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TOPIC HIGHLIGHT

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Iron overload and cofactors with special reference to alcohol, hepatitis C virus infection and steatosis/insulin resistance

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Abstract

There are several cofactors which affect body iron metabolism and accelerate iron overload. Alcohol and hepatic viral infections are the most typical examples for clarifying the role of cofactors in iron overload. In these conditions, iron is deposited in hepatocytes and Kupffer cells and reactive oxygen species (ROS) produced through Fenton reaction have key role to facilitate cellular uptake of transferrin-bound iron. Furthermore, hepcidin, antimicrobial peptide produced mainly in the liver is also responsible for intestinal iron absorption and reticuloendothelial iron release. In patients with ceruloplasmin deficiency, anemia and secondary iron overload in liver and neurodegeneration are reported. Furthermore, there is accumulating evidence that fatty acid accumulation without alcohol and obesity itself modifies iron overload states. Ineffective erythropoiesis is also an important factor to accelerate iron overload, which is associated with diseases such as thalassemia and myelodysplastic syndrome. When this condition persists, the dietary iron absorption is increased due to the increment of bone marrow erythropoiesis and tissue iron overload will thereafter occurs. In porphyria cutanea tarda, iron is secondarily accumulated in the liver.

Key words: Iron overload; Cofactors; Alcohol; Chronic hepatic C; Non-alcoholic steatohepatitis; Insulin resistance; Hepatocellular carcinoma

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INTRODUCTION

In hereditary hemochromatosis, patients having HFE trait are more susceptible to iron overload when cofactors such as alcohol, hepatitis viruses, and abnormal porphyrin metabolism are present. Even in the absence of hereditary hemochromatosis, there are several conditions associated with secondary iron overload in which iron deposition is rather mild[1]. For example, in alcoholics and patients with chronic hepatitis C, intrahepatic iron is increased and liver injury is accelerated, followed by development of fibrosis, cirrhosis and hepatocellular carcinoma (HCC). In addition, abnormal copper metabolism and several causes for iron-loaded anemia are also important cofactors which influence the background iron overload. Furthermore, there is accumulating evidences that fatty acid accumulation without alcohol and obesity itself modifies insulin resistance through iron[2] and fibrogenesis of the liver[3]. In this review, the role of cofactors on iron overload will be discussed in three categories such as alcohol, hepatitis C virus infection and steatosis with obesity, the most common cofactors in liver iron overload.

COFACTORS AFFECTING BODY IRON METABOLISM AND IRON OVERLOAD

There are several factors which affect body iron metabolism and accelerates iron overload. Table 1 lists cofactors and disease conditions which are known to accelerate hepatic iron accumulation independent from responsible genes for hereditary hemochromatosis. Alcoholic and hepatic viral infections are the most typical examples for clarifying the role of cofactors in iron overload. In addition, abnormal copper metabolism and several causes for iron-loaded anemia such as thalassemia and myelodysplastic syndrome are also important factors which influence the background iron overload. When this condition persists, the dietary iron absorption is increased due to the increment of bone marrow erythropoiesis[4] and tissue iron overload will occur thereafter. These patients are usually anemic in spite of increased body iron stores (iron-

Table 1 Cofactors of iron overload

1	Alcohol (Alcoholic liver disease)
2	Infection (Hepatitis C virus infection, *etc*)
3	Obesity and insulin resistance (Nonalcoholic steatohepatitis)
4	Copper (Ceruloplasmin deficiency)
5	Porphyrin (Porphyria)
6	Ineffective erythropoiesis (Thalassemia, myelodysplastic syndrome)
7	Others

loaded anemia), and require frequent blood transfusions, which further exaggerate secondary iron overload, in which conditions of new oral iron chelators are effective[5]. In patients with ceruloplasmin deficiency, anemia and secondary iron overload in liver and neurodegeneration are reported[6]. Furthermore, there are accumulating evidences that fatty acid accumulation without alcohol and obesity itself modifies iron overload states. Ineffective erythropoiesis is also an important factor to accelerate iron overload. This condition is associated with diseases such as thalassemia, aplastic anemia, and myelodysplastic syndrome. In porphyria cutanea tarda, iron is secondarily accumulated in the liver and phlebotomy and oral iron chelators are effective as well as in hemochromatosis.

