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ORIGINAL RESEARCH

Serum Hepcidin-25 and All-Cause Mortality in Patients Undergoing Maintenance Hemodialysis

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Background: Hepcidin plays an important role in iron homeostasis, inhibits intestinal iron absorption and iron release from hepatocytes and macrophages, while its clinical utility remained unclear. This study aimed to investigate the associations between hepcidin-25 and mortality in MHD patients.

Methods: This was a prospective observational cohort of 161 MHD patients, with 2-year follow-up. We investigated the relationships between the variables in our dataset, including serum hepcidin-25, demographic characteristics as well as other clinical parameters.

Results: The median value of baseline serum hepcidin-25 was 31.0 (12.1, 57.3) ng/mL; therefore, the patients were stratified into two groups (low-level hepcidin-25 group, and high-level hepcidin-25 group). The serum iron, serum ferritin, transferrin saturation (TSAT), and hsCRP were higher, pre-dialysis creatinine and albumin were lower, and the scores of health-related qualities of life were worse in the high-level hepcidin-25 group than in the low-level hepcidin-25 group. Maximal information-based nonparametric exploration analysis suggested that serum hepcidin-25 was associated with ferritin, TSAT, and all-cause mortality. The patients with hepcidin-25<31 ng/mL had better survival outcomes than those with hepcidin-25≥31 ng/mL during the 24-month follow-up (Log rank test, P = 0.0017). For per 10ng/mL increase of serum hepcidin-25, the hazard ratio (HR) for all-cause mortality was 1.225 (95% confidence interval [CI]1.085–1.382, P<0.001), which remained significant after multivariate adjustments.

Conclusion: Serum hepcidin-25 was associated with ferritin and TSAT, and could be an independent predictor for all-cause mortality in MHD patients. Further research with larger sample size and longer-term follow-up is still needed.

Keywords: hepcidin, mortality, hemodialysis, survival analysis, ESRD

Introduction

Anemia is mainly caused by decreased production of erythropoietin and increased loss of red blood cell in patients with end-stage renal disease (ESRD). Iron-deficient usually co-existed in the patients undergoing maintenance hemodialysis (MHD).[1] Iron homeostasis is maintained by absorption of dietary iron in duodenum making up for daily iron loss. In patients receiving MHD, increased blood losses and compromised gastrointestinal iron absorption result in absolute iron deficiency. While reticuloendothelial cell blocks the delivery of its storage iron to marrow for erythropoiesis, which causes functional iron deficiency.[2]

Hepcidin, encoded by the HAMP gene, is a key regulator of iron utilization, which inhibits intestinal iron absorption and iron release from hepatocytes and macrophages.[3] The promoter of hepcidin contains several binding sites for hypoxia-inducible factors

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(HIFs), therefore, hepcidin could be down-regulated by hypoxia and HIF stabilization.[4] Previous studies demonstrated that serum hepcidin was reduced in patients who received the HIF stabilizer roxadustat.[5,6] In addition, hepcidin could also be affected by iron stores, erythropoiesis, inflammation, as well as decreased renal clearance.[3,7] Hepcidin is an 84-amino acid prepropeptide, and usually cleaved into three peptide types, hepcidin-20, hepcidin-22, and hepcidin-25, of which, hepcidin-25 is the active form and plays important roles in regulating functional iron deficiency.[8]

Previous studies demonstrated that hepcidin-25 could help to evaluate the iron status and anemia,[9,10] and participated in the pathophysiology of atherosclerosis and cardiovascular events in patients receiving MHD.[11,12] However, one study of 56 patients receiving MHD showed that serum hepcidin-25 could not predict the hematopoietic response to the therapy of intravenous iron plus erythropoiesis-stimulating agent (ESA).[13] A similar conclusion was reported in another study of 61 patients with non-dialysis chronic kidney disease (CKD).[14] One study of 50 patients receiving MHD suggested that hepcidin-25 was not related to mortality in the 12-month follow-up.[15] All the above findings were based on studies of small sample size and short-term follow-up. Therefore, the clinical utility of hepcidin-25 remained uncertain, this study aimed to investigate the associations between hepcidin-25 and mortality in patients receiving MHD.

Materials and Methods

Participants

This was a prospective observational study in the clinically stable patients receiving MHD at the Xuzhou Central Hospital (Xuzhou, China). Inclusion criteria: 1) patients with ESRD; 2) aged 18–80 years; 3) duration of dialysis treatment ≥ 3 months. Exclusion criteria: 1) had infection, inflammation, or malignant diseases; 2) hospital admission for any cause within the preceding 3 months; 3) planned to receive kidney transplant or transfer to other facilities in 2 years. The cohort was established in January 2016. All patients were followed up until death or the end date of the study (December 2017). This study followed the International Conference on Harmonized guidelines for good clinical practice and was conducted in accordance with the Helsinki Declaration. The agreement was approved by the Ethics Committee of Xuzhou Central Hospital.

