ISSN 0975-2366
DOI:https://doi.org/10.31838/ijpr/2021.13.01.127

Research Article

Assessment of Erythropoietin Levels and Correlation with Cytokines in Patients with Chronic Heart Failure.

GADAEV A.G.[1], TURAKULOVR.I.[2],QURBONOV A.K.[3], SABIROV M.A.[4], SAYFULLAYEV M.B.[5], GADAEVA N.A.[6]

[1]Professor of department of Internal medicine №3, Tashkent Medical Academy, Doctor of Medicine, M.D.

[2]Assistant of department of Internal medicine №3, Tashkent Medical Academy, Doctor of Philosophy in Medicine, PhD

[3]Assistant of department of Internal medicine №1, Tashkent Medical Academy, Doctor of Philosophy in Medicine, PhD

[4]Head of department of therapeutic subjects №2,Tashkent State Dental Institute, Doctor of Medicine, M.D.,

[5]Physician in multifield hospital of Tashkent Medical Academy.

[6]Physician in multifield hospital of Tashkent Medical Academy.

E-mail: abgadaev@yahoo.com[1], Rustam_434@mail.ru[2], a.qurbonov@apgmu.uz[3]

Received: 03.08.20, Revised: 15.09.20, Accepted: 07.10.20

ABSTRACT

Aim of the study: To study interdependence between pro-inflammatory cytokines and erythropoietin in chronic heart failure (CHF) patients with anemia.

Materials and methods:

For the study 95 patients with CHF that developed as a result of ischemic heart disease were chosen.

Patients were divided into three groups. The first group consisted of 30 CHF patients with iron deficiency anemia. The second group consisted of 35 CHF patients with anemia of chronic disease. The control group consisted of 30 CHF patients without anemia. In all patients serum levels of ferrokinesis, erythropoietin and levels of pro-inflammatory cytokines (IL-1, IL-6 and α-TNF) were measured.

Results: the study showed that in CHF patients with anemia of chronic disease (the 2nd group) serum levels of erythropoietin was lower than other groups with 6,4±1,12 mIU/ml (p<0,05).

Only in limited number of studies correlation between erythropoietin levels and pro-inflammatory cytokines was examined, and they had contradictory results[3, 4, 8]. Statistically significant correlation was found between pro-inflammatory cytokines and erythropoietin levels. In CHF patients with anemia of chronic disease (2ndgroup) there was weak negative correlation (r= -0.24 P<0.05) between erythropoietin and IL-1, strong negative correlation (r= -0.52, P<0.001) between IL-6 and erythropoietin, and average negative correlation (r= -0.37, P<0.05) betweenTNF-αand erythropoietin. In CHF patients with iron deficiency anemia and without anemia weak positive correlation was found between erythropoietin and pro-inflammatory cytokines.

Conclusion: The study found, that in patients with iron deficiency and anemia of chronic disease serum ferritin, transferrin, hepcidin and erythropoietin levels were different, and in the last group erythropoietin levels were reliably low (6.4±1.12 mIU/ml, p<0.05) compared to other groups;

There was a negative correlation between cytokines and erythropoietin levels in disease development.

Keywords: Chronic heart failure, anemia, pro-inflammatory cytokines, erythropoietin.

INTRODUCTION

In recent years, special attention is given to anemia as a leading factor that affects life expectancy and quality of life in patients with chronic heart failure (CHF). «In developed countries anemia is found in 10-70% of CHF patients, the percentage increases in tandem with functional class (FC) of CHF, and in IV FC it reaches 80% » [1, 4, 5, 8]. At the same time, anemia is seen as an independent risk factor that negatively affects the course, results and outcome of the main disease. There are different approaches in explaining the course and pathogenesis of anemia in CHF patients, requiring serious study of the problem. Distinctive course of CHF in anemic patients and doubled morbidity compared to CHF patients without anemia make early diagnosis, effective treatment and prevention of this pathology one of the pressing problems of medicine [10, 12, 13].

Mechanisms that cause chronic kidney disease (CKD) and anemia CHF activate renin-

angiotensin-aldosterone and sympathic nervous system, oxidation stress стресс and inflammation factors. In development of kidney failure negative correlation between serum erythropoietin levels and hemoglobin is disturbed. As a result, erythropoietin synthesis will not increase in accordance with degree of anemia. Ineffective erythropoiesis that consists of bone marrow hemolysis and shorter erythrocyte lifespan, develops [9].

