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Higher Intra-Dialysis Serum Phosphorus Reduction Ratio as a Predictor of Mortality in Patients on Long-Term Hemodialysis

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Background: Rapid shifting between extracellular and intracellular phosphorus can occur during dialysis sessions, which can cause aberrant intracellular signaling in long-term hemodialysis (LTHD) patients. However, the effect of these intra-dialysis fluctuations of phosphorus on clinical outcomes has not been examined. Therefore, we investigated the relationship between intradialysis serum phosphorus reduction ratio (IDSPRR) and mortality in LTHD patients.

Material/Methods: This was a retrospective, observational cohort study to assess the predictive power of IDSPRR (>0.63 vs. ≤0.63) on mortality in a total of 805 LTHD patients. All these fatal events were analyzed using the Cox proportional hazards regression model.





Results: After multivariable analysis, baseline IDSPRR higher than 0.63 was significantly predictive of all-cause mortality (hazard ratio [HR]: 1.58; 95% confidence interval [CI]: 1.10–2.26), but not for cardiovascular (CV) mortality (HR: 1.41; 95% CI: 0.91–2.18). However, when time-varied IDSPRRs were applied, a value greater than 0.63 was not only significantly predictive of all-cause mortality (HR: 1.74, 95% CI: 1.16–2.63), but also CV mortality (HR: 2.04, 95% CI: 1.23–3.40).

Conclusions: High IDSPRR (>0.63) is independently associated with increased all-cause and CV mortality, which shows the negative effect of rapid intracellular phosphorus-shifting on LTHD patients.

MeSH Keywords: **Fetal Mortality • Hemodialysis Units, Hospital • Phosphorus**

Abbreviations: **LTHD** – long-term hemodialysis; **DOPPS** – Dialysis Outcome and Practice Patterns Study; **CV** – cardiovascular; **CKD** – chronic kidney disease; **MDRD** – Modification of Diet in Renal Disease; **KEEP** – Kidney Early Evaluation Program; **HD** – hemodialysis; **IDPRR** – intradialysis serum phosphorus reduction ratio; **CTR** – cardiothoracic ratio; **Kt/V** – urea kinetics; **DM** – diabetes mellitus; **RAS** – renin-angiotensin system; **IQR** – interquartile range; **ANOVA** – analysis of variance

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Background

Patients undergoing long-term hemodialysis (LTHD) have a substantially reduced life expectancy, mostly due to fatal cardiovascular disease (CVD) [1–3]. Identifying risk factors of mortality in LTHD patients may aid early intervention and improve outcomes. Several studies have suggested that tight control of serum phosphorus levels may be one such approach [4,5]. In fact, hyperphosphatemia is quite common in LTHD patients. According to the Dialysis Outcome and Practice Patterns Study (DOPPS), more than one-fourth of LTHD patients experienced hyperphosphatemia [6]. This hyperphosphatemia is not only due to impaired renal clearance, but also to bone resorption, which can then lead to secondary hyperparathyroidism [7–9], endothelial dysfunction [10], vascular or soft tissue calcification [11], atherosclerosis [12], and cellular or tissue injury [13].

Data from the United States [14], Taiwan [15], and DOPPS [6] disclosed an association between high serum phosphorus levels and all-cause or cardiovascular (CV) mortality in patients on LTHD. This association was also found in pre-dialysis chronic kidney disease (CKD) patients, transplant recipients [16], and the normal population [17]. Moreover, serum phosphorus levels within the normal range were also demonstrated to be significantly associated with poor clinical outcomes in pre-dialysis CKD patients [18], transplant recipients [19], and the normal population [20,21]. Although this association has been reported, several studies found conflicting results. For example, 2 controlled studies (Modification of Diet in Renal Disease [MDRD] and the Kidney Early Evaluation Program [KEEP]), found that high serum phosphate level was not an independent risk factor for mortality in pre-dialysis CKD patients [22]. Furthermore, in contrast to the adjustment for case-mix and serum albumin, Rivara et al. observed a reverse association between serum phosphorus level and all-cause and CV mortality in unadjusted analysis in patients on either hemodialysis (HD) or peritoneal dialysis [24]. These controversial findings suggest that serum phosphorus level is profoundly influenced by multiple confounding factors and is not consistently related to poor outcomes in long-term dialysis patients. Therefore, biomarkers other than serum phosphorus level are needed to predict outcomes.

