Role of Hepcidin in the Regulation of Iron Metabolism in Patients with Chronic Liver Diseases

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Abstract

Chronic liver diseases, especially alcoholic fatty liver disease (AFLD), nonalcoholic fatty liver disease (NAFLD) and chronic hepatitis C (CHC), are frequently associated with iron overload. Hepcidin is the iron-regulatory hormone, which is synthesized mainly in the liver and plays an important role in iron homeostasis. **The aim** of this study was to assess the serum levels of hepcidin and to evaluate its relationships with other parameters of iron metabolism in patients with various chronic liver diseases. Material **and Methods:** A total of 186 patients with chronic liver disease (CLD), divided into six comparable groups were studied (115- male, 71 – female; mean age 50.41 ± 12.85 y), and 60 healthy controls were studied. Laboratory parameters of liver function and indices of iron metabolism were monitored. After liver biopsy, the presence of iron deposition and the histological grades of steatosis and inflammation, and stage of fibrosis were also evaluated in patients with CLD. The serum level of hepcidin was determined by ELISA test / DRG International Inc. (USA). **Results:** Hepcidinwas significantly lower in the whole group of patients with CLD (82.9±40.74 ng/ml)compared to the controls (99.14±32.94 ng/ml, p=0.005). According to the type of liver disease, decreased serum values of hepcidin were found significantly more frequently in AFLD, NAFLD and CHCin comparison with controls (p=0.02, p=0.001 and p=0.01 respectively), CHB (p=0.034, p=0.003 and p=0.023 respectively) and chronic autoimmune hepatitis and primary biliary cirrhosis (p=0.023, p=0.005 and p=0.022 respectively), but without difference when compared between them. There was a reverse relationship between the levels of hepcidin by one hand and the values of iron, ferritin, liver enzymes, some of the parameters of liver function, as wells as with degree of the deposition of iron in the liver and severity of steatosis, inflammation and fibrosis (0.01-0.001). **In conclusion**, our results show decreased serum levels of hepcidin in patients with nonalcoholic and alcoholic fatty liver diseases, and chronic hepatitis C. The relationships with the parameters of iron metabolism and the severity of liver disease prove the importance of serum hepcidin as a surrogate marker for evaluation of iron overload in patients with chronic liver diseases.

Keywords: serum hepcidin, iron overload, chronic liver diseases

Introduction

The syndrome of iron overload is due to genetic reasons and acquired disorders. Unlike primary hemochromatosis, it is common in patients with chronic liver diseases, particularly hepatitis C, alcohol and non-alcoholic steatosis and steatosis hepatitis and liver cirrhosis.

In case of overload, iron has the ability to generate free oxygen radicals with damaging effects on many tissues and organs. In this connection efforts to study the overall iron homeostasis and the regulatory mechanisms to maintainher are made. Particular attention is paid to ferritin, transferrin and transferrin receptors and the hormone hepcidin(1,3). It has been shown that distortions in the synthesis of hepcidin cause iron overload, which defines it as an important factor in the regulation of iron metabolism. It is known that the synthesis of hepcidin may increase, regardless of iron metabolism and activity of erythropoiesis, by the action of bacterial lipopolizaharids and proinflammatory cytokines, there by creating conditions for redistribution of iron stores(4-6). While the physiological and pathophysiological role of hepcidin in the main processes of the body is well studied in experimental conditions, data on serum hepcidin, both in healthy subjects and patients with various diseases are still insufficient and contradictory.

The aim of this study was t oidentify and assess the level of hepcidinin serum of patients with various chronic liver diseases and seek connection with other surrogate serum markers reflecting iron metabolism and the type and characteristics of liver disease.

Material and Methods

The study included a total of 246 persons - 186 patients with chronic liver disease (CHLD, -115 men and women-71, the average age of 50.41 ± 12.85, from 23to 77) and 60 healthy controls (male 30,female- 30, the average age 50.5 ± 11.3, from 29 to 83years).Respondents are distributes in the following seven groups:
Group I - 38 patients with primary non-alcoholic fatty liver disease (NAFLD) - non-alcoholic steatosis (NAS, n = 23) and non-alcoholic steatohepatitis (NASH, n = 15) with histological diagnosis in 30 cases.

