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Exploring the Link of Serum Hepcidin, Ferritin, and Iron Levels in Patients of Chronic Hepatitis C of Hyderabad District

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ABSTRACT

Background: In Pakistan, Hepatitis C Virus (HCV) is the second most prevalent infectious disease. Approximately 20% of individuals infected with HCV progress to liver fibrosis and hepatocellular carcinoma. Iron overload is a major contributing factor to disease progression, leading to a subsequent decrease in hepcidin levels. **Objective:** The present study was designed to investigate any link between serum ferritin and hepcidin concentrations with disease advancement in chronic hepatitis C individuals. **Study Design:** Cross-sectional study. **Settings:** Physiology department of LUMHS, in cooperation with the Medical wards, Pakistan. **Duration:** February 22, 2022, to September 22, 2022. **Methods:** Patients aged 20 to 45 years, with confirmed diagnoses of chronic hepatitis C, were enrolled. Data were obtained through a self-structured questionnaire, and samples of blood were taken for the analysis of serum hepcidin and ferritin concentrations. **Results:** 116 participants were included in the study, including 49 males (42.24%) and 68 females (57.75%), with a mean age of 47.7 years. Serum hepcidin levels indicated a non-significant negative link with ferritin ($r = -0.059$, $P > 0.005$) and a significant inverse correlation with iron levels ($r = -0.477$ and a p-value of < 0.005). **Conclusion:** The findings suggest that patients with chronic hepatitis C often exhibit reduced hepcidin levels due to iron overload. This study evaluates the potential of hepcidin as a prognostic biomarker for monitoring iron accumulation in individuals with chronic HCV infection.

Keywords: *Hepcidin regulation, Iron overload, Chronic hepatitis C, Liver cirrhosis, Hepatocellular cancer.*

INTRODUCTION

Hepcidin was identified in 2001, initially detected in human urine and plasma. The hormone derives its name from its site of synthesis—hepatocytes (*hep-*)—and its antimicrobial properties (*-cidin*).[1] Hepcidin plays a role in systemic iron regulation and is primarily synthesized in the liver. It decreases the iron absorption from the intestine and reduces iron release from hepatocytes and macrophages. Iron homeostasis is tightly regulated through a complex interplay of molecular sensors and feedback loops that modulate the production of key regulatory proteins. Any significant alteration in iron metabolism is closely associated with corresponding changes in hepcidin levels.[2]

Iron overload is the common finding in chronic hepatitis C, with approximately 35% exhibiting markedly elevated iron stores and suppressed hepcidin levels. This dysregulation is considered a central pathophysiological mechanism contributing to the progression of liver disease in these patients. Hepatic iron accumulation is observed in approximately 10–36% of individuals with CHC, with a higher prevalence in those with advanced or end-stage liver cirrhosis. Emerging evidence suggests that transferrin receptor 2 (Tfr2) may be involved in this process, although further research is needed to delineate its exact role in hepatic iron overload and symptom exacerbation.[3,4]

HCV-induced liver damage, particularly fibrosis, has been linked to reactive oxygen species (ROS). These ROS impair the binding of transcription factors such as CCAAT/enhancer-binding protein (C/EBP) and signal transducer and activator of transcription 3 (STAT3) to the *HAMP* gene promoter, thereby reducing hepcidin expression.[5] Interestingly, the suppressed hepcidin response in HCV-infected patients appears to be more closely related to overall iron status than to viral load or fibrosis stage. This suggests that oxidative stress may disrupt the normal regulatory mechanisms of hepcidin expression, potentially limiting the body's ability to counteract viral replication through iron sequestration.[6]

This study aims to establish hepcidin as a reliable biomarker for assessing disease progression in chronic hepatitis C. Monitoring serum hepcidin levels could provide valuable insights into the degree of liver damage and facilitate earlier diagnosis and intervention. Ultimately, the findings of this research may enhance clinical decision-making, leading to improved management and prognosis of hepatitis C in Pakistan, while also contributing to a reduction in CHC-related morbidity and mortality.

