Evaluation of Serum Hepcidin Level in Iraqi Patients with Chronic Hepatitis C and Relation to Serum Iron Status

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Abstract

Background: Persistent hepatitis C virus (HCV) infection is a significant cause of chronic liver disease and has often enhanced liver iron, causing iron overload connected with liver damage. Hepcidin is the primary regulator of iron metabolism and inhibits iron absorption and erythrophagocytosis recycling. Decreasing hepcidin in CHC is a possible pathophysiological iron overload mechanism. Chronic hepatitis C virus (HCV) infection is often associated with markers of elevated iron stores.

Objective: Estimate the amount of serum hepcidin in patients with chronic hepatitis C (CHC) and investigate the effect of the hepcidin level on iron status.

Methods: The research was included 54 patients diagnosed with hepatitis C virus infection as HCV positive, antibody identification of HCV (anti-HCV) and viral load by quantitative real-time PCR as well as 50 healthy subjects registered in the research. Hepcidin concentrations were determined by the research group. The parameter of serum iron was determined along with liver functions.

Results: Among the studied fifty-four of HCV positive patients hepcidin level was a normal when compared with healthy controls. Serum hepcidin level was found to be reduced in CHC patients as compared to controls (P<0.001). Serum iron was a highly significant decrease in healthy controls when compared with HCV patient. As regards serum ferritin and Transferrin saturation (TS%) were a highly significant increase in HCV patients when compared with healthy controls. Serum TIBC in controls was substantially higher than inpatient groups. Liver function was a Significant increase level of liver enzymes as AST, ALT, ALP, and TSB detected in group of patient when compared with healthy controls, (P < 0.001).

Conclusion: These results, indicate that Hepcidin levels in patients with CHC were significantly lower the suppression of this hormone by hepatitis C virus is likely an important factor in liver iron accumulation.

Keywords*: chronic hepatitis C, serum ferritin, serum hepcidin, serum iron, serum total iron-binding capacity*

Introduction

Around 180 million people globally were infected with hepatitis C .[(1)] It is well known that within 20 years of infection, roughly 20% of chronic hepatitis C (CHC) patients will advance to cirrhosis, the estimated annual threat of hepatocellular carcinoma (HCC) is 3–7 percent.[(2)] Patients with CHC often create mild to moderate iron overload. [(3)] Many experimental and clinical studies show that excessive CHC iron is a cofactor that encourages the growth of hepatic damage and reduces the risk of fibrosis, cirrhosis, and HCC .[(4)] The hepatic iron concentration was inversely associated with the antiviral response.[(5)] Phlebotomy iron removal increases liver function testing and histology [(6)], increases the probability of ongoing hepatitis C (HCV) eradication of antiviral treatment[(7)], and decreases HCC in CHC patients.[(8)] The elucidation of CHC's iron accumulation processes can, therefore, provide new tools to manage CHC or prevent its complications .[(9)] The liver emerged as the main organ in systemic homeostasis regulation when hepcidin was discovered

.[10] Hepcidin is a peptide hormone of 25 amino acids primarily synthesized by hepatocytes and negatively controls two critical measures of iron homeostasis: duodenal absorption and release of iron recycling iron from macrophages. [11] At the molecular level, hepcidin binds to ferroportin, a membrane iron exporter heavily expressed by enterocytes and macrophages. [12] These results in ferroportin internalization and degradation decreased the entry of iron into the plasma compartment. [13] Hepcidin expression is modulated by iron stores and therefore decreases the iron deficiency to encourage iron absorption, While replenishment of iron increases to prevent pathological overload. Inflammation also causes expression of hepcidin and suppresses hypoxia and anemia .[10]

Subjects and Methods

The study was included fifty-four samples of Al-Karama Teaching Hospitals and General Public Health Central Laboratory hepatitis C infected patients (HCV) and fifty controls. This research was performed between February 2018 and April 2019 in Kut City.In these samples. Mini VIDAS system (enzyme immunoassay) ferritin level assay, The saturation of transferrin was calculated by separating the serum iron concentration by complete iron-binding ability and multiplying by 100. The iron and TIBC were evaluated using colorimetric iron measurement and TIBC was performed .[14,15] Serum hepcidin has been determined usingSinoGeneClon Biotech (China) commercial ELISA kits by Human Reader Systems, (Germany) It is a strong stage enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. The microtiter wells are covered with an antigenic monoclonal antibody.

Statistical Analysis

IBM Social Science Statistical Package (SPSS) version 23 (USA) was conducted to identify all statistical relationships between the category group. The mean and standard mean error (SE) were designated as parametric variables. The differences for all relations were based on two-tailed *P*-value at significant level≤0.05.

