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Associations of NT-proBNP and hepcidin levels with clinical and laboratory parameters in patients with heart failure with various severity of left ventricular systolic dysfunction

Podzolkov V. I., Dragomiretskaya N. A., Stolbova S. K., Rusinov I. S.
I. M. Sechenov First Moscow State Medical University. Moscow, Russia

Data on hepcidin levels in patients with heart failure (HF) are contradictory and do not make clear its contribution to the progression of multiple organ failure. There remain a number of issues about the prognostic significance of the N-terminal pro-brain natriuretic peptide (NT-proBNP) in HF with preserved ejection fraction (EF). The authors suggested the relationships between these markers in decompensated HF, as well as their associations with other clinical and laboratory parameters.

Aim. To identify the association of NT-proBNP and hepcidin levels with clinical and laboratory parameters in patients with HF with various severity of left ventricular (LV) systolic dysfunction.

Material and methods. The study included 68 patients (29 women, 39 men; mean age — 72,3±11,7 years) hospitalized due to decompensated HF. Patients were divided into three groups: reduced (HFrEF) (n=20), mid-range (HFmrEF) (n=23), and preserved EF (HFpEF) (n=24). Upon admission, along with standard diagnostic tests, all patients were examined for NT-proBNP and hepcidin levels by enzyme-linked immunosorbent assay. Statistical processing was carried out using the software package Statistica 8.0.

Results. NT-proBNP levels in the entire sample was 315,9 [129,9; 576,1] pg/ml. Significantly higher concentrations of NT-proBNP were found in patients with lower EF: 433,05 [346,8-892,6] pg/ml for HFrEF, 289,97 [185,9-345,3] pg/ml for HFmrEF pg/ml and 214,98 [207,37-562,31] pg/ml for HFpEF ($p<0,05$). At the same time, hepcidin levels in the HFrEF group (31,63 ng/ml [22,0; 71,6]) was significantly higher than in the HFmrEF (23,89 ng/ml [21,1; 27,9]) ($p<0,05$) and HFpEF (26,91 ng/ml [18,6; 31,1]) ($p<0,05$). In HFpEF patients, there was a correlation of hepcidin level with body mass index ($r=0,47$, $p<0,05$) and chronic obstructive airway diseases ($r=0,44$, $p<0,05$). A correlation of hepcidin level with cardiac arrhythmias ($r=0,61$, $p<0,05$) was revealed in the HFmrEF group. In the HFrEF group, there were correlations of a significantly increased level of NT-proBNP (median — 433,05; 95% confidence interval: 346,8-892,6) with indicators of disease severity and multiple organ dysfunction: decrease in systolic blood pressure, cardiorenal syndrome, decrease in hemoglobin level and mean corpuscular hemoglobin concentration, characteristic of iron-deficiency anemia.

Conclusion. Patients with lower EF showed higher NT-proBNP values and a trend towards higher hepcidin levels. Relationships of hepcidin and NT-proBNP levels with following clinical parameters were found: body mass index, presence of obstructive airway diseases, cardiac arrhythmias, as well as low cardiac output syndrome, cardiorenal syndrome and anemia.

Key words: heart failure, hepcidin, NT-proBNP, clinical and laboratory associations.

Relationships and Activities: none.

Podzolkov V. I. ORCID: 0000-0002-0758-5609, Dragomiretskaya N. A.* ORCID: 0000-0002-6531-6255, Stolbova S. K. ORCID: 0000-0002-6686-099X, Rusinov I. S. ORCID 0000-0003-3414-9834.

*Corresponding author: dragomiretskaya_natalia@mail.ru

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The prevalence of heart failure (HF), despite the improved treatment and prevention strategy, is steadily growing, reaching the scale of a non-infectious pandemic [1]. According to the Russian population-based study EPOCHA-CHF, today no more than 8,5% of the population of the European Russia suffer from HF [2]. Its prevalence specifies the need to search for new methods and markers for assessing the patients' condition in order to form clinical phenotypes of HF and determine a personalized strategy.

The role of brain natriuretic peptide and N-terminal pro-brain natriuretic peptide (NT-proBNP) in the pathogenesis of HF has been well known since the beginning of 2000s, while the serum determination of these biomarkers is currently the gold standard of laboratory diagnosis of HF [1, 3-4]. However, the issue of the prognostic significance of an increase in these markers remains poorly understood, especially in patients with preserved left ventricular ejection fraction (LVEF) [5-6]. The study of the predictor significance of NT-proBNP decrease in patients hospitalized with decompensated HF continues, as well as the search for effective therapy aimed at reducing the level of this biomarker [7].

