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Original Article

ORIGINAL ARTICLE

Association of calciprotein particles with serum phosphorus among patients undergoing conventional and extended-hours haemodialysis

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ABSTRACT

Background and hypothesis. Extended-hours haemodialysis (HD) is associated with better clinical outcomes than conventional HD. We investigated whether extended-hours HD and conventional HD have varying effects on blood levels of calciprotein particles (CPPs) and phosphorus, which have been identified as major pathogenic molecules for vascular calcification.

Methods. Patients who underwent conventional or extended in-centre daytime HD between January and March 2020 were included. Plasma CPP levels, representing only secondary CPPs (CPP-II), were measured in pre-dialysis samples. Linear and non-linear associations between CPPs and serum phosphorus levels were examined across dialysis modalities.

Results. A total of 382 participants (185 undergoing extended-hours HD and 197 undergoing conventional HD) were included in the analysis. The median age of participants was 71 years, 65% of the patients were men and the mean phosphorus level was 5.4 mg/dl. Plasma CPP (CPP-II) levels were lower in the extended-hours HD group than in the conventional HD group [40 018 (arbitrary units) AU versus 75 728 AU; $P < .01$]. Multivariable linear regression analysis showed that extended-hours HD was associated with lower natural logarithmic plasma CPP (CPP-II) levels: −0.64 (95% confidence interval −0.74 to −0.55). A restricted cubic spline function indicated that extended-hours HD was associated with lower plasma CPP (CPP-II) levels across levels of serum phosphorus, with significant differences observed between groups, especially in hyperphosphataemic conditions (P for interaction <.01).

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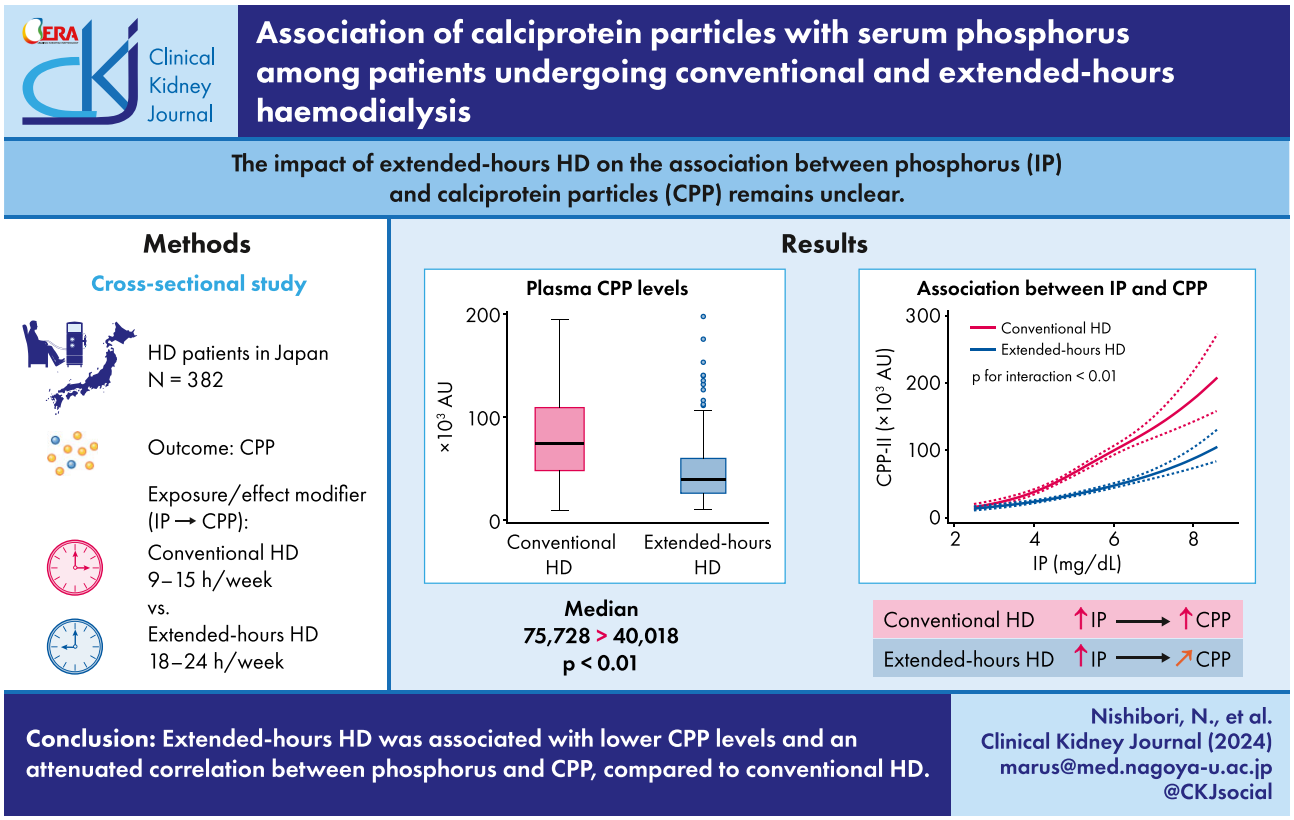
Conclusions. The extended-hours HD group had lower CPP levels than the conventional HD group despite no significant differences in serum phosphorus levels, which may contribute to better clinical outcomes in patients on extended-hours HD.

