

Is there a link between Hepcidin, inflammation and atherosclerosis in patients with chronic kidney disease

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Abstract

Objective: The major cause of death and morbidity in chronic kidney disease (CKD) patients is atherosclerotic cardiovascular disease. Iron build-up is enhanced in atherosclerotic lesions in arterial macrophages. The inhibition of hepcidin by binding and inactivating the iron export cell iron ferroportin from macrophages and iron absorption from Enterocytes. In this study, the relationship between hepcidin-25 serum and subclinical atherosclerosis is clarified in CKD patients.

Methods: 40 non hemodialysis CKD (non HD-CKD) and 40 hemodialysis CKD (HD-CKD) patients were registered in this study in addition to 20 subjects as a control group. In HD-CKD patients, the sample was collected at start of hemodialysis session after overnight fast. In non HD-CKD patients and the controls, the venous blood sample was also collected from a peripheral vein after overnight fast. These samples were centrifuged for 15 minutes at approximately 3000 rpm and stored at -70°C for the measurement of serum levels of hepcidin-25, TNF- α , IL-6, hs-CRP, ferritin, iron, and total iron binding capacity.

Results: In the non HD-CKD and HD-CKD groups compared to controls ($P < 0.001$ for each group) hepcidin-25 serum has been considerably elevated. In contrast to the non-HD-CKD group ($P = 0.005$) it was considerably elevated in HD-CKD groups. In non-HD-CKD, compared to control group ($P = 0.002$), CIMT has been considerably raised. In HD-CKD, compared with the control group ($P = 0.001$), it was also considerably elevated. In non HD-CKD and HD-CKD groups serum levels were raised substantially by hs-CRP ($P < 0.001$ for each group), TNF- α ($P < 0.001$, for each group), and IL-6 ($P < 0.001$ for each group) compared to the control group.

Conclusion: Our data suggest that in individuals with CKD hepcidin may be involved in cardiovascular disease and atherosclerotic pathogenesis.

Keywords: CKD; Hepcidin-25; HD

Introduction

The bioactive kind is Hepcidin-25, a 25 amino acid peptide (1). Systematic hepcidin is generated mostly in the liver but also in the heart, retina, fat, lumbar, brain, stomach and pancreas, as well as in monocytes, neutrophils and macrophages. Diet iron is absorbed into the duodenum and upper jejunum. The FPN facilitates cellular iron efflux into the systemic circuit at the enterocyte basolateral location. Hepcidin bonds with FPN to membranes of cells that export iron, such macrophages, and enterocytes to cause ferroportin endocytosis and lysosomal breakdown, decrease plasma iron supply and decrease the supply of iron to the plasma⁽²⁾.

Chronic kidney disease (CKD) is characterised as renal structural or function abnormalities that occur for three months or more (3). Recurring inflammation and high pro-inflammatory markers occur in CKD patients. The chronic inflammation of CKD is induced by persistent imbalances of prooxidants and antioxidants (4).

Hepcidin is eliminated by hemodialysis and peritoneal dialysis in patients reaching dialysis (5). Anemia is among the most frequent CKD co-morbidities and is caused by a range of causes, including decreases in the synthesis of endogenous erythropoietin in the kidneys, decreases in erythrocyte survival and nutritive deficiencies (folate and vitamin B12) (6).

The risk of early death is increased in patients with CKD, mostly due to cardiovascular reasons (7). Age, fumes, diabetes mellitus, hypertension, dyslipidemia and insulin resistance are traditional risk factors of atherosclerosis. Oxidative stress, inflammation, gut dysbiosis, endothelial dysfunction, secondary hyperparathyroidism, progressive end products of glycemia and vascular calcification are non-traditional risk factors (8). Dyslipidemia is a frequent feature in CKD patients with a highly atherogenic profile with low HDL cholesterol levels and high LDL cholesterol triglycerides and oxidation (9).

Aim of the work

The study clarified the relationship between hepcidin-25, inflammatory markers and atherosclerosis of serum in individuals with CKD.

Subjects and Methods

In the period of October 2019 to July 2020, the study was place in the Urology and Nephrology University Hospital of Minia University, Egypt. The Committee on Hospital Ethics authorised this study and each case got written permission. Malignant patients, DM, trauma history, acute infection, chronic liver disease, heart failure, recent heart attack, and stroke were not included. All patients and controls were clinically examined, including a comprehensive heart examination, a complete abdomen examination, chest and neurology.⁽¹⁰⁾.