The value of hepcidin, a regulator of systemic iron homeostasis, has been well studied in patients with HF and anemia [8-10]. Currently, its diagnostic and prognostic role is studied as a new marker of liver damage in HF [9]. Despite the fact that it is produced by many body cells, including adipocytes, macrophages, pancreatic β-cells, cardiomyocytes, the main synthesis site is the liver, which expresses 15-1500 times more of

hepcidin than other cells of the body [9-13]. The main mechanism for controlling iron metabolism by hepcidin is its binding to the ferroportin, a transmembrane transporter of iron located on the surface of enterocytes, macrophages and hepatocytes, and its inactivation. This leads to a decrease in iron absorption in the small intestine and release from macrophages, resulting in iron deficiency and anemia. With a lack of hepcidin, there is an uncontrolled absorption of iron and accumulation in tissues, primarily in the liver, where it has a cytotoxic effect through oxidative stress [10].

Previous research on hepcidin role in the anemia in HF patients did not give an unambiguous answer about the change in hepcidin levels with the disease progression, while the place of this marker in the continuum of cardio-renal-hepatic anemia syndrome in decompensated HF in patients with different systolic dysfunction is also not identified [3, 9-13].

The aim was to identify the association of NT-proBNP and hepcidin levels with clinical and laboratory parameters in HF patients with LV systolic dysfunction of different severity.

Material and methods

This non-randomized uncontrolled study included 68 patients (29 women, 39 men) with coronary artery disease and/or hypertension hospitalized due to decompensated HF. All patients signed informed consent. This study was performed in accordance with the Helsinki declaration and Good Clinical Practice standards. The medical ethics committee approved this study. There were following inclusion criteria: age >18 years, presence of class II-IV HF at least 6 months, NT-proBNP of 125 pg/ml upon admission to the hospital. The exclusion criteria were primary liver (viral, toxic, etc.) and biliary tract disorders, cancer, dialysis-requiring renal failure.

Along with the standard diagnostic tests, all patients were studied for NT-proBNP using Biomedica BI-20852W kit (BNP-fragment (Austria)) and serum hepcidin-25 using CEB979Hu 96 Tests Enzyme-linked Immunosorbent Assay Kit For Hepcidin (Hepcidin ELISA) (Cloud-Clone Corp, USA). According to echocardiography, the patients were divided into 3 groups: with preserved LVEF (>50%) — 25 patients, mid-range LVEF (40-50%) — 23 patients, and reduced LVEF (<40%) — 20 patients.

Statistical evaluation was carried out using the SPSS and Statistica 8.0 software. The distribution normality was assessed using the Shapiro-Wilk test. In normal distribution, differences between groups were analyzed using the parametric Student's t-test. In non-normal distribution, the nonparametric Mann-Whitney U-test or the nonparametric Jonckheere-Terpstra test was used. To study a correlation between the variables, we used the linear Pearson's correlation coefficient with a normal distribution and Spearman's correlation coefficient with a non-normal distribution. To compare the frequency, the Chi-squared test and Fisher's exact test were used. Differences were considered significant at $p<0,05$.

Results

The mean age of the examined patients was 72,3±11,7 years (LVEF — 46,3±11,3%). All patients had clinical manifestations of NYHA class III-IV HF. The most common cause of HF in patients with mid-range and reduced LVEF was old myocardial infarction. The general clinical characteristics of patients are presented in Table 1.

The NT-proBNP level in all patients included in the study exceeded the threshold value of 125 pg/ml [1] and amounted to 315,9 [129,9; 576,1] pg/ml. Moreover, in patients with LVEF <40%, the NT-proBNP and hepcidin levels were higher than in patients with preserved and mid-range EF (Table 2).

Correlation analysis in the general sample of patients with HF showed the negative correlations of NT-proBNP level with LVEF ($r=-0,3$, $p<0,05$) and body mass index (BMI) ($r=-0,4$, $p<0,05$), as well as its positive correlations with laboratory parameters reflecting the severity of multiple organ dysfunction in patients with HF (Table 3).

