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IMPORTANCE OF DETECTION OF HEPSIDINE AND INTERLEUKINS IN IRON DEFICIENCY ANEMIA

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ABSTRACT

The paper presents our own data on the study of the content of hepcidin in the blood in iron deficiency anemia and in anemia that developed in patients with rheumatoid arthritis and bacterial infections. Our own data on the content of hepcidin in practically healthy people are presented. The results of the study indicate an extremely low level of hepcidin in iron deficiency anemia, while in patients with anemia of chronic diseases, the content of hepcidin is significantly high. There were no differences in the levels of hepcidin in people with ACD in rheumatoid arthritis and in bacterial infections of various localizations. The study of the level of hepcidin in the blood serum can be used in the algorithm for the differential diagnosis of iron deficiency anemia and functional iron deficiency.

KEYWORDS: *Iron Deficiency Anemia; Anemia Of Chronic Diseases; Hepcidin; Cytokines.*

INTRODUCTION

A number of proteins are involved in the regulation of iron metabolism, which control its absorption from food in the small intestine and the recycling of iron from macrophages. Proteins responsible for iron metabolism are expressed according to the body's need. About 20 regulatory molecules have been discovered that control this highly organized process. In recent years, the role of hepcidin as a key regulator of iron metabolism has been widely discussed **[1, 2].** Hepcidin is a cysteine-rich polypeptide (molecular weight 470 kDa). Its precursor, prepropeptide (84 amino acids), is converted into the prohormone prohepcidin (60 amino acids), which is proteolytically cleaved to the bioactive hepcidin, hepcidin-25. Hepcidin is synthesized mainly by hepatocytes and excreted by the kidneys.

MAIN PART

For the first time, hepcidin was isolated from urine and described by S.N. park et al. **[3].** Subsequently, this peptide was also isolated from plasma. Hepcidin has strong antibacterial properties. A.A. Levina et al. **[4]** found that in humans, the expression of hepcidin in the liver is observed as early as the 5th week of intrauterine development. The same authors showed that in fetuses that died due to a bacterial infection, the expression of the peptide was ten times higher than in fetuses without signs of infection. At the same time, in fetuses that died from a viral infection, hepcidin expression increased slightly, on average, by about 1.5 times, which confirms the predominantly antibacterial orientation of this link of innate immunity. In recent years, it has been found that the role of hepcidin in the body is much more multifaceted than just antibacterial protection, since disturbances in the expression of the hepcidin gene are associated with clinical abnormalities in iron metabolism, as well as with anemia **[5].** The relationship between hepcidin and iron metabolism was first presented by C. pigeon et al. **[6],** who proved that excess iron promotes the expression of the hepcidin synthesis gene, and it was shown that mRNA is expressed not only under the influence of an iron-rich diet, but also under the influence of lipopolysaccharides.

Studies conducted both in model experiments on transgenic mouse lines and in humans with infectious diseases and inflammation have shown that hepcidin overproduction during infection and inflammation causes hypoferremia and may be responsible for anemia in chronic diseases **[7,8].** It was shown that 3 hours after the administration of an inflammatory agent, the valuesof the pro-inflammatory cytokine, interleukin-6, increase, and already after 6 hours, the peak of hepcidin expression and a decrease in the level of iron in the serum are determined. The leading role of il-6 in the regulation of hepcidin production is confirmed by the data that treatment with monoclonal antibodies to the interleukin-6 receptor in patients rapidly reduced the level of hepcidin **[9]** Hepcidin levels significantly affect the treatment of anemia in chronic kidney disease, when inflammation and possibly a decrease in hepcidin clearance leads to an increase in its plasma level, which helps to limit the participation of iron in erythropoiesis and resistance to erythropoietin. Accordingly, a high level of hepcidin dictates the need for parenteral administration of iron to prevent impaired erythropoiesis and increased doses to suppress hepcidin production. A low hepcidin level may be indicative of a better response to iron supplementation. It is likely that the level of hepcidin can become a unique marker that determines the tactics of iron therapy**. [10]**

