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CLINICAL STUDIES

Prevalence of hepatic iron overload and association with hepatocellular cancer in end-stage liver disease: results from the National Hemochromatosis Transplant Registry

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Abstract

Background: It is unclear whether mild to moderate iron overload in liver diseases other than hereditary haemochromatosis (HH) contributes to hepatocellular carcinoma. This study examined the association between hepatic iron grade and hepatocellular carcinoma in patients with end-stage liver disease of diverse aetiologies. *Methods:* The prevalence of hepatic iron overload and hepatocellular carcinoma was examined in 5224 patients undergoing liver transplantation. Explant pathology reports were reviewed for the underlying pathological diagnosis, presence of hepatocellular carcinoma and degree of iron staining. The distribution of categorical variables was studied using χ^2 tests. *Results:* Both iron overload and hepatocellular carcinoma were the least common with biliary cirrhosis (1.8 and 2.8% respectively). Hepatocellular carcinoma was the most common in patients with hepatitis B (16.7%), followed by those with hepatitis C (15.1%) and HH (14.9%). In the overall cohort, any iron overload was significantly associated with hepatocellular carcinoma ($P = 0.001$), even after adjustment for the underlying aetiology of liver disease. The association between hepatic iron content and hepatocellular carcinoma was the strongest in patients with biliary cirrhosis ($P < 0.001$) and hepatitis C ($P < 0.001$). *Conclusions:* Iron overload is associated with hepatocellular carcinoma in patients with end-stage liver disease, suggesting a possible carcinogenic or cocarcinogenic role for iron in chronic liver disease.

Background

The incidence of hepatocellular cancer (HCC) is rising, and it is currently the sixth most common malignancy worldwide (1). HCC carries a poor prognosis and is the major cause of liver-related death in patients with compensated cirrhosis, accounting for almost 600 000 deaths annually (1). Various aetiological factors associated with HCC include advanced age, male gender, non-Caucasian ethnicity, hepatitis B or C infection, alcoholic cirrhosis, hereditary haemochromatosis (HH) and exposure to aflatoxin (1–14).

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Epidemiological studies also suggest a positive relationship between elevated body iron stores and cancer (15, 16). This supports the concept that iron may have a generalized role in carcinogenesis (17, 18). Excess iron is also considered a potential carcinogen in the liver. For example, the risk of HCC is markedly increased in patients with HH. Several studies from referral centres have reported an approximately 8–10% incidence of HCC among patients with HH, mostly in cirrhotic patients (19–22), and have estimated a 100–200-fold increase in risk over the general population. In a population-based study using death certificate data, the prevalence of liver cancer was increased 24-fold in association with a death certificate diagnosis of HH (23). A Swedish study found a 1.7-fold increase in the incidence of HCC in patients with HH (24). Additional risk factors such as hepatitis B, older age and alcohol abuse may be synergistic with iron overload in increasing the risk for HCC among cirrhotic patients with HH (25).

Excess hepatic iron is common in patients with end-stage liver disease from aetiologies other than HCC, such as hepatitis C or alcohol use (26–30). Some have hypothesized that hepatic iron overload may also be an independent risk factor for development of hepatocellular carcinoma in these patients (31). HCC can develop in non-cirrhotic patients with HH, and increased hepatic iron has been reported in HCC patients without cirrhosis or HH, suggesting that iron may play an independent role in hepatic carcinogenesis (32–34). Supporting this hypothesis, Borgna-Pignatti *et al.* (35) reported that patients with transfusional iron overload owing to underlying β-thalassaemia are at increased risk of HCC compared with the general population. Similar findings have been reported in patients with African iron overload, even after adjustment for age, gender, hepatitis B and C, alcohol, aflatoxin and the presence of portal fibrosis or cirrhosis (36, 37). However, the role of mild to moderate hepatic iron deposition in hepatic carcinogenesis in patients with non-HH liver disease remains unclear.

The aims of this study were to determine the prevalence of hepatic iron overload in patients with end-stage liver disease of varying aetiologies and to examine the association of hepatic iron overload with hepatocellular carcinoma. Understanding this relationship may provide valuable data for understanding hepatic carcinogenesis and for development of strategies to treat iron overload and screen for HCC in patients with end-stage liver disease.

