History of Medicine, 2023, 9(1): 1650–1655
DOI: 10.17720/2409-5834.v9.1.2023.206

Hepcidin, Iron Regulatory Hormone: Association with Patients with Heart Failure and Anemia

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Received: 20 January 2023 **Accepted:** 15 April 2023

Citation: Al-razaq SFA, Alkatib SR, Al Naffi KO (2023) Hepcidin, Iron Regulatory Hormone: Association with Patients with Heart Failure and Anemia. History of Medicine 9(1): 1650−1655. https://doi.org/10.17720/2409-5834.v9.1.2023.206

Abstract

Background: hepcidin (HCN) is a key regulatory protein of iron homeostasis. Heart failure (HF) get increased incidence, and the prevalence rate reaches about 1.5% of the US population. Chronic HF and iron deficiency are the commonest disorders universally. Current evidence has confirmed that they are related. This present study targeted to evaluate the association of HCN with heart failure patients and anemia Methodology: This study is a case-control, involving 80 patients with HF and 80 healthy subjects. All subjects underwent echocardiography (LVEF %), biochemical and hematological assays including ferritin, HCN, and iron status parameters (serum iron, TIBC, TSAT%, MCV, MCHC, HCT, and Hb). All variables were matched between the two study groups. Results: A highly significant difference was observed between the groups in the mean HCN and EF% levels (P<0.0001). A non-significant difference in HF patients between non-anemic and anemic patients regarding HCN was detected. HCN show highly significant differences among classes (P<0.001). HCN and ferritin significantly correlated with LVEF% Conclusion: the HCN levels were significantly reduced in cases with advanced HF. HCN and ferritin serum levels revealed a highly significant correlation with the levels of LVEF%.

Keywords

hepcidin, heart failure, anemia, ferritin, iron, EF%,

Hepcidin (HCN) is a hepatic hormone analogous to ferritin, discovered first as an antimicrobial polypeptide, but later research exposed its key regulatory role in iron homeostasis [1, 2]. Other body cells are also expressed HCN including the cardiocytes, but, at lower levels [3]. HCN is a homeostatic key regulatory protein of iron absorption by enhancing iron uptake from the intestinal mucosa and macrophages along with the secretion of hepatic stores of iron [4]. HCN can regulate intestinal iron absorption through a mechanism of HCN-ferroportin interaction. Moreover, HCN promotes ferroportin degradation, thereby decreasing bone marrow iron and reducing circulatory iron concentrations [5].

In recent years, cardiovascular diseases (CVD) have gained increasing attention globally, as it was responsible for cumulative deaths in patients with very complex pathogenesis [6-9]. Hence, finding out new factors for clarifying the etiopathophysiology of CVD and eventually discovering new therapies for CVD are desirable. About 1/5th deaths in the US died from heart disease in 2020 [10], and the prevalence rate of heart failure (HF) is 1.5% of the total population [11].

Chronic HF and iron deficiency are the commonest disorders universally. Current studies have confirmed that they are related. In addition, interventional trials have proved the advantages of the administration of iron supplements in HF [12]. Upregulation of liver HCN has been shown to play a major role in the pathophysiology of anemia and iron deficiency, which are linked to many illnesses. The limited and conflicting available data on the specific iron status in HF is alarming. As well, the series of processes in iron status taking place in the classical history of heart failure and the mechanisms causing iron deficiency in HF remain unexplored.

Because there have been a few clinical studies examining the link between HCN and iron status in patients with HF and anemia, this study was intended.

Material and methods

Study design and sampling

This study was a case-control, carried out at Al-Hussein Teaching Hospital during a period from 20 February 2021 to 16 April 2022. The study was conducted on 160 individuals (aged 20-75yrs), 95 were males. 80 patients were diagnosed with heart failure (based on echocardiographic findings) and 80 as a control group. All patients were examined and classified for four NYHA classes of HF [13]. Individuals with a history of diabetes mellitus, renal disease, stroke, thyroid disease, malignancy, smokers, autoimmune disease, and pregnancy.

