Iron Load and Serum Hepcidin in Hepatitis C Virus-Related Hepatocellular Carcinoma

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) is a major cause of death worldwide, and chronic inflammatory stress caused by hepatitis viruses plays a major role in HCC carcinogenesis. The aim of the present study was to investigate the expression of serum hepcidin and its correlation with iron overload in HCC.

Study design: The study was carried out on 50 hepatitis C virus (HCV)-related HCC cirrhotic patients (Group I) and 20 age-matched non-HCC liver cirrhosis patients as control (Group II). Serum hepcidin was measured in all samples with a commercial immunoassay. The extent of iron deposition was evaluated in liver histochemically.

Results: There was no relationship between the serum hepcidin level and the histological grade of HCC (p = 0.1492), or multiplicity of focal lesion (p = 0.0719), however, hepatic iron deposition was significant higher in HCC than non-HCC cirrhotic patients (p < 0.005).

Conclusion: The current study suggests a positive association of hepatic iron score, but the role of hepcidin in this context remains to be elucidated.

Abbreviations: HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; ROS: Reactive oxygen species; ESR: Erythrocytes sedimentation rate; TIBC: Total iron-binding capacity; HIS: Hepatocytic iron score; SIS: Sinusoidal iron score; PIS: Portal iron score; TIS: The total iron score.

Keywords: Hepatocellular carcinoma, Serum hepcidin level, HCV, Iron deposition, Liver.

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INTRODUCTION

Hepatitis C virus (HCV) is a major cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC).1 Over 20% HCV-infected patients would develop liver cirrhosis with an estimated annual risk of 1 to 4% among patients with cirrhosis to develop HCC. Elucidating the mechanism of HCV pathogenesis will provide new targets to treat HCV and its complications. Iron is an essential metal that functions as a component of oxygen-carrying proteins and of redox enzymes in cellular metabolism. In addition, excessive deposition of iron evokes inflammatory cytokines, reactive oxygen species (ROS), and fibrosis by activating inflammatory cells and hepatic stellate cells.2,3 Furthermore, ROS causes oxidative damage to lipids, proteins and nucleic acids.4 Excessive iron is a presumptive factor that contributes to insulin resistance, steatosis, liver fibrosis and hepatic carcinogenesis, which are common sequelae of chronic HCV infection.

Chronic HCV infection is also characterized by iron deposition in the liver, which contributes to liver injury.4,5 Withdrawal of iron by phlebotomy improves liver function tests6 and decreases HCC development in HCV-infected patients.7 Furthermore, combining antiviral therapy with phlebotomy increases cases of successful HCV eradication,8 which in turn attenuates hepatic iron accumulation in addition to histological improvement.9 Hepcidin is a key negative regulator of iron availability.10 Transgenic mice over expressing hepcidin exhibit iron-deficient anemia, whereas hepcidin-deficient mice exhibit iron overload in many organs.11,12 Consistent with studies in genetically modified animals, iron overload induces hepcidin, whereas iron deficiency suppresses hepcidin expression as negative feedback.13 Chronic hepatitis C patients and HCV transgenic mice have low hepcidin levels with elevated iron accumulation in the liver,14 suggesting that HCV inappropriately suppresses hepcidin expression.

The aim of the present study was to estimate serum hepcidin level and to study its relation with iron overload in HCV-induced HCC patients.

SUBJECTS AND METHODS

The study included 50 HCV-related HCC patients (25 females and 25 males), aged from 48 to 62 years (group I) and 20 non-HCC liver cirrhosis patients as controls (group II). The patients of both groups were age-matched. This study was done after approval from the institutional ethical committee and the subjects included in the study gave their written informed consent prior to participation in the study. Patients were proved to be HCV-positive after detection of HCV antibodies by ELISA using the commercially available ELISA kit.

