

# Serum Hepcidin Predicts Uremic Accelerated Atherosclerosis in Chronic Hemodialysis Patients with Diabetic Nephropathy

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## Abstract

**Background:** Hepcidin, as a regulator of body iron stores, has been recently discovered to play a critical role in the pathogenesis of anemia of chronic disease. Atherosclerotic cardiovascular disease is the most common complication and the leading cause of death in chronic hemodialysis (CHD) patients. In the current study, we aimed to explore the relationship between serum hepcidin and uremic accelerated atherosclerosis (UAAS) in CHD patients with diabetic nephropathy (CHD/DN).

**Methods:** A total of 78 CHD/DN and 86 chronic hemodialyzed nondiabetic patients with chronic glomerulonephritis (CHD/non-DN) were recruited in this study. The level of serum hepcidin-25 was specifically measured by liquid chromatography–tandem mass spectrometry. Serum levels of interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) were measured by enzyme-linked immunosorbent assay.

**Results:** High serum level of hepcidin-25 was seen in CHD patients. Serum hepcidin-25 in CHD/DN was significantly higher than that in CHD/non-DN patients. Serum hepcidin-25 was positively correlated with ferritin, high-sensitivity C-reactive protein (hs-CRP), TNF- $\alpha$ , and IL-6 in CHD/DN patients. CHD/DN patients exhibited higher common carotid artery intima media thickness (CCA-IMT), hs-CRP, and hepcidin-25 levels than that in CHD/non-DN patients. Moreover, in CHD/DN patients, CCA-IMT was positively correlated with serum hepcidin, hs-CRP, and low-density lipoprotein-cholesterol. On multiple regression analysis, serum hepcidin and hs-CRP level exhibited independent association with IMT in CHD/DN patients.

**Conclusions:** These findings suggest possible linkage between iron metabolism and hepcidin modulation abnormalities that may contribute to the development of UAAS in CHD/DN patients.

**Key words:** Atherosclerosis; Hemodialysis; Hepcidin; Iron Status

## INTRODUCTION

Renal anemia is one of the major complications that occurs in end-stage renal disease (ESRD) patients who have even received erythropoiesis-stimulating agent (ESA) therapy,<sup>[1]</sup> which contributes significantly to the morbidity and mortality of ESRD patients.<sup>[2]</sup> Despite the widespread use of ESA, near 50% ESRD patients do not reach the target hemoglobin (Hb) levels.<sup>[3]</sup> Iron deficiency<sup>[4]</sup> and inflammation<sup>[5]</sup> are the most common reasons for hyporesponsiveness to ESA therapy in ESRD patients. Serum iron, transferrin saturation (TSAT), the ratio of serum iron to total iron-binding capacity (TIBC), multiplied by 100, and serum ferritin levels are commonly used to evaluate iron status. While serum iron concentration and TSAT reflect the amount of iron available for erythropoiesis, serum ferritin level is the only marker of

total body iron stores.<sup>[6]</sup> Hepcidin, as a regulator of body iron stores, has been recently discovered to play a critical role in the pathogenesis of anemia of chronic disease.<sup>[7]</sup>

Atherosclerotic cardiovascular disease (ACVD) is the most common complication and the leading cause of death in chronic hemodialysis (CHD) patients.<sup>[8,9]</sup> And the mortality caused by CVD in death of ESRD patients accounted for 45–50%.<sup>[10]</sup> This condition, with its age of onset being younger and the progress faster than that of the conventional ACVD, is referred to as uremic accelerated atherosclerosis (UAAS).<sup>[11,12]</sup> Dysregulated iron status has been suspected to be linked to anemia of chronic disease and to ACVD. Several recent studies have reported a strong relationship between hepcidin and ACVD and atherosclerosis in different patients, such as nonalcoholic fatty liver disease,<sup>[13]</sup> metabolic syndrome alterations,<sup>[14]</sup> hemodialysis (HD) patients,<sup>[15]</sup> and so on. Martinelli *et al.*<sup>[16]</sup> recently found that hepcidin may worsen insulin resistance contributing to the ACVD. But the role of iron metabolism remains unclear in this UAAS process in

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CHD patients with or without diabetic nephropathy (DN). Therefore, we evaluated the relationship between serum hepcidin and atherosclerosis in CHD patients with DN and without DN.