METHODS

This present cross-sectional research was conducted in the Physiology department, Liaquat University of Medical and Health Sciences, Jamshoro, in collaboration with the Medicine and Gastroenterology Wards. The study was carried out over seven months, from February 22, 2022, to September 22, 2022, following ethical approval from the Ethical Review Committee of LUMHS (Approval Letter No: LUMHS/REC/-41, dated 22/02/2022). All procedures were conducted according to the research guidelines of LUMHS.

The sample size was determined using Epi Info software, and participants were recruited through a non-probability convenience sampling technique. Consent was taken from all the individuals before conducting the present study.

The study population included patients aged between 40 and 60 years who had been diagnosed with Hepatitis C. The exclusion criteria included: patients under 40 and over 60 years of age; individuals with diabetes mellitus, hypertension, alcohol consumption, non-alcoholic fatty liver disease (NAFLD), hepatic malignancies, hemochromatosis, or other iron metabolism disorders; and those who declined to participate. As this was not a clinical trial, a trial registration number was not applicable.

Participants were recruited from both the outpatient department and inpatient wards of the Medicine and Gastroenterology Units at Civil Hospital, LUMHS. All individuals were thoroughly briefed about the study's objectives, potential risks, and expected benefits. Informed consent was obtained before data collection. Participants who declined participation were not coerced and were excluded without any prejudice. Eligible participants go through a comprehensive medical history and physical examination. Personal and demographic details, including age and gender, were recorded using a structured case report form. Confidentiality and anonymity of participant data were strictly maintained throughout the study. For biochemical analysis, 10 ml of venous blood was collected under aseptic conditions. The serum was separated and analyzed for hepcidin levels using the Enzyme-Linked Immunosorbent Assay (ELISA) method, employing a commercially available ELISA kit.

Anthropometric Measurements: Height was measured in centimeters using a wall-mounted stadiometer while the participant was in a standing position. Body weight was recorded in kilograms using a calibrated beam balance, with participants wearing light indoor clothing and no footwear.

Blood Sample Collection

1. A 10 ml sample of venous blood was collected from a prominent vein in the forearm or the dorsum of the hand using aseptic techniques. The sample was then distributed into three pre-labelled collection vials.
2. The vial containing a clot activator was left undisturbed at room temperature (22–26°C) for 60 minutes to allow for coagulation. After clot formation, the sample was centrifuged at 5000 RPM for 10 minutes to separate the serum. The resulting serum was transferred into a sterile Eppendorf tube for the biochemical estimation of serum hepcidin.

Descriptive statistics, including means, medians, standard deviations, and frequencies, were calculated for all study variables, with stratification based on age, gender, and body mass index (BMI). Pearson correlation was conducted to estimate the relationships among key variables, including serum hepcidin, serum iron, and ferritin levels. The results were also represented graphically to illustrate trends and associations.

All data were analyzed through SPSS software, version 23.0. Qualitative variables were presented as frequencies and percentages. Quantitative variables such as age, serum hepcidin, and serum ferritin levels were expressed as mean ± standard deviation. The Chi-square test was used to determine associations between categorical variables, with a p-value of < 0.05 considered statistically significant. Additionally, independent samples t-tests and one-way ANOVA were employed to compare means across different groups.

RESULTS

Table 1 presents the basic characteristics of the study participants. The mean age of participants was 46.9 ± 6.9 years. The mean systolic and diastolic blood pressure was recorded as 116.6 ± 7.1 mmHg, 76.89 ± 8.95 mmHg. The mean serum hepcidin level was 7.02 ± 4.9 ng/ml, the mean serum iron level was 116.90 ± 43.89 mg/dl, and the mean serum ferritin level was 108.87 ± 73.9 ng/l. Additionally, the mean serum albumin was 2.79 ± 0.8 g/dl, the mean gamma-glutamyl transferase (GGT) was 68.0 ± 22.1 IU/l, and the mean total bilirubin level was 4 ± 1.0 mg/dl.