Additionally, interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor alpha (α-TNF) decrease erythropoietin concentration and increase sensitiveness of erythropoiesis line cells to it, which may lead to the development of relative resistance to erythropoietin [11, 12, 13].

Nakhodnova gives in her study that in CHF with iron deficiency anemia (IDA) and anemia of chronic disease (ACD) serum erythropoietin levels are high and it is one of the predictors of the course of the disease. In contrast, Zokhidova shows in her studies, that in CHF patients with ACD erythropoietin levels decrease, and therefore use of iron compounds alone is not sufficient in effective treatment of anemia of this type. She also observed, that in CHF patients with IDA erythropoietin levels increased as a compensation and after the threatment of anemia erythropoietin levels returned to normal ranges [12].

In addition, it needs an attention, thatvadministration of angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) for a long time in CHF patients may itself cause decreased erythropoiesis and development of anemia [11]. According to De Silva R. and coauthors, medication used in treatment of CHF patients, such as diuretics, ARBs and aldosterone antagonists, also negatively affect kidneys, causing anemia [11]. Life expectancy of CHF patients with anemia decreases even more when combined with CKD [2, 3].

As mentioned above, anemia is considered an independent risk factor of kidney insufficiency. Presence of CHF, anemia and diabetes additionally affects the kidney function. This in turn worsens the anemiани even more and leads in decreaseof erythropoietin levels.

Additionally, number of conducted clinical studies show, that it is diagnostically important to examine serum erythropoietin levels in CHF patients. Erythropoietin levels is not always related to kidney damage in CHF patients.

All reviewed material indicates, that anemia is one of the leading factors that increase mortality in CHF patients. CKD induced anemia deteriorates the course and prognosis of CHF. If the cause of anemia is not determined when found and additional measurements included in complex treatment of CHF, itmay become refractory.

There is information about effectiveness of intravenous iron and recombinant erythropoietin (rEPO) use in European Society of Cardiology guidelines for the diagnosis and management of CHF (2012) [1, 10]. Taking this into consideration, number of trials were conducted concerning erythropoietin use in CHF patients with anemia [7, 10]. Initially, RED–HF multi-centered randomized clinical trial showed improvement of clinical condition, decreased dyspnoe, increased tolerance to physical exercise was found in CHF patients with anemia after erythropoietin treatment [1, 3]. Unfortunately, final results of the trial did not confirm initial theory. Erythropoietin (darbopoietin–alpha) use did not lead to decrease in hospitalization and morbidity levels in CHF patients with hemoglobin less than 120 mg/l [1, 3, 7]. At the same time thromboembolic complications increased 3.5, strokes rose 1.7times in these patients. Similar cardiovascular complications were found after erythropoietin use in CKD and 2nd type diabetes mellitus patients [7].

Before, when optimal levels of hemoglobin was considered 120–140 mg/l, such complications were thought to be related with hemoglobin levels higher than 145 mg/l [1, 6]. But in RED–HF trial reached hemoglobin levels were on average 130 mg/l, despite this risk of complications kept increasing [1]. It was concluded, that even in low levels of hemoglobin of CHF patients, use of erythropoietin is not recommenden in treatment of anemia (recommendation class III, level of evidence A) [1].

In conclusion, getting good results by using of erythropoietin in treating anemia of CHF patients is not proved and further research in this field is required.

Aim of the study

To study interdependence between pro-inflammatory cytokines and erythropoietin in CHF patients with anemia.

MATERIALS AND METHODS

For the study 95 patients with CHF that developed as a result of ischemic heart disease were chosen. Patients were divided into three groups. The first group consisted of 30 CHF patients with iron deficiency anemia. The average age of the patients was 64.8±1.31 years, 11 (37.5%) of them were men and 19 (62.5%) were women. According to functional class of CHF, 9 (30.5%) of the patients had II FC and 21(69.5%) had III FC.

The second group consisted of 35 CHF patients with anemia of chronic disease. The average age of the patients was 65.3±1.48 years, 14 (39.5%) of them were men and 21 (60.5%) were women. According to functional class of CHF, 10 (28.5%) of the patients had II FC and 25(71.5%) had III FC.

The control group consisted of 30 CHF patients without anemia. The average age of the patients was 63.4±1.13 years, 16 (54.5%) of them were men and 14 (45.5%) were women. According to functional class of CHF, 12 (39.5%) of the patients had II FC and 18 (61.5%) had III FC.