Sample Size Estimation

To ensure that the sample size was sufficient, the formula was as following:

$$N = Z^2_{1-\frac{\alpha}{2}} p(1-p)/d^2 \quad (1)$$

Assuming α value of 0.05, $Z_{1-\alpha/2}$ value of 1.96, d value of 0.1, and p is set as 0.5, therefore, N=96. Considering the design effect as 1–3 and the drop-out rate as 10%, the sample size should be from 107 (if design effect=1) to 320 (if design effect=3). We calculated that this study needed to enroll at least 107 participants.

Data Collection and Measurements

Baseline demographic and clinical parameters were recorded, including age, gender, body mass index (BMI), etiology of ESRD, comorbidities, and laboratory measures. Questionnaires of the 36-Item Short-Form Health Survey (SF-36) and the Pittsburgh Sleep Quality Index (PSQI) were self-administered to all patients. The plasma and serum were centrifuged and frozen at –80°C until laboratory measurements were made in a certified laboratory (Dian Diagnostics, Nanjing, China). The single-pool Kt/V (spKt/V) was calculated by two-point urea modeling based on the intradialytic reduction of blood urea and weight loss.[16] The ESA responsiveness (ERI) was determined by the ratio of weekly ESA dose to hemoglobin (Hb).[17] The duration of dialysis treatment was defined as the time since dialysis was initiated. Serum hepcidin-25 was measured using competitive enzyme-linked immunosorbent assay kits[18] (Cat. CSB - E14239h, Cusabio, China), with a coefficient of variation (CV) < 10% in both intra- and inter-assay precision analyses.

Statistical Analysis

Patients' baseline demographic and clinical characteristics, as well as laboratory parameters, were summarized as proportions, mean (±SD), or median (interquartile range), and analyzed using one-way analysis of variance, Fisher exact test, and Kruskal–Wallis test depending on the data type. We applied the maximal information-based nonparametric exploration (MINE) statistics, and its maximal information coefficient (MIC) with scores, roughly equal to the coefficient of determination (R^2) in identifying the relationships between variables in large datasets,[19] were applied to identify novel relationships using R software, version 3.4.3 (https://www.r-project.org). Non-parametric Kaplan-Meier plot was used to evaluate the effect of hepcidin-25 on predicting all-cause mortality. Multivariate Cox proportional

hazard models were performed to identify risk factors for all-cause mortality, and results were expressed as a hazard ratio (HR) for all-cause mortality with per 10 ng/mL increase of serum hepcidin-25, with 95% confidence interval (CI). Cox proportional hazard regression model based on restricted cubic spline was applied to explore the non-linear association between serum hepcidin-25 and mortality, adjusted by different confounding factors. A two-sided P-value<0.05 was defined as statistically significant. Statistical analyses were performed using the SAS system, version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Baseline Demographics of the Cohort Stratified by Serum Hepcidin-25 Levels

A total of 161 patients receiving MHD were enrolled in this study with complete data and were assigned to 2 groups by the median value of baseline serum hepcidin-25, low-level hepcidin-25, and high-level hepcidin-25 groups (shown in Figure 1). The median hepcidin-25 was 31.0 (12.1, 57.3) ng/mL. Table 1 showed the baseline characteristics of the cohort, comparing with the low-level hepcidin-25 group, the patients in the high-level hepcidin-25 group had older age, higher levels of serum iron, ferritin, TSAT, and hypersensitive C-reactive protein (hs-CRP), lower levels of pre-dialysis creatinine and albumin, as well as worse scores of the SF-36 and PSQI.

Associations Between Hepcidin-25 and Other Variables

There was a total of 86 variables, involving 9 categories in our dataset. We observed the 216 top-scoring relationships between the 72 variables, with MIC $\geq$ 0.3 (shown in Figure 2). Among them, the hepcidin-25 had strong