Methods

The National Hemochromatosis Transplant Registry (NHTR) is the result of an ongoing collaboration between 22 liver transplant centres in the US with data compilation beginning in 1990. Fourteen of the 22 centres in the NHTR participated in this study (Baylor University, Cleveland Clinic, Columbia University, California Pacific Medical Center, Mayo Clinic, Medical College of Virginia, University of Michigan, Rush University, Mount Sinai, Saint Louis University, Stanford University, University of California Los Angeles, University of California San Francisco and University of Nebraska). Anonymized explant pathology reports without any private health information of all patients undergoing a first liver transplantation for end-stage liver disease were sent to the Data Repository at the University of Washington during the inclusive period of this study (1 January 1990–December 1996). This study was approved by the Institutional Review Board at the University of Washington, and each individual centre complied with local institutional guidelines to provide data to the NHTR.

We conducted a retrospective review of explant pathology reports in the NHTR database. Patients undergoing their first liver transplant between 1990 and 1996 were considered for this study. Patients were excluded if: (i) age < 18 years at the time of transplantation, (ii) acute or fulminant hepatic failure without cirrhosis, (iii) undergoing retransplantation, (iv) primary indication for transplantation was amyloidosis, polycystic liver disease or liver metastasis from non-hepatobiliary cancer and/or (v) insufficient data in the registry database. We also excluded patients in whom hepatic iron quantification was not performed. From the eligible pathology reports, we extracted data about year of transplantation, pretransplant diagnosis of liver disease, post-transplant diagnosis from the explant pathology report and degree of hepatic iron deposition. The presence or absence of HCC was also determined from the pathology reports. Because data for this study were obtained from the explant pathology reports, data about age, gender and prior blood transfusions were not always available for analysis. For this study, subjects' liver disease was determined from the pathology report and classified into:

1. Biliary cirrhosis – primary biliary cirrhosis, primary sclerosing cholangitis and other biliary disorders including biliary atresia, Caroli's disease, secondary cholestasis, cystic fibrosis, systemic lupus erythematosus, triaditis and Von Meyenburg disease.

2. Viral cirrhosis – hepatitis C, hepatitis B, hepatitis B and hepatitis C co-infection and other unspecified viral infection.
3. Alcoholic cirrhosis – alcohol alone, or alcohol with viral coinfection.
4. Autoimmune hepatitis.
5. Metabolic – α-1-antitrypsin deficiency, HH, Wilson's disease.
6. Other chronic liver disease – cryptogenic, non-alcoholic fatty liver disease, other/unspecified (including fibrosis, metastatic non-hepatic malignancy, haemangioma, hepatoportal sclerosis, ischaemia, portal vein thrombosis).
7. Cancer without other liver disease – hepatocellular carcinoma, cholangiocarcinoma.

Hepatic iron content was documented on the pathology reports by a histological semi-quantitative grade on a scale of 0–4+ where 0 represented a complete absence of stainable iron and 4+ represented hepatic iron visible on slides to the naked eye (38). Within each aetiological category of liver disease, hepatic iron staining was subclassified into three categories: no iron (0); mild iron (1–2+); and moderate to severe iron (3–4+). Because the time period of this study predates the identification of the *HFE* gene, *HFE* mutation status in the study subjects was unknown. Hepatic iron staining, where available, was performed using Perl's prussian blue and scored as described previously (38)

Data analysis

We examined the pattern of iron deposition in the liver in patients with and without HCC, both in the overall population and within categories of liver disease aetiology. The distribution of categorical variables was expressed as a binomial proportion. Differences in the distribution of categorical variables were compared using the χ^2 test. A *P*-value < 0.05 was considered to be statistically significant. We used logistic regression analysis to examine the association of iron staining with HCC after adjustment for other variables.

Results

Of the 5320 subjects who underwent orthotopic liver transplantation in the study period, 96 were excluded and data were extracted from each of the 5224 remaining reports. The post-transplantation diagnoses, degree of iron staining and proportion of patients with HCC are shown in Table 1. Viral hepatitis was the most common aetiology of liver disease, followed by alcoholic liver disease (with or without coexisting viral infection) and biliary cirrhosis. HH was uncommon in this study population.

