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Serum Hepcidin Level in Different Stages of Hepatitis C Induced Chronic Liver Disease

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ABSTRACT

Background: Patients with chronic hepatitis C (CHC) often have increased liver iron, a condition associated with more rapid progression to cirrhosis. Hepcidin decrease is a possible pathophysiological mechanism of iron overload in these patients.

Objective: The aim of the present work was to assess serum hepcidin in patients with chronic hepatitis C as an iron-regulating hormone.

Patients and Methods: This study included 45 patients with CHC as patients group and 15 healthy individuals who served as a control group. All were subjected to full history taking, clinical examination, pelvi-abdominal ultrasound, laboratory investigations such as liver function tests, kidney function tests, complete blood count, prothrombin time, INR, C-reactive protein, serum iron, ferritin, transferring saturation and serum hepcidin level by ELISA.

Results: Serum hepcidin (ng/ml) was highly significantly lower in CHC patients than in controls. Serum iron (µg/dl), serum ferritin (µg/l) and serum transferrin saturation (%) were significantly higher in CHC patients than in controls.

Conclusion: Hepcidin levels in patients with CHC were significantly lower than that in HCV- negative individuals. It is an important factor in iron abnormalities and is detected in such cases in which serum iron levels, serum ferritin and transferrin saturation were significantly high in CHC patients compared to HCV- negative healthy individuals. The suppression of this hormone by hepatitis C virus is likely an important factor in liver iron accumulation.

Keywords: Chronic hepatitis C, Serum hepcidin, Serum iron, Serum ferritin, Transferrin saturation.

INTRODUCTION

Globally, an estimated 71 million people have chronic hepatitis C virus infection. Hepatitis C virus causes both acute and chronic infection. New HCV infections are usually asymptomatic. Around 30% (15–45%) of infected persons spontaneously clear the virus within 6 months of infection without any treatment. The remaining 70% (55–85%) of persons will develop chronic HCV infection. Of those with chronic HCV infection, the risk of cirrhosis ranges between 15% and 30% within 20 years [(1)]. CHC patients frequently develop mild to moderate iron overload. Many experimental and clinical studies suggested that excessive iron in CHC is a cofactor promoting the progression of liver damage [(2)]. With the discovery of hepcidin, the liver has emerged as the central organ in the regulation of systemic iron homeostasis [(3)]. Hepcidin is 25-amino acid peptide hormone primarily synthesized by hepatocytes. Hepcidin expression is modulated by iron stores. Hepcidin expression is also induced by inflammation, infection and suppressed by hypoxia and anaemia [(4, 5)]. Each part of iron metabolism including absorption, restoration, recycling and utilization is regulated by hepcidin and any abnormalities in the process of iron metabolism may lead to excessive iron burden. In chronic hepatitis C patients, excessive iron deposition has been found in both hepatocytes and reticuloendothelial cells although the mechanism is not fully clarified [(6)]. Iron is toxic to liver leading to ROS (reactive oxygen species) production and progression of liver disease [(7)]. Some previous studies aimed to detect serum hepcidin level in different causes of liver diseases and their results need further validation [(8)]. In our study, we tried to detect serum hepcidin level using ELISA technique in different stages of chronic HCV-induced liver disease.

PATIENTS AND METHODS:

This study was conducted on 60 participants. These participants were classified into two main groups; control group of healthy individuals and patients group .Control group included 15 healthy individuals of matched age and sex. In control group, 8 (53.3%) were males & 7 (46.7%) were females. Their ages ranged from 18 to 57 years, with a mean age of 36.20 ± 11.68 years old. Patients group was subdivided into 3 subgroups according to modified Child Pugh classification. These subgroups were A, B and C and included 45 patients with CHC. In patients group, 21 (48.9%) were males & 24 (51.1%) were females. Their ages ranged from 19 to 57 years, with a mean age of 40.77 ± 10.91 years old.

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Ethical approval:

Approval from the Local Research Ethical Committee 0f Zagazig University was obtained. Informed consent from each patient was obtained before enrolment in the study.

Exclusion criteria

Associated hepatitis B virus infection, α1 antitrypsin deficiency, Wilson's disease, haemochromatosis, alcoholic liver disease, autoimmune hepatitis, associated HCC, acute inflammatory disorder, previous treatment with antiviral agents, associated HIV infection and CKD.

