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ORIGINAL ARTICLE

Assessment of the Serum Hepcidin as a Biomarker of Iron Status in Pediatric Congestive Heart Failure

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ABSTRACT

Background: Hepcidin plays a vital role in controlling iron homeostasis. Functional iron deficiency, as well as anemia, are common in heart failure, and it is hypothesized that hepcidin plays a significant role in both conditions. This study aimed to assess the relationship between hepcidin and anemia with the correlation of cardiac functions among congestive heart failure (CHF) pediatric cases.

Methods: Our case-control study was performed in the Pediatrics department at Zagazig University Hospital. It included 42 participants divided into Group I: 21 cases of congestive heart failure, and Group II: 21 healthy controls. Serum hepcidin level was assessed a mong all participants.

Results: A statistically significant decrease was found in hepcidin levels among studied CHF cases ($p<0.001$). High statistically significant positive correlations were found between hepcidin level and hemoglobin, red blood cell (RBCs) count,serum iron and ejection fraction (EF) of the studied cases ($p<0.001$, 0.008, <0.001, <0.001 respectively). There was a higher statistically significant decrease in hepcidin level assessed among severe CHF cases (ROSS grade 4) than cases of grade II and III ($p<0.001$). The sensitivity of hepcidin level in the prediction of CHF was 95.2% at a level less than 30.8 with the ability to exclude 90.5% of healthy cases and 92.9% test accuracy. The sensitivity of hepcidin level in the prediction of severe CHF was 85.7% at a level less than 27.6 with the ability to exclude 78.6% of mild cases and 83.3% test accuracy.

Conclusions: In children with congestive heart failure, serum hepcidin level is a promising biomarker for anemia, and it can predict the severity of heart failure.

Keywords: Hepcidin, Iron Status, Pediatric, Congestive Heart Failure.

INTRODUCTION

Hepcidin plays a vital role in controlling iron homeostasis. Anemia and functional iron deficiency are common in heart failure, and it is hypothesized that hepcidin plays a significant role in both conditions. Because it blocks iron from being absorbed in the intestine and released from macrophage reserves, an excess of the hepatocyte-produced hormone hepcidin leads to iron deficiencies and anemia [1]. Multiple variables induce and regulate hepcidin expression. Hepcidin synthesis is suppressed in conditions of anemia and hypoxia but is repressed via bone morphogenetic protein-6 when serum holo-transferrin and iron concentrations are increased [2]. Patients with congestive heart failure who also suffer from anemia are at higher risk for hospitalization and

death from any cause. Congestive heart failure patients often suffer from anemia for unknown and likely complex reasons. Possible causes include increased plasma volume, anemia, decreased bone marrow activity and inflammation. Congestive heart failure has been linked to inflammatory immunological activation. Patients with congestive heart failure may have increased serum hepcidin concentrations [3].

Hepatocyte-produced hepcidin interacts with ferroportin, the principal cellular iron export protein, to regulate systemic iron homeostasis. Serum iron levels drop because hepcidin prevents iron from being exported by macrophages and intestinal absorptive cells. Hepcidin levels rise in response to elevated iron and inflammation but fall in response to hypoxia, anemia, and depleted iron reserves [4]. To regulate iron levels, hepcidin is the primary hormone. Hepcidin is internalized and degraded by lysosomal activity after being secreted into the circulation and binding to ferroportin (iron transporter) on macrophages as well as enterocytes. Hypoferretinemia and iron-restricted erythropoiesis develop despite normal iron storage (functional iron deficiency) and anemia of chronic disease, and the latter can progress to anemia of chronic disease plus real iron deficiency when chronic inflammation is present. Conversely, low levels of hepcidin expression have been linked to iron overload [5].

So, we aimed in this study to assess the relationship between hepcidin and anemia with the correlation of cardiac functions among congestive heart failure pediatric cases.

METHODS

From October 2022 to July 2023, this case-control study was carried out in the pediatric departments of Zagazig University Hospitals.

Inclusion Criteria: Participatants in the study were 42 children from both sexes ranging in age from 2 months to 10 years (120 months); they were divided into 2 groups:

Group I (cases): included 21 cases hospitalized for heart failure with any symptoms or history of the following: high jugular venous pressure, ascites, pleural effusion, abdominal discomfort, hepatomegaly, pedal edema, orthopnea, dyspnea, rales by auscultation for pulmonary edema, nausea, fatigue, vomiting, abdominal discomfort, and eating intolerance, tachycardia, hypotension, and hypoperfusion. There were 11 males and 10 females. ***Group II (control group):*** Included 21 children, sex as well as age-matched, healthy controls who were at the outpatient clinic for routine care visits. There were 10 males and 11 females.

