have examined its association with CKD-MBD. We examined the association between proteinuria and CKD-MBD using data from the Japan Chronic Kidney Disease Database Extension (J-CKD-DB-Ex). Among the patients registered in the J-CKD-DB-Ex, 30,977 with CKD stages G2–G5 who had serum creatinine, albumin, calcium, and phosphate concentrations measured at least once and urinalysis performed were included. The patients were divided into four groups (negative, 1+, 2+, and 3+) according to the degree of proteinuria. The association between proteinuria and CKD-MBD was examined by a logistic regression analysis. In a model adjusted for age, sex, diabetes, and the estimated glomerular filtration rate (eGFR), the odds ratio of the 3+ group compared with the negative group significantly increased to 2.67 (95% confidence interval, 2.29–3.13) for hyperphosphatemia, 2.68 (1.94–3.71) for hypocalcemia, and 1.56 (1.24–1.98) for hypomagnesemia. Proteinuria is associated with hyperphosphatemia, hypocalcemia, and hypomagnesemia in patients with CKD independently of eGFR.

Keywords CKD-MBD, Proteinuria, Hyperphosphatemia, Hypocalcemia, Hypomagnesemia, J-CKD-DB-Ex

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Disturbances in mineral and bone metabolism are widespread in chronic kidney disease (CKD). These disturbances are an important cause of morbidity, extraskeletal calcification, and are associated with increased cardiovascular mortality^{1–3}. Disturbances in mineral and bone metabolism have traditionally been referred to as renal osteodystrophy and classified based on a bone biopsy. Subsequently, mineral metabolism disorders associated with CKD became recognized as systemic diseases, and the concept of CKD-mineral and bone disorder (CKD-MBD) was proposed to describe broader clinical manifestations⁴. This concept has been adopted internationally and has contributed to the global development of evidence-based clinical practice guidelines. These guidelines recommend target ranges for managing serum concentrations of calcium, phosphate, and parathyroid hormone (PTH), which are the main parameters in CKD-MBD. Appropriate management of these disorders is important to improve the prognosis of patients with CKD⁵. Recently, the importance of magnesium in managing CKD-MBD has also been recognized⁶.

Proteinuria is a highly sensitive marker of various kidney diseases and vascular endothelial disorders, and is recognized as a risk factor for CKD progression and mortality in patients with CKD^{7,8}. CKD-MBD and proteinuria are important factors associated with a poor prognosis in patients with CKD, but few studies have examined their association. This study was performed to clarify the relationship between CKD-MBD (especially dysmetabolism of calcium, phosphate, and magnesium) and proteinuria in patients with CKD using data from the Japan Chronic Kidney Disease Database Extension (J-CKD-DB-Ex).

Materials and methods

Study design and population

The J-CKD-DB-Ex is a longitudinal extension of the existing Japan Chronic Kidney Disease Database (J-CKD-DB) (UMIN trial number, UMIN000026272). The J-CKD-DB-Ex is a multicenter, automatically extracted comprehensive database of patients with CKD from 21 Japanese university hospitals^{9,10}. In this study, we used data on patients with CKD from four university hospitals from which we collected prior data. The J-CKD-DB was initiated in December 2014, and this database contains information on all inpatient and outpatient encounters, prescriptions, diagnostic codes, and laboratory measurements. J-CKD-DB inclusion criteria were as follows: (1) age ≥ 18 years; (2) proteinuria $\geq 1+$ (dipstick test), and/or estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m². The facilities participating in the J-CKD-DB were required to have electronic health record systems that incorporated Standardized Structured Medical Information eXchange 2 (SS-MIX2) storage and a structured data entry function that could transfer data to the SS-MIX2 storage system¹¹. Patient records were anonymized and informed consent was obtained in an opt-out method on the website of each participating university hospital in accordance with the Ethical Guidelines for Medical and Health Research Involving Human Subjects in Japan. The J-CKD-DB-Ex study was conducted in accordance with the principles of the Declaration of Helsinki under the supervision of the Ethics Committee of Kawasaki Medical School. The J-CKD-DB-Ex was supported by grants from the Japan Agency for Medical Research and Development, Practical Research Project for Life-Style related Diseases including Cardiovascular Diseases and Diabetes Mellitus.