Phosphorus is ubiquitously distributed in the body, with 85% stored in bone and teeth, 14% in the intracellular space, and less than 1% in the extracellular fluid. As less than 0.5% of total body phosphorus content is found in the blood, serum phosphorus level is maintained within a narrow range and is regarded as a biomarker for phosphorus homeostasis and for clinical outcomes in dialysis patients. Approximately 600–700 mg of phosphorus are removed during a conventional HD session; however, 90% of this may come from intracellular spaces [23]. Using magnetic resonance spectroscopy to evaluate

phosphorus kinetics, Lemoine et al. showed that intracellular phosphorus contents increased and extracellular phosphorus decreased during a 3-h HD session in an animal study [24]. Since intracellular phosphorus is responsible for the phosphorylation of various proteins, such shifting between extracellular and intracellular phosphorus contents can induce abnormal cellular signaling and thus lead to cell and tissue damages [13]. Rapid changes in serum phosphorus level and shifting of intracellular phosphorus contents may occur during dialysis, and such negative effects can be repeated 2 to 3 times per week in patients undergoing LTHD.

We postulated that changes in the intradialysis serum phosphorus reduction ratio (IDSPRR), a surrogate for dynamic intracellular phosphorus shifting occurring during dialysis sessions, can be used as a predictor of mortality in patients on LTHD. The effect of this dynamic intracellular phosphorus shifting on clinical outcomes in LTHD patients has not been previously assessed; therefore, we conducted the present study.

Material and Methods

Study design and patients

This retrospective, observational, cohort study was based on the records obtained from patients who underwent HD for at least 3 months between December 2006 and December 2012 in a medical center. Patients enrolled had to be clinically stable and without hospitalization due to acute events related to cardiovascular, cerebrovascular, infectious, or other active diseases 3 months preceding the beginning of data collection. The follow-up for patient's outcomes was up to December 2013 (Figure 1). This study was performed in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Shin-Kong Wu Ho-Su Memorial Hospital (protocol No. 20160809R). Informed consent was waived because the study was based on medical chart review. Patient information was anonymized and de-identified prior to analysis.

Demographic and laboratory data

Demographic and biochemical data were acquired from medical records, which included age; sex; HD vintage; cardiothoracic ratio (CTR); levels of blood urea nitrogen, serum creatinine, albumin, uric acid, total cholesterol, triglyceride, hemoglobin, intact parathyroid hormone, ionized calcium, serum phosphorus before and after HD, and alkaline phosphatase; iron profile; urea kinetics (Kt/V); history of diabetes mellitus (DM), hypertension, coronary artery disease, or cerebrovascular disease; and prescription of renin-angiotensin system (RAS) blockers, lipid-lowering agents, beta-blockers, or anti-platelet agents.

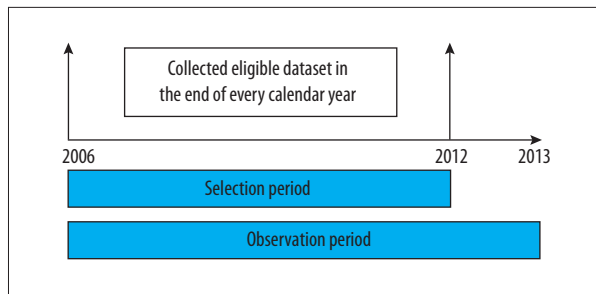


Figure 1. Selection of records of patients on long-term hemodialysis.

We defined CVD based on a documented history of coronary artery or cerebrovascular disease. Coronary artery disease was diagnosed according to history of exertional angina, significant arterial occlusive disease disclosed by angiogram, past myocardial infarction, or a history of coronary artery intervention. Cerebrovascular disease was defined according to the history of cerebrovascular accidents, either hemorrhage or infarction. Fasting blood samples were collected for biochemical analysis before and immediately after the dialysis session, which were measured using an autoanalyzer (Beckman, Lane Cove, NSW, AU). The intact parathyroid hormone was measured using the Roche Elecsys assay (Roche Diagnostic, Penzberg, Germany). IDSPRR was calculated according to the following formula:

$$\text{IDSPRR} = \frac{\text{Serum phosphorus level before dialysis} - \text{Serum phosphorus level after dialysis}}{\text{Serum phosphorus level before dialysis}}$$

The IDSPRR was classified into 4 quartiles for further analysis.

Statistical analysis

Data are expressed as mean \pm standard deviation or median and interquartile range (IQR) as appropriate for continuous data and number (%) for categorical data. The Kruskal-Wallis test or analysis of variance (ANOVA) was used to compare the means of continuous variables according to whether the normal distribution assumptions were violated or not, and the χ^2 test was used for categorical variables between groups. Survival curves were estimated by using the Kaplan-Meier method and tested by the log-rank test. Moreover, a Cox proportional regression model with/without time-dependent covariates was used to determine the risk of death. A bivariate parameter of IDSPRR (>0.63 vs. ≤ 0.63) was chosen for survival analysis due to the results of Kaplan-Meier survival curves of IDSPRR. The assumption of proportionality was not violated by testing for interaction between time and variables. We also performed subgroup analysis for the following variables: sex, age (≤ 65 and >65 years), DM, serum phosphorus level (≤ 5 and >5 mg/dL), and CTR (≤ 0.5 and >0.5). A two-tailed P value of <0.05 was considered statistically significant. All statistical

Table 1. Baseline characteristics of study population.

