Review

Hepatic Iron Overload and Hepatocellular Carcinoma

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Key Words

Cirrhosis · Dietary iron overload · Excess body iron · Hepatocellular carcinoma · Hereditary hemochromatosis

Abstract

In recent years it has become increasingly evident that excess body iron may be complicated by the supervention of hepatocellular carcinoma (HCC). Hereditary hemochromatosis (HH) was the first condition in which hepatic iron overload was shown to predispose to the development of HCC. The inherited predisposition to excessive absorption of dietary iron in HH is almost always the result of homozygosity of the C282Y mutation of the *HFE* gene, which causes inappropriately low secretion of hepcidin. HCC develops in 8–10% of patients with HH and is responsible for approximately 45% of deaths in the HCC patients. Cirrhosis is almost always present when HCC is diagnosed. Dietary iron overload is a condition which occurs in rural-dwelling Black Africans in southern Africa as a result of the consumption, over time, of large volumes of alcohol home-brewed in iron containers and having, as a consequence, a high iron content. Iron loading of the liver results and may be complicated by malignant transformation of the liver (relative risk of approximately 10.0). Accompanying cirrhosis does occur but is less common than that in HH. The development of HCC as a consequence of increased dietary iron, and the fact that it may develop in the absence of cirrhosis, has been confirmed in an animal model. Drinking water with a high iron content might contribute to the high incidence of HCC in parts of Taiwan. The metabolic syndrome [obesity, insulin resistance type 2 (or diabetes mellitus type 2), non-alcoholic fatty liver or non-alcoholic steatohepatitis] has in recent years become a major public health problem in some resource-rich countries. A link between excess body iron and insulin resistance or the metabolic syndrome has become apparent. The metabolic syndrome may be complicated by the supervention of HCC, and recent evidence suggests that increased body iron may contribute to this complication.

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Iron is ubiquitous in human cells and is essential for their normal functioning. But, in excess, iron cannot be disposed of and is harmful to cells. Only a small fraction of the total body iron enters or leaves the body on a daily basis [1–4], with both iron absorption and loss being finely regulated and balanced [5]. Intestinal absorption of the metal is the most important determinant of the amount of iron in the body at any one time, and hepatocytes are the main storage sites of that iron [1–6]. Iron is stored in the core of ferritin as ferric oxyhydroxyapatite, with one molecule of ferritin binding as many as 4,500 molecules of iron [1–3]. Hepcidin, the most important regulator of systemic iron metabolism, is influenced by body iron stores, erythropoietic activity, and hypoxia, and plays the dominant role in controlling iron absorption [6, 7]. MicroRNAs also contribute to the regulation of iron homeostasis [7]. The divalent metal transporter (previously named Nramp2) may be the primary intestinal transporter of the metal [3], and ferroportin exports iron across the basolateral membrane of enterocytes into the circulation after it has been oxidized by membrane-bound ferroxidase [8]. All circulating plasma iron is bound to transferrin [3], and cellular uptake of transferrin-bound iron depends upon the number of membrane-transferring receptors [8].

Most of the iron in humans exists either in storage as ferritin [3] or as heme (iron protoporphyrin IX) [5]. Iron is an essential metal in oxygen transport mediated by hemoglobin. Hemoglobin iron in erythrocytes makes up more than two-thirds of the total iron pool, with storage in the liver accounting for most of the remainder [5]. Much of the body iron resulting from the breakdown of effete erythrocytes by macrophages of the reticuloendothelial system is recycled [3]. An increase in metabolic demand for iron results in both increased intestinal absorption of the metal and the mobilization of iron from tissue stores [5]. No biological mechanisms exist for the excretion of iron in excess of physiological requirements [9], and sloughing of intestinal mucosal cells and menstrual blood loss are normally the main processes by which the metal is lost from the body [1–3]. Body iron stores accumulate insidiously with aging [9, 10].