The study population comprised 34,628 patients from the J-CKD-DB-Ex registry with CKD stages G2–G5 who had serum creatinine, albumin, calcium, and phosphate concentrations measured at least once and urinalysis. Of the 34,628 patients, 1,012 were excluded from our study because of taking phosphate binders and/or vitamin D receptor activators. In addition, to minimize the impact of acute kidney injury (AKI) on the results, 2,639 patients with onset of AKI were excluded. The criteria for AKI were an increase in serum creatinine concentration of 0.3 mg/dL or more within 48 h or an increase of 1.5 times the basal value, based on the KDIGO classification¹². We enrolled the remaining 30,977 patients in this cross-sectional study. The flowchart of patients enrolled in this study is shown in Fig. 1. When extracting the data used in this study from the database, patients with simultaneous measurements of four laboratory parameters (i.e., serum creatinine, albumin, calcium, and phosphate) were selected, and the lowest eGFR point was used as the reference point when multiple data were available for the same patient as performed in the previous study¹³. Inpatients and outpatients were included, and patients undergoing kidney replacement therapy (i.e., hemodialysis, peritoneal dialysis, and kidney transplantation) were not excluded. This study protocol was approved by the Clinical Research Ethics Committee of the Institutional Review Board at Kyushu University (Approval Number: 21166–02).

Outcomes and covariates

The primary outcome of the study was the presence of the following mineral metabolism disorders (i.e., hyperphosphatemia, hypophosphatemia, hypercalcemia, and hypocalcemia), and the secondary outcome of the study was the presence of abnormal magnesium metabolism (i.e., hypermagnesemia, and hypomagnesemia). Mineral metabolism disorders were defined as follows. Hyperphosphatemia and hypophosphatemia were defined as serum phosphate concentrations ≥ 4.5 mg/dL and < 2.5 mg/dL, respectively^{13–15}. Hypercalcemia and hypocalcemia were defined as serum corrected calcium concentrations ≥ 10.3 mg/dL and < 8.4 mg/dL, respectively^{13,14}. Hypermagnesemia and hypomagnesemia were defined as serum magnesium concentrations ≥ 2.5 mg/dL and < 1.8 mg/dL, respectively^{6,16}. The serum corrected calcium concentration was calculated using the serum albumin concentration based on Payne's formula: corrected calcium = serum calcium + $(4 - \text{serum albumin})$, only when the serum albumin concentration is < 4 g/dL¹⁷.

The primary exposure was the degree of proteinuria by the dipstick test. A urinalysis by the dipstick method was performed on spot urine specimens. The urine dipstick results were interpreted by the medical staff in each hospital and recorded as negative, 1+, 2+, and 3+. In accordance with the policy of the Japanese Committee for Clinical Laboratory Standards (<https://www.jccls.org/>), the urine dipstick results are manufactured to be displayed according to urine protein concentrations as follows: 0–29 mg/dL for negative, 30–99 mg/dL for 1+, 100–299 mg/dL for 2+, and ≥ 300 mg/dL for 3+. More precisely, the Japanese Committee for Clinical Laboratory