Characteristic	All (n=805)
Age (years)	63.3 \pm 13.2
Male gender (%)	396 (49)
Duration of dialysis (years)	1.9 (0.7, 7.1)
Diabetes mellitus (%)	342 (43)
Cardiovascular disease (%)	226 (28)
Hypertension (%)	336 (42)
Uric acid (mmol/L)	0.40 \pm 0.14
Albumin (g/L)	41 \pm 4
Triglyceride (mmol/L)	1.41 (0.92, 2.23)
Cholesterol (mmol/L)	4.53 \pm 1.14
Low density lipoprotein (mmol/L)	2.72 \pm 0.93
High density lipoprotein (mmol/L)	1.21 \pm 0.44
Kt/V	1.34 \pm 0.24
Hemoglobin (g/L)	103 \pm 14
Transferrin saturation (%)	34.4 \pm 15.6
Ionized calcium (mmol/L)	1.15 \pm 0.13
Phosphate pre-dialysis (mmol/L)	1.68 \pm 0.48
Phosphate post-dialysis (mmol/L)	0.71 \pm 0.19
Alkaline phosphatase (U/L)	80 (60, 109)
iPTH (ng/L)	102 (48, 222)
Cardiothoracic ratio (%)	50.7 \pm 6.8
Medications	
Anti-platelet agents (%)	285 (36)
RAS blockaders (%)	278 (35)
Beta-blocker (%)	159 (20)
Lipid-lowering agents (%)	150 (19)

Kt/V – urea kinetics; IDPRR – intra-dialysis serum phosphate reduction ratio; iPTH – intact parathyroid hormone; RAS – renin-angiotensin system

analyses were performed using SAS for Windows version 9.4 (SAS Institute, Inc., Cary, NC, USA).

Results

With a mean duration of 4.4 \pm 2.3 years follow-up in 805 LTHD patients, the mean age, HD vintage, and CTR were 62.3 \pm 13.2 years, 1.9 (IQR, 0.7–7.1) years, and 50.7 \pm 6.8%, respectively. Among them, 49% were male, 43% had DM, 42% had hypertension, and 28% had CVD. The mean pre-dialysis phosphorus

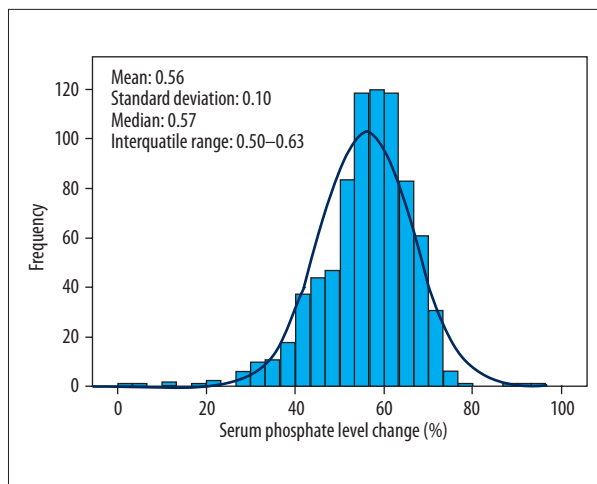


Figure 2. The distribution of intradialysis serum phosphorus reduction ratios in patients on long-term hemodialysis.

level was 5.2 ± 1.5 mg/dL and it decreased to 2.2 ± 0.6 mg/dL immediately after dialysis (Table 1). The distribution of IDSPRR is shown in Figure 2, with a mean value of 0.56 ± 0.10 .

The comparison between quartile of IDSPRR

As shown in Table 2, the difference across 4 subgroups (IDSPRR, ≤ 0.50 ; $0.51-0.57$; $0.58-0.63$; >0.63) was statistically significant with respect to sex, HD vintage, high-density lipoprotein, ionized calcium, serum phosphorus, and uric acid levels, dialysis adequacy (Kt/V), alkaline phosphatase, CTR, and the use of anti-platelet agents and beta-blockade medication (all $P < 0.05$). Moreover, non-significant factors across subgroups included age; history of DM, CAD, and hypertension; medications consumed for RAS blockade and lipid-lower agents; and levels of serum albumin, total cholesterol, triglyceride, low-density lipoprotein, hemoglobin, transferrin saturation, and intact parathyroid hormone.