The exclusion criteria included patients (1) with HCC unrelated to HCV, (2) family history of hemochromatosis or classic clinical expression of the disease (skin pigmentation, cardiac failure, diabetes, hypogonadism, arthritis), (3) hemolytic disease, porphyria cutanea tarda, and (4) patient who had recent history of bleeding.
After obtaining a detailed clinical history of illness and assessment of clinical status, venous blood samples were obtained and analyzed for complete blood picture, erythrocytes sedimentation rate (ESR), coagulation profile, liver function tests, kidney function tests and alpha-fetoprotein. Serum hepcidin-25 level, iron, total iron binding capacity (TIBC) and ferritin, were measured in blood samples collected from all patients. Serum hepcidin-25 concentration was measured using the commercially available ELISA kit (Hepcidin Prohormone ELISA kit, Catalog Number: 20-HEPHU-E01, ALPCO Diagnostics, 26 Keewayain Drine, Salem, NH03079).

**Tissue Specimens**

The liver specimens of at least 1 cm in length were collected from HCC patients using a percutaneous method. Specimens were fixed in alcoholic Bouin’s liquid, embedded in paraffin, cut into 5 µm thick slices, and stained with hematoxylin-eosin, Masson’s trichrome, Sirius red and Perls’ staining. Each biopsy specimen was examined by the same pathologist with no previous knowledge of the corresponding group. Histopathology and staging of fibrosis was determined with hematoxylin-eosin and Masson trichrome staining respectively. Histological iron content was graded semi quantitatively according to that described by Deugnier et al.\textsuperscript{11} in the three areas of the Rappaport lobule. Iron deposits were assessed using three different scores: Hepatocytic iron score (HIS), 0 to 36; sinusoidal iron score (SIS), 0 to 12; and portal iron score (PIS), 0 to 12. The total iron score (TIS) 0 to 60, was defined as the sum of these three scores (TIS = HIS + SIS + PIS). To quantify heterogeneous iron distribution, a coefficient of heterogeneity was evaluated (screening at ×4 magnification) according to Turlin et al.\textsuperscript{14} This coefficient of heterogeneity was 1/3 when iron distribution was very heterogeneous (iron deposits in 1/3 of the nodules), 2/3 when iron distribution was heterogeneous (iron deposits in 1/3 to 2/3 of the nodules), and 3/3 when liver siderosis was homogeneously distributed (iron deposits in more than 2/3 of the nodules). Liver siderosis was finally quantified by the corrected total iron score: CTIS=TIS × coefficient of heterogeneity. Large liver cell dysplasia was screened for in all samples according to the morphological criteria proposed by Anthony et al.\textsuperscript{17}

**Statistics**

A predesigned SPSS (Statistical Package for Social Science Version 11.01) file was used for data entry and analysis. The following tests were used student’s t-test, unpaired t-test, Pearson’s correlation, with 95% confidence intervals (95% CI) and significant p-values at <0.05.

**RESULTS**

The study was done on 70 subjects (50 cases and 20 controls). All of them were infected with HCV. Group I included 50 patients with HCV-related HCC and group II included 20 HCV-infected non-HCC related cirrhotic patients. The clinical profiles of the patients have been shown in Table 1. Patients with HCC had progressive liver diseases compared with non-HCC patients (Table 1).

As shown in Table 2, the levels of iron and ferritin were significantly higher in patients with of group I with HCC compared to patients with group II without HCC. However, the levels of hepacidin were not statistically different between these two groups (Table 2).

Also, the levels of serum hepcidin showed no significant relation with the levels of differentiation and histopathology of HCC nodules (Table 3). Also, serum hepcidin did not show any significant correlation in HCC patients of group I and non-HCC patients of group II (data not shown).

