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Effect of Serum Hepcidin on Predicting Mortality in Hemodialysis Patients: A Prospective Cohort Study

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Abstract

Background: Hepcidin is a key regulator of iron homeostasis, while the clinical utility of hepcidin remains uncertain in hemodialysis (HD) patients.
Objectives: Our study aimed to evaluate the predictive effect of serum hepcidin-25 on mortality in HD patients.
Methods: A prospective observational cohort study of chronic HD patients were conducted at Xuzhou Central Hospital, Jiangsu, China, during years 2015 - 2017. The data on demographic factors, dialysis vintage, comorbidities, and laboratory measures were collected. Kaplan-Meier survival analysis was used to compare the effect of serum hepcidin-25 levels on mortality. Logistic regression models and multivariate Cox proportional hazard models were performed to identify the predictors of all-cause mortality in HD patients.
Results: A total of 159 patients were included in this cohort, who were stratified into three groups by tertiles of hepcidin-25 values, and their 2-year overall mortality rate was 11.94%. The Kaplan-Meier analysis showed that patients with the highest tertile of serum hepcidin-25 had significantly higher all-cause mortality than in the two lower tertiles ($P < 0.001$). Serum hepcidin-25 was an independent risk factor for all-cause mortality after multivariate adjustments using logistic regression models and Cox proportional hazard models.
Conclusions: A higher level of serum hepcidin-25 in chronic HD patients could be associated with increased mortality. Further studies are needed in a larger size of HD patients with a longer term of follow up.

Keywords: End-Stage Renal Disease, HAMP Gene, Hemodialysis, Hepcidin, Homeostasis, Mortality

1. Background

Anemia is mainly caused by decreased red blood cell survival and reduced renal erythropoietin (EPO) production in patients with end-stage renal disease (ESRD). Meanwhile, iron-deficient widely exists in patients undergoing hemodialysis (HD) (1). Disorders of iron homeostasis in HD patients turn the management of anemia into a multifactorial therapeutic task, where iron dose must be properly balanced to achieve the desired outcome without exposing patients to the risks of serious adverse events (2).

Normal iron homeostasis is maintained through duodenal absorption of dietary iron, which compensates for the daily loss of iron. In HD patients, the compromised gastrointestinal iron absorption and increased blood losses result in absolute iron deficiency. Moreover, reticuloendothelial cell iron blockade causes the defect of delivering iron to marrow for erythropoiesis, even in presence of sufficient iron, and that is defined as functional iron deficiency (3, 4).

Hepcidin is a protein produced in the liver, encoded by the HAMP gene, performs a negative regulator of iron utilization through inhibiting iron release from macrophages and hepatocytes, and reducing intestinal absorption of iron (5). Hepcidin expression can be impacted by a variety of factors, such as iron status, hypoxia, inflammation, erythropoiesis, as well as decreased renal clearance in patients with chronic kidney disease (CKD) (3, 6, 7). Hepcidin is initially synthesized as an 84-amino acid prepropeptide, then cleaved into three peptide types, hepcidin-20, -22 and -25, of which, hepcidin-25 is the active form and plays essential roles in functional iron deficiency (8).

Current diagnostic tests are not enough to accurately assess iron status and its toxicity risk; therefore, novel

biomarkers are still needed (3). Several studies reported that hepcidin-25 was helpful in the assessment of iron status and management of anemia (9), and took part in the pathophysiology of cardiovascular events in HD patients (10-13). While some studies showed that serum hepcidin-25 was not predictive of hematopoietic response to intravenous iron therapy in patients receiving erythropoiesis-stimulating agent (ESA) (14, 15), and it was not related with mortality (16). All previous studies were based on data from small sizes of the population and short periods of follow-up; thus, the clinical utility of hepcidin-25 remains unclear.

2. Objectives

The purpose of this study was to investigate the relation between serum hepcidin-25 and mortality in HD patients.

3. Methods

3.1. Sample Size Calculation

The sample size formula is as follows:

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

Assuming α value of 0.05, $Z_{1-\frac{\alpha}{2}}$ 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 is from 107 (if design effect = 1) to 320 (if design effect = 3).

3.2. Participants

This prospective observational cohort study conducted at a single blood purification center in Xuzhou, China. The subjects were recruited from the adult patients who received HD routinely for at least 6 months. Exclusion criteria were: 1) had malignant diseases, or overt infection/inflammation; 2) hospital admission within the preceding 3 months for any cause; 3) had < 3h of HD per session; 4) planned to received kidney transplantation or peritoneal dialysis in 2 years; 5) refused to provide written consent. The cohort was established in October 2015. All patients were followed up until death or December 31st, 2017 (the end of the study). 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, Xuzhou Medical University (approval No. ZXXY-LJ-20150115-001).