## METHODS

### Data sources

A total of 164 CHD patients from January 2014 to March 2014 were recruited in the Department of Blood Purification of Beijing Chao-Yang Hospital, Capital Medical University, China. The inclusion criteria of this study were age more than 18 years and maintained HD for more than 3 months. Patients were not included in the study if they had residual renal function, heart failure, a recent acute coronary event, cancer, autoimmune disease, and active infection. According to primary disease of uremia, the 164 CHD patients were divided into two groups, 78 CHD/DN patients and 86 chronic hemodialyzed nondiabetic patients with chronic glomerulonephritis (CHD/non-DN). Patients were considered DN if they were on insulin, oral hypoglycemic agents, diagnosed as diet controlled diabetes or had a fasting blood glucose of  $\geq 7$  mmol/L, and/or 2 h blood glucose after an oral glucose load test of  $\geq 11.1$  mmol/L. A standard questionnaire was used for every participant to obtain systematic information regarding conventional cardiovascular risk factors, including hyperlipidemia, hypertension, diabetes, and family history of CVD.

The study was approved by the Ethics Committee of Beijing Chao-Yang Hospital, Capital Medical University, China and written informed consent was obtained from each participant.

### Sample collection and measurement of parameters

Blood samples were collected at the time of starting the second HD session of the week. The levels of Hb, blood urea nitrogen (BUN), creatinine (Cr), total protein, albumin, total cholesterol (TC), triglycerides (TG), and iron were measured by standard laboratory methods using an autoanalyzer. Serum intact-parathyroid hormone was determined by immunoradiometric assay. Transferrin was measured by the Nitro-so-PSAP test, and TSAT was calculated as serum iron-TIBC. The Kt/V was calculated by formal urea kinetic modeling. Serum levels of interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) were measured by enzyme-linked immunosorbent assay (human IL-6 and human TNF- $\alpha$  immunoassay kit; Biosource International Inc., CA, USA). Serum levels of hepcidin-25 were determined by liquid chromatography-tandem mass spectrometry, as previously described.<sup>[17]</sup>

### Common carotid artery ultrasonography

Carotid artery ultrasonography was performed by an experienced specialist physician who was specifically trained for the vascular ultrasonography. Carotid B-mode ultrasound measurements were performed using HDI 5000 equipped with a 5–12 MHz linear array transducer (Philips, USA). Subjects were examined in the supine position with the head turned 45° contralateral to the side of scanning. Intima media

thickness (IMT) was defined as the distance between the lumen-intima and the media-adventitia ultrasound interfaces and measured as the distance between the two parallel echogenic lines on the far wall of artery in longitudinal plane image frozen in the screen by electronic calipers.<sup>[18]</sup> The IMT on the far wall of the bilateral common carotid artery (CCA) about 10 mm proximal to the bifurcation of the carotid artery was measured manually as previously described.<sup>[19]</sup> Mean IMT was calculated as the average of the three readings of bilateral carotid arteries. Plaque was defined as localized thickening of IMT  $\geq 1.2$  mm that did not uniformly involve the whole wall of carotid artery.

### Statistical analysis

The SPSS (SPSS for Windows, USA) version 13.0 statistics package was employed for the statistical analysis. Measurement data was presented as mean  $\pm$  standard deviation (SD). Comparisons were performed using independent-samples *t*-test or Chi-square test. In addition, bivariate correlation analysis and multiple regression analysis were performed. A  $P < 0.05$  was regarded as statistically significant.

## RESULTS

### Subject characteristics

A total number of 164 patients (78 CHD/DN, 86 CHD/non-DN) with a mean age of  $48.6 \pm 11.6$  years (range, 20–71 years) and a mean dialysis period of  $41.3 \pm 17.8$  months (range, 5–84 months) were included in this study. CHD/DN group consisted of 35 men and 43 women; the mean age was  $48.7 \pm 11.3$  years and average dialysis period was  $40.3 \pm 18.3$  months. CHD/non-DN group consisted of 39 men and 47 women; the mean age was  $48.4 \pm 12.0$  years and average dialysis period was  $44.2 \pm 17.2$  months. There was no significant difference between the two groups in terms of age, sex ratio, dialysis duration, smoking, body mass index, Kt/V, ESA dose, the percent of intravenous (IV) iron replacement therapy, iron dose, Hb, serum Cr, BUN, TG, TC, etc. [Table 1].