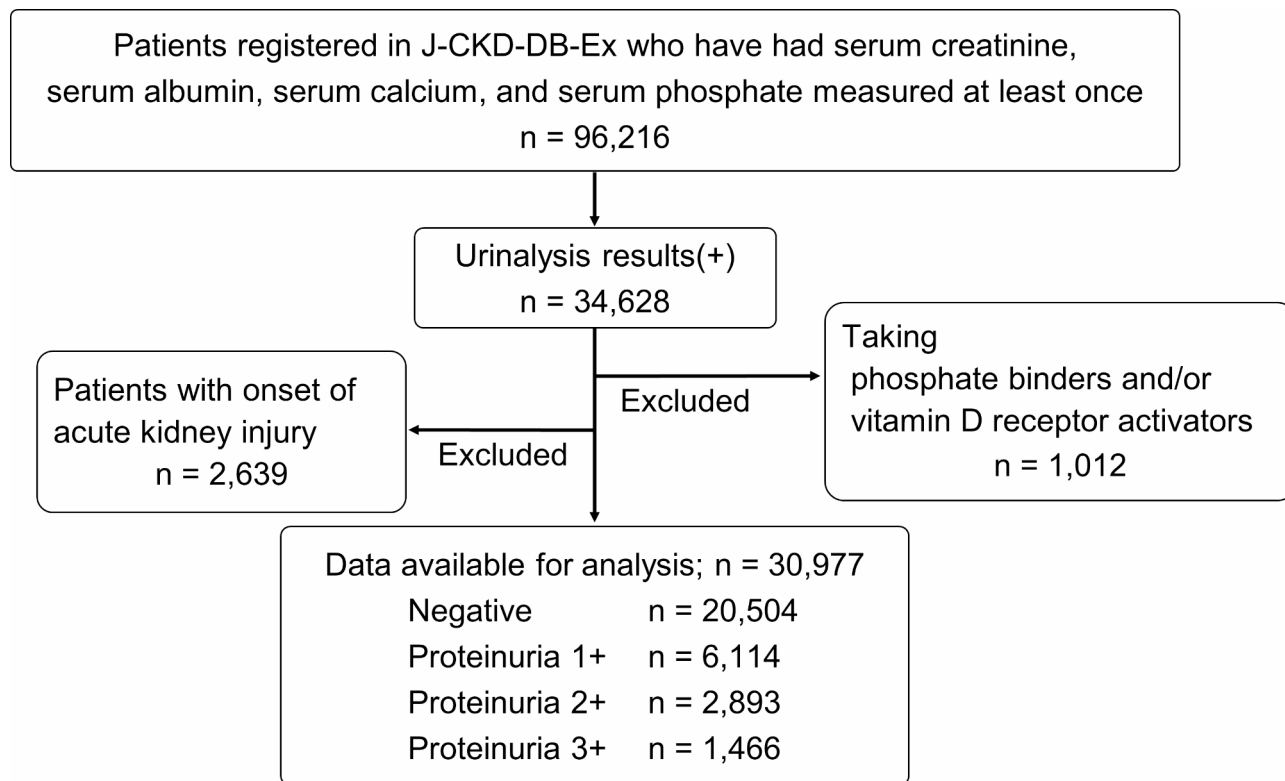


Fig. 1. Flowchart of patients enrolled in this study.

Standards defines a urine protein concentration $\geq 1,000$ mg/dL as 4+. However, the J-CKD-DB defines ≥ 300 mL/dL as 3+ because some urine test paper manufacturers do not have a 4+ setting. The patients were divided into four groups according to the degree of proteinuria: negative ($n=20,504$), 1+ ($n=6,114$), 2+ ($n=2,893$), and 3+ ($n=1,466$). The presence or absence of diabetes was obtained from the medical records. Serum PTH concentrations were measured using whole or intact PTH assays. The values measured by the two different assays were converted using the following formula: intact PTH (pg/mL) = $1.7 \times$ whole PTH (pg/mL)¹⁸.

Statistical analysis

Continuous variables and categorical data are described as the median (interquartile range) and percentage, respectively. The distribution of the baseline characteristics stratified by the degree of proteinuria was compared using the following trend analyses. The Cochran–Armitage and Jonckheere–Terpstra tests were used for categorical and continuous variables, respectively. Age, sex, diabetes, and the eGFR were selected as confounding factors. Unadjusted and adjusted logistic regression models were used to estimate the odds ratio (OR) and 95% confidence interval (CI) for the outcomes. A two-tailed P -value < 0.05 was considered statistically significant in all analyses. Several subgroup sensitivity analyses were performed, including stratifying patients according to the presence or absence of diabetes because this has a major effect on proteinuria. Additionally, analyses in which patients were stratified by CKD stages were performed to examine the effect of kidney function. Finally, subgroup analyses were performed with PTH, a factor with high missing value, added as a confounding factor. Statistical analyses were performed with JMP pro version 17.0 (SAS Institute, Cary, USA) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R version 3.6.2 (www.r-project.org)¹⁹.

Results

Baseline characteristics according to the degree of proteinuria

The median age of the 30,977 patients was 71 years and 56.8% were men. The baseline characteristics of this study population stratified by the degree of proteinuria are shown in Table 1. Patients with higher levels of proteinuria showed the following: (1) younger age; (2) higher proportion of men and diabetes; (3) higher serum concentrations of creatinine and phosphate; (4) lower serum concentrations of albumin, corrected calcium and magnesium; and (5) a lower eGFR.

Distribution of mineral metabolism disorders according to the degree of proteinuria

The distribution of mineral metabolism disorders according to the degree of proteinuria is shown in Fig. 2. In particular, 26.1% (382/1,466) of patients with proteinuria 3+ had hyperphosphatemia. The proportion of patients