In our study 100mg iron (III) hydroxide saccharose complex (Venofer ®) was given intravenously to patients of 1st and 2nd groups in addition to standard CHF treatment in in-patient conditions. In order to correct iron deficiency total amount of the compound was calculated using special formula created for Venofer ® (general iron deficiency = body mass, kg x (150 - hemoglobin level of the patient Hb, g/l) x 0.24 + 500mg). Patients of the control group received standard CHF treatment. All patients were observed for six months. Sufficient information about CHF was given to the patients, and they were taught principles of self control.

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). In all patients serum levels of ferrokinesis, erythropoietin and levels of pro-inflammatory cytokines (IL-1, IL-6 and α-TNF) were measured.

Inclusion criteria

Ischemic heart disease induced chronic heart failure patients with anemia.

Exclusion criteria

Patients with acute myocardial infarction and instable angina pectoris, arterial hypertension, serious arrhythmias, II-III level AV blockade, congenital heart defects, stroke, autoimmune and diffuse connective tissue diseases, acute and chronic inflammatory diseases in recurrence stage, developed chronic kidney disease, liver failure, severe respiratory failure caused by bronchial asthma and COPD recurrence, oncologic diseases, psychiatric conditions, alcohol abuse and other serious comorbidities.

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

In order to study how anemia affects CHF patients, theirhematologic levels were measured before the treatment.

Analysis showed, that there were defferences in hematologic indicators between CHF groups with and without anemia, between patients with anemia of chronic disease and iron deficiency anemia.

Before the treatment, in CHF patients with iron deficiency anemia (first group) average 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, of hepcidin - 10.6±1.3 ng/ml and of erythropoietin was 52.67±4.3 mIU/ml.

In CHF patients with anemia of chronic disease (second group) average 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, of hepcidin - 23.3±3.5 ng/ml and of erythropoietin was 6.4±1.12 mIU/ml.

In CHF patients without anemia (control group) average 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, of hepcidin - 7.7±1.02 ng/ml and of erythropoietin was 20.76±6.2 mIU/ml.

After six month of treatment, significant improvement took place in hematologic indicators of patients of the first group compared to initial state: average levels of hemoglobin increased from 101.4±2.1 g/l to 126.6±4.7 g/l (P<0.001). In the second group levels of hemoglobin increased from 104.3±4.8 g/l to 118.1±3.9 g/l (P<0.001). Additionally, in patients of the first group serum iron levels increased from 7.94±0.21 mmol/l to 22.9±2.8 mmol/l (P<0.001), ferritin levels rose from 85.9±8.5 to 318.4±5.46 (P<0.001), transferrin decreased from 5.2±1.21 g/l to 3.1±0.14 (P<0.05), hepcidin levels changed from

10.6±1.3 ng/ml to 3.2±0.05 ng/ml, and erythropoietin levels decreased from 52.67±4.3 mIU/ml to 32.4±2.1 mIU/ml (P< 0.001).

In the second group patients serum iron levels increased from 6.14±1.12 mmol/l to 16.1±2.12 mmol/l (P<0.001), ferritin levels rose from 167.5±8.54 to 259.6±6.5 (P<0.001), transferrin decreased from 6.9±2.28 to 4.4±0.3 (P<0.001), hepcidin levels decreased from 23.3±3.5 ng/ml to 19.2±0.06 ng/ml (P<0.001), and erythropoietin levels changed from 6.4±1.12 mIU/ml to 11.2 ± 1.32 mIU/ml (P<0.01).

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

Table 1: Hematologic changes before and after six month of treatments in CHF patients with and without anemia

Indicator	Treatment stages	Main group		P	Control group
		1st subgroup (30)	2nd subgroup (35)		3rd group (30)
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
Эритроцитлар, $\cdot 10^{12}$/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^^^	<0.001	4.2±0.05
	After	5.2±0.15^^^	5.0±0.16^^^	>0.05	5.5±0.08
Serum iron, 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^^^	<0.001	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^^^	<0.001	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
Erythropoietin mIU/ml	Before	52.67±4.3***	6.4±1.12*	<0.001	20.76±6.2
	After	32.4±2.1^^^	11.2 ± 1.32^^	<0.001	18.6±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)

Pro-inflammatory cytokines in serum were also measured in all patients.