GRAPHICAL ABSTRACT



Keywords: calcification, chronic haemodialysis, end-stage kidney disease, mineral bone disorder, nutrition

KEY LEARNING POINTS

What was known:

- Patients receiving maintenance haemodialysis (HD) have a high prevalence of vascular calcification, which increases the risk for cardiovascular disease and mortality; calciprotein particles (CPPs) have been considered as a causative agent of vascular calcification.
- Compared with conventional HD, extended-hours HD reduces serum phosphorus levels, which may be associated with lower mortality.
- Secondary CPPs (CPP-II) induce calcification in cultured vascular smooth muscle cells, whereas primary CPPs (CPP-I) do not; therefore, it could be important to evaluate the quality and quantity of CPP.

This study adds:

- Plasma CPP (CPP-II) and serum CPP (CPP-I + II) levels were lower in patients receiving extended-hours HD than in those in patients receiving conventional HD, despite the lack of significant differences in serum phosphorus and calcium levels.
- Plasma CPP (CPP-II) levels were elevated exponentially with elevation of phosphorus levels in both the conventional and extended-hours HD groups, but more gradually in the extended-hours HD group.

Potential impact:

- In this study, patients receiving extended-hours HD exhibited better CPP status than those receiving conventional HD; therefore, extended-hours HD may be a promising treatment option for reducing cardiovascular disease progression.
- The acceptable upper limit of serum phosphorus levels in patients receiving extended-hours HD may differ from those receiving conventional HD.

INTRODUCTION

Patients undergoing maintenance haemodialysis (HD) have a high prevalence of vascular calcification, which is strongly associated with cardiovascular disease (CVD) and mortality [1, 2]. Elevated phosphorus and calcium levels are associated with vascular calcification and mortality in patients with chronic kidney disease (CKD) and end-stage kidney disease (ESKD) [3–5]. Recent studies have reported that calciprotein particles (CPPs) are causative agents of vascular calcification and reflect extraosseous calcification stress in CKD, and higher serum CPP levels are associated with CVD events in patients with ESKD [6–8]. When the concentrations of calcium and phosphate ions exceed their solubility in the blood, amorphous calcium phosphate (CaPi) is precipitated, which is adsorbed by the protein fetuin-A and forms colloidal mineral–protein complexes called primary CPPs (CPP-I) [9]. CPP-I undergoes aggregation to form secondary CPPs (CPP-II), which is associated with the transition of CaPi from an amorphous to a crystalline phase [10]. CPP-II induces calcification in vascular smooth muscle cells (VSMCs), whereas CPP-I does not exhibit this pathogenic activity [11]. Hence it is plausible that CPPs containing crystalline CaPi or CPP-II is a reasonable risk factor for vascular calcification and mortality in patients undergoing maintenance HD [12–14].

Extended-hours HD, including nocturnal HD (typically three to four times per week, 6–8 hours per treatment), has been shown to enhance the removal of serum phosphorus and other uraemic solutes, which are potential mediators in the pathophysiological pathway between ESKD and cardiovascular events [15–17]. Previous studies have shown that extended-hours HD is associated with lower mortality than conventional HD (typically three times per week, 3–5 hours per treatment) [18–21]. We have reported that extended-hours HD without strict dietary restriction, which we call a liberalized diet, was associated with improved survival compared with conventional HD, and that patients whose body mass index (BMI) was maintained or increased for 1 year after the induction of extended-hours HD had a better prognosis than those whose BMI decreased [22, 23]. Although a previous study revealed that liberalized phosphorus intake among conventional HD patients was associated with better nutritional indicators and survival [24], clinicians often have difficulty balancing concerns about vascular calcification with phosphorus intake and dietary liberalization. Therefore, investigating the impact of extended-hours HD with a liberalized diet on CPPs and mineral and bone disorder (MBD)-related indices is relevant for dialysis providers.

We examined the association between CPPs and phosphorus, along with other parameters related to MBD in patients who received conventional HD and extended-hours HD. To the best of our knowledge, this is the largest study to examine the impact of extended-hours HD on CPPs compared with conventional HD.

MATERIALS AND METHODS

Study design and data source

We performed a cross-sectional study of 197 patients who underwent conventional HD and 185 patients who underwent extended-hours HD between January and March 2020. In the conventional HD group, participants received daytime in-centre HD for 3–5 hours per treatment session, three times per week, at specialized facilities for conventional HD in central Japan. In the extended-hours HD group, participants received daytime in-centre extended-hours HD, defined as >6 hours per session, three or four times per week, at specialized facilities for extended-hours HD in northeastern Japan. Patients in the conventional HD group received dietary counselling for salt, potassium and phosphorus restrictions. The patients in the extended-hours HD group were instructed to consume their preferred diet. Baseline patient characteristics were obtained from electronic medical records and all analytical data were anonymized. This study complied with the Declaration of Helsinki and was approved by the institutional review boards of all sites (approval no. 2014-0422). All the participants provided written informed consent.