	Groups by the degree of proteinuria				P for trend
	Negative (n = 20,504)	Proteinuria 1+ (n = 6,114)	Proteinuria 2+ (n = 2,893)	Proteinuria 3+ (n = 1,466)	
Age, years	71 (61-79)	71 (57-80)	71 (57-80)	69 (54-78)	<0.001
Male	53.9	61.4	62.3	67.9	<0.001
Diabetes	50.1	46.1	54.7	60.6	<0.001
Serum albumin, g/dL	4.1 (3.7-4.4)	3.9 (3.3-4.2)	3.8 (3.3-4.1)	3.4 (2.8-3.9)	<0.001
Serum creatinine, mg/dL	0.95 (0.79-1.16)	1.00 (0.80-1.35)	1.22 (0.91-1.98)	1.68 (1.05-3.33)	<0.001
Albumin-corrected serum Ca, mg/dL	9.4 (9.1-9.6)	9.3 (9.0-9.6)	9.3 (9.0-9.6)	9.4 (9.0-9.7)	<0.001
Serum phosphate, mg/dL	3.4 (3.0-3.8)	3.3 (2.9-3.8)	3.5 (3.0-4.1)	3.7 (3.2-4.5)	<0.001
Serum magnesium, mg/dL	2.1 (2.0-2.2)	2.1 (1.9-2.2)	2.1 (1.9-2.3)	2.0 (1.9-2.3)	<0.001
eGFR, mL/min/1.73m ²	54.6 (44.5-63.1)	53.7 (37.5-68.0)	42.8 (24.5-59.7)	30.8 (14.6-51.8)	<0.001
CKD stage G2	31.5	38.2	24.6	16.6	<0.001
CKD stage G3a	42.6	26.8	22.3	16.2	<0.001
CKD stage G3b	18.8	18.6	21.4	18.8	0.098
CKD stage G4	6.3	11.4	16.9	22.9	<0.001
CKD stage G5	0.9	5.0	14.9	25.6	<0.001

Table 1. Baseline characteristics stratified by the degree of proteinuria. Baseline data are expressed as median (interquartile range) or percentage. The Cochran-Armitage test was used to determine *P* for trend of categorical variables. The Jonckheere-Terpstra trend test was used to determine *P* for trend of continuous variables. A two-tailed *P*-value of <0.05 was considered statistically significant. *Ca* calcium, *eGFR* estimated glomerular filtration rate, *CKD* chronic kidney disease.

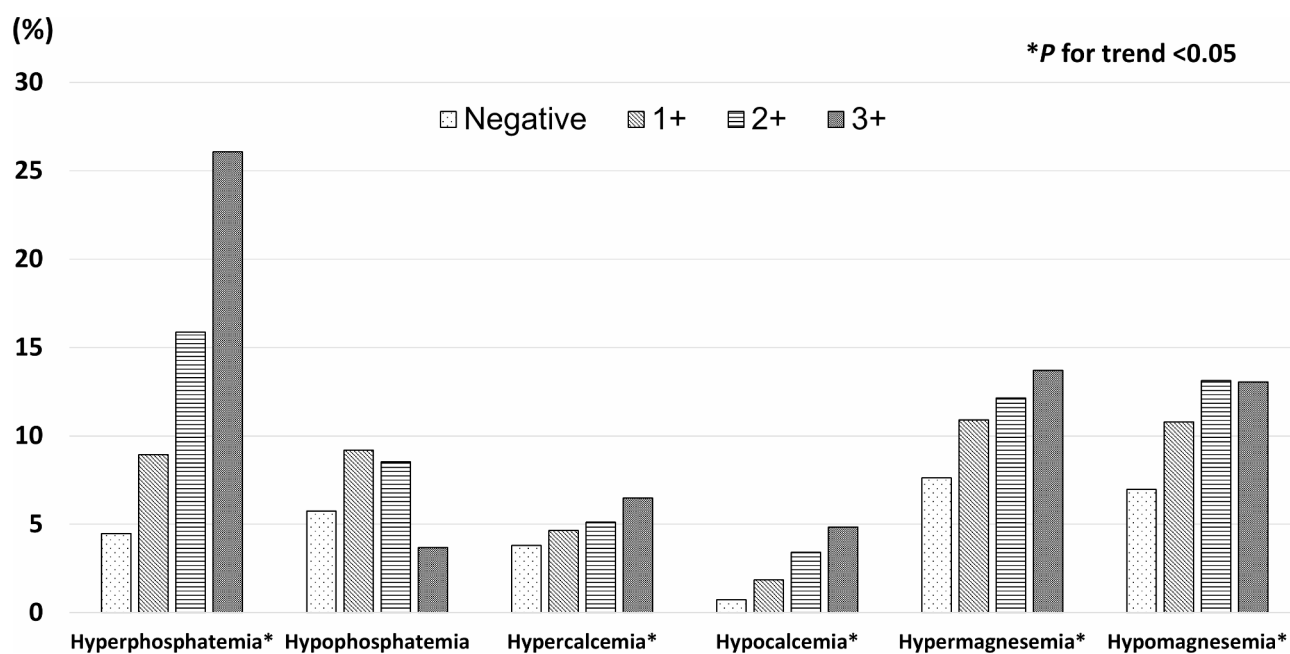


Fig. 2. Distribution of mineral metabolism disorders according to the degree of proteinuria.

with each mineral metabolism disorder tended to increase as proteinuria increased in hyperphosphatemia, hypercalcemia, hypocalcemia, hypermagnesemia, and hypomagnesemia.

