

The Role of Iron and Iron Overload in Chronic Liver Disease

Milić, Sandra; Mikolašević, Ivana; Orlić, Lidija; Devčić, Edita; Starčević-Čizmarević, Nada; Štimac, Davor; Kapović, Miljenko; Ristić, Smiljana

Source / Izvornik: **Medical Science Monitor, 2016, 22, 2144 - 2151**

Journal article, Published version

Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

<https://doi.org/10.12659/MSM.896494>

Permanent link / Trajna poveznica: <https://urn.nsk.hr/urn:nbn:hr:184:825437>

Rights / Prava: [Attribution-NonCommercial-NoDerivatives 4.0 International/Imenovanje-Nekomercijalno-Bez prerada 4.0 međunarodna](#)

Download date / Datum preuzimanja: **2024-07-27**



Repository / Repozitorij:

[Repository of the University of Rijeka, Faculty of Medicine - FMRI Repository](#)



Received: 2015.10.30

Accepted: 2015.11.24

Published: 2016.06.22

The Role of Iron and Iron Overload in Chronic Liver Disease

Authors' Contribution:

Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF G 1 Sandra Milic
ABCDEF G 1,2 Ivana Mikolasevic
ABCDEF G 2 Lidija Orlic
ABCDE 1 Edita Devcic
ABCD 3 Nada Starcevic-Cizmarevic
ABCDE 1 Davor Stimac
ABCD 3 Miljenko Kapovic
ABCDEF 3 Smiljana Ristic

1 Department of Gastroenterology, UHC Rijeka, Rijeka, Croatia
2 Department of Nephrology, Dialysis and Kidney Transplantation, UHC Rijeka, Rijeka, Croatia
3 Department of Biology and Medical Genetics, Faculty of Medicine, Rijeka, Croatia





Corresponding Author: Sandra Milic, e-mail: smilic05@gmail.com

Source of support: This work was supported by University of Rijeka grants to S. Milic, to N. Starcevic-Cizmarevic, to S. Ristic

The liver plays a major role in iron homeostasis; thus, in patients with chronic liver disease, iron regulation may be disturbed. Higher iron levels are present not only in patients with hereditary hemochromatosis, but also in those with alcoholic liver disease, nonalcoholic fatty liver disease, and hepatitis C viral infection. Chronic liver disease decreases the synthetic functions of the liver, including the production of hepcidin, a key protein in iron metabolism. Lower levels of hepcidin result in iron overload, which leads to iron deposits in the liver and higher levels of non-transferrin-bound iron in the bloodstream. Iron combined with reactive oxygen species leads to an increase in hydroxyl radicals, which are responsible for phospholipid peroxidation, oxidation of amino acid side chains, DNA strand breaks, and protein fragmentation. Iron-induced cellular damage may be prevented by regulating the production of hepcidin or by administering hepcidin agonists. Both of these methods have yielded successful results in mouse models.

MeSH Keywords: **End Stage Liver Disease • Hepcidins • Iron Metabolism Disorders**

Full-text PDF: <http://www.medscimonit.com/abstract/index/idArt/896494>

 4291  1  —  67



Background

According to epidemiological data, the incidence of chronic liver disease is increasing [1,2]. Chronic liver disease includes various causes of liver failure that lead to progressive destruction and regeneration of the liver parenchyma, ending in fibrosis and cirrhosis. Cirrhosis is the final stage of liver fibrosis and often leads to portal hypertension and hepatic failure; patients with end-stage cirrhosis often require liver transplantation. Pathologically, cirrhosis disrupts the liver architecture and is associated with fibrotic bands, parenchymal nodules, and vascular distortion. The most common causes of chronic liver disease are metabolic, viral, toxic, and autoimmune [3–5]. In recent years, nonalcoholic fatty liver disease (NAFLD), the hepatic expression of metabolic syndrome (MS), has reached epidemic proportions. Nowadays, with the increasing prevalence of obesity, diabetes mellitus, and MS in the general population, NAFLD has become the most common cause of chronic liver disease in Western countries [6]. Contributing factors to this may include the increasingly sedentary lifestyle of the population and increased consumption of a high-fat diet and high fructose corn syrup [5]. However, therapeutic options to treat chronic liver disease are limited and new approaches are necessary. Recent studies have highlighted the role of iron in the pathophysiology of chronic liver disease. Iron accumulation has been noted not only in patients with hereditary hemochromatosis (HH), but also in those with acquired metabolic disorders and viral infections [7]. Interestingly, iron perturbations are often observed in patients with obesity, insulin resistance (IR), or NAFLD. The peptide hepcidin is produced mainly in the liver, although macrophages, pancreatic islet cells, and adipose tissue can also excrete it. Hepcidin was recently found to be a key regulator of iron metabolism. Thus, it may be of great importance in the development of new therapeutic approaches for chronic liver disease [8,9]. The present review highlights current knowledge about hepatic iron overload status in chronic liver diseases, the impact of hepatic iron overload on diseases progression, its relevance to hepatocarcinogenesis, and possible new therapeutic options.

Role of Liver in Iron Metabolism

Iron is an essential micronutrient for the human body. It has important roles in oxygen transport, oxidative phosphorylation, and other enzymatic functions [10,11]. The human body has an average of 2–4 g of iron, of which 80% is bound in hemoglobin. There is tight control of systemic iron levels by means of iron absorption, storage, and recycling. Iron deficiency causes a decrease in hemoglobin production and can consequently lead to anemia, while iron overload burdens the body and results in excess tissue iron, which can cause cell damage [8,12,13].

