Hepcidin mRNA Level as A Parameter of Disease Progression in Chronic Hepatitis C and Hepatocellular Carcinoma

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ABSTRACT

Background: Hepcidin, a key regulator of iron metabolism, is synthesized by the liver. Hepcidin binds to the iron exporter ferroportin to regulate the release of iron into plasma from macrophages, hepatocytes, and enterocytes.

Aim: To study hepcidin expression in liver tissue of patients with hepatocellular carcinoma (HCC), chronic hepatitis C (CHC) and normal human liver biopsies and to compare its level with serum and liver iron indices.

Patients and Methods: Liver biopsies from 66 patients (36 HCC and 30 CHC) were analysed as well as normal human liver biopsies obtained from 20 healthy liver transplant donors as a control group. Liver function tests, AFP, hepatitis markers, HCV-RNA levels, hemoglobin concentration and serum iron parameters were analyzed. Hepcidin mRNA was quantified in all liver biopsies of patients and controls by real-time PCR. Liver iron concentration (LIC) was evaluated and hepatic iron index (HII) was calculated by dividing LIC in µmol/gm dry weight by the patient’s age.

Results: The mean level for hepcidin mRNA in HCC, CHC and healthy controls were 2351±505, 5735±2403 and 16308±2194 copies/ml, respectively; with significant decrease in cancerous (HCC) than non-cancerous (CHC) and control liver tissues. The level was significantly lower in patients with multiple tumour masses. Hepcidin mRNA had a significant positive correlation with synthetic function of the liver (serum albumin and prothrombin concentration) and haemoglobin. In contrast, hepcidin mRNA was negatively correlated with parameters of iron stores as (serum ferritin and HII) and grade of liver fibrosis in both patient groups.

Conclusion: The expression of hepcidin mRNA is decreased in liver tissues of CHC patients and more suppressed in the liver tissues of patients with HCC, suggesting that hepcidin expression appears to be appropriately responsive to iron status and disease progression in cirrhosis and hepatocarcinogenesis.

Key Words: HCC – Hepcidin – mRNA – Real-time PCR

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant tumours, however, the molecular mechanisms of early hepatocarcinogenesis are far from clear [1]. Chronic inflammatory stress caused by hepatitis viruses B and C plays a major role in HCC carcinogenesis [2]. It has been indicated that iron overload is a common feature of chronic liver disorders, it has been linked with oxidative DNA damage, insulin resistance and liver steatosis, and with triggering of hepatic stellate cells thus inducing liver fibrosis and HCC [3].

Recently, a key iron regulatory hormone, hepcidin, was discovered. It is synthesized in the liver from its precursor protein, prohepcidin [4,5]. Hepcidin is a peptide molecule playing a key role in iron homeostasis. It is produced by the liver, and inhibits intestinal iron absorption by enterocytes in the duodenum through its binding to ferroportin and inducing its internalization and degradation [6,7]. It also causes a release of iron by macrophages and hepatocytes [8]. These mechanisms result in a decrease of serum iron concentration and increased intracellular iron content [9].

Hepcidin mRNA expression is modulated in response to body iron stores, anaemia, hy-
poxia, inflammatory and infectious stimuli involving, at least in part cytokines secreted by macrophages such as IL-6 and IL-1 [10], whereas it is decreased by iron deficiency and erythropoiesis, leading to iron accumulation in the body [11].

There is a considerable body of evidence that expression of hepcidin is altered in various types of diseases. Excessive hepcidin production explains the relative deficiency of iron during inflammatory states, eventually resulting in the anaemia of chronic inflammation [7,12]. On the other hand, hepcidin deficiency has been reported in hereditary haemochromatosis and attributed to mutation in each of HFE (gene of haemochromatosis), transferrin receptor-2 and hemojuvelin genes [13].

Although, hepcidin mRNA expression was detected in surgical specimens from patients with colorectal cancer [14], and renal cell carcinoma [15], few studies investigated the expression of hepcidin in HCC.