3.3. Data Collection and Measurements

The study consisted of 2 study phases, the first phase (3 months) was used to collect and record baseline demographic and clinical data, including age, gender, body mass index (BMI), etiology of ESRD, comorbidities and laboratory measures; and the second phase (the subsequent 2 years) was used to evaluate the time-dependent risk of mortality. The primary endpoint of the study was all-cause mortality during the follow-up period from January 1, 2016, to December 31, 2017. Vital status of the participants was assessed by searching the electronic medical records, and confirmed by telephone interviews of their families.

Hematological measurements were made using fresh venous blood with EDTA and clotted blood in a certified laboratory (Dian Diagnostics, Nanjing, China). The plasma and serum were centrifuged and frozen at -80°C until further laboratory analysis. The single-pool Kt/V (spKt/V) was determined by two-point urea modeling based on the intradialytic reduction in blood urea and intradialytic weight loss (17). The weekly dose of ESA to hemoglobin ratio was calculated as an index of ESA responsiveness (ERI) (18). Serum hepcidin-25 levels were measured using competitive enzyme-linked immunosorbent assay kits (19) (Cat. CSB-E14239h, Cusabio, China), with a coefficient of variation (CV) < 10% in both inter- and intra-assay precision analyses.

3.4. Statistical Analysis

Patients' baseline demographics, clinical characteristics, and laboratory measurements were summarized. Continuous values were expressed as mean ($\pm$ SD) and analyzed using one-way analysis of variance, categorical values were expressed as percentages and analyzed by Fisher exact test. Both logistic regression analysis and multivariate Cox proportional hazard model were performed to determine how independent variables predicted the mortality in HD patients. Log-log survival plots examined the Cox regression assumption of each variable. The Kaplan-Meier curve was performed to detect the influence of hepcidin-25 on the subjects censored for death. A two-sided P value < 0.05 was defined as statistically significant. All statistical analyses were performed using the SPSS system, version 23.0 (SPSS, Inc., Chicago, IL).

4. Results

A total of 320 participants were selected randomly from the patients received HD treatment routinely for at least 6 months at Xuzhou Central Hospital in October 2015, and 161 of them were excluded from participation in this study for a variety of reasons (Figure 1). The mean age of the entire study population was 52.11 $\pm$ 14.93 years, and 42.77% of the subjects were female. The baseline characteristics of the participants were shown in Table 1. The median hepcidin-25 concentration was 35.17 (26.04) ng/mL. The participants were divided into three groups by tertile of hepcidin-25 values: the lowest tertile (< 19.35 ng/mL), a middle tertile (19.35 to < 44.97 ng/mL), and the highest tertile ($\geq$ 44.97 ng/mL). Comparing with patients in the middle and lowest tertiles, the patients in the highest tertile of hepcidin-25 were much older. The levels of serum ferritin, serum iron, TSAT and hypersensitive C-reactive protein (hs-CRP) increased, while pre-dialysis serum creatinine, urea, and albumin decreased in three tertiles with the increase of serum hepcidin-25.

During the 2-year follow-up, there were 19 deaths (11.94%), and therefore, 140 censored (88.1%). Cerebrovascular events, such as intracerebral hemorrhage and ischemic stroke, were ranked as the most common cause of death, accounting for 21.05% of deaths. Across all strata of baseline serum hepcidin-25, the highest mortality risk was observed in the patients with the highest tertile of hepcidin-25, with 15 deaths reported over the follow-up period (log-rank test, P < 0.001) (Figure 2).

Multiple logistic regression models were established to discuss the potential predictors of all-cause mortality (Table 2). The variables, those were significantly different across the three tertiles of hepcidin-25 (P < 0.05 in Table 1), or possibly associated with serum levels of hepcidin-25, were used as independent variables for multivariate logistic regression analyses, including age, hemoglobin, ferritin, TSAT, ERI, hsCRP, predialysis creatinine, albumin, and intact PTH. The baseline serum hepcidin-25 was significantly associated with all-cause mortality in univariate logistic regression analysis [odds ratio (OR): 1.035, 95% CI: 1.015 - 1.056, P < 0.001), which was consistent with the results of multivariate logistic regression analyses (OR: 1.026 - 1.037, all P < 0.05). Furthermore, Cox proportional hazard models with adjustment for multivariate factors were also used to evaluate mortality risk. The patients with higher level of serum hepcidin-25 had a higher risk of all-cause mortality [unadjusted hazard ratios (HR): 1.021, 95% CI: 1.009 - 1.033, P < 0.001], which remained significant after multivariate adjustments using multiple models (HR: 1.021 - 1.027, all P < 0.05) (Table 3).