Biochemical and hematological assays

The biochemical analyses of Hepcidin were achieved by "Hepcidin ELISA kit Elabscience Biotechnology Incor-USA", normal serum levels of HCN is 27.6-61.4 (ng/ml). For the measurement of other blood parameters including "[hematocrit (HCT), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH)]", Hematology analyzer (Abbott, USA) was used. The hematological evaluation of iron and ferritin was performed by Iron and Ferritin ELISA kits, Shenzhen New Industries, China-Maglumi. The total iron binding capacity (TIBC) was estimated using a "TIBC kit from Biolabo-SAS". Anemic patients were diagnosed if hemoglobin levels Hb<13g/dl in males and Hb <12g/dl in females) according to the WHO criteria. Transferrin saturation (TSAT %) estimation was measured as "serum iron value/serum TIBC value Ч100".

Echocardiography assessment

All participants underwent an echocardiographic study using "The vivid E9 ultrasound machine, GE Healthcare USA". Two separate specialists echocardiographer, who were blind to the study protocol, examined the participants and the "left ventricular ejection fraction (LVEF %)" assessed to measure and evaluate the class and degree of HF according to NYHA classification. Those with LVEF ≤50% are labeled as heart failure cases [14].

Statistical examinations

Measures are presented as means±SD. Statistical analysis was done using the available statistical soft ware (SPSS/25, USA). Variations between the study groups were evaluated with the χ2 test for categorical variables. Variations in continuous variables were evaluated with unpaired Student-T and ANOVA tests. The correlations among variables of interest were verified by linear regression analysess. A P-value below 0.05 was statistically significant.

Ethical issue

All participants provided informed permission in advance for their involvement, and the ethics committee at the health institution permitted the study protocol.

Results

Main characteristics of the participants

Table 1 demonstrates the characteristics of the two studied groups. The mean ages and BMI of the control and patients were comparable. A highly significant difference was detected between the HF patients and the controls in the mean HCN and EF% levels (P<0.0001). There were substantial differences in the levels of TIBC between control and patients (P<0.05); whereas there were non-significant changes regarding ferritin, iron, as well as TSAT% (P>0.05). Highly significant differences were found between HF cases and controls in the MCV and MCH (P<0.001). While, no significant differences were present concerning Hb, HCT, and MCHC.

Table 1: Characteristics of the two studied groups (mean ±SD)

Parameter		Control	Patient	p-value
Age (yrs)		60.42± 8.01	62.51±9.12	P>0.05
Sex	Males	47 (58.75%)	48 (60%)	P >0.05
	Females	33 (41.25%)	32 (40%)	
Body mass index Kg/mI		26.61±3.4	25.49±3.01	P>0.05
EF%		66.79 ±7.3	37.55±9.5	P<0.0001
Ferritin µg /dl		102.0005 ± 56.56	96.14±66.2	P>0.05
S. Iron µg /dl		85.46±41.07	83.24 ± 39.48	P>0.05
TIBC µg /dl		317.402 ± 58.24	347.11±67.9	P<0.05
TSAT%		47.73 ±15.1	43.28 ± 7.5	P>0.05
HB g/dl		12.09 ± 1.9	12.47±1.7	P>0.05
MCV fL		81.98±17.5	96.54±12.7	P<0.0001
MCH pg		25.57 ± 3.9	28.402±2.6	P<0.0001
MCHC g/dl		30.92 ± 4.1	29.65 ± 3.5	P>0.05
HCT g/dl		38.69 ± 8.1	39.27±8.8	P>0.05
Hepcidin ng/ml		44.61 ± 9.7	70.83±28.2	P<0.0001

"EF=ejection fraction, TIBC=Total iron binding capacity, TSAT=Transferrin saturation, Hb=

Hemoglobin, MCV= Mean cell volume, MCH= mean corpuscular hemoglobin, MCHC= Mean corpuscular hemoglobin concentration, HCT= hematocrit"

Differences in the Hepcidin, hematological, and iron status parameters between anemic and non-anemic patients with heart failure

Table 2 exposed a non-significant difference in heart failure patients between the non-anemic and anemic patients regarding Hepcidin, MCV, and MCHC (P>0.05), while other parameters (ferritin, Iron, TIBC, and Hb) were highly significant differ (P<0.001).