Association of the degree of proteinuria with mineral metabolism disorders

The ORs and 95% CIs using the unadjusted and adjusted logistic regression models for mineral metabolism disorders according to the degree of proteinuria are shown in Table 2. In the model adjusted for age, sex, diabetes, and the eGFR, the ORs for the 3+ group compared with the negative group were significantly increased to 2.67 (95% CI, 2.29–3.13) for hyperphosphatemia, 2.68 (1.94–3.71) for hypocalcemia, and 1.56 (1.24–1.98) for hypomagnesemia. Adjusted ORs and 95% CIs for every one-category increase in proteinuria were 1.40 (1.33–1.46) for hyperphosphatemia, 1.17 (1.11–1.24) for hypophosphatemia, 1.43 (1.30–1.58) for hypocalcemia, and 1.25 (1.18–1.33) for hypomagnesemia. In hypophosphatemia, ORs for the 1+ and 2+ group compared with the

	Number of patients/total number	Unadjusted model			Adjusted model		
		OR (95% CI)	P-value	P for trend	OR (95% CI)	P-value	P for trend
<i>Hyperphosphatemia</i>							
Negative	916/20,504	1.00 (reference)			1.00 (reference)		
Proteinuria 1+	546/6,114	2.10 (1.88–2.34)	<0.001		1.65 (1.47–1.85)	<0.001	
Proteinuria 2+	459/2,893	4.03 (3.58–4.55)	<0.001	<0.001	1.98 (1.73–2.27)	<0.001	<0.001
Proteinuria 3+	382/1,466	7.53 (6.59–8.62)	<0.001		2.67 (2.29–3.13)	<0.001	
Every 1 category increase		1.97 (1.90–2.05)	<0.001		1.40 (1.33–1.46)	<0.001	
<i>Hypophosphatemia</i>							
Negative	1,179/20,504	1.00 (reference)			1.00 (reference)		
Proteinuria 1+	561/6,114	1.66 (1.49–1.84)	<0.001		1.59 (1.43–1.76)	<0.001	
Proteinuria 2+	247/2,893	1.53 (1.49–1.84)	<0.001	<0.001	1.72 (1.49–1.99)	<0.001	<0.001
Proteinuria 3+	54/1,466	0.63 (0.47–0.83)	0.001		0.79 (0.59–1.04)	0.09	
Every 1 category increase		1.10 (1.05–1.16)	<0.001		1.17 (1.11–1.24)	<0.001	
<i>Hypercalcemia</i>							
Negative	780/20,504	1.00 (reference)			1.00 (reference)		
Proteinuria 1+	285/6,114	1.24 (1.08–1.42)	0.003		1.19 (1.04–1.37)	0.015	
Proteinuria 2+	148/2,893	1.36 (1.14–1.63)	<0.001	<0.001	1.06 (0.87–1.28)	0.57	0.09
Proteinuria 3+	95/1,466	1.75 (1.41–2.18)	<0.001		1.18 (0.93–1.49)	0.18	
Every 1 category increase		1.19 (1.13–1.28)	<0.001		1.06 (0.99–1.13)	0.09	
<i>Hypocalcemia</i>							
Negative	151/20,504	1.00 (reference)			1.00 (reference)		
Proteinuria 1+	114/6,114	2.56 (2.00–3.27)	<0.001		2.02 (1.57–2.60)	<0.001	
Proteinuria 2+	99/2,893	4.78 (3.70–6.17)	<0.001	<0.001	2.60 (1.97–3.43)	<0.001	<0.001
Proteinuria 3+	71/1,466	6.86 (5.15–9.14)	<0.001		2.68 (1.94–3.71)	<0.001	
Every 1 category increase		1.95 (1.80–2.12)	<0.001		1.43 (1.30–1.58)	<0.001	
<i>Hypermagnesemia</i>							
Negative	690/9,044	1.00 (reference)			1.00 (reference)		
Proteinuria 1+	371/3,399	1.48 (1.30–1.69)	<0.001		1.39 (1.21–1.59)	<0.001	
Proteinuria 2+	186/1,532	1.67 (1.41–1.99)	<0.001	<0.001	1.13 (0.94–1.36)	0.20	0.08
Proteinuria 3+	106/774	1.92 (1.54–2.39)	<0.001		1.06 (0.83–1.34)	0.66	
Every 1 category increase		1.28 (1.20–1.35)	<0.001		1.06 (0.99–1.13)	0.08	
<i>Hypomagnesemia</i>							
Negative	630/9,044	1.00 (reference)			1.00 (reference)		
Proteinuria 1+	367/3,399	1.62 (1.41–1.85)	<0.001		1.59 (1.39–1.83)	<0.001	
Proteinuria 2+	201/1,532	2.02 (1.70–2.39)	<0.001	<0.001	1.80 (1.51–2.15)	<0.001	<0.001
Proteinuria 3+	101/774	2.00 (1.60–2.51)	<0.001		1.56 (1.24–1.98)	<0.001	
Every 1 category increase		1.34 (1.26–1.42)	<0.001		1.25 (1.18–1.33)	<0.001	

