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ABSTRACT

Objectives: To study the effect of hepcidin on the course of chronic heart failure with anemia. Methods: 115 patients with CHF caused by ischemic heart disease (IHD) were chosen. All of them were treated in in-patient conditions and were followed in out-patient conditions. The patients were divided into two main and one control groups. Main group A consisted of 40 CHF patients with iron deficiency anemia. Main group B consisted of 35 CHF patients with anemia of chronic disease. 40 CHF patients without anemia were chosen for the control group. To patients of the main groups 200mg of Iron (III) hydroxide (Venofer®) was administered intravenously once in two days, along with standard CHF treatment. Results: Our study found reliable increase of serum levels hepcidin in CHF patients with anemia of chronic disease compared to CHF patients with iron deficiency anemia (45,4%) and control group (33%) (P<0,001). This, in turn, shows that hepcidin is an important indicator in differential diagnosis between anemia of chronic disease and iron deficiency anemia. Statistical analysis of pro-inflammatory cytokines and hepcidin levels was done in order to find any associations in patients of our study. Following was found: in group B weak positive correlation between hepcidin and IL-1 (r=0,24 P<0,05), strong positive correlation between hepcidin and IL-6 (r=0,52, P<0,001), and moderate positive correlation between hepcidin and TNF-α (r=0,37, P<0,05). Conclusion: In CHF patients with anemia of chronic disease (Group B) hepcidin levels were reliably higher than normal ranges and correlation between pro-inflammatory cytokines and hepcidin during deterioration of the disease.

Introduction

Chronic heart failure (CHF) is one of the most signicifant socio-economical problems of the healthcare and one of the leading causes of morbidity and mortality worldwide [13].

Early diagnosis and adequate treatment of aggravating factors, including anemia, is important in increasing the quality of life and life expectancy of CHF patients. It was found in number of multi-center studies (ELITE II, ValHeFT, COPERNICUS, VEST, COMET and others) that prevalence of anemia in CHF varies from 10 to 55%. Anemia is viewed as an independent risk factor negatively affecting course, prognosis and complications of CHF [18]. SOLVD trial found direct assotiation between hematocrit levels CHF. According to 33 month long observation in patients with hematocrit levels of 40-44, 35-39 and 35%, mortality was respectively 22, 27 and 39% [11]. It was found in a

meta-analysis by Groenveld and coauthors that mortality was two times higher in CHF patients with anemia compared to the patients with normal hemoglobin levels [14]. In all of above mentioned studies criteria of anemia recommended by World Health Organisation was used (hemoglobin is <130g/l in men, <120 g/l in women) [1].

Etiology and pathogenesis of anemia in CHF is not fully studied [4]. Hemodilution, disorders of kidney (cardiorenal anemia syndrome) and iron metabolism, activation of pro-inflammatory cytokines (Tumor Necrosis Factor – α (α-TNF), interleukin-6 (IL-6) and others) and factors are thought to be the mechanisms of development of anemia in CHF [4]. There is also a possibility of drug induced anemia in CHF patients after continuous use of angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor (AR) blockers (suppression of erythropoiesis) [21].

Data about the share of different type of anemias in CHF patients varies. According to J. Ezekowitz in CHF patients with anemia 21% of cases was due to iron deficiency anemia (IDA), other deficiencies – 8%, anemia of chronic disease (ACD) 58% and 13% was due to other causes [8]. In a Canadian study conducted on 12065 CHF patients with anemia similar results were found (27% iron deficiency, 8% vitamin B12 deficiency, 7% folate deficiency, 58% - anemia of chronic disease) [8, 21]. Contrary to them, according to Stavros G. Drakos and coauthors share of iron deficiency anemia was 73%, anemia of chronic disease - 18,9%, hemodilution - 5,4% and drug induced anemia was 2,7% [19, 21]. These numbers show that prevalence of anemia among CHF patients, as well as that the entire subject itself is not fully studied.

According to a Polish study decrease of ferritin levels (less than 100mcg/l) and ferritin saturation (less than 15% when ferritin is 100-300mcg/l) was found in 101 of 218 CHF patients (46%). Among the anemic patients 61% had iron deificiency and 43% had normal hemoglobin levels [12]. Serum ferritin levels correctly represent iron metabolism in reticuloendothelial system, however it is not sensitive in anemia of chronic disease [12, 15].