Exclusion criteria: we excluded from our study patients suffering from renal failure, history of severe hemorrhage, hematological diseases, chronic liver disease, inherited hemochromatosis and other iron overload disorders, blood transfusion, or iron replacement during the preceding six months, autoimmune diseases and hypothyroidism.

Informed consent and ethics committee/IRB approval:

This study followed the guidelines [the World Medical Association's Code of Ethics (Declaration of Helsinki) for human studies]. All parents of participants provided informed and written consent. The Institutional Review Board has approved this research (#9994/19-10-2022).

All the included children were subjected to:

Entire history taking including personal, complaint, present, past, perinatal, developmental, and dietetic history. The patients were classified according to the symptoms bases on Modified ROSS classifiction of heart failure [6] :Class I Asymptomatic, Class II Mild tachypnea or diaphoresis with feeding in infants Dyspnea on exertion in older children ,Class III Marked tachypnea or diaphoresis with feeding in infants Marked dyspnea on exertion Prolonged feeding times with growth failure ,Class IV Symptoms such as tachypnea, retractions, grunting, or diaphoresis at rest. The general and local examinations were done on all participants with particular emphasis on measurements of weight and height, as well as a full body checkup covering the cardiovascular, gastrointestinal

and nervous systems. Echocardiography was done on all subjects for valves, chamber visualization and congenital anomalies with the assessment of ejection fraction (EF).

Laboratory investigations included complete blood count (CBC); subjects had two milliliters of blood taken into a potassium EDTA tube for analysis with a cell counter. Indicated by the model number ABX Pentra XL 80 (Horiba, France). C-reactive protein (CRP), as well as serum iron, were done for all participants.

Detection of serum hepcidin level: Serum hepcidin was measured using enzyme-linked immunosorbent assay (ELISA), using BioVision's Human Hepcidin ELISA Kits (48 kits). At a wavelength of 450 nm, spectrophotometry was used to determine the optical density (OD). By comparing the OD of the samples to the standard curve, we could determine the hepcidin concentration in the samples, as the OD value was proportionate to the concentration. Human hepcidin concentration was calculated by graphing the optical density O.D of the sample against concentration (Ct). Multiplying the dilution factor back to the initial concentration.

Statistical analysis

Information gathered from a patient's medical history, physical examination, and laboratory tests were coded, processed, and analyzed in Microsoft Excel. To conduct statistical analysis, the gathered data were loaded into SPSS 20.0 (Statistical Package for the Social Sciences). The indicated differences between qualitative variables were computed using the Chi-square (2) test , Z score and the Fisher exact test. For non-parametric quantitative variables, the Mann-Whitney test was employed to determine the significance of the gap between the two groups.

RESULTS

There was a statistically non-significant difference among both studied groups regarding age and sex. Cyanosis was the most common complaint among studied CHF cases (23.8%), followed by tachycardia (14.3%), difficult breathing(14.3%) and general weakness (14.3%) (Table 1). Statistically significant increases were found in HR, RR, and body temperature among CHF cases ($p<0.001$, $p<0.001$, $p=0.005$). Both groups were matched as regard anthropometric measurements (Table 2).

A statistically significant decrease in hemoglobin, RBCs count, serum iron, and serum hepcidin level among CHF cases than the other group ($p<0.001$) and a significant increase in WBCs and CRP ($p=0.01$, $p=0.008$) (Table 3). There was a statistically significant decrease in EF among studied CHF groups, and the most common ECHO finding was PHT (33.3%) and VSD (28.6%), followed by 23.8% AVC (Table 4). We found a high statistically significant positive correlation between hepcidin level, hemoglobin, RBC count, and EF of studied cases (Table 5). A higher statistically significant decrease was found in hepcidin level assessed among severe CHF cases (ROSS grade 4) than in cases of grades II and III (Table 6). The sensitivity of hepcidin level in the prediction of CHF was 95.2% at a level less than 30.8 with the ability to exclude 90.5% of healthy cases and 92.9% test accuracy (Table 7, Figure 1). The sensitivity of hepcidin level in the prediction of severe CHF was 85.7% at a level less than 27.6 with the ability to exclude 78.6% of mild cases and 83.3% test accuracy (Table 8 and Figure 2).

