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Review

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Association between hereditary hemochromatosis and hepatocellular carcinoma: a comprehensive review

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Abstract

Hepatocellular carcinoma (HCC) is a significant global health problem with high morbidity and mortality. Its incidence is increasing exponentially worldwide with a close overlap between annual incidence and death rates. Even though significant advances have been made in HCC treatment, fewer than 20% of patients with HCC are suitable for potentially curative treatment. Hereditary hemochromatosis (HH) is an important genetic risk factor for HCC. HH is an autosomal recessive disorder of iron metabolism, characterised by elevated iron deposition in most organs including the liver, leading to progressive organ dysfunction. HCC is a complication of HH, nearly always occurring in patients with cirrhosis and contributes to increased mortality rates. Identifying the susceptibility of development of HCC in HH patients has gained much traction. This review summarises the current knowledge with regard to the association of HH and HCC in order to encourage further research. In this review, we focus particularly on *HFE* gene-related HH. Herein, we highlight and discuss emerging clinical research which addresses the prevalence of HCC in HH patients and the coincidence of HH with other risk factors for HCC development. We also focus on the therapeutic tools in the management of HCC associated with HH.

Keywords: Hepatocellular carcinoma, hereditary hemochromatosis, *HFE* gene, *C282Y* mutation, *H63D* mutation, liver cirrhosis

INTRODUCTION

Hepatocellular carcinoma (HCC) is considered to be the most frequent primary liver cancer accounting for 80%-90% of cases[1]. HCC has become a leading cause of cancer-related death globally in the last decades, accounting for approximately 800,000 deaths annually[2]. The incidence of HCC continues to escalate by 3%-9% cases annually worldwide with a nearly equal proportion of deaths[3]. Surgical resection, trans-arterial chemoembolization radiofrequency ablation and liver transplantation remain the treatments of choice for HCC patients and are beneficial for patients in the early stages of the disease[4]. The prognosis for HCC patients in the advanced stages is poor due to the limited efficacy of current therapy[5].

Implementing HCC surveillance among at-risk populations is imperative to identify HCC at early stages amenable to curative treatments. HCC mostly develops in patients with underlying chronic hepatic disease[6-8]. Several at-risk populations for HCC have been identified including patients with cirrhosis, hepatitis B and hepatitis C viral infection, alcoholism, aflatoxin, non-alcoholic steatohepatitis, type 2 diabetes, obesity and Wilson's Disease[7]. Hemochromatosis is also an important risk factor for HCC.

Hereditary hemochromatosis (HH) is a common inherited iron metabolism disorder, characterised by increased deposition of iron in the liver and other organs. If left untreated, hepatic iron overload in HH patients can result in liver injury, which can progress to cirrhosis and subsequently HCC[9,10]. Further clarification of the risk factors in individual HH patients for HCC development remains an area of unmet clinical need. Herein, we review the current literature concerning the association between HH and HCC with a focus on *HFE* gene-related HH. This review highlights and discusses clinical studies that address the prevalence of HCC in HH and risk factors linked with the development of HCC in patients with HH.

HEREDITARY HEMOCHROMATOSIS

HH comprises a number of inherited diseases of iron metabolism[11]. Although its geographic distribution is worldwide, HH is one of the most common genetic disorders in individuals of Northern European ancestry, particularly Nordic or Celtic ancestry. In this population, the frequency of homozygous HFE mutation is approximately 1 in 200-250 individuals[9,12-14]. There is considerable phenotypic diversity in HH and associated biochemical changes are more common than the clinical manifestations of iron overload-related disease. Indeed, advanced clinical expression of HFE-related hemochromatosis is rare[15]. This autosomal recessive condition is characterised by excessive iron absorption by the small intestine. This leads to progressive iron loading over many years of the affected individual's adult life. As hepatocytes store most of the excess iron, the liver is the organ mostly afflicted by iron overload[16,17]. Long-term effects of excessive iron loading include liver fibrosis, cirrhosis, HCC, cardiomyopathy, diabetes mellitus, hypogonadism and arthropathy[10,11]. Early studies suggested that liver disease was the most frequent cause of death in HH individuals[18]. However, more readily available genetic testing, greater public awareness and an improved understanding of the natural history of the condition means that most affected subjects are now diagnosed before significant target organ injury occurs.