The revealed correlations between the NT-proBNP and total bilirubin levels ($r=0,3$, $p<0,05$), as well as the international normalized ratio (INR) allow to consider an increased NT-proBNP concentration as a marker of cardiohepatic syndrome. At the same time, the relationship between NT-proBNP and urea nitrogen

Table 1

Clinical characteristics of patients

Parameter, n (%)	HFpEF (n=25)	HFmrEF (n=23)	HFrEF (n=20)
Gender, M/F	10/15 (40%/60%)	17/6 (74%/26%)	14/6 (70%/30%)
Age, years	75,88±11,45	72,13±12,05	68,15±11,19
NYHA class III-IV HF	23 (92%)	19 (79,1%)	20 (100%)
Type 2 diabetes	10 (40%)	11 (45,8%)	4 (20%)
Old myocardial infarction	9 (36%)	15 (62,5%)	13 (65%)
Anemia	3 (12%)	9 (37,5%)	6 (30%)
Pneumonia	12 (48%)	15 (62,5%)	10 (50%)
CKD G3-4 (CKD-EPI GFR <60 ml/min/1,73 m^2)	15 (60%)	11 (45,8%)	12 (60%)

Note: M/F — males/females.

Table 2

NT-proBNP and hepcidin levels in patients with HFpEF, HFmrEF and HFrEF

Parameter	HFpEF (n=25)	HFmrEF (n=23)	HFrEF (n=20)	HFpEF (n=25)	p_{2-3}	p_{1-3}
NT-proBNP, pg/ml (median, [Q1; Q3])	214,98 (207,37-562,31)	289,97 (185,9-345,3)	NA	433,05 (346,8-892,6)	0,01	0,006
Hepcidin, ng/ml (median, 95% CI)	26,9 (20,8-28,9)	23,9 (21,7-27,0)	NA	31,6 (22,2-69,6)	NA	NA

Table 3

Correlation coefficients between the NT-proBNP level and parameters reflecting the severity of the condition and multiple organ dysfunction in the general sample of HF patients

Parameter	Patients with HF (n=68)
	NT-proBNP level
Systolic blood pressure, mm Hg	-0,18
Urea nitrogen, mmol/l	0,29*
Creatinine, μmol/l	-0,02
Total bilirubin, μmol/l	0,3*
Albumin, mmol/l	-0,335
INR	0,29*
Platelets, $x10^9$/l	0,29*
Hemoglobin, g/l	-0,22
Mean corpuscular hemoglobin, pg	-0,21
BMI, kg/m^2	-0,38*
LVEF, %	-0,27*

Note: * — significant (p<0,05) Spearman's correlation coefficients.

Table 4

Correlation coefficients between the NT-proBNP level and parameters reflecting the severity of the condition and multiple organ dysfunction in patients with HFrEF

Parameter	Patients with HFrEF (n=20)
	NT-proBNP level
Systolic blood pressure, mm Hg	-0,34*
Urea nitrogen, mmol/l	0,55*
Creatinine, μmol/l	-0,015
Total bilirubin, μmol/l	0,026
Albumin, mmol/l	-0,5
INR	0,19
Platelets, $x10^9$/l	0,32
Hemoglobin, g/l	-0,65*
Mean corpuscular hemoglobin, pg	-0,44*

Note: * — significant (p<0,05) Spearman's correlation coefficients.

(r=0,3, p<0,05) serve as markers of the cardiorenal syndrome.

Taking into account that 95% CI for the median hepcidin in the group of HF with mid-range EF (HFmrEF) (40-50%) fits into the 95% CI for the median hepcidin in the group of HF with preserved EF (HFpEF) (>50%), it can be assumed that in itself a LVEF decrease with hypoperfusion of the liver and kidneys stimulates its synthesis. At the same time, a large scatter of hepcidin levels within one group, as well as the absence of significant differences between groups with different HF phenotypes (p=0,131), may indicate the predominance of different regulation mechanisms in patients with different severity of systolic dysfunction.

Therefore, we analyzed the relationship of NT-proBNP and hepcidin levels with clinical and laboratory parameters within each of the groups.

In patients with HFpEF, differences in hepcidin level in the presence of chronic obstructive lung diseases were revealed — 50,34 ng/ml [46,66; 54,02] and, in their absence — 25,99 ng/ml [17,93; 28,89] (p<0,05), as well as its positive correlation with BMI. At the same time, a negative correlation was found between BMI and NT-proBNP (r=-0,48, p<0,05).