Table 2: Iron status and hematological parameters (Mean ± SD) among cases with heart failure, according to the iron status (anemic and non-anemic)

Iron deficiency patients	Hepcidin ng/ml	Ferritin µg/dl	Iron µg/dl	TIBC µg/dl	Hb g/dl	MCHC g/dl	MCV fL
Non-anemic	72.4 ± 31.1	129.3±60.1	106.4±35.7	318.9±54.6	13.6±0.9	29.9± 3.7	93.2±12.8
Anemic	76.9 ± 23.5	55.2±45.9	53.94±20.6	387.4 ±64.6	10.84±1.4	30.03±3.3	99.9±10.9
P-value	P>0.05	P<0.001	P<0.001	P<0.001	P<0.001	P>0.05	P>0.05

"TIBC=total iron binding capacity, Hb=Hemoglobin, MCV=Mean cell volume, MCHC=Mean corpuscular hemoglobin concentration"

Comparison of Hepcidin, hematological, and iron status parameters among patients according to NYHA classes of heart failure

ANOVA test was used to compare the result of the four classes of heart failure regarding HCN, hematological, and iron status parameters (Table 3). The Hepcidin show highly significant differences among classes (P<0.001). Serum values of ferritin and iron, Hb, TIBC, MCHC, and MCV results demonstrated that only TIBC showed significant differences among classes (P<0.05), while other parameters were not significantly differing (P>0.05).

Table 3: Multiple comparisons of Hepcidin and hematological parameters among patients according to "NYHA classification of heart failure"

Parameters	Class I	Class II	Class III	Class IV	P-value
Hepcidin ng/ml	88.5±23.3	68.9±22.6	54.04 ±20.8	30.2±5.9	P<0.001
Ferritin µg/dl	111.1±69.5	88.3±75.2	87.9±53.1	102.05±21.3	P>0.05
Iron µg/dl	96.2±43.1	74.2±41.6	66.4±26.7	94.05±2.7	P>0.05
TIBC µg/dl	329.5±58.3	380.1±56.9	377.6±60.9	290.3± 97.9	P<0.05
Hb g/dl	12.7±2.1	12.6±1.2	11.9±1.6	12.52±0.7	P>0.05
MCHC g/dl	30.1±3.9	30.4±3.7	29.5± 3.3	29±0.6	P>0.05
MCV fl	96.2±14.9	92.6±10.0	97.3±8.3	98.97±7.5	P>0.05

"Post Hoc comparisons" between each pair of heart failure classes (according to NYHA classification) regarding Hepcidin, hematological, and iron parameters were shown in Table 4. The Hepcidin revealed significant differences between (class I vis III and IV) and (class II vis IV). While there were non-significant differences between other pairs of classes. There were no significant differences between (classes I vis II, III, IV), (classes II vis III and IV), and (class III vis IV) regarding ferritin, Iron, Hb, MCHC, and MCV. TIBC reveals significant differences only between classes II vis IV and III vis class IV, while other classes did not significantly differ.

Table 4: Multiple comparisons of hematological and iron status parameters among patients according to NYHA classification of heart failure

Parameters	Class I vis II	Class I vis III	Class I vis IV	Class II vis III	Class II vis IV	Class III vis IV
Hepcidin ng/ml	N/S	S	S	N/S	S	N/S
Ferritin µg/dl	N/S	N/S	N/S	N/S	N/S	N/S
Iron µg/dl	N/S	N/S	N/S	N/S	N/S	N/S
TIBC µg/dl	N/S	N/S	N/S	N/S	S	S
HB g/dl	N/S	N/S	N/S	N/S	N/S	N/S
MCHC g/dl	N/S	N/S	N/S	N/S	N/S	N/S
MCV fL	N/S	N/S	N/S	N/S	N/S	N/S

Correlation between hepcidin, and ferritin with the ejection fraction

Table 6 shows the correlation between HCN and ferritin with the EF%, which shows a significant positive correlation between them among patients with heart failure.