All patients were treated by epoetin- $\alpha$ . Mean dose of epoetin- $\alpha$  was  $116.5 \pm 36.8$  U·kg<sup>-1</sup>·w<sup>-1</sup>. Furthermore, 79.9% of patients were treated with iron ( $186.6 \pm 68.4$  mg/month) in this study.

### Hepcidin and common carotid artery intima media thickness in patients with chronic hemodialysis/diabetic nephropathy and chronic hemodialysis/nondiabetic nephropathy

Serum hepcidin-25 level in CHD/DN group ( $34.43 \pm 4.75$  ng/ml) was significantly higher than that in CHD/non-DN group ( $26.63 \pm 10.12$  ng/ml). Furthermore, in CHD/DN patients, serum hepcidin-25 level was positively correlated with serum ferritin ( $r = 0.835$ ,  $P = 0.000$ ), high sensitivity C-reactive protein (hs-CRP) ( $r = 0.849$ ,  $P = 0.000$ ), TNF- $\alpha$  ( $r = 0.929$ ,  $P = 0.000$ ) and IL-6 ( $r = 0.919$ ,  $P = 0.000$ ) [Table 2]. It was interesting to note that the serum level of hepcidin-25 was no correlated with serum

ferritin ( $r=0.130, P=0.233$ ), hs-CRP ( $r=0.006, P=0.955$ ), TNF- $\alpha$  ( $r=0.118, P=0.279$ ) and IL-6 ( $r=0.110, P=0.314$ ) in CHD/non-DN patients [Table 3].

On the other hand, Table 4 also shows CHD/DN patients exhibited higher CCA-IMT, hs-CRP, and higher serum hepcidin-25 levels than in CHD/non-DN patients [Table 4].

### Correlations between common carotid artery intima media thickness and risk factors for atherosclerosis and hepcidin-25 in patients with chronic hemodialysis/diabetic nephropathy

In CHD/DN patients, bivariate correlation analysis showed that CCA-IMT was positively correlated with serum hepcidin-25 ( $r=0.942, P=0.000$ ), hs-CRP ( $r=0.909, P=0.000$ ), TNF- $\alpha$  ( $r=0.944, P=0.000$ ), IL-6 ( $r=0.961, P=0.000$ ), ferritin ( $r=0.781, P=0.000$ ), and low-density lipoprotein (LDL) ( $r=0.876, P=0.000$ ). No correlation with age, dialysis duration, serum TG and TC [Table 5]. However, in CHD/non-DN patients, bivariate correlation analysis showed that CCA-IMT was only positively

**Table 1: Characteristics of patients in both study groups**

Items	CHD/DN group (n = 78)	CHD/non-DN group (n = 86)	t/ $\chi^2$	P
Age, years	48.7 ± 11.3	48.4 ± 12.0	0.190	0.850
Gender, male/female	35/43	39/47	0.004	0.951
Dialysis duration, months	40.3 ± 18.3	44.2 ± 17.2	1.376	0.171
BMI, kg/m <sup>2</sup>	23.6 ± 2.3	23.1 ± 1.4	1.184	0.241
Smoking, n (%)	20 (25.6)	16 (18.6)	1.182	0.277
RASI, n (%)	56 (71.8)	66 (76.7)	0.526	0.468
IV iron, n (%)	60 (76.9)	71 (82.6)	0.808	0.369
Iron dose, mg/week	62.9 ± 20.0	58.9 ± 16.9	1.385	0.168
ESA dose, U·kg <sup>-1</sup> ·w <sup>-1</sup>	118.4 ± 39.1	114.8 ± 34.1	1.374	0.172
SBP, mmHg	139.4 ± 7.1	141.1 ± 10.1	1.192	0.235
DBP, mmHg	80.0 ± 6.9	81.6 ± 7.4	1.357	0.177
Kt/V	2.34 ± 0.29	2.36 ± 0.31	0.347	0.729
Hb, g/L	116.0 ± 7.7	115.8 ± 8.0	0.136	0.892
TSAT (%)	32.9 ± 8.9	30.9 ± 9.7	1.374	0.171
Ferritin, mmol/L	326.3 ± 159.5	354.8 ± 125.6	1.277	0.204
Alb, g/L	34.7 ± 2.7	35.2 ± 2.8	1.231	0.220
Creatinine, $\mu$ mol/L	881.5 ± 101.0	896.6 ± 102.4	0.947	0.345
BUN, mmol/L	23.4 ± 6.1	24.0 ± 5.2	0.689	0.492
TG, mmol/L	1.46 ± 0.66	1.31 ± 0.68	1.337	0.183
TC, mmol/L	4.11 ± 1.08	3.92 ± 0.87	1.300	0.196
LDL-C, mmol/L	2.28 ± 0.66	2.21 ± 0.60	0.670	0.504
Glucose, mmol/L	5.19 ± 0.75	5.15 ± 0.78	0.348	0.728
PTH, pg/ml	212.8 ± 68.3	224.4 ± 61.6	1.144	0.254