In the HFmrEF group, the hepcidin level in patients with cardiac arrhythmias (atrial fibrillation and high-grade ventricular premature contractions) was 26,55 ng/ml [23,03; 43,91], while in patients without arrhythmias — 21,06 ng/ml [18,56; 21,4], (p<0,05). NT-proBNP values in these subgroups were 343,78 pg/ml [151,21; 504,20] and 381,41 pg/ml [217,89; 711,82] (p=0,181), respectively. Correlations of the NT-proBNP level with INR (r=0,6, p<0,05) can be considered as an indicator of cardiohepatic syndrome even with mid-range EF.

In the group of patients with HF with reduced EF (HFrEF) (<40%), there was a relationship between a significantly increased NT-proBNP (median, 433,05; 95% CI, 346,8-892,6 pg/ml) and severe clinical condition and multiple organ dysfunction (Table 4).

Discussion

Recently, not only an active study of new CF markers, but also an assessment of their influence on remodeling of internal organs and the development of multiple organ dysfunction is being carried out.

The diagnostic and predictive role of an increased NT-proBNP level has been well studied in patients with HFrEF. However, there are still questions about the significance of NT-proBNP in HFpEF [6-7]. High NT-proBNP values in patients with reduced LVEF are an independent marker of the HF phenotype, characterized by an unfavorable prognosis, and is combined with other unfavorable prognostic factors, such as hypotension and weight loss, as well as anemia, cardiorenal and cardiohepatic syndromes.

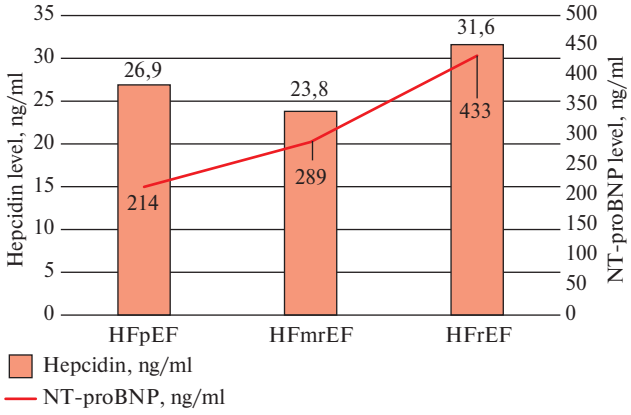

Figure 1. NT-proBNP and hepcidin levels in patients with different HF phenotypes.

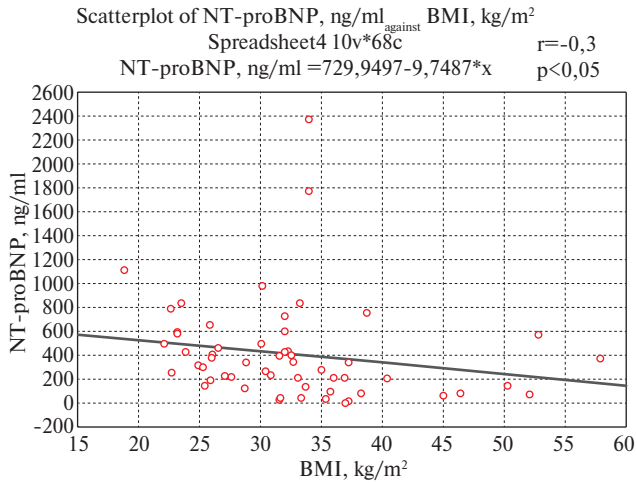

Figure 2. Correlation of NT-proBNP level and BMI in patients with HF.

The change in the hepcidin levels in patients with HF is due to many regulatory mechanisms. According to previous studies, the main mechanisms for regulating the hepcidin level are iron-deficiency, which suppresses its synthesis by negative feedback, and inflammation, which induces its formation in the liver and in immunocompetent cells [6, 10]. In patients with HF, along with the indicated mechanisms, there are a number of other factors that can have a significant effect on the hepcidin synthesis: hypoxia, impaired synthetic liver function, diabetes, obesity, chronic kidney disease (CKD) [6-9].

In the present study, despite the absence of a linear correlation between NT-proBNP and hepcidin, both in the general sample and in the groups of patients with different LV systolic dysfunction ($r<-0,14$, $p>0,05$), a tendency towards higher hepcidin values in patients with HFrEF was revealed, accompanied by a significant increase in NT-proBNP (Figure 1).

The revealed effect of obstructive lung diseases on the hepcidin level is due to two key factors: on the one hand, this group of diseases is accompanied by an inflammatory reaction, and on the other, it makes an additional contribution to the progression of right ventricular failure, increasing venous stasis in the systemic circulation [14-16].