HH is classified into 6 groups depending on the nature of the underlying genetic mutation[17]. Mutations in the high iron gene (*HFE*) (Hemochromatosis Type 1, i.e., *C282Y* homozygosity, *C282Y/H63D* compound heterozygosity and other *HFE*-related genotypes, e.g., *S65C*) are responsible for the majority of hemochromatosis cases[19]. Rarer forms of non-*HFE* associated HH have been attributed to mutations in hemojuvelin (*HJV*) (Hemochromatosis Type 2a), hepcidin (*HAMP*) (Hemochromatosis Type 2b), transferrin receptor 2 (*TFR2*) (Hemochromatosis Type 3), ferroportin (*SLC40A1*) (Hemochromatosis Type 4) and ferritin heavy chain 1 (*FTH1*) (Hemochromatosis Type 5) gene[17]. These latter conditions are thought to account for most of the non-HFE forms of HH[9].

Hemochromatosis type 1

HFE is located on chromosome 6p21.3, has seven exons and five introns and encodes for a 343-amino acid protein that is similar to human leucocytes antigen class I molecules[20]. The *HFE* gene is important for normal iron metabolism. Feder *et al.*[19] first identified that a mutation in this gene caused HH. The three most common mutations at exons 2 (187C→G and 193A→T) and exon 4 (845G→A) of the *HFE* gene were linked with HH[19]. However, not all HH patients harbour these mutations, as outlined above[21].

C282Y (845G→A) homozygotes

About 80%-85% of individuals with HH are *C282Y* (845G→A) homozygotes. This founder mutation leads to a single-base change, resulting in the substitution of tyrosine (Y) for cysteine (C) in the amino acid sequence of the HFE protein at position 282[10]. HFE-related HH is an adult onset disorder and in expressing patients is characterised by increased transferrin saturation and serum ferritin levels compared to the healthy population[22]. Moreover, *C282Y* homozygotes are at particular risk of cirrhosis if serum ferritin levels are greater than 1000 μg/L[23]. Males and *C282*Y homozygotes of family members affected by HH exhibit a higher penetrance of homozygous *C282Y* gene[22]. However, due to the variable phenotypic expression of this mutation, only some of these patients develop cirrhosis or HCC[22]. The frequency of the *C282Y* allele is high in European populations and the reported frequency varies from 0%-3% in South Europe to 4%-10% in North Europe[20,24].

This HFE mutant protein has reduced cell surface expression and undergoes rapid degradation. The mechanisms by which HFE protein regulates iron homeostasis at the cellular level are beginning to emerge. Earlier studies proposed that the HFE protein binds the transferrin receptor 1 (TFR1) to form a stable complex, which in turn decreases its binding affinity for transferrin. The HFE mutant protein does not form this complex, which results in transferrin binding to the transferrin receptor, leading to increased cellular uptake of iron and subsequently causing iron overload[25,26]. Recent studies have demonstrated that the HFE protein is an upstream regulator of the hormone hepcidin in hepatocytes[27,28]. Hepcidin is synthesised and secreted by hepatocytes and is a master regulator of iron homeostasis in the body. Hepcidin negatively regulates dietary iron uptake by the intestine[29]. Under physiological conditions, hepatic hepcidin expression is regulated by proteins that are predominantly expressed in hepatocytes, including HFE, transferrin receptor 2 (TFR2), HJV, bone morphogenetic protein 6 (BMP6), matriptase-2 and transferrin[29,30]. HH patients harbouring the *HFE* mutations have low levels of hepcidin protein allowing a slow accumulation of iron over the individual's lifetime, resulting in iron overload[31]. Iron overload induces reactive oxygen species (ROS) formation, which causes DNA damage and somatic mutations that may play a role in the subsequent development of HCC over time[32].