In recent studies of ACD pathogenesis great attention is given to acute phase protein hepcidin, which blocks iron metabolism in different parts of organism, particularly enterocytes and macrophages [7, 12]. Correlation was found between the hepcidin and liver iron and hemoglobin levels [9, 16]. When there is excess of iron in organism, hepcidin production increases under the influence of inflammation factor IL-6 [16]. Increased hepcidin levels results in decreased absorbtion of iron from GI tract and decreased release of iron from macrophages [11]. Hepcidin suppresses the erithropoesis as well [11]. Number of other studies found consistent high levels of hepcidin in anemia of chronic disease [3, 5]. But there is not enough available information concerning hepcidin levels in CHF patients with anemia.

As was mentioned before, mortality is two times higher in CHF patients with anemia compared to the patients with normal hemoglobin levels [17]. Treatment of anemia in the patients results in better quality of life and prolonged life expectancy. Therefore, correction of anemia should be included in the treatment complex of CHF patients.

Existence of anemia in CHF worsens the prognosis, considerable decreases life quality of patients. Thus, further study of the subject, development of adequate methods of correction of anemia along with standard therapy remains actual.

Methods

115 patients with CHF caused by ischemic heart disease (IHD) were chosen. All of them were treated in in-patient conditions and were followed in out-patient conditions.

The patients were divided into two main and one control groups. Main group A consisted of 40 CHF patients with iron deficiency anemia. Main group B consisted of 35 CHF patients with anemia of chronic disease. 40 CHF patients without anemia were chosen for the control group.

To patients of the main groups 200mg of Iron (III) hydroxide (Venofer®) was administered intravenously once in two days, along with standard CHF treatment. Control group patients received standard CHF therapy. All patients were observed for six months. They were taught about CHF and principles of self-monitoring.

Diagnosis of CHF and classification of the disease according to functional classes in involved patients was made based on patients complaints, anamnesis, clinical examination, laboratory and instrumental investigations and New – York Heart Association (1964) criteria.

For classification of anemia World Health Organisation (WHO) criteria were used (hemoglobin is <13,0 g/dl in men and <12,0 g/dl in women).

Inclusion criteria: patients with CHF caused by ischemic heart disease

Exclusion criteria: Miocardial infarction, unstable angina, arterial hypotonia, severe arrythmia, II-III degree AV blocade, congenital heart diseases, stroke, autoimmune and diffuse connective tissue diseases, acute inflammation diseases or recurrence of chronic inflammation diseases, chronic kidney disease, liver failure, severe bronchial asthma and COPD, cancer, psychiatric conditions, alcohol dependence and other severe comorbid conditions.

Laboratory tests. 9ml venous blood was taken from fasting patients. The blood was centrifuged for 15 minutes in order to separate serum. Next, in Cobas e601 analysator (Germany) hepcidin levels were measured using “BCM diagnostics” protocols, ferritin levels - «ORGENTEG» and transferrin using «ELISA» protocols.

Data analysis. For data processing MS Excel (2013) computer program was used. Arithmetic mean and standard deviation (M±m) of all data in following tables were calculated. Student’s paired and unpaired t-tests were used to determine significance of difference between groups. Correlation analysis was done using Pearson’s correlation coefficient and confidence tables.

Ethical statement. The study was conducted according to scientific research plan of Tashkent Medical Academy, within “Development of new methods of diagnosis, prophylaxis and treatment of internal diseases” (2015-2017) parameters. Written informed consent was taken from all patients before the study.

Results

For the study 115 patients with CHF caused by ischemic heart disease were chosen from cardiology and cardiorehabilitation departments of Tashkent Medical Academy Number 3. Their age was from 50 to 80 years, the mean age was 64,6±4,9 years.

Hematologic indicators were examined before and after the treatment in order to study the influence of anemia in CHF patients and effectiveness of treatment (Table-1).

Comparative analysis showed differences in hematologic indicators between CHF patients with and without anemia, and between patients with anemia of chronic disease and iron deficiency anemia.

In CHF patients with iron deficiency anemia (Group A) before the treatment, mean level of hemoglobin was 101,4±3,1 g/l, of ferritin - 85,9±8,5 mcg/l, of transferrin - 5,2±1,21 g/l, of serum iron - 7,94±0,21 mmol/l and of hepcidin - 10,6±1,3 ng/ml.