Iron overload, generally defined as an increase in total body iron exceeding 5 g [7], can occur in a number of ways and results in a variety of illnesses. When the level of safe sequestration of the metal is exceeded, the storage protein is denatured, releasing large amounts of iron into the cytoplasm of the hepatocytes. Accordingly, the liver is the organ most likely to be afflicted by iron overload. Excess iron in the liver was first well-documented in two human diseases: hereditary hemochromatosis (HH) and African dietary iron overload (previously named Bantu visceral siderosis). The detrimental effects on the liver in other conditions affecting iron storage – thalassemia major, sideroblastic anemia, and spherocytosis – have received less attention. Evidence continues to accumulate that, independently of any underlying liver disease, iron accumulation in the liver is an important risk factor for the development of hepatocellular carcinoma (HCC).

Hepatic Iron Overload in Hereditary Hemochromatosis

HH is a not uncommon autosomally recessive inherited disorder in individuals of Celtic descent. It is characterized by increased absorption of dietary iron that results in progressive deposition of the metal in multiple organs, but particularly in the liver [11]. The inherited predisposition to excessive absorption of the metal is the consequence of mutations in a number of genes concerned with iron absorption [7, 9–13]. HCC complicating HH is most commonly attributed (in 70–95% of patients) to homozygosity for the C282Y mutation of the *HFE* gene [7, 9–13]. This mutation impairs the expression of HFE protein on cell membranes, thereby compromising iron sensing by hepatocytes and the transcription and

release of hepcidin [14]. Inappropriately low secretion of hepcidin is postulated to be the mechanism for the consequent iron overload [6]. The characteristic biochemical abnormalities in patients with HH are raised serum ferritin concentrations and transferrin-iron saturation. Missense mutations in other genes (e.g., hemojuvelin and ferroportin genes) involved in the regulation of hepcidin release, transcription, and biologic activity, are responsible for rarer forms of HH [5, 6].

As a consequence of the iron overload in HH, the ability of hepatocytes to safely sequester the metal is exceeded. Denaturation of ferritin subunits then occurs, leading to ionic iron accumulation in the hepatocyte cytoplasm [12]. Over time, this results in hepatocyte damage and dysfunction and the development of fibrosis, the latter in part by activated hepatic stellate cells [12]. This is followed, in due course, by cirrhosis and finally HCC.

Hepatocellular Carcinoma in Hereditary Hemochromatosis

HCC accounts for as many as 45% of deaths in patients afflicted with HH [11–14]. A relative risk of tumor formation in the iron-loaded liver of between 20 and 200 has been calculated [11–14]. The incidence of HCC in patients with HH is approximately 8–10% in most surveys, but figures as low as 1.7% have been recorded [14–20]. Long-term survival in patients with HH and cirrhosis is significantly shorter than that in matched controls, a phenomenon attributed mainly to the development of HCC. Cirrhosis is present in almost all patients who develop HCC, but whether or not the risks of developing HCC are greater in patients with cirrhosis complicating HH than in those with other causes of cirrhosis remains uncertain [14, 20]. Also uncertain is whether patients with HH are at increased risk of developing cancers in organs other than the liver [14, 20].

Iron is a transition metal capable of causing oxidative tissue damage by catalyzing the formation of free radicals. The hepatotoxic and hepatocarcinogenic potential of excessive iron is based upon its ability, by way of the Fenton reaction, to generate reactive oxygen intermediates and oxidative stress, which damage DNA, lipids, and proteins and result in necrosis and apoptosis of hepatocytes [14–18]. Iron-catalyzed oxidative stress causes lipid peroxidation, protein modification, and DNA damage, with consequent exhaustion of anti-oxidant defenses and promotion of mutagenesis [12, 17]. The amount of excess iron and the duration of exposure to this excess is crucial to the development of hepatic damage, and, with few exceptions, patients with HH who develop HCC do so in the presence of cirrhosis [17, 18]. In addition, there is a close link between increased iron stores and insulin resistance type 2, which promotes hepatocarcinogenesis [5] (see the section devoted to the metabolic syndrome).