Group II - 45patients with histologically confirmed alcoholic liver disease (ALD, n = 13) and alcoholic steatohepatitis (ASH, n = 32) with absolute alcohol intake above 40g /Living room.
Group III - 35 patients with chronic hepatitis B (HHB proven viral replication and histologically confirmed diagnosis)
Group IV - 38patients with chronic hepatitis C (HHCwith proven viral replication and histologically confirmed diagnosis)
Group V - 13 patients with histologically confirmed chronic hepatitis B and D (HHB and D)
Group VI -17 patients with histologically confirmed autoimmune liver disease –primary biliar cirrhosis (PBC, n = 7) and chronic autoimmune hepatitis(CHAH, n =10).
Group VII - 60 healthy controls (HC).

The diagnosis of liver disease is on the basis of the standard used for each of these criteria. In addition to the laboratory and instrumental investigations (abdominal ultrasound with Doppler and / or other imaging studies fibrogastroscopy etc.). The following additional parameters for assessing iron overload syndrome - serum iron (men: 12.5 to 26 mmol / L; women: 10.5 to 23 mmol / L), total iron-binding capacity (JAC, 44 to 66 mmol / L), and serum ferritin (men: 20 to 280 mg / L females: 10 to 140 mg / L). It was calculated and the saturation of transferrin (with reference range 20-40%). Serum levels of these indicators are shown in **Table. 1.** Hepcidin hormone levels in the serum was determined by ELISA test / DRG International Inc. (USA) under the following reference ranges: males - 88.7 to 135 ng / ml; women - from 57.5 to 123 ng / ml. Venous samples were obtained from cubital vein using vacuum systems for collection of blood in the morning after 12 hours of fasting.
Histological examination was carried out on material from percutaneous or surgical liver biopsy. It was evaluated the histological activity and stage of fibrosis and the degree of steatosis, respectively, by the method of Brunt (steatosis and steato hepatitis), METAVIR (chronic viral hepatitis) and others. As evidence of iron in liver tissue was used Ishak stain and Perl's Prussian-blue. It was assessed the type and extent of postponement. Static analysis of the results included Mann-Whitney test, ANOVA and parametric and nonparametric correlation analysis.

Results

In 12 healthy subjects studied (20%) were found deviation from the normal range of serum hepcidin. In the group with chronic liver disease serum hepcidin was reduced in 76 patients (41%). Mean serum hepcidin was significantly lower in patients with CHLD(82,9± 40.74 ng / ml) compared with controls (99,14 ± 32.94 ng / ml, p = 0.005). There is no significant difference in the level of hepcidinin men than women in both the normal subjects(94,80 ± 34,26s /t103,47 ± 31,54, p = 0,312),and the group CHLD(79,79 ± 40,36s /t87,94 ± 41,14 ng / ml,p =0.185). In both groups we found no relationship between age (under and over 45 years) and the level of hepcidin. In healthy people over 45 -the age serum hepcidin were 94.35 ± 35.15 ng / ml, and in young controls - 108,71± 26.24 ng / ml (p = 0.112). In group CHLDvalues were respectively 79.05 ± 41.24 ng / ml and 89,83± 35.46 ng / ml (p = 0.092). Decreased serum hepcidin there was most often in cases of non-alcoholic and alcoholic fatty disease and in patients with chronic hepatitis C (**Fig. 1**). Serum hepcidin was significantly lower in patients with NASD|, and ALD and CHHC compared with controls (p = 0.02, respectively, p = 0.001 and p = 0.01), CHHB (respectively p = 0.034, p = 0.003 and p = 0.023) and CHAH and PBC (respectively p = 0.023, p = 0.005 and p = 0.022), but no statistically significant difference in the comparison between them.

In patients with non-alcoholic steatohepatitis and alcoholic serum hepcidin was significantly lower compared with non-alcoholic cases ($p = 0.001$) and alcohol ($p = 0.021$) steatosis. There is no significant difference in the serum hepcidin of patients with HHB and CHAH and PBC compared with controls and with each other. In healthy subjects, we found a high inverse correlation between serum levels of hepcidin and transferrin saturation ($r = -0.675$, $p = 0.0001$). In patients with CHLD serum hepcidin also showed strong feedback (Pearson Correlation) with transferrin saturation ($r = -0.842$, $p = 0.0001$), serum iron ($r = -0.788$, $p = 0.0001$), ferritin ($r = -0.674$, $p = 0.0001$) and IBC ($r = -0.471$, $p = 0.001$).

Sought connection between the values of hepcidin levels and the presence and extent of deposition of iron in the liver. Positive reaction for iron in liver tissue we reported only in patients with NASD (47%) ALD (17%) and CHHC (8%). Deposition of iron was mostly mild to moderate in Kupfer cells. We found significantly lower values of hepcidin in cases with histologically proven deposition of iron in the liver tissue ($F = 27.54$, $p = 0.0001$). Semi-quantitative determination of iron in tissue histology showed that marked iron overload hepcidin values are lower compared with no or mild iron deposition, ($F = 9.211$, $p = 0.0001$).

