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Hepcidin-25 a well iron bio-marker with prognostic implications in chronic kidney diseases

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ABSTRACT

AIM, DATA, RESTULT, CONCLUSION

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 to quantificate serum hepcidin levels in healthy control group (n=60) and patients with chronic kidney disease (n=34). 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 13.1 ± 8.7 µg/L to 90.7 ± 74.2 µg/L. Ferritin levels and hemoglobin concentration in reticulocytes correlated significantly to serum hepcidin levels ($0.3 < r < 0.5$, $p < 0.010$). Transferrine 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.

KEYWORDS

Hepcidin;
Iron deficiency anemia;
Reference ranges;
Chronic kidney disease.

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

Regular Paper

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.

Aim

This study describes statistically significant differences in hepcidin serum quantification between control group with no evidence of iron metabolism disorders and patients with chronic kidney disease (CKD).

MATERIALS AND METHODS

Subjects

This study included 60 healthy controls and 34 patients with CKD. The study was approved by the ethics committees of the participating institution. Informed consent was obtained from all healthy controls in accordance with to the Declaration of Helsinki (Directive 2001/20/ÅÎ) .

60 serum samples from healthy volunteers 29 males (age 38.2 ± 10.2) and 31 females (age 40.5 ± 8.9) were collected. 34 serum samples from patients with CKD 17 males (age 67.3 ± 10.1) and 17 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).

Data analysis

our parameter curve was 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 was used t-test and Pearson correlation.

RESULTS

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.

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 13.1 ± 8.7 µg/l and b) 90.7 ± 74.2 µg/l.

We found a differences in serum hepcidin levels

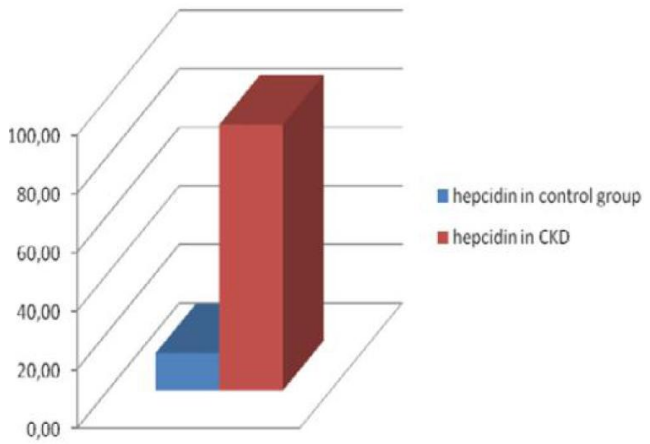

Figure 1 : Serum hepcidin results

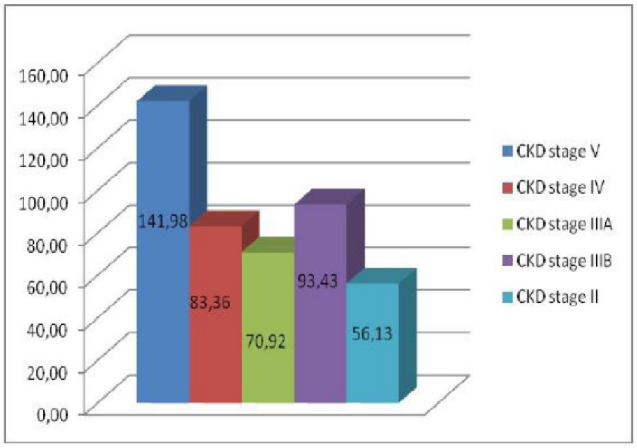

Figure 2 : Hepcidin levels in CKD stages

Regular Paper

TABLE 1 : Hepcidin correlation between CKD stages

r=	p=	
0,517184	0,175095	V/IV
0,948621	0,276692	IV/IIIA
0,854534	0,097752	V/II
-0,55286	0,448274	IIIA/IIIB
-0,67793	0,307601	IIIA/II

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.

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.

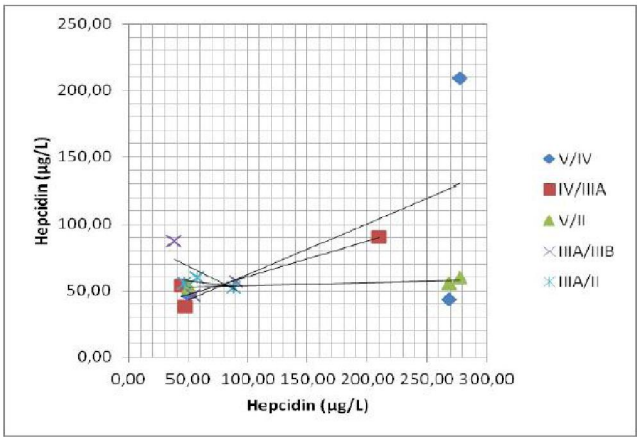

Figure 3 : Hepcidin correlation between CKD stages

Positive and negative correlation between different CKD stages.

We tried to find a correlation between serum hepcidin levels and measured parameters.

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**		**MVC**	
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

Significantly high correlation between serum hepcidin levels and eGFR, hsCRP and red blood cells was found in all CKD stages ($p < 0.005$).

Regular Paper

A significant correlation was found between serum hepcidin levels and ferritin (TABLE 2).

DISCUSSION

The present study describes a immunological assay for hepcidin quantification in human serum, based on the use of a recombinant hepcidin peptide and a polyclonal antibody.

We found that serum hepcidin levels correlate significantly between two groups 13.1 ± 8.7 μg/L vs. 90.7 ± 74.2 μg/L.

A high correlation between CKD stages II and V was found.

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.

High correlation between eGFR between all groups was found.

For other measured parameters we found no statistically significant correlation.

ACKNOWLEDGMENTS

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