All patients were subjected to the following:

Full history taking, full clinical examination, pelvi-abdominal ultrasound, routine laboratory investigations such as liver function tests, kidney function tests, complete blood count, prothrombin time, INR,C-reactive protein, serum iron, serum ferritin, transferring saturation and serum hepcidin level by ELISA. Serum iron (µg/dl) was evaluated using Iron Ferro Zine Colorimetric method. Serum ferritin (ng/ml) was evaluated using Abcam's ferritin competitive human in- vitro ELISA kit.

Statistical analysis

Recorded data were analyzed using the statistical package for social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage. Independent-samples t-test of significance was used when comparing between two means. Chi-square (x^2) test of significance was used in order to compare proportions between two qualitative parameters. The confidence interval was set to 95% and the margin of error accepted was set to 5%. The p-value was considered significant as the following: P-value <0.05 was considered significant.

- P-value <0.001 was considered as highly significant.
- P-value >0.05 was considered insignificant.

RESULTS

Serum hepcidin (ng/ml) was detected in both main groups. There was a highly significant decrease in CHC patients when compared to healthy controls, with a mean of 49.3 ± 13.4 and 102.6 ± 10.6, respectively (P < 0.001) as shown in Table (1).

Table (1): Comparison between the studied groups regarding serum hepcidin (ng/ml)

Item	Controls (n= 15)	Patients (n=45)	t-	P
Serum hepcidin (ng/ml): Mean ± SD	102.6 ± 10.6	49.3 ± 11.4	13.966	0.001(S)

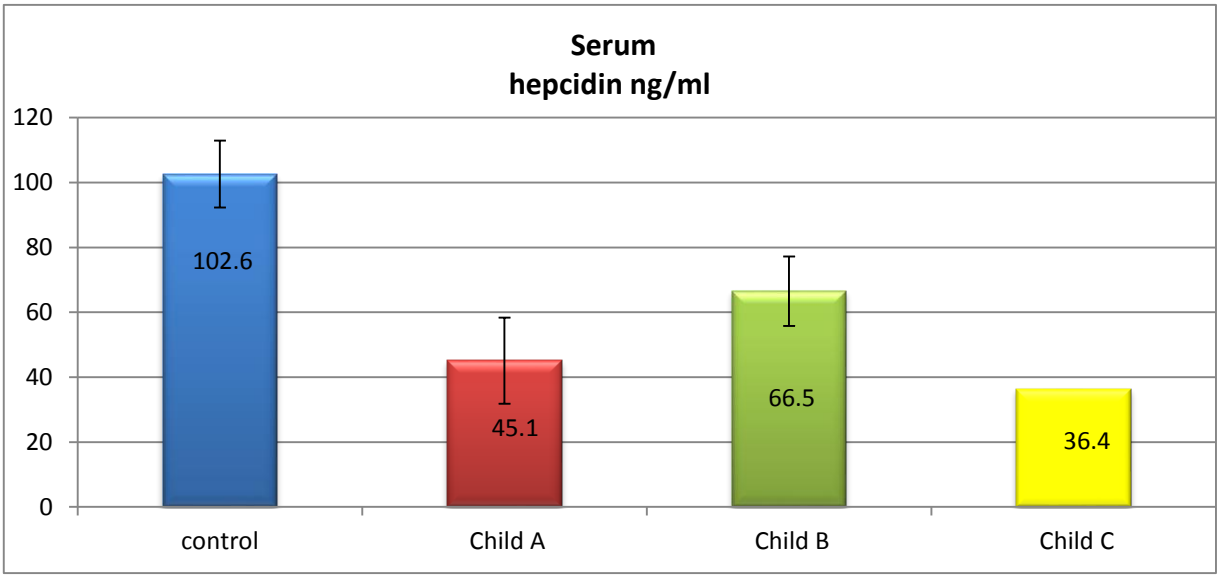

Fig. (1): Comparison of serum hepcidin (ng/ml) between the studied groups

Serum hepcidin (ng/ml) in CHC patients' subgroups was 45.1 ± 3.6 in group A, 66.5 ± 5.7 in group B and 36.4 ± 2.7 in group C as shown in Figure (1).

As regards serum iron (µg/dl) in both groups, there was a highly significant increase in CHC patients when compared to healthy control, with a mean of 119.7± 9.9 and 95.9 ± 4.9 respectively (P < 0.001) as shown in Table (2).