Biomarkers and biochemical data

Blood samples were collected from all participants before the first HD session. The plasma samples were frozen at −80°C promptly after centrifugation using sodium–heparin blood collection tubes, whereas the serum samples were frozen at −80°C after coagulation and centrifugation using blood collection tubes with separating gel. Serum creatinine, albumin, calcium, phosphorus, magnesium, zinc, C-reactive protein (CRP) and intact parathyroid hormone (iPTH) levels were measured centrally at the laboratory of LSI Medience, Tokyo, Japan. We used commercially available enzyme-linked immunosorbent assay kits (BI-20700, Biomedica, Vienna, Austria) to measure intact fibroblast growth factor 23 (iFGF23) in plasma samples, following the manufacturer's instructions.

Plasma and serum CPP levels were measured in the same samples using a previously reported 'gel filtration method' at the Division of Anti-Aging Medicine, Center for Molecular Medicine, Jichi Medical University, Shimotsuke, Japan [25]. In brief, plasma or serum samples were inoculated with OsteoSense (PerkinElmer, Chicago, IL, USA) that bound to calcium phosphate crystals, incubated for 60 minutes at 25°C, and then transferred to a gel filtration spin column to remove unbound OsteoSense. The fluorescence intensity of the CPP-containing flow-through was quantified using an infrared fluorescence scanner and designated as the CPP level. As this assay uses a fluorescent probe that binds to crystalline CaPi, CPP levels in plasma samples reflect CPP-II levels *in vivo*. In contrast, CPP levels in serum samples represent total CPP levels (CPP-I and CPP-II) because blood coagulation accelerates the amorphous-to-crystalline phase transition of CaPi in CPP-I (Fig. 1) [14, 25].

Statistical analysis

Continuous variables were expressed as mean [standard deviation (SD)] if normally distributed or median [interquartile range (IQR)] if non-normally distributed. Categorical variables were expressed as numbers (%). Skewness and kurtosis tests were performed to assess the normality of the data, and skewed data (CRP, iPTH, iFGF23 and CPP levels) underwent natural logarithmic (ln) transformation to achieve a normal distribution in the multivariable analysis. For comparisons between the conventional and extended-hours HD groups, normally distributed variables were subjected to Student's *t*-test and non-normally distributed variables were subjected to the Mann–Whitney U test. Linear relationships between phosphorus, calcium levels, calcium–phosphorus products and ln plasma CPP and ln serum CPP levels were evaluated using Pearson's correlation coefficient.

Multivariable linear regression analysis was performed to assess the impact of extended-hours HD on CPP levels; independent factors incorporated into the multivariable analysis in-

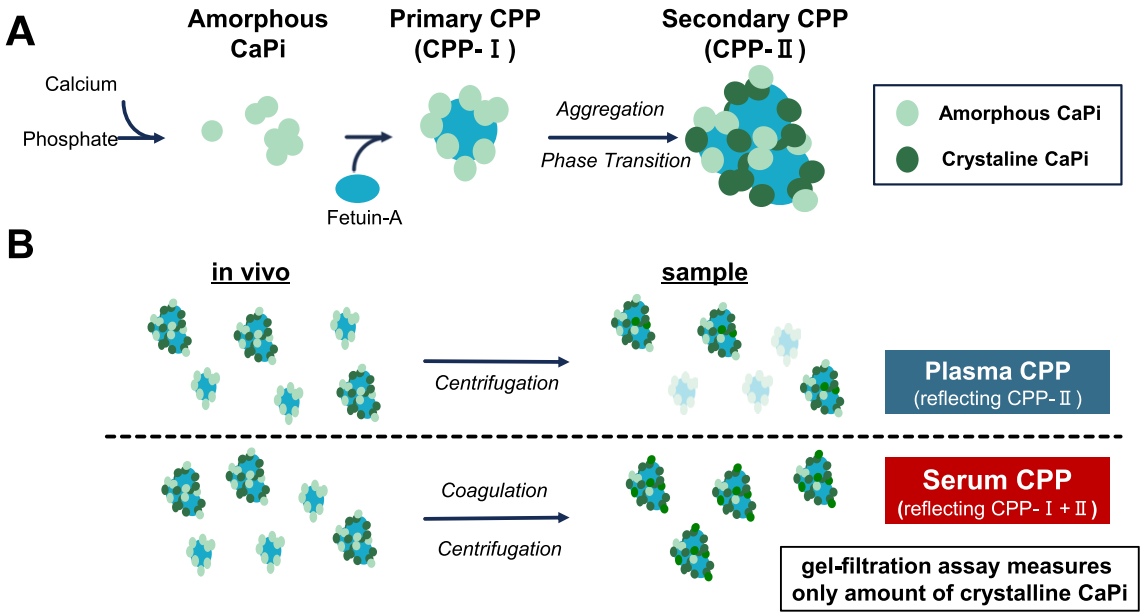


Figure 1: Changing properties of CPPs. **(A)** A schematic diagram of CPP maturation. Amorphous CaPi precipitates bind to fetuin-A protein to form primary CPP (CPP-I) . In the process of CPP-I undergoing aggregation and becoming secondary CPP (CPP-II), CaPi transitions from the amorphous phase to the crystalline phase. **(B)** The difference between plasma CPPs and serum CPPs. Blood coagulation promotes phase transition from the amorphous phase to the crystalline phase of CaPi and aggregation of CPP-I. Therefore, serum CPP (CPP-I + II) levels are higher than plasma CPP (CPP-II) levels because the gel filtration assay measures the amount of crystalline CaPi (CPP-II).

cluded dialysis modalities (conventional or extended-hours HD), sex, age, dialysis duration, history of diabetes mellitus, medication (use of phosphate binders, vitamin D receptor activators and calcimimetics) and laboratory data (albumin, creatinine, phosphorus, calcium, magnesium, zinc, iPTH, CRP and iFGF23).