Statistical analysis

The obtained data have been evaluated statistically using version 25 of the SPSS programme. For the mean numerical data, the standard deviation (SD), the lowest and maximum range, descriptive statistics were carried out, whilst the number and the percentage for categorical data were carried out.

Results

In the research there were 40 patients with GFR estimated CKD stage 3-5, so no hemodialysis was performed. In non-HD-CKD patients, 70% (28), 57.5% (23) and 35% (7) of individuals were diagnosed with hypertension. In patients with chronic renal disorders whether dialysis or not there has been a substantial rise in inflammatory markers. In both groups, serum ferritin was considerably elevated despite these modifications. (Tables 1-4; Figure 1)

Table 1: Demographic features of the controls and patients' groups (* P < 0.05)

Variable	Group I Control n = 20	Group II Non HD CKD n = 40	Group III HD CKD n = 40	I vs II	I vs III	II vs III
				P value		
Age (years) Range Mean ± SD	40 – 53 45.95 ± 4.01	41 – 69 49.70 ± 6.76	40 – 57 47.75 ± 5.04	0.06	0.203	0.261
Sex Male Female	12 (60%) 8 (40%)	18 (45%) 22 (55%)	17 (42.5%) 23 (57.5%)	0.41	0.26	0.22
HTN HTN No HTN	7 (35%) 13 (65%)	28 (70%) 12 (30%)	23 (57.5%) 17 (42.5%)	0.013*	0.17	0.35
BMI (kg/m²) Range Mean ± SD	22.32 – 26.81 23.71 ± 0.94	20.69 – 34.65 22.31 ± 0.88	20.03 – 23.73 21.97 ± 0.64	< 0.001***	< 0.001***	0.06

Table 2: Inflammatory markers in the controls and patients' groups

Variable	Group I Control n = 20	Group II Non HD CKD n = 40	Group III HD CKD n = 40	I vs II	I vs III	II vs III
				P value		
Serum hs-CRP (mg/L) Range Mean ± SD	6 – 24 10.20 ± 5.87	12 – 102 41.13 ± 23.91	12 – 102 43.21 ± 21.26	< 0.001***	< 0.001***	> 0.999
Serum IL-6 (pg/ml)						

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Range	1.7 – 46	3 – 71	6.2 – 101	< 0.001***	< 0.001***	> 0.999
Mean ± SD	7.36 ± 9.28	26.79 ± 21.44	29.04 ± 19.24			
Serum TNF-α (pg/ml)						
Range	3.8 – 11	7.5 – 59.5	9.5 – 57.5	< 0.001***	< 0.001***	> 0.999
Mean ± SD	7.12 ± 2.02	26.85 ± 15.72	29.04 ± 15.04			

Table 3: Iron profile in the controls and patients' groups

Variable	Group I Control n = 20	Group II Non HD CKD n = 40	Group III HD CKD n = 40	I vs II	I vs III	II vs III
				P value		
Serum iron (µg/dl)						
Range	66 – 180	58 – 247	96 – 341	0.195	0.293	< 0.001***
Mean ± SD	136.15±28.61	120 ± 48.51	172.02 ± 61.13			
Serum TIBC (µg/dl)						
Range	210 – 340	218 – 410	129 – 390	> 0.999	0.047*	0.011*
Mean ± SD	278.85 ± 2.13	284.70 ± 41.85	245.85 ± 54.48			
Serum ferritin (ng/ml)						
Range	39 – 200	130 – 760	239 – 730	< 0.001***	< 0.001***	> 0.999
Mean ± SD	88.55 ± 37.41	347.97 ± 111.57	354.40± 110.92			
TAST (%)						
Range	21.29 - 80.90	14.39 – 105.11	35.52 – 237.04	0.356	0.073	< 0.001***
Mean ± SD	49.68 ± 14.04	44.18 ± 21.98	76.16 ± 41.82			
HB (gm/dl)						
Range	11.9 – 14	6 – 12.7	6.2 – 15.5	< 0.001***	< 0.001***	> 0.999
Mean ± SD	12.95 0.48	9.64 1.77	9.83 1.96			