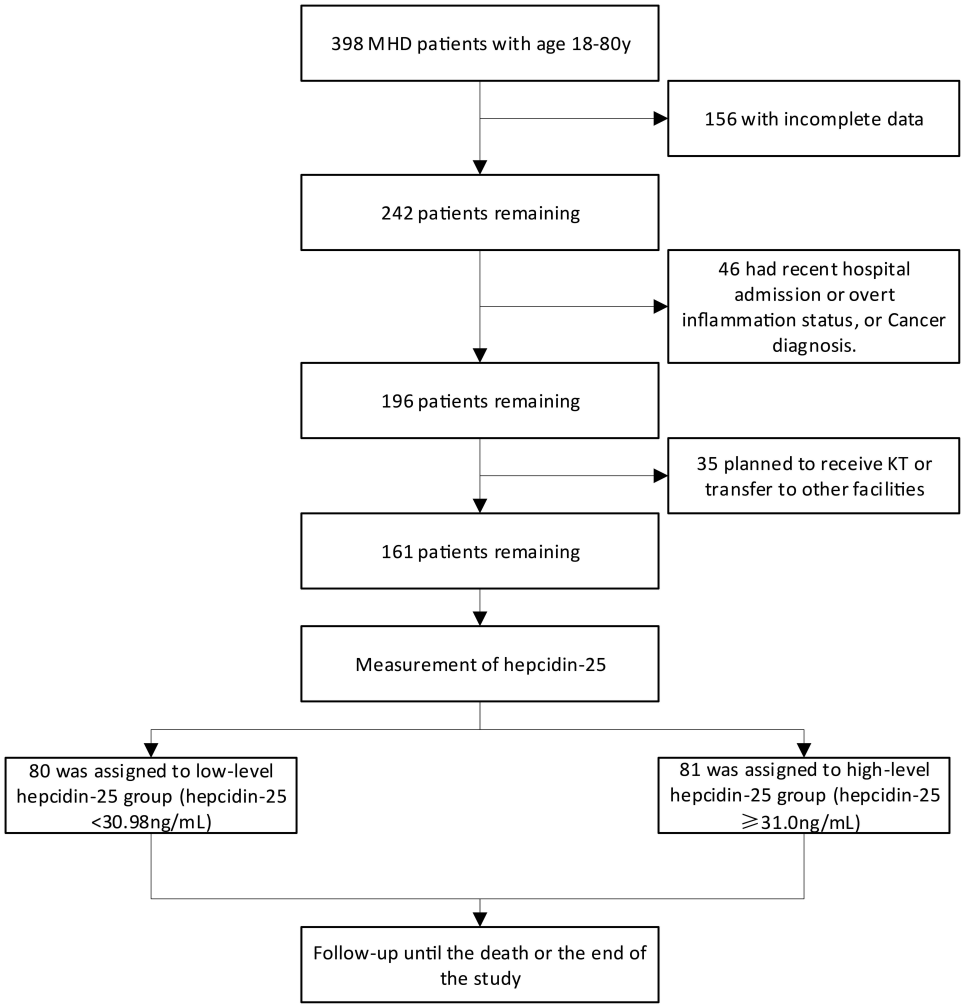

Figure 1 Flow diagram showing the creation of the main dataset, reasons for exclusions, and assignment according to hepcidin-25 at study entry.
Abbreviations: MHD, maintenance hemodialysis; KT, kidney transplant.

Table 1 Baseline Characteristics Stratified by Baseline Level of Hepcidin-25 in 161 MHD Patients