Mild and excess iron staining were found in 5.6 and 2.8% of the study population respectively (Table 1). Iron staining was the least common in patients with biliary cirrhosis (2.6%), but the most common in patients with HH (91.9%) and alcoholic cirrhosis (14.3%).

Hepatocellular cancer was the most common in patients with viral infection (15.6%), and the least common in patients with biliary cirrhosis (1.8%) (Table 1).

Table 2 shows the grade of iron staining in patients with or without HCC. There was a strong trend towards increased iron staining in patients with HCC in the overall population ($P = 0.06$), and a significant association of HCC with increased iron staining in patients with biliary cirrhosis ($P < 0.001$) or viral hepatitis ($P = 0.008$). The association of stainable iron with HCC was the strongest in patients with hepatitis C infection ($P < 0.001$), but was also present in patients with primary biliary cirrhosis ($P = 0.01$), other biliary tract disease ($P = 0.02$) and in patients with cryptogenic cirrhosis ($P = 0.04$). The apparent lack of association of iron with HCC in the HH category may be because of the high prevalence of iron overload in HH patients without HCC.

Finally, we used logistic regression to examine whether hepatic iron is a risk factor for HCC, independent of the underlying liver disease (Table 3). Our results showed that the risk of HCC was increased with both mild (odds ratio 1.59; 95% confidence interval 1.07, 2.38) and excess (odds ratio 2.10, 95% confidence interval 1.25, 3.42) iron deposition, even after adjustment for the aetiology of underlying liver disease. Consistent with prior data, the prevalence of HCC was the highest in patients with viral cirrhosis (compared with biliary cirrhosis: odds ratio 9.92, 95% confidence interval 6.35, 15.5).

Discussion

We found that the prevalence of hepatic iron overload at transplantation differs by the aetiology of liver disease, and that hepatic iron overload is associated with an increased prevalence of HCC. To our knowledge, this is the largest study examining the relationship between iron overload and HCC in patients with diverse aetiologies of liver disease. Similar to previous reports, we found a low prevalence of iron overload in biliary cirrhosis compared with non-biliary cirrhosis (37, 39, 40). Also in concordance with previous

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Table 1. Stainable iron or hepatocellular carcinoma in explant livers classified by pathological diagnosis

Category of pathological diagnosis (*N*)	Iron, *N* (%)			Hepatocellular carcinoma present, *N* (%)
	No iron*	Mild iron†	Excess iron‡	
All patients (5224)	4788 (91.6)	291 (5.6)	145 (2.8)	482 (9.2)
Biliary cirrhosis (1212)	1179 (97.2)	27 (2.2)	7 (0.6)	22(1.8)
PBC (541)	527 (97.4)	11 (2.0)	3 (0.6)	16 (3.0)
PSC (527)	517 (98.1)	8 (1.5)	2 (0.4)	3 (0.6)
Other biliary (144)	134 (93.1)	8 (5.6)	2 (1.4)	2 (1.4)
Viral cirrhosis (1367)	1305 (95.5)	39 (2.8)	23 (1.7)	214 (15.6)
HCV (1052)	1031 (98.0)	11 (1.0)	10 (1.0)	159 (15.1)
HBV (300)	260 (86.7)	28(9.3)	12 (4.0)	50 (16.7)
HCV+HBV (4)	4 (100.0)	0 (0.0)	0 (0.0)	4 (100.0)
Viral unspecified (11)	10 (90.9)	0 (0.0)	1 (9.1)	1 (9.1)
Alcoholic cirrhosis (870)	746 (85.7)	86 (9.9)	38 (4.4)	46 (5.3)
Alcohol (663)	569 (85.8)	67 (10.1)	27 (4.1)	33 (5.0)
Alcohol+viral (207)	177 (85.5)	19 (9.2)	11(5.3)	13 (6.3)
Autoimmune hepatitis (192)	184 (95.8)	5 (2.6)	3 (1.6)	1 (0.5)
Metabolic (214)	130 (60.8)	38 (17.8)	46 (21.5)	16 (7.5)
A1AT deficiency (102)	90 (88.2)	9 (8.8)	3 (2.9)	5 (4.9)
Haemochromatosis (74)	6 (8.1)	27 (36.5)	41 (55.4)	11 (14.9)
Wilson's disease (38)	34 (89.5)	2 (5.3)	2 (5.3)	0 (0.0)
Other chronic liver disease (1282)	1160 (90.5)	94 (7.3)	28 (2.2)	114 (8.9)
Cryptogenic (1175)	1064 (90.6)	86 (7.3)	25 (2.1)	113 (9.6)
NASH (35)	32 (91.4)	2 (5.7)	1 (2.9)	1 (2.9)
Other (72)	64 (88.9)	6 (8.3)	2 (2.8)	0 (0.0)
Cancer only (86)	84 (97.7)	2 (2.3)	0 (0.0)	69 (80.2)
HCC (69)	68 (98.6)	1 (1.4)	0 (0.0)	69 (100)
Cholangiocarcinoma (17)	16 (94.1)	1 (5.9)	0 (0.0)	0 (0.0)