This study aimed to investigate the expression of hepcidin mRNA level in liver tissues of HCC and CHC patients in comparison with normal healthy controls, and to find its correlation with iron parameters, liver functions and grades of liver fibrosis.

**PATIENTS AND METHODS**

The current study was conducted on 66 patients (46 males and 20 females, age range 35-62 years) attending the outpatient and inpatient clinics of the Hepatology Department-National Liver Institute, Menoufiya University; National Cancer Institute-Cairo University and Internal Medicine Department of Al Zahraa University Hospital from March 2008 till May 2009. Thirty six patients had HCC (25 males and 11 females, age range 40-62 years with a mean of 53.3±6.8), and 30 patients had CHC (21 males and 9 females, ages range 35-54 years with a mean of 47.7±7.03). Twenty healthy control group (16 males and 4 females, ages range 32-46 years with a mean of 39±3.5) who were negative for serological markers of HBV and HCV and with normal histopathology of their liver biopsies. The study was approved by the local ethical committee in university hospitals and informed consent was obtained from the patients.

Patients were divided into two groups: The first group included 36 patients with HCC. Those were diagnosed according to clinical examination, laboratory and radiological investigations including abdominal ultrasonography and triphasic C.T. abdomen. Clinical data of HCC patients were collected to determine tumour characteristics as tumour number and vessel involvement. All HCC patients were newly diagnosed cases and did not receive prior chemotherapy. The second group included 30 patients with CHC, selected according to the following criteria: The presence of anti HCV antibodies, detection of serum HCV RNA, negative results for hepatitis B surface antigen and the histopathological features in liver biopsy samples.

Exclusion criteria for both groups: Patients with known HFE genetic hemochromatosis as those are expected to exhibit abnormal hepcidin regulation, previous history of any malignancy, autoimmune diseases or any genetic diseases as well as patients with chronic liver disease other than HCV related.

Normal human liver tissue samples obtained from 20 healthy liver transplant donors were selected as a control. They were negative for serological markers of HBV and HCV and with normal histopathology of their liver biopsies. The study was approved by the local ethical committee in university hospitals and informed consent was obtained from the patients.

The patients and controls were subjected to: Laboratory investigations including serum levels of AST and ALT, albumin, total and direct bilirubin, iron and TIBC using Integra-400 (Roche-Germany). Prothrombin concentration was done by Fibrintimer (Roche-Germany). Complete blood cell counts were measured by Sysmix K-21 automatic cell counter (Japan). AFP and serum ferritin were measured by an automated chemiluminescences using ACS-180SE (Chiron, Diagnostic-Germany). HCV antibodies were assayed by EIA (COBAS-AmpliCure, Germany). HCV-RNA levels were analyzed by reverse transcriptase polymerase chain reaction (RT-PCR) using a commercial kit (Roche Diagnostic, Branchburg, NJ) according to the manufacturer’s instructions.

Real-time PCR was performed using non-cancerous and cancerous liver biopsies and control groups. Part of liver sample was stored in liquid nitrogen immediately after the operation and kept at -80ºC until RNA extraction and
the other part was fixed in 10% neutral buffered formalin and embedded in paraffin for histological studies. The sections were stained with haematoxylin and eosin. Histological grading and staging were performed blindly using a modified Knodell scoring system by a pathologist [16]. Biopsies were scored for hepatic fibrosis stage (from 0-6) according to modified Knodell score [17].

Liver iron concentration (LIC) was evaluated in liver tissues as described previously [18]. The fresh liver tissue fragment (approximately 15 mm) was weighed and dried at 85°C for 2 hours in decontaminated quartz vessel and then digested in concentrated nitric oxide (2ml). The resulting solution was then diluted to 5ml with deionized water. Quantitative analysis was performed by inductively coupled plasma-atomic emission spectrometry to determine hepatic iron content. Hepatic iron index (HII) was calculated by dividing LIC in μmol/gm dry weight by the patient’s age. Any value higher than 1.9 for hepatic iron index, was considered positive for hemochromatosis [19].