According to the results of the study, in II-III FC CHF patients without anemia (control group) average serum IL-1 levels were respectively 16.4±0.72 (II) and 18.6±1.93ng/ml (III), levels of IL-6 were respectively17.2±1.78 (II) and 18.9±1.3 ng/ml (III), and that of TNF-α were respectively 15.9±0.72 (II) and 17.4±1.93 ng/ml (III).

In II-III FC CHF patients with iron deficiency anemia (first group) average serum IL-1 levels were respectively 17.9±2.1 (II) and 20.8±1.3ng/ml (III), levels of IL-6 were respectively20.4±1.8 (II) and 22.6±1.3 ng/ml (III), and that of TNF-α were respectively 19.6±0.62 (II) and 21.8±1.64 ng/ml (III).

In II-III FC CHF patients with anemia of chronic disease (second group)average serum IL-1 levels were respectively 18.2±0.72 (II) and 21.1±1.93ng/ml (III), levels of IL-6 were respectively26.6±1.7 (II) and 29.7±1.3 ng/ml (III), and that of TNF-α were respectively 20.2±0.72 (II) and 24.7±1.93 ng/ml (III).(Table 2)

Table 2: Dynamics of serum pro-inflammatory cytokines levels after the treatment

Indicator	Treatment stages	Group A		Control group	
		CHF FC II, n=9	CHF FC III, n=21	CHF FC II, n=12	CHF FC III, n=18
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^ ^ ^
TNF-α	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	
		CHF FC II, n=10	CHF FC III, n=25	CHF FC II, n=12	CHF FC III, n=18
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^ ^ ^
TNF-α	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)

After the treatment in CHF patients with iron deficiency anemia (Group I A) serum levels of pro-inflammatory cytokines reliably decreased. In CHF patients with anemia of chronic diseases serum levels of pro-inflammatory cytokines, although decreased after the treatment, remained higher compared to other groups. Dynamics of serum pro-inflammatory cytokines levels after 6 months of treatment is given in the Table 2.

After the treatment in CHF patients without anemia pro-inflammatory cytokines changed as following: in FC II patients there was reliable

decrease of IL-1 levels from 16,4 to 7,4 ng/ml ($p<0,001$), of IL-6 levels from 17,2 to 7,7 ng/ml ($p<0,001$), of TNF-α levels from 15,9 to 6,8 ng/ml ($p<0,001$); in FC III patients there was positive change in IL-1 levels from 18,6 to 8,7 ng/ml ($p<0,001$), in IL-6 levels from 18,9 to 8,6 ng/ml ($p<0,001$), in TNF-α levels from 17,4 there was reliable decrease 8,2 ng/ml ($P<0,001$).

Only in limited number of studies correlation between erythropoietin levels and pro-inflammatory cytokines was examined, and they had contradictory results[3, 4, 8]. Statistically significant correlation was found between pro-inflammatory cytokines and erythropoietin levels. In CHF patients with anemia of chronic disease (2nd group) there was weak negative correlation ($r= -0.24$ $P<0.05$) between erythropoietin and IL-1, strong negative correlation ($r= -0.52$, $P<0.001$) between IL-6 and erythropoietin, and average negative correlation ($r= -0.37$, $P<0.05$) betweenTNF-αand erythropoietin. In CHF patients with iron deficiency anemia and without anemia weak positive correlation was found between erythropoietin and pro-inflammatory cytokines.

DISCUSSIONS

In chronic heart failure patients some authors consider iron deficiency anemia as a prevalent form of anemia [2, 3, 4, 9], while others see anemia of chronic diseases as the main type [9, 12]. Number of studies showed, that duration of CHF is one of the main causes of anemia of chronic diseases [9, 11, 12]. Gastrointestinal disorders (antacid gastritis), blood lost due to anticoagulant and antiaggregant administration, as well as vitamin B12 and folic acid deficiency are also among factors that cause anemia of chronic diseases.

Anemia of chronic diseases has characteristics of normocytic anemia. And number of studies showed that it is the prevalent form of anemia in CHF patients [5, 6, 12].

Course and outcome of CHF are closely related to hemoglobin levels.Therefore anemia is considered as an independent factor in severe cases of CHF [3, 9]. According to some sources [2, 4, 9] in patients with iron deficiency anemia blood levels of hemoglobin, serum levels of iron and ferritin are decreased, while transferrin levels are increased compared to normal ranges. In patients with anemia of chronic diseases, on the other hand, levels of hemoglobin and iron are decreased, while serum levels ferritin which indicate iron reserves, are within normal ranges.