Table 4: Serum hepcidin-25 and carotid atherosclerosis in the studied groups

Variable	Group I Control n = 20	Group II Non HD CKD n = 40	Group III HD CKD n = 40	I vs II	I vs III	II vs III
				P value		
Serum hepcidin-24 (ng/ml)						
Range	3.9 - 8.9	10.8 – 48.5	13.5 – 98	< 0.001***	< 0.001***	0.005**
Mean ± SD	6.44 ± 1.48	24.89 ± 12.55	48.44 ± 27.84			

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Carotid atherosclerosis						
CIMT (mm)						
Range	0.5 – 0.7	0.5 – 1.5	0.5 ± 1.6			
Mean ± SD	0.6 ± 0.07	0.90 ± 0.38	0.91 ± 0.37	0.002**	0.001**	0.8
Atherosclerotic plaque	0	12	14			
No atherosclerotic plaques	20	28	26	0.005**	0.002**	0.811

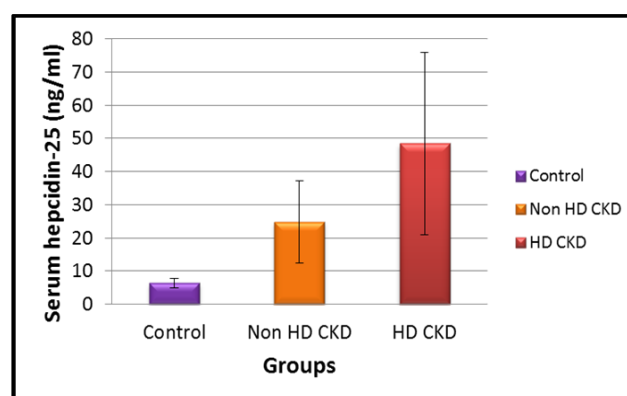


Figure 1: Serum hepcidin-25 level in the studied groups

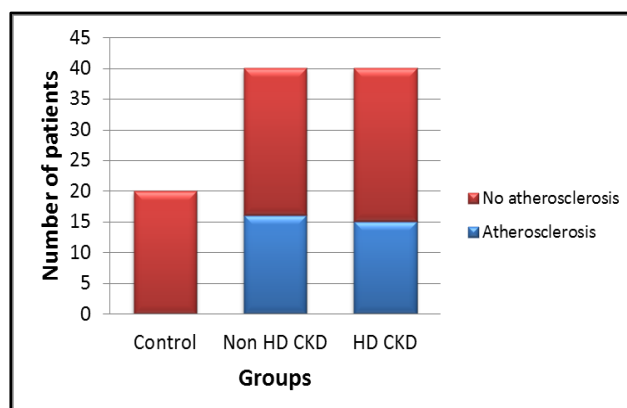


Figure 2: Carotid atherosclerosis in the studied groups

Table 5: Correlation of serum hepcidin-25 with laboratory and demographic parameters in non HD-CKD and HD-CKD groups

Variable	Non HD-CKD		HD-CKD	
	R	P value	R	P value
CIMT	0.816	<0.001***	0.914	<0.001***
Serum hs-CRP	0.698	<0.001***	0.664	<0.001***

Serum IL-6	0.725	<0.001***	0.756	<0.001***
Serum TNF-α	0.836	<0.001***	0.852	<0.001***
Serum TC	0.717	<0.001***	0.705	<0.001***
Serum TG	0.811	<0.001***	0.889	<0.001***
S. LDL-C	0.600	<0.001***	0.612	<0.001***
S. HDL-C	-0.371	0.018*	-0.637	<0.001***
S. iron	0.011	0.947	-0.070	0.669
S. TIBC	0.026	0.873	0.065	0.692
S. ferritin	0.380	0.016*	-0.090	0.581
TSAT	0.038	0.818	-0.102	0.533
Duration of HD			-0.032	0.843
Age	0.169	0.354	0.914	0.280

Discussion

In non-HD-CKD and HD-CKD groups, BMI was substantially lower than controls. It accords with Kikuchi et al. who showed that the BMI in CKD patients was considerably lower and was related with CKD progression (11). This is comparable to the Dai et al. research, which found that BMI in HD-CKD patients is substantially lower than in controls (12). BMI has been reduced by increased protein catabolism and protein deficiency in CKD and ESRD patients. Metabolism acidosis, bowel dysbiosis, systemic inflammation and uremic toxin accretion might lead to this (13).