Values are mean ± SD, unless specified otherwise. BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; Hb: Hemoglobin; TSAT: Transferrin saturation; Alb: Albumin; BUN: Blood urea nitrogen; TG: Triglyceride; TC: Total cholesterol; LDL-C: Low density lipoprotein-cholesterol; RASI: Renin angiotensin system inhibitor; CHD: Chronic hemodialysis; DN: Diabetic nephropathy; IV: Intravenous; ESA: Erythropoiesis-stimulating agent; PTH: Parathyroid hormone; SD: Standard deviation.

**Table 2: Simple correlation coefficients between hepcidin-25 and other variables in CHD/DN patients**

Variables	r	P
Ferritin	0.835	0.000
hs-CRP	0.849	0.000
TNF- $\alpha$	0.929	0.000
IL-6	0.919	0.000

CHD: Chronic hemodialysis; DN: Diabetic nephropathy; hs-CRP: High-sensitivity C-reactive protein; TNF- $\alpha$ : Tumor necrosis factor- $\alpha$ ; IL-6: Interleukin-6.

**Table 3: Simple correlation coefficients between hepcidin-25 and other variables in CHD/non-DN patients**

Variables	r	P
Ferritin	0.130	0.233
hs-CRP	0.006	0.955
TNF- $\alpha$	0.118	0.279
IL-6	0.110	0.314

CHD: Chronic hemodialysis; DN: Diabetic nephropathy; hs-CRP: High-sensitivity C-reactive protein; TNF- $\alpha$ : Tumor necrosis factor- $\alpha$ ; IL-6: Interleukin-6.

**Table 4: Serum hepcidin-25 level and CCA-IMT in CHD/DN and CHD/non-DN patients**

Items	CHD/DN group (n = 78)	CHD/non-DN group (n = 86)	t	P
CCA-IMT, mm	1.16 ± 0.10	0.91 ± 0.10	9.446	0.000
Hepcidin-25, ng/ml	34.43 ± 4.75	26.63 ± 10.12	6.604	0.000
hs-CRP, mmol/L	2.17 ± 0.92	1.71 ± 1.06	2.954	0.004
TNF- $\alpha$ , pg/ml	25.72 ± 6.50	23.29 ± 5.31	2.630	0.009
IL-6, pg/ml	13.89 ± 5.30	10.24 ± 5.47	4.335	0.000

CCA-IMT: Common carotid artery intima-media thickness; CHD: Chronic hemodialysis; DN: Diabetic nephropathy; hs-CRP: High-sensitivity C-reactive protein; TNF- $\alpha$ : Tumor necrosis factor- $\alpha$ ; IL-6: Interleukin-6.