In our study, in order to differentiate CHF patients with anemia we used above mentioned laboratory finding differences between iron deficiency anemia and anemia of chronic diseases. We also measured serum levels of hepcidin, useful in diagnosing anemia of chronic diseases. After diagnosing patients were divided into groups. The 1st group of our study consisted of CHF patients with iron deficiency anemia (IDA). In this group 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. Data gathered during the study indicate statistically reliable decrease in levels of hemoglobin, serum iron and ferritin (which indicate iron reserves in organism). This in turn mean, that those patients had clear iron deficiency. The 2nd group consisted of CHF patients with anemia of chronic diseases (ACD). In this group 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, of serum level 6,14±1,12 mmol/l and of hepcidin 23,3±3,5 ng/ml.

Control group consisted of CHF patients without anemia. In those patients indicators of iron metabolism were within normal ranges. As can be seen, there is a difference in laboratory findings between IDA and ACD. Our study confirmed, that measuring serum levels of iron, transferrin, ferritin and hepcidin is important in diagnosing IDA and ACD.

Moreover, in the 1st group patients erythropoietin levels were higher compared to other groups ($p<0,001$). Scientific literature also confirms increased erythropoietin production in iron deficiency anemia patients [4, 12]. In anemia of chronic diseases according to some authors erythropoietin levels decrease [5, 6], while according to others [8, 9] it increases. Besides, in CHF patients with anemia, increase in serum erythropoietin levels is independent risk factor in development of unwanted outcomes [6, 9, 12] and aggravating course of the disease [3, 9]. In our study mean serum level of erythropoietin of the 2nd group patients (CHF patients with anemia of chronic diseases) was 6,4±1,12 mIU/ml, which is considerable lower compared to the other groups ($p<0,05$).

Comparative analysis of laboratory finding of CHF patients with iron deficiency anemia and anemia of chronic disease show the following: before the treatment patients of 1stgroup patients had decreased serum iron and ferritin levels, as well as increased transferrin levels, which indicates absolute iron deficiency in these patients. 2nd group patients had decreased serumида iron levels and decreased transferrin levels, while ferritin which indicates iron reserves was within normal ranges. This means that 2nd group patients had functional iron deficiency. As

known, functional iron deficiency is one of the key mechanisms of ACD pathogenesis. Although ferritin levels correctly represent iron reserves of reticuloendothelial system, it is not specific enough in assessing anemia of chronic disease. Discovery of acute phase protein that regulates iro levels – hepcid in recent years, allowed to understand iron homeostasis disorder in ACD development. Therefore, in our study hepcidin levels were measured in all patients, and in 2nd group patients hepcidin levels were reliably higher (P<0.001) compared to 1st and control groups (45.4% and 33% difference respectively). This in turn means that hepcidin is important in differentiating anemia of chronic disease and iron deficiency.

Erythropoietin levels were reliably higher (p<0.001) in CHF patients with iron deficiency anemia (1st group) compared to other groups. Literature on the subject also shows increased erythropoietin production in iron deficiency anemia [4, 9, 12], which was also found in our study. In anemia of chronic disease, on the other hand, some authors [5, 6] found decreased erythropoietin levels, while other authors [8, 9] had opposite results. Additionally, it was found, that in CHF patients with anemia increased erythropoietin levels may be independent risk factor in causing complications [5, 9] and deteriorating the course of the disease [6, 8]. In our study, reliably low erythropoietin levels (6.4±1.12 mIU/ml p<0.05) in the second group patients compared to other groups.

Based on literature review, it can be concluded, that in progression of CHF and development of complications systemic hypoxic process, activation of neurohumoral local tissue hormones induce expression of pro-inflammatory cytokines. Increase in expression of cytokines is directly proportional with functional class and severity of CHF. In all patient groups of our study cytokine levels increased from normal ranges in tandem with functional classes of CHF. The dependence was reliably higher in patients with anemia compared to patients without anemia.

In the 1st group patients IL-1 levels were 9.1 – 11.8% (P<0.05), that of IL-6 were 18.6 – 19.5 % (P<0.01), and of TNF-α were 23.2–25.0% (P<0.01) higher compared to the control group. In the 2nd group patients IL-1 levels were 10.9 – 13.4% (P<0.05), that of IL-6 were 54.6 – 57.1% (P<0.001), and of TNF-αwere 27.1–41.0% (P<0.01) higher compared to the control group.