	Total		Hepcidin-25 (ng/mL)				
			<31		≥31		P-value
N (%)	161	(100.0)	80	(49.7)	81	(50.3)	
Clinical Characteristic							
Female, n (%)[a]	68	(42.2)	30	(37.5)	38.00	(46.9)	0.2281
Age, years[b]	52.2	(14.9)	48.9	(14.4)	55.5	(14.7)	0.0047
Catheter, n (%)	17	(10.6)	1	(2.5)	11.0	(13.6)	0.1060
Duration of dialysis treatment, months	50.0	(32.5)	51.8	(32.3)	48.3	(32.8)	0.5002
Dialysis frequency (per week)	2.5	(2,3)	2.5	(2,3)	2.5	(2,3)	0.4497
HDF frequency (per week)	1	(0.5,1)	1	(0.5,1)	1	(0.5,1)	0.5185
Body mass index, kg/m2	21.6	(20.1,24.1)	21.8	(20.1,25.3)	21.6	(19.8,23.6)	0.5294
Comorbid illnesses							
Hypertension, n (%)	143	(88.8)	73	(91.3)	70.00	(86.4)	0.3323
Diabetes mellitus, n (%)	34	(21.1)	14	(17.5)	20.00	(24.7)	0.1331
Gastrointestinal bleeding, n (%)	18	(11.2)	4	(10.0)	11.00	(13.6)	0.3323
Laboratory data							
Hemoglobin (g/dL)	99.3	(20.6)	100.8	(19.4)	97.9	(21.7)	0.3667
Ferritin (ng/mL)	152.3	(58.9,987.2)	103.9	(56.4,180.8)	580.8	(152.3,3721)	<0.0001
TSAT (%)	30.6	(20.3,46.2)	28.1	(17.3,39.7)	43.6	(22,59.4)	<0.0001
Serum iron (umol/L)	12.6	(9.2,17.6)	11.7	(8.3,17.0)	15.0	(9.8,21.3)	0.0011
ERI (U/kg/week/g/dL)	13.0	(8.5,16.9)	12.0	(7.8,16.4)	13.2	(9.7,17.0)	0.1556
Vitamin B12 (ng/L)	586	(353,2000)	548	(359,2000)	592	(353,2000)	0.1682
Folic acid (ug/L)	4.6	(2.8,6.5)	4.8	(2.8,6.5)	4.5	(2.8,6.5)	0.4142
hsCRP (mg/dL)	1.6	(0.7,4.2)	1.2	(0.5,2.2)	2.7	(1.5,6.3)	<0.0001
Predialysis Creatinine (mg/dL)	974	(349)	1073	(362)	877	(308)	0.0003
Albumin (g/dL)	38.7	(3.7)	39.1	(3.5)	37.7	(3.8)	0.0408
Calcium (mg/dL)	2.3	(2.2,2.4)	2.3	(2.2,2.5)	2.3	(2.2,2.4)	0.2279
Intact PTH (pg/mL)	346	(156,476)	347.0	(198,510)	331	(106,451)	0.1074
Fasting glucose (mmol/L)	5.3	(4.4,5.3)	5.3	(4.5,5.3)	5.3	(4.4,5.3)	0.3941
LDL-C (mg/dL)	2.3	(2.2,2.4)	2.3	(2.3,2.4)	2.3	(1.7,2.4)	0.0691
Potassium (mmol/L)	5.1	(0.8)	5.2	(0.8)	5.0	(0.8)	0.0656
Magnesium (mmol/L)	1.2	(0.2)	1.2	(0.2)	1.2	(0.2)	0.2266
Cardiothoracic ratio	0.5	(0.5,0.6)	0.5	(0.5,0.6)	0.5	(0.5,0.7)	0.1912
Ejection fraction	0.5	(0.5,0.6)	0.6	(0.5,0.6)	0.5	(0.5,0.6)	0.1137
36-Item Short Form Health Survey, SF-36							
Physical Functioning	55	(45,75)	65	(50,80)	50	(35,70)	0.0009
Role-Physical	0	(0,50)	12.5	(0,50)	0	(0,25)	0.0143
Pain	52.0	(31,80)	62	(41.5,100)	42	(31,74)	0.0070
General health	30.0	(15,45)	35	(20,46)	20	(10,45)	0.0085
Vitality	55.0	(35,75)	55	(37.5,77.5)	50	(35,65)	0.0149
Social Function	37.5	(12.5,62.5)	43.75	(25,75)	37.5	(12.5,62.5)	0.0150
Role-Emotional	0	(0,33)	0	(0,33)	0	(0,33)	0.1421
Mental Health	60.0	(36,80)	68	(40,84)	52	(36,76)	0.0085
Reported Health Transition	50.0	(25,75)	50	(25,75)	50	(0,75)	0.0115
Pittsburgh Sleep Quality Index, PSQI							
Sleep quality	2.0	(1,2)	1	(1,2)	2	(1,2)	0.0221
Sleep latency	1.0	(1,2)	1	(1,2)	2	(1,2)	0.0246
Sleep duration	2.0	(0,3)	1	(0,3)	2	(0,3)	0.0479
Habitual sleep efficiency	2.0	(1,2)	1	(0.5,2)	2	(1,2)	0.0049
Sleep disturbances	1.0	(1,2)	1	(1,2)	1	(1,2)	0.1293
Use of sleeping medication	0.0	(0,2)	0	(0,1.5)	1	(0,3)	0.0160
Daytime dysfunction	2.0	(1,3)	2	(1,3)	2	(1,3)	0.0350
Global PSQI score	9.0	(5,17)	7	(5,15)	13	(6,17)	0.0023

Notes: [a]Discrete values expressed as number (percentage). [b]Continuous values expressed as means (SD) if normally distributed or median (interquartile range) if skewed.
Abbreviations: MHD, maintenance hemodialysis; HDF, hemodiafiltration; TSAT, transferrin saturation; hsCRP, high sensitivity C-reactive protein; ERI, erythropoiesis-stimulating agents (ESA) resistance index; PTH, parathyroid hormone; LDL-C, low-density lipoprotein cholesterol.