For sensitivity analysis, an inverse probability weighting propensity score (IPW-PS) analysis was performed to minimize the influence of indication bias in assessing the impact of extended-hours HD on CPPs. We predicted the propensity score (PS) using a logistic regression model with sex, age, dialysis duration, comorbidities (diabetes mellitus, hypertension and dyslipidaemia) and past medical history (coronary artery disease, stroke and peripheral artery disease) for extended-hours HD. The weights were 1/PS for participants receiving extended-hours HD and 1/(1 − PS) for participants receiving conventional HD [26, 27]. We used C-statistics to assess the validity of the PS and predicted absolute standardized difference values to assess the fit of the patients' backgrounds.

The association between phosphorus and CPP levels was evaluated using a restricted cubic spline (RCS) function [28, 29]. In the RCS analysis, we truncated the subjects at the 1st and 99th percentiles of phosphorus levels (2.5 and 8.6 mg/dl), set three knots at the 10th, 50th and 90th percentiles of the phosphorus values and adjusted for sex, age, dialysis duration, history of diabetes mellitus, medication and laboratory data. The non-linear association between phosphorus levels and CPP was expressed as a spline curve combined with cubic polynomials and linear terms. Wald's test was also performed to assess differences in the association between phosphorus levels and CPP parameters (plasma and serum CPP levels) between the conventional and extended-hours HD groups.

Statistical significance was set at $P < .05$ (two-tailed). All statistical analyses were conducted using the Stata version 15.1 (StataCorp, College Station, TX, USA).

RESULTS

Baseline characteristics

The baseline characteristics of the entire study population and the conventional and extended HD groups are shown in Table 1. Patients who received extended-hours HD were younger, had higher BMI and albumin levels, and had fewer comorbid hypertension, dyslipidaemia, and peripheral artery disease than those who received conventional HD. Despite the liberalized diet and lower use of phosphate binders in the extended-hours HD group, calcium and phosphorus levels were similar to those in the conventional HD group.

Correlation of CPP and CKD-MBD parameters

The median plasma and serum CPP levels were 40 018 arbitrary units (AU) (IQR 26 245–59 895) and 66 524 AU (IQR 39 519–98 642), respectively, in the extended-hours HD group and 75 728 AU (IQR 48 137–109 087) and 100 812 AU (IQR 67 201–139 209), respectively, in the conventional HD group. The mean of serum phosphorus, calcium levels and calcium–phosphorus products was 5.4 mg/dl, 8.5 mg/dl and 45.9 mg^2/dl^2, respectively, in the extended-hours HD group and 5.3 mg/dl, 8.4 mg/dl and 44.8 mg^2/dl^2, respectively, in the conventional HD group. Plasma CPP (CPP-II) levels were significantly lower in the extended-hours HD group, but phosphorus levels were not significantly different according to the Mann–Whitney U test (Fig. 2). Serum CPP (CPP-I + II) levels were also significantly lower in the extended-hours HD group, despite no significant differences in calcium or calcium–phosphorus product levels (Supplementary Fig. S1).

Figure 3 shows the correlations between ln plasma CPP, ln serum CPP and other laboratory parameters (phosphorus, calcium and calcium–phosphorus products). In both the extended-hours and conventional HD groups, serum phosphorus

Table 1: Baseline characteristics.