Table 1

Changes in hematologic indicators in CHF patient with and without anemia before and after treatment

Indicator	Treatment	Main group		P	Control group
		A group	B group		Without anemia
Hemoglobin, g/l	Before	101,4±3,1***	104,3±4,8***	>0,05	126,0±6,5
	After	126,6±4,7^^^	120,1±3,9*^^	<0,05	126,4±5,2
Hematocrit, %	Before	35,6±0,62**	34,8±0,82**	>0,05	40,9±0,37
	After	40,1±0,19^^	39,7±0.17*^	>0,05	41,6±0,45
Erythrocytes, $\cdot 10^{12}$/l	Before	3,6±0,04***	3,7±0,02**	>0,05	4,3±0,04
	After	4,1±0,02^^	4,0±0,01^	>0,05	4,2±0,05
Leukocytes	Before	6,3±0,24	8,9±0,22*	<0,05	6,1±0,21
	After	6,0±0,14	7,2±0,12	>0,05	6,3±0,14
Platelets	Before	217,8 ±2,98*	229±4,12	>0,05	230,2±2,74
	After	224,6±2,51	231,7±3,79	>0,05	225,2±2,62
ESR mm/h	Before	15,7±1,09	21,6±0,75*	<0,05	12,5±1,07
	After	11,6±0,33	14,4±0,51	>0,05	11,2±0,53
ALT	Before	29,3±1,72	34,0±4,67	>0,05	29,2±1,45
	After	22,6±1,30	30,9±1,60	>0,05	28,2±0,97
AST	Before	23,2±1,78	28,7±4,39	>0,05	22,6±1,56
	After	22,5±1,51	26,5±1,68	>0,05	24,6±0,78
Creatinine, mmol/l	Before	79,9±3,27	77,4±2,46	>0,05	69,9±3,21
	After	78,7±16,4	79,6±0,97	>0,05	72,8±1,31
Glucose, mmol/l	Before	5,1±0,32	5,9±0,4	>0,05	5,7±0,4
	After	5,2±0,15	5,0±0,16	>0,05	5,5±0,08
Serum, mmol/l	Before	7,94±0,21***	6,14±1,12***	>0,05	25,8±4,81
	After	22,9±2,8^^	16,1±2,12*^	<0,05	24,7±3,72
Ferritin, mcg/l	Before	85,9±8,5***	167,5±8,54	<0,001	289,5±11,6
	After	318,4±5,46^^^	259,6±6,5^	>0,05	286,6±10,9
Transferrin, g/l	Before	5,2±1,21	6,9±2,28	>0,05	4,1±1,8
	After	3,1±0,14	4,4±0,3	>0,05	3,9±0,10
Transferrin saturation,%	Before	10,1±8,2**	11,6±6,8*	>0,05	26,3±8,6
	After	28,6±8,2^	22,4±5,2*^	<0,05	30,3±6,9
Hepcidin ng/ml	Before	10,6±1,3	23,3±3,5***	<0,001	5,7±1,02
	After	3,2±0,05	19,2±0,06**	<0,001	2,7±0,03

Note: ** - differences are significant compared to control group (*- P <0,05, ** - P <0,01, *** - P<0,001*

^ - differences are significant after treatment compared to before treatment (^ - P <0,05, ^^ - P <0,01, ^^^ - P<0,001)

In CHF patients with anemia of chronic disease (Group B) before the treatment, mean level of hemoglobin was 104,3±4,8 g/l, of ferritin - 167,6±8,54 mcg/l, of transferrin - 6,9±2,28 g/l, of serum iron - 6,14±1,12 mmol/l and of hepcidin - 23,3±3,5 ng/ml.

In CHF patients without anemia (Control group) mean level of hemoglobin was 126,0±6,5 g/l, of ferritin - 289,5±11,6 mcg/l, of transferrin - 4,1±1,8 g/l, of serum iron 25,8±4,81 mmol/l and of hepcidin was 7,7±1,02 ng/ml. Changes in hematologic indicators of main and control groups are given in Table 1.