The table also outlines the distribution of participants by gender and location. Out of 116 participants, 48 (41.3%) were male and 68 (58.6%) were female. Regarding residential background, 45 participants (38.79%) were from urban areas, while 71 (61.20%) resided in rural regions. Based on Body Mass Index (BMI), 21 participants (18.1%) were categorized as underweight, 34 (29.3%) normal weight, 30 (25.86%) overweight, and 31 (26.72%) as obese. In terms of PCR quantitative viral load, 12 participants (10.3%) had low viremia, 43 (37.06%) had moderate viremia, and 61 (52.58%) exhibited a high viremia load. Regarding the stage of liver fibrosis, 39 participants (33.62%) had mild fibrosis, 40 (34.48%) had moderate fibrosis, and 37 (31.89%) were diagnosed with severe fibrosis.

Table 1: Basic characteristics of the participants

Variable		Mean ± SD	
Age (Years)		46.9 ± 6.9	
SBP (mmHg) **DBP (mmHg)**		116.6 ± 7.1 76.89 ± 8.95	
Seum Hepcidin (ng/ml)		7.02 ± 4.9	
Serum Iron (mg/dl)		116.90 ± 43.89	
Serum Ferritin (ng/l)		108.87 ± 73.9	
Serum Albumin		2.79 ± 0.8 g/dl	
GGT		68.0 ± 22.1 IU/l	
Total Bilirubin level		4 ± 1.0 mg/dl	
		N	**%**
Distribution of the Participants	Male	49	42.24%
	Female	67	57.75%
Sociodemographic characteristics	Urban	45	38.76%
	Rural	71	61.20%
BMI	< 20	21	18.1%
	25	34	29.3%
	>25	30	25.86%
	>30	31	26.72%
PCR	Decreased viremia	12	10.3%
	Moderate viremia	43	37.06%
	Increased viremia	61	52.58%
Stages of Liver Fibrosis	Mild	39	33.62%
	Moderate	40	34.48%
	Severe	37	31.89%

Table 2 illustrates the link between serum hepcidin levels and the stages of liver fibrosis among the individuals. In individuals with mild fibrosis (n = 39), the mean serum hepcidin level was 7.61 ± 7.52 ng/ml. Participants with moderate fibrosis (n = 42) exhibited a mean level of 7.67 ± 3.95 ng/ml, while those with severe fibrosis (n = 35) had a lower mean serum hepcidin level of 5.54 ± 2.79 ng/ml. The overall mean hepcidin level across all participants (n = 116) was 7.02 ± 4.9 ng/ml.

Table 2: Relationship between hepcidin levels & liver fibrosis

Stages	n	Mean ± SD	95% Confidence Interval		P-value
			Lower Bound	Upper Bound	
Mild	39	7.61±7.52	4.67	9.54	0.166
Moderate	42	7.67± 3.95	6.46	8.89	
Severe	35	5.54 ± 2.79	4.58	6.50	
Total	116	7.02 ± 4.9	5.93	7.80	

Table 3 presents a gender-based comparison of serum hepcidin, ferritin, and iron levels among the participants. The mean serum hepcidin level was 6.96 ± 6.0 ng/l in males (n = 48) and 6.79 ± 4.2 ng/ml in females (n = 68). The mean serum ferritin level was 116.77 ± 63.63 ng/l, in males was 116.77 ± 63.63 ng/l, compared to 104.84 ± 81.02 ng/l in females. Similarly, the mean serum iron level was higher in males (123.40 ± 43.25 mg/dl) than in females (113.51 ± 44.79 mg/dl).

Table 3: Gender based distribution of serum hepcidin, ferritin, and iron

		N	Mean	95% Confidence Interval		P Value
				Lower Bound	Upper Bound	
Serum Hepcidin	Male	49	6.96 ± 6.0	5.19	8.72	.865
	Female	67	6.79 ± 4.2	5.76	7.83	
	Total	116	7.02 ± 4.9	5.93	7.80	
Serum Ferritin	Male	48	116.77 ± 63.63	98.29	135.25	.396
	Female	68	104.84 ± 81.02	85.23	124.45	
	Total	116	108.87 ± 73.9	96.12	123.43	
Serum Iron	Male	48	123.40 ± 43.25	110.84	135.95	.238
	Female	68	113.51 ± 44.79	102.67	124.36	
	Total	116	116.90 ± 43.89	109.47	125.74	

Figure 1 illustrates the Pearson correlation analysis to reveal the relationship between serum hepcidin and iron levels. A significant inverse link was observed in individuals with chronic Hepatitis C, with a correlation coefficient of r = -0.477 and a p-value of < 0.005, indicating an inverse link between serum hepcidin and iron levels.

