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SERUM HEPCIDIN QUANTIFICATION IN DIFFERENTIATION OF ANEMIA

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ABSTRACT

Hepcidin is a 25-amino peptide hormone that regulates iron homeostasis. Its serum quantification helps to provide the right therapeutic choice in iron-deficiency anemia and anemia in chronic diseases. We determined serum hepcidin levels using ELISA assay in 125 patients with chronic kidney diseases (CKD) [stages from II to V] (n=60), rheumatoid arthritis (n=30), β-talasemia major (n=15) and iron-deficiency anemia (IDA; n=20) for a period 2012 – 2014 year. We compare their results to control group. The reference ranges for serum hepcidin in Bulgarian population are 3.052 - 37.750 µg/L. We found statistically significant differences in serum hepcidin levels between measured groups: CKD stages II to IV – 89.9 ± 9.2 µg/L; CKD stage V – 321.4 ± 20.5 µg/L; β-talasemia major – 0.78 ± 0.9 µg/L; ; IDA – 1.14 ± 1.2 µg/L; RA – 27.9 ± 8.7 µg/L; $P < 0.001$. Our results may support the right choice of a therapeutic approach to the anemia in patients with different disorders of iron homeostasis.

INTRODUCATION

Maintaining a balance of iron in the body is critical to the state of health. Identifying hepcidin as a key hormone of iron dramatically improves our understanding of the molecular control mechanisms of iron homeostasis and allows more detailed understanding of the pathophysiology in clinical disorders. Recent studies highlight the role of hepcidin as a useful diagnostic tool and therapeutic target in various diseases with impaired exchange of iron. Hepcidin regulates systemic iron homeostasis - absorption in the duodenum, recycling from erythrocytes and control release from hepatocytes. It is a peptide which is synthesized by the liver in response to a series of signals according to the iron needs of the body. The biological action of hepcidin is mediated by its binding to the receptor ferroportin, which is the only known iron exporter that presents in the duodenum, macrophages, hepatocytes, placenta. The hormone binds to the receptor in general complex is internalized and unlocks lysosomal ferroportin structure (McDonald, 2010; Nancy, 2008; Hentze *et al*., 2010 and Nemeth *et al*., 2004). Anemia of chronic disease is an acquired disorder of iron homeostasis. This condition is associated with infection, malignancy, organ failure, severe trauma, or other causes of inflammation. Anemia is usually mild or moderate; erythrocytes cannot show typical characteristics of iron deficiency. Underlying etiology associated with iron is obvious: impaired recycling of iron from macrophages, impaired intestinal absorption of iron, but

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erythropoesis is impaired due to insufficient iron. The characteristics of this type of anemia associated with induced hepcidin synthesis under the influence of interleukin-6 and other cytokines. According to some authors normocytic erythrocytes in anemia of chronic diseases are the result of the action of two opposite effects: on the one hand insufficient iron, the other still unknown in detail tends to macrocytosis probably due to impaired homeostasis of folate in response to inflammation (Nancy, 2008). In patients with iron deficiency anemia, and those with low serum ferritin latent and pre-latent form of deficiency without anemia accompanying establish very low hepcidin, often even below the lower limit of detection of the methods used for quantitative analysis of indicator (Ganz *et al*., 2008). In healthy subjects, an increase occurs in the concentration of hepcidin in serum and urine during oral intake of iron-containing preparations (Joyce *et al*., 2011). 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 (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.

MATERIALS AND METHODS

For a period 2012 – 2014 year we determined serum hepcidin levels using ELISA assay in 125 patients with chronic kidney diseases (CKD) [stages from II to IV, without dialysis] (n=30), CKD, stage V, on dialysis (n=30), rheumatoid arthritis (n=30), β-talasemia major (n=15) and iron-deficiency anemia (IDA; n=20). All results were comparing to the reference ranges for Bulgarian population, established in earlier researches (Manolov *et al*., 2014). All healthy volunteers and patients included were signing the informed consent according to the Declaration of Helsinki (Directive 2001/20/EC). All hematology parameters were measured on ADVIA 2120 (Siemens Healthcare Diagnostics); biochemical parameter on Cobas Integra 400 (Roche Diagnostics), immunochemical parameters on Elecsys 2010 (Roche Diagnostics) and serum iron levels were measured on AAS (Perkin Elemer). For statistical analysis we used Student's paired t-test, Pearson's correlation, ROC curves. Diagnostic reliability were established for each group included (Shipkovenska *et al*., 2002).