Table 1: Basic characteristics and complain among both studied groups.

		Group I N=21		**Group II N=21**		**MW test**	**P**
Age\ months Median (Range)		60 (2 – 120)		60 (2 – 120)		0.276	0.831 NS
		N	**%**	**N**	**%**	**X^2**	**P value**
Gender	**Male**	11	52.4	10	47.6	0.092	0.725

	female	10	47.6	11	52.4		NS

	Group I N=21 N (%)
Cyanosis	5 (23.8%)
Difficulty of breathing	3 (14.3%)
General weakness	3 (14.3%)
Heart failure	2 (9.5%)
Respiratory distress	2 (9.5%)
Tachycardia	3 (14.3%)
Fever	2 (9.5%)
Down	1 (4.8%)

NS: P-value>0.05 is not significant HS: P-value<0.001 is high significant

Table 2: Vital signs &anthropometric measurements among both studied groups.

	Group I N=21 N (%)	**Group II N=21 N (%)**	**t-test X^{2*}**	**P**
HR Mean ± SD	135.2 ± 18.7	93.8 ± 9.04	9.13	<0.001 (**)
RR Mean ± SD	37.9 ± 8.14	20.7 ± 2.33	9.29	<0.001 **
Temperature Mean ± SD	37.7 ± 1.11	37.03 ± 0.16	2.99	0.005 *
BMI (kg\m^2) Median (Range)	15.5 (10.9- 20)	18.3 (8.9 –22.2)	1.71	0.081
Z -score of BMI Median (Range)	1.6 (-2.7 – 3.82)	2.3 (-4.7 – 3.55)	1.05	0.271
Weight\ kg Median (Range)	14 (3.5 –45)	25 (3.7 -50)	0.692	0.421
Z -score of weight Median (Range)	-0.54 (-2.61- 3.94)	1.2 (-2.71- 3.57)	1.54	0.119
Height\ cm Median (Range)	100 (50 – 150)	115 (50- 150)	0.277	0.781
Z -score of height Median (Range)	-0.24 (-4.39 – 5.4)	-0.23 (-8.6– 4.26)	0.059	0.961

*: P-value<0.05 is significant **: P-value<0.001 is high significant
HR: Heart rate, RR: respiratory rate, BMI: body mass index

Table 3: Laboratory data among both studied groups.

	Group I N=21	**Group II N=21**	**t-test\ MW***	**P value**
Hemoglobin (g\dl) Mean ±SD	10.3 ± 0.82	11.8 ± 0.79	6.12	<0.001 HS
WBCs (10^3/ul) Mean ±SD	12.4 ± 3.31	10.2 ± 2.64	2.44	0.01 S
RBCs (10^6/ul)				<0.001

	Group I N=21	Group II N=21	t-test\ MW*	P value
Mean ±SD	3.59 ± 0.34	4.49 ± 0.58	5.82	**
Platelet count (10^3/ul) Mean ±SD	297.6 ± 99.6	306.3 ± 97.2	0.287	0.771
CRP (mg/L) Mean ±SD	11.9 ± 15.8	3.13 ± 0.93	2.54*	0.008 *
Serum iron(µg/dL)	56.4 ± 4.2	85.1 ±9	13.24	<0.0001 **
MCV(fL) Mean ±SD	83.8 ± 5.22	81.9 ± 5.28	1.18	0.244 NS
MCH(pg/cell) Mean ±SD	28.7 ± 1.85	29.4 6.69	0.405	0.689
Hepcidin (ng/ml) Mean ±SD	25.9 ± 6.26 (13.1- 31.7)	55.9 ± 17.2 (29.3 – 92)	7.48	<0.001 **

**: P-value<0.001 is high significant : P-value>0.05 is not significant
P-value <0.05 is significant
WBCs: white blood cells, RBCS: red blood cells: C reactive protein, MCV: Mean corpuscular volume, MCH:Mean corpuscular hemoglobin

Table 4: Etiological causes of heart failure among studied patient .

