Original Article

Hepcidin as a Marker of Anemia in Chronic Hepatitis C Infections

Anemia in Chronic Hepatitis C

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ABSTRACT

Objective: To determine the level of serum hepcidin and iron studies in patients of chronic hepatitis C with anemia and to calculate sensitivity, specificity, negative predictive value ,positive predictive value, diagnostic accuracy of serum hepcidin and to differentiate between patients of iron deficiency anemia and anemia of chronic disease.

Study Design: Diagnostic Study.

Place and Duration of Study: This study was conducted at the Department of Pathology, Post Graduate Medical Institute (PGMI) Lahore. Subjects were taken from Medical Outdoor / Liver Clinic, Lahore General Hospital from 01.06.2015 to 30.06.2016.

Materials and Methods: The study included 70 diagnosed patients of chronic hepatitis C which were divided into IDA group and ACD group. The IDA group comprised of 17(73.91%) males and 6(26.09%) females, while in ACD group there were 39(82.98%) males and 8(17.02%) females. The parameters studied were blood hemoglobin, total iron binding capacity, serum iron, percent transferrin saturation, serum ferritin and serum hepcidin and subsequent data was recorded. Mean ± standard deviation, frequency distribution and percentages were calculated. Using serum ferritin as a standard 2x2 table was made to determine diagnostic accuracy of serum hepcidin.

Results: In the present study mean hemoglobin, MCV, serum iron, TIBC, ferritin and hepcidin in IDA and ACD groups were 7.21 ± 1.29 g/dL ,8.69±0.86 g/dL, p-value < 0.001, 63.30 ± 8.04 fL and 86.51±6.61, p-value < 0.001, 30.98 ± 13.50, 34.36 ± 17.53 µg/dl, p-value > 0.05, 431.91±145.05 µg/dl and 230.51 ± 89.74 µg/dl, p-value < 0.001, 20.37± 4.08 and 154.07 ± 144.01, p-value < 0.001, 2.39 ± 1.39 and 15.17 ± 7.21 ng/ml, p-value < 0.001 respectively. The Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value of serum hepcdin levels was 91.3%, 87.23%, 77.78% and 95.35% respectively. Diagnostic accuracy of serum hepcdin levels was 88.57% with positive regression coefficient (beta) and hepcidin Odds ratio for Hepcidin was 71.750.

Conclusion: Low levels of serum hepcidin levels are significantly associated with decreased iron parameters in iron deficient patients of chronic hepatitis C and hepcidin could be a useful indicator of iron deficiency anemia and to differentiate iron deficiency anemia from anemia of chronic disease. Patients with decreased hepcidin level might get benefit by the use of iron therapy.

Key Words: ACD anemia of chronic disease, IDA Iron deficiency anemia, serum hepcidin, serum ferritin

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INTRODUCTION

Hepatitis C virus is a single-stranded enveloped RNA virus having positive polarity. This virus is a member of the hepaci viruses genus which belongs to Flaviviridae family.

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This is one of the most essential infectious agents for the human which is able to cause varying degree of liver disease. This virus is seen in almost all the regions of the world and almost 170 million are infected this comprises for greater than 3% of the world's population. The Hepatitis C virus can reside in the host for a long period.[1]

HCV spreads mainly by blood contact accompanying intravenous drug use, transfusions and ill sterilized instruments. According to one estimate about 130-200 million people of the world are diseased by Hepatitis C virus.[2]

The iron is kept in its soluble state by the attachment of transferrin and which also acts as a main transporter for the release of iron to the cells through transferrin receptor, TfR1 and synthesis of toxic radicals is prevented by it. Almost 30% Transferrin in human is iron saturated. A level of saturation less than 16% entitles iron deficiency, and saturation more than 45% is a mark for overload of the iron. And when saturation level is more than 60%, the iron which is not attached

to the transferrin initiates accumulation and may cause damage of the parenchyma cells.[3]