Figure 1: Serum hepcidin and iron: correlation shown through scatter plot

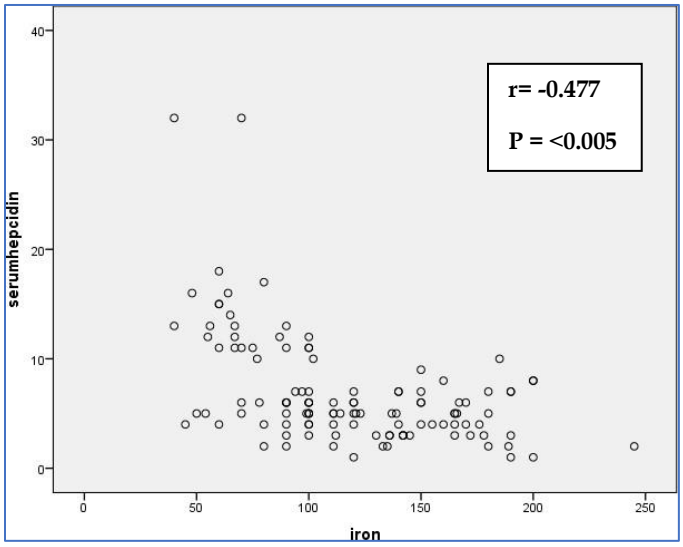
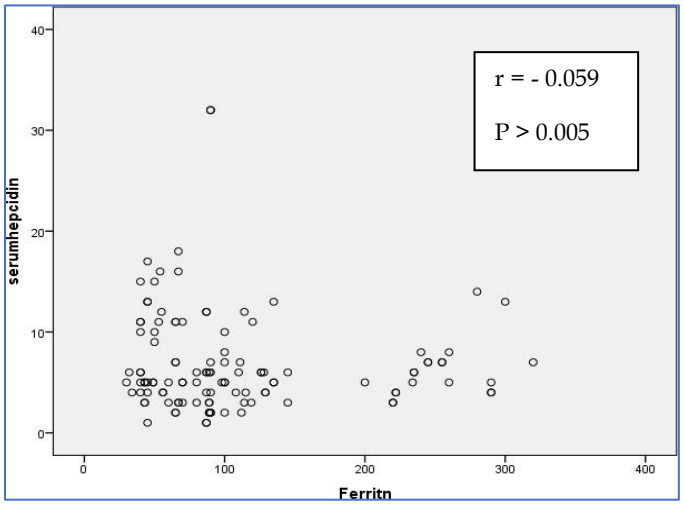


Figure 2 presents the Pearson correlation analysis between serum hepcidin and serum ferritin levels. A negative, though statistically not significant, relationship was observed (r = -0.059, P > 0.005), suggesting no meaningful relationship between the two variables.

Figure 2: Analysis of Pearson Link between Serum hepcidin and ferritin Levels



DISCUSSION

On the liver, the effects of Hepatitis C virus infection can range from mild changes to inflammation and scarring, potentially leading to liver carcinoma. The hepcidin levels in biological fluids are a predictive marker of the severity of inflammatory diseases. In patients of chronic hepatitis C (CHC), mild to moderate iron deposits within hepatocytes are frequently observed. Hepcidin is a crucial hormone for regulating iron homeostasis by monitoring the transmission of iron from iron-transferring tissues into the bloodstream.[7] Current studies have demonstrated that hepcidin may bind to ferroprotein, the key protein responsible for exporting iron from cells, causing its internalization and degradation.[8] As a result, iron efflux from enterocytes is reduced, highlighting hepcidin's vital role in maintaining iron balance in the body.[9, 10]