All-cause mortality in patients on LTHD

In the follow-up period, 272 episodes of all-cause deaths were ascertained, which included 187 fatal CV events, 31 cerebrovascular events, 15 malignancies, 10 infectious diseases, 8 gastrointestinal bleeding events, and 21 of unknown etiology. Results of a Cox proportional hazards regression analysis for serum phosphorus level and mortality are shown in Table 3. In this study, baseline serum phosphorus levels and time-dependent variables were not significantly related to all-cause mortality in LTHD patients. However, when adjusting demographic data, comorbidity, dialysis-related parameters, and medications consumed, adjusted hazard ratio (aHR) of the baseline, and time-dependent IDSPRR (>0.63 vs. ≤ 0.63) for all-cause mortality were significant (1.58; 95% CI: 1.10–2.26 and 1.74; 95% CI: 1.16–2.63, respectively), but aHR of the

time-dependent IDSPRR (>0.63 vs. ≤ 0.63) remained higher (1.74 vs. 1.58). Kaplan-Meier survival curves of all-cause mortality in the tertiles of IDSPRR is delineated in Figure 3A. Only the IDSPRR >0.63 had significant difference in all-cause mortality compared to the other 3 groups ($\chi^2=3.97$, $P=0.046$ to 1st quartile; $\chi^2=9.21$, $P=0.002$ to 2nd quartile; $\chi^2=4.55$, $P=0.033$ to 3rd quartile) by using the log-rank test.

Cardiovascular mortality in patients on LTHD

In the follow-up period, 187 fatal CV events were ascertained. According to the results of a Cox proportional hazards regression analysis, baseline serum phosphorus levels and time-dependent variables were not associated with CV mortality in LTHD patients (Table 3). Furthermore, after adjusting demographic data, comorbidity, dialysis-related parameters, and medications consumed, only aHR of the time-dependent IDSPRR (>0.63 vs. ≤ 0.63), but not baseline IDSPRR, for CV mortality was significant (aHR, 2.04; 95% CI: 1.23–3.40) (Table 4). Kaplan-Meier survival curves of CV mortality in the tertiles of IDSPRR are delineated in Figure 3B. Only the IDSPRR quartile between >0.63 and $0.50-0.57$ had a significant difference in CV mortality ($\chi^2=5.76$, $P=0.016$) by using the log-rank test.

Subgroup analysis

We also analyzed the association of IDSPRR (>0.63 vs. ≤ 0.63) with CV mortality stratified by covariates, including history of DM, age (≤ 65 and >65 years), sex, phosphorus level (≤ 5 and >5 mg/dL), and CTR (≤ 0.5 and >0.5). As shown in Figure 4, after multivariable adjustments, the aHR of time-dependent IDSPRR (>0.63 vs. ≤ 0.63) for all-cause mortality was significant only in LTHD patients who were male (2.95; 95% CI: 1.27–6.33), age older than 65 years (2.13; 95% CI: 1.11–4.09), non-diabetic (3.77; 95% CI: 1.80–7.90), with pre-dialysis phosphorus 5 mg/dL or less (2.36; 95% CI: 1.13–4.50), and CTR less than 0.5 (2.24; 95% CI: 1.02–4.92). The aHR of time-dependent IDSPRR (>0.63 vs. ≤ 0.63) for CV mortality was also significant in LTHD patients who were male (2.65; 95% CI: 1.397–5.05), age older than 65 years (1.72; 95% CI: 1.01–2.93), non-diabetic (2.07; 95% CI: 1.170–3.66), and with pre-dialysis phosphorus 5 mg/dL or less (1.86; 95% CI: 1.03–3.35).

Discussion

In this retrospective, observational, cohort study of 805 patients on LTHD, with a follow-up period up to 7 years, high IDSPRR (>0.63) was independently associated with increased all-cause and CV mortality, even after adjusting for the pre-dialysis serum phosphorus levels in the time-dependent Cox regression model. However, baseline and time-varying serum phosphorus levels in our LTHD patients were not significantly

Table 2. The comparison of baseline characteristic among quartiles of IDSPRR.