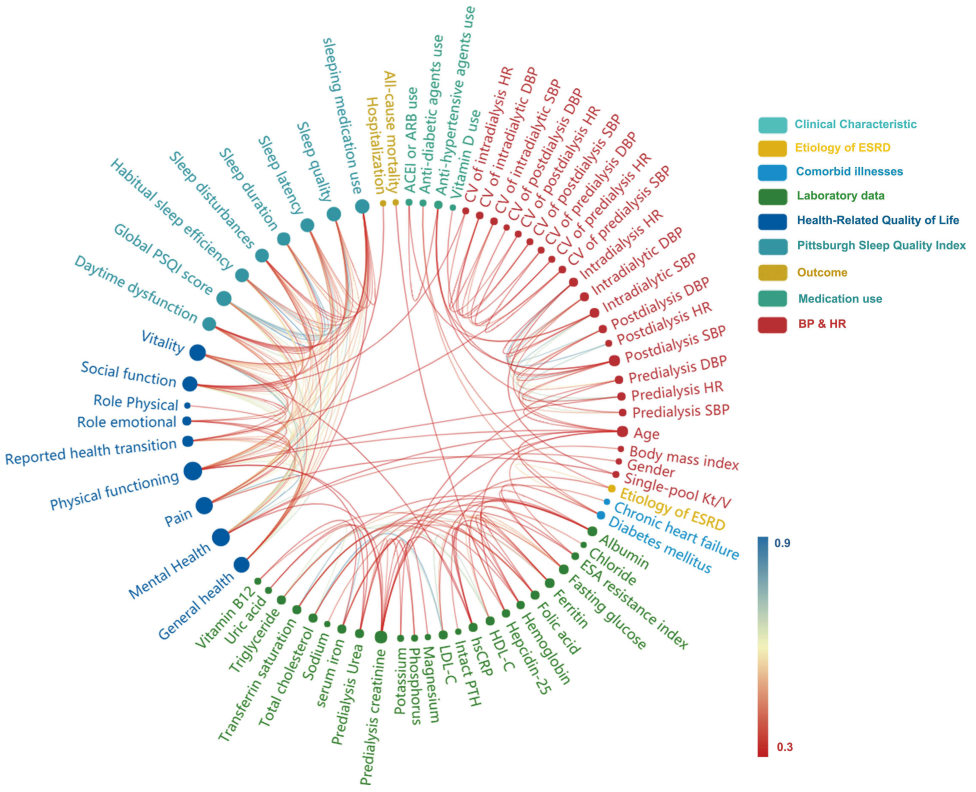

Figure 2 Associations between the variables in the dataset of patients receiving MHD. A topological graph in which nodes correspond to variables and edges correspond to the top 216 relationships. Node size is proportional to the number of these relationships involving the variable. The colors of the edges represent the values of MIC between variables. MIC assigns a perfect score of 1 to all never-constant noiseless functional relationships, scores that tend to 1 for a large class of noiseless relationships and a score of 0 to statistically independent variables.

Abbreviations: ESRD, end-stage renal disease; ESA, erythropoietin stimulating agents; hsCRP, high sensitivity C-reactive protein; PTH, parathyroid hormone; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; PSQI, Pittsburgh Sleep Quality Index; ARB, angiotensin II receptor blocker; ACEI, angiotensin-converting enzyme inhibitor; SBP, systolic blood pressure; DBP, diastolic blood pressure; CV, coefficient of variation; HR, heart rate.

associations with ferritin (MIC, 0.46), transferrin saturation (TSAT) (MIC, 0.36), age (MIC, 0.31), and all-cause mortality (MIC, 0.30).

Hepcidin-25 Levels and Mortality

The mean follow-up duration was 22.81 ± 3.7 months; during this period, 19 deaths (11.8%) occurred, of which, 16 death occurred in the high-level hepcidin-25 group. Cerebrovascular events (21.1%, ischemic stroke, and intracerebral hemorrhage) ranked as the first cause leading to death. Patients with hepcidin-25<31ng/mL had better survival outcomes than those with hepcidin-25≥31ng/mL during the 24-month follow-up (Log rank test, P = 0.0017) (Figure 3A). Similarly, patients in the high-level hepcidin-25 group also had a longer duration of dialysis treatment, which meant longer intervals from the first dialysis session to the death or the end of the study (Log rank test, P = 0.0019) (Figure 3B). Meantime, for per 10ng/mL increase of serum hepcidin-25, the unadjusted HR for all-cause mortality was 1.225 (95% CI 1.085–1.382, P<0.001), the HR remained significant after multivariate adjustments (Table 2). Furthermore, restricted cubic spline showed that the curves of adjusted HR for all-cause mortality were relatively stable at hepcidin-25<31ng/mL, and then the curves increased significantly at hepcidin-25≥31ng/mL, adjusted by age together with hemoglobin (Figure 4A), ferritin (Figure 4B), TSAT (Figure 4C), ERI (Figure 4D), hsCRP (Figure 4E), predialysis creatinine (Figure 4F), albumin (Figure 4G), intact PTH (Figure 4H), respectively.