It is currently not known whether the hepatocarcinogenic effect of HH is solely attributable to the iron molecule per se, by virtue of its capacity to generate oxidative stress and to form mutagenic hydroxyl radicals and suppress host defense, or whether this effect occurs indirectly through the induction of chronic inflammation leading to cirrhosis and hence to HCC [10, 21–23]. Oxidative stress leads to lipid peroxidation of unsaturated fatty acids in membranes of cells and organelles. Cytotoxic by-products of lipid peroxidation, such as malonaldehyde and 4-hydroxy-2'-nonenol, are produced, and these impair cellular function and protein synthesis and induce DNA damage [21–23]. Iron is also thought to be involved in the beta cleavage of lipid hydroperoxide, producing biogenic aldehydes that interact with DNA. In addition, deoxyguanosine residues of DNA are hydroxylated by reactive oxygen intermediates to form 8-hydroxy-2'-deoxyguanosine, a major promutagenic adduct that causes G:C to T:A transversions, DNA unwinding, and strand breaks. Iron is also thought to be involved in the beta cleavage of lipid hydroperoxides, producing biogenic aldehydes that interact with

DNA. In addition, free iron induces immunologic abnormalities that may decrease immune surveillance for malignant transformation [21–23].

A direct role for excess hepatic iron in the etiology and pathogenesis of HCC has recently been confirmed in an animal model in which pre-neoplastic nodules and HCC developed in the absence of cirrhosis, or even fibrosis, in rats fed a high-iron diet [24]. Moreover, in HCC cell lines and murine models, exogenous iron administration is accompanied by enhanced tumor development and growth [25]. HCC has been documented to occur in iron-overloaded individual patients in the absence of cirrhosis [26–29], supporting the belief that increased hepatic iron may also cause or promote malignant transformation of hepatocytes directly. The degree of iron overload was mild in more than 50% of these patients [27]. Iron overload may also act synergistically with other risk factors for HCC, such as chronic hepatitis B virus (HPB) infection and alcohol abuse, in causing the tumor [30].

Dietary Iron Overload and HCC in Black Africans

Dietary iron overload has been reported to occur in Black Africans living in as many as 17 countries in southern and central sub-Saharan Africa. Within these countries, the condition, previously referred to as Bantu visceral siderosis but now called African dietary iron overload, is virtually confined to rural areas, where 80% of the black population resides [31]. Consumption, over time, of large volumes of an iron-rich traditional beer that is home-brewed in cast iron drums or pots is the cause of the iron overload [32–34]. During the process of fermentation of sorghum or other locally-grown crops, the pH of the ferment falls to very low levels (3.5–3.8), leaching iron from the container into its contents [34]. In consequence, the beer has a high iron content (46–82 mg/L) compared with the low content (<0.5 mg/L) in commercial beers [35]. Moreover, the iron in the beer is in an ionized, highly bioavailable form [35]. Because the alcohol content of the beer is low (approximately 3%), large volumes of the beverage are often consumed in order to achieve the desired effects. More than two-thirds of adult males (and fewer females) consume the beer, and dietary iron overload may affect as many as 15% of rural Black African males [34]. Histological evidence of traditional alcoholic liver disease is rarely evident.

Consumption over time of large volumes of the home-brewed beer results in hepatic iron concentrations comparable with those in HH, and which may be complicated by portal fibrosis or, less often, by cirrhosis [36]. Concentrations of the metal in the liver of iron-loaded individuals range between 32 and 519 $\mu\text{mol/g}$ dry weight, with a geometric mean of 128 $\mu\text{mol/g}$ (equivalent to >2.0% dry weight) in heavy iron overload [36]. The iron accumulation affects both hepatocytes and macrophages [36, 37]. Serum ferritin levels are typically greater than 700 pg/L and later in the accumulation process, transferrin saturations become greater than 55% [38]. Non-transferrin-bound iron may also be present, and its presence correlates with transferrin saturation and the ferritin concentration [39]. The hepatic fibrosis and cirrhosis that complicate the more severe degrees of iron overload do so far less frequently than they do in HH [34, 36].

Because not all Black Africans who consume large volumes of iron-rich beer accumulate iron in the liver, and because iron absorption is normally genetically controlled, it seems likely that a genetic predisposition plays a role in the pathogenesis of African dietary iron overload. However, a putative gene (or genes) has yet to be identified. A mutation in the *SLC4A0A1* gene, whose product ferroportin 1 is the main iron export protein, was detected in a minority of African Americans with ‘primary’ iron overload [40], but this mutation was

not found in southern Black Africans with dietary iron overload more often than in unaffected family members [41].