Characteristics	Overall ($N = 382$)	Extended-hours HD ($n = 185$)	Conventional HD ($n = 197$)	P-value
Sex (male), *n* (%)	248 (65)	127 (68)	121 (61)	.16
Age (years), median (IQR)	71 (63–78)	66 (58–75)	73 (67–79)	<.01
Body mass index (kg/m^2), median (IQR)	22.2 (19.9–24.8)	23.1 (20.9–26.0)	21.0 (18.9–23.2)	<.01
Duration of dialysis (months), median (IQR)	81 (34–171)	109 (45–192)	67 (25–143)	.01
Primary cause of ESKD, *n* (%)				.30
Diabetic nephropathy	170 (45)	77 (42)	93 (47)	
Nephrosclerosis	50 (13)	21 (11)	29 (15)	
Glomerulonephritis	107 (28)	56 (30)	51 (26)	
Polycystic kidney disease	14 (3.7)	7 (3.8)	7 (3.6)	
Others	17 (4.5)	12 (6.5)	5 (2.5)	
Unknown	24 (6.3)	12 (6.5)	12 (6.1)	
Comorbidities, *n* (%)				
Diabetes mellitus	196 (51)	90 (49)	106 (54)	.36
Hypertension	253 (66)	86 (47)	167 (85)	<.01
Dyslipidaemia	124 (33)	34 (18)	90 (46)	<.01
Coronary artery disease	108 (28)	44 (24)	64 (33)	.07
Stroke	92 (24)	47 (25)	45 (23)	.63
Peripheral artery disease	88 (23)	27 (15)	61 (31)	<.01
Medication, *n* (%)				
Phosphate binders	267 (70)	100 (54)	167 (85)	<.01
Calcium based	184 (48)	71 (38)	113 (57)	<.01
Non-calcium based	160 (42)	35 (19)	125 (63)	<.01
Vitamin D receptor activators	257 (67)	100 (54)	157 (80)	<.01
Calcimimetics	141 (37)	74 (40)	67 (34)	.24
Intravenous iron	99 (26)	18 (9.7)	81 (41)	<.01
Erythropoietin-stimulating agent	312 (82)	134 (72)	178 (90)	<.01
Classes of antihypertensive drugs, median (IQR)	1 (0–2)	0 (0–1)	2 (1–3)	<.01
Biochemical variables				
Albumin (g/dl), mean (SD)	3.3 (0.3)	3.5 (0.3)	3.2 (0.3)	<.01
Creatinine (mg/dl), mean (SD)	9.8 (2.5)	10.0 (2.5)	9.6 (2.4)	.090
Calcium (mg/dl), mean (SD)	8.4 (0.6)	8.5 (0.6)	8.4 (0.5)	.19
Phosphorus (mg/dl), mean (SD)	5.4 (1.2)	5.4 (1.4)	5.3 (1.1)	.64
Magnesium (mg/dl), mean (SD)	2.3 (0.3)	2.3 (0.4)	2.3 (0.3)	.110
Zinc (μg/dl), mean (SD)	61.1 (13.5)	63.3 (14.0)	59.0 (12.6)	<.01
Intact PTH (pg/ml), median (IQR)	188 (117–266)	188 (122–269)	189 (114–263)	.74
C-reactive protein (mg/dl), median (IQR)	0.12 (0.04–0.34)	0.09 (0.04–0.29)	0.14 (0.06–0.39)	.03
Intact FGF23 (pg/ml), median (IQR)	2375 (877–7025)	1952 (598–6245)	2851 (1196–7800)	.02

levels were significantly correlated with ln plasma CPP (CPP-II) and ln serum CPP (CPP-I + II) levels (Fig. 3A and D). Serum calcium levels were correlated with ln plasma CPP (CPP-II) and ln serum CPP (CPP-I + II) levels in the extended-hours HD group, but not in the conventional HD group (Fig. 3B and E). Calcium–phosphorus products correlated with ln plasma CPP (CPP-II) and ln serum CPP (CPP-I + II) levels in both the extended-hours and conventional HD groups (Fig. 3C and F).

The results of the multivariate linear regression analysis are presented in Table 2. Extended-hours HD was significantly associated with lower plasma CPP (CPP-II) and serum CPP (CPP-I + II) levels, independent of serum phosphorus levels, calcium levels and medications for CKD-MBD, i.e. phosphate binders, vitamin D receptor activators and calcimimetics. In the IPW-PS analysis, extended-hours HD was associated with lower plasma CPP (CPP-II) and serum CPP (CPP-I + II) levels (Supplementary Table S1). For the fit of PS analysis, the C-statistic of logistic regression analysis for PS was 0.817, and absolute standardized difference values were <0.1 for all covariates (Supplementary Table S2).

Impact of extended-hours HD on the association between CPP and phosphorus levels

The RCS functions showed a non-linear association between phosphorus and CPP levels, with a significant *P*-value for the interaction between extended-hours HD and serum phosphorus levels for CPP. Plasma CPP (CPP-II) levels increased exponentially with the increase in phosphorus levels in both the conventional and extended-hours HD groups, but more gradually in the extended-hours HD group, with a significant interaction (Fig. 4). Serum CPP (CPP-I + II) levels also increased exponentially with elevated phosphorus levels in both the conventional and extended-hours HD groups, without a significant interaction (Supplementary Fig. S2). The results of the IPW-PS analysis were similar for plasma CPP (CPP-II) and serum CPP (CPP-I + II) levels (Supplementary Fig. S3).

DISCUSSION

This is the first study to compare CPP levels between patients undergoing extended-hours HD and those receiving conventional