Real-time PCR for quantification of hepcidin mRNA:

For real-time PCR, liver tissues were stored in liquid nitrogen immediately after the operation, and kept at -80°C until RNA extraction. Total RNA from each sample was isolated using a Total RNA Isolation Kit (Roche - Mannheim, Germany). Reverse transcription reactions were performed using a Rever Tra Ace alpha-First Strand cDNA Synthesis Kit (Toyobo, Osaka, Japan). Briefly, 1μg of total RNA, 1μL of oligo dT-primer, and 2μL dNTPs were incubated at 65°C for 5 min, then 10μL of a cDNA synthesis mixture were added and this mixture was incubated at 50°C for 50 min. The reaction was terminated by adding 1μL of RNaseH and incubating the mixture at 37°C for 20 min.

Real-time quantification of hepcidin mRNA transcripts was performed using Roche LightCycler-2.0 TM (Mannheim, Germany). The PCR reaction was carried out in a tube containing 2μL cDNA, 12.5μL 2X SYBR Green (Applied Biosystems), 0.5μL of 25nM sense and antisense primers, and H₂O up to final volume of 25 μL. The PCR conditions consisted of 40 cycles at 95°C for 15 seconds and 60°C for 60 seconds. The sequences of primers were as follows [20]:

**GAPDH:** Sense-primer 5’-CCACCCAG-AAGACTGTGGAT-3’
Anti-sense 5’-TTCACTTCAGGGATG-ACCTT-3’

**Hepcidin:** Sense-primer 5’-CACAACAGC-GGGACAACC-3’
Anti-sense 5’-GCAGCAGAAATGCAGATG-3’

The level of expression was calculated using the formula:

Relative expression (t) = (Copy number of target molecule / Copy number of GAPDH) x 1000 [20].

Samples were assayed in duplicate. Means and standard deviations were calculated from the data obtained. The t-value was calculated from the mean of two different assays (Fig. 1).

**Statistical analysis:** Statistical package for SPSS (statistical package for social science) program version 13 for windows and Epi info computer program was used for data analysis. Quantitative variables were summarized using Mean±SD. Student’s t-test was done to compare two normally distributed variables and ANOVA test to compare three or more of normally distributed variables. Kruskal-Wallis test was done to compare three or more of nonnormally distributed variables. Tamhane test is a post hoc test used for variables of significant difference of more than two groups of not normally distributed data after Kruskal-Wallis test to detect the significant difference between either groups. Correlation coefficients (r) were calculated using the Pearson’s correlation analysis. p-value was significant at <0.05 level.

**RESULTS**

Results of the study are illustrated in tables 1-4, and figures 2-4. The comparison between liver function tests in the studied groups revealed a significant increase in ALT, AST, ALP, GGT, T. Bilirubin and AFP in patients with CHC and HCC compared to controls, with the highest value in HCC group. While the serum albumin and prothrombin concentration were significantly decreased (Table 1).

The mean levels for hepcidin mRNA in tumorous (HCC cases) and non-tumorous tissues (CHC cases) were 2351±505 and 5735±
2403 copies/ml; respectively. Whereas, its level in the liver tissues of healthy control was 16308±2194 (Fig. 2). Expression of hepcidin mRNA was significantly decreased in tumorous than non-tumorous tissues (p<0.01) and control tissues (p<0.01) (Table 2).

The mean values for hepcidin mRNA in patients who had single and multiple tumor masses were 2509±514 and 1992±234 copies/ml; respectively. It was significantly decreased with multiple than single tumor mass (p<0.05). In contrast, the mean values for hepcidin mRNA in patients with negative or positive vessel invasion were 2213±499 and 2067±408 copies/ml; respectively, the difference was not statistically significant (p>0.05) (Table 3).