Patients suffering from inflammatory and infectious diseases, including CHC, often exhibit increased hepcidin production due to iron overload. In contrast, decreased hepcidin levels in CHC individuals may be associated with oxidative stress-induced epigenetic alterations that impact hepcidin expression.[11] Excess uninhibited iron within liver cells excites the generation of reactive oxygen species (ROS), leading to liver cell injury, fibrosis, and the potential progression of liver cancer. This notion is supported by findings from Blas-García *et al*, who suggested that excess iron in liver cells promotes ROS production, which damages liver cells and exacerbates fibrosis, further elevating markers like 8-hydroxy-2'-deoxyguanosine, contributing to hepatocellular carcinoma (HCC).[12,13]

Our study found that CHC patients exhibited significantly lower serum hepcidin levels compared to the control group. This observation suggests that the downregulation of hepcidin may contribute to the accumulation of iron in the liver, with HCV infection possibly influencing hepcidin expression through ROS production. These findings are consistent with those of El Sharkawy *et al*, who reported that individuals with chronic hepatitis C had significantly inferior serum hepcidin levels compared to healthy individuals, attributing this suppression to HCV's ability to induce ROS-mediated downregulation of hepcidin.[14]

Our study also reported a significant link between serum hepcidin and serum iron levels, which is in line with observations by C *et al*. and others.[15] The findings further indicate that ROS induced by HCV infection stimulates histone deacetylase (HDAC) activity, leading to the deacetylation of transcription factor binding sites and histones. This process contrasts with weak oxidative stress, which induces histone hyperacetylation. Research by Diab *et al* has demonstrated that chronic hepatitis C is often related to elevated liver markers and iron storage. In patients of CHC, a reduced hepatic hepcidin expression, proportional to the level of iron increase, plays a vital role in the pathophysiology of high iron overload.

These results align with studies by Nemeth *et al*, who established that hepatic hepcidin manifestation increases in response to iron increase in chronic hepatitis C patients. However, Olmez *et al* documented a non-significant link between hepcidin and levels of iron, offering a contrast to the current study's findings. In

contrast, our study strongly suggests a correlation between serum hepcidin levels and the extent of iron overload in CHC individuals, consistent with past research.[16]

Liu established that serum hepcidin concentration was significantly inferior in CHC patients compared to healthy individuals, further supporting our findings. Our study suggests that reduced serum hepcidin levels in CHC patients may enhance the uptake of iron by increasing ferroprotein expression in the duodenum.[17] The immune responses are also related to Hepatitis C infection, permitting it to persist in the host. One potential strategy for HCV is the suppression of hepcidin expression, which may facilitate increased iron availability necessary for HCV replication. In this context, the upregulation of hepcidin expression in response to iron status in chronic HCV infection is observed, but it is insufficient to counteract the effects of oxidative stress and HCV replication.[18]

Additionally, studies have revealed that HCV infection impairs the binding of hepcidin up-regulators to the hepcidin antimicrobial peptide (HAMP) gene promoter, leading to decreased hepcidin expression.[19] Increased oxidative stress induced by HCV infection is one potential mechanism for this impairment. Furthermore, research has shown that hepcidin may act as a cofactor in HCV replication, suggesting its pivotal role in the viral lifecycle.[20]

CONCLUSION

The present study was limited by its sample size, which may affect the findings for the broader population of Chronic Hepatitis C individuals. Furthermore, the cross-sectional study limits the ability to reveal links between serum hepcidin, ferritin, and iron levels. Other factors, like dietary intake, genetic variants, and coexisting medical conditions, were not extensively controlled, which might have influenced the observed biochemical parameters. Lastly, our study was conducted in a one district, and the results were applied to other geographical regions.

LIMITATIONS

The present study was limited by its small sample size, which may affect the findings for the broader population of Chronic Hepatitis C patients. Furthermore, the cross-sectional study limits the ability to reveal associations between serum hepcidin, ferritin, and iron levels. Other factors, such as dietary intake, genetic variants, and coexisting medical conditions, were not extensively controlled, which might have influenced the observed biochemical parameters. Lastly, this study was conducted in a one district (Hyderabad) that may limit the applicability of the results to other geographic regions.

SUGGESTIONS / RECOMMENDATIONS

Serum iron, ferritin, and hepcidin levels should be regularly assessed in CHC patients to monitor iron overload and prevent progression to cirrhosis or hepatocellular carcinoma.

CONFLICT OF INTEREST / DISCLOSURE

No conflict of interest.

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