Dietary iron is in the ferric form and must be reduced by the apical ferric reductase duodenal cytochrome b (DcytB, also known as CYBRD1) to ferrous iron for transport into enterocytes via the apical iron transporter divalent metal transporter-1 (DMT-1, also known as SLC11A2). Iron is exported through ferroportin (SLC40A1), which is located on the basolateral side of enterocytes. Once in the bloodstream, iron is bound to transferrin (TF). Prior to the binding of iron to TF, it is oxidized back to the ferric form by the ferroxidases hephaestin (HEPH) and ceruloplasmin (CP) [10,12,13]. Most of the transferrin-bound iron is used by erythroblasts to synthesize hemoglobin. Aging erythrocytes undergo erythrophagocytosis, during which iron is recovered and routed back to circulation via ferroportin [12,13].

Under conditions in which there is excess iron, cells can secrete Fe^{2+} via ferroportin or secrete heme through the putative heme exporter feline leukemia virus subgroup C cellular receptor 1 (FLVCR). Cells can also store iron within ferritin. Ferritin is a multi-subunit protein that comprises a variable ratio of heavy and light chains, depending on the type of tissue. Hepatocytes serve as a major site of ferritin synthesis, which is controlled through a posttranscriptional mechanism by the iron response element (IRE)/iron regulatory protein (IRP) network. Hypoxia, oxidative stress, IL1, and TNF also regulate ferritin expression [10,13].

Iron stored within ferritin is thought to be bioavailable; thus, hepatocytes have a crucial role in the mobilization of iron to satisfy metabolic requirements [10]. The liver produces the majority of proteins involved in iron metabolism, including hepcidin and transferrin. The main characteristic of transferrin is its ability to reversibly bind iron, which allows it to be a cellular iron donor or iron acceptor [10,12].

Hepcidin is an antimicrobial peptide with a key role in iron metabolism. Its expression is regulated by the bone morphogenetic protein (BMP) and JAK2/STAT3 signaling pathways. The level of iron in the bloodstream serves as a stimulus for hepcidin synthesis. Hepcidin regulates iron delivery via ferroportin. Once it binds to the cell, hepcidin triggers ferroportin degradation by endocytosis and proteolysis of the ligand-receptor complex. This results in restriction of iron flow into the plasma and blockage of dietary iron absorption, resulting in hypoferrremia [14–17].

Disruptions in the BMP signaling pathway result in lower hepcidin levels and, consequently, iron overload. BMP binds to a BMP receptor complex and activates the SMAD signaling pathway [10,17]. Research in a mouse model showed that BMP6, produced in sinusoidal endothelial cells, has a crucial role in maintaining iron metabolism, which cannot be compensated for by other BMPs [17].

Hepatic hemojuvelin (HJV, also known as HFE2) is a coreceptor for BMP6 and interacts with neogenin to induce hepcidin expression. Hemojuvelin is also synthesized in skeletal and heart muscles and can be released into the bloodstream as soluble HJV. It is still unclear if soluble HJV suppresses hepcidin expression by taking the place of hepatic HJV and binding to BMP6. Recently, 2 specific BMP type I receptors, ALK2 (ACVR1) and ALK3 (BMPR1A), were identified as important for hepcidin regulation [16–18].

Researchers do not yet know definitively how hereditary hemochromatosis protein (HFE) and transferrin receptor 2 (TFR2) regulate iron homeostasis. A new hypothesis suggests that HFE and TFR2 interact with HJV, particularly under conditions in which there is high transferrin saturation, and that this complex facilitates hepcidin expression induced by HJV [18].

Matriptase-2 (MT2) is a membrane serine protease encoded by the gene *TMPRSS6* and found mainly in the liver. It releases HJV from hepatocytes; by doing so, it lowers the ability of HJV to act as a coreceptor. Thus, MT2 is the key suppressor of hepcidin expression [10,16,18].

Hypoxia and erythropoietic activity also suppress hepcidin expression. During tissue hypoxia, hypoxia inducible factors-1 and -2 (HIF-1 and HIF-2) activate a variety of genes involved in hypoxia adaptation. It remains unknown whether hepcidin release is suppressed directly by HIF-1 and HIF-2 or if it depends upon increased erythropoietic activity [16,18,19].

During inflammation, there is an increase in hepcidin transcription mediated by IL6. Binding of IL6 to its receptors activates the STAT pathway, which works in synergy with the BMP pathway to stimulate hepcidin synthesis. Other cytokines, including IL22 and oncostatin M, are also suspected of having the same effect [16,20].

Changes in Iron Metabolism in Patients with Chronic Liver Disease

Considering the critical role played by the liver in iron metabolism, it follows that liver diseases affect iron homeostasis. Iron accumulation has been noted not only in patients with HH, but also in those with NAFLD, alcoholic liver disease (ALD), and hepatitis C virus (HCV) infection [21–23].