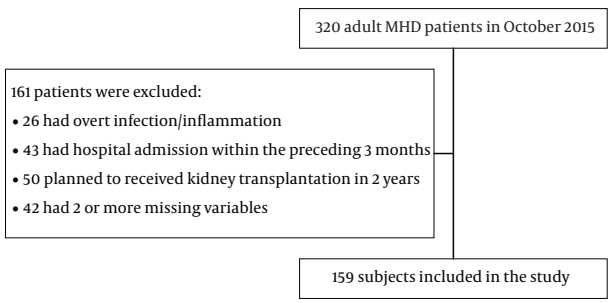

Figure 1. Study participant selection flow diagram.

5. Discussion

Anemia develops in most patients with ESRD. Iron deficiency is one of the leading causes, particularly in HD patients. Iron is not only an essential element for all living organisms, but also produces toxic oxidants. Thus, iron supplementation is double-edged and should be monitored and adjusted precisely to achieve optimal hemoglobin targets and minimize its side effects (20). Recent guidelines on anemia in CKD patients recommend that iron status should be evaluated periodically by hemoglobin, serum ferritin, TSAT, and hs-CRP (21). However, none of these parameters is sensitive or specific for functional iron deficiency. Hepcidin-25 has emerged as a molecule that regulates iron metabolism, through binding its receptor (ferroportin), inhibiting intestinal iron absorption and iron efflux from hepatocytes and macrophages (9, 22). Hepcidin-25 takes part in the pathogenesis of anemia in CKD patients and could be a better biomarker for functional iron deficiency than conventional iron indices (9-11, 23).

KNOW-CKD study (19) enrolled 1677 non-dialysis CKD patients and demonstrated that serum hepcidin was associated with more severe anemia in patients with eGFR < 45 mL/min/1.73 m^2. Serum hepcidin was reported to be positively correlated with ferritin; however, had no relationship with TSAT and inflammatory cytokines (2, 24, 25). Our results showed that the serum levels of ferritin, TSAT, serum iron, and hsCRP gradually increased in the three groups stratified by hepcidin-25 levels, with opposite trends of albumin and pre-dialysis creatinine. These findings suggested that hepcidin-25 levels might be associated with iron stores, inflammation and protein-energy