ALCOHOL

Alcohol is one of the most important cofactors to modify or enhance iron accumulation in the liver. Excess intake of alcohol induces alcoholic liver diseases (ALD) such as fatty liver, fibrosis, hepatitis, and cirrhosis, in which iron overload is frequently associated[7]. By Perls' iron stain, excess iron accumulation was found in hepatic tissues with ALD, but not in any normal hepatic tissues[8]. In ALD, iron is deposited in both hepatocytes and reticuloendothelial (Kupffer) cells. In advanced cases of ALD, which is also called as "alcoholic siderosis", the reticuloendothelial iron deposition is dominant. In earlier stages of ALD such as fatty liver and fibrosis, iron deposition is mild and is preferentially present in hepatocytes rather than in Kupffer cells, which finding is more frequently observed in Japanese patients who have mild clinical phenotype comparing with those in US[9].

The reactive oxygen species (ROS) produced play an important role in the development of ALD[10]. The expression of 4-hydroxy-2-nonenal (HNE)-protein adducts, which is a lipid peroxidative product is increased in oxidized hepatocytes[11]. Chronic alcohol ingestion in experimental animals is associated with oxidative stress as reflected by increased hepatic levels of lipid peroxidation products such as malondialdehyde and HNE, both of which have been implicated in hepatic fibrogenesis in the intragastric ethanol infusion model[12]. Furthermore, lipid peroxidation products induce gene expression of procollagen α-1 (I) and increase collagen production by several folds in cultured hepatic stellate cell[13]. In human ALD, there is a positive correlation between iron deposition and histological intensity of HNE-protein adduct[14]. As shown in Figure 1, the distribution of HNE-protein adducts and iron granules appeared to be identical, suggesting that iron may be associated with the production of HNE-protein adduct. As hepatic iron is visualized by Perls' reaction as an insoluble protein-bound iron such as hemosiderin, this form of iron may be inactive for the production of ROS. But, the free iron responsible for Fenton reaction should be present close to the protein-bound iron, and may be involved in the production of HNE-protein adducts. There are two pathways to generate ROS through ethanol metabolism. Oxidation of ethanol by alcohol dehydrogenase to form acetaldehyde, which is subsequently oxidized to acetate and ultimately carbon dioxide and water. During the oxidation process of acetaldehyde involving aldehyde oxidase and xanthine oxidase, superoxide (O_2^-) is produced[15]. In addition, cytochrome P450 is involved in the metabolism of ethanol, in which ROS are also generated in microsomes[16]. Among ROS, hydroxy radical (OH^-) is most potent, which is produced via Fenton reaction in the presence of free iron and the resulted OH^- can easily cause cell damage by oxidizing lipid, proteins, and nucleic acids. In an intragastric infusion mouse model of ALD, supplementation of carbonyl iron advanced peri-venular fibrosis to bridging fibrosis and cirrhosis[17]. Oxidative stress arising from hepatocytes and macrophage activates hepatic stellate cells by increasing the production of cytokines such as transforming growth factor-β (TGFβ), directly or indirectly. The dietary iron supplementation was associated with increased NF-κB activation[18], and the up