Significant changes in hematologic indicators occurred compared to initial state in Group A students after six month of treatment. Hemoglobin levels rose from 101,4±2,1 g/l to 126,6±4,7 g/l (P<0,001). In group B patients hemoglobin levels changed from 104,3±4,8 g/l to 120,1±3,9 g/l (P<0,01). Moreover, in Group A patients serum iron level increased from 7,94±0,21 mmol/l to 22,9±2,8 mmol/l (p<0,01), ferritin from 85,9±8,5 to 318,4±5,46 (P<0,001), transferrin from 5,2±1,21 g/l to 3,1±0,14 (P>0,05). Hepcidin levels decreased from 10,6±1,3 ng/ml to 3,2±0,05 ng/ml (P> 0,05).

In Group B patients serum iron levels increased from 6,14±1,12 mmol/l to 16,1±2,12 mmol/l (P<0,05), ferritin levels rose from 167,5±8,54 to 259,6±6,5 (P<0,05), transferrin decreased from 6,9±2,28 to 4,4±0,3 (P>0,05). The study found change in serum hepcidin levels from 23,3±3,5 ng/ml to 19,2±0,06 ng/ml (P>0,05).

In control group CHF patients without anemia serum iron, ferritin, transferrin saturation, transferrin and hepcidin levels were within normal ranges (Table 1).

The following was found in comparative analysis of laboratory results of CHF patients with iron deficiency anemia and anemia of chronic disease. Presence of decreased serum iron, ferritin levels and increased transferrin levels in Group A patients proves absolute iron deificiency in them. Presence of decreased serum iron levels, increased transferrin, and normal amount of iron reserve indicator – ferritin in Group B patients suggests functional iron deificiency. Functional iron deificiency is an important part of the ACD pathogenesis. While serum ferritin levels correctly represents iron reserves in reticular-endothelial system, it is ineffective in evaluation of anemia of chronic disease. Recent discovery of acute phase protein – hepcidin that regulates iron metabolism gave an insight into pathogenesis of anemia of chronic disease. Our study found reliable increase of serum levels in CHF patients with anemia of chronic disease compared to CHF patients with iron deficiency anemia (45,4%) and control group (33%) (P<0,001). This, in turn, shows that hepcidin is an important indicator in differential diagnosis between anemia of chronic disease and iron deficiency anemia.

Correlation between hepcidin levels and pro-inflammatory cytokines was also studied.

Immune inflammation (i.e. cytokines and reticulo-endothelial cells that regulate iron homeostasis, proliferation of erythroid progenitor cells, erythropoietin production, and lifespan of erythrocytes) is the basis of pathogenesis of anemia of chronic disease. Hypoxic process in organism triggers increased hepcidin production through pro-inflammatory cytokines [20].

According to results of our study in control group of CHF patients without anemia levels of serum IL-1 were respectively 16,4±0,72 – 18,6±1,93 ng/ml, of IL-6 were 17,2±1,78 - 18,9±1,3 ng/ml, of TNF-α were 15,9±0,72 – 17,4±1,93 ng/ml in II-III functional classes.

In group A CHF patients with iron deficiency anemia serum levels of IL-1 were respectively 17,9±2,1 – 20,8±1,3 ng/ml, of IL-6 were 20,4±1,8 - 22,6±1,3 ng/ml, of TNF-α were 19,6±0,62 – 21,8±1,64 ng/ml in II-III functional classes.

In group B CHF patients with anemia of chronic disease serum levels of IL-1 were respectively 18,2±0,72 - 21,1±1,93 ng/ml, of IL-6 were 26,6±1,7 - 29,7±1,3 ng/ml, of TNF-α were 20,2±0,72 – 24,7±1,93 ng/ml in II-III functional classes (Table 2).