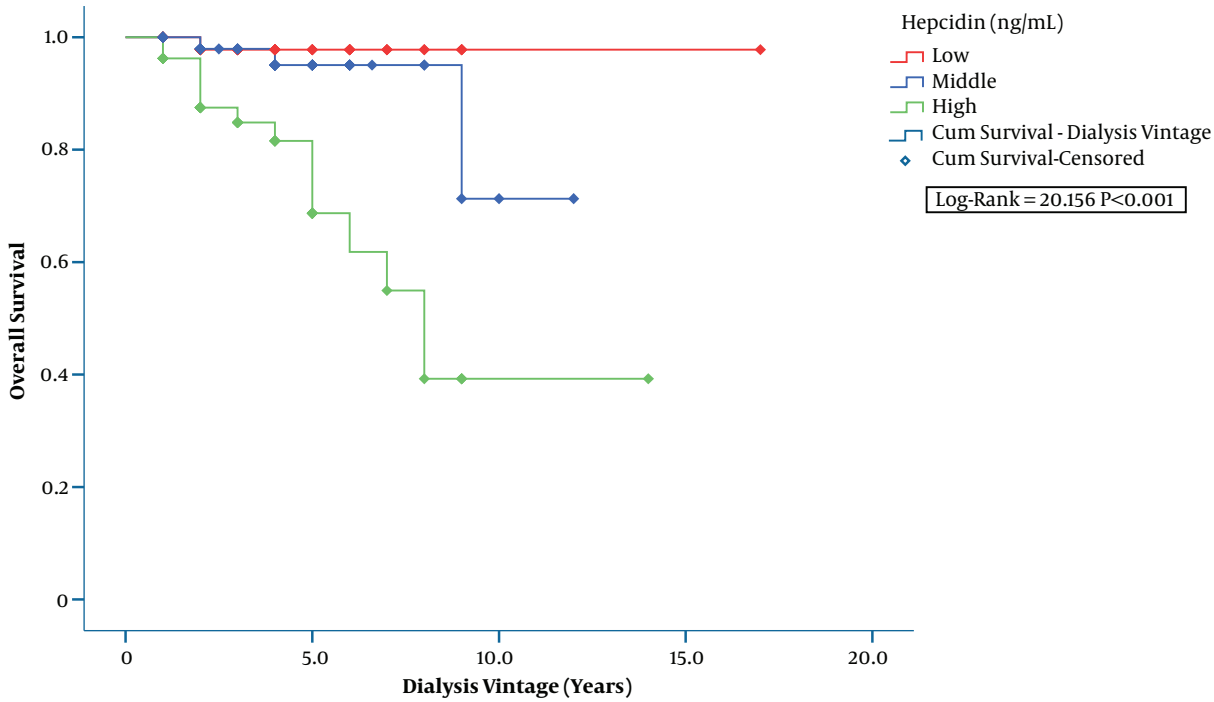

Figure 2. Kaplan-Meier plot of overall survival. Patients were classified into three groups by the baseline values of serum hepcidin-25. Dialysis vintage defined as the interval from the first dialysis session to the time of death.

wasting (PEW), and therefore, hepcidin-25 could be a good biomarker for iron status in HD patients without apparent inflammation.

Few studies were conducted to reveal the relationship between serum hepcidin-25 and mortality in HD patients. A previous study with 50 HD patients reported that hepcidin was not related to mortality (16). While our study enrolled 159 HD patients, which suggested that baseline serum hepcidin-25 was associated with all-cause mortality in HD patients. The survival time of the patients with the highest tertile of serum hepcidin-25 was significantly shorter than patients with middle and lowest tertiles of hepcidin-25. Monitoring hepcidin-25 might be helpful in clinical practice, not only for the management of anemia but also for predicting the survival prognosis in HD patients.

In conclusion, serum hepcidin-25 could be an independent predictor of all-cause mortality in HD patients. Further studies are needed to confirm the predictive effect of hepcidin on mortality in a larger size of HD patients with a longer term of follow up.References

Footnotes

Authors' Contribution: Ling Sun conceived and designed the experiments and wrote the paper. Ling Sun, Yan Lu, Na Deng and Hui-Xin Wang performed the experiments. Ling Sun and Lu-Xi Zou analyzed the data and revised the manuscript.

Conflict of Interests: None of the authors has any form of conflict of interest related to this paper.

Ethical Approval: The protocol was approved by the Ethical Committee of the Xuzhou Central Hospital, Xuzhou Medical University (approval No. ZXXY-LJ-20150115-001).

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Patient Consent: It is not declared by the authors.

Table 1. Baseline Characteristics Stratified by Baseline Levels of Hepcidin-25 in 159 MHD Patients