RESULTS

Demographic details of included healthy volunteers and patients are presented in Table 1. We used a sandwich ELISA method for serum hepcidin quantification that uses recombinant human hepcidin (Manolov *et al*., 2014). The reference ranges for serum hepcidin in Bulgarian population are 3.052 - 37.750 μg/L. We found statistically significant differences in serum hepcidin levels between measured groups: CKD stages II to IV – 89.9 ± 9.2 μg/L; CKD stage V – 321.4 ± 20.5 μg/L; β-talasemia major – 0.78 ± 0.9 μg/L; ; IDA – 1.14 ± 1.2 μg/L; RA – 27.9 ± 8.7 μg/L; $P < 0.001$. Results are presented in Table 2 and given as average ± standard deviation (SD). According to serum hepcidin levels and using soluble transferrin receptors, ferritin, serum iron and total iron-binding capacity we calculate indexes that helped for differentiation between iron-deficiency anemia and anemia of chronic disease (Kroot *et al*., 2010). Calculated indexes are presented in Table 3. According to soluble transferrin receptors to ferritin index patients with RA were divided into group with IDA and a group with ACD (Van Santen *et al*., 2011).

Table 1. Demographic details of included healthy volunteers and patients

Group	n=	Age ± SD
Control	180	34.9 ± 12.5
CKD II to IV	30	58.3 ± 10.1
CKD V	30	64.5 ± 13.1
RA	30	51.2 ± 8.4
IDA	20	25.6 ± 10.2
β-thalassemia major	15	30.0 ± 4.8

Table 2. Serum hepcidin levels in included groups

Group	Hepcidin (μg/L) ± SD
Control	18.4 ± 9.2
CKD II to IV	89.9 ± 10.9
CKD V	321.4 ± 20.5
RA with IDA	1.11 ± 0.4
RA with ACD	75.6 ± 7.9
IDA	1.14 ± 1.2
β-thalassemia major	0.78 ± 0.9

Table 3. Calculated indexes

Group	sTfr/Ferritin (mg/ng)	Hepcidin/Ferritin (μg/ng)	TSAT/Hepcidin (%/μg)
Control	0.108	0.363	2.272
CKD II to IV	0.1	1.316	0.321
CKD V	0.014	1.64	0.088
RA	0.292	0.312	6.735
IDA	1.864	0.23	11.66
β-thalassemia major	0.01	0.002	n.a.

DISCUSSION

Patients with inflammatory and reduced hepcidin are expected to have an iron deficiency. In contrast, those with high level of hepcidin are diagnosed with ACD. Using serum hepcidin levels would help in assessing the need for the application of preparations containing iron. The results suggest that patients with IDA may be subjected to treatment with such drugs, while patients with ACD do not need them. In patients with β-thalassemia major we found highest levels of serum iron and ferritin compared to the control group. The observed variations in both indicators are due to iron overload due to frequent blood transfusions and inefficient erythropoesis with increased hemolysis of erythrocytes. This finding, coupled with ultra-low levels of serum hepcidin suggests application of hepcidin agonists in the treatment of disease in order to reduce the toxicity of a significantly increased iron stores in tissues and cells, and blocking in hepatocytes (De Domenico *et al*., 2010). In patients with RA have been established strong correlations between serum hepcidin levels with ferritin and index of soluble transferrin receptor, but not the parameters for the hemoglobin content in reticulocytes. Serum hepcidin presents well as a diagnostic test of the deficiency of iron, even in the presence of inflammation and differentiate groups with iron deficiency anemia and a combination of iron deficiency/ anemia of chronic disease compared to the group of patients with ACD (Van Santen *et al*., 2011).

It is known that serum hepcidin not increase significantly in patients with impaired renal function who do not require dialysis. Hemodialysis patients, however, show significantly higher hepcidin levels than patients with chronic kidney insufficiency. The concentration of serum ferritin is a significant predictor of levels hepcidin (Peters *et al*., 2010).

Determination of serum hepcidin is still a novelty in Bulgarian medical practice. The introduction of a reliable routine method for the study of hepcidin in biological fluids is a step forward in the treatment of diseases with impaired iron homeostasis. Our study in patients with RA and different anemia confirms the ability of verified immunochemical method to differentiate the increase and decrease in serum hepcidin in patients with RA. It provides a basis for choosing the correct therapeutic approach in the treatment of anemia.

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