The correlation between the expression of hepcidin mRNA and other studied parameters in HCC and CHC patient groups were listed in (Table 4). There was no significant correlation between the expression of hepcidin mRNA and either liver enzymes, AFP levels or serum iron (p>0.05) for each. But, hepcidin mRNA showed a significant positive correlation with synthetic function of the liver (serum albumin, p<0.05 and prothrombin concentration, p<0.01). Also, hepcidin mRNA was positively correlated with haemoglobin (p<0.01).

On the contrary, hepcidin mRNA was negatively correlated with parameters of iron stores as (serum ferritin and HII, p<0.01 for each) and grade of liver fibrosis in both patient groups (p<0.01) (Table 4, Figs. 3, 4).

Hepcidin mRNA Level as A Parameter of Disease Progression

Table (1): Liver function tests in the studied groups.

<table>
<thead>
<tr>
<th>Studied variables</th>
<th>HCC (N=36) Mean ± SD</th>
<th>CHC (N=30) Mean ± SD</th>
<th>Control (N=20) Mean ± SD</th>
<th>Kruskal Wallis test</th>
<th>p-value</th>
<th>Post Hoc p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>195.3±107.1</td>
<td>74.8±21.3</td>
<td>21.1±4.79</td>
<td>57.84</td>
<td>&lt;0.01</td>
<td>p&lt;0.01 p&lt;0.01 p3&lt;0.01</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>152.8±140.4</td>
<td>69.3±16.05</td>
<td>21.85±6.78</td>
<td>56.63</td>
<td>&lt;0.01</td>
<td>p&lt;0.01 p&lt;0.01 p&lt;0.01</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>143.3±103.1</td>
<td>76.7±21.8</td>
<td>40.05±10.95</td>
<td>52.51</td>
<td>&lt;0.01</td>
<td>p&lt;0.01 p&lt;0.01 p3&lt;0.01</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>145.05±140.8</td>
<td>39.7±11.97</td>
<td>27.35±10.16</td>
<td>54.14</td>
<td>&lt;0.01</td>
<td>p&lt;0.01 p&lt;0.01 p3&lt;0.01</td>
</tr>
<tr>
<td>T.Bil (mg/dl)</td>
<td>4.38±2.82</td>
<td>2.07±0.87</td>
<td>0.57±0.2</td>
<td>65.28</td>
<td>&lt;0.01</td>
<td>p&lt;0.01 p&lt;0.01 p3&lt;0.01</td>
</tr>
<tr>
<td>Alb (g/dl)</td>
<td>2.65±0.62</td>
<td>3.17±0.49</td>
<td>4.46±0.48</td>
<td>71.79*</td>
<td>&lt;0.01</td>
<td>p&lt;0.01 p&lt;0.01 p3&lt;0.01</td>
</tr>
<tr>
<td>Proth. Con. (%)</td>
<td>55.8±12.3</td>
<td>63.7±13.2</td>
<td>97.8±2.78</td>
<td>93.86*</td>
<td>&lt;0.01</td>
<td>p&lt;0.05 p&lt;0.01 p3&lt;0.01</td>
</tr>
<tr>
<td>AFP (ng/ml)</td>
<td>1038±149</td>
<td>4.77±2.64</td>
<td>1.97±0.7</td>
<td>65.62</td>
<td>&lt;0.01</td>
<td>p&lt;0.01 p&lt;0.01 p3&lt;0.01</td>
</tr>
</tbody>
</table>

* ANOVA test. p1 between HCC and CHC, p2 between HCC and Control, p3 between CHC and Control.
Table (2): Iron parameters, Hb and hepcidin mRNA level in the studied groups.