	Overall (N = 159)	Hepcidin-25 (ng/mL)			P Value
		< 19.35 (N = 53)	19.35 - 44.97 (N = 53)	≥ 44.97 (N = 53)	
Clinical characteristic					
Female[a]	68 (42.77)	19.00 (35.85)	25.00 (47.17)	24.00 (45.28)	0.4532
Age, y	52.11 (14.93)	47.87 (14.13)	51.25 (14.21)	57.21 (15.16)	0.0044
Body mass index, kg/m^2	22.27 (3.26)	22.62 (3.72)	22.45 (3.10)	21.74 (2.90)	0.3435
spKt/V	1.23 (0.22)	1.28 (0.24)	1.23 (0.20)	1.17 (0.21)	0.0251
Dialysis vintage, y[b]	4.18 (2.71)	4.36 (2.96)	4.23 (2.47)	3.96 (2.72)	0.7480
Dialysis frequency, /wk[a]	2.51 (0.46)	2.48 (0.45)	2.58 (0.42)	2.46 (0.49)	0.3298
Catheter	17 (10.69)	3.00 (5.66)	7.00 (13.21)	7.00 (13.21)	0.3509
Comorbid illnesses					
Hypertension[a]	141 (88.68)	50.00 (94.34)	46.00 (86.79)	45.00 (84.91)	0.2705
Diabetes mellitus[a]	33 (20.75)	8.00 (15.09)	9.00 (16.98)	16.00	0.1146
Chronic heart failure[a]	47 (29.56)	10.00 (18.87)	14.00 (26.42)	23.00 (43.40)	0.0185
Stroke[a]	29 (18.24)	7.00 (13.21)	7.00 (13.21)	15.00 (28.30)	0.0684
Arrhythmia[a]	5 (3.14)	1.00 (1.89)	3.00 (5.66)	1.00 (1.89)	0.4401
Gastrointestinal bleeding[a]	18 (11.32)	6.00 (11.32)	7.00 (13.21)	5.00 (9.43)	0.8296
Laboratory data					
Hepcidin-25, ng/mL	35.17 (26.04)	8.32 (5.39)	30.05 (6.97)	66.13 (15.93)	< 0.0001
Hemoglobin, g/dL	99.08 (20.56)	101.45 (17.93)	100.08 (19.95)	95.70 (23.39)	0.3245
Ferritin, ng/mL	1344.98 (2576.16)	247.36 (529.10)	864.55 (1812.45)	2923.03 (3549.48)	< 0.0001
TSAT, %	38.30 (24.21)	27.13 (13.59)	37.01 (19.88)	50.75 (30.24)	< 0.0001
Serum iron, umol/L	14.45 (6.79)	11.93 (4.94)	14.58 (5.55)	16.84 (8.52)	0.0008
ERI, U/kg/week/g/dL	13.70 (7.25)	12.49 (6.09)	13.68 (7.17)	14.92 (8.25)	0.2272
Vitamin B12, ng/L	1024.86 (774.25)	850.51 (711.42)	1057.85 (780.58)	1166.23 (808.44)	0.1023
Folic acid, ug/L	6.70 (6.74)	6.48 (6.58)	5.72 (5.03)	7.91 (8.17)	0.2396
HsCRP, mg/dL	3.48 (5.00)	1.52 (1.99)	2.58 (2.79)	6.34 (7.14)	< 0.0001
Predialysis Creatinine, mg/dL	974.98 (350.66)	1126.47 (368.98)	946.87 (303.38)	851.60 (325.14)	0.0002
Predialysis Urea, mg/dL	26.95 (7.64)	29.36 (6.20)	26.78 (6.66)	24.70 (9.13)	0.0065
Albumin, g/dL	38.29 (3.56)	38.82 (3.16)	38.74 (3.78)	37.32 (3.58)	0.0489
Calcium, mg/dL	2.45 (1.61)	2.69 (2.77)	2.37 (0.16)	2.28 (0.22)	0.3958
Phosphorus, mg/dL	1.99 (0.58)	2.14 (0.59)	1.88 (0.54)	1.95 (0.60)	0.0567
Intact PTH, pg/mL	383.11 (322.06)	416.37 (313.74)	374.08 (303.01)	358.87 (350.85)	0.6382
Fasting glucose, mmol/L	5.33 (1.73)	5.29 (1.56)	5.34 (1.78)	5.35 (1.87)	0.9804
Triglyceride, mg/dL	1.75 (0.89)	1.76 (0.87)	1.73 (0.79)	1.75 (1.01)	0.9810
Total cholesterol, mg/dL	3.85 (0.71)	4.03 (0.66)	3.73 (0.65)	3.81 (0.80)	0.0856
HDL-C, mg/dL	1.16 (0.86)	1.33 (1.41)	1.12 (0.32)	1.03 (0.31)	0.1823
LDL-C, mg/dL	2.25 (0.60)	2.37 (0.48)	2.14 (0.61)	2.23 (0.67)	0.1435
Uric acid, mg/dL	420.06 (97.49)	442.72 (103.90)	416.16 (96.70)	401.29 (88.53)	0.0851
Sodium, mmol/L	137.76 (3.68)	137.78 (4.32)	138.04 (3.50)	137.46 (3.17)	0.7253
Chloride, mmol/L	96.16 (3.99)	95.97 (4.23)	96.94 (3.17)	95.58 (4.40)	0.1932
Potassium, mmol/L	5.12 (0.79)	5.27 (0.75)	5.05 (0.71)	5.03 (0.89)	0.2045
Magnesium mmol/L	1.17 (0.19)	1.21 (0.18)	1.16 (0.18)	1.15 (0.21)	0.2658
Cardiothoracic ratio	0.54 (0.12)	0.52 (0.14)	0.53 (0.11)	0.57 (0.12)	0.1233
Ejection fraction	0.49 (0.11)	0.52 (0.08)	0.49 (0.11)	0.48 (0.13)	0.1199

Abbreviations: ERI, erythropoiesis-stimulating agents (ESA) resistance index; HDL-C, high-density lipoprotein cholesterol; hsCRP, high sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; MHD, maintenance hemodialysis; PTH, parathyroid hormone; spKt/V, single-pool Kt/V; TSAT, transferrin saturation.

