Research Article

Hepcidin-25 and its Implication in Chronic Kidney Diseases

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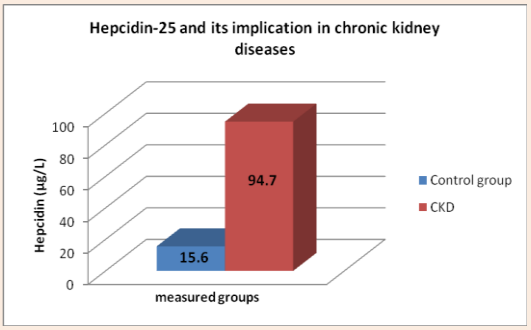
Abstract

Hepcidin is a 25-aminoacid cysteine-rich iron regulating peptide. Hepcidin quantification in human serum provides new topics for the pathogenesis of disorders of iron homeostasis and its treatment. This study describes ELISA immunoassay for hepcidin quantification in human serum in chronic kidney disease. We use a sandwich ELISA method for quantification of serum hepcidin levels in healthy control group (n = 50) and patients with chronic kidney disease (n = 50). Including criteria for control group was no evidence of iron metabolism disorders. The sandwich ELISA was highly specific for hepcidin-25. We found that serum hepcidin levels correlate significantly between two groups 15.6 ± 4.9 µg/L to 94.7 ± 19.9 µg/L. Ferritin levels and hemoglobin concentration in reticulocytes correlated significantly to serum hepcidin levels ($0.3 < r < 0.5$, $P < 0.010$). Transferrin levels showed negative and no significant correlation to hepcidin in serum ($r = -0.111$). The use of 2 monoclonal antibodies in a sandwich ELISA format provides a reliable, reproducible and not very expensive method for measuring serum concentrations of the bioactive form of hepcidin in laboratory practice.



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Introduction

The essential nature of iron for humans is known from XIX century [1]. Recently, it has been found that a key regulator of iron metabolism is hepcidin 25. It is synthesized by hepatocytes as 25-amino acid peptide, which is a biologically active form [2]. Various physiological and pathological processes regulate the synthesis of the hormone hepcidin [3]. Hepcidin acts in duodenal enterocytes and macrofages with ferroportin (an iron intracellular exporter) [4-7]. The introduction of an analytical method with sufficient sensitivity and specificity for accurate quantification of significant concentrations of hepcidin in biological fluids causes a marked interest in its investigation in different biomedical sciences. Patients with chronic kidney disease are in chronic inflammatory condition. As a result of the synthesis of hepcidin inflammation is mediated by IL-6 induction and coupling of signal transducer and activator of transcription 3 (STAT 3) to the promoter of hepcidin [8]. The level of serum hepcidin in the body is closely

associated with the iron, which is due to microinflammatory patients on maintenance hemodialysis and lead to new potential targets for therapy.

Experimental

Subjects

This study included 50 healthy controls and 50 patients with CKD. Patients were diagnosed in Clinic of Nephrology, "Aleksandrovska" hospital, Bulgaria. Informed consent was obtained from all healthy controls in accordance with to the Declaration of Helsinki (Directive 2001/20/EO).

50 serum samples from healthy volunteers 27 males (age 51.9 ± 5.2) and 23 females (age 44.2 ± 8.9) were collected. 50 serum samples from patients with CKD 27 males (age 67.3 ± 10.1) and 23 females (age 58.3 ± 14.5) were collected. All samples were collected, stored, and deidentified to protect patient privacy. CKD patients were separated into disease stage according to eGFR CKD-EPI Creatinine Equation (2009). Samples were stored at −70 °C before analysis of hepcidin levels. Ferritin analysis was performed by using ECLIA immunoassay (Roche Diagnostics). Transferrin levels were analyzed on Cobas Integra 400 (Roche Diagnostics). For hemoglobin concentration in reticulocytes we use Advia 2120 hematology analyzer (Siemens Healthcare Diagnostics). For serum hepcidin quantification we used verified ELISA method [9-11].

Data analysis

Four parameter curves were used for the calibration curve. The distribution of the data analysis was defined by REFVAL programme according to IFCC/CLSI C28-A3 2008 year. For statistical significance, Student's t-test and Pearson's correlation are used.