Discussion

Iron deficiency is a common cause of anemia in patients receiving MHD. Iron is essential for all living organisms, but iron overload could produce toxic oxidants and cause multiple organ damage. Therefore, iron supplementation is a double-edged sword and should be managed carefully to achieve the guideline-recommended target of hemoglobin and avoid its side effects.[20] Recent guidelines recommend that iron status should be monitored periodically through Hb, TSAT, serum ferritin, and hs-CRP.[21] However, none of them is specific or

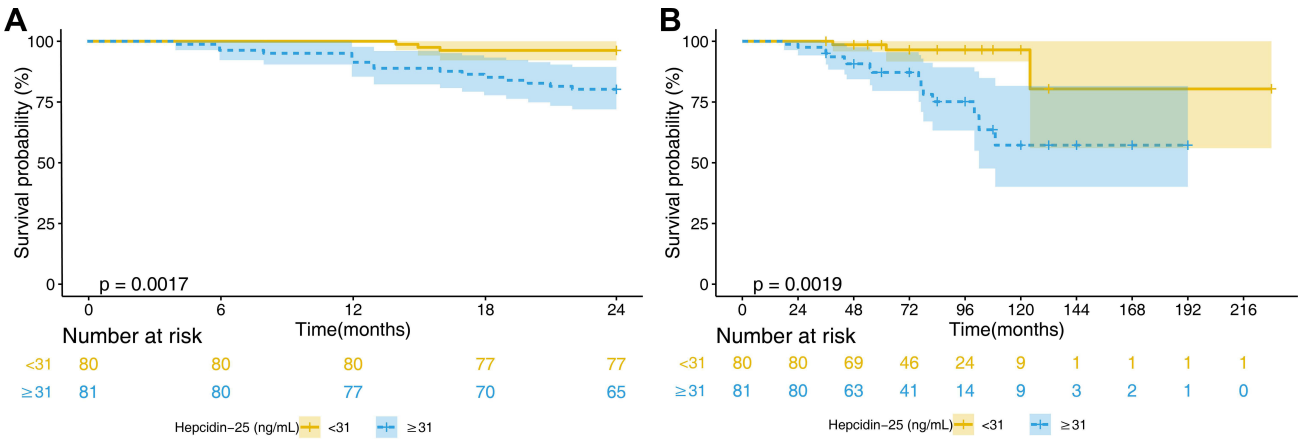

Figure 3 Kaplan-Meier curve of overall survival. The patients receiving MHD were classified into two groups by the baseline serum hepcidin-25. (**A**) Patients with hepcidin-25<31 ng/mL had better survival outcomes than those with hepcidin-25≥31 ng/mL during the 24-month follow-up. (**B**) Patients with hepcidin-25<31 ng/mL had longer duration of dialysis treatment than those with hepcidin-25≥31 ng/mL (Log rank test, P = 0.0019). Here, duration of dialysis treatment was defined as the time since dialysis initiated.

sensitive for the regulation of iron metabolism. Hepcidin-25 could regulate iron metabolism through binding ferroportin, inhibiting iron release from hepatocytes and macrophages, and reducing intestinal iron absorption.[10] Therefore, hepcidin-25 could be a supplement for evaluating functional iron deficiency to conventional iron indices in patients receiving MHD.[22]

KNOW-CKD study[23] demonstrated that high hepcidin-25 was associated with anemia in patients with non-dialysis CKD. Serum hepcidin was positively correlated with ferritin but had no relationship with inflammatory cytokines and TSAT.[24,25] Our results showed that the levels of serum iron, TSAT, serum ferritin, and hsCRP were higher in the high-level hepcidin-25 group, with opposite trends of pre-dialysis creatinine and albumin, indicating malnutrition-inflammation complex syndrome in these patients. The inflammation could promote hepcidin expression through several pathways of inflammatory cytokines, such as IL-6 and IL-1β. Then the increased hepcidin-25 could result in functional iron deficiency, and influence iron status.[7]

In addition, we introduced a novel statistical method "maximal information-based nonparametric exploration (MINE)", to identify potential relationships between pairs of variables in our dataset, the higher value of MIC, the stronger associations between the variables. As far as we know, there is no previous authoritative literature that can be referred to, thus we chose 0.3 as the cutoff point of MIC to display the variables that might have associations with each other. Consistent with the results in Table 1, the MINE analysis suggested that the circulating hepcidin-25 was associated with ferritin, TSAT, and all-cause mortality.