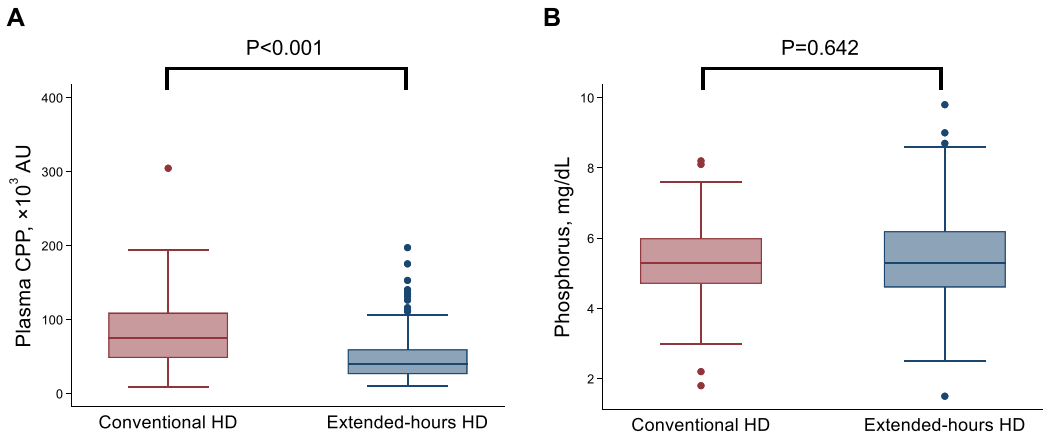


Figure 2: Differences in plasma CPP and phosphorus levels between the extended-hours and conventional HD groups. Box and whisker diagrams representing differences in plasma CPP and serum phosphorus levels between the extended-hours HD and conventional HD groups. The centre line and box indicate the median and the first and third quartiles. The whiskers on both sides indicate the minimum and maximum values, and the points outside the whiskers are outliers. P is the *P*-value according to the Mann–Whitney U test for comparisons between the extended-hours and conventional HD groups. **(A)** Plasma CPP (CPP-II) levels in the extended-hours HD group were significantly lower than in the conventional HD group. **(B)** Serum phosphorus levels were not significantly different between the extended-hours and conventional HD groups.

Table 2: Multivariable linear regression models for CPP parameters.

	ln plasma CPP (AU)			ln serum CPP (AU)		
Variables	Coefficient	95% CI	*P*-value	Coefficient	95% CI	*P*-value
Sex (male)	−0.09	−0.18 to −0.00	.04	−0.05	−0.14–0.04	.29
Age (+1 year)	0.00	0.00–0.00	.71	0.00	−0.01–0.00	.26
Extended-hours HD (versus conventional HD)	−0.64	−0.74 to −0.55	<.01	−0.42	−0.51 to −0.32	<.01
Duration of dialysis (years)						
0–1.9		Reference			Reference	
2–9.9	0.00	−0.11–0.10	.94	0.03	−0.08–0.13	.63
≥10	−0.04	−0.15–0.07	.47	−0.02	−0.13–0.10	.76
Diabetes mellitus	−0.02	−0.10–0.05	.52	−0.04	−0.12–0.03	.29
Phosphate binders use	−0.05	−0.14–0.05	.33	0.01	−0.09–0.10	.90
Vitamin D receptor activators use	−0.09	−0.17–0.00	.05	−0.05	−0.14–0.03	.24
Calcimimetics use	−0.06	−0.15–0.02	.15	0.00	−0.09–0.08	.97
Albumin (+1 g/dl)	0.06	−0.09–0.20	.44	−0.01	−0.16–0.13	.82
Creatinine (+1 mg/dl)	0.02	0.00–0.04	.07	0.00	−0.02–0.02	.82
Calcium (+1 mg/dl)	0.13	0.05–0.21	<.01	0.20	0.12–0.28	<.01
Phosphorus (+1 mg/dl)	0.38	0.34–0.42	<.01	0.37	0.33–0.41	<.01
Magnesium (+1 mg/dl)	0.10	−0.02–0.21	.11	0.05	−0.07–0.17	.42
Zinc (+1 μg/dl)	0.00	0.00–0.00	.55	0.00	0.00–0.00	.21
ln iPTH (pg/ml)	−0.01	−0.06–0.05	.83	−0.01	−0.07–0.04	.59
ln iFGF23 (pg/ml)	0.03	−0.00–0.06	.09	0.01	−0.02–0.04	.42
ln CRP (mg/dl)	0.00	−0.02–0.03	.75	0.00	−0.03–0.03	.96

HD. This study showed that patients on extended-hours HD had significantly lower CPP levels than those on conventional HD regardless of phosphorus and calcium levels. Multivariate analysis showed a stronger effect of phosphorus on plasma CPP (CPP-II) and serum CPP (CPP-I + II) than calcium, and the relationship between CPP and phosphorus levels differed among HD groups. Plasma CPP (CPP-II) levels, which have pathogenic activity by inducing calcification and immune responses [14], increased more gradually with increasing phosphorus levels in the extended-hours HD group than in the conventional HD group. These results suggest that phosphorus-induced vascular calcification is less likely to occur in patients on extended-hours HD, which may partially explain their favourable clinical outcomes.

CPP-I is 50–100 nm in diameter and CPP-II is even larger [30], thus extended-hours HD is not expected to increase CPP removal. Extended-hours HD was associated with lower serum CPP (CPP-I + II) levels regardless of phosphorus and calcium levels, indicating that prolonged dialysis treatment time combined with a liberalized diet may inhibit CPP production from serum phosphorus and calcium. Formation of CPP-I depends on the presence of fetuin-A, in addition to serum calcium phosphate [31], and low fetuin-A levels are a risk factor for CVD in ESKD