Results and Discussion

The established serum hepcidin levels for control group and patients with CKD are showed in **Figure 1**. Quantification of serum hepcidin levels in different CKD groups is showed in **Figure 2**. The correlation between different CKD groups is shown in **Table 1** and **Figure 3**.

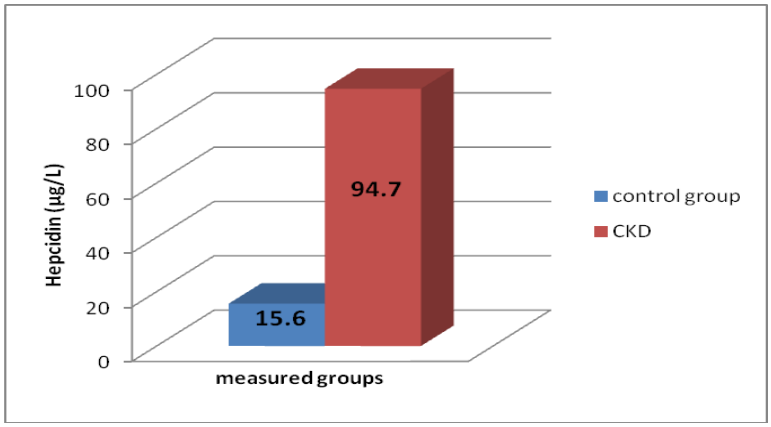

Figure 1 Serum hepcidin results

We found that there is a significant difference between serum hepcidin-25 levels in healthy control group compared to all stages of CKD. Values as described: a) for control group 15.6 ± 4.9 µg/l and b) 94.7 ± 19.9 µg/l. We found differences in serum hepcidin levels between CKD groups. The obtained results are: a) for stage II CKD (eGFR 61 – 90) – 56.13 µg/l; b) for stage IIIA CKD (eGFR 46 – 60) – 70.92 µg/l; c) for stage IIIB CKD (eGFR 31 – 45) – 93.43

µg/l; d) for stage IV CKD (eGFR 16 – 30) – 83.36 µg/l; e) for stage V CKD (eGFR < 15, without dialysis) – 141.98 µg/l.

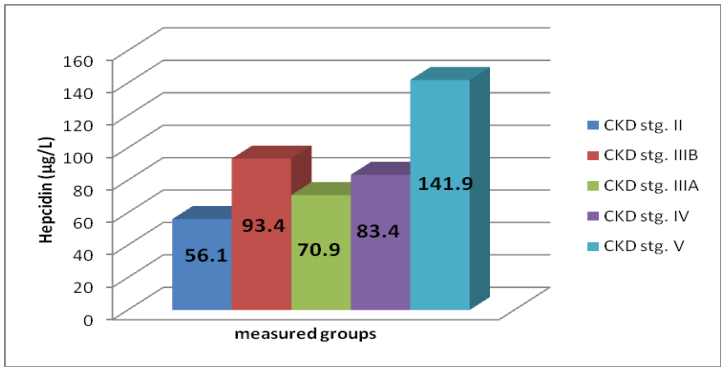

Figure 2 Hepcidin levels in CKD stages

Table 1 Hepcidin correlation between CKD stages

r	p	stages
0,517	0,175	V/IV
0,949	0,277	IV/IIIA
0,855	0,098	V/II
-0,553	0,448	IIIA/IIIB
-0,678	0,308	IIIA/II

We found a high correlation in serum hepcidin levels between CKD stages V and II (P < 0.1). A negative correlation was established between CKD stages IIIA and IIIB; CKD stages II and IIIA.

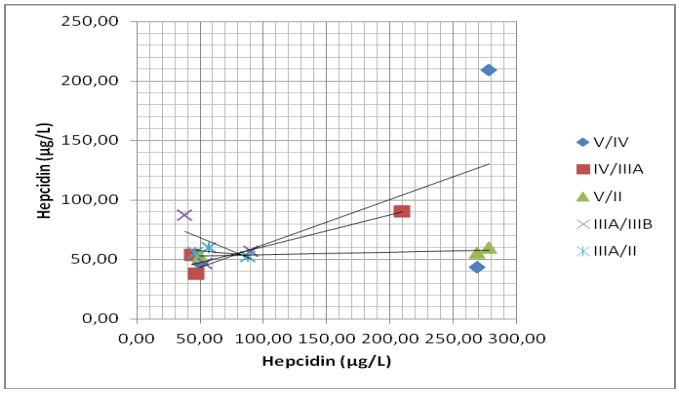

Figure 3 Hepcidin correlation between CKD stages

Positive and negative correlation between different CKD stages

We tried to find a correlation between serum hepcidin levels and measured parameters. A significant correlation was found between serum hepcidin levels and ferritin (Table 2).