Result

In both patient groups and controls, serum hepcidin (µg / L) was identified. There was a highly significant decrease in HCV patient when compared with healthy controls, with a mean ±SD of 57.4±39.64 and 85.8 ±56.75 , respectively ($P < 0.001$). As regards serum iron (µg/dl) in both groups patients and controls, there was a highly significant decrease in healthy controls when compared with HCV patient, with a mean ±SD of 84.6 ±29.44 and 123.6±26.59 respectively ($P < 0.001$). As regards In both patient groups and controls, serum ferritin (ng / ml) increased significantly in HCV patients compared to healthy controls, with a mean ±SD of 1540±1771and127.7 ±40.29 , respectively ($P < 0.001$). Serum TIBC (µg/dl) was significantly higher in controls than in group of patient, with a mean ±SD of 213.3±43.89 and 173.3±41.6 , respectively ($P < 0.001$). Transferrin saturation (TS%) in group patient and controls there was a highly significant increase in HCV patients when compared with healthy controls, with a mean ±SD of 47.5±12.8 and 24.3 ±7.63 , respectively ($P < 0.001$). There was a high significant difference of AST, ALT, ALP, and TSB detected in group of patient when compared with healthy controls, ($P < 0.001$) as shown in Table 1.

Table 1 - Serum Iron parameters and Liver function test in HCV patients and control.

Mean ±SD			
Item	**HCV n=54**	**Control n=50**	**p-value≤**
IRON/µg/dl	123.6±26.59	84.6 ±29.44	0.001
TIBC/µg/dl	270.6 ±52.03	321 ±53.19	0.001
TS%	47.5±12.8	24.3 ±7.63	0.001
S.F ng/Ml	1540 ±1771	127.7 ±40.29	0.001

Cont... **Table 1 - Serum Iron parameters and Liver function test in HCV patients and control.**

Hepcidin µg/L	57.4 ±39.64	85.8 ±56.75	0.001
AST u/L	61.95±37.44	18.09±10.24	0.001
ALT u/L	63.97±44.49	19.14±10.02	0.001
ALP u/L	192.19±90.69	73.2±17.57	0.001
TSB mg/dL	1.9±0.98	0.88±1.2	0.001

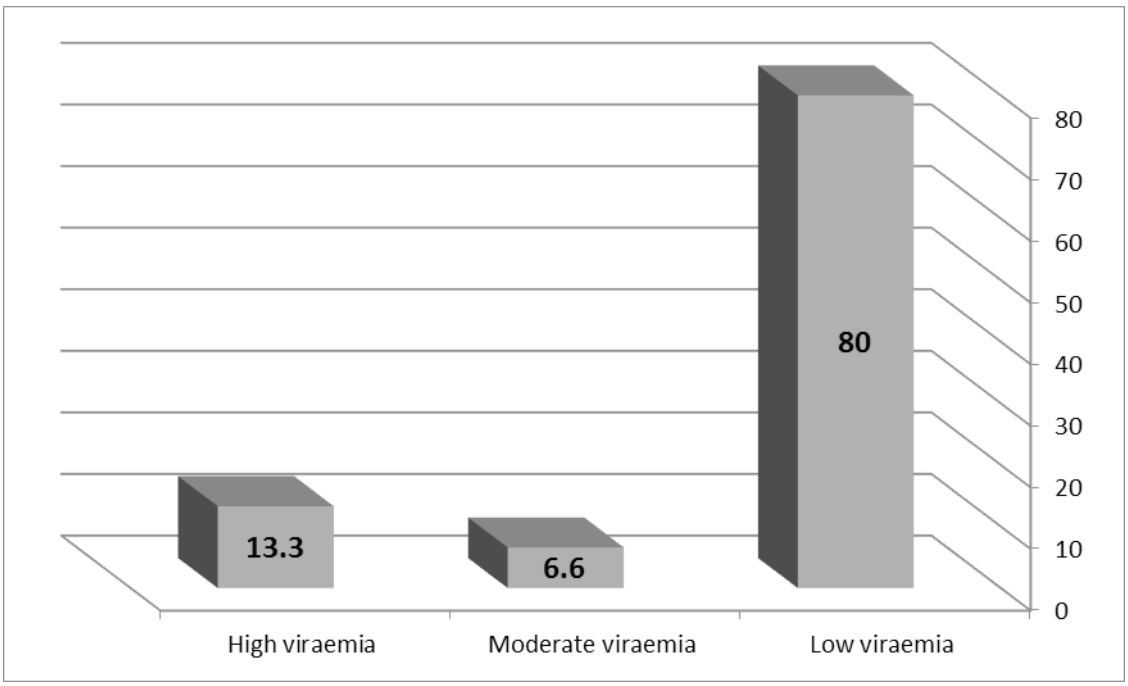

Fig-1 PCR levels of hepatitis C virus RNA

Viral load detection .