In CHLD group, but not in normal subjects, serum hepcidin showed feedback (Pearson Correlation) with the following parameters: age ($r = -0.152$, $p = 0.039$), BMI ($r = -0.279$, $p = 0.001$) and abdominal circumference ($r = -0.355$, $p = 0.001$); liver enzymes AST ($r = -0.248$, $p = 0.001$), ALT ($r = -0.227$, $p = 0.002$), GGT ($r = -0.222$, $p = 0.002$) , Overall ($r = -0.183$, $p = 0.012$) and direct ($r = -0.233$, $p = 0.001$) bilirurin, immunoglobulin A ($r = -0.192$, $p = 0.009$), glucose ($r = -0.177$, $p = 0.015$) , triglycerides ($r = -0.293$, $p = 0.001$) and uric acid ($r = -0.193$, $p = 0.008$), and positively correlated with platelet count ($r = 0.234$, $p = 0.001$) and prothrombin time (%) , ($r = 0.234$, $p = 0.001$). We also found a negative relation to history and the amount of alcohol consumed ($r = -0.209$, $p = 0.023$). In terms of histological changes, we found significantly lower values of hepcidin in the presence of steatosis ($F = 13.134$, $p = 0.0001$) and especially pronounced histological grade of steatosis ($F = 6.785$, $p = 0.0001$). We found a similar relationship with the level of activity ($F = 7.475$, $p = 0.0001$). Lower values of hepcidin also observed in the presence ($F = 14.554$, $p = 0.0001$) and advanced fibrosis ($F = 14.088$, $p = 0.0001$).

Discussion

Besides hereditary hemochromatosis, there are non-hereditary metabolic iron diseases in the body that are associated with mild hepatic iron overload, such as viral hepatitis, particularly associated with HCV infection, excessive alcohol consumption, non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatohepatitis (NASH) (1-7). The importance of HFE-mutations in the development of iron overload in these conditions is probably minimal (8, 9, 10-16). Hepcidin regulatory hormone plays an important role in the metabolism violations of iron in the body. Hepsidin is produced mainly by hepatocytes but also by macrophages (17) and fat cells (18). Hepcidin internalize and subsequently degrade ferroportin, which is present on the cell surface of macrophages and enterocytes and is responsible for transporting iron cage. Hepcidin formation increases during inflammation, with increased levels of iron in the body and inhibits at high erythropoietic activity. These regulatory pathways are responsible for protecting the body from proliferating extracellular pathogens and harmful effects of iron overload. The aim is to ensure that the presence of iron in the body is correlated with the needs of the body.

The results of our study show that the deviation from the normal range of serum hepcidin is found in 20% of healthy individuals. As a whole, the rate of reduction of hepcidin the reference limits established in 41% of the surveyed patients with chronic liver disease and serum levels were significantly lower compared to control healthy subjects. It is not proven a significant differences between gender and age of the subjects, both in health and in liver disease. If we look at changes in serum levels of hepcidin in the different groups of chronic liver disease, are identified several changes. The difference between healthy volunteers and patients with liver disease is mainly due to the decrease in the proportion of serum hepcidin in the case of non-alcoholic and alcoholic fatty disease, especially with steatosis hepatitis and chronic hepatitis C. In addition to the healthy controls, levels of serum hepcidin in these groups was significantly lower in comparison with that of patients with chronic hepatitis B and D, chronic autoimmune hepatitis and primary biliary cirrhosis, but no difference between them. On the other hand we found significant changes in hepsidin compared to control in patients with chronic hepatitis B and D, and autoimmune diseases - autoimmune hepatitis and primary biliary cirrhosis, as well as between them.

Analysis of factors associated with the decrease in serum hepcidin shows different correlations in health and disease. While in healthy individuals only strong inverse correlation between serum hepcidin and transferrin saturation was found, then with chronic liver disease hepcidin correlates strongly and negatively not only with transferrin saturation, but also with other serum parameters of iron metabolism such as serum iron and IBC and ferritin. Furthermore, the reduction in serum hepcidin is in connection with the deposition of iron and its level in the hepatic parenchyma. Unlike healthy people in chronic liver disease the serum hepcidin feedback with age and some features of metabolic abnormalities such as BMI and waist circumference, glucose, triglycerides and uric acid. We proved a negative correlation with the amount of alcohol consumed and the history of immunoglobulin A, and correlation with liver enzymes (AST, ALT and GGT) and liver function as a general and direct bilirurin, prothrombin time and platelet count. In terms of histological changes we found lower values of hepcidin in the presence of steatosis, particularly pronounced degree, high activity in the presence of fibrosis, especially in advanced stages. According to our data, similar relationships exist with other surrogate markers for assessing hypersaturation in iron in liver disease.