Characteristic	Intra-dialysis serum phosphate reduction ratio				P value
	0.50 (n=204)	0.51–0.57 (n=193)	0.58–0.63 (n=209)	>0.63 (n=199)	
Age (years)	62.7±14.5	62.4±13.8	63.4±12.8	64.9±11.7	0.256
Male gender (%)	136 (67)	106 (55)	96 (46)	58 (29)	<0.001
Duration of dialysis (years)	0.9 (0.4, 4.3)	2.3 (0.8, 8.5)	2.2 (0.7, 6.4)	3.2 (0.9, 9.7)	<0.001
Diabetes mellitus (%)	93 (46)	85 (44)	86 (41)	78 (40)	0.598
Cardiovascular disease (%)	68 (34)	58 (30)	57 (27)	43 (22)	0.067
Hypertension (%)	96 (47)	80 (41)	77 (37)	83 (42)	0.217
Uric acid (mg/dL)	6.1±2.8	7.0±1.6	7.0±2.2	6.9±2.1	<0.001
Albumin (g/L)	41±04	42±4	41±4	41±4	0.322
Triglyceride (mmol/L)	1.47 (0.96, 2.27)	1.49 (0.95, 2.57)	1.39 (0.90, 2.03)	1.37 (0.89, 2.09)	0.432
Cholesterol (mmol/L)	4.51±1.14	4.53±1.17	4.48±1.11	4.58±1.11	0.849
LDL (mmol/L)	2.72±0.98	2.69±0.85	2.69±0.90	2.72±0.93	0.984
HDL (mmol/L)	1.21±0.39	1.27±0.44	1.32±0.41	1.42±0.49	<0.001
Kt/V	1.24±0.22	1.32±0.18	1.34±0.20	1.48±0.27	<0.001
Hemoglobin (g/L)	102±16	105±14	104±16	101±12	0.131
Transferrin saturation (%)	33.6±15.6	35.2±15.4	34.4±17.1	34.7±14.0	0.801
Ionized calcium (mmol/L)	1.13±0.10	1.15±0.10	1.15±0.13	1.18±0.10	0.001
Phosphate pre-HD (mmol/L)	1.36±0.71	1.62±0.68	1.84±0.45	1.97±0.48	<0.001
Phosphate post-HD (mmol/L)	0.74±0.23	0.74±0.16	0.71±0.16	0.65±0.16	<0.001
Alkaline phosphatase (U/L)	86 (62, 117)	77 (58, 101)	86 (64, 115)	77 (58, 105)	0.017
iPTH (ng/L)	101 (46, 188)	90 (51, 202)	102 (48, 223)	123 (46, 249)	0.868
Cardiothoracic ratio (%)	49.5±6.7	49.7±6.1	51.1±7.4	52.3±6.6	0.001
Medications					
Anti-platelet agents (%)	89 (44)	61 (32)	72 (35)	63 (32)	0.037
RAS blockaders (%)	69 (34)	64 (33)	71 (34)	74 (38)	0.803
Beta-blocker (%)	50 (25)	25 (13)	48 (23)	36 (18)	0.015
Lipid-lowering agents (%)	41 (20)	39 (24)	33 (26)	37 (19)	0.640

Kt/V – urea kinetics; IDSPRR – intra-dialysis serum phosphate reduction ratio; iPTH – intact parathyroid hormone; RAS – renin-angiotensin system

associated with mortality. In our subgroup analysis, high time-varying IDSPRR was significantly associated with all-cause and CV mortality only in patients who were male, age over 65 years, non-diabetic, and with serum phosphorus level 5 mg/dL or less. Accordingly, we speculated that IDSPRR increases the risk of death through mechanisms other than high serum phosphorus level itself. In fact, the same serum phosphorus

level may arise from different confounding factors that have various impacts on clinical outcomes. In contrast, IDSPRR can avoid these confounding influences and simply reflects the absolute changes of extracellular and intracellular phosphorus during HD sessions. This novel finding expands our understanding of the association between aberrant phosphorus homeostasis during dialysis and outcomes in patients on LTHD.

Table 3. Multivariable Cox regression analysis of the risk factor of serum phosphate level for mortality in patients with chronic hemodialysis.

	Pre-dialysis phosphate level		Post-dialysis phosphate level	
	aHR (95% CI)	P value	aHR (95% CI)	P value
All-cause mortality				
Baseline model	1.00 (0.90–1.12)	0.958	0.88 (0.64–1.21)	0.444
Time-vary model	0.99 (0.97–1.13)	0.893	0.69 (0.47–1.02)	0.068
Cardiovascular death				
Baseline model	1.11 (0.97–1.26)	0.105	1.17 (0.80–1.70)	0.421
Time-vary model	1.09 (0.93–1.27)	0.299	0.83 (0.52–1.33)	0.452

Multivariable model is adjusted for age*, gender*, hemodialysis vintage*, diabetes mellitus*, cardiovascular disease*, hypertension*, cardiothoracic ratio, high dense lipoprotein, albumin, hemoglobin, KT/V, ionized calcium, phosphate, alkaline phosphatase, antiplatelet, renin angiotensin blockade, beta-blocker and lipid-lowering agents. * Indicates invariable parameter in time-vary Cox regression model. aHR – adjusted hazard ratio; CI – confidence interval; Kt/V – urea kinetics.