<table>
<thead>
<tr>
<th>Studied variables</th>
<th>HCC (N=36) Mean ± SD</th>
<th>CHC (N=30) Mean ± SD</th>
<th>Control (N=20) Mean ± SD</th>
<th>Kruskal Wallis test</th>
<th>p-value</th>
<th>Post Hoc</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron (µg/dl)</td>
<td>107.4±34.3</td>
<td>105.3±31.7</td>
<td>73.5±14.9</td>
<td>9.3*</td>
<td>&lt;0.01</td>
<td>p₁≥0.05</td>
<td></td>
</tr>
<tr>
<td>Ferittin (µg/dl)</td>
<td>682.7±288.2</td>
<td>316.2±51.6</td>
<td>107.6±21.8</td>
<td>60.7</td>
<td>&lt;0.01</td>
<td>p₁=0.01</td>
<td></td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>10.7±1.5</td>
<td>11.5±1.2</td>
<td>13.8±0.87</td>
<td>40.07*</td>
<td>&lt;0.01</td>
<td>p₁=0.05</td>
<td></td>
</tr>
<tr>
<td>HII (µmol/gm/age)</td>
<td>0.93±0.35</td>
<td>0.79±0.36</td>
<td>0.38±0.09</td>
<td>41.15</td>
<td>&lt;0.01</td>
<td>p₁=0.05</td>
<td></td>
</tr>
<tr>
<td>Hepcidin mRNA (copies/ml)</td>
<td>2351±505</td>
<td>5735±2403</td>
<td>16308±2194</td>
<td>395.6</td>
<td>&lt;0.01</td>
<td>p₁=0.01</td>
<td></td>
</tr>
</tbody>
</table>

* ANOVA test. p₁ between HCC and CHC, p₂ between HCC and Control, p₃ between CHC and Control.

Table (3): Tumour characters and hepcidin mRNA level in the studied groups.

<table>
<thead>
<tr>
<th>Studied variables</th>
<th>Tumor number</th>
<th>t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Multiple (n=13) Mean ± SD</td>
<td>Single (n=23) Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Hepcidin mRNA (copies/ml)</td>
<td>1992±234</td>
<td>2509±514</td>
<td>3.87</td>
</tr>
</tbody>
</table>

* p<0.05 is statistically significant; p>0.05 is statistically non significant.

Vascular involvement

|                  | Yes (n=11) Mean ± SD | No (n=25) Mean ± SD |        |
| Hepcidin mRNA (copies/ml) | 2213±499 | 2067±408 | 2.08 | >0.05  |

Table (4): Correlation between Hepcidin and all studied variables in studied groups.

<table>
<thead>
<tr>
<th>Studied variables</th>
<th>Hepcidin in CHC</th>
<th></th>
<th>Hepcidin in HCC</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p-value</td>
<td>r</td>
<td>p-value</td>
</tr>
<tr>
<td>AST</td>
<td>-0.26</td>
<td>&gt;0.05</td>
<td>-0.31</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>ALT</td>
<td>-0.13</td>
<td>&gt;0.05</td>
<td>-0.32</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>ALP</td>
<td>-0.24</td>
<td>&gt;0.05</td>
<td>-0.19</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>GGT</td>
<td>0.02</td>
<td>&gt;0.05</td>
<td>-0.16</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.52</td>
<td>&lt;0.05*</td>
<td>0.73</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>Proth. Conc.</td>
<td>0.86</td>
<td>&lt;0.01**</td>
<td>0.59</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>Hb</td>
<td>0.71</td>
<td>&lt;0.01**</td>
<td>0.86</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>Iron</td>
<td>-0.33</td>
<td>&gt;0.05</td>
<td>0.28</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>AFP</td>
<td>-0.09</td>
<td>&gt;0.05</td>
<td>-0.02</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Ferritin</td>
<td>-0.76</td>
<td>&lt;0.01**</td>
<td>-0.81</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>HII</td>
<td>-0.67</td>
<td>&lt;0.01**</td>
<td>-0.76</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>Liver fibrosis</td>
<td>-0.96</td>
<td>&lt;0.01**</td>
<td>-0.73</td>
<td>&lt;0.01**</td>
</tr>
</tbody>
</table>