*No stainable iron.

†Mild stainable iron, 1–2+.

‡Moderate to severe iron, 3–4+.

A1AT, α-1-antitrypsin; HBV, hepatitis B virus; HCC, hepatocellular cancer; HCV, hepatitis C virus; NASH, non-alcoholic steatohepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.

epidemiological reports, HCC was most prevalent in cirrhotics with viral hepatitis B, hepatitis C and HH but uncommon in biliary cirrhosis (41). Our findings suggest that excess iron may assist in carcinogenesis in the cirrhotic liver.

Various carcinogenic or cocarcinogenic mechanisms for iron have been proposed. Non-transferrin-bound free iron may promote carcinogenesis by promoting production of reactive oxygen species, leading to point mutations or structural damage to chromosomes (strand breaks) (42–44). Inactivation of tumour suppressor genes such as p53 may thus occur (45, 46). Because experimental iron overload does not induce hepatic cancer in normal laboratory animals, it is possible that iron may not cause but may assist carcinogenesis (47). Non-transferrin-bound free iron and ferritin may also impair lymphocyte proliferation (47) and decrease tumoricidal activity of macrophages (48). As an essential substrate for cellular proliferation, addition of iron promotes DNA synthesis in rat hepatocyte cell cultures (49). Iron also increases cell proliferation in hepatoma cell lines, while iron depletion causes tumour cell death (50). Other possible carcinogenic mechanisms include increased production of nitric oxide synthase 2 observed in HH patients with HCC (45). Excess production of nitric oxide by this enzyme may initiate or aid carcinogenesis by enhancing lipid peroxidation and impairing DNA repair (51, 52). Furthermore, iron overload may indirectly promote hepatic carcinogenesis by enhancing fibrogenic effects of lipid peroxidation and activating stellate cells, thus causing accelerated progression to cirrhosis (52).

In our study, the presence of iron overload was associated with HCC in patients with viral and biliary cirrhosis, but was not significantly associated in patients with alcoholic liver disease or HH. However, some patients with cirrhosis from viral or other aetiologies may have had concomitant alcoholic liver disease not noted on the pathology reports, and we did

Table 2. Degree of hepatic iron staining in patients with or without hepatocellular carcinoma