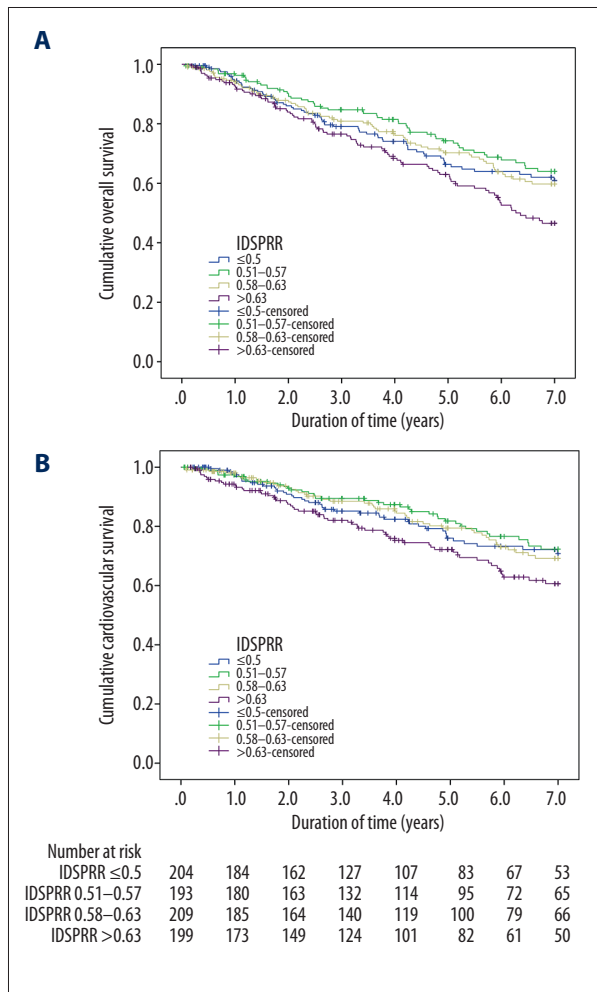


Figure 3. Kaplan-Meier cumulative survival plots of (A) overall mortality (B) cardiovascular death in the study population according to groups of intradialysis serum phosphorus reduction ratios (IDSPRR). A significant difference in mortality was noted between the groups of the IDSPRR >0.63 and other 3 groups (log-rank test: $\chi^2=3.97$, $P=0.046$ to 1st quartile; $\chi^2=9.21$, $P=0.002$ to 2nd quartile; $\chi^2=4.55$, $P=0.033$ to 3rd quartile) in overall mortality; and between >0.63 and 0.50–0.57 (log-rank test: $\chi^2=5.76$, $P=0.016$) in cardiovascular death.

Physiologically, phosphorus acts as an essential molecule in cell-signaling activities and serves as an energy provider through the formation of ATP, through which normal cellular functions are maintained. Therefore, an aberrant phosphorus homeostasis

may impair cell-signaling activities and cause further cell and tissue damages. Using radio-labeled phosphorous magnetic resonance spectroscopy, Lemoine et al. found that removal of extracellular phosphorus induced an elevation of intracellular phosphorus contents in the gluteal muscle region of pigs undergoing HD. This intracellular phosphorus retention may result in a decrease of β ATP and increase of cellular stress; both changes are toxic to mitochondria [24]. Moreover, elevated extracellular phosphorus can also enhance mitochondrial membrane potential by increasing the permeability of the transition pore, thus generating superoxide formation and inducing apoptosis [25]. Therefore, we hypothesized that the rapid decrease in extracellular phosphorus and shifting of intracellular phosphorus contents during dialysis can jeopardize normal cellular functions and induce apoptosis, which may explain the negative impact of IDSPRR on mortality in patients on LTHD.