**p<0.01 is statistically highly significant; *p<0.05 is statistically significant; p>0.05 is statistically non significant.
**DISCUSSION**

Hepcidin is a pivotal regulator of iron metabolism because it controls the efflux of iron from enterocytes, hepatocytes, and macrophages by internalization and degradation of the iron exporter (ferroportin), and also regulates the plasma iron level [9,10]. The role of hepcidin in human cancer deserves to be studied, since there have been only few reports in this context [14,21]. It is well known that HCC develops in more than 40% of patients with hemochromatosis [22]. On the other hand, iron is an essential nutrient for cell growth, and cancer cells in particular require iron in order to proliferate [23].

Hepcidin mRNA was quantified in liver biopsies of HCC, CHC patients and controls by real-time PCR, liver iron concentration (LIC) was evaluated and hepatic iron index (HII) was calculated. Liver function tests, hepatitis markers and serum iron parameters were also done.

The present study revealed that, the expression of hepcidin mRNA was significantly decreased in the liver tissue of CHC patients compared with control group ($p<0.01$). Our result indicating that, the suppression of hepcidin hormone by hepatitis C virus is likely to be an important factor of liver iron accumulation in this condition [24].

In favor of our results, hepcidin expression is not induced in either human or murine hepa-
tocytes exposed to iron in vitro; in fact, hepcidin synthesis is suppressed at high iron levels [25,26]. Thus, the mechanisms regulating hepcidin do not appear to be directly responsive to iron; expression of hepcidin in the body is complicated and indirect.

In anemia of chronic diseases, hepcidin mRNA expression is influenced by acute-phase reactant cytokines as IL-6 [11], while HCV infection is a cell-mediated immune response that is characterized by a T helper-1 immune response with secretion of IL-2, IL-4, IL-10, tumor necrosis factor-α, and interferon-γ [27]. Thus hepcidin expression may be less influenced by T helper-1 cytokines in chronic HCV infection.

Moreover, hepcidin mRNA was significantly decreased in cancerous liver tissues of patients with HCC compared to CHC and control groups (p<0.01). Its level was significantly lower in patients with multiple tumour masses (p<0.05), with no difference in patients who were negative or positive for vascular invasion.

Although the mechanism responsible for suppression of hepcidin mRNA expression in HCC remains unclear, suppression of hepcidin transcription contradicts the previously proposed scheme for iron homeostasis in cancer cells, because cancer cells must retain iron in order to proliferate. However, suppression of hepcidin is rational because duodenal enterocytes transfer iron to plasma, resulting in an increase of total body iron content. One explanation was provided by Weizer-Stern, et al. [28] who reported that activation of the tumor suppressor gene p53 stimulates the expression of hepcidin. The promoter region of the hepcidin gene (HAMP) contains a putative p53 response element. Inactivation or mutation of the p53 gene has been detected in various types of human cancer [29], including HCC [30]. Suppression of hepcidin expression may be linked to the altered expression and function of p53.

Kamai, et al. [15], suggested that hepcidin might have differing roles in different cancers. Hepcidin might have a pro-oncogenic role by internalization and degradation of the cellular iron export protein ferroportin. In addition, iron overload has been linked with oxidative DNA damage, insulin resistance and liver steatosis, and with triggering of hepatic stellate cells thus inducing liver fibrosis and HCC [3].

Kijima, et al. [21], reported that hepcidin is produced in patients with HCC, from non-cancerous liver tissue, even though production is inhibited in cancerous tissue. Moreover, hepcidin mRNA expression was not related to the histological grade, vascular invasion, or recurrence of HCC.

In a more recent study by His-Huang [31], it was reported that hepcidin is down-regulated in HCC but still negatively correlated with hepatic iron stores. The altered expression of iron-regulatory genes accompanying HCC may disturb patient’s iron balance leading to decreased hepcidin expression in theses cases. This finding suggests that, hepcidin may play a role in defending the body against HCC development.