Category of pathological diagnosis (*N*)	No hepatocellular carcinoma (*n* = 4742), *N* (%)			Hepatocellular carcinoma (*n* = 482), *N* (%)			
	No iron*	Mild iron†	Excess iron‡	No iron*	Mild iron†	Excess iron‡	*P*-value
All patients (5224)	4359 (91.9)	258 (5.4)	125 (2.6)	429 (89.0)	33 (6.9)	20 (4.1)	0.06
Biliary cirrhosis (1212)	1160 (97.4)	24 (2.0)	7 (0.6)	19 (84.4)	3 (13.6)	0 (0.0)	0.001
PBC (541)	513 (97.7)	9(1.7)	3 (0.6)	14 (87.5)	2 (12.5)	0 (0.0)	0.01
PSC (527)	514 (98.1)	8 (1.5)	2 (0.4)	3 (100.0)	0 (0.0)	0 (0.0)	0.97
Other biliary (144)	133 (93.7)	7 (4.9)	2 (1.4)	1 (50.0)	1 (50.0)	0 (0.0)	0.02
Viral cirrhosis (1367)	1109 (96.2)	29 (2.5)	15 (1.3)	196 (91.6)	10 (4.7)	8 (3.7)	0.008
HCV (1052)	884 (99.0)	4 (0.4)	5 (0.6)	147 (92.4)	7 (4.4)	5 (3.1)	< 0.001
HBV (300)	216 (86.4)	25 (10.0)	9 (3.6)	44 (88.0)	3 (6.0)	3 (6.0)	0.51
HCV+HBV (4)	–	–	–	4 (100.0)	0 (0.0)	0 (0.0)	–
Viral unspecified (11)	9 (90.0)	0 (0.0)	1 (10.0)	1 (100.0)	0 (0.0)	0 (0.0)	0.74
Alcoholic cirrhosis (870)	703 (85.3)	83 (10.1)	38 (4.6)	43 (93.5)	3 (6.5)	0 (0.0)	0.21
Alcohol (663)	538 (85.4)	65 (10.3)	27 (4.3)	31 (93.9)	2 (6.1)	0 (0.0)	0.33
Alcohol+viral (207)	165 (85.0)	18 (9.3)	11 (5.7)	12 (92.3)	1 (7.7)	0 (0.0)	0.66
Autoimmune hepatitis (192)	183 (95.8)	5 (2.6)	3 (1.6)	1 (100)	–	–	0.98
Metabolic (214)	124 (62.6)	35 (17.7)	39 (19.7)	6 (37.5)	3 (18.8)	7 (43.7)	0.06
A1AT deficiency (102)	85 (87.6)	9 (9.3)	3 (3.1)	5 (100.0)	0 (0.0)	0 (0.0)	0.70
Haemochromatosis (74)	5 (7.9)	24 (38.1)	34 (54.0)	1 (9.1)	3 (27.3)	7 (63.6)	0.78
Wilson's disease (38)	34 (89.5)	2 (5.3)	2 (5.3)	–	–	–	–
Other chronic liver disease (1282)	1064 (91.1)	81 (6.9)	23 (2.0)	96 (84.2)	13 (11.4)	5 (4.4)	0.05
Cryptogenic (1175)	969 (91.2)	73 (6.9)	20 (1.9)	95 (84.1)	113 (11.5)	5 (4.4)	0.04
NASH (35)	31 (91.2)	2 (5.9)	1 (2.9)	1 (100.0)	0 (0.0)	0 (0.0)	0.95
Other (72)	64 (88.9)	6 (8.3)	2 (2.8)	–	–	–	–
Cancer only (86)	16 (94.1)	1 (5.9)	0 (0.0)	68 (98.6)	1 (1.4)	0 (0.0)	0.28
HCC (69)	–	–	–	68 (98.6)	1 (1.4)	0 (0.0)	–
Cholangiocarcinoma (17)	16 (94.1)	1 (5.9)	0 (0.0)	–	–	–	–

*No stainable iron.

†Mild stainable iron, 1–2+.

‡Moderate to severe iron, 3–4+.

P-values compare distribution of iron staining in patients with and without hepatocellular carcinoma.

A1AT, α-1-antitrypsin; HBV, hepatitis B virus; HCC, hepatocellular cancer; HCV, hepatitis C virus; NASH, non-alcoholic steatohepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.

Table 3. Logistic model of risk factors for hepatocellular carcinoma

	Odds ratio	95% CI	*P*-value
Iron			0.02
None	Referent	–	
Mild	1.59	1.07, 2.38	
Excess	2.10	1.25, 3.52	
Disease category			< 0.001
Biliary	Referent	–	–
Viral	9.92	6.35, 15.5	
Alcohol	2.79	1.66, 4.69	
Autoimmune	0.28	0.04, 2.09	
Metabolic	3.34	1.69, 6.65	
Other	5.06	3.181, 8.06	

CI, confidence interval.

not have data about alcohol consumption in our patients. The lack of association of hepatic iron with HCC in patients with HH may be owing to the high overall prevalence of iron overload in these patients. The small number of patients with metabolic liver disease other than HH likely limited our statistical power to detect an association. Within the patients with viral hepatitis, the association of iron and HCC was seen primarily in patients with hepatitis C, while there was no significant association in patients with hepatitis B. It is notable that iron overload was a risk factor for HCC in patients with biliary cirrhosis, despite the low prevalence of both HCC and iron overload in this population. Likewise, HCC was less common in patients with other chronic liver diseases (primarily cryptogenic cirrhosis), but even so iron overload appeared to be associated with HCC in this patient group.