The CTR, which is the ratio of cardiac and thoracic diameters, is estimated according to chest X-ray [26], and is usually regarded as a predictor of left ventricular systolic dysfunction in LTHD patients [27]. We previously reported that CTR was not only related to peripheral arterial disease [28], but also had a negative impact on long-term survival in our LTHD

Table 4. Multivariable Cox regression analysis of the risk factor of IDSPRR >0.63 (versus ≤0.63) for mortality in patients with chronic hemodialysis

	Baseline model		Time dependent model	
	aHR (95% CI)	P value	aHR (95% CI)	P value
All-cause mortality				
Model 1	1.58 (1.18, 2.11)	0.002	1.42 (1.05, 1.93)	0.023
Model 2	1.47 (1.09, 1.97)	0.010	1.34 (0.99, 1.82)	0.058
Model 3	1.54 (1.07, 2.21)	0.018	1.70 (1.13, 2.55)	0.010
Model 4	1.58 (1.10, 2.26)	0.013	1.74 (1.16, 2.63)	0.008
Cardiovascular death				
Model 1	1.55 (1.09, 2.21)	0.014	1.63 (1.12, 2.36)	0.009
Model 2	1.38 (0.97, 1.96)	0.077	1.48 (1.02, 2.14)	0.040
Model 3	1.33 (0.86, 2.06)	0.196	1.98 (1.20, 3.26)	0.007
Model 4	1.41 (0.91, 2.18)	0.127	2.04 (1.23, 3.40)	0.005

Multivariate model 1 is adjusted for age*, gender*, hemodialysis vintage* and pre-dialysis phosphate. Multivariate model 2 comprises model 1 as well as adjustments for diabetes mellitus*, cardiovascular disease*, and hypertension*. Multivariate model 3 comprises model 2 as well as adjustments for cardiothoracic ratio, high dense lipoprotein, albumin, hemoglobin, KT/V, ionized calcium, phosphate, alkaline phosphatase. Multivariate model 4 comprises model 3 as well as adjustments for medications of antiplatelet, renin angiotensin blockade, beta-blocker and lipid-lowering agents. * indicates invariable parameter in time-dependent Cox regression model. IDSPRR – intradialysis serum phosphate reduction ratio; aHR – adjusted hazard ratio; CI – confidence interval; Kt/V, urea kinetics.

patients [3,29,30]. An association between CTR and inflammation or malnutrition in non-diabetic patients who underwent HD was shown by Chen et al. [31]. In the present study, we found that patients with elevated IDSPRR had higher CTR value, suggesting these phosphorus changes are involved in cardiac remodeling, and contribute to poor CV outcome.

In our subgroup analysis, high IDSPRR (>0.63) was found to have significant predictive power for either all-cause or CV mortality only in LTHD patients who were male, aged over 65 years, not diabetic, and with pre-dialysis serum phosphorus level 5 mg/dL or less. Based on the observation of a sex-specific difference in the slope of chronic kidney disease progression, sex hormones may regulate phosphorus levels [32,33]. Further research is needed to assess their ability to protect against cell damage. As for the impact of age, it is reasonable to attribute this aging process to poor responses to phosphorus-induced injury. In addition, poor dialysis quality, history of DM, and CTR greater than 0.5 are all strong predictive factors of increased mortality in patients on LTHD, these might then negate the impact of IDSPRR on outcomes [34]. Even our results were not in agreement with the previous reports that higher pre-dialysis phosphorus levels are harmful to LTHD patients; instead, we found that high IDSPRR (>0.63) was significantly associated with deaths in LTHD patients, especially in patients whose pre-dialysis serum phosphorus levels were 5 mg/dL or less. Thus, our findings extend our ability to identify

high-risk LTHD patients whose serum phosphorus levels remain within normal range.

There are several strengths to our study. First, our follow-up period was long enough to include enough patients reaching the primary outcomes. Second, either serum phosphorus levels or IDSPRR was adjusted for several dialysis-related and time-varied factors rather than just adopted baseline parameters, thus providing more precise estimations. Despite its strength, our study has several potential limitations. First, this was a single-center, retrospective study, so that the findings could not be representative of all the patients undergoing LTHD. Multicenter studies or further studies that include different ethnicities are needed to confirm our findings. Second, although it is reasonable to assume that extracellular phosphorus fluctuations and rapid shifting of intracellular phosphorus contents may result in aberrant cell signaling and thus are toxic to cells, we still lack solid evidence in the literature to confirm these impacts in LTHD patients. The evidence we cited on intracellular phosphorus kinetic changes came a single animal study [24]; however, this group is now conducting a similar study in humans (clinicaltrials.gov identifier NCT03119818). Finally, IDSPRR is a ratio and not equal to the total quantity of phosphorus removed during an HD session. Nevertheless, it can be easily applied in clinical practice as a surrogate for intracellular phosphorus changes and as a predictor of mortality in LTHD patients.