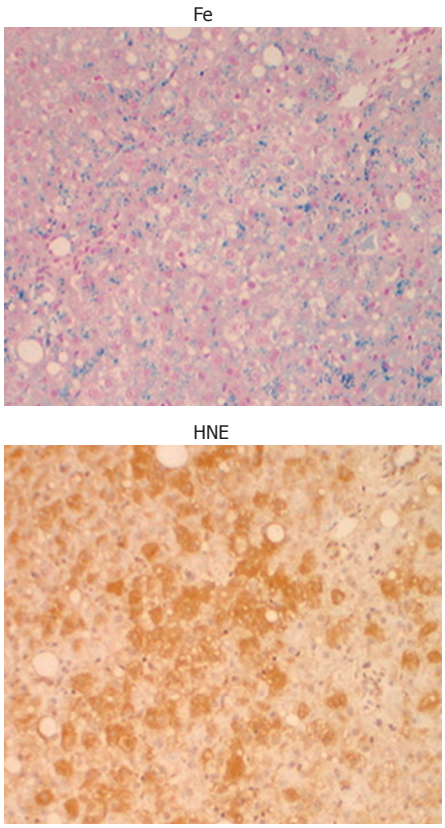

Figure 1 Iron staining and immunohistochemical staining of 4-hydroxy-2-nonenal-modified protein (HNE-protein) adducts in human alcoholic liver disease. The localization of HNE-protein adducts and iron in hepatocytes appeared to be identical (from ref. 14 with some modifications).

regulation of NF-κB responsive proinflammatory genes such as IL-1β, TNFα, and MIP-1[19].

In advanced cases of ALD, iron is accumulated more prominently in Kupffer cells than in hepatocytes, mainly due to repeated endotoxemia and hyper-cytokinemia of TNFα and IL-1β[20]. These cytokines induced hepatic uptake of transferrin iron *in vitro*[21] and *in vivo*[22]. In mild cases of ALD, iron is preferentially stained in hepatocytes, rather than in Kupffer cells, suggesting that hepatocyte is the main site of early iron storage in the liver. However, it is not clear why iron is accumulated in liver parenchymal cells of alcoholics in such conditions. Two possibilities can be drawn: one is the increased uptake of iron in hepatocytes, and another is the increased iron absorption through hepcidin, which is a newly found antimicrobial peptide, and is a negative regulator of iron absorption and reticuloendothelial iron releases[23]. Hepatocytes have several pathways for iron uptake. Iron in serum is usually bound to transferrin and iron-bound transferrin is taken up via transferrin receptor (TfR) with high affinity or via other unknown mechanism with greater capacity, but low affinity independent of high affinity receptor[24]. There are two molecules of transferrin receptor: transferrin receptor 1 (TfR1) and transferrin receptor 2 (TfR2). TfR1 has a high affinity to serum transferrin and considered to be functional, while the function of TfR2 is not clear yet, even though the TfR2 gene is responsible for genetic hemochromatosis[25]. In normal hepatocytes, TfR2 is constitutively expressed. But, TfR1 is down-regulated, suggesting that TfR1 does not contribute to the steady state hepatic iron uptake. Recently, Wallace *et al*[26] reported that homozygous TfR2 knockout mice had no TfR2 associated with typical iron overload, and there was no upregulation of hepcidin mRNA, suggesting that TfR2 is required to iron regulated expression and is involved in a pathway to HFE and hemojuvelin. In addition, DMT1 may be involved when serum iron concentration exceeds transferrin iron binding capacity[27]. It is noteworthy that TfR1 is regulated by cellular iron levels or oxidative stresses post-transcriptionally and it is possible that ethanol may augment TfR1 expression by producing oxidative stresses. According to immunohistochemical investigation, TfR1 expression was increased in hepatocytes in 80% of hepatic tissues with ALD, but was not detected in any normal hepatic tissues[28]. It is noteworthy that the mean duration of abstinence of patients who demonstrated positive TfR1 expression in hepatocytes was significantly shorter than that of patients who demonstrated negative TfR1 expression.

Ethanol exposure in the presence of iron to the primary cultured-hepatocytes demonstrated an increase of TfR expression, and this augmentation was suppressed by the inhibitor of alcohol dehydrogenase, 4-methyo pyrazole, but enhanced by a inhibitor of acetaldehyde dehydrogenase, cyanamide, suggesting that ethanol metabolite acetaldehyde itself is involved for the induction of TfR1 by ethanol[29]. By functional uptake assay using ^{59}Fe-transferrin, the additional ethanol exposure increased transferrin-iron uptake into hepatocytes, while non-transferrin-bound iron (NTBI) uptake[30] was not increased. It has been reported that TfR1 expression was up-regulated both transcriptionally[31] and posttranscriptio nally[32]. This regulation is induced either by iron deficiency state or oxidative stress such as H_2O_2 and nitric oxide via iron regulatory protein, IRP[33]. In addition to the direct cell toxicity, acetaldehyde produces free radicals[34] and free radicals modify IRP activity[35,36].