The restricted cubic spline is an important method for multivariate survival analysis to reveal nonlinear relationships.[26] Considering the nonlinear associations between variables in our dataset, the application of restricted cubic spline in the Cox proportional hazard regression model was more suitable than the typical Cox proportional hazard regression model. Therefore, the restricted cubic spline was applied to explore the adjusted non-linear associations between serum hepcidin-25 and mortality, the adjusted HR of hepcidin-25 for mortality showed nonlinear upward trends. The results of these two statistical methods were similar, showing that the risk of mortality increased with the growth of serum levels of hepcidin-25, and the increasing trends were more obvious in higher hepcidin-25 level groups.

Limitations and Strengths

This study has strengths. First, this is the first attempt of using MINE in clinical medical research until now, the MINE analysis can detect not only the linear but also non-linear novel relationships between variables in a large dataset,[19] therefore, it could have a wider application than the traditional analysis, such as Pearson and Spearman correlation coefficient. Next, there was only one published literature of 50 patients receiving MHD reported the correlation between serum hepcidin-25 and mortality, which showed that hepcidin-25 was not related to mortality during 12 months of follow-up.[15] Our study enrolled 161 patients receiving MHD with 24 months of follow-up and found that high serum hepcidin-25 was associated with all-cause mortality risk.

Table 2 Hazards Ratio (HR) and Predictors of All-Cause Mortality in Multivariate Cox Proportional Hazard Models

	Unadjusted	Adjusted	Adjusted	Adjusted	Adjusted	Adjusted	Adjusted	Adjusted	Adjusted
	HR	HR	HR	HR	HR	HR	HR	HR	HR
	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)
Hepcidin (ng/mL)	1.225***	1.262*	1.276**	1.294**	1.220*	1.221*	1.253*	1.243*	1.247**
	(1.085–1.382)	(1.049–1.517)	(1.067–1.526)	(1.080–1.549)	(1.025–1.453)	(1.018–1.465)	(1.055–1.489)	(1.048–1.474)	(1.072–1.451)
Age		1.062**	1.073***	1.07***	1.06**	1.064**	1.070**	1.066**	1.05**
		(1.023–1.103)	(1.033–1.115)	(1.031–1.11)	(1.02–1.101)	(1.021–1.108)	(1.026–1.117)	(1.02–1.114)	(1.01–1.093)
Hemoglobin (g/dL)		0.982							
		(0.962–1.003)							
Ferritin (ng/mL)			1.000						
			(1.000–1.000)						
TSAT (%)				0.993					
				(0.975–1.012)					
ERI (U/kg/week/g/dL)					1.037				
					(0.983–1.095)				
hsCRP (mg/dL)						1.024			
						(0.957–1.094)			
Predialysis Creatinine (mg/dL)							1.000		
							(0.998–1.002)		
Albumin (g/dL)								0.977	
								(0.849–1.124)	
Intact PTH (pg/mL)									0.997
									(0.994–1.001)

Notes: *P<0.05, **P<0.01, ***P<0.001

Abbreviations: TSAT, transferrin saturation; ERI, erythropoiesis-stimulating agents (ESA) resistance index; hsCRP, high sensitivity C-reactive protein; PTH, parathyroid hormone.

This study had limitations. This was a single-center study, therefore, the selection of patients could have introduced bias. We excluded unstable MHD patients, the hepcidin expression could be regulated by multiple factors, such as acute inflammation, therefore, the conclusions here are not applicable for unstable MHD patients, especially