Our results confirm previously published studies examining iron overload and HCC. For example, Chapoutot *et al*. (46) estimated hepatic iron in liver biopsies from 104 hepatitis C cirrhotics – 48 with HCC

and 56 controls without HCC. Hepatic iron deposition was more prevalent and more significant in patients with HCC than in control patients without HCC, suggesting that iron excess may be a cofactor in hepatic carcinogenesis (46). Ito *et al.* (53), evaluated magnetic resonance images for diffuse hepatic iron deposition and iron deposition in regenerative hepatic nodules in 196 cirrhotic patients with and without HCC ($n = 80$ and 116 respectively). HCC was more common in patients with siderotic regenerative nodules than in patients without iron in regenerative nodules. They hypothesized that iron may be causally linked to HCC in this population. In contrast, Ebara *et al.* (54) studied the association of copper, zinc and iron with HCC in cirrhotics with hepatitis C. Metal content was quantified in non-HCC liver parenchyma and in malignant nodules of 112 patients with HCC, in seven patients with dysplastic nodules and in 12 patients without HCC. No significant differences in hepatic iron concentrations were found in patients with and without HCC, although the relatively small number of patients without HCC may have impaired the statistical power of this study. In this study, iron was quantified using particle-induced X-ray emission, rather than using semiquantitative measures as in our study. However, this method likely measures all types of hepatic iron, rather than just haemosiderin, which appears to be most closely associated with HCC.

Our study has some limitations. Because the data collection predates HFE genotyping, genotype data were not available for analysis. We did not have direct measurements of hepatic iron concentrations, but instead relied on semiquantitative iron grading in explant specimens. Because we relied primarily on pathology reports, additional clinical and laboratory data such as age, gender, history of blood transfusions, history of haemolytic diseases, duration of liver disease, prior alcohol intake and routine laboratory measurements were not consistently available. We therefore could not include these variables in our model, and some of the associations of iron and HCC in our study may be related to confounding of our results by these variables. For example, excess iron deposition in the liver may occur with longer disease duration, which could also increase the risk of HCC.

We were only able to study patients with end-stage liver disease who survived to liver transplantation, and our results may not necessarily apply to other patients with end-stage liver disease. Our study dates back to 1990, and it is possible that hepatitis C infection was under-diagnosed in this cohort. Finally, there is significant variability in hepatic iron deposition in end-stage liver disease (55). We were able to obtain tissue from explanted livers, minimizing sampling variability, but we cannot account for biological variability in iron deposition. Nevertheless, the large size of our tissue samples and the similarity of our results to prior reports (27) suggest that such variability did not significantly affect our results.

In summary, our findings support earlier evidence that iron overload is associated with HCC in patients with end-stage liver disease of diverse aetiologies. The association between iron overload and HCC was the strongest in patients with viral hepatitis and biliary tract disease. Similar to previous studies, we found that iron overload is common in patients with alcoholic liver disease, but relatively uncommon in patients with biliary cirrhosis. Additional studies are necessary to identify the mechanistic role of iron in initiating or promoting HCC in cirrhotic patients, and to determine whether iron acts independently of potential confounders such as age, alcohol intake and disease duration. Because HCC is common and incurs high morbidity and mortality, measures to prevent HCC in patients with end-stage liver disease are clearly needed. Our results also suggest that treatment of iron overload, such as with phlebotomy, may have some benefits in decreasing the burden of HCC in this patient population. Additional prospective studies will further clarify the relationships among iron deposition, HFE genotypes and hepatocellular carcinoma in patients with diverse aetiologies of liver disease.

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