Table 6: Correlation between hepcidin, ferritin, with EF% among the patients with heart failure

Variables	r	Significance
Ferritin	0.168	P<0.001
Hepcidin	0.473	P<0.001

Discussion

The objective of this cross-sectional study was to evaluate the association between HCN and iron status among anemic patients with HF. The main findings of this study were: highly significant elevation of serum HCN levels among the patients compared to the controls, highly significant reduction of the HCN levels with the worsening of heart failure, and finally highly significant correlation of both HCN and ferritin serum levels with the mean levels of LVEF%.

The association between HCN and CVD has been previously inspected [15]. HCN is primarily recognized as a key regulatory protein of iron metabolism, and knowing the ways HCN body changes facilitate understanding the mechanisms leading to iron derangement that characterizes HF patients. In the presence of HCN, ferroportin is degraded and cellular iron is blocked from leaving the cells. Thus, leads to reduced iron absorption by the duodenum with the retaining of iron in the reticuloendothelial system. Hence decreasing both iron accessibility to target tissues and serum iron levels [16]. Nevertheless, the physiological role of HCN is larger than orchestrating homeostasis of iron but, is also the regulator of effective iron-replete erythropoiesis, and has antimicrobial activity [16, 17].

Our findings of (i) highly significant elevated serum HCN levels among the HF patients compared to the controls and (ii) non-significant differences in HF patients between the non-anemic and anemic patients regarding HCN and variable iron status parameters are fascinating in the context of preceding studies. In a study involving about 100 patients with LVEF of <40%, there were certainly no major variations between HCN concentrations in cases with HF and anemia compared to HF with normal HCT. Researchers examining analogous associations between HF and HCN have found equivocal results [18, 19]. Divakaran et al. found no differences between HF subjects with and without anemia in serum or urine hepcidin (97 patients in total) [18], while in Matsumoto et al. study, 36-HF subjects with anemia had decreased plasma HCN compared with 25 healthy controls [19].

These biological behaviors of HCN among HF patients may be beneficial. A reduction in intestinal iron absorption and/or iron sequestration reduces the labile iron pool and diminishes the unfavorable impacts of excess serum iron. Alternatively, suppressing innate immunity may inhibit the progress of inflammation. As free radicals and inflammation are crucial pathways underlying cardiovascular damage, hepcidin upregulation may provide an adaptive response to prevent their progression [20]. The regulatory mechanism of liver HCN expression is very multifaceted and certainly not so far entirely agreed upon, thus, the role of other factors, not considered here, may also be desired.

Remarkably, our data appear to be somewhat conflicting, as in cases with HF, circulatory HCN was linked to the presence of neither anemia nor HCT levels (irrespective of whether these tests were done individually in NYHA HF-classes: I, II, III, or IV). Concordant to these conclusions were the results described by Ewa A. et al. [18, 20]. However, these results appear to be contrasting with the prevalent pieces of literature [4] and merit to be discussed.

In line with previous studies [20], we found lower levels of TIBC, TSAT%, and iron status parameters among patients with heart failure. An iron deficiency per se constrains HCN expression and its hepatic release into the blood, thus causing the gradual decline of serum hepcidin in HF and the accompanying depletion of iron body stores (low plasma ferritin), leading to a negative tissue iron homeostatic balance (i.e. low TSAT%, TIBC, and serum iron), and iron restricted hematopoiesis (decreased HCT and Hb).

A highly significant correlation of both HCN and ferritin serum levels with the mean levels of LVEF% were inconsistent with the outcomes of Erwin H. et al. [21], who revealed that hypoxia and anemia that may accompany the progression of HF and reduced LVEF%, can reduce HCN production and its blood level.

In the current study, similar to the Divakaran V. et al., findings, serum HCN levels were significantly reduced with the worsening of HF. Two explanations can be postulated to explain this finding. Firstly, when HF subjects become anemic, possibly the anemia down-regulates hepatic production of HCN, which reduces serum levels of HCN [18]. This may be a potential reason for the decreased HCN levels in anemic HF cases. Secondly, in patients with HF high erythropoietin levels could reduce HCN levels and outweighs the effect of inflammatory cytokines [19].