**Table 5: Correlation coefficients for CCA-IMT and other variables in CHD/DN patients**

Variables	r	P
Hepcidin-25	0.942	0.000
hs-CRP	0.909	0.000
TNF- $\alpha$	0.944	0.000
IL-6	0.961	0.000
Ferritin	0.781	0.000
Age	0.186	0.103
Dialysis durations	-0.015	0.899
TG	0.064	0.580
TC	0.182	0.111
LDL-C	0.876	0.000

CCA-IMT: Common carotid artery intima-media thickness; CHD: Chronic hemodialysis; DN: Diabetic nephropathy; hs-CRP: High-sensitivity C-reactive protein; TNF- $\alpha$ : Tumor necrosis factor- $\alpha$ ; IL-6: Interleukin-6; TG: Triglycerides; TC: Total cholesterol; LDL-C: Low density lipoprotein-cholesterol.

correlated with serum hs-CRP ( $r = 0.255$ ,  $P = 0.018$ ) and LDL-cholesterol (LDL-C) ( $r = 0.683$ ,  $P = 0.000$ ) [Table 6].

It was interesting to note that after adjustment for age, gender, dialysis durations, smoking, IV iron, TG, TC and serum TNF- $\alpha$ , serum hepcidin-25, ferritin, LDL-C, hs-CRP, and IL-6 level exhibited independent association with CCA-IMT in CHD/DN patients on multiple regression analysis [Table 7]. However, in CHD/non-DN patients, after adjustment for age, gender, dialysis durations, IV iron, serum TNF- $\alpha$ , IL-6, hepcidin-25, ferritin, TG and TC, smoking and serum LDL-C level exhibited independent association with CCA-IMT [Table 8].

## DISCUSSION

Cardiovascular diseases remain the leading cause of morbidity and mortality in patients with chronic kidney disease (CKD), especially with diabetes mellitus.<sup>[20,21]</sup> The prevalence of cardiovascular death in CKD patients, especially in those on dialysis therapy is 10–20 times greater than the age, gender, and race-matched general population.<sup>[22,23]</sup> Some studies have demonstrated that atherosclerosis could induce an increase of the arterial IMT and arterial stiffening, and eventually lead to luminal obstruction with consequent ischemic events, such as myocardial infarction and stroke.<sup>[24,25]</sup> In the 1970s, Lindner *et al.*<sup>[26]</sup> proposed that atherosclerosis was the main cause of CVD in patients with CKD and that its progression was accelerated by long-term dialysis. Subsequent investigations elucidated abnormal atherosclerosis pathology in patients with CKD may be classified as atherosclerosis, arteriosclerosis, and vascular calcification.<sup>[27-30]</sup>

Hepcidin, a small antimicrobial peptide synthesized by the liver, is the principal effector of the modulation of iron metabolism, via its ability to bind ferroportin-1 (Fp-1) on cellular surface blocking its iron transport activity, and to increase Fp-1 degradation.<sup>[31]</sup> In enterocytes, Fp-1 internalization on the basolateral surface causes the retention of absorbed iron with subsequent loss by desquamation while the same process in macrophages causes the failure to release iron.<sup>[32]</sup> Hepcidin plays an important role in iron accumulation in several types of cells, binding to and degrading the iron export protein Fp-1, potentially leading to iron sequestration in various tissues and organs, including vascular cells.<sup>[17]</sup> Inflammation accounts for accelerated atherosclerosis in ESRD patients. It had already been demonstrated that TNF- $\alpha$  and IL-6 induced oxidative stress and vascular calcification.<sup>[33]</sup> Our study found that CHD/DN patients exhibited higher hepcidin-25 levels than that in CHD/non-DN patients. Recent study reported that it was established a link between iron metabolism and insulin resistant states, including diabetes mellitus.<sup>[16]</sup> A cellular research showed that hepcidin binding to Fp is able to activate Janus kinase 2/Signal Transducer and Activator 3 signaling in mice models.<sup>[34]</sup> Interestingly, we also found that serum hepcidin-25 level was positively correlated with serum ferritin, hs-CRP, TNF- $\alpha$ , and IL-6 in CHD/DN patients, which was not similar to that in CHD/non-DN patients. These findings suggest possible linkage between iron metabolism and inflammation that may contribute to the development of UAAS in CHD/DN.

