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Original Research Article

Anemia in chronic dialysis patients – the right therapeutic choice?

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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 dialysis patients. We use a sandwich ELISA method to quantificate serum hepcidin levels in healthy control group (n=55) and patients on chronic dialysis (n=32). 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 262.5 ± 53.5 µg/L. Ferritin levels and hemoglobin concentration in reticulocytes correlated significantly to serum hepcidin levels ($0.3 < r < 0.5$, $P < 0.001$). 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 Bulgarian laboratory practice.

Keywords: Hepcidin, Iron Deficiency Anemia, Reference Ranges

INTRODUCTION

Essential nature of iron for humans is known from XIX century (McDonald, 2010). Iron is the most intensive studies currently micronutrients.

In recent years, 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 (Nancy, 2008).

Various physiological and pathological processes regulate the synthesis of the hormone hepcidin (Hentze et al., 2010).

Hepcidin acts in duodenal enterocytes and macrofages with ferroportin (an iron intracellular exporter) (Nemeth et al., 2004; De Domenico et al., 2005; Delaby et al., 2005; Ramey et al., 2010).

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 undergoing continuous dialysis 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 (Ganz and Nemeth, 2011). 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

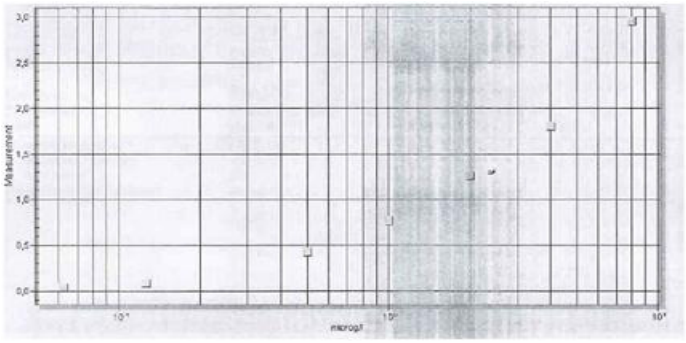

Figure 1. Calibration curve; X-logarythmic, Y-linear

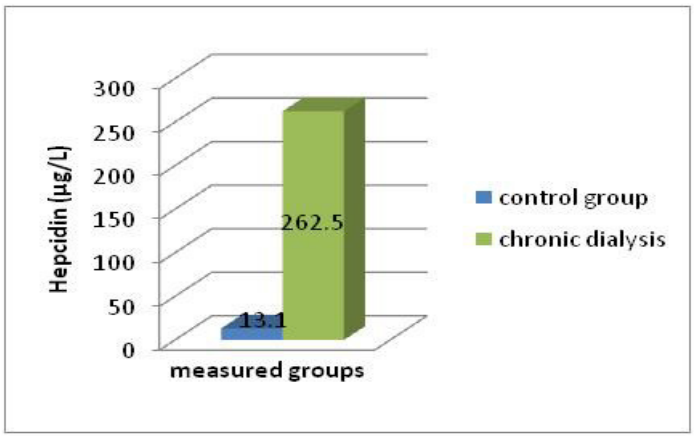

Figure 2. Hepcidin results

hepcidin quantification in human serum between control group with no evidence of iron metabolism disorders and patients on chronic dialysis.

MATERIALS AND METHODS

Subjects

This study included 55 healthy controls and 32 patients on chronic dialysis. 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/EO).

55 serum samples from healthy volunteers 23 males (age 40.2 ± 9.1) and 22 females (age 41.6 ± 9.2) were collected. 32 serum samples from patients on chronic dialysis 17 males (age 64.5 ± 13.1) and 15 females (age 54.3 ± 14.7) were collected. All samples were collected, stored, and deidentified to protect patient privacy. 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

Four 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 (Manolov et al., 2014). For statistical significance was used t-test and Pearson correlation.

RESULTS

The sandwich ELISA method produces a typical calibration curve for the recombinant hepcidin25-His (Figure 1). The measurement range was 0.0625 – 8 µg/L.

Clinical evaluation in hepcidin levels

The serum hepcidin levels were for the control group 13.1 ± 8.7 µg/L and for patients on chronic dialysis 262.5 ± 53.5 µg/L ($P < 0.001$). (Figure 2)

We tried to find a correlation between serum hepcidin

Table 1. Correlation between hepcidin and measured parameters

RBC	**r=-0.39 p<0.001**	**HGB**	**r=-0.26 p<0.001**	**CHr**	**r=0.26 p<0.001**
Retic	r=-0.27 p<0.001	Fe	r=0.20 p<0.001	TIBC	r=-0.23 p<0.001
TRSF	r=-0.41 p<0.001	Ferrit	r=0.57 p<0.001	TSAT	r=0.34 p<0.001
CRP	r=-0.01 p<0.001	Crea	r=-0.20 p<0.001	eGFR	r=-0.08 p<0.001
MCV	r=0.37 p<0.001	MCH	r=0.34 p<0.001	MCHC	r=0.04 P=0.09

levels and measured parameters.

A significant correlation was found between serum hepcidin levels and ferritin (Table 1).

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.

Upon verification of basic analytical characteristics of ELISA method we established:

- ✓ Reproducibility in series and between series of analyzes in three concentration areas - reference and clinically relevant are comparable with those established by the manufacturer;
- ✓ Very good accuracy (analytical detection), consistent with the declared by the manufacturer;
- ✓ We received a calibration curve and it's check meets international requirements and demonstrated the applicability of the method for clinical purposes.

We found that serum hepcidin levels correlate significantly between two groups 13.1 ± 8.7 µg/L to 262.5 ± 53.5 µg/L ($P < 0.001$). Future of hepcidin is related to the possibility of its antagonists as a therapeutic agent in the treatment of ACD. Reducing of hepcidin levels or countering biological effects of hepcidin could overcome the negative effects of inflammation on erythropoesis by enhancing the mobilization of stored iron and increase intestinal absorption of element. These new therapeutic approaches could reduce or eliminate all toxic effects of parenteral iron and Co-reduction of erythropoetin stimulating agents (ESAs) needs. In these cases, serum hepcidin would be therapeutic target in the management of therapy in CKD.

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CONCLUSION

Patients with elevated serum hepcidin levels do not require further application of iron replacement therapy due to functional blockade of iron homeostasis. New knowledge on the role of hepcidin in the development of anemia in chronic inflammation can significantly contribute to the correct choice of therapeutically approach. Implementation in clinical laboratory practice routine method for the study of serum hepcidin concentrations is a step forward in the treatment of impaired iron homeostasis.

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