The molecular quantitative technique with Real-Time PCR was used to confirmed the serological diagnosis and for measurement of the viral loads (concentrations) in the blood of 54 HCV patients .The results revealed that 45(83.3%) of seropositive patients were gave positive results for HCV RNA, and 9 (16.6) was undetectable for HCV RNA . Viral load level CHC was 36(80%) of patients had low viraemia (HCV RNA level: <100 000 IU/ml),3 (6.6%) of patients had moderate viraemia (HCV RNA level: 100 000–1 000 000 IU/ml) and 6 (13.3%) of patients had high viraemia (HCV RNA level: >1 000 000 IU/ml), as shown in (Fig. 1)

Discussion

In the current study, we demonstrated that HCV patients had a highly significantly lower hepcidin concentrations than those of matched controls. This is consistent with the results Fujita, et al. and Farid, et al. hepcidin levels in the liver tissue of chronic hepatitis C virus infection(CHC) were significantly lower than controls.[16],[17] Fujita, et al., postulated that hepcidin indices in HCV patients were considerably lower. Lehleh, et al., demonstrated that CHC patients had a highly significantly lower hepcidin concentrations than those of matched controls. Hepcidin down regulation is likely to contribute to liver iron accumulation in this condition, and HCV infection may directly modulate hepcidin expression as it induces reactive oxygen species (ROS) through increased histone deacetylase activity.[18]

This is in agreement with the findings of [19], [16],[20],[21] Whose stated that serum hepcidin was significantly lower in CHC patients than in controls. Serum hepcidin, there was highly significant decrease in S. hepcidin level in CHC patients This goes in agreement with *Mohamed et al*[22,23] who stated that Serum hepcidin was significantly lower in CHC cirrhotic patients than in controls and hepcidin level was significantly decreased in HCC cases than liver cirrhosis, CHC and controls. *Pietrangelo et al.* [24] reported a decrease in serum hepcidin along with increased serum ferritin in decompensated cirrhotic patients and the levels also correlated with the severity of the disease. These results were agreement with Mohamed *et al.* [23] Who found that serum iron in patients with CHC was considerably greater than the control group and Fujita, *et al*.[16] who concluded that serum iron levels and transferrin saturation in HCV+ patients were significantly higher than those without viral infection.Our results were consistent *Oikonomou et al.* [25] who made a study on 192 patients with decompensated cirrhosis. They found that high serum ferritin is associated with worse outcomes in patients with decompensated cirrhosis. Also, *Finkenstedt et al.* [26]confirmed in their study that high serum ferritin and transferrin saturation are associated with poor survival in liver cirrhosis.In addition to that, TBIC was significantly decreasing in HCV patients than in the control group. These results were an agreement with Vagu, et al. [27] who concluded that observed significantly elevated levels of serum iron ($P < 0.05$) and ferritin ($P < 0.001$), associated with lower levels of TIBC ($P < 0.05$) in patients CHC. In this study the transferrin saturation in HCV patients were significantly higher compared to the control group. Our results were in agreement with Fujita, et al.[16] who concluded that transferrin saturation in HCV+ patients was significantly higher than those without viral infection, and total iron score was significantly higher in the HCV+ group than in the HBV+ or HCV– HBV– groups star and Dbaibo. [28]reported that mild-to-moderate iron overload is a common finding among patients with chronic HCV infection; indeed, up to 30–40% of them may show increased serum transferrin-iron saturation and serum ferritin or increased hepatic iron concentration.

Our study has shown that iron burden affects liver function in thalassemic patients. The level of serum AST, ALT , ALP and TSB in β-thalassemia with HCV patients increased significantly $P<0.001$in compared to the controls. Our result is agreement with the finding of .[29] There is an important correlation between the degree of SGPT, SGTOT, and GGT represented by saturation transferrin and SF concentrations. Signs of abnormal liver function, i.e., high levels of SGPT, SGTOT, GGT, and alkaline phosphatase, were commonly observed in patients with phosphatase compared to patients with a lower level of phosphatase. Wanachiwanawin *et al.* reported that subjects with positive anti-HCV results generally had higher levels of ALT and AST compared to those with negative anti-HCV results.[29] Other factors such as use of iron chelation and frequency of transfusion may also affect the serum ferritin and aminotransferase level. Higher serum concentrations were severely associated with liver disease, particularly infectious with HCV. In reality, greater transferrin saturation values and serum concentrations were combined with high ALT, AST, and ALP activity.

Conclusion

These results, indicate that Hepcidin levels in patients with CHC were significantly lower the suppression of this hormone by hepatitis C virus is likely an important factor in liver iron accumulation.Liver diseases are associated with significantly higher serum levels ,ferritin and transferrin saturation values. The iron overload and HCV infection increase oxidative stress, which causes or increases liver inflammation and fibrosis. However, a still unresolved issue is whether higher iron levels increase the risk for developing chronic hepatitis C, or, conversely, whether liver damage causes increases in iron levels. Regardless of which occurs first, it is likely that increased iron levels contribute to the progression of hepatic injury and fibrosis.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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