	Group I N=21	Group I I N=21	P value
EF Mean ± SD	**36.2 ± 2.71**	**66.1 ± 5.51**	**<0.001 ****
	Group I		
ECHO findings	**N**	**%**	
VSD	**6**	**28.6**	
ASD	**3**	**14.3**	
AVC	**5**	**23.8**	
TGA	**3**	**14.3**	
PDA	**2**	**9.5**	
PHT	**7**	**33.3**	
DCM	**2**	**9.5**	
TR	**1**	**4.8**	
MR	**1**	**4.8**	

**: P-value<0.001 is high significant
VSD: Ventricular septal defect, ASD: Atrial septal defect, AVC: Atrioventricular Canal, TGA: Transposition of the great arteries, PDA: Patent ductus arteriosus, PHT: Pulmonary hypertension, DCM: dilated cardiomyopathy, TR: tricuspid regurgitation, MR: Mitral regurgitation

Table 5: Correlation between hepcidin level and laboratory data among studied cases.

Variables	Hepcidin level	
	R	P
Age	0.267	0.08
Weight	0.331	0.03*
Z score of weight	0.259	0.09
Height	0.227	0.124
Z score of height	-0.091	0.905
BMI	0.375	0.02*
Z score of BMI	0.210	0.822
Hemoglobin	0.685	<0.001**
RBCs	0.477	0.008*
WBCs	-0.244	0.09
Platelet count	0.137	0.641
CRP	-0.227	0.311
EF	0.734	<0.001**
Serum iron	-0.496	<0.004**
MCV	-0.148	0.322
MCH	0.221	0.198

*P-value<0.05 is significant **P-value<0.001 is high significant

BMI: Body mass index, RBCs: red blood cells, WBCS: white blood cells, CRP: C reactive protein, EF: Ejection fraction, MCV: Mean corpuscular volume, MCH:Mean corpuscular hemoglobin

Table 6: Relation between ROSS grading of CHF cases and laboratory data.

	Grade II N=7	Grade III N=11	Grade IV N=3	F test KW*	P value
Hemoglobin Mean ±SD	10.2 ± 0.56	10.5 ± 1.13	10 ± 0.51	0.675	0.301
Hepcidin Mean ±SD	31.5 ± 2.88	25.4 ± 3.99	15.3 ± 2.85	22.1	<0.001 **
RBCs Mean ±SD	3.56 ± 0.36	3.64 ± 0.36	3.63 ± 0.35	0.033	0.986
WBCs Mean ±SD	12.8 ± 1.58	12.9 ± 4.13	9.9 ± 2.92	1.01	0.136
Platelet count Mean ±SD	270.7 ± 82.4	300.6 ± 98.3	347 ± 47.5	0.691	0.546
CRP Mean ±SD	11.7 ± 16.7	14.1 ± 17.5	3.63 ± 1.35	1.49*	0.496

**: P-value<0.001 is high significant : P-value<0.05 is not significant

RBCs: red blood cells, WBCS: white blood cells, CRP: C reactive protein

Table 7: Validity data of ROC analysis for hepcidin level as diagnostic parameters of CHF.

Variable(s)	AUC	Cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy	P
Hepcidin	0.976	<30.8	95.2%	90.5%	90.9%	95%	92.9%	<0.001

AUC: Area under curve, PPV: positive predictive value, NPV: Negative predicted value

Table (8): Validity data of ROC analysis for Hepcidin level as predictor of severe CHF.

Variable(s)	AUC	Cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy	P
Hepcidin	0.959	<27.6	85.7%	78.6%	81.8%	85%	83.3%	0.001

Figure (1): Receiver operating characteristics (ROC) curve analysis of Hepcidin level as a diagnostic parameter for CHF (according to ROSS grade).

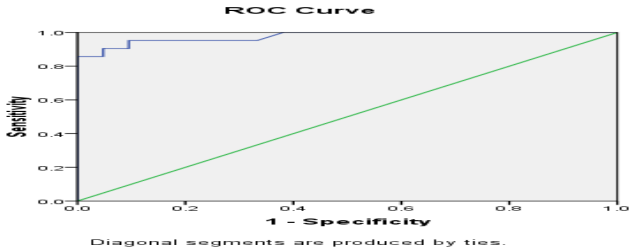
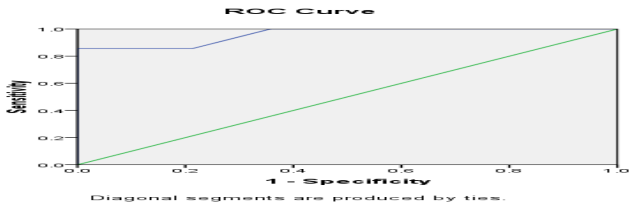


Figure (2): Receiver operating characteristics (ROC) curve analysis of hepcidin level as a predictor of severe CHF cases (according to ROSS grade).