In non-HD-CKD patients, serum TG and Serum LDL-C in comparison to controls were substantially higher. In non-HD-CKD, serum HDL-C compared with controls was substantially lower. This is consistent with the results of BEHERA and Mohanty et al. which have reported a significant increase in serum TC, serum TG and serum LDL-C than the controls in patients without diabetic CKD. In nondiabetic CKD patients, they observed that serum HDL-C was much reduced than in controls ⁽¹⁴⁾⁽¹⁵⁾. In comparison to controls, Serum TC, Serum TG, Serum LDL-C and HD-CKD patients have been considerably elevated in our study. The HDL-C serum dropped substantially in HD-CKD compared to control patients. Maurya et al. have indicated that serum TC, serum TG and serum LDL-C have been raised substantially compared to non-regular patients with HD-CKD in regular patients with HD-CKD. He also discovered that serum HDL-C in regular HD-CKD was much lower than in non-regular individuals with HD-CKD. ⁽¹⁶⁾ Due to a number of reasons, HDL-C levels are low. First, the major HDL elements are frequently low apolipoprotein A-I and A-II in individuals with reduced renal function. Furthermore, lecithin-cholesterol acyltransferase activity is decreased in CKD patients as an enzyme involved in the esterification of free cholesterol in HDL. The metabolism of LDL and IDL in CKD patients is severely affected, leading to high particulate concentrations ⁽¹⁷⁾. Hypertriglyceridemia is prevalent in individuals with CKD due

to the increased content in triglyceride-rich lipoproteins. Hypertriglyceridemia is caused by delayed catabolism and increasing the formation of triglyceride-rich lipoproteins ⁽¹⁸⁾.

In the Non HD-CKD patients compared to controls, serum HS-CRP, serum IL-6 and serum TNF- α were substantially elevated. This is in accordance with Gupta et al. who observed a substantial rise in serum hs-CRP, serum TNF- α and serum IL-6 in CKD patients with low eGFR compared to controls non-dialysis ⁽¹⁹⁾. In our study patients with HD-CKD, HS-CRP Serum hs-C, IT-6 Serum and TNF- α Serum compared to control topics were substantially elevated. This is in keeping with that in ESRD patients on hemodialysis the blood hs-CRP and serum TNF- α were significantly higher (20, 21). The reduction in renal clearance clearly results in higher circulating cytokines, while production has also risen. CKD patients are more prone to viral infections, particularly those on dialysis, which induce further inflammatory reactions. Blood stream, site infections, IV thrombosis and grafts catheter-related infections, ⁽²²⁾.

In non-HD-CKD patients and HD-CKD patients serum ferritin was considerably elevated in comparison with controls. The same is true for Kamal et al. who have shown that the amount of serum ferritin in non-HD-CKD and HD-CKD patients in comparison to controls is considerably greater (23). In HD-CKD patients serum iron was much higher than in controls. Serum TIBC reduced markedly compared to controls in HD-CKD patients. In HD-CKD patients, it has also dropped considerably compared to people without HD-CKD. ⁽²⁴⁾.

In non-HD-CKD and HD-CKD groups, hepcidin-25 serum was substantially greater than control patients. In HD-CKD patients, it was considerably greater than in non-HD-CKD individuals. This is consistent with the reports of Ali et al. and Kamal et al. that hepcidine-25 serum in non- HD-CKD patients and in HD-CKD patients was substantially greater than in controls. In HD-CKD patients, hepcidin-25 has also been shown to be substantially elevated compared to patients without HD-CKD (23) (25). It is not agreed that blood hepcidin-25 levels between the chronic hemodialysis patients and controls are not substantially different in Pelusi et al' ⁽²⁶⁾. ⁽²⁷⁾.

In non-HD-CKD patients, compared with the controls, CIMT was considerably elevated. It coincides with the findings of Kim et al. and Kajitani et al. that the CIMT in nondiabetic CKD patients compared with control participants has increased substantially (28). (29). In our study HD-CKD patients compared to the controls, CIMT was considerably enhanced. This has been agreed with Paul et al. and J. Paul et al. who have shown that hemodialysis CRF patients had higher CIMT than normal people (30, 31).

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