Hemochromatosis

HH is a genetic disorder that causes dysregulation of hepcidin homeostasis and, consequently, iron overload (24). There are 4 types of HH. Type 1 is the most common. It is caused by a missense mutation of cysteine 282 to tyrosine (Cys282Tyr) in

the HFE gene (*HFE*). HFE binding to TFR1 (also known as TFR2) is thought to be the mechanism by which HFE blocks hepcidin expression. This binding is increased in patients with the type 1 HFE mutation, enabling HFE to interact with TFR2 and thus facilitate hepcidin expression. Juvenile hemochromatosis, or HH types 2a and 2b, is a rare condition; mutations in *HFE2* and *HAMP*, in types 2a and 2b, respectively, result in almost complete loss of hepcidin synthesis. Type 3 hemochromatosis is characterized by TFR2 mutations, and type 4 involves mutation of *SLC40A1*, which encodes the iron exporter ferroportin. The severity of iron overload depends upon the level of hepcidin deficiency. Iron hyperabsorption and rapid iron release from macrophages can occur. As the result of large iron flux, the iron-binding capacity of transferrin is exceeded, causing nontransferrin-bound iron (NTBI) levels to rise in the bloodstream. NTBI accumulates in the liver and other parenchyma [8,10,24,25].

Alcoholic liver disease

Alcoholic liver disease is one of the major liver diseases in the developed countries and is characterized by hepatic iron overload in approximately 50% of all patients. According to the literature, chronic alcohol consumption in moderate to excessive amounts is associated with elevation of serum ferritin concentration and transferrin saturation, and can result in increased hepatic iron stores. Additionally, increased intestinal iron absorption has been documented in patients with ALD. Iron and ethanol each cause oxidative stress and lipid peroxidation and the cumulative effects of ethanol and iron on liver cell damage, in patients with ALD, exacerbate liver injury. Therefore, iron overload is an independent factor of disease progression in hepatocellular carcinoma and it determines patient survival [26,27].

ALD includes a spectrum of liver disorders ranging from fatty liver and hepatic inflammation and necrosis (alcoholic hepatitis) to progressive fibrosis (alcoholic cirrhosis) [28]. Serum iron markers are elevated in alcohol drinkers from an early age [11]. The characteristics of iron distribution disorders are shown in Table 1. In patients with ALD, ferritin levels are often markedly elevated, and serum iron and transferrin saturation can also be greatly elevated. Additionally, in these patients low hepcidin levels are usually found along with iron deposition in macrophages. There are 2 opposing, hepcidin-regulating, stimuli that occur in ALD. On the one hand, alcohol-induced endoplasmic reticulum (ER) stress and inflammation leads to hepcidin upregulation, while on the other, ethanol downregulates the mRNA- and DNA-binding activity of CCAAT/enhancer binding protein (C/EBP), alpha (C/EBP α , also known as CEBPA), which is the main transcription factor regulating hepcidin expression. According to the literature, the down-regulation of hepcidin expression leads to up-regulation

Table 1. The characteristics of iron distribution disorders.

Liver disease	Ferritin	Serum iron	TfS	Hepcidin	M	Parenchyma
ALD	↑↑	↑,↑↑	↑,↑↑	↓	+	+
NAFLD	↑	↔	↑, ↔	↑	-	+
HCV	↑, ↔	↑, ↔	↑, ↔	↓	-	+

ALD – alcoholic liver disease; NAFLD – nonalcoholic fatty liver disease; HCV – hepatitis C virus infection; TfS – transferrin saturation; M – macrophages – spleen, bone marrow, liver Kupffer cells; ↑ – elevated; ↑↑ – markedly elevated; ↔ – unchanged; ↓ – decreased); + present; – absent.

of iron transporters expression in the duodenum. This affects ferroportin and DMT1 and leads to increased iron absorption in the duodenum. These observations could explain iron metabolism disturbances in ALD [11,27,28], and were recently confirmed by Dostalikova-Cimburowa et al. [27] in patients with ALD. Alcohol consumption very probably causes suppression of hepcidin expression in patients with ALD. Taken together, iron overload observed in patients with ALD is mediated by regulatory mechanisms, and the alcohol-mediated down-regulation of hepcidin synthesis in the liver may be one of the dominant underlying mechanisms of iron overload. Interestingly, new reports indicate that the presence of iron may be predictive of death and hepatocellular carcinoma (HCC) development in patients with alcoholic cirrhosis [11,28].

Nonalcoholic fatty liver disease

As the prevalence of obesity and MS rises, NAFLD with or without associated MS has become the most prevalent form of chronic liver disease. It is commonly considered as a hepatic manifestation of MS and is thought to be involved in the pathogenesis of cardiovascular diseases [11,29,30]. Nonalcoholic steatohepatitis (NASH), a more severe form of NAFLD, is characterized by chronic and progressive liver pathology and may lead to fibrosis, liver cirrhosis, end-stage liver disease, and hepatocellular carcinoma [5]. Perturbations of iron homeostasis are often seen in NAFLD patients. Characteristic findings in NAFLD patients are elevated iron stores as indicated by hyperferritinemia, with normal or mildly elevated transferrin saturation and mostly mild liver iron deposition in liver biopsies (hemosiderosis). Serum ferritin levels range from mild elevations to 1000–1500 ng/ml. Its concentrations increase with the number of MS components. Transferrin saturation is in the upper range or normal or mildly elevated (45–50%) (Table 1). On the other hand, in patients with hemochromatosis, high ferritin levels are associated with markedly elevated transferrin saturation [31]. A recently introduced term, dysmetabolic or insulin-resistance hepatic iron overload syndrome (DIOS or IR-HIO, respectively) has been used in cases of unexplained hepatic iron overload characterized by high serum ferritin levels, normal serum iron, and associated metabolic