Table (2): Comparison between the studied groups about serum iron (mcg/dl)

Item	Controls (n=15)	Patients (n=45)	t-	P
Serum iron (mcg/dl): Mean ± SD	95.9 ± 4.9	119.7±9.9	-8.924	0.001(S)

As regards serum iron (µg/dl) in CHC patients' subgroups, group A was 117 ± 4.5, group B was 110.4 ± 2.7 and group C was 131.7 ± 4.9 as shown in Figure (2).

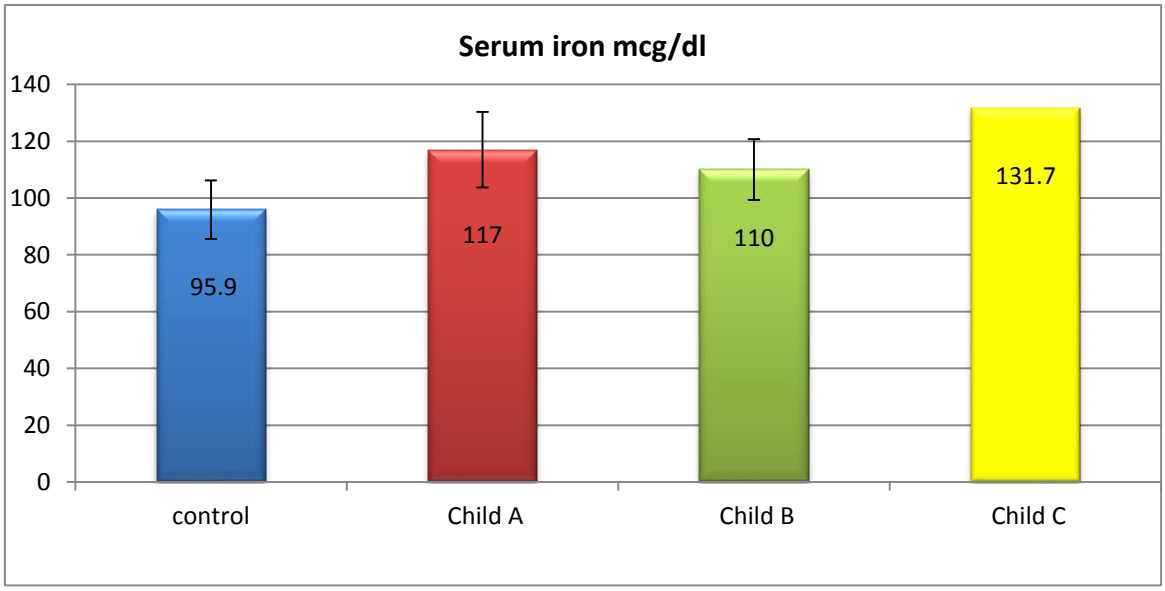

Fig. (2): Comparison of serum iron (mcg/dl) between the studied groups

As regards serum ferritin (µg /l) in both groups, there was a highly significant increase in CHC patients when compared to healthy controls, with a mean of 134.4 ± 17 and 85.3 ± 12.2 respectively (P < 0.001) as shown in Table (3).

Table (3): Comparing between the studied groups regarding ferritin

Item	Control (n=15)	Patients (n=45)	t-	P
Ferritin (µg/l): Mean ± SD	85.3 ± 12.2	134.4 ± 17	-10.252	0.001(S)

Serum Transferrin saturation (%) was significantly higher in CHC patients than in controls, with a mean of 32.5 ± 3.7 and 27.7 ± 1.5 respectively (P < 0.001).

As regards hepcidin/ferritin ratio, we found that there was a high statistically significant difference between CHC patients and healthy controls, as it was lower in CHC group (0.031 ± 0.008 and 0.161 ± 0.041 respectively) as shown in Table (4).

Table (4): Hepcidin/ferritin ratio of the studied groups.