CONCLUSION

The study found, that in patients with iron deficiency and anemia of chronic disease serum ferritin, transferrin, hepcidin and erythropoietin levels were different, and in the last group erythropoietin levels were reliably low (6.4±1.12 mIU/ml, p<0.05) compared to other groups;

In II and III functional classes of CHF with anemia of chronic disease levels of pro-inflammatory cytokinesincreased in accordance with functional class of the disease.

There was a negative correlation between cytokines and erythropoietin levels in disease development.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

Financial support

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

ACKNOWLEDGMENTS

All authors participated in the research process and data collection.

REFERENCES

1. Anand I., Gupta P. How I treat anemia in heart failure. *Blood* 2020;136:7: 790–800.
2. Dinatolo E, Dasseni N, Metra M, Lombardi C, von Haehling S.J. Iron deficiency in heart failure. Cardiovasc Med (Hagerstown). 2018;19(12):706-716.
3. Cunha GJL, Rocha BML, Menezes Falcão L. Iron deficiency in chronic and acute heart failure: A contemporary review on intertwined conditions. Eur J Intern Med. 2018;52:1-7.
4. von Haehling S, Ebner N, Evertz R, Ponikowski P, Anker SD. Iron Deficiency in Heart Failure: An Overview. JACC Heart Fail2019;7:36-46.
5. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, et al.; ESC Scientific Document Group. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur Heart J. 2016 Jul; 37(27):2129–200.
6. Van Veldhuisen DJ, Ponikowski P, van der Meer P, Metra M, Böhm M, Doletsky A, Voors AA, Macdougall IC, Anker SD, Roubert B, Zakin L. for EFFECT-HF Investigations Effect of ferric carboxymaltose on exercise capacity in patients with chronic heart failure and iron deficiency. *Circulation* 2017; 136: 1374– 1383.
7. Jankowska EA, Tkaczyszyn M, Suchocki T, Drozd M, von Haehling S, Doehner W,Banasiak

W, Filippatos G, Anker SD, Ponikowski P. Effects of intravenous iron therapy in iron deficient patients with systolic heart-failure: a meta-analysis of randomized controlled trials. *Eur J Heart Fail* 2016; 18: 786– 795.

8. Anker S. D, KirwanB.A., van Veldhuisen D.J., Filippatos G, Comin-Colet J,Ruschitzka F, Lüscher TF, Arutyunov GP, Motro M, Mori C, Roubert B. (2018) Effects of ferric carboxymaltose on hospitalizations and mortality rates in iron-deficient heart failure patients: an individual patient data meta-analysis. *Eur J Heart Fail* 20:125- 133.
9. Jacob C, Altevers J, Barck I, Hardt T, Braun S, Greiner W. Retrospective analysis differences in heart failure patients with and without iron deficiency or anaemia. *ESC Heart Fail* 2019 Aug; 6: 840– 855.
10. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr, Drazner MH, Fonarow GC,Geraci SA, Horwich T, Januzzi JL, Johnson MR. 2017 ACC/ AHA/HFSA Focused update of the 2013 ACCF/ AHA guideline for the management of heart failure: a report of the American College of Cardiology foundation/American Heart Association Task Force on Practice Guidelines and the Heart Failure Society of America. *J Am Coll Cardiol* 2017; 70: 776– 803.
11. Qurbonov A.K., Gadayev A. G., Turaqulov R. I. The Urgency of Firotic Processes in Chronic Heart Failure. World Healthcare Providers & Multidisciplinary medical journal. - Alabama, USA. 2017;8. -№:6:77-80. ISSN: 2154-0918.
12. Turaqulov R. I., Gadayev A. G. Dynamics of cytokines and level of hepsidine in patients with chronic heart failure with anemia// European science review 2018; 3-4:193-195. ISSN: 2310-5577.
13. Bobamuratov T.A, Akramova N.T., Bobamuratova D.T., Aliyeva K.K., Saidrasulova G.B., Matkuliyeva S.R. Cardiovascular System Involvement in Patients with Ankylosing Spondyloarthritis. *International* Journal of Pharmaceutical Research 2020; 12 :3:883-888 DOI:https://doi.org/10.31838/ijpr/2020.12.03.123