The contributory role of inflammation in the evolution and progression of HF is undeniable and several works of literature have confirmed this association. Tumor necrosis factor (TNF) [22, 23], myeloperoxidase [22, 24], and several interleukins (IL)-1 family, besides IL-6 [25, 26], are a few proinflammatory cytokines that have been linked to

HF progression. These cytokines have been found to increase the hepatic synthesis of HCN [18], and their merit is discussed further in future studies.

In agreement with our findings, clear shreds of evidence from the past literature exposed a positive correlation of both HCN and ferritin serum levels with the mean levels of LVEF%. Along similar lines, high ferritin levels were related to an accentuated EF% deterioration described recently by Crischentian B. et al. [27]. However, contrary results were also reported that revealed serum ferritin concentrations negatively correlated with the LVEF% [28].

Hepcidin concentrations were recently investigated by Jankowska, et al. in a study of 387 individuals, comprising 321 HF patients and 66 healthy controls [29]. Harmonious to our outcomes, they claimed that the high HCN level was unrelated to anemia and inflammation and instead was a sign of early HF. They observe the link between developing iron deficiency and deteriorating serum hepcidin concentrations caused by worsening LFEF%. Low hepcidin levels were considered to be independent unfavorable findings.

Conclusion

This study concluded a highly significant elevation of serum HCN levels among the HF patients compared to the controls, with a highly significant reduction of the HCN levels with the worsening of HF. In addition, a highly significant correlation between both HCN and ferritin serum levels with the mean levels of LVEF%.

References

Park, C.H., et al., *Hepcidin, a urinary antimicrobial peptide synthesized in the liver.* J Biol Chem, 2001. **276**(11): p. 7806-10.

Xu, Y., et al., *RelatioN/Ship between serum hepcidin levels and cardiovascular disease in patients with maintenance hemodialysis.* Physiol Int, 2020. **107**(4): p. 491-500.

van Breda, G.F., et al., *Cardiac Hepcidin Expression Associates with Injury Independent of Iron.* American Journal of Nephrology, 2016. **44**(5): p. 368-378.

Borku Uysal, B., et al., *The effect of anemia on serum hepcidin levels in patients with heart failure.* Journal of Cardiology and Cardiovascular Medicine, 2019. **4**: p. 159-163.

Di Lullo, L., et al., *Left Ventricular Hypertrophy in Chronic Kidney Disease Patients: From Pathophysiology to Treatment.* Cardiorenal Med, 2015. **5**(4): p. 254-66.

Amir A., A.-H.H., *Hyperuricemia has a Deleterious Role in Patients with Acute Coronary Syndrome Presented with Poor Oral Hygiene.* International Journal of Pharmaceutical Research, 2020. **Jan-Jun**(1): p. 7.

Hajir K., D.F., Al-Aaraji A., Abdul-Amir H., Mazin J., *Biochemical causal-effect of circulatory uric acid, and HSCRP and their diagnostic correlation in admitted patients with ischemic heart diseases.* Journal of Cardiovascular Disease Research 2020. **11**(2): p. 25-31.

Hayder A., M.J., Thekra A., Ahmed S., Saja A., *On Admission Levels of High Sensitive C- Reactive Protein as A Biomarker in Acute Myocardial Infarction: A Case-Control Study.* Indian Journal of Public Health Research & Development 2019. **10**(4): p. 5.

Qayssar J., R.T., Abdul-Amir H., *Evaluation of Practice of Prescribing and Monitoring Anticoagulants Among Hospitalized Iraqi Patients.* HIV Nursing, 2022. **22**(1): p. 71-76.

Tsao, C.W., et al., *Heart Disease and Stroke Statistics—2022 Update: A Report From the American Heart Association.* 2022. **145**(8): p. e153-e639.

Ponikowski, P., et al., *Heart failure: preventing disease and death worldwide.* 2014. **1**(1): p. 4-25.