Recently, Improving Global Outcomes guidelines recommended IV iron administration for HD patients and either oral or IV iron for patients in CKD stages 1–5 who are anemic.<sup>[3]</sup> Iron state should be determined not only at the start of ESA therapy but also during ESA treatment in this population.<sup>[3]</sup> According to the KDOQI guidelines, TSAT <20% and serum ferritin concentrations <100 ng/ml were regarded as absolute iron deficiency, and TSAT <20% and serum ferritin levels >100 ng/ml was regarded as functional iron deficiency. For this reason, most ESRD patients require IV iron to replete iron stores.<sup>[35]</sup> But, unfortunately, IV administered iron is often used routinely with inadequate attention to the body iron stores or severity of systemic inflammation. Only a minute amount (3–4 mg)

**Table 6: Correlation coefficients for CCA-IMT and other variables in CHD/non-DN patients**

Variables	<i>r</i>	<i>P</i>
Hepcidin-25	0.073	0.505
hs-CRP	0.255	0.018
TNF- $\alpha$	0.021	0.851
IL-6	0.071	0.513
Ferritin	0.018	0.867
Age	0.138	0.206
Dialysis durations	-0.163	0.133
TG	0.156	0.151
TC	0.012	0.914
LDL-C	0.683	0.000

CCA-IMT: Common carotid artery intima-media thickness; CHD: Chronic hemodialysis; DN: Diabetic nephropathy; hs-CRP: High-sensitivity C-reactive protein; TNF- $\alpha$ : Tumor necrosis factor- $\alpha$ ; IL-6: Interleukin-6; TG: Triglycerides; TC: Total cholesterol; LDL-C: Low density lipoprotein-cholesterol.

**Table 7: Variables predicting IMT in multiple regression analysis in CHD/DN patients**

Variables	Standardized coefficient $\beta$	SE	<i>t</i>	<i>P</i>	95% CI	
					Lower bound	Upper bound
Hepcidin-25	0.215	0.001	3.792	0.000	0.002	0.007
hs-CRP	0.182	0.005	3.822	0.000	0.009	0.030
TNF- $\alpha$	0.021	0.001	0.291	0.772	-0.002	0.003
IL-6	0.353	0.001	4.486	0.000	0.004	0.010
Ferritin	0.096	0.000	2.786	0.005	0.000	0.000
Age	0.007	0.000	0.360	0.720	0.000	0.000
Gender	-0.029	0.006	-1.019	0.312	-0.017	0.005
Dialysis durations	0.020	0.000	1.025	0.309	0.000	0.000
TG	0.001	0.003	0.074	0.941	-0.006	0.006
TC	0.016	0.002	0.775	0.441	-0.005	0.002
LDL-C	0.218	0.006	5.345	0.000	0.020	0.045
Smoking	0.031	0.007	1.008	0.317	-0.007	0.021
IV iron	0.021	0.006	0.763	0.448	-0.008	0.018

IMT: Intima-media thickness; CHD: Chronic hemodialysis; DN: Diabetic nephropathy; SE: Standard error; CI: Confidence interval; hs-CRP: High-sensitivity C-reactive protein; TNF- $\alpha$ : Tumor necrosis factor- $\alpha$ ; IL-6: Interleukin-6; TG: Triglycerides; TC: Total cholesterol; LDL-C: Low density lipoprotein-cholesterol; IV: Intravenous.



**Table 8: Variables predicting CCA-IMT in multiple regression analysis in CHD/non-DN patients**

Variables	Standardized coefficient $\beta$	SE	$t$	$P$	95% CI	
					Lower bound	Upper bound
Hepcidin-25	0.002	0.001	0.027	0.979	-0.002	0.002
hs-CRP	0.126	0.010	1.731	0.088	-0.003	0.038
TNF- $\alpha$	0.044	0.002	0.505	0.772	-0.002	0.005
IL-6	0.023	0.002	0.332	0.741	-0.004	0.003
Ferritin	0.032	0.000	0.482	0.631	0.000	0.000
Age	0.124	0.001	1.560	0.123	-0.003	0.000
Gender	0.182	0.028	1.920	0.059	-0.002	0.110
Dialysis durations	0.004	0.001	0.065	0.949	-0.001	0.001
TG	0.018	0.016	0.237	0.814	-0.029	0.036
TC	0.100	0.012	1.392	0.168	-0.007	0.041
LDL-C	0.399	0.021	4.756	0.000	0.058	0.141
Smoking	0.482	0.035	5.257	0.000	-0.251	0.113
IV iron	0.149	0.031	1.881	0.064	-0.003	0.119