Table 2. Odds ratios and 95% confidence intervals for mineral metabolism disorders according to the degree of proteinuria. Unadjusted and adjusted ORs and 95% CIs were analyzed by the logistic regression models. Age, sex, diabetes, and eGFR were selected as confounding factors in adjusted models. A two-tailed *P*-value of < 0.05 was considered statistically significant. *OR* odds ratio, *CI* confidence interval, *eGFR* estimated glomerular filtration rate.

negative group were significant; however, the significant difference disappeared for the 3+ group, which had ORs of 0.79 (0.59–1.04) compared with the negative group. In contrast, no consistent associations were found for hypercalcemia or hypermagnesemia. Adjusted ORs for the 3+ group compared with the negative group were 1.18 (0.93–1.49) for hypercalcemia and 1.06 (0.83–1.34) for hypermagnesemia; adjusted ORs and 95% CIs for every one-category increase in proteinuria were 1.06 (0.99–1.13) for hypercalcemia and 1.06 (0.99–1.13) for hypermagnesemia. The ORs and 95% CIs for mineral metabolism disorders for each confounding factor are shown in Supplementary Table 3.

Subgroup analyses

Subgroup analyses were performed by stratifying patients according to the presence or absence of diabetes. The ORs and 95% CIs using the adjusted logistic regression models for mineral metabolism disorders according to the degree of proteinuria stratified by the presence or absence of diabetes are shown in Table 3. A significant association was observed between proteinuria and hypercalcemia and hypermagnesemia in the group without