We have conducted a study of the content of hepcidin in the blood serum in hypochromic anemia of various origins in order to assess the role of hepcidin in the formation of anemic syndrome and the possibility of using this indicator in the differential diagnosis of hypochromic anemia. Material and methods. The study included women (n = 375) aged 16 to 60; 79 of them were practically healthy and made up the control group, 296 people had anemic syndrome, of which 103 women suffered from iron deficiency anemia (IDA), 193 women suffered from anemia of chronic disease (ACD). 121 women with ACD were diagnosed with anemia that developed against the background of autoimmune diseases of the connective tissue (rheumatoid arthritis), 72 women with anemia of chronic diseases with bacterial infections (chronic tonsillitis, bacterial endocarditis, chronic pyelonephritis). The nature of anemia was established on the basis of the results of a study of iron metabolism parameters, taking into account clinical and hematological data. In women with ACD on the background of rheumatoid arthritis (RA), mild anemia was recorded in 69 cases, moderate anemia in 52 patients. The duration of RA ranged from 1.5 to 15 years, the onset of the disease in all patients was subacute and subsequently took a protracted progressive course. Extra-articular symptoms involving the kidneys, heart and other organs were found in 95 patients, 69 had a moderate (ii) and 52 had a high (iii) degree of activity with functional insufficiency of the joints of stage ii–iii. In 46 women with ACD with infectious and inflammatory diseases, anemia was mild, 26 had moderate anemia. The inflammatory reaction was confirmed by a high level of acute phase proteins: C-reactive protein, α1-acid glycoprotein, neopterin. A pre-laboratory clinical examination was carried out using a questionnaire, which included sections on complaints, anamnesis of life and illness, the presence of concomitant diseases, and objective examination data. Research methods included the evaluation of indicators of the peripheral link of erythron, iron metabolism, levels of cytokines and hepcidin. The study of indicators of the peripheral link of erythron and iron metabolism was carried out by standard conventional methods. Iron reserves were assessed by the level of serum ferritin, which was studied by enzyme immunoassay using test systems from orgentec diagnostika (Germany). The cytokine status (il-6, TNFα, IFN-γ) was studied using test systems from Vector-Best by enzyme immunoassay. Hepcidin-25 in the serum of the examined patients was determined by ELISA using test systems from Peninsula Laboratories, llc (USA). **[11]**

Statistical processing of the obtained data was carried out using the software packages ms-eXcel, ms-Word, biosTaT, Version 4.03. The results of the studies were processed by the method of variation statistics, to assess the reliability of the results of the studies, the Student's t-test was used. The normal distribution was determinedusing the Shapiro–Wilk test. The critical level of significance when testing statistical hypotheses was taken equal to 0.05. **[12]**

TABLE-1 INDICATORS OF THE PERIPHERAL LINK OF ERYTHRON IN HYPOCHROMIC ANEMIA OF VARIOUS ORIGINS.

Indicator	Control group	ACHZ in infectious and inflammatory processes	ACP in RA	IDA
Erythrocytes, 1012/l	4,18±0,05	3,23±0,28*	3,52±0,13*	3,57±0,07*

Hemoglobin, g/l	135,88±3,08	91,00±7,55*	103,00±3,87*, **	90,14±1,99*
Hematocrit, %	36,88±1,1	25,4±2,89*	27,02±1,35*	29,09±0,71*
mcv, fl	89,15±1,38	76,00±3,88*	76,67±2,99*	74,8±1,18*
mch, pg	33,15±0,31	26,8±1,97*	27,99±1,22*, **	23,46±0,64*
mchc, g/dl	37,44±0,55	34,1±0,89*, **	35,22±0,047*, **	31,28±0,6*
rdW, %	11,09±0,11	16,53±0,92*, **	15,59±0,62*	14,29±0,4*

N o t e . * - reliability of differences in indicators compared with the control group at $p < 0.05$; ** - reliability of differences in indicators in patients with ACD compared with those in IDA at $p < 0.05$.

Indicators of iron metabolism in patients with ACD and IDA

Table-2.

N o t e . Here and in Table. 4: * - reliability of differences in indicators compared with the indicator of the control group; ** - significance of differences in indicators in patients with ACD compared with those in IDA.