Recent evidence has shown that the basis of interference in the synthesis of hepcidin is not a hereditary defect. Indirect evidence of a link between hepcidin, glucose and lipid metabolism come from case reports of hepatic adenomas producing hepcidin with severe anemic syndrome and impaired metabolism of glycogen and fat(18, 21). It is also interesting that many of the diseases that are part of the metabolic syndrome have abnormal iron homeostasis- ie. diabetes (1, 2, 3.4, 8), hypertension (22) and increased body mass index(23). These data are consistent with our established association between reduction of hepcidin, hypersaturation syndrome with non-alcoholic steatosis and chronic hepatitis C who are closely associated with the metabolic syndrome.

Alcoholic liver disease, non-alcoholic steatosis and steatohepatitis and chronic viral hepatitis are the most typical examples of acquired mild iron overload. Liver biopsies in patients with these diseases show iron deposits in hepatocytes and Kupfer cells(5, 19, 24), which is confirmed by our results. Iron can cause oxidative stress, which leads to destruction of the tissue. Some scientists believe that the increased liver iron deposition is associated with more advanced forms of the disease, including liver fibrosis, cirrhosis and hepatocellular carcinoma(9, 11, 25-29, 30, 31), but this was not confirmed by other authors(12,32 to 35). Our data also strengthens the relationship between syndrome hypersaturation of iron and fibrosis. Fugita N et al. (36) found that patients with chronic hepatitis C has low hepcidin in the liver as an expression of iron overload in the body, but they can be restored upon termination of viral replication. In addition, they found that levels in liver tissue hepcidin reflects of the circulating levels of serum hepcidin. This data suggest that serum hepcidin could be a surrogate marker for assessing the level of liver hepcidin as a useful indicator of diagnostic precision and therapeutic decision making in patients with acquired forms of mild hepatic iron overload in chronic liver disease.

Finally, the analysis of the data from our study showed a decrease in serum hepcidinin non-alcoholic fatty disease and alcohol liver disease, and chronic hepatitis C compared to healthy subjects and other chronic liver disease associated with CHHB, CHHD or autoimmune pathogenesis. This decrease is directly related to increased accumulation of iron in the body and correlated with liver enzyme showing activity in liver function, severity of steatosis and hepatitis, and the presence and stage of fibrosis. All of this demonstrates the importance of hepcidin as another surrogate markers oldered better assessment of increased iron overload in chronic liver disease.

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Table 1. Serum parameters of iron metabolism in healthy subjects and chronic liver diseases

	NAFLD (n=38)	**AFLD** (n=45)	**Chronic hepatitis C** (n=45)	**Chronic hepatitis B** (n=35)	**Chronic hepatitis B+D** (n=13)	**Chronic autoimmune hepatitis** (n=17)	**Chronic Liver diseases** (n=186)	**Controls** (n=60)
Serum Iron								
Mean value	24.27	26.21	22.72	16.99	21.99	18.20	22.34	18.30
Standard deviation	1.47	1.26	1.30	0.68	1.47	1.12	0.59	0.60
Minimum value	12.30	9.16	12.03	11.50	16.80	12.60	9.16	10.80
Maximum value	37.50	36.40	37.10	28.60	37.90	31.90	37.90	30.20
Serum Ferritin								
Mean value	280.65	346.54	244.12	122.06	72.92	93.04	227.62	147.32
Standard deviation	36.05	37.83	32.17	14.41	13.01	16.69	15.46	11.82
Minimum value	47.00	31.70	23.50	51.00	29.00	28.00	23.50	68.00
Maximum value	709.00	812.00	697.00	527.00	219.00	310.00	812.00	397.00
Transferin Saturation								
Mean value	32.14	33.86	33.28	32.56	29.46	26.12	32.13	31.15
Standard deviation	1.34	1.28	1.27	0.90	1.49	1.16	0.55	0.69
Minimum value	20.00	20.80	21.00	22.00	24.00	20.16	20.00	21.00
Maximum value	47.00	45.00	44.10	44.00	43.00	37.00	47.00	45.00

Figure 1. Frequency of low serum hepcidin in different groups of patients with CHLD and healthy individuals.