Item	Controls(n=15)	Patients (n=45)	t-	P
Hepcidin/ferritin ratio Mean ± SD	0.161 ± 0.041	0.031 ± 0.008	71.32	0.001(S)

DISCUSSION

Hepatitis C virus causes both acute and chronic infection. New HCV infections are usually asymptomatic. Around 30% (15–45%) of infected persons spontaneously clear the virus within 6 months of infection without any treatment. The remaining 70% (55–85%) of persons will develop chronic HCV infection. Of those with chronic HCV infection, the risk of cirrhosis ranges between 15% and 30% within 20 years [(1)]. The liver is the major iron storage organ in the body. Chronic hepatitis C is one of the liver diseases that show excess hepatic iron accumulation [(9)]. Elevated iron-related serum markers and increased hepatic iron accumulation are relatively common and correlate with the severity of hepatic inflammation and fibrosis in patients with chronic hepatitis C. Oxidative stress and increased iron levels strongly favor DNA damage, genetic instability, and tumorgenesis [(10)]. The mechanisms underlying hepatic iron accumulation in chronic hepatitis C infection have not been fully understood. The hepatic peptide hormone hepcidin is the major regulator of iron metabolism and reduction of the hepcidin transcription activity by hepatitis C virus

(HCV)-induced reactive oxygen species may account for that [11].

The regulation of hepcidin is very complex and may depend on many variables, including the particular stage of the systemic and/or hepatic inflammatory conditions and the circulating transferrin-bound iron and intracellular iron stores. This might explain the variations in hepatic iron concentrations reported among patients with HCV-related chronic liver disease [12]. Animal and cellular models have suggested that HCV infection may directly modulate hepcidin expression. HCV-induced reactive oxygen species (ROS) have been shown to raise iron level by reducing hepcidin transcription in animals [13]. Hepcidin has a central role in iron homoeostasis, as it decreases iron release from macrophages and iron absorption from intestinal enterocytes. Its correlation to CHC, hepatic iron load and fibrosis is controversial between different studies [14].

In control group, there were 8 (53.3%) males & 7 (46.7%) females. Their ages ranged from 18 to 57 years, with a mean age of 36.20 ± 11.68 years old. Patients' group was subdivided into 3 subgroups according to modified Child Pugh classification. These subgroups (A, B and C) included 45 patients with CHC. In patients' group 21 (48.9%) were males & 24 (51.1%) were females. Their ages ranged from 19 to 57 years, with a mean age of 40.77 ± 10.91 years old. There was no significant difference between the two groups as regards age and sex, indicating no bias in this study. However, there was a statistically significant difference between the studied groups as regards laboratory findings (P < 0.05).

In our study, the mean age in control group was 34.6 ±1 0.3 and in CHC patients as follow: A group was 45.6 ± 13.3, B group was 54.5 ± 10.7 and C group was 59.1 ± 9.5. There was no statistically significant difference between the two main groups. Statistically significant difference was detected between patients' subgroups (Group B Vs C) as p value was < 0.05.

In our study, there was a statistically significant difference (P > 0.05) between the studied groups as regards alanine aminotransferase (ALT) and aspartate aminotransferase (AST) with a mean of 64 ± 15 and 67.57 ± 15.5, respectively, in CHC patients' group versus 29.8 ± 4.3 and 25.9 ± 6.5 in control group. This is in agreement with the findings of **Marzouk *et al.*** [15].

In our study, there was a high statistically significant difference between groups regarding blood picture (P-value < 0.05) and no statistically significant difference was detected between patients' subgroups (Group B Vs. C).

Our study revealed that there was a high statistically significant difference between groups regarding serum Bilirubin (mg/l) with a mean of 1 ± 0.2 in control group and in CHC patients group with a mean of 1 ± 0.6 in A, 2 ± 0.8 in B and 4.95 ± 0.5 in C.

In the current study, there was a high statistically significant difference between groups regarding serum Albumin (gm/l) levels with a mean of 4.2 ± 0.5 in control group and in CHC patients group with a mean of 4.3 ± 0.42 in A, 3.1 ± 0.5 in B and 2.3 ± 0.9 in C.

In the present work, there is a high statistically significant difference between groups regarding serum creatinine (mg/dl) levels with a mean of 0.9 ± 0.5 in control group and in CHC patients group with a mean of 0.951 ± 0.4 in A, 1.1 ± 0.43 in B and 1.9 ± 1.4 in C.

Our study revealed that there was a high statistically significant difference between groups regarding serum urea (mg/dl) levels with a mean of 30 ± 9 in control group and in cases group with a mean of 23.9 ± 7.8 in A, 45.3 ± 15.5 in B and 104.1 ± 70.2 in C.

Our study revealed that there was a high statistically significant difference between groups regarding coagulation profile (PT) with a mean of 12 ± 1.2 in control group and in CHC patients' group with a mean of 14.5 ± 1.5 in A, 19.9 ± 1.9 in B and 25 ± 4.9 in C.