Table 2

Levels of pro-inflammatory cytokines before the treatment

Indicator	Main group A CHF patients with iron deficiency anemia (n=40)		Main group B CHF patients with anemia of chronic disease (n=35)		Control group, CHF patients without anemia (n=40)	
	CHF Functional class II, n=14	CHF Functional class III, n=26	CHF Functional class II, n=12	CHF Functional class III, n=28	CHF Functional class II, n=16	CHF Functional class III, n=24
IL-1	17,9±2,1	20,8±1,3	18,2±0,72	21,1±1,93*	16,4±0,72	18,6±1,93
IL-6	20,4±1,8	22,6±1,3*	26,6±1,7**	29,7±1,3***^	17,2±1,78	18,9±1,3
α - TNF	19,6±0,62	21,8±1,64*	20,2±0,72*	24,7±1,93**	15,9±0,72	17,4±1,93

Note: ** - differences are significant compared to control group (*- P <0,05, ** - P <0,01, *** - P<0,001*
^ - differences are significant after treatment compared to before treatment (^ - P <0,05, ^^ - P <0,01, ^^^ - P<0,001)

In Group A patients levels of IL-1 were increased to 9,1 – 11,8 % (P<0,05), of IL-6 to 18,6 – 19,5 % (P<0,01), of α - TNF to 23,2–25,0 % (P<0,01) compared to the control group. In Group B patients levels of IL-1 were increased to 10,9 – 13,4 % (P<0,05), of 54,6 – 57,1% (P<0,001), of α - TNF to 27,1–41,0 % (P<0,01) compared to the control group.

Only part of conducted studies found correlation between hepcidin and pro-inflammatory cytokines, and data was mostly controversial [6, 19, 22]. Statistical analysis of pro-inflammatory cytokines and hepcidin levels was done in order to find any associations in Group B patients of our study. Following was found: weak positive correlation between hepcidin and IL-1 (r=0,24 P<0,05), strong positive correlation between hepcidin and IL-6 (r=0,52, P<0,001), and moderate positive correlation between hepcidin and TNF-α (r=0,37, P<0,05).

Our study found reliable decrease of serum pro-inflammatory cytokines levels in CHF patients with iron deficiency anemia and the control group after the treatment. Although serum pro-inflammatory cytokines level decreased in Group B CHF patients with anemia of chronic disease after the treatment, the levels remained high compared to other groups (Table 3).

In functional class II CHF patients without anemia (control group) there was statistically significant decrease in levels of IL-1 from 16,4 to 7,4 ng/ml (p<0,01), IL-6 from 17,2 to 7,7 ng/ml (p<0,001), α - TNF from 15,9 to 6,8 ng/ml (p<0,001) after the treatment. In functional class III CHF patients of the same group levels of IL-1 decreased from 18,6 to 8,7 ng/ml (p<0,001), of IL-6 from 18,9 to 8,6 ng/ml (p<0,001), of α - TNF from 17,4 to 8,2ng/ml (P<0,01) after the treatment.

Table 3

Levels of pro-inflammatory cytokines after the treatment

Indicator	Treatment	Group A		Control group	
		CHF Functional class II, n=12	CHF Functional class III, n=28	CHF Functional class II, n=16	CHF Functional class III, n=24
IL-1	Before	17,9±2,1	20,8±1,3	16,4±0,72	18,6±1,93
	After	9,6±1,2^^	10,8±1,23^^^	7,4±1,3^^	8,7±0,92^^^
IL-6	Before	20,4±1,8	22,6±1,3*	17,2±1,78	18,9±1,3
	After	10,3±0,98^^^	12,2±1,43^^^	7,7±1,43^^^	8,6±1,2^^^
α - TNF	Before	19,6±0,62	21,8±1,64*	15,9±0,72	17,4±1,93
	After	9,2±1,33^^^	11,4±1,67^^	6,8±2,1^^^	8,2±2,3^^

		Group B		Control group	
IL-1	Before	18,2±0,72	21,1±1,93*	16,4±0,72	18,6±1,93
	After	16,2±1,12***	18,3±1,21***	7,4±1,3^^	8,7±0,92^^^
IL-6	Before	26,6±1,7**	29,7±1,3***	17,2±1,78	18,9±1,3
	After	19,7±0,98***^	22,8±1,32***	7,7±1,43^^^	8,6±1,2^^^
α - TNF	Before	20,2±0,72*	24,7±1,93**	15,9±0,72	17,4±1,93
	After	15,6±1,42**^	19,3± 0,89***^	6,8±2,1^^^	8,2±2,3^^

Note: ** - differences are significant compared to control group (*- P <0,05, ** - P <0,01, *** - P<0,001*
^ - differences are significant after treatment compared to before treatment (^ - P <0,05, ^^ - P <0,01, ^^^ - P<0,001)

In functional class II CHF patients with iron deficiency anemia (Group A) there was statistically significant decrease in levels of IL-1 from 17,9 to 9,6 ng/ml (P<0,01), IL-6 from 20,4 to 10,3 ng/ml (p<0,001), α - TNF from 19,6 дан 9,2 ng/ml (p<0,001) after the treatment. In functional class III CHF patients of the same group levels of IL-1 decreased from 20,8 to 10,8 ng/ml (p<0,001), of IL-6 from 22,6 to 12,2 ng/ml (p<0,001), of α - TNF from 21,8 to 11,4ng/ml (P<0,01) after the treatment.