Lakhal-Littleton, S., et al., *204 The cardiac hepcidin/ferroportin axis is essential for cardiac iron homeostasis and function.* Heart, 2017. **103**: p. A137.2-A137.

The Criteria Committee of the New York Heart Association., *Nomenclature, and Criteria for Diagnosis of Diseases of the Heart and Great Vessels.* 9th ed. Boston, Mass: Little, Brown & Co, 1994: p. 253-256.

Schwinger, R.H.G., *Pathophysiology of heart failure.* Cardiovasc Diagn Ther, 2021. **11**(1): p. 263-276.

Özlem Yayar, B.E., Harun Kılıç, *Relation between high serum hepcidin-25 level and subclinical atherosclerosis and cardiovascular mortality in hemodialysis patients.* Anatol J Cardiol, 2018. **19**: p. 6.

Babitt, J.L. and H.Y. Lin, *Molecular mechanisms of hepcidin regulation: implicatioN/S for the anemia of CKD.* Am J Kidney Dis, 2010. **55**(4): p. 726-41.

Ganz, T. and E. Nemeth, *Hepcidin and iron homeostasis.* Biochim Biophys Acta, 2012. **1823**(9): p. 1434-43.

Divakaran, V., et al., *Hepcidin in anemia of chronic heart failure.* Am J Hematol, 2011. **86**(1): p. 107-9.

Matsumoto, M., et al., *Iron regulatory hormone hepcidin decreases in chronic heart failure patients with anemia.* Circ J, 2010. **74**(2): p. 301-6.

Jankowska, E.A., et al., *Iron status in patients with chronic heart failure.* Eur Heart J, 2013. **34**(11): p. 827-34.

Kemna, E.H., et al., *Hepcidin: from discovery to differential diagnosis.* Haematologica, 2008. **93**(1): p. 90-7.

Ahmed Khalid Aldhalmi, A.J.H.A.-A., Hayder AA Makki, *Association of Tumor Necrosis Factor-α and Myeloperoxidase enzyme with Severe Asthma: A comparative study.* Reports of Biochemistry & Molecular Biology, 2022. **11**(2): p. 239-46.

Hayder MA, Mazin J. Mousa Enas K. Alkhazraji, A.A.A., Mahir Abdulkhadhum Alzughaibi, *Positive correlation of circulating tumor necrosis factor-alpha (TNFα) with spirometric pulmonary tests in adult asthmatic patients.* Biochem. Cell. Arch., 2021. **21**: p. 2605-2610.

Shaymaa Obaid Abdullah, G.M.R., Al-Hindy HA., Mazin Jaafar Mousa, Amir Al-Mumin, Safa Jihad, Suhad Hafidh, Yaqeen Kadhum, *Serum Myeloperoxidase as A Biomarker of Asthma Severity Among Adults: A Case-Control Study.* Reports of Biochemistry & Molecular Biology 2022. **11**(1): p. 182-190.

Hayder AM, Al-Athari A., Mazin J., Hameed Safa J., Obeed S., *The Utility of Serum IL-18 and CRP Together with Fractional Exhaled Nitric Oxide in the Diagnosis of Asthma in Adults.* NeuroQuantology, 2021. **19**(8): p. 119-124.

Amjed H. Abbas, M.A.R., Al-Hindy H., Mazin J. Mousa and Hadeel Abd Ameir Al-Shalah, *The Role of Serum IL-18 in Combination with Fractional Exhaled Nitric Oxide in the Diagnosis of Adult Bronchial Asthma.* NeuroQuantology, 2021. **19**(9): p. 13-19.

Brinza, C., et al., *The Prognostic Performance of Ferritin in Patients with Acute Myocardial Infarction: A Systematic Review.* Diagnostics (Basel), 2022. **12**(2).

Wongtong, N., et al. *RelatioN/Ship between Serum Ferritin Level and Left Ventricular Function in Thalassemia Patients*. 2016.

Jankowska, E.A., et al., *Iron status in patients with chronic heart failure.* European Heart Journal, 2012. **34**(11): p. 827-834.