Our study revealed that CHC patients have highly significantly lower hepcidin (ng/ml) concentrations than those of matched control. Mean serum hepcidin in control group was 102.6 ± 10.6 and in patients group was 49.3 ± 13.4. There was a high statistically significant difference between the studied groups being higher in the control group. This is in agreement with the findings of **Nishina *et al.*** [13], **Nagashima *et al.*** [16], **Fujita *et al.*** [17] and **Girelli *et al*** [18] who stated that serum hepcidin was significantly lower in CHC patients than in controls. They attributed this to the suppressive effect of ROS, which is induced by HCV on hepcidin production. However, our study is not in agreement with the findings of **Wrighting and Andrews** [19] and **Trinder *et al.*** [20] who attributed that to the upregulation of hepcidin production by proinflammatory cytokines, particularly interleukin- 6 that counteracts ROS-induced hepcidin suppression.

Our study revealed that serum hepcidin level in B group w2as 66.5 ± 5.7 and this is higher than patients in A and C while slightly lower than control level. This is in agreement with **Tsochatzis *et al*** [21] who had a midway view seeing that HCV infection down regulates serum hepcidin, whereas increased inflammation and/or fibrosis tend to restore its levels.

Our study revealed that the mean serum iron (mcg/dl) was statistically significantly higher in CHC patients' group than those of matched controls were. In control group, it was 95.9 ± 4.9 and in patients' group was 119.7 ± 9.9 (in A group 117 ± 4.5, in B group 110.4 ± 2.7 and in C group 131.7 ± 4.9). There is a high statistically significant difference between the two groups. This is in agreement with **El Wakil *et al*** [22] who found that serum iron levels were higher in the CHC group than in the control group, however there was no statistically significant difference between the two studied groups. This is in disagreement with **Marzouk *et al*** [15] who found a highly statistically significant difference between the studied groups, as it was lower in the CHC group than in the control group.

Our study revealed that CHC patients had high significantly higher ferritin (µg/l) concentrations than those of matched controls. In control group, it was 85.3 ± 12.2 and in patients' group was 134.4 ± 17 (A group 117.4 ± 10.72, in B group 135.6 ± 9.5 and in C group 151.4 ± 10.8). There was a high statistically significant difference between the two groups (P-value < 0.05). This is in agreement with **Fujita *et al.* (17)** who found that serum ferritin was significantly higher in the CHC group than in the control group. In addition, **Sugimoto *et al.* (23)** found that serum ferritin was significantly higher in the CHC group than in the control group (P < 0.001). **El Wakil *et al.* (22)** found that serum ferritin was significantly higher in the CHC group than in the control group (P < 0.05) and **Marzouk *et al.* (15)** found that serum ferritin was significantly higher in the CHC group than in the control group (P < 0.001).

Our study revealed that the mean transferrin saturation (%) is higher in CHC patients group than those of matched controls. In control group, it was 27.7 ± 1.5 and in patients group, it was 32.5 ± 3.7 (in A group 32.6 ± 1.7 in B group 28.4 ± 1.4 and in C group 36.5 ± 1.8). There was a high statistically significant difference between the two main groups (P-value < 0.05). This is in agreement with the findings of **Fujita *et al.* (17), Sugimoto *et al.* (23)** and **Aoki *et al.* (24)** who found no statistically significant relation between serum hepcidin level and the severity of inflammatory activity (P > 0.05).

CONCLUSION

Hepcidin levels (ng/ml) in patients with CHC were significantly lower than that in HCV- negative individuals. It is an important factor in iron abnormalities and is detected in such cases in which serum iron (µg/dl) levels, serum ferritin (µg /l) and Transferrin saturation % were significantly high in CHC patients compared to HCV- negative healthy individuals. Hepcidin regulation by iron stores is maintained in CHC, the suppression of this hormone by hepatitis C virus is likely an important factor in liver iron accumulation in this condition.

RECOMMENDATION

1) Large-scale studies for Hepcidin and iron are needed in different etiologies of chronic liver disease.
2) Further studies that depend on much more accurate methods for detection of Hepcidin level in chronic liver disease.