Iron homeostasis in mammals is mainly controlled by hepcidin. Hepcidin is a 25-amino acid peptide hormone with a multifaceted linkage of 4 disulfide bonds.[4] Ferroportin enables the outward movement of iron from intestinal cells, liver cells and macrophages after hepcidin binding, leads to reduced release of iron.[5] A large diversity of factors, like hemojuvelin, transferrin receptor-2, bone morphogenetic protein 6, transferrin, the HFE gene, hypoxia and inflammation are involved in the regulation of hepcidin level.[6]

Hepcidin transcription is also regulated by inflammation. Cytokines such as IL-6 facilitate this reaction by prompting of hepcidin mRNA transcription by a signal transducer and activator of transcription 3 (STAT3), which then attaches to a STAT-responsive element inside the promoter of hepcidin. It has been reported that in patients with anemia of chronic inflammation or due to any severe inflammatory disorder, a 100-fold increase in urinary excretion of hepcidin observed. An infusion of IL-6 in human may prompts excretion of urinary hepcidin within two hours resulting in hypoferremia. This indicates that up-regulation of hepcidin which is mediated by IL-6 as a result of inflammation, have a key role in imbalance of iron homeostasis which is detected in acute as well as in chronic inflammatory diseases.[7] A noteworthy relationship between hepcidin and serum ferritin has been observed as hepcidin transcription is mainly controlled according to concentration of iron in these patients. In this way conflicting the effects of HCV made despotic factors of hepcidin and hepcidin inspiration factors due to raised iron which are possibly included in the direction of hepcidin transcription in these patients of hepatitis C. [8]

Decreased or undetectable level of hepcidin is seen in iron deficiency, increased level in conditions like transfusion-induced iron overload and inflammatory diseases, and also showed high association with serum ferritin levels. It has been observed by Girelli, Nemeth and Swinkels that the patients of anemia of chronic disease are also threatened for having deficiency of iron. [10] According to Tomas Ganz hepcidin is controlled in response to both by the erythropoietic requirements for iron and by iron concentrations. In mouse models, hepcidin-1 mRNA was shown to be suppressed by anemia and hypoxia and induced by iron loading.[9]

The patients with chronic disorders like chronic renal failure, inflammatory bowel disease, or using drugs like nonsteroidal anti-inflammatory drugs or antithrombotic drugs often present with chronic blood loss. In such situation hepcidin can distinguish iron deficiency anemia and anemia of chronic disease better than traditional marker such as iron studies. Patients having both iron deficiency combined with inflammatory disorders characteristically present with reduced serum hepcidin as compared to those patients presenting with only anemia of chronic disease. [10]

AIM OF THE STUDY

The aim of this study is to demonstrate the role of serum Hepcidin in progress of anemia and in the control of iron homeostasis in chronic hepatitis C patients pointing the differentiation of the types of anemia in these patients.

Therefore this project is envisaged to study anemia in these patients and their evaluation by using serum Hepcidin as a new diagnostic tool to differentiate anemia in chronic hepatitis C patients, which will lead to better evaluation and management of these patients.

MATERIALS AND METHODS

The study was conducted at the Department of Pathology, Post Graduate Medical Institute (PGMI) Lahore. Subjects were taken from Medical Outdoor / Liver Clinic, Lahore General Hospital from 01.06.2015 to 30.05.2016 for 12 months. Non probability convenience sampling technique was used. The study was performed on 70 subjects. The patients were divided into two groups according to the results. Group1 or Iron deficiency anemia (IDA) group: Inclusion criteria for this group was: low Hb (male<13 g/dl and female <12 g/dl), TSAT <20% and Ferritin concentrations <30 ng/ml. Group 2 or Anemia of chronic disease (ACD) group: Inclusion criteria for this group was: Hb concentration <13 g/dl for male and <12 g/dl for female and low TSAT <20% and normal 30-100 ng/dl or increased Serum Ferritin concentration >100ng/ml.