Analysis of the relationship between hepcidin mRNA and other clinical data in the current study led to identify significant correlations, involving iron status, hematologic parameters, and hepatic functional status. Thus, HII and serum ferritin appeared to be inversely correlated with hepcidin mRNA, as reported by previous studies [32,33].

Also, Kulaksiz, et al. [34], and Nagashima, et al. [35] found that, serum prohepcidin levels were inversely correlated with serum ferritin levels, and total iron score in chronic HCV infection. But serum prohepcidin was positively correlated with these parameters in patients with chronic HBV infection and healthy controls, although its levels were lower in chronic HBV patients than in healthy controls. These results suggest that chronic HCV infection possibly affects hepcidin expression by a mechanism that differd from chronic HBV infection.

On the contrary to our results, Ottar, et al. [36] noticed a highly significant correlation between hepcidin transcript levels and LIC in the HCV patients. Aoki, et al. [37] stated that hepcidin was up-regulated in the liver in response to elevated iron stores and served as a signal to down-regulate iron absorption and increase iron storage. As opposed to what is observed in patients with hereditary hemochromatosis, patients with HCV and elevated iron stores have
an increased expression of hepcidin mRNA in the liver. Courselaud, et al. [38] mentioned that hepcidin transcription appeared to be regulated by a CCAAT/enhancer-binding protein (C/EBP) element in the 5' flanking region of the mouse and human hepcidin genes. Interestingly, iron loading increases C/EBP-alpha, which may in turn lead to induction of hepcidin.

The present work also demonstrates a relationship between haemoglobin level and hepcidin mRNA expression, supporting the hypothesis of an impact of anaemia or hypoxia (or both) on hepcidin mRNA expression. The same findings were reported by previous studies [11,33].

Bridle, et al. [13] and Dé tivaud, et al. [33], demonstrated a relationship between hepatic iron and hepcidin mRNA levels. However, in our cases the hepcidin mRNA did not significantly correlate with either serum iron concentration or liver enzymes. In accordance with our results, hepcidin expression was not induced in either human or murine hepatocytes exposed to iron in vitro; in fact, hepcidin synthesis was suppressed at high iron levels [25-26]. Thus, the mechanisms regulating hepcidin do not appear to be directly responsive to iron; expression of hepcidin in the body is complicated and indirect.

Finally, we found that the parameters reflecting synthetic hepatic functions are correlated with hepcidin levels. Thus, serum albumin and prothrombin concentrations were positively correlated with hepcidin mRNA levels, whereas fibrosis status was negatively correlated with hepcidin mRNA levels, our findings were in agreement with Dé tivaud, et al. [33] and Olmez, et al. [39] who found that, significantly lower levels of hepcidin correlate with liver fibrosis in chronic hepatitis C virus infection. A recent study by, Flisiak [5], indicates that in situations of liver function impairment, hepcidin synthesis as well as activity or expression of converting enzymes might be altered and suggesting HCV interference with hepcidin synthesis at the level of prohormone synthesis or maturation in the liver. These results suggest that, the progression of liver fibrosis affects synthesis of hepcidin and the inadequate hepcidin production can explain the majority of iron overload, which play a pivotal role in liver fibrosis.

Taken together, these results suggest, that hepcidin expression appears to be appropriately responsive to iron status and disease progression in cirrhosis and HCC patients. Hepatic function and the effects of disease processes on hepatocytes could modulate iron metabolism.

Conclusion:
In conclusion, expression of hepcidin mRNA is strikingly suppressed in HCC cancerous more than in non-cancerous CHC and normal liver tissues, irrespective of iron overload. Moreover, there is a positive relationship between hepcidin mRNA levels and synthetic liver function and an inverse correlation with grade of liver fibrosis, suggesting that a uniform suppression of hepcidin may be linked to disease progression and development of HCC. Further analysis is still required to evaluate its usefulness in routine pathological diagnosis including biopsy diagnosis and also a marker for early detection of HCC.

REFERENCES
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