DISCUSSION

Twenty to forty percent of patients with heart failure have anemia, making it one of the leading causes of morbidity and mortality worldwide and a significant socioeconomic concern in the healthcare system. Concurrent renal failure is one potential confounder that could explain the increased prevalence of anemia in heart failure patients [7]. Hepcidin has been recognized as the molecule responsible for anemia of inflammation over the previous 8 years. Although hepcidin was first identified as an antibiotic, it has now been shown to play a crucial function in the regulation of iron metabolism, especially in the context of infection and inflammation. Hepcidin is mainly produced in the liver and then secreted into the blood stream [8]. Acute inflammatory responses are characterized by a rapid increase in plasma hepcidin content by several folds, leading to a dramatic decline in plasma iron concentration [9]. Intestinal iron absorption is reduced by hepcidin, and iron release from macrophages that recycle iron from senescent erythrocytes is likewise suppressed. The iron required for erythropoiesis cannot reach the erythroid progenitor cells as a result. Abnormal erythropoiesis leads to chronic anemia if the stimulation for producing hepcidin persists, as in a chronic inflammatory disease [10].

Our results showed that there was a statistically non-significant difference among both studied groups regarding age and sex. Our results were supported by Okoromah et al. [11], who tried to identify the incidence,

demographics and risk factors for severe malnutrition among children with congenital heart defects. Their study included 90.4% of cases of congenital heart defects and 21.1% of controls, children aged 3–192 months, with non-statistically significant differences between the study participants. Uysal et al. [12] aimed to investigate if the anemia among heart failure cases was related to the concentration of hepcidin. Thirty-three males (or 66% of the total) and seventeen females (34% of the total) participated in the study. There were only 6 men in the control group (30%) and 14 women (70%). Male patients were overrepresented in the case group compared to the control group ($p< 0.05$). The mean age of the case group was 62.5 ± 10.5 years, while it was 51.7 ± 9.2 years in the control group, with a statistically significant difference between both groups ($p < 0.05$). The mean weight of cases was 73.7 ± 8.0, while it was 68.4 ± 10.3 in the control group.

We found that cyanosis was the most common complaint among studied CHF cases (23.8%), followed by tachycardia (14.3%),difficult breathing(14.3%) and general weakness (14.3%). Varma et al. [13] demonstrated that cyanosis was the most common complaint among studied CHF cases, followed by tachycardia and general weakness.

Our findings showed a statistically significant increase in HR, RR, and body temperature among CHF cases; 33.3% of them had PHT ,both groups were matched as regards anthropometric measurements and Z score.

We observed a statistically significant decrease in hemoglobin, serum iron, and RBC count among CHF cases in the other group and a significant increase in WBCs and CRP. Our results were like Sarhan et al. [14], who demonstrated that hemoglobin, differed significantly between the studied groups. Furthermore, Gadayev et al. [15] reported that hemoglobin differed significantly between both groups.

Our results showed that there was a high statistically significant decrease in hepcidin levels among studied CHF cases ($p<0.001$). Our findings are consistent with Sarhan et al. [14], who reported that hepcidin differences across the groups were statistically significant. Xu et al. [16] found that patients with cardiovascular disease had greater serum hepcidin levels compared to those without cardiovascular disease ($p< 0.05$). Also, Gadayev et al. [15] aimed to investigate how hepcidin affects the course of anemia in patients with chronic heart failure. They found that a significant difference was found between the studied groups regarding hepcidin. In contrast with Uysal et al. [12], who reported that There was no statistically significant difference in the hepcidin levels between the case and control groups($p > 0.05$). Otherwise, There was a statistically significant difference between the hepcidin concentrations of patients with anemia and those without anemia in heart failure ($p< 0.05$).

Our current study showed that EF decreased significantly among studied CHF groups($p<0.001$); the most common ECHO finding was PHT (33.3%) and VSD (28.6%), followed by 23.8% AVC. Our findings were supported by Sarhan et al. [14], who observed that Variables such as ejection fraction, fractional shortening, early diastolic velocity, and left ventricular mass index varied significantly between the groups. Also, Matsumoto et al. [17] found that Patients with heart failure and anemia had decreased serum hepcidin levels compared to other groups, suggesting that inflammation is a secondary cause of anemia in this population.