[a] Values are expressed as No. (%).

[b] Dialysis vintage defined as the interval from the first dialysis session to the entry of the study.

Table 3. Hazards Ratio (HR) and Predictors of All-Cause Mortality on Cox Multivariate Analysis

Variables	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Adjusted OR (95% CI)	Adjusted OR (95% CI)	Adjusted OR (95% CI)	Adjusted OR (95% CI)	Adjusted OR (95% CI)	Adjusted OR (95% CI)	Adjusted OR (95% CI)
Hepcidin, ng/mL	1.021 (1.009 - 1.033)[a]	1.024 (1.006 - 1.043)[b]	1.025 (1.007 - 1.044)[b]	1.027 (1.008 - 1.045)[b]	1.021 (1.003 - 1.039)[c]	1.021 (1.002 - 1.039)[c]	1.023 (1.006 - 1.041)[b]	1.023 (1.006 - 1.04)[b]	1.023 (1.007 - 1.038)[b]
Age, y		1.062 (1.023 - 1.103)[b]	1.074 (1.033 - 1.116)[a]	1.07 (1.032 - 1.11)[a]	1.06 (1.021 - 1.102)[b]	1.064 (1.022 - 1.108)[b]	1.071 (1.026 - 1.118)[b]	1.067 (1.022 - 1.115)[b]	1.050 (1.01 - 1.092)[b]
Hemoglobin, g/dL		0.983 (0.962 - 1.004)							
Ferritin, ng/mL			1.000 (1.000 - 1.000)						
TSAT, %				0.993 (0.975 - 1.011)					
ERI, U/kg/week/g/dL					1.036 (0.981 - 1.094)				
hsCRP, mg/dL						1.023 (0.957 - 1.093)			
Predialysis creatinine, mg/dL							1.000 (0.998 - 1.002)		
Albumin, g/dL								0.983 (0.853 - 1.131)	
Intact PTH, pg/mL									0.997 (0.994 - 1.001)

Abbreviations: ERI, erythropoiesis-stimulating agents (ESA) resistance index; hsCRP, high sensitivity C-reactive protein; PTH, parathyroid hormone; TSAT, transferrin saturation.
[a] $P < 0.001$.
[b] $P < 0.01$.
[c] $P < 0.05$.

Table 2. Odds Ratio (OR) and Predictors of All-Cause Mortality on Logistic Regression Analysis

Variables	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Adjusted OR (95% CI)	Adjusted OR (95% CI)	Adjusted OR (95% CI)	Adjusted OR (95% CI)	Adjusted OR (95% CI)	Adjusted OR (95% CI)	Adjusted OR (95% CI)
Hepcidin, ng/mL	1.035 (1.015 - 1.056)[a]	1.037 (1.013 - 1.062)[b]	1.030 (1.007 - 1.054)[b]	1.035 (1.012 - 1.058)[b]	1.032 (1.009 - 1.056)[b]	1.026 (1.002 - 1.049)[c]	1.033 (1.012 - 1.055)[b]	1.034 (1.012 - 1.056)[b]	1.035 (1.013 - 1.057)[b]
Age		1.084 (1.034 - 1.137)[a]	1.081 (1.031 - 1.133)[a]	1.083 (1.034 - 1.135)[a]	1.082 (1.034 - 1.131)[b]	1.075 (1.024 - 1.128)[b]	1.077 (1.026 - 1.131)[b]	1.075 (1.023 - 1.13)[b]	1.071 (1.021 - 1.124)[b]
Hemoglobin, g/dL		0.971 (0.944 - 0.999)							
Ferritin, ng/mL			1 (1 - 1)						
TSAT, %				0.999 (0.977 - 1.02)					
ERI, U/kg/week/g/dL					1.112 (1.029 - 1.202)				
hsCRP, mg/dL						1.087 (0.993 - 1.19)			
Predialysis creatinine, mg/dL							0.999 (0.997 - 1.001)		
Albumin, g/dL								0.937 (0.801 - 1.096)	
Intact PTH, pg/mL									0.998 (0.995 - 1.001)

Abbreviations: ERI, erythropoiesis-stimulating agents (ESA) resistance index; hsCRP, high sensitivity C-reactive protein; PTH, parathyroid hormone; TSAT, transferrin saturation.
[a] $P < 0.001$.
[b] $P < 0.01$.
[c] $P < 0.05$.

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