Table 2 Speculated effects of iron on HCV

1	Immunological modification (Immunological escape of HCV)
	Decrease of Th1 activity
	Impaired function of macrophage and Kupffer cells
	Decrease of innate immunity (Natural resistance macrophage protein 2)
2	Increase of liver toxicity by iron-mediated radical formation
	Reactive oxygen production through fenton reaction
	Induction of apoptosis
	Acceleration of fibrinogenesis
	DNA damage and carcinogenesis
3	Effect on cell signalling
	Decrease of interferon responsiveness by NFκB activation
4	HCV proliferation
	Activation of translation initiation factor 3 (eIF3)
	Suppression of HCV RNA polymerase (NS5B) activity

Body iron homeostasis is strictly regulated by a balance between the processes such as dietary iron absorption in enterocytes, iron transport by transferrin in circulation, iron utilization and storage in bone marrow and liver. The increase of intestinal iron absorption was one of the mechanisms of the hepatic iron deposition in alcoholics[37]. In patients with hereditary hemochromatosis, serum pro-hepcidin is lower than that of normal controls, suggesting that iron absorption is increased in spite of high iron storage[38]. It is speculated that down-regulation of hepcidin might be one of important factors for pathogenesis of iron overload in ALD[39]. Serum pro-hepcidin concentration in ALD was significantly lower than that in healthy subjects, and pro-hepcidin/ferritin ratios in ALD were lower than healthy subjects[40]. In the ethanol-loaded mouse model which has a mild steatotic change, the hepcidin mRNA and protein expression were significantly lower than that of control. In addition, alcohol-loading might disrupt the sensing signal of inflammatory cytokines, and then down-regulate hepcidin expression, following the increased iron absorption from small intestine. Recently, the mechanism of hepcidin downregulation by alcohol has been elucidated: a decreased hepcidin expression in mouse liver is accompanied with an increase of DMT1 and ferroportin1, and a decrease of hepcidin promoter activity and DNA-binding activity of CCAAT/enhancer-binding, protein α (C/EBPα)[41].

HEPATITIS C VIRUS INFECTION

Hepatitis C virus infection is one of the most common disorders in liver diseases involving chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). Table 2 summarizes the effect of iron on hepatitis C virus infection. In the Third National Health and Nutrition Examination Survey, HCV infection is significantly associated with higher serum levels of ferritin and iron in the US population[42]. The mean serum levels of

ferritin and iron were significantly higher among subjects with HCV infection than among subjects without liver disease[43]. In addition, serum ferritin levels were directly and significantly correlated with serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyl transpeptidase, whereas platelet counts were inversely correlated with serum ferritin. It is also found that lipid peroxidative products such as malondialdehyde are increased in hepatic tissues with CH-C[44]. In 1994, an initial report was published that phlebotomy was effective in improving the serum ALT level in patients with CH-C[45] and a national prospective study confirmed the results[46]. Since then, it was reported that hepatic iron accumulation in CH-C predict a response to interferon (IFN) therapy[47], and phlebotomy before and during IFN therapy improved virological and histological response to short-term IFN therapy evaluated at the end-of-treatment[48]. This observation is reasonable considering the finding that oxidative stress impairs interferon alpha signal by blocking JAK-STAT pathway[49]. The standard therapy for hepatitis C is now a combined therapy of interferon-α and ribavirin, in which patients with viral response to treatment seemed to develop higher soluble transferrin receptor levels[50] with decline in serum iron and ferritin than non-responders, revealing intracellular reduction of iron store depending on the result of treatment including hemolytic reaction by ribavirin[51]. This is an interesting observation that decrease of iron status may be an additional effect of the combination therapy with interferon and ribavirin. Moreover, HFE mutations are also associated with increased sustained virologic responses by antiviral long-term treatment, while it is well known that HFE mutations are associated with increased iron loading[52]. However, some reports suggest that iron depletion was unable to trigger interferon response, so that there are conflicting data. It should be further investigated whether hepatic iron content modify the response to interferon[53,54]