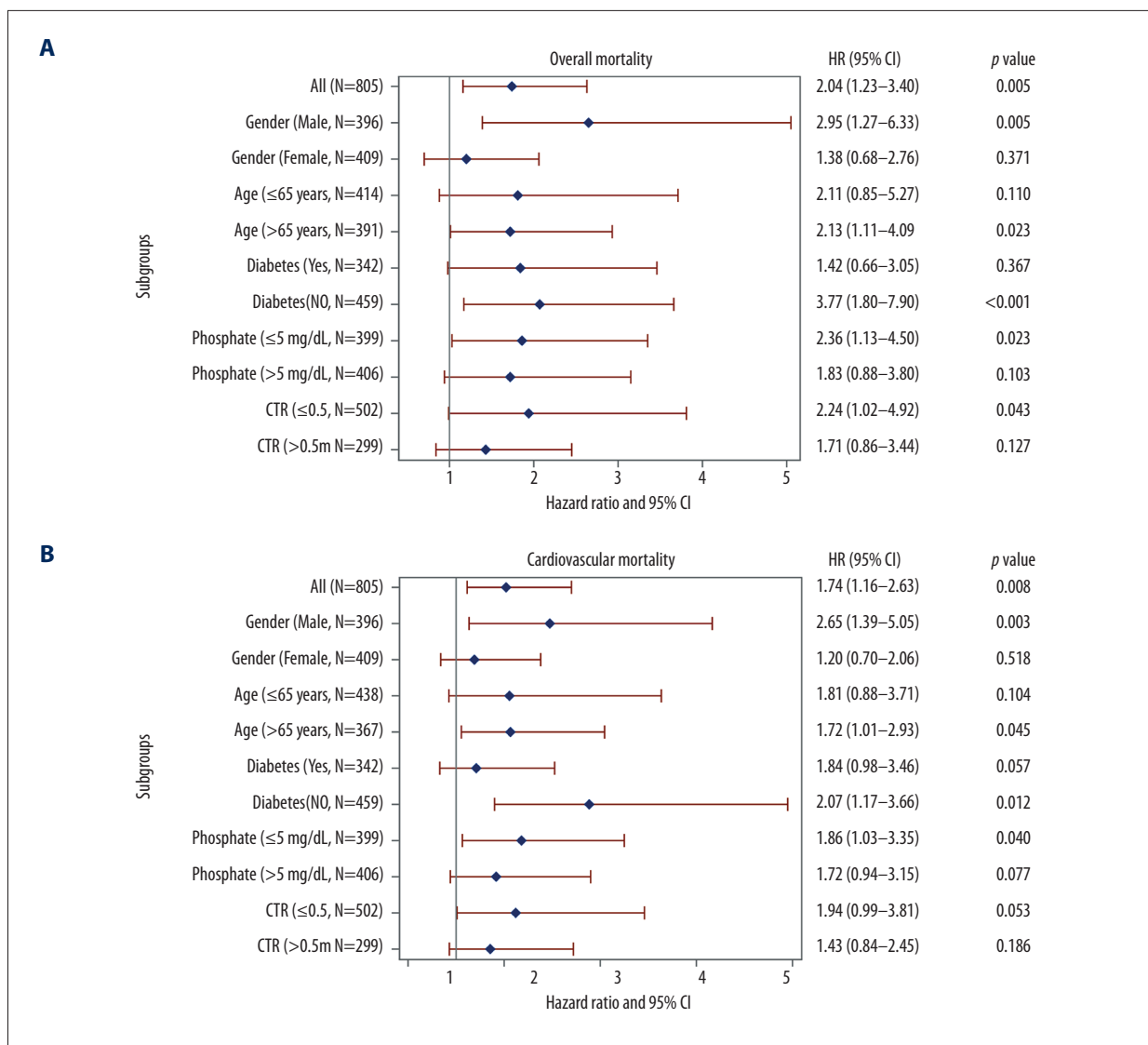


Figure 4. Subgroup analysis. The effect of intradialysis serum phosphorus reduction ratio (>0.63 vs. ≤0.63) on **(A)** overall mortality and **(B)** cardiovascular death among patients on long-term hemodialysis using time-dependent Cox regression model. The full model comprised adjusted variables as model 4 in Table 3.

Conclusions

We identified high IDSPRR (>0.63) as an independent predictor for all-cause and CV mortality in patients undergoing LTHD, especially in patients whose serum phosphorus levels were normal. In contrast to high serum phosphorus level, which may arise from various confounding factors, high IDSPRR usually indicates not only the higher pre-dialysis serum phosphorus

level, but also reflect the absolute changes of serum and intracellular phosphorus contents during HD sessions. Therefore, in addition to consistently controlling the pre-dialysis phosphorus level, avoiding elevated IDSPRR may be another issue that must be addressed to reduce mortality in LTHD patients. However, its mechanisms and the details of the signaling pathway changes during HD session warrant further exploration.

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