Hepatocellular Carcinoma in Dietary Iron Overload

Mandishona et al. in 1998 reported a relative risk of HCC development of 10.6 (95% confidence intervals 1.5–76.8) in black patients in southern Africa with dietary iron overload, after adjusting for the confounding effects of chronic HBV or hepatitis C virus (HCV) infections, cirrhosis, and dietary exposure to aflatoxin B₁ [42]. A similar observation was made in two additional sub-Saharan African studies after allowing for the confounding effects of cirrhosis, although the confounding effects of HBV and HCV were not taken into consideration in these studies [43, 44].

In recent years, persuasive evidence has accumulated that excess hepatic iron may be directly hepatocarcinogenic, in addition to causing neoplastic transformation in the presence of cirrhosis. A number of case reports of HCC developing in patients with HH in the absence cirrhosis attest to this conclusion [45].

HCC is known to occasionally complicate hepatic iron accumulation in patients with thalassemia major, sideroblastic anemias, or spherocytosis [46–48].

Until recently HCC had not been reported in animal models that had been exposed to dietary iron overload for durations of less than 15 months [49, 50]. But in a recent study, HCC developed in Wistar rats that became heavily iron-overloaded after receiving 16 months or more of a diet rich in iron, and it did so in the absence of cirrhosis [25]. In these rats, the iron accumulated in hepatic parenchymal cells and macrophages, a pattern similar to that seen in African dietary iron overload. By 20 months, altered iron-free hepatic foci were present in many of the animals. By 28 months these foci had become plentiful, and the foci had changed in character, becoming indistinguishable from the iron-free preneoplastic nodules described by Deugnier and colleagues in humans with iron overload [51], and the foci ultimately developed into typical neoplastic nodules with an expansive pattern [25].

Cirrhosis is less common in patients with dietary iron overload than in those with HH [31, 35]. Nevertheless, the association between cirrhosis and dietary iron overload supports the belief that iron-induced cirrhosis may contribute to the pathogenesis of HCC caused by dietary iron overload. The mechanisms by which non-transferrin-bound iron induces malignant transformation have yet to be fully characterized. The ability of iron to function as both an electron donor and an electron acceptor with an environmentally tolerable redox potential renders iron potentially toxic. Under aerobic conditions the Fenton and Haber-Weiss reactions are operative and catalytic amounts of free iron are sufficient to generate noxious reactive oxygen intermediates, disrupting the redox balance of the cell and generating chronic oxidative stress, which damages DNA, lipids, and proteins in hepatocytes and leads to necrosis and apoptosis of these cells [52, 53]. Oxidative stress also leads to increased lipid peroxidation of unsaturated fatty acids in membranes and cells. The mechanisms by which the cytotoxic and genotoxic by-products contribute to hepatocarcinogenesis have been mentioned in the section on iron overload in HH [53–55].

Iron Levels in Drinking Water and Occurrence of HCC in South-Western Taiwan

Studies in the south-western coastal townships of Taiwan, which have serious ground subsidence problems as a result of over-pumping of groundwater for aquaculture, have identified both high concentrations of iron in the groundwater and a high incidence of HCC. Such studies have shown a statistically significant correlation between the development of HCC and the high concentrations of iron in the drinking water [56]. As a consequence of the shortage of surface water in these regions, groundwater is the major source of drinking water [57]. Iron concentrations of untreated water pumped from groundwater in these regions is significantly higher (1.04 ± 0.20 mg/L) than in regions with no serious land subsidence (0.34 ± 0.05 mg/L) [56] (concentrations of iron in drinking water are normally less than 0.3 mg/L) [57]. These observations raise the possibility that the long-term residents of these areas with serious land subsidence may have been consuming large volumes of groundwater with high iron concentrations [57], and that this may explain the high rates of HCC in these regions.

Alternatively, other causes of HCC in Taiwan – chronic HBV infection and/or exposure to the fungal toxin aflatoxin B₁ – could be responsible for the malignant transformation. Information concerning these risk factors needs to be included in the work-up of the patients to prove or disprove this putative etiological association between the consumption of iron-rich drinking water and the high incidence of HCC.