Conflict of interest: NO

Financial disclosure: NO

REFERENCES

1. **WHO (2019):** Hepatitis C.https://www.who.int/news-room/fact-sheets/detail/hepatitis-c
2. **Büyükaşik N, Büyükaşik Y (2016):** Chronic liver diseases and iron. Turk J Med Sci., 46: 1281-1291.
3. **Pietrangelo A (2007):** Hemochromatosis: an endocrine liver disease. Hepatology, 46: 1291–1301.
4. **El- Shafie A, El- Mashad G, Hegran H *et al.* (2015)**: Egypt Health Issues Survey. https:// dhsprogram. com/pubs/pdf/FR313/FR313.pdf
5. **Ganz T, Nemeth E (2012):** Hepcidin and iron homeostasis. Biochim Biophys Acta., 1823: 1434–43.
6. **Zou D, Sun W (2017):** Relationship between hepatitis C virus infection and iron overload. Chinese Medical Journal, 130 (7): 866-871.
7. **Milic S, Mikolasevic I, Orlic L *et al.* (2016):** Role of iron and iron overload in CLD. Med Sci Moint., 22: 2144 -2151.
8. **Wang J, Dong A, Liu G *et al.* (2016):** Correlation of serum hepcidin level with disease progression in HBV-related disease. Scientific Reports Journal, 6: 34252-5.
9. **Hino K, Nishina S, Hara Y (2013):** Iron metabolic disorder in chronic hepatitis C: mechanisms and relevance to hepatocarcinogenesis. J Gastroenterol Hepatol., 4: 93-8**.**
10. **Lambrecht R, Sterling R, Naishadham D *et al.* (2011):** Iron levels in hepatocytes and portal tract cells predict progression and outcomes of patients with advanced chronic hepatitis C. Gastroenterolog., 140: 1490–500.
11. **Deicher R, Hörl W (2006):** New insights into the regulation of iron homeostasis. European Journal of Clinical Investigation, 36: 301-9.
12. **Kato J, Miyanishi K, Kobune M (2007**): Long-term phlebotomy with low-iron diet therapy lowers risk of development of hepatocellular carcinoma from chronic hepatitis C. J Gastroenterol., 42: 830–6.
13. **Nishina S, Hino K, Korenaga M *et al.* (2008):** Hepatitis C virus- induced reactive oxygen species raise hepatic iron level in mice by reducing hepcidin transcription. Gastroenterology, 134: 226–238.
14. **Zumrutdal A, Sezgin N (2012):** The relationship between anemia, liver disease and hepcidin levels in hemodialysis patients with hepatitis. Indian J Nephrol., 22 (6): 415-8.
15. **Marzouk H, Azayed N, Al- Ansary M *et al.* (2013):** Hepcidin levels in Egyptian patients with chronic hepatitis C. World Appl Sci J., 22: 1145-8.
16. **Nagashima M, Kudo M, Chung H *et al.* (2006):** Regulatory failure of serum prohepcidin levels in patients with hepatitis C. Hepatol Res., 36: 288–293.
17. **Fujita N, Sugimoto R, Takeo M *et al.* (2007):** Hepcidin expression in the liver: relatively low level in patients with chronic hepatitis C. Mol Med., 1: 97–104.
18. **Girelli D, Pasino M, Julia B *et al.* (2009):** Reduced serum hepcidin levels in patients with chronic hepatitis C. J Hepatol., 51: 845–852.
19. **Wrighting D, Andrews N (2006):** Interleukin- 6 induces hepcidin expression through STAT3. Blood, 108: 3204–3209.
20. **Trinder D, Ayonrinde O, Olynyk J (2008):** HCV, iron, and oxidative stress: the new choreography of hepcidin. Gastroenterology, 134: 348–351.
21. **Tsochatzis E, Bosch J, Burroughs A (2014):** Liver cirrhosis. The Lancet, 383 (9930): 1749-1761.
22. **El Wakil R, Mokhles M, Tohamy A *et al.* (2012):** Hepcidin and chronic hepatitis C: exploring the controversy. Aust J Basic Appl Sci., 6: 558–565.
23. **Sugimoto R, Fujita N, Tomosugi N *et al.* (2009):** Impaired regulation of serum hepcidin during phlebotomy in patients with chronic hepatitis. Hepatol Res., 39: 619–624.
24. **Aoki C, Rossaro L, Ramsamooj R *et al.* (2005):** Liver hepcidin mRNA correlates with iron stores, but not inflammation, in patients with chronic hepatitis C. J Clin Gastroenterol., 39: 71–74.