With an increase in BMI, the proportion of adipose hormonally active tissue increases, which not only contributes to the maintenance of a high level of proinflammatory cytokines that enhance hepcidin production, but also directly synthesizes it by itself [10, 17]. At the same time, it is known that an increase in BMI in patients with severe HF is considered by some researchers as a factor of a favorable prognosis, described as the obesity paradox [18]. However, in recent years, controversy has resumed regarding the considering obesity as an adaptive mechanism [19]. Inverse correlation between BMI and NT-proBNP in the general sample, which is especially significant in patients with HFrEF, confirms the presence of this phenomenon (Figure 2).

It is known that the presence of severe arrhythmias further worsens organ perfusion in HF, aggravating hypoxia. *In vitro* experiments have shown that hypoxia inhibits the synthesis of hepcidin [3, 6], and therefore more expected was a negative relationship between its level and arrhythmias. The increase in hepcidin in patients with cardiac arrhythmias made it possible to consider other factors as a probable cause of this, including those leading to an increase in pro-inflammatory cytokines, which, as shown by previous studies, have a stimulating effect on hepcidin synthesis [5, 7, 20].

To confirm this hypothesis, a more detailed analysis of the clinical characteristics of this group was carried out, in which it was revealed that 15 (62,5%) patients had a history of acute myocardial infarction, 11 (45,8%) suffered from type 2 diabetes. All patients had signs of congestion in both circulations, with a decrease in exercise tolerance (NYHA class III-IV HF) in 19 (79,1%) patients, of which 15 (62,5%) had clinical, instrumental and laboratory signs of pneumonia, while 4 subjects had clinical and instrumental signs of ascites. Also, 11 (45,8%) patients had CKD with a decrease in the glomerular filtration rate (GFR) >60 ml/min/1,73 m^2, while 9 (37,5%) patients suffered from anemia.

In patients with LVEF <40%, along with an increase in NT-proBNP level, there was an increase in low cardiac output syndrome, manifested by a decrease in systolic blood pressure, cardiorenal and anemic syndromes.

It is known that hepcidin plays an important role in the development of anemia in HF, the median level of which in patients with HFrEF was higher than in other groups — 31,6 ng/ml (95% CI, 22,2-69,6). At the same time, there were no significant correlations between the level of hepcidin and other clinical parameters, including with the levels of creatinine, urea and GFR, which allows to consider a hepcidin increase in patients with LVEF <40% as one of the markers of multiple

organ dysfunction in HF and an additional factor of unforable prognosis.

Conclusion

In the examined patients with different HF phenotypes, selected depending on severity of LV systolic dysfunction, various associations were revealed between the NT-proBNP and hepcidin levels and clinical and laboratory parameters. In patients with HFrEF, a high level of NT-proBNP correlated with low body weight, cardiorenal and cardiohepatic syndromes, as well as anemia.

The revealed tendency to a hepcidin increase in HFrEF without correlations of its level with other clinical and laboratory parameters does not allow one to determine its independent role in HF progression, since the regulation of hepcidin levels in HF patients largely depends on various metabolic parameters and comorbid conditions, which complicates its assessment as a diagnostic and prognostic marker. Being an acute-phase protein, hepcidin also reflects the different severity of systemic inflammation characteristic of HF, which also complicates the interpretation. The results of this study coincide with the study by Jankowska EA, et al. with 321 HF patients, which did not demonstrate the association of hepcidin levels with either anemia or inflammation [12]. On the contrary, in the study of Solomakhina NI, et al., in patients with HF and anemia, a high level of hepcidin positively correlated with high values of proinflammatory cytokines, and negatively — with hemoglobin, which allowed the authors to consider inflammation as the cause of hepcidin increase in elderly and senile patients [13].

Relationships and Activities: none.