In functional class II CHF patients with iron deficiency anemia (Group A) IL-1 levels decreased from 18,2 to 16,2 ng/ml (P>0,05), IL-6 from 26,6 to 19,7 ng/ml (p<0,05), α - TNF from 20,2 to 15,6 ng/ml (p<0,05) after the treatment. In functional class III CHF patients of the same group levels of IL-1 decreased from 21,1 to 18,3 ng/ml (P>0,05), of IL-6 from 29,7 to 22,8 ng/ml (P>0,05), of α - TNF from 24,7 to 19,3ng/ml (P>0,05) after the treatment.

Discussions

Comparative analysis of laboratroy results of CHF patients with iron deficiency anemia and anemia of chronic disease in our prospective study showed following results. Mean level of hemoglobin was 101,4±3,1 g/l in Group A CHF patients with IDA. Mean levels of ferritin was 85,9±8,5 mcg/l, of transferrin - 5,2±1,21 g/l, and of serum iron was 7,94±0,21 mmol/l. Decreased levels of all iron metabolism indicators, particularly the serum iron and ferritin that represents iron reserves, proves the presence of overt iron deficiency in Group A patients. In Group B CHF patients with anemia of chronic disease

mean level of hemoglobin was 104,3 ±5,8 g/l, of ferritin - 167,6±8,5 mcg/l, of transferrin - 6,9±2,28 g/l, and of serum iron was 6,14±1,12 mmol/l. The laboratory results of Group B patients show that although hemogobin and serum iron levels were decreased, amount of ferritin, that represents iron reserves, remained within normal ranges. This suggests the presence of functional iron deficiency in the patients. Functional iron deficiency lays on the basis of anemia of chronic disease and is caused by inflammation process (cytokines and reticulo-endothelial cells that regulate iron homeostasis, proliferation of erythroid progenitor cells, erythropoietin production, and lifespan of erythrocytes) [6]. Discovery of acute phase protein – hepcidin that affects iron metabolism, enabled the understanding of disturbance of iron homeostasis its connection with immune mechanisms in pathogenesis of ACD. Increased synthesis of hepcidin occurs in liver under the influence of IL-6 and in turn results in decreased absorption of iron from GI tract and blocking of iron release from macrophages. This process is the main cause of development ofБу жараён CHFда anemia of chronic disease in CHF [18].

Therefore measuring of hepcidin enables the diagnosis of ACD in patients, including CHF patients.

In Group A CHF patients with IDA serum level of hepcidin was 10,6±1,3 ng/ml. In CHF patients with ACD level of hepcidin was 23,3±3,5 ng/ml. In control group CHF patients without anemia hepcidin levels were within normal ranges.

It should be stressed, that differential diagnosis of causes of anemia in CHF in hospital conditions is important. Because it is difficult to do adequate prognosis or to design optimal treatment tactics without knowing exact cause of anemia. Correction of hemoglobin levels is an integral part of treatment of CHF patients.

There are number of randomized trials on use of erythropoietine with and without iron medications in treatment of CHF anemia. According to results of these trials, administration of erythropoietine in CHF anemia increases the life quality of patients [2], but negatively affects life expectancy [13]. Because of increased risk of thrombosis during erythropoietine use, European and the USA associations of cardiologists are discouraging the use of these medications in treatment of anemia in CHF patients.