<table>
<thead>
<tr>
<th>Table 1: Clinical features of the patients</th>
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<tbody>
<tr>
<td><strong>Group I</strong></td>
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<tr>
<td><strong>(N = 50)</strong></td>
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<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Child-Pugh class [numbers (%)]</td>
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<td></td>
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<td></td>
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<tr>
<td>Large esophageal varices [numbers (%)]</td>
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<tr>
<td>Interferon treatment [numbers (%)]</td>
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<tr>
<td>Hemoglobin (gm/dl)</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl )</td>
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<tr>
<td>Direct bilirubin (mg/dl)</td>
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<tr>
<td>Albumin (gm/dl)</td>
</tr>
<tr>
<td>PC (prothrombin concentration)</td>
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<tr>
<td>INR (International normalized ratio)</td>
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<tr>
<td>Alanine aminotransferase (U/L)</td>
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<td>Alkaline phosphatase (U/L)</td>
</tr>
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*p < 0.05, compared with group II
Univariate analysis revealed that iron deposits (cTIS >0) were found histologically in 80% HCC patients compared with 55% controls (p = 0.0049).

Localization of sinusoidal iron deposits was different between the two groups (Table 4). Sinusoidal iron was exclusively at the periphery of nodules in 29.1% of HCC patients compared with 15% of controls. The centrilobular type of iron deposition was detected in 7.3% of HCC patients compared with 15% of controls. The perinodular iron overload in patients with liver diseases. This might provide valuable data for understanding hepatic iron overload with HCC. Understanding this relationship may provide insights if iron levels may have any prognostic marker in HCC.

In our study, serum iron and ferritin were higher in HCC patients (group I) than non-HCC liver cirrhosis (group II). These results are in agreement with Selby and Friedman, who found that a positive relationship between elevated body iron liver stores and cancer.

Our study revealed positive correlation between serum iron and severity of the liver cirrhosis because serum iron in child A was lower than child C, although some deviation from this observation was also seen. This data what that has been reported by Morrison et al who found a significant association between serum iron and HCV disease severity. However, the extent of this relation needs further studies.

It may be assumed that cirrhosis itself is of central importance in the carcinogenic process, but whether or not iron acts as an additional risk factor in this process is still elusive.

We also failed to show a significant correlation between serum hepcidin and laboratory data neither in group I or II. In contrast Tschantzis et al have reported that serum hepcidin was significantly lower in patients with HCC than healthy controls. In patients with HCC, serum hepcidin correlated positively with aspartate aminotransferase (r = 0.334, p = 0.001) and had a trend for correlation with alanine aminotransferase (r = 0.197, p = 0.057) and serum hemoglobin (r = 0.188, p = 0.067) but not with ferritin. Kijima et al have stated that serum concentration of hepcidin was significantly correlated with serum concentration of iron, ferritin. However, Kijima et al stated that expression of hepcidin mRNA was not correlated with serum concentration of hepcidin in noncancerous or cancerous tissues. So the detection of hepcidin mRNA may be a more accurate measure to detect iron overload in liver than serum hepcidin level measurement.

Our study revealed that although there was no relationship between the serum hepcidin-25 level and the histological grade of HCC (p = 0.1492), or multiplicity of focal lesion (p = 0.0719), however, hepatic iron deposition was significant higher in HCC than non-HCC cirrhotic patients (p < 0.005). Also, we showed that iron deposits (cTIS > 0) were found histologically in 80% HCC patients compared with 55% controls (p = 0.0049).

The current study seems to suggest a possibility of an association of increased hepatic iron score with HCC. These results may have important implications, if they are confirmed by other studies. Liver biopsy should not only be practiced to confirm a diagnosis of cirrhosis but there is also a need to assess hepatic iron deposits. The second implication is that phlebotomy therapy could be proposed in these patients to reduce the risk of HCC. Finally, serum hepcidin-25 level was correlated with neither hepatic iron overload non-HCC. We recommend that detection of hepcidin mRNA expression may be a more accurate measure

### DISCUSSION

The aims of this study were to determine the prevalence of hepatic iron overload in patients with end-stage liver disease caused by HCV and to examine the association of hepatic iron overload with HCC. Understanding this relationship may provide valuable data for understanding hepatic carcinogenesis and for development strategies to treat iron overload in patients with liver diseases. This might provide insights if iron levels may have any prognostic marker in HCC.

In our study, serum iron and ferritin were higher in HCC patients (group I) than non-HCC liver cirrhosis (group II).
to detect iron overload in liver than serum hepcidin level estimation.

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REFERENCES