abnormalities [32–35]. IR-HIO is detected in one-third to half of patients with NAFLD [31,32]. It is important to note that hyperferritinemia in patients with NAFLD and/or the MS overestimates the degree of iron overload compared to hemochromatosis. It is assumed that in NAFLD patients, the iron overload, characterized by mild degree of body iron excess compared to often markedly elevated serum ferritin levels, is a consequence of a combination of alimentary and inflammatory driven iron loading and retention [31]. Thus, elevated ferritin may be found in the MS without evidence of iron excess [9]. The origin of excess iron in NAFLD is still uncertain. Insulin was suggested to cause increased liver iron uptake due to its ability to redistribute transferrin receptors from an intracellular membrane compartment to the cell surface [31,32,36]. The first report of an impact of iron stores in non-hemochromatotic metabolic diseases was described in 1981 by Sullivan [37]. He observed that postponed occurrence of cardiovascular diseases in women compared to men and the subsequent postmenopausal increase could be caused by low premenopausal iron stores [31,37]. The association of higher iron store with several clinical manifestations of IR and MS has been repeatedly confirmed in other studies [9,38]. Moreover, iron overload can worsen IR by interfering with insulin receptor signaling and inhibiting the ability to burn carbohydrates in the liver and muscle. It is well documented that liver and peripheral IR increase and pancreatic insulin secretion decrease as level of body iron rises [9].

Some authors found an association of iron with more progressed stages or higher incidence of NAFLD. In the study by Kowdley et al. [39], iron deposition in NAFLD liver biopsies was associated with more advanced stages of fibrosis and disease severity. Additionally, excess liver iron may also be involved in the pathogenesis of hepatocellular carcinoma in NASH patients [9]. Recently, Meli et al. [40] reported early impairment of iron metabolism in the initial stage of steatosis that contributes to disease progression. They hypothesized that the concept of “multiple hits” in the progression of inflammation in NAFLD patients might include the early alteration of iron metabolism. In summary, the prevailing body of evidence suggests that excess iron is a contributing factor for progression

of steatosis to NASH, cirrhosis, or liver carcinoma. Besides directly inducing liver damage, excess iron is involved in the pathogenesis of MS by inducing adipose tissue IR and modifying the release of adipokines [41]. Interestingly, iron removal was found to improve coronary vascular dysfunction and endothelial function in patients with diabetes and coronary artery disease with hemochromatosis. These findings are important, considering the recent observations regarding the increased cardiovascular morbidity and mortality in NAFLD patients [31].

Recent research has underlined the role of hepcidin in NAFLD. Increased adipose tissue expression of hepcidin is associated with inflammation in obese individuals, independent of steatosis and nonalcoholic steatohepatitis (NASH). Furthermore, leptin expression is correlated with hepcidin levels in obese children and appears to enhance hepcidin mRNA expression *in vitro*. Additionally, leptin was found to up-regulate hepcidin transcription in hepatocyte cultures via JAK2/STAT3-dependent signaling pathways. Therefore, leptin-induced hepcidin synthesis may favor iron perturbations in NAFLD patients [9,42]. On the other hand, necrosis can lead to iron leakage, which, in combination with phagocytosis by liver macrophages and increased levels of hepcidin (induced by inflammatory cytokines), results in iron accumulation in Kupffer's cells. Moreover, an *in vivo* study reported a highly significant correlation in patients with DIOS between hepcidin levels and TNF- α , indicating that proinflammatory milieu also contribute to hepcidin production in NAFLD patients. From the clinical point of view, in NAFLD patients increased hepcidin and proinflammatory cytokines can be derived from adipose tissue and the liver [9,43]. Mesenchymal iron deposition is more common than hepatocellular iron accumulation, but usually both compartments are affected. This is in contrast to tissue iron deposition in hemochromatosis, in which the iron is almost exclusively found in the hepatocellular compartment and macrophages are iron deficient [31]. Similar to the mechanism described for ALD, hepcidin downregulation may be a consequence of oxidative stress [5,11,32–35,44]. In NAFLD patients with liver iron on histology, the duodenal iron exporter ferroportin is lower in comparison to NAFLD patients without liver iron accumulation and controls [45]. In summary, both iron and inflammation contribute to hepcidin synthesis in NAFLD patients.

Hepatitis C virus infection

Approximately 170 million people worldwide are infected with hepatitis C virus [46]. Infection with HCV is often asymptomatic, but can lead to severe liver damage. The pathogenesis of HCV infection is not yet fully understood, but according to research data, a chronic inflammation state and excess iron play an important role in the pathogenesis of chronic hepatitis C, as well as in the hepatocarcinogenesis [46,47]. For example, Kato et al. [48] found that phlebotomy procedures are