CCA-IMT: Common carotid artery intima-media thickness; CHD: Chronic hemodialysis; DN: Diabetic nephropathy; SE: Standard error; CI: Confidence interval; hs-CRP: High-sensitivity C-reactive protein; TNF- $\alpha$ : Tumor necrosis factor- $\alpha$ ; IL-6: Interleukin-6; TG: Triglycerides; TC: Total cholesterol; LDL-C: Low-density lipoprotein cholesterol; IV: Intravenous.

of the total body iron (3–4 g in an adult man) resides in the plasma bound to transferrin, which serves as a safe vehicle for iron transport in the circulation. IV iron products are generally administered as bolus injections of 100–1000 mg, which far exceeds the available pool of free transferrin and represents a huge quantity compared to the intestinal iron absorption of 1–2 mg/d in the course of 3–4 meals. Administration of these products results in an increased plasma level of catalytically active nontransferrin bound iron and the rise in the biomarkers of oxidative stress and inflammation.<sup>[36]</sup> IV iron bypasses the biological safeguards for the transport and handling of iron and helps to intensify CKD-associated oxidative stress and inflammation. Ng *et al.*<sup>[37]</sup> found that iron treatments result in a fall in A1C, which is independent of glycemic changes in patients with diabetes and CKD Stage IIIb and IV. But indiscriminate use of IV iron can accelerate CVD, promote microbial infections and worsen diabetes and diabetic complications in such patients.

Given that high-frequency B-mode ultrasonography is a noninvasive and effective method of detecting carotid artery wall and provides measurement of IMT and presence of plaques,<sup>[38]</sup> and the increased IMT as well as formation of carotid artery plaque can predict future events of coronary heart disease and systemic subclinical atherosclerosis,<sup>[39,40]</sup> we evaluated the CCA-IMT in CHD patients. According to our results obtained in this study, the CHD patients with dialysis were further grouped into CHD/DN and CHD/non-DN group, and the level of serum hepcidin in these two groups were compared. We found that CHD/DN patients exhibited higher CCA-IMT and higher

hepcidin levels than in CHD/non-DN patients. In CHD/DN patients, CCA-IMT was positively correlated with serum hepcidin, hs-CRP, and LDL-C. After adjustment for age, gender, dialysis durations, smoking, IV iron, TG, TC, and TNF- $\alpha$ , serum hepcidin-25, LDL-C, hs-CRP, and IL-6 level exhibited independent association with IMT in CHD/DN patients on multiple regression analysis. However, it was interesting to note that in CHD/non-DN patients, after adjustment for age, gender, dialysis durations, IV iron, serum TNF- $\alpha$ , IL-6, hepcidin-25, TG, and TC, smoking and serum LDL-C level exhibited independent association with CCA-IMT. With respect to the relationship between hepcidin and UAAS in CHD/DN patients, we suspected that iron sequestration in vascular cells might be linked to arterial stiffness.

The exact mechanism by which hepcidin promotes atherosclerosis has not been clarified. However, several reports have demonstrated a relationship between iron accumulation and arterial alteration,<sup>[41]</sup> including generation of oxidized LDL,<sup>[42]</sup> endothelial cell dysfunction<sup>[43]</sup> and arterial smooth muscle proliferation.<sup>[44]</sup> Reis *et al.*<sup>[45]</sup> found the significant correlations between cIMT and serum ferritin or IV iron administration doses in 60 CHD patients, suggesting that excessive administration or accumulation of iron may account for accelerated arteriosclerosis in CHD patients. Moreover, using electron paramagnetic resonance spectroscopy and inductively coupled plasma–mass spectroscopy, Stadler *et al.*<sup>[46]</sup> confirmed that there is a significantly greater accumulation of iron in arteriosclerotic lesions than in healthy vessels in non-CKD patients. These reports suggest that iron accumulation might be associated with the development and/or progression of arteriosclerosis. Similarly, our current study further confirmed that the increase of serum hepcidin in CHD patients, especially with diabetes mellitus compared with chronic hemodialyzed nondiabetic patients and general population, which suggests that increase of hepcidin might be an important cardiovascular risk factor in CHD/DN.

In conclusion, our results suggest that elevated levels of serum hepcidin may be closely associated with CCA-IMT or possibly the development of uremic accelerated in CHD patients.

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