Ten ml of venous sample was collected under aseptic measures. About 3 ml blood sample was placed into EDTA vacutainer for hemoglobin determination. The remaining quantity of blood was collected in yellow vials and left for a while without anticoagulant to allow to clot. Then serum sample was obtained by centrifugation at room temperature at 3000 rpm/10 minutes to assay serum iron, serum ferritin, TIBC serum transferrin saturation and serum hepcidin. The serum was stored at -20 °C until analysis.

Inclusion criteria:

1. Patients with age range of 20-60 years and of both sexes.
2. All patients with chronic Hepatitis C positive determined by PCR-HCV RNA for 6 months.
3. Hemoglobin level <12g/dl females and <13g/dl males determined by hematology analyzer.

Exclusion criteria:

1. Patients with a history of repeated and / or recent blood transfusions, hematinic/parenteral iron therapy.
2. Significant gastrointestinal bleeding, alcoholism.
3. Coexisting HBV or human immunodeficiency virus infection.

4. De-compensated cirrhosis, liver disorders other than HCV infection.
5. Current or previous antiviral therapy.
6. Connective tissue disorders such as Rheumatoid Arthritis, Systemic Lupus Erythematosus.
7. Evidence of renal disease.
8. Evidence of malignancy.

RESULTS

Distribution of Age in the Study Groups: The minimum age in the IDA study group was 28 years and maximum age was 57 years the Mean ± SD 42.91±7.01. The minimum age in the ACD study group was 25 years and maximum age was 60 years the Mean ± SD 41.701±9.12 (Table 3.1). The mean age of patients was 42.10 ± 8.45 where as in IDA and ACD group mean age was 42.91±7.01 years and 41.70 ± 9.12 years, the mean age in both groups was the same.

Table-No.1: Comparison of Age in both groups

IDA	ACD	Total
17(73.91%)	39(82.98)	56
6(26.09)%	8(17.02)	14
23	47	70

Key: IDA = Iron deficiency anemia, ACD = Anemia of chronic disease, n = frequency and %= Percentage

Table No.2: Comparison of Hb (g/dL) in both groups

		Mean	S.D
Hb g/dL	IDA (n=23)	7.21	1.29
	ACD (n=47)	8.69	0.86
	Total (n=70)	8.20	1.23

Key: IDA = Iron deficiency anemia, ACD = Anemia of chronic disease, Hb = Hemoglobin, n = frequency and % = Percentage, Hb, a. Independent sample test was applied

Table No.3: Comparison of MCV in both groups

		Mean	S.D
MCV	IDA (n=23)	63.30	8.04
	ACD (n=47)	86.51	6.61
	Total (n=70)	78.89	13.05

Key: IDA = Iron deficiency anemia, ACD = Anemia of chronic disease, Hb = Hemoglobin, n = frequency and % = Percentage, MCV = Mean cell volume, b. Mann Whitney U test was applied

Table No.4: Comparison of TIBC (µg/dl) in both groups

		Mean	S.D
TIBC µg/dl	IDA (n=23)	431.91	145.05
	ACD (n=47)	230.51	89.74
	Total (n=70)	296.69	145.45

Key: IDA = Iron deficiency anemia, ACD = Anemia of chronic disease, TIBC= Total Iron Binding Capacity, n = frequency and % = Percentage, b. Mann Whitney U test was applied**.**

Table-5: Comparison of S/Ferritin ng/ml in both groups

		Mean	S.D	Minimum	Maximum	p-value
S/Ferritin ng/ml	IDA (n=23)	20.37	4.08	14.30	28.00	<0.001 [b]
	ACD (n=47)	154.07	144.01	34.00	690.00	
	Total (n=70)	110.14	133.54	14.30	690.00	

Key: IDA = Iron deficiency anemia, ACD = Anemia of chronic disease, n = frequency, b. Mann Whitney U test was applied.