associated with lower risk of progression to hepatocellular carcinoma in patients affected with chronic hepatitis C virus infection. This data indicate a critical role of iron in hepatocellular carcinoma in patients with HCV [46]. However, the mechanisms linking hepatic iron overload with disease progression and its contribution to hepatocarcinogenesis in chronic hepatitis C are not fully understood [46]. Iron accumulation in the liver and iron overload have been found mainly in patients infected with HCV rather than in those infected with other types of viral hepatitis. It has been showed that about 30–40% of HCV patients have elevated serum iron and ferritin, as well as transferrin saturation (Table 1) [49,50]. According to the literature, hepatic iron content in patients with HCV is approximately 2–5 times the normal hepatic iron content if the liver weight is estimated to be 1500 g, which is much less than in hereditary hemochromatosis [46,50]. It is unclear whether iron predominantly accumulates in hepatocytes or the reticuloendothelial system (Kupffer cells) in HCV patients. Some authors have reported that iron was mainly localized in the Kupffer cells, whereas others reported its localization in hepatocytes [46,51,52]. However, iron accumulation in hepatocytes can indicate potential DNA damage and genetic instability in association with HCV-induced oxidative stress. On the other hand, iron deposition in Kupffer cells can contribute to cytokine release, leading to inflammation or fibrosis [46]. Recent findings have highlighted that hepcidin transcription is suppressed in the presence of HCV infection. HCV decreases hepcidin levels through histone acetylation. The HCV core protein induces the production of reactive oxygen species, increases hepatic expression of CCAAT/enhancer-binding protein (C/EBP) homology protein (CHOP), and subsequently reduces DNA binding activity of C/EBP α , which leads to reduction of hepcidin transcription. Consequently, decreased hepcidin expression increases ferroportin expression in the enterocytes and reticuloendothelial macrophages, resulting in increased duodenal iron transport and macrophage iron release, which lead to liver iron accumulation. Additionally, inflammation can also have the opposing effects of stimulation and suppression of hepcidin transcription through the interleukin (IL)-6/signal transducer and activator of transcription (STAT) pathway and reactive oxygen species pathway, respectively. In summary, relative suppression of hepcidin expression is a possible mechanism responsible for the hepatic iron accumulation in patients with HCV [46]. Researchers have also found that hepcidin attenuates HCV expression and reduces HCV replication in cell cultures via STAT3 activation. STAT3 itself is involved in hepcidin regulation; this suggests that a positive feedback system boosts the antiviral effects of hepcidin. In chronic HCV infection, hepatic steatosis is correlated with higher serum iron concentrations and transferrin saturation [53,54]. The hypothesis for this finding is that iron and HCV work together to cause fibrosis. Interestingly, recent studies have shown an improved clinical response in patients who were treated with combined

phlebotomy and interferon monotherapy compared to those treated with interferon alone. Other recent studies indicate that iron reduction maintained over several years results in histopathological improvement and reduced risk of HCC [55,56].

Autoimmune liver diseases

Very recently, Lyberopoulou et al. [57] published about a study on liver hepcidin gene expression, serum hepcidin levels, and hepcidin/ferritin ratios in patients with various chronic liver diseases, and including for the first time patients with autoimmune liver disorders. They correlated these measurements with the clinical, histological, and laboratory data of their patients. In their analysis both serum hepcidin and the serum hepcidin/ferritin ratio were significantly lower in autoimmune hepatitis patients and patients with autoimmune cholestatic diseases (primary biliary cirrhosis and primary sclerosing cholangitis) compared to patients with chronic hepatitis B and C virus infection and compared to those with NAFLD, and correlated negatively with serum alkaline phosphatase levels. Patients with autoimmune hepatitis and those with autoimmune cholestatic diseases maintained low serum hepcidin during the course of their 2-year treatment. The authors concluded that parallel determination of hepcidin expression levels in liver biopsies and sera of patients with different liver diseases has revealed that serum hepcidin levels and their corresponding ratios to ferritin are lower in patients with autoimmune liver diseases in comparison to other investigated liver diseases [57].

Oxidative Stress and Iron Deposit Placement

The liver is the main organ affected by the oxidative stress caused by iron-overload toxicity. When transferrin saturation is increased by more than 75%, NTBI begins to accumulate [54]. NTBI is potentially toxic due to its high propensity to induce reactive oxygen species (ROS), and it can cause cellular damage both at the plasma level and in intracellular organelles. Liver injury during iron overload may be caused by NTBI and iron deposits [58,59].

ROS are normally produced by metabolic functions in the cell. Iron, in conjunction with ROS, leads to an increase in hydroxyl radicals and, consequently, cellular damage. Through Haber-Weiss and Fenton reactions, iron generates ROS [59,60]. The reduction of ferric iron to ferrous iron is mediated by the superoxide radical, which then reacts with hydrogen to form highly reactive hydroxyl radicals. Hydroxyl radicals cause an increase in phospholipid peroxidation, oxidation of amino acid side chains, DNA strand breaks, and protein fragmentation [10,61].

Iron deposits are localized in 1 of 3 possible histological patterns: hepatocellular (HC) or parenchymal, reticuloendothelial

system cells (RES), or mesenchymal and mixed RES/HC. In early HH, iron is localized within hepatocytes and distributed in a decreasing gradient from the periportal to the centrolobular areas. This represents a typical parenchymal iron overload pattern. With time, periportal sideronecrosis occurs, resulting in macrophage activation followed by fibrosis and the redistribution of iron to non-parenchymal cells. Iron accumulation in patients with DIOS and ALD is characterized by a mixed RES/HC pattern and is associated with milder iron overload [62,63]. During HCV infection, iron deposits are found mostly in mesenchymal and endothelial cells. Iron deposits in patients with HCV infection are associated with activation of hepatic stellate cells, leading to their proliferation and differentiation into myofibroblast-like cells. This process results in liver damage [64,65]. Research by Nelson et al. identified a relationship between the placement of hepatic iron deposits and the histological severity of NAFLD. According to Nelson et al., percentages of advanced fibrosis, portal inflammation, hepatocellular ballooning, and definitive NASH were higher in histological samples with an RES iron-staining pattern [63].