Our current study observed there was a high statistically significant positive correlation between hepcidin level, hemoglobin, RBC count, and EF of studied cases ($p<0.001$, 0.008, <0.001, respectively). Our results were in agreement with Sarhan et al. [14], who reported that Serum hepcidin was positively correlated with iron, transferrin saturation, and ferritin levels. Anemia and heart dysfunction had a positive and statistically significant association. Also, they found a significant positive correlation between serum hepcidin and FS, the E/Ea ratio, and LVMI. Also, Uysal et al. [12]

demonstrated that Hepcidin concentration was positively correlated with hemoglobin and hematocrit ($p<0.05$). Otherwise, they showed that hepcidin levels and ejection fraction were not significantly linked ($p > 0.05$). In the same line, Xu et al. [16] reported a high statistically significant positive correlation between serum hepcidin level and hemoglobin.

Our results showed that there was a higher statistically significant decrease in hepcidin level assessed among severe CHF cases (ROSS grade 4) than cases of grades II and III. Furthermore, we found that the sensitivity of hepcidin level in the prediction of CHF was 95.2% at a level less than 30.8 with the ability to exclude 90.5% of healthy cases and 92.9% test accuracy. The sensitivity of hepcidin level in the prediction of severe CHF was 85.7% at a level less than 27.6 with the ability to exclude 78.6% of mild cases and 83.3% test accuracy. Our findings were following Sarhan et al. [14], who demonstrated that the optimal cutoff for serum hepcidin was 158.5 ng/ml, with an AUC of 0.741, a sensitivity of 78.8%, and a specificity of 52.9% (p-value 0.021). Furthermore, Xu et al. [16] reported that hepcidin may be a potential biomarker and therapeutic target for cardiovascular disease because of its association with cardiovascular disease in maintenance hemodialysis cases with elevated serum hepcidin levels.

LIMITATIONS

There are certain limitations in our study. Firstly, the sample size might be relatively small, with 21 subjects in each group. The results may not apply to a broader population. Secondly, since the study was conducted in a single outpatient clinic of a specific hospital.

CONCLUSIONS

In children with congestive heart failure, serum hepcidin level is a promising biomarker for anemia, and it can predict the severity of heart failure.

Conflict of interest

The authors declared that they have no conflicts of interest with respect to the authorship and publication of this article.

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Supplmentary Table & Figure

Table S1: Validity data of ROC analysis for hepcidin level as predictor of severe CHF.

Variable(s)	AUC	Cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy	P
Hepcidin	0.959	<27.6	85.7%	78.6%	81.8%	85%	83.3%	0.001

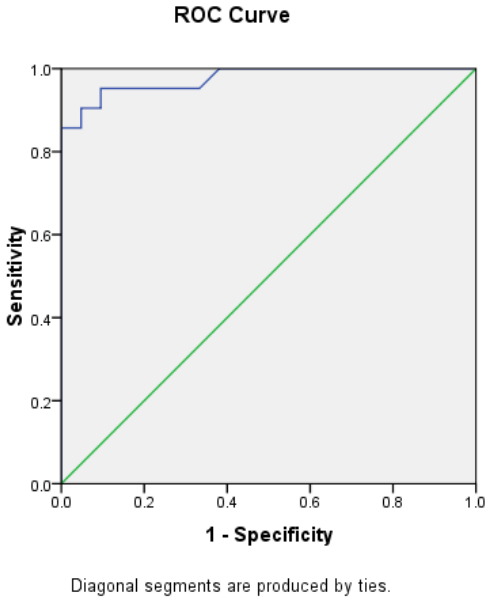

Figure S1: Receiver operating characteristics (ROC) curve analysis of hepcidin level as a diagnostic parameter for CHF.

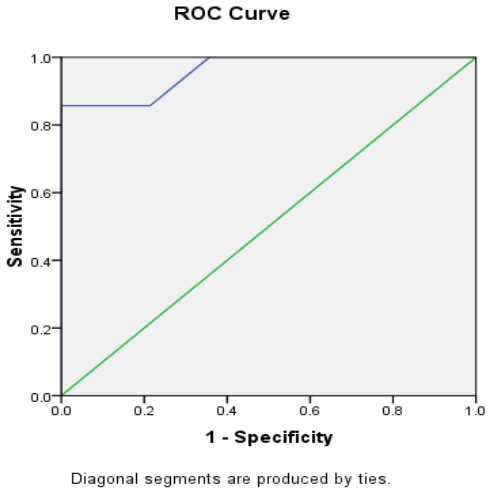

Figure S2: Receiver operating characteristics (ROC) curve analysis of hepcidin level as a predictor of severe CHF cases (according to ROSS grade).