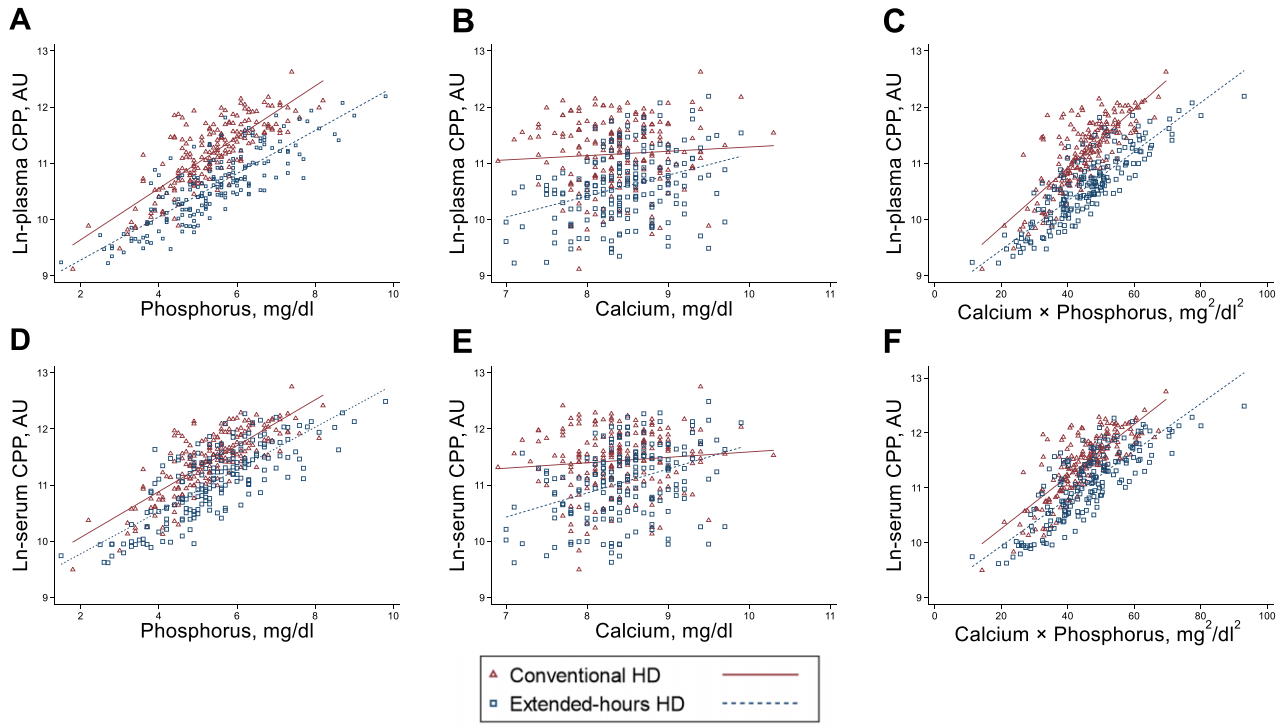


Figure 3: Correlation between CPP and serum parameters. Triangles outlined in red indicate values in the conventional HD group and squares outlined in blue indicate in the extended-hours HD group. The solid red line represents the approximate curve for the conventional HD group and the dotted blue line represents the extended-hours HD group, and these approximate curves express the correlation between CPPs and each serum parameter. **(A)** Correlation between serum phosphorus and ln transformation of plasma CPP (CPP-II) levels (conventional HD group: $r = 0.80$; extended-hours HD group: $r = 0.83$). **(B)** Correlation between serum calcium and ln plasma CPP (CPP-II) levels (conventional HD group: $r = 0.07$; extended-hours HD group: $r = 0.34$). **(C)** Correlation between calcium–phosphorus products and ln plasma CPP (CPP-II) levels (conventional HD group: $r = 0.79$; extended-hours HD group: $r = 0.86$). **(D)** Correlation between serum phosphorus and ln serum CPP (CPP-I + II) levels (conventional HD group: $r = 0.79$; extended-hours HD group: $r = 0.78$). **(E)** Correlation between serum calcium and ln serum CPP (CPP-I + II) levels (conventional HD group: $r = 0.10$; extended-hours HD group: $r = 0.38$). **(F)** Correlation between calcium–phosphorus products and ln serum CPP (CPP-I + II) levels (conventional HD group: $r = 0.80$; extended-hours HD group: $r = 0.82$).

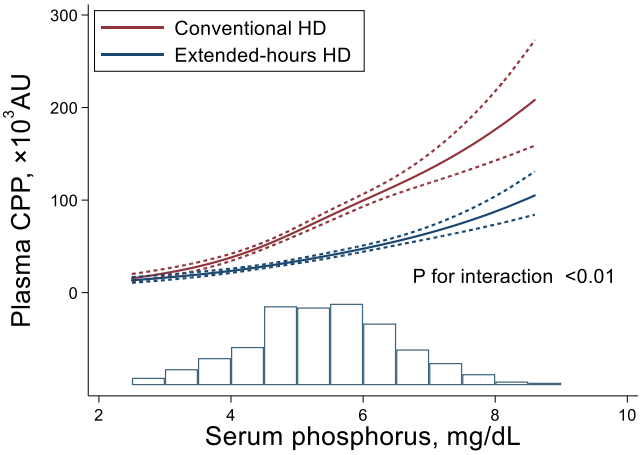


Figure 4: Association between plasma CPP and phosphorus levels. Restricted cubic spline curves with 95% confidence intervals show the non-linear association between serum phosphorus and plasma CPP levels. The red lines are for the conventional HD group and the blue lines are for the extended-hours HD group. The histograms show the distribution of serum phosphorus levels. Extended-hours HD and serum phosphorus levels had significant interactions for plasma CPP (CPP-II); plasma CPP (CPP-II) increased more gradually with elevated phosphorus levels in the extended-hours HD group than in the conventional HD group.