Future Perspectives

To date, iron overload has been treated with phlebotomy and iron chelators. Disadvantages of phlebotomy are concurrent anemias, difficulties for people with poor vascular access, adverse physiological response to phlebotomy, fear, religious beliefs, and lack of long-term convenient access to phlebotomy centers. Iron chelators have multiple adverse effects, and compliance is often suboptimal. Hepcidin deficiency plays a key role in iron overload; thus, influencing hepcidin production or administering hepcidin substitutes could result in improved treatment of conditions resulting in iron overload [8].

Recent discoveries have yielded promising results. Problems associated with the use of natural hepcidin have been obviated by the use of synthetic minihepcidins. Minihepcidins are small peptides that mimic hepcidin activity and have been used to ameliorate iron overload in mice. Natural hepcidin is expensive and has unfavorable pharmacological properties. Bioactive hepcidin has 4 disulfide bonds, and a high dosage is required for optimal therapeutic effects, which would make therapy with these molecules costly. From a pharmacological perspective, natural hepcidin has a short half-life due to rapid kidney excretion and a low per oral absorption because of its large size. By contrast, minihepcidins are small peptides of 7–9 amino acids, which can be administered orally. They were rationally designed based on the region of hepcidin that interacts with ferroportin. Experiments conducted on mouse models of HH showed a significant decrease in iron load after intraperitoneal injections of minihepcidin. Redistribution of iron from the liver to the spleen and macrophages was observed.

Unlike hepatocytes, macrophages are relatively resistant to iron-related oxidative stress [8,66].

Another therapeutic approach is to potentiate endogenous hepcidin production. Two tactics that have been explored are inactivation of TMPRSS6 and administration of BMP6 agonists. Inactivation of TMPRSS6 has thus far been accomplished either via antisense oligonucleotides (ASOs) or small interfering RNAs (siRNAs). ASOs trigger the degradation of their target mRNA based on the RNaseH mechanism, while siRNAs inhibit expression of target genes through the RNA interference pathway. Experiments on mice yielded positive results with both approaches. BMP6 agonists are associated with increased BMP signaling and consequently increased hepcidin expression. However, peritoneal calcification has been observed in experimental models. Researchers are now shifting their focus to BMP type I receptor inhibitors (e.g., LDN-193189) and soluble forms of ALK3 (e.g., ALK3-Fc), which could be used to

treat microcytic anemia by preventing increased hepcidin expression [8,16,67].

Conclusions

Iron homeostasis is crucial for normal functioning in humans. The liver plays a critical role in the regulation of iron levels, particularly through production of hepcidin. In patients with chronic liver disease, iron metabolism changes result in iron overload. This is believed to occur mostly due to low levels of hepcidin. Iron deposits and NTBI are responsible for further damage to the liver by inflicting oxidative stress on hepatocytes. These findings suggest that future studies should focus on the production of the hepcidin agonists minihepcidins and on manipulating various metabolic pathways to increase endogenous production of hepcidin.

References:

- Kim Y, Ejaz A, Tayal A et al: Temporal trends in population-based death rates associated with chronic liver disease and liver cancer in the United States over the last 30 years. *Cancer*, 2014; 120: 3058–65
- Milic S, Stimac D: Nonalcoholic fatty liver disease/steatohepatitis: epidemiology, pathogenesis, clinical presentation and treatment. *Dig Dis*, 2012; 30: 158–62
- Liu Y, Meyer C, Xu C et al: Animal models of chronic liver diseases. *Am J Physiol Gastrointest Liver Physiol*, 2013; 304: G449–68
- Ramachandran P, Iredale JP: Liver fibrosis: a bidirectional model of fibrogenesis and resolution. *QJM*. 2012;105: 813-817.
- Basaranoglu M, Basaranoglu G, Sentürk H: From fatty liver to fibrosis: A tale of “second hit”. *World J Gastroenterol*, 2013; 19: 1158–65
- Valenti L, Fracanzani AL, Dongiovanni P et al: A randomized trial of iron depletion in patients with nonalcoholic fatty liver disease and hyperferritinemia. *World J Gastroenterol*, 2014; 20: 3002–10
- Abu Rajab M, Guerin L, Lee P, Brown KE: Iron overload secondary to cirrhosis: a mimic of hereditary haemochromatosis? *Histopathology*, 2014; 65: 561–69
- Fung E, Nemeth E: Manipulation of the hepcidin pathway for therapeutic purposes. *Haematologica*, 2013; 98: 1667–76
- Datz C, Felder TK, Niederseer D, Aigner E: Iron homeostasis in the metabolic syndrome. *Uer J Clin Invest*, 2013; 42: 215–24
- Anderson ER, Shah YM: Iron homeostasis in the liver. *Compr Physiol*, 2013; 3: 315–30
- Corradini E, Pietrangeli A: Iron and steatohepatitis. *J Gastroenterol Hepatol*, 2012; 27(Suppl.2): 42–46
- Muñoz M, Villar I, García-Erce JA: An update on iron physiology. *World J Gastroenterol*, 2009; 15: 4617–26
- Wang J, Pantopoulos K: Regulation of cellular iron metabolism. *Biochem J*, 2011; 434(Pt 3): 365–81
- Meynard D, Babitt JL, Lin HY: The liver: Conductor of systemic iron balance. *Blood*, 2014; 123: 168–76
- Nelson JE, Brunt EM, Kowdley KV: Lower serum hepcidin and greater parenchymal iron in nonalcoholic fatty liver disease patients with C282Y HFE mutations. *Hepatology*, 2012; 56: 1730–40
- Zhao N, Zhang AS, Enns CA: Iron regulation by hepcidin. *J Clin Invest*, 2013; 123: 2337–43
- Andriopoulos B Jr, Corradini E, Xia Y et al: BMP6 is a key endogenous regulator of hepcidin expression and iron metabolism. *Nat Genet*, 2009; 41: 482–87
- D'Alessio F, Hentze MW, Muckenthaler MU: The hemochromatosis proteins, HFE, TFR2, and HJV form a membrane-associated protein complex for hepcidin regulation. *J Hepatol*, 2012; 57: 1052–60
- Bridle KR, Frazer DM, Wilkins SJ et al: Disrupted hepcidin regulation in HFE-associated haemochromatosis and the liver as a regulator of body iron homeostasis. *Lancet*, 2003; 361: 669–73
- Ravasi G, Pelucchi S, Trombini P et al: Hepcidin expression in iron overload diseases is variably modulated by circulating factors. *PLoS One*, 2012; 7(5): e36425
- Dostalíkova-Cimbuřova M, Kratka K, Stranský J et al: Iron overload and HFE gene mutations in Czech patients with chronic liver diseases. *Dis Markers*, 2012; 32: 65–72
- Hentze MW, Muckenthaler MU, Galy B, Camaschella C: Two to tango: Regulation of Mammalian iron metabolism. *Cell*, 2010; 142: 24–38
- Sebastiani G, Pantopoulos K: Disorders associated with systemic or local iron overload: From pathophysiology to clinical practice, *Metallomics*, 2011; 3: 971–86
- Taddei T, Mistry P, Schilsky ML: Inherited metabolic disease of the liver. *Curr Opin Gastroenterol*, 2008; 24: 278–86
- Swinkels DW, Fleming RE: Novel observations in hereditary hemochromatosis: Potential implications for clinical strategies. *Haematologica*, 2011; 96: 485–88
- Mueller S, Rausch V: The role of iron in alcohol-mediated hepatocarcinogenesis. *Adv Exp Med Biol*, 2015; 815: 89–112
- Dostalíkova-Cimbuřova M, Balusikova K, Kratka K et al: Role of duodenal iron transporters and hepcidin in patients with alcoholic liver disease. *J Cell Mol Med*, 2014; 18: 1840–50
- Costa-Matos L, Batista P, Monteiro N et al: Hfe mutations and iron overload in patients with alcoholic liver disease. *Arq Gastroenterol*, 2013; 50: 35–41
- Fujita N, Takei Y: Iron overload in nonalcoholic steatohepatitis. *Adv Clin Chem*, 2011; 55: 105–32
- Sumida Y, Yoshikawa T, Okanou T: Role of hepatic iron in non-alcoholic steatohepatitis. *Hepatol Res*, 2009; 39: 213–22
- Aigner E, Weiss G, Datz C: Dysregulation of iron and copper homeostasis in nonalcoholic fatty liver. *World J Hepatol*, 2015; 7: 177–88
- Dongiovanni P, Fracanzani AL, Fargion S, Valenti L: Iron in fatty liver and in the metabolic syndrome: a promising therapeutic target. *J Hepatol*, 2011; 55: 920–32
- Barisani D, Pelucchi S, Mariani R et al: Hepcidin and iron-related gene expression in subjects with Dysmetabolic Hepatic Iron Overload. *J Hepatol*, 2008; 49: 123–33