Increased Hepatic Iron and HCC in Patients with the Metabolic Syndrome

Obesity is an increase in body fat to the point of having a body weight of more than 20 percent above the normal [58]. Obesity is determined in a number of ways, based on the ratio of body weight to body height (body/mass index), waist circumference to body height, waist circumference to hip circumference, or other ratios [58]. In recent years, obesity has become a major public health problem in many resource-rich countries, with incidences increasing in adults and even in children. For example, in the U.S.A. today nearly two-thirds of the adult population and an increasing number of children are considered to be obese [58].

Non-alcoholic fatty liver disease (NAFLD), a component of the metabolic syndrome, is currently the most common form of chronic liver disease in resource-rich countries. It, and the more aggressive but less common non-alcoholic steatohepatitis (NASH), another component of the metabolic syndrome, are increasing in incidence in many of these countries [59]. The metabolic syndrome comprises obesity, insulin resistance type 2 (with or without type 2 diabetes mellitus), cirrhosis, and NAFLD or NASH. In the general populations of these countries, the prevalence of NAFLD ranges from 9–37% and that of NASH from 5–7%. Moreover, in patients with NASH and elevated hepatic enzymes in the absence of serological markers, the values may be as high as 34–40% [60]. The development of NAFLD has been shown to be associated with preceding significant weight gain or frank obesity [61].

Evidence continues to accumulate of a link between body iron excess, insulin resistance, and the metabolic syndrome [4, 62–64]. Increased serum ferritin levels are present in more than 30% of patients with NAFLD and have been reported to correlate with insulin resistance and the other metabolic abnormalities defining the metabolic syndrome [64, 65]. Moderate iron accumulation has been observed in NAFLD, and down-regulation of the liver iron exporter, ferroportin-1, has been described [66].

Hepatocellular Carcinoma in the Metabolic Syndrome

Over the past approximately 20 years, HCC has increased in frequency in parallel with the increasing incidence of obesity in resource-rich countries [59]. For example, in the U.S.A. the incidence of the tumor has increased by 80% over this time. Recent evidence indicates that an increased hepatic iron content may contribute to the hepatocarcinogenesis complicating NASH and, to a much lesser extent, NAFLD [60]. The cause of the increased hepatic iron in patients with NASH and NAFLD remains to be determined, but there is increasing evidence that, in association with other insults such as chronic HCV infection or excess alcohol intake, even slightly increased amounts of iron in the liver cause hepatic injury in these patients [4, 61, 62].

Iron overload has been hypothesized to induce insulin resistance by catalyzing oxidative stress in the liver. The iron may fuel oxidative stress-driven cell toxicity or activate signaling pathways involved in fibrogenesis or carcinogenesis occurring in the metabolic syndrome [63]. Although the exact link to insulin resistance remains unexplained, it is thought to be related to dysregulation of hepcidin transcription and of molecules involved in cellular export of iron. Hyperinsulinemia is a risk factor for the development of NAFLD and NASH, and the co-existence of excess iron may contribute to the development of insulin resistance [64]. In support of the latter hypothesis is the observation that iron removal by phlebotomy can improve the insulin resistance and liver function in patients with NAFLD [64]. Metabolic derangement and progression of liver disease are more severe with NASH than with NAFLD, and hepatic iron deposition has been shown to increase the risk of malignant transformation in NASH-derived cirrhosis [65, 67, 68].

Increased Hepatic Iron in Patients with HCV Infection

Mild to moderate iron loading is common in patients chronically infected with HCV [69]. Moreover, people over the age of 40 years with chronic HCV infection are three times more likely to have type 2 diabetes mellitus than those without HCV infection [70]. Shintani and co-workers have shown direct experimental evidence for the contribution of HCV to the development of insulin resistance [71]. The cause of hepatic iron overload in the presence of the virus is not known, although low hepcidin levels have been shown to trigger hepatic iron accumulation in these patients [72]. HCV suppresses hepcidin expression, increasing duodenal iron transport and macrophage iron release [73, 74]. Over-expression of transferrin receptor type 1 could be another factor responsible for the accumulation of hepatic iron in hepatitis C virus-infected individuals [75].

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