patients owing to the inability to form CPP-I [32]. Low fetuin-A levels could have been associated with lower serum CPP levels, however, patients in the extended-hours HD group who were younger and had lower CRP and higher albumin levels could be more likely to have higher fetuin-A levels than patients in the conventional HD group [32, 33]. Therefore, serum calcium phosphate saturation or metabolism might differ between patients in the extended-hours and conventional HD groups.

This study showed that plasma CPP levels, representing CPP-II *in vivo*, were increased more gradually with elevated phosphorus levels in the extended-hours HD group. In contrast, when we used serum samples to measure CPPs, we did not find a statistically significant interaction in CPP levels, representing total CPP (CPP-I and CPP-II), between phosphorus levels and extended-hours HD. These results indicate that extended-hours HD may inhibit phase conversion from CPP-I to CPP-II. Magnesium, calcium, phosphorus, fetuin-A and pH levels have been reported as factors affecting phase transitions of CPP [31]. In the multivariable analysis adjusted for magnesium, calcium, phosphorus and other variables, extended-hours HD was associated with lower plasma CPP (CPP-II) levels (Fig. 4 and Table 2). These results suggest that extended-hours HD may have inhibited CPP phase transition via higher fetuin-A levels or improving acidosis. Given that the association between CPP and phosphorus levels differed among HD groups, the acceptable upper and lower limits for serum phosphorus in patients undergoing

extended-hours HD could differ from those in patients undergoing conventional HD. A previous study reported that both hyper- and hypophosphataemia in patients on maintenance HD were associated with higher mortality; however, the association between hypophosphataemia and mortality was attenuated when adjusted for malnutrition–inflammation–cachexia syndrome [34]. These changes in CPP dynamics may result in the liberalization of dietary intake and may be one of the possible mechanisms for improved outcomes in patients receiving extended-hours HD.

We initially hypothesized that patients undergoing extended-hours HD would exhibit lower CPP levels than those undergoing conventional HD, which was attributed to decreased phosphorus levels. Previous studies have shown that a prolonged dialysis treatment time is associated with lower phosphorus levels [21, 35]; however, in this study, phosphorus and calcium levels were similar between the extended-hours and conventional HD groups. The first reason is that patients in the extended-hours HD group were instructed to consume their preferred diet and may have had a higher phosphorus intake than those in the conventional HD group. Second, clinicians in both HD groups adhered to similar target levels for phosphorus and calcium, which are set at 3.5–6 mg/dl and 8.4–10 mg/dl, respectively, for patients with maintenance HD in Japan [36]. The fact that 70% of the patients in both groups had phosphorus and calcium levels within this target range indicated high guideline compliance and increased the generalizability of this study.

Our study had several limitations. First, there might be an indication bias regarding the patients' selection of conventional or extended-hours HD. However, the extended-hours HD facilities in this study were located in rural areas with limited healthcare resources, limiting the choice of other dialysis modalities for patients on extended-hours HD. In addition, we addressed potential confounders using IPW-PS as a sensitivity analysis to ensure that the results were consistent. Second, the pathophysiological mechanisms underlying the effects of extended-hours HD on CPP remain unknown. Third, we did not assess whether the association between extended-hours HD and lower CPP affected mortality. Fourth, the levels of fetuin-A and pH values in the same sample were unavailable. Further research on various biomarkers, including uraemic retention solutes or metabolites, is needed to improve the clinical outcomes in patients undergoing maintenance HD.

In conclusion, extended-hours HD was associated with lower plasma CPP (CPP-II) levels than conventional HD, especially under hyperphosphataemic conditions. CPP levels were increased exponentially with the elevation of phosphorus levels in both extended-hours and conventional HD groups, but more gradually in the extended-hours HD group. Our results suggest that the acceptable upper limit of serum phosphorus levels in patients undergoing extended-hours HD may differ from that in patients undergoing conventional HD.

SUPPLEMENTARY DATA

Supplementary data are available at *ckj* online.

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AUTHORS' CONTRIBUTIONS

N.N., T.I., M.O. and M.H. were responsible for the research and study design. N.N., M.O., H.K. and F.K. were responsible for data acquisition. N.N., M.K. and Y.M. measured iFGF23 and CPPs. N.N. and T.I. were responsible for the statistical analysis. N.N., T.I., M.O., M.H. and S.K. were responsible for the data analysis and interpretation. T.I., M.O., S.K., T.K., N.K. and S.M. were responsible for supervision and mentorship. All the authors approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data underlying this article will be shared upon reasonable request to the corresponding author.

CONFLICT OF INTEREST STATEMENT

T.I. received a research grant from Kyowa Kirin and consulting fees from GlaxoSmithKline. No conflicts of interest (financial or otherwise) are declared by the other authors.

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