34. Trombini P, Paolini V, Pelucchi S et al: Hepcidin response to acute iron intake and chronic iron loading in dysmetabolic iron overload syndrome. *Liver Int*, 2011; 31: 994–1000
35. Riva A, Trombini P, Mariani R et al: Reevaluation of clinical and histological criteria for diagnosis of dysmetabolic iron overload syndrome. *World J Gastroenterol*, 2008; 14: 4745–52
36. Manco M, Alisi A, Fernandez Real JM et al: Early interplay of intra-hepatic iron and insulin resistance in children with non-alcoholic fatty liver disease. *J Hepatol*, 2011; 55: 647–53
37. Sullivan JL: Iron and sex difference in heart disease risk. *Lancet*, 1981; 1: 1293–94
38. Wood RJ: The iron-heart disease connection: is it dead or just hiding? *Ageing Res Rev*, 2004; 3: 355–67
39. Kowdley KV, Belt P, Wilson LA et al: Serum ferritin is an independent predictor of histologic severity and advanced fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology (Baltimore, MD)*, 2012; 55: 77–85
40. Meli R, Mattace Raso G, Irace C et al: High fat diet induces liver steatosis and early dysregulation of iron metabolism in rats. *PLoS One*, 2013; 8(6): e66570
41. Valenti L, Fracanzani AL, Dongiovanni P et al: A randomized trial of iron depletion in patients with nonalcoholic fatty liver disease and hyperferritinemia. *World J Gastroenterol*, 2014; 20: 3002–10
42. Chung B, Matak P, McKie AT, Sharp P: Leptin increases the expression of the iron regulatory hormone hepcidin in HuH7 human hepatoma cells. *J Nutr*, 2007; 137: 2366–70
43. Aigner E, Theurl I, Theurl M et al: Pathways underlying iron accumulation in human nonalcoholic fatty liver disease. *Am J Clin Nutr*, 2008; 87: 1374–83
44. Zumerle S, Mathieu JR, Delga S et al: Targeted disruption of hepcidin in the liver recapitulates the hemochromatotic phenotype. *Blood*, 2014; 123: 3646–50
45. Barisani D, Pelucchi S, Mariani R et al: Hepcidin and iron-related gene expression in subjects with Dysmetabolic Hepatic Iron Overload. *J Hepatol*, 2008; 49: 123–33
46. Hino K, Nishina S, Hara Y: Iron metabolic disorder in chronic hepatitis C: Mechanisms and relevance to hepatocarcinogenesis. *J Gastroenterol Hepatol*, 2013; 28(Suppl.4): 93–98
47. Tanaka H, Fujita N, Sugimoto R et al: Hepatic oxidative DNA damage is associated with increased risk for hepatocellular carcinoma in chronic hepatitis C. *Br J Cancer*, 2008; 98: 580–86
48. Kato J, Miyanishi K, Kobune M et al: Long-term phlebotomy with low-iron diet therapy lowers risk of development of hepatocellular carcinoma from chronic hepatitis C. *J Gastroenterol*, 2007; 42: 830–36
49. Georgopoulou U, Dimitriadis A, Foka P et al: Hepcidin and the iron enigma in HCV infection. *Virulence*, 2014; 4: 465–64
50. Rulyak SJ, Eng SC, Patel K et al: Relationships between hepatic iron content and virologic response in chronic hepatitis C patients treated with interferon and ribavirin. *Am J Gastroenterol*, 2005; 100: 332–37
51. Bonkovsky HL, Banner BF, Rothman AL: Iron and chronic viral hepatitis. *Hepatology*, 1997; 25: 759–68
52. Brunt EM: Pathology of hepatic iron overload. *Semin Liver Dis*, 2005; 25: 392–401
53. Nishina S, Korenaga M, Hidaka I et al: Hepatitis C virus protein and iron overload induce hepatic steatosis through the unfolded protein response in mice. *Liver Int*, 2010; 30: 683–92
54. Sikorska K, Stalke P, Romanowski T et al: Liver steatosis correlates with iron overload but not with HFE gene mutations in chronic hepatitis C. *Hepatobiliary Pancreat Dis Int*, 2013; 12: 377–84
55. Liu H, Trinh TL, Dong H et al: Iron regulator hepcidin exhibits antiviral activity against hepatitis C virus. *PLoS One*, 2012; 7(10): e46631
56. Ryan Caballes F, Sendi H, Bonkovsky HL: Hepatitis C, porphyria cutanea tarda and liver iron: an update. *Liver Int*, 2012; 32: 880–93
57. Lyberopoulou A, Chachami G, Gatselis NK et al: Low serum hepcidin in patients with autoimmune liver diseases. *PLoS One*, 2015; 10(8): e0135486
58. Brisson P, Ropert M, Le Lan C, Loréal O: Non-transferrin bound iron: A key role in iron overload and iron toxicity. *Biochim Biophys Acta*, 2012; 1820: 403–10
59. Thompson JW, Bruick RK: Protein degradation and iron homeostasis. *Biochim Biophys Acta*, 2012; 1823: 1484–90
60. Ucar F, Sezer S, Erdogan S et al: The relationship between oxidative stress and nonalcoholic fatty liver disease: Its effects on the development of non-alcoholic steatohepatitis. *Redox Rep*, 2013; 18: 127–33
61. Ivanov AV, Bartosch B, Smirnova OA et al: HCV and oxidative stress in the liver. *Viruses*, 2013; 5: 439–69
62. Deugnier Y, Turlin B: Pathology of hepatic iron overload. *Semin Liver Dis*, 2011; 31: 260–71
63. Nelson JE, Wilson L, Brunt EM et al: Relationship between the pattern of hepatic iron deposition and histological severity in nonalcoholic fatty liver disease. *Hepatology*, 2011; 53: 448–57
64. Maliken BD, Nelson JE, Klintworth HM et al: Hepatic reticuloendothelial system cell iron deposition is associated with increased apoptosis in nonalcoholic fatty liver disease. *Hepatology*, 2013; 57: 1806–13
65. Martinelli AL, Ramalho LN, Zucoloto S: Hepatic stellate cells in hepatitis C patients: Relationship with liver iron deposits and severity of liver disease. *J Gastroenterol Hepatol*, 2004; 19: 91–98
66. Preza GC, Ruchala P, Pinon R et al: Minihepcidins are rationally designed small peptides that mimic hepcidin activity in mice and may be useful for the treatment of iron overload. *J Clin Invest*, 2011; 121: 4880–88
67. Poli M, Asperti M, Ruzzenenti P et al: Hepcidin antagonists for potential treatments of disorders with hepcidin excess. *Front Pharmacol*, 2014; 5: 86