	Presence of diabetes				Absence of diabetes			
	Number of patients/Total number	Adjusted OR (95% CI)	P-value	P for trend	Number of patients/total number	Adjusted OR (95% CI)	P-value	P for trend
<i>Hyperphosphatemia</i>								
Negative	500/10,267	1.00 (reference)			416/10,237	1.00 (reference)		
Proteinuria 1+	259/2,819	1.32 (1.11–1.56)	0.002		287/3,295	2.02 (1.72–2.38)	<0.001	
Proteinuria 2+	253/1,583	1.47 (1.22–1.78)	<0.001	<0.001	206/1,310	2.69 (2.21–3.26)	<0.001	<0.001
Proteinuria 3+	250/889	2.03 (1.65–2.50)	<0.001		132/577	3.50 (2.74–4.46)	<0.001	
Every 1 category increase		1.25 (1.17–1.33)	<0.001			1.57 (1.47–1.68)	<0.001	
<i>Hypophosphatemia</i>								
Negative	508/10,267	1.00 (reference)			671/10,237	1.00 (reference)		
Proteinuria 1+	223/2,819	1.64 (1.39–1.94)	<0.001		338/3,295	1.55 (1.35–1.78)	<0.001	
Proteinuria 2+	117/1,583	1.71 (1.38–2.12)	<0.001	<0.001	130/1,310	1.73 (1.42–2.12)	<0.001	<0.001
Proteinuria 3+	28/889	0.77 (0.51–1.14)	0.19		26/577	0.79 (0.53–1.18)	0.25	
Every 1 category increase		1.15 (1.07–1.25)	<0.001			0.79 (0.53–1.18)	<0.001	
<i>Hypercalcemia</i>								
Negative	437/10,267	1.00 (reference)			343/10,237	1.00 (reference)		
Proteinuria 1+	137/2,819	1.09 (0.89–1.33)	0.39		148/3,295	1.33 (1.09–1.62)	0.005	
Proteinuria 2+	68/1,583	0.81 (0.62–1.07)	0.14	0.71	80/1,310	1.42 (1.10–1.85)	0.008	<0.001
Proteinuria 3+	55/889	1.05 (0.77–1.44)	0.76		40/577	1.45 (1.01–2.07)	0.041	
Every 1 category increase		0.98(0.90–1.08)	0.71			1.17 (1.07–1.29)	<0.001	
<i>Hypocalcemia</i>								
Negative	71/10,267	1.00 (reference)			80/10,237	1.00 (reference)		
Proteinuria 1+	48/2,819	1.64 (1.12–2.41)	0.011		66/3,295	2.33 (1.67–3.24)	<0.001	
Proteinuria 2+	50/1,583	1.89 (1.26–2.83)	0.002	<0.001	49/1,310	3.36 (2.29–4.91)	<0.001	<0.001
Proteinuria 3+	49/889	2.16 (1.40–3.35)	<0.001		22/577	2.77 (1.66–4.62)	<0.001	
Every 1 category increase		1.29 (1.13–1.48)	<0.001			1.54 (1.34–1.77)	<0.001	
<i>Hypermagnesemia</i>								
Negative	357/4,343	1.00 (reference)			333/4,701	1.00 (reference)		
Proteinuria 1+	162/1,420	1.19 (0.97–1.46)	0.10		209/1,979	1.57 (1.30–1.89)	<0.001	
Proteinuria 2+	90/747	0.91 (0.70–1.19)	0.49	0.22	96/785	1.36 (1.05–1.76)	0.018	<0.001
Proteinuria 3+	55/429	0.76 (0.54–1.07)	0.11		51/345	1.52 (1.08–2.14)	0.016	
Every 1 category increase		0.94 (0.86–1.04)	0.22			1.19 (1.09–1.30)	<0.001	
<i>Hypomagnesemia</i>								
Negative	341/4,343	1.00 (reference)			289/4,701	1.00 (reference)		
Proteinuria 1+	176/1,420	1.61 (1.32–1.95)	<0.001		191/1,979	1.60 (1.32–1.94)	<0.001	
Proteinuria 2+	105/747	1.69 (1.32–2.16)	<0.001	<0.001	96/785	1.96 (1.53–2.51)	<0.001	<0.001
Proteinuria 3+	63/429	1.49 (1.09–2.04)	0.012		38/345	1.63 (1.13–2.35)	0.009	
Every 1 category increase		1.22 (1.12–1.33)	<0.001			1.29 (1.18–1.42)	<0.001	

Table 3. Adjusted odds ratios and 95% confidence intervals for mineral metabolism disorders according to the degree of proteinuria and stratified by the presence or absence of diabetes. Adjusted ORs and 95% CIs were analyzed by the logistic regression models. Age, sex, and eGFR were selected as confounding factors in adjusted models. A two-tailed *P*-value of < 0.05 was considered statistically significant. *OR* odds ratio, *CI* confidence interval, *eGFR* estimated glomerular filtration rate.

diabetes, while this association disappeared in the group with diabetes. The results for the other mineral metabolism disorders were generally consistent with the main analysis of all subjects.

ORs and 95% CIs using adjusted logistic regression models for mineral metabolism disorders according to the degree of proteinuria stratified by CKD stage are shown in Supplementary Table 1. We focused on hyperphosphatemia, hypocalcemia, and hypomagnesemia, which were shown to have significant results in Table 2. In hyperphosphatemia, the results were similar to those shown in Table 2, except for CKD stages G3b and G4. In hypocalcemia, the number of patients in the 3+ group could not be adequately analyzed because stratification considerably reduced the number in this group. In hypomagnesemia, stratification by kidney function eliminated any significant association, suggesting that kidney function may have a significant impact on the association between proteinuria and hypomagnesemia.

Additional analyses were performed to determine the effect of PTH on mineral metabolism disorders (Supplementary Table 2). However, the number of subjects available for this analysis decreased from 30,977 to

1,062 because of the large number of missing PTH values. The extremely low number of outcomes may have destabilized the statistical model when multivariate analysis was performed.

Discussion

In this cross-sectional study of patients from the J-CKD-DB-Ex registry, we showed that the proportion of patients with mineral metabolism disorders tended to increase as proteinuria increased. In logistic regression models adjusted for age, sex, and the eGFR, we found that the increase in proteinuria was significantly associated with hyperphosphatemia, hypocalcemia, and hypomagnesemia. In this analysis using real-world data from the J-CKD-DB-Ex, we were able to show an association between proteinuria and CKD-MBD, both of which are risk factors for progression and mortality in patients with CKD.