References

1. Mareev VY, Fomin IV, Ageev FT, et al. Russian Heart Failure Society, Russian Society of Cardiology. Russian Scientific Medical Society of Internal Medicine Guidelines for Heart failure: chronic (CHF) and acute decompensated (ADHF). Diagnosis, prevention and treatment. Kardiologiia. 2018;58(6S):8-158. (In Russ.) doi:10.18087/cardio.2475.
2. Fomin IV. Chronic heart failure in Russian Federation: what do we know and what to do. Russian Journal of Cardiology. 2016;(8):7-13. (In Russ.) doi:10.15829/1560-4071-2016-8-7-13.
3. Zaphiriou A, Robb S, Murray-Thomas T, et al. The diagnostic accuracy of plasma BNP and NT-proBNP in patients referred from primary care with suspected heart failure: Results of the UK natriuretic peptide study. Eur J Heart Fail. 2005;7(4):537-41. doi:10.1016/j.ejheart.2005.01.02286.
4. Maisel A, Mueller C, Adams K, et al. State of the art: Using natriuretic peptide levels in clinical practice. Eur J Heart Fail. 2008:10(9):824-39. doi:10.1016/j.ejheart.2008.07.014.
5. Leto L, Testa M, Feola M. The predictive value of plasma biomarkers in discharged heart failure patients: role of plasma NT-proBNP. Minerva Cardioangiol. 2016; 64(2):157-64.
6. Govender AP, Rodseth RN, Biccard BM. Natriuretic peptide-directed medical therapy: a systematic review. Perioper Med (Lond). 2020;9:5. doi:10.1186/s13741-019-0134-y.
7. Stienen S, Salah KCS, Moons AH, et al. NT-proBNP (N-Terminal pro-B-Type Natriuretic Peptide)-Guided Therapy in Acute Decompensated Heart Failure: PRIMA II Randomized Controlled Trial (Can NT-ProBNP-Guided Therapy During Hospital Admission for Acute Decompensated Heart Failure Reduce Mortality and Readmissions?). Circulation. 2018;137(16):1671-83. doi:10.1161/CIRCULATIONAHA.117.029882.
8. Ganz T, Nemeth E. Hepcidin and iron homeostasis. Biochim Biophys Acta 2012;1823:1434-43. doi:10.1016/j.bbamcr.2012.01.014.
9. Divakaran V. Hepcidin in anemia of chronic heart failure. Am J Hematol. 2011;86(1):107-9. doi:10.1002/ajh.21902.
10. Wang CY, Babitt JL. Hepcidin regulation in the anemia of inflammation. Curr Opin Hematol. 2016;23(3):189-97. doi:10.097/MOH.0000000000000236.
11. Saprina VT, Zima AP, Musina NN, et al. Pathogenetic aspects of hepcidin metabolism and ferrocinetics dysregulation in carbohydrate metabolism disorders. Diabetes mellitus. 2018;6(21): 506-12. (In Russ.) doi:10.14341/DM9378.
12. Jankowska EA, Malyszko J, Ardehali H, et al. Iron status in patients with chronic heart failure. Eur Heart J. 2013;34(11):827-34. doi:10.1093/eurheartj/ehs377.
13. Solomakhina NI, Nakhodnova ES, Gitel EP, Belenkov YN. Hepcidin and its relationship with inflammation in old and older patients with anemia of chronic disease associated with CHF. Kardiologiia. 2018;58(2S):4-11. (In Russ.) doi:10.18087/cardio.2457.
14. Agusti AG. Systemic effects of chronic obstructive pulmonary disease. Proc Am Thorac Soc. 2005;2:367-70. doi:10.1513/pats.200504-026SR.
15. Karoli NA, Borodkin AV, Rebrov AP. Features of the clinic and diagnosis of chronic heart failure in patients with chronic obstructive pulmonary disease. Kardiologiia. 2019;59(2S):47-55. (In Russ.) doi:10.18087/cardio.2486.
16. Ohno Y, Hanawa H, Jiao S, et al. Liver congestion in heart failure contributes to inappropriately increased serum hepcidin despite anemia J Exp Med. 2015;235:69-79. doi:10.1620/tjem.235.69.
17. Vuppalanchi R, Troutt JS, Konrad RJ, Ghabril M. Serum hepcidin levels are associated with obesity but not liver disease. Obesity. 2014;22(3):836-41. doi:10.1002/oby.20403.
18. Horwich TB, Fonarow GC, Clark AL. Obesity and the Obesity Paradox in Heart Failure. Prog Cardiovasc Dis. 2018;61(2):151-6. doi:10.1016/j.pcad.2018.05.005.
19. Elagizi A, Kachur S, Lavie CJ, et al. An Overview and Update on Obesity and the Obesity Paradox in Cardiovascular Diseases. Prog Cardiovasc Dis. 2018;61(2):142-50. doi:10.1016/j.pcad.2018.07.003.
20. Jamali R, Razavizade M, Arj A, Aarabi MH. Serum adipokines might predict liver histology findings in non-alcoholic fatty liver disease. World J Gastroenterol. 2016;22(21):5096-103. doi:10.3748/wjg.v22.i21.5096.