In our study, intravenous infusion of saccharose complex Iron (III) hydroxide (Venofer®) as a part of standard therapy in CHF patients under hospital conditions resulted in normalization of hemoglobin levels and replenishment of iron reserves. In CHF patients with iron deficiency anemia mean levelof hemoglobin increased from 101,4±3,1 g/l to 126,6±4,7 g/l (P<0,001), ferritin and transferrin levels reached normal ranges. In CHF patients with anemia of chronic disease hemoglobin levels increased from 104,3±5,8 g/l to 120,1±3,9 g/l (P<0,05), ferritin and transferrin levels also reached normal ranges. But in the latter group the changes were less visible compared to the first group. Comparative analysis of effectiveness of the standard antianemic treatment between Groups A and B showed reliable increase of hemoglobin and serum iron levels in Group A patients. Although there was increase of hemoglobin and serum levels in Group B patients, it was less reliable compared to Group A.

Follow up after 6 month showed lasting effect of Венофер in Group A patients according to laboratory findings.

As known, cytokines participate in intercellular connection and regulation of number of biological processes (hemopoiesis, immune reaction of lymphoid and mesenchymal cells, tissue reparation, angiogenesis, inflammationни and others). Cytokine induced damage of myocardium is realized through direct toxic effect, decreased contractility, increased growth of connective tissue and apoptosis of

cardiomyocytes and peripheric muscle cells. Moreover cytokines are one of the causes of myocardial hypertrophy and cardiac remodeling. Disruption of endothelium dependent arteriole dilation causes high and continuous increase in general peripheric resistance. This in turn increases oxygen demand of heart and decreases its contraction. Recently, cytokine induced apoptosis of cardiomyocytes is being seen as a fundamental mechanism of irreversible changes of cardiac contractility in CHF patients [18]. Lon term effects of cytokines include restructuring of intracellular collagen matrix, hypertrophy, dilation and myocardial remodeling. Thus, activation of cytokines in CHF negatively affects the inotropic function of heart, decreases ejection fraction of left ventricle, increases diastolic pressure in heart chambers, limits the physical capabilities of patients and deteriorates the disease [12, 18].

According to current views, hepcidin secretion increases due to pro-inflammatory cytokines, particularly IL-6 [12]. Rise in hepcidin levels blocks the iron export from macrophages. Thus macrophages are “closed” and they cannot participate in erythropoiesis.

Moreover, IL-1 and α-TNF decreases concentration of erythropoietin and and increases sensitivity of erythropoiesis line to it, causing relative resistance to erythropoietin [11, 18].

The following was found. In functional class II CHF patients without anemia (control group) IL-1 level was 16,4±0,72ng/ml, IL-6 was 17,2±1,78 ng/ml, α - TNF was 15,9±0,72 ng/ml. In functional class III CHF patients of the same group levels of IL-1 was 18,6±1,93 ng/ml, of IL-6 was 18,9±1,3 ng/ml, of α - TNF was 17,4±1,93 ng/ml. In Group A CHF patients with iron deficiency anemia levels of IL-1 were increased 9,1 – 11,8% ($P<0,05$), of IL-6 were 18,6 – 19,5% ($P<0,01$), of α - TNF were increased 23,2–25,0% ($P<0,01$) in II and III functional classes respectively. In Group B CHF patients with anemia of chronic disease levels of IL-1 were increased 10,9 – 13,4% ($P<0,05$), of IL-6 were 54,6 – 57,1% ($P<0,001$), of α - TNF were increased 27,1–41,0% ($P<0,01$) in II and III functional classes respectively.

Correlation between hepcidin and pro-inflammatory cytokines was found only in part of conducted studies, and overall data is controversial [12, 20].

Statistical analysis of hepcidin levels and pro-inflammatory cytokines of patients in or study. Following was found in CHF patients with anemia of chronic disease (Group B): weak positive correlation between hepcidin and IL-1 ($r=0,24$ $P<0,05$), strong positive correlation between hepcidin and IL-6 ($r=0,52$, $P<0,05$), moderate positive correlation between hepcidin and α-TNF ($r=0,37$, $P<0,05$).

Comparative analysis of of data was done to evaluate effect of standard antianemic treatment to cytokine profile in main and control groups.

In Functional class II CHF patients without anemia (Control group) there was reliable ($p<0,001$) decrease of IL – 1 levels from 16,4 to 7,4 ng/ml, of IL – 6 from 17,2 to 7,7 ng/ml, of α - TNF from 15,9 to 6,8 ng/ml after the treatment. In functional class III CHF patients of the same grop there was reliable ($p<0,01$) decrease of IL – 1 levels from 18,6 to 8,7 ng/ml, of IL – 6 from 18,9 to 8,6 ng/ml, of α - TNF from 17,4 to 8,2ng/ml after the treatment. This show the effectiveness of standard CHF therapy.