Table No.6 Comparison of S/Hepcidin ng/ml in both groups

		Mean	S.D	Minimum	Maximum	p-value
S/Hepcidin ng/ml	IDA (n=23)	2.39	1.39	1.50	7.10	<0.001 [b]
	ACD (n=47)	15.17	7.21	2.30	29.10	
	Total (n=70)	10.97	8.48	1.50	29.10	

Key: IDA = Iron deficiency anemia, ACD = Anemia of chronic disease, n = frequency, b. Mann Whitney U test was applied.

Table No.7: Diagnostic comparison of S/Hepcidin ng/ml in both groups

		Study Group		Total
		IDA	ACD	
S/Hepcidin ng/ml	≤ 2.7	21	6	27
	> 2.7	2	41	43
Total		23	47	70

The Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value of serum hepcdin levels was 91.3%, 87.23%, 77.78% and 95.35% respectively. Diagnostic accuracy of serum hepcdin levels was 88.57% with positive regression coefficient (beta) and hepcidin Odds ratio for was 71.750 that shows there 71.750 time more chances of IDA in cases having Hepcidin ≤ 2.7.

DISCUSSION

In both anemia and state of hyposideremia, the raised level of serum hepcidin helps to differentiate anemia of chronic disease from iron deficiency anemia. A condition of mixed anemia can arise in conditions with chronic inflammatory diseases due to bleeding with or

without malnutrition. Under such conditions, the hyposideremia could neutralize the inflammation mediated hepcidin increase. A true iron deficiency may develop from non-intestinal absorption by hepcidin, when inflammation is present for a long time [11]. In such settings, it is difficult to detect iron deficiency using traditional iron biomarkers, but hepcidin may be helpful. Patients with inflammatory disorders and concomitant iron deficiency typically have reduced serum hepcidin levels as compared with those with pure anemia of chronic disease. [10]

In the present study the mean MCV in IDA group was 63.30 ± 8.04 fL and in mean MCV in ACD group was 86.51±6.61 fL with significantly lower MCV in IDA groups, p-value< 0.001. According to Naqvi et al., 2014 MCV in his iron deficiency anemia patients was 78.15 ± 2.71 fL.[12] The results of the present study were similar to Duru et al., 2014 who showed MCV levels in iron deficient anemia patients and anemia of chronic disease patients to be63.30 ± 5.9 and 75.32 ± 8.50 fL with a p-value < 0.001.[13] In one study by van Santen et al., 2011 mean MCV value in iron deficient anemia patients and anemia of chronic disease patients were 82.9 (80.8–87.3) and 91.4 (89.9–96.6) with a p-value < 0.03.[14]

In the present study the mean total iron binding capacity (TIBC) µg/dl was significantly higher in IDA group 431.91±145.05 µg/dl as compared to ACD group 230.51 ± 89.74 µg/dl, p-value < 0.001.This was similar to Naqvi et al., 2014 where he reported mean TIBC in his iron deficiency anemia patients to be 470 ± 4.3 mg/dl.[12] Our results were in accordance with Duru et al., 2014 who showed mean serum TIBC levels in iron deficiency anemia patients and anemia of chronic disease patients to be 443.46 ± 43.96 and 310.30 ± 94.7 mg/dl with a p-value < 0.001.[13]

In the present study mean Ferritin in IDA and ACD group was 20.37± 4.08 and 154.07 ± 144.01 ng/ml with significant difference, p-value< 0.001. According to Naqvi et al., 2014 mean ferritin level in his iron deficiency anemia patients was 9.09 ± 1.35 ng/mL.[12] Duru et al., 2014 showed that mean serum ferritin levels in iron deficiency anemia patients and anemia of chronic disease patients were 4.10 ± 3.29 and 111.8 ± 158.2 ng/ml, p-value < 0.001.[13] According to Van Santen et al., 2011 mean Ferritin levels in iron deficient anemia patients and anemia of chronic disease patients were 11.5 (8.3–22.8) and 191 (102–262), p-value < 0.001 which was similar to our results.[14] In the study conducted by Eun et al., 2015, he found the serum ferritin levels in iron deficient anemia patients and anemia of chronic disease patients to be 11.38 ± 10.13 and 111 ± 54.95 ug/ml which are again similar to our results.[15]