From these observations, iron and related molecules seem to be key factors in the hepatocytes to influence the disease condition of CH-C, and also development of cirrhosis and maybe hepatocellular carcinoma. Clinical data on phlebotomy on CH-C generally indicates that phlebotomy does not influence the viral load in vivo. On the other hand, in vitro study on HCV replication is controversial: iron promotes HCV translation by up-regulating expression of the translation initiation factor eIF3 by reporter assay[55], whereas iron suppresses HCV replication by inactivating the RNA polymerase NS5B[56].

As previously described, hepatocytes have two iron uptake systems, transferrin-mediated and nontransferrin-bound iron-mediated pathway. Transferrin and TfR1 are molecules involved in the classical pathway of cellular iron uptake, but are faintly expressed in normal hepatocytes, and is down-regulated in iron-loaded hepatic tissues with hemochromatosis. Concerning the post-transcriptional regulation of TfR1, two mechanisms are postulated through the activity change of IRP which is already mentioned. In CH-C, TfR1 expression was up-regulated and DMT1 expression was down-regulated in the condition of hepatic excess iron accumulation, suggesting that regulation of DMT1 expression is iron-dependent, but that of TfR1 expression is iron-independent in CH-C[57]. In patients with CH-C, serum values of inflammatory cytokines such as IL-1β, IL-6, and TNFα have been reported to be high in comparison with those in normal controls. In addition, TfR1 was up-regulated by IL-1β, IL-6, and TNFα in HepG2. Administration of IL-6 augments hepatic uptake of transferrin-bound iron (^{59}Fe), and this is mainly mediated through hepatocytes, but not through Kupffer cells. These results suggest that the up-regulation of TfR1 expression in CH-C might be caused by increase of inflammatory cytokines that proceeded from HCV infection, although there is a possibility that the components of HCV themselves may induce TfR1 expression directly or indirectly.

Like wise, the up-regulated TfR1 might act as a key molecule for hepatic excess iron accumulation in CH-C; however, there are several candidate molecules which cause this condition. For instance, each mutant of HFE, TfR2, hepcidin, hemojuvelin and ferroportin1 (also known as Ireg1 or MTP1) with substitution of amino acid causes the similar phenotype of hemochromatosis. That is, these facts indicate that at least 5 molecules are involved in the familiar hemochromatosis[58]. In hepatocytes, TfR2 predominantly expresses in the normal condition[59] and the disruption of TfR2 gene caused the hepatic iron overload, a phenotype of hemochromatosis, suggesting that TfR2 should also have important role in hepatic iron metabolism[60]. This receptor might act as a sensor of iron status because hepatic TfR2 protein level was increased in iron loaded rats and was decreased in iron deficient rats. Recently, Takeo *et al*[61] reported that in CH-C TfR2 protein expression is increased parallel with ferroportin1, although the meaning of this TfR2 elevation is still to be elucidated[62].

In addition, there was a significant correlation of hepcidin mRNA expression in the liver with hepatic iron concentration and serum ferritin, but did not correlate with ALT, AST, HAI, or viral load. In inflammatory conditions, hepcidin is regulated transcriptionally by IL-6[63] and IL-1β[64] independent of liver iron content. It is noteworthy that, in contrast to other inflammatory states, hepcidin mRNA expression in the liver was independent of markers of inflammation in hepatitis C, suggesting that iron stores in patients with hepatitis C regulate hepcidin expression, and that iron loading in chronic hepatitis C is not due to inappropriate hepcidin expression[65]. However, there is still a controversial result concerning the hepcidin metabolism in chronic hepatitis C that serum pro-hepcidin is down-regulated[66]. The role of hepcidin in chronic hepatits C seems to need further consideration.