In Functional class II CHF patients with iron deficiency anemia (Group A) there was reliable ($p<0,001$) decrease of IL – 1 levels from 17,9 to 9,6 ng/ml, of IL – 6 from 20,4 to 10,3 ng/ml, of α - TNF from 19,6 to 9,2 ng/ml after the treatment. In functional class III CHF patients of the same grop there was reliable ($p<0,01$) decrease of IL – 1 levels from 20,8 to 10,8 ng/ml, of IL – 6 from 22,6 to 12,2 ng/ml, of α - TNF from 21,8

to 11,4ng/ml after the treatment. As these results show, anemia worsens hypoxia, already present in CHF patients. Increased hypoxia stimulates the production of pro-inflammatory cytokines, which in turn damages the myocardium and deteriorates the course of CHF. Adequately treated anemia reverses these processes.

In Functional class II CHF patients with anemia of chronic disease (Group B) there was decrease of IL – 1 levels from 18,2 to 16,2 ng/ml, of IL – 6 from 26,6 to 19,7 ng/ml, of α - TNF from 20,2 to 15,6 ng/ml after the treatment. In functional class III CHF patients of the same grop there was decrease of IL – 1 levels from 21,1 to 18,3 ng/ml, of IL – 6 from 29,7 to 22,8 ng/ml, of α - TNF from 24,7 to 19,3ng/ml after the treatment. Iron medications were not effective in Group B patients compared to iron deficiency anemia patients. Although there was decrease of cytokines after the treatment, their levels remained high, compared to other groups. Therefore, revision of treatment strategy of CHF patients with anemia of chronic disease is needed.

Follow up after 6 month showed significant improvement in intracardiac hemodynamic indicators in control group and Group B patients. In group B pateints although positive changes took place, they were not significant.

Comparative analysis of main and control groups show, that antianemic treatment was effective in CHF patients with iron deficiency anemia where the hematological indicators changed positively. In CHF patients with anemia of chronic disease effectiveness of antianemic treatment was not reliable. In our opinion, hepcidin levels decreased the effectiveness of the treatment. According to the literature of the subject there is a positive correlation between iron levels in organism and amount of hepcidin. Excessive amounts of iron causes the increased production of hepcidin, which in turn blocks the metabolism of the microelement. But no correlation was found between serum iron and hepcidin in our study. Moreover, effectiveness of iron medications was not proved.

Conclusion

In CHF patients with iron deificiency (Group A) and anemia of chronic disease (Group B) levels of serum ferritin transferrin, hepcidin were various. In Group B patients hepcidin levels were reliably higher than normal ranges (23,3±3,5 ng/ml, $p<0,001$);

Results of the study proves that there is difference in laboratory findings between iron deficiency anemia and anemia of chronic disease. Measuring of serum levels of hepcidin plays an important role in diagnosis of ACD and IDA.

In functional class II - III CHF patients with anemia of chronic disease levels of pro-inflammatory cytokines increase in accordance with the functional class of the disease. There was a correlation between pro-inflammatory cytokines and hepcidine during deterioration of the disease ($r=0,24$ $P<0,05$ between hepcidin and IL-1, $r=0,52$, $P<0,05$ between hepcidin and IL-6, $r=0,37$, $P<0,05$ between hepcidin and TNF-α)**;**

Systemic hypoxic process, neurohumoral activation of local tissue hormones stimulates the expression of pro-inflammatory cytokines, which plays in progression of CHF and development of adverse complications. Expression of cytokines positively correlates with functional classes of degree of severity of CHF. In patients involved in our study cytokine levels increased in accordance with functional class of CHF in both main and control groups. In anemic patients (Group A and B) increase was higher compared to the control group.

Serum levels of pro-inflammatory cytokines were increased in CHF patients of all groups. The levels were more significant in higher functional classes of CHF, in anemic patients, and particularly in patients with anemia of chronic diseaseнинг. It can be

concluded that, reason for this is the continuos systemic hypoxia and activation of local hormones.

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