Parameter	Control group	ACD in infectious and inflammatory processes	ACD in RA	IDA
SF, µmol/l	20,4±1,02	11,07±1,9*	9,52±1,0*	8,44±0,32*
TIBC, µmol/l	65,68±1,83	53,24±4,5*, **	49,65±6,56*, **	80,96±1,25*
LZhSS, µmol/l	44,53±1,87	42,91±4,38**	38,42±8,02**	71,41±1,45*
CST, %	32,32±1,84	14,99±2,73*	16,74±2,02*, **	11,4±0,52*
SF, ng/ml	33,55±2,59	178,59±75,52*, **	238,38±64,16*, **	4,91±0,66*
Hepcidin-25, ng/ml	8,07±0,2	39,33±0,38*, **	45,05±0,38*, **	0,25±0,02*

RESULTS

The results of the study of the peripheral link of erythron in individuals with anemic syndrome are presented in table. 1. When assessing the iron metabolism in all examined patients with iron deficiency anemia, a significant decrease in the level of serum iron and CST was noted with significantly increased TIBC and LVVR compared with those in healthy individuals (p = 0) (Table 2). The level of serum ferritin in patients with iron deficiency anemia averaged 4.91±0.66 ng/ml and was significantly lower compared to that in the control group (p = 0), which, in combination with clinical data, the results of hematological studies and indicators iron metabolism testified to the microcytic, hypochromic, iron deficiency nature of anemia. Impaired iron metabolism in patients with ACD was characterized by a low level of serum iron, a reduced transferrin saturation coefficient, and a high level of serum ferritin (see Table 2). An increase in the level of serum ferritin in patients with anemia of chronic diseases on the background of infectious inflammatory processes and in rheumatoid arthritis occurs in parallel with an increase in the levels of acute phase plasma proteins (C-reactive protein, α-acid glycoprotein, neopterin).

In practically healthy people, the level of hepcidin varied from 5 to 12 ng/ml, averaging 8.07±0.2 ng/ml. When using the Gaussian distribution, normal laboratory values are the average values for a healthy population ± 2 standard deviations (± 2sd). In our studies, ± 2sd corresponded to the value of ± 3.74; accordingly, the range of normal values of hepcidin25 in healthy subjects ranged from 4.33 to 11.81 ng/ml. In patients with IDA with verified iron deficiency, a significant decrease in the level of hepcidin was revealed. The content of hepcidin in the blood serum of patients with IDA averaged 0.25±0.02 ng/ml against that in the control group - 8.07±0.2 ng/ml. In patients with ACD, the level of hepcidin was significantly higher compared to that in healthy people and in patients with IDA (p = 0), and the increase in hepcidin level did not depend on the etiology of the disease and the localization of the inflammatory process. There were no significant differences in the levels of hepcidin in patients with ACD associated with rheumatoid arthritis and ACD with bacterial infections of various localizations (see Table 2).

We found the most pronounced increase when assessing the level of il-6 with a significant increase in the level of TNFα and interferon-γ in ACD. Thus, the average values of il-6 in the groups with ACD on the background of RA and in infectious and inflammatory diseases were significantly higher than in the control group and amounted to 43.39±11.93 pg/ml (p = 0.005) and 48.27, respectively. ±12.86 pg/ml (p = 0) versus 2.78±0.23 pg/ml in healthy people (see Table 4). We see that in ACD there is a direct correlation between the serum concentrations of il-6 and hepcidin. This is consistent with modern ideas about the activation and increased synthesis under the influence of il-6 of the iron-regulatory protein hepcidin, which plays the role of a negative mediator in the regulation of iron metabolism and its incorporation into erythroid cells, which leads to iron-deficient erythropoiesis and anemia [10]. The obtained results of the study indicate that hepcidin serves as a regulator of iron metabolism and can be used in the algorithm for the differential diagnosis of ACD (functional iron deficiency) and true iron deficiency - iron

deficiency anemia. An increase in hepcidin-25 over 11.81 ng/ml indicates anemia of chronic diseases, and a value of less than 4.33 ng/ml indicates iron deficiency anemia (4.33–11.81 ng/ml is the range of hepcidin-25 levels in practically healthy). **[13,14]**

CONCLUSION

The decrease in the level of hepcidin in IDA is quite understandable in terms of the role of hepcidin in iron metabolism and the desire of the body to replenish iron reserves to ensure the synthesis of hemoglobin in the erythroid cells of the bone marrow and replenish the number of erythrocytes. A low level of hepcidin can serve as an indicator of latent iron deficiency when there is no change in other indicators of iron metabolism, such as ferritin levels. This is of great practical importance: incorrect interpretation of a patient with ACD as having an iron deficiency leads to ineffective iron therapy with a risk of complications. **[15]** Modulation of the biological activity of hepcidin, which is a key factor in the regulation of iron homeostasis, determines the clinical possibilities for the treatment of patients with anemia of chronic diseases and other hypochromic anemias associated with impaired iron metabolism.

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