The role of iron on the hepatocellular carcinoma (HCC) development in patients with chronic hepatitis C is another major concern. In primary hemochromatosis, iron could be involved in the development of HCC in associated with cirrhosis, suggesting a strong link between heavy iron overload and HCC development. In cases of chronic hepatitis C, it is also known that HCC are developed 20 to 30 years after the infection of hepatitis C virus through the progression of the disease from chronic hepatitis and cirrhosis. In Long-Evans Cinnamon (LEC) rat, an animal model of human Wilson

disease which spontaneously developed hepatitis and liver fibrosis, HCC is frequently developed after the rats have recovered from initial fulminant hepatitis and subsequent liver fibrosis. This is considered to relate to progressive iron accumulation in the animal[67], and iron depletion prevents their development of hepatic cancer[68]. Even though the iron deposition in chronic hepatitis C is mild compared with that in hemochromatosis, iron may be an independent factor on the risk of HCC. It is reported that liver fibrosis is a favorable environment of proliferation of cancer cells by releasing transforming growth factor β, and there is a strong link between liver fibrosis and liver iron deposition. In clinical trials of phlebotomy, the hepatic content of 8-OH deoxyguanosine is decreased and fibrotic score is improved. An important issue in hepatocaricinogenesis in chronic hepatitis C is the closely related sustained production of ROS during inflammation and fibrosis. Moriya *et al*[69] reported that HCC developed in HCV core transgenic mice after the age of 16 mo, and showed high hepatic lipid peroxidation levels in old (more than 16 mo) core transgenic mice, than in control. However, the association of HCV transgenic mice, and HCC development disappeared with advanced passaging of animals, suggesting that HCC development in HCV transgenic mice cannot be simply explained by HCV infection, but requires additional cofactors. A recent study by Furutani *et al*[70] clearly showed that hepatic iron overload induces HCC in transgenic mice expressing HCV polyprotein. Transgenic animal carrying full length polyprotein-coding region (core to NS5B, nts 342-9378) by using pAlb promoter/enhancer was fed with excess iron diet. After 6 mo feeding, the transgenic mice showed marked steatosis and increased 8 hydroxy-2'deoxyguanosine content in association with the hepatic iron accumulation. Twelve months after feeding, 45% of transgenic mice developed hepatic tumors including HCC. It is noteworthy that the steatosis does not accompany with inflammation but a remarkable ultrastructural alteration of mitochondria associated with decreased degradation activity of fatty acids.

STEATOSIS AND INSULIN RESISTANCE

Nonalcoholic steatohepatitis (NASH) is a clinical entity characterized by the development of histopathological changes in the liver that are nearly identical to those induced by excessive alcohol intake, but in the absence of alcohol abuse; the presence of macrovesicular steatosis and mixes inflammatory infiltrate associate with varying amounts of Mallory's hyaline, glycogenated nuclei, and focal hepatocyte ballooning degeneration. Clinical features of NASH include obesity, hyperlipidemia, diabetes mellitus, and hypertension. In US population, approximately 25% is obese, and at least 20% of the obese individuals have hepatic steatosis. Thus, non-alcoholic liver disease (NAFLD) is the most common cause of liver dysfunction, and it is believed that NASH becomes a cause of cryptogenic cirrhosis and hepatocellular carcinoma (HCC). In patients with homozygote of HFE-related hemochromatosis, obesity and steatosis affect liver disease progression, and will be cofactors for iron overload. There is one study of Australia that showed that the prevalence of abnormal genotype of HFE in NASH is 31% compared to a normal prevalence of 13% in the general population, sugget that excess iron might be important. A study on North American subjects showed similar results that the prevalence of the *HFE* gene mutation associated with hereditary hemochromatosis are increasing in patients with NASH[71]. In the study dealing Japanese NASH patients, who had no *HFE* gene mutations, a significant staining of liver iron and increased level of thioredoxin, a marker of oxidative stress in addition to the increase of serum ferritin, was observed.