Significantly high correlation between serum hepcidin levels and eGFR, hsCRP and red blood cells was found in all CKD stages ($P < 0.005$). We found that serum hepcidin levels correlate significantly between two groups 15.6 ± 4.9 µg/L vs. 94.7 ± 19.9 µg/L. A high correlation between CKD stages II and V was found. High correlation between eGFR between all groups was found. With disease progression occurs an increase in serum hepcidin levels.

We found a significant correlation for RBC, CRP and creatinine between CKD II and V stages; serum iron levels between CKD IIIA and II groups; CRP between CKD IV and V stages. Significant correlation between serum CRP and hepcidin levels is due to the etiology of the anemia - in chronic inflammation. For other measured parameters we found no statistically significant correlation.

Table 2 Correlation between hepcidin and measured parameters

CKD stages	RBC		HGB		CHr		PCR	
V/IV	r=-0,41*	p>0.05	r=-0,39*	p>0.05	r=-0,99^		r=0,42*	
IV/IIIA	r=-0,16		r=-0,94^		r=-0,02		r=-0,17	
V/II	r=-0,73^	p<0.005	r=-0,98^	p<0.05	r=-0,99^	p>0.05	r=0,99^	p>0.05
IIIA/IIIB	r=0,88*	p<0.05	r=-0,99^	p>0.05	r=0,98^		r=-0,84^	
IIIA/II	r=0,78*		r=-0,17		r=-0,03		r=-0,99^	
CKD stages	Retic		Fe		TIBC		MCV	
V/IV	r=-0,11		r=-0,99^		r=0,86^	p>0.05	r=-0,87^	
IV/IIIA	r=0,09		r=0,18	p>0.05	r=-0,69^	p<0.05	r=-0,59^	
V/II	r=-0,32	p>0.05	r=-0,94^		r=0,62^		r=0,79^	p>0.05
IIIA/IIIB	r=-0,38		r=-0,83^	p<0.05	r=-0,98^	p>0.05	r=0,89^	
IIIA/II	r=-0,99^		r=0,78^	p<0.005	r=-0,47*		r=0,95^	
CKD stages	TRSF		FERRIT		TSAT		MCH	
V/IV	r=0,79^	p>0.05	r=-0,73^		r=-0,97^	p>0.05	r=-0,99^	
IV/IIIA	r=0,55^		r=0,48*		r=-0,81^	p<0.05	r=-0,23	
V/II	r=0,04	p<0.05	r=-0,38	p>0.05	r=-0,61^	p>0.05	r=0,77^	p>0.05
IIIA/IIIB	r=0,10	p>0.05	r=0,98^		r=-0,67^		r=-0,48*	
IIIA/II	r=0,92^		r=0,27		r=0,69^	p<0.05	r=0,99^	
CKD stages	CRP		Crea		eGFR		MCHC	
V/IV	r=0,70^	p<0.005	r=0,62^	p<0.05	r=0,87^		r=0,25	
IV/IIIA	r=0,87^	p>0.05	r=0,99	p>0.05	r=0,87^	p<0.005	r=-0,84^	p>0.05
V/II	r=0,95^	p<0.005	r=0,84^	p<0.005	r=1^		r=-0,06	
IIIA/IIIB	r=-0,62^	p>0.05	r=-0,67^	p<0.05	r=1^		r=-0,86^	p<0.05
IIIA/II	r=-0,65^		r=-0,69	p>0.05	r=1^	p<0.05	r=0,15	p>0.05

*0.3<r<0.5 – significant correlation; ^0.7<r<1 – strong correlation; statistically significant (p<0.005); statistically significant (p<0.05)

Acknowledgments

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