In the present study the mean S/Hepcidin ng/ml in IDA group was 2.39 ± 1.39 and 15.17 ± 7.21 ng/ml with lower Hepcidin values in IDA when compared to ACD group, p-value < 0.001.This was in accordance with van Santen et al., 2011 and Choi et al., 2012.[14,16] Results of Van Santen et al., 2011 study showed mean hepcidin levels in his iron deficient anemia patients and anemia of chronic disease patients to be 0.4 (0.4–0.8) and 7.4 (2.6–11.0) nmol/liter , p-value < 0.001.[14] Choi et al., 2012 has stated a serum hepcidin level of 2.07 ± 2.30 ng/ml, p- value < 0.0001 for his iron deficient anemia patient.[16] Eun et al., 2015 found the serum hepcidin levels in iron deficient anemia patients and anemia of chronic disease patients to be 2.31 ± 3.24 and 10.52 ± 11.6 ug/ml which were similar to our results.[15]

According to van Santen et al., 2011 serum hepcidin at < 2.4 nmoles/liter had a sensitivity of 89% and a specificity of 88% to distinguish iron deficiency anemia from anemia of chronic disease (combined state) from anemia of chronic disease similar to our present study results.[14] Choi et al., 2012 showed serum hepcidin level of 2.07 ± 2.30 ng/ml, p- value < 0.0001 for his iron deficient anemia patient and according to his study hepcidin at 6.895ng/mL had a sensitivity of 79.2% and specificity of 82.8% to make the diagnosis of iron deficiency.[16] Pasricha et al., 2011 has reported that serum hepcidin less than 8ng/mL showed a sensitivity of 41.5% and a specificity of 97.6% while hepcidin less than 18ng/ml showed a sensitivity of 79.2% and a specificity of 85.6% for the diagnosis of iron deficiency anemia.[17]

There remains substantial uncertainty for the precise cutoffs of these indices to define iron deficiency and state of chronic inflammation. According to Pasricha et al., 2011 anemic patients without inflammation with hepcidin less than 0.5 nmol/l were considered to have iron deficiency anemia. And the patients with inflammation and hepcidin below 2.4 nmol/l or in case with hepcidin between 2.4 and 7.6 nmol/l combined with reduced reticulocyte hemoglobin, were considered to have combined iron deficiency anemia and anemia of chronic disease. Patients with inflammation and hepcidin above 7.6 nmol/l were considered to have anemia of chronic disease.[17]

Van Santen et al., 2011 has confirmed the use of hepcidin for the identification of iron deficiency anemia either alone or with concomitant inflammation in his patients with rheumatoid arthritis.[14]

CONCLUSION

In conclusion, the discovery of hepcidin has increased the possibility of understanding the disturbances of iron homeostasis especially in iron deficiency anemia and anemia of chronic disease. Our study demonstrated that

1. Low levels of serum hepcidin were significantly associated with decreased iron parameters including serum ferritin in iron deficient patients of chronic hepatitis C which could be a useful indicator of iron deficiency anemia.

2. Serum hepcidin and serum ferritin were normal in anemia of chronic disease group which was useful to label the anemia of chronic disease in patients of chronic hepatitis C.
3. The diagnostic comparison of serum hepcicin and serum ferritin showed a high sensitivity, specificity and diagnostic accuracy of serum hepcidin for the diagnosis of iron deficient patient and to differentiate these patients from anemia of chronic disease in chronic hepatitis C.

Author's Contribution:

Concept & Design of Study:	Hafiz Ather Farooq
Drafting:	Ayesha Samad Dogar
Data Analysis:	Sadia Ijaz
Revisiting Critically:	Hafiz Ather Farooq
Final Approval of version:	Hafiz Ather Farooq

Conflict of Interest: The study has no conflict of interest to declare by any author.

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