As diabetes and obesity were background conditions of NAFLD, and is thought to be a initial triggering factor, insulin resistance is now considered the fundamental operative mechanism. Insulin resistance is probably the "first step" in NASH, and a close correlation between insulin resistance and iron is speculated. Even though it is not still clear whether secondary iron accumulation increases insulin resistance, or vice versa, oxidative stress may be the elusive "second" hit of possibly multiple steps in the progression of steatosis to fibrosing steatohepatitis[72]. This may be due to the activation of stellate cells[73].

Because hepatic iron promotes oxidative stress, it seems that iron is a contributory cofactor in NASH. This proposal is strengthened by an association with hepatic fibrosis with NASH[74] and was confirmed by measuring serum markers of oxidative stress[75-77]. Excess hepatic iron also occur in insulin resistance-associated iron overload (IRHIO), characterized by hyperferritinemia with normal to mild increases in transferrin saturation. There is an interesting clinical study that venesections and restricted diet are effective in patients with IRHIO[78]. As in IRHIO, restriction of dietary calories, fat and iron improved NAFLD in addition the decrease of levels of serum aminotransferases and ferritin[79]. It seems that the simultaneous disorder of iron and glucose and/or lipid metabolism, in most cases associated with insulin resistance, is responsible for persistent hyperferritinemia and identifies patients at risk for NASH[80]. However, it is still unclear why iron is deposited in IRHIO and NAFLD. There is an interesting report by Bekri *et al*[81] that there is an increase of hepcidin in adipose tissue of the severely obese but of liver, suggesting that severe obesity itself cause hypoferremia due to the overproduction of hepcidin in the adipocytes. This finding may explain the hypoferremia in severe obese patients, but does not show the mechanism of hepatic iron deposition in IRHIO and NASH. Further studies are needed to clarify this issue, including an increase of transferrin iron influx into hepatocytes in NAFLD.

In patients with NASH, increased transferrin saturation correlated positively with the severity of fibrosis in univariate analysis, although it became insignificant when age, obesity, diabetes, and AST/ALT ratio were controlled. A recent study showed improvement in insulin sensitivity with the use of venesection in 11 patients with NASH. Biweekly phlebotomy until serum ferritin concentration became lower than or equal to 30 ng/mL reduced mean serum ALT activity without a significant change of

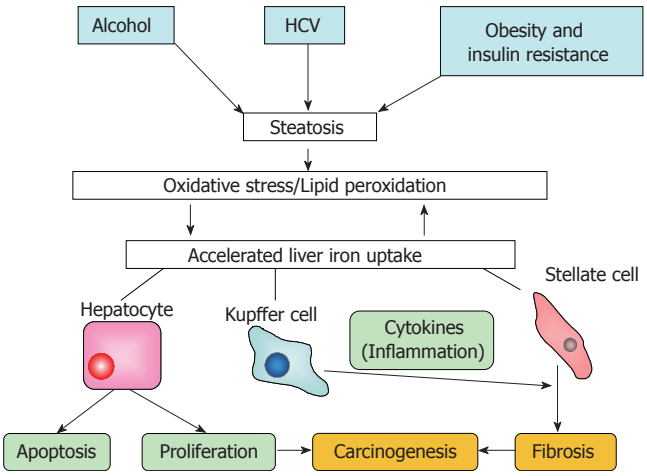

Figure 2 Postulated schema of liver damage occurred by alcohol, HCV infection, obesity and insulin resistant. A common pathway through steatosis/oxidative stress may be responsible for the development of liver fibrosis and carcinogenesis by iron.

body weight, suggesting that iron reduction therapy by phlebotomy will be one of the promising therapies for NASH[82], although this approach cannot be implemented without extensive review.

The natural history of NASH is still unclear, but some patients follow advanced liver fibrosis progressing to cirrhosis and sometimes HCC[83]. It is also known that diabetes increases the risk of hepatocellular carcinoma in US[84]. Further studies are needed to clarify this issue, especially the relation between hepatocarcinogenesis from mild iron accumulation in NASH.

As shown in Figure 2, a common pathway through steatosis/oxidative stress may be present for the development of liver fibrosis and carcinogenesis by iron.

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