Among the mineral metabolism disorders found in this study, hyperphosphatemia is a strong predictor of mortality and cardiovascular events, and an association between hyperphosphatemia and proteinuria has been reported. A cross-sectional study of 1,738 patients with CKD reported that albuminuria was significantly associated with higher plasma phosphate concentrations independent of the GFR²⁰. To understand the mechanisms underlying these observations, the authors of this previous study conducted experiments using puromycin aminonucleoside (PAN)-induced nephrosis rats, which is a model that shows high levels of proteinuria. The expression of NaPi-IIa, a sodium-phosphate cotransporter in proximal tubules, was increased in PAN rats, which suggested that phosphate reabsorption in the tubules was enhanced. PAN rats also showed decreased phosphorylation of fibroblast growth factor (FGF) receptor substrate 2a, which is a major FGF-23 receptor substrate. This finding suggested that the urinary excretion of phosphate by FGF-23 may have been suppressed. Another cohort study also reported that proteinuria was associated with decreased FGF-23 biological activity and increased serum phosphate concentrations²¹. These previous results support our findings.

An association between proteinuria and the serum vitamin D status has been observed, which may explain the associations between proteinuria and hypocalcemia and hypomagnesemia. In experiments using PAN rats, vitamin D binding protein, to which the most serum vitamin D is bound, leaked into the urine as proteinuria, resulting in a decrease in serum 25-hydroxyvitamin D concentrations²². Similarly, a multicenter, cross-sectional study of patients with CKD reported that patients with a high amount of proteinuria were at higher risk of having low serum 25-hydroxyvitamin D concentrations²³. Decreased 25-hydroxyvitamin D may result in decreased production of active vitamin D in the kidneys. The reduced action of active vitamin D suppresses calcium and magnesium absorption from the gastrointestinal tract, resulting in hypocalcemia and hypomagnesemia. Another cross-sectional study reported that proteinuria led to renal magnesium wasting through tubular injuries²⁴. Because serum vitamin D is also involved in phosphate absorption, the aforementioned decrease in active vitamin D may explain the trend toward higher hypophosphatemia with increased proteinuria observed in this study.

Our study had two main strengths. First, the data used in this study were automatically extracted from medical records and are real-world data from actual clinical practice. Second, this study used a larger data set than previous studies^{20,25}, increasing the possibility of generating better quality medical evidence regarding the association between proteinuria and CKD-MBD. Although urinalysis is frequently performed in clinical practice, it is rare to investigate mineral metabolism disorders, especially serum phosphate and magnesium disorders, in departments other than nephrology. However, given that this study has demonstrated an association between proteinuria and CKD-MBD, it may be advisable to evaluate patients with positive proteinuria using blood tests for hidden disorders in serum calcium, phosphate, and magnesium to help with early detection.

Our study also had several limitations. First, this study was conducted on patients at university hospitals, which may have led to selection bias and may not be widely applicable to patients with CKD. Second, of the data collected in the J-CKD-DB-Ex, some factors had high missing values (e.g., PTH) and some potential confounding factors were not collected (e.g., alkaline phosphatase). These factors could not be used in the multivariate analysis and their effect on the results could not be verified. Third, this was a cross-sectional study and causal relationships could not be discussed. Finally, unmeasured or unknown confounding factors could lead to a null hypothesis. Despite these limitations, we believe that the present observational study provides useful insights into proteinuria and mineral metabolism disorders in patients with CKD.

Conclusion

This study shows that proteinuria is associated with hyperphosphatemia, hypocalcemia, and hypomagnesemia in patients with CKD. Hopefully, the associations shown in this study will lead to further research and ultimately to further improvements in the management of proteinuria and CKD-MBD.

Data availability

The data analyzed in this study are not publicly available, but are available from the corresponding author on reasonable request.

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Author contributions

Sho Shimamoto: Conceptualization, Methodology, Formal analysis, Visualization, Writing- Original draft preparation. Takako Nakahara: Investigation, Data curation, Visualization, Writing- Reviewing and Editing. Shunsuke Yamada: Validation, Writing- Reviewing and Editing. Hajime Nagasu: Data curation, Writing- Reviewing and Editing. Seiji Kishi: Data curation, Validation. Naoki Nakashima, Kazuhiko Tsuruya, Hirokazu Okada, Kouichi Tamura, Ichiei Narita, Shoichi Maruyama, Yuichiro Yano, Takashi Yokoo, Takashi Wada, Jun Wada, Eiichiro Kanda, Hiromi Kataoka, and Masaomi Nangaku: interpretation of data, final approval of the submitted manuscript. Naoki Kashihawa: Project administration. Toshiaki Nakano: Conceptualization, Validation, Supervision.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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