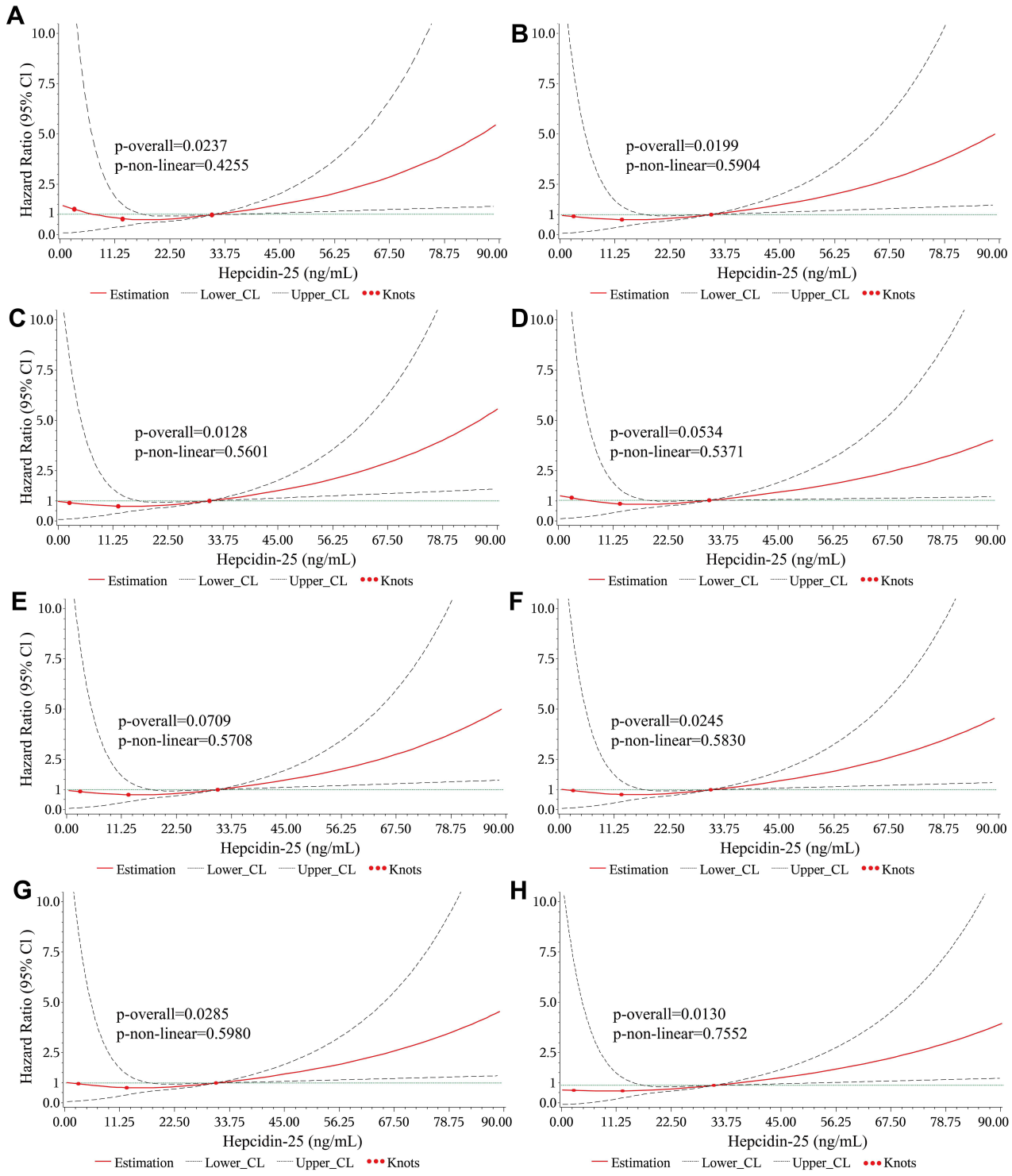

Figure 4 Association between hepcidin-25 and hazard ratio (HR) of all-cause mortality using restricted cubic spline, allowing for non-linear effects, with 95% confidence intervals (CIs). The reference hepcidin-25 for these plots (with HR fixed as 1.0) was 31 ng/mL. Cox regression models were adjusted for age together with hemoglobin (**A**), ferritin (**B**), TSAT (**C**), ERI (**D**), hsCRP (**E**), predialysis creatinine (**F**), albumin (**G**), intact PTH (**H**), respectively.

Abbreviations: TSAT, transferrin saturation; ERI, erythropoiesis-stimulating agents (ESA) resistance index; hsCRP, high sensitivity C-reactive protein; PTH, parathyroid hormone.

the patients with infection, inflammation, malignant diseases. There were very few events of all-cause mortality, and we had to perform the Cox proportional hazard models with potential confounding factors separately. However, to our knowledge, this study had the largest sample size and longest follow-up to investigate the associations between hepcidin-25 and mortality, thus these limitations may be acceptable.

In summary, serum hepcidin-25 was associated with ferritin and TSAT, and could be an independent predictor for all-cause mortality in patients receiving MHD. Monitoring hepcidin-25 could be helpful in the forecast of the survival prognosis in patients receiving MHD. Further research with larger sample size and longer-term follows up was needed.

Ethics and Consent

The study involved Human Participants and it was performed at the Xuzhou Central Hospital. The study adhered to the International Conference on Harmonization guidelines for Good Clinical Practice and was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the ethical committee of the Xuzhou Central Hospital (Approval No. ZXXY-LJ-20150115-001). All participants provided written informed consent.

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Disclosure

The authors have no conflicts of interest to declare.

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