HFE compound heterozygotes

Two other mutations in the *HFE* gene, namely 187C→G (*H63D*) and 193A→T (*S65C*), have been identified[33,34]. These additional mutations alone have not been implicated in iron overload. However, the co-occurrence of these mutations together with a *C282Y* mutation forming a compound heterozygote (*C282Y/H63D* or *C282Y/S65C*) has been implicated in iron overload[35]. *HFE* compound heterozygotes might have increased iron indices but iron overload-related disease is most uncommon[36,37]. Clinical disease may develop in HFE compound heterozygotes in conjunction with comorbid factors such as obesity, excess alcohol consumption or diabetes[37]. The Netherlands and the Iberian Peninsula have a high frequency of the *H63D* allele that varies from 7.9% to 17.5% in the general population[38]. The frequency of S65C allele is low, 0%-1% in European and Brazilian populations[12,34,39]. Approximately 5% of individuals with a clinical diagnosis of HH are compound heterozygotes[40,41].

THE ASSOCIATION BETWEEN HH AND HCC

HCC develops in HH individuals and contributes to the increased mortality[42-47]. Although the biological and physiological functions of *HFE* gene within the liver are not fully understood, several case-control

and population-based studies have confirmed that *HFE* mutations confer increased risk for HCC development[48-57]. The exact incidence and prevalence of HCC varies considerably between different studies, which is probably explained by the heterogeneity of the study cohorts. The characteristics of the study cohort is critical in the interpretation of such studies given the variable phenotype of HH and also because cirrhosis is such a critical risk factor for the development of HCC. Some studies report on cohorts where a large proportion of patients had underlying cirrhosis. Other studies have clear referral bias due to the authors' interest in disorders of iron metabolism, are retrospective in nature or are from liver transplant programs where one would expect cirrhosis and HCC to dominate the study cohort.

Population-based studies provide a more realistic assessment of the overall incidence and prevalence of HCC in HH. In one such study, the US National Centre for Health Statistics reported a close association between HH and HCC. In this study, patients who were diagnosed with HH and who died were 23-fold more likely to have HCC in comparison with individuals without a diagnosis of HH[47]. A further study conducted in Sweden reported the risk of HCC in HH individuals to be approximately 20-fold higher than in the general population[42]. At 10 years of follow up, the absolute risk of HCC among HH men was 6%, which was higher than the risk in women (1.5%)[42]. Willis *et al.*[58] found that HCC patients had a 7% prevalence of the *C282Y* homozygous mutation. In a similar study, Sánchez-Luna *et al.*[59] found that, among 118 *C282Y* homozygotes, eight homozygotes developed HCC, representing 1.8% of patients with HCC.

Meta-analyses have recently been conducted to clarify the effect of *HFE* polymorphisms on the susceptibility to HCC. Ellervik *et al.*[60] conducted a meta-analysis to examine associations between *C282Y* and *H63D* mutations with HCC. An odds ratio of 11 for HCC occurrence was reported for *C282Y* homozygotes (YY *vs.* CC). A further study conducted on 43 published articles (5758 cases and 14,741 controls) demonstrated that the *HFE C282Y* homozygous mutation was significantly associated with increased risk of HCC compared to the overall population[61]. Another meta-analysis including nine studies based on European populations (1102 HCC cases and 3766 controls) showed an association between the Y allele of *C282Y* and HCC risk overall as well as in alcohol-related cirrhosis patients but not in viral-related cirrhosis patients[51]. There are also contrary reports showing no association with the risk of developing HCC[23,33,55,62,63], possibly reflecting the low penetrance of the *C282Y* mutation in the populations studied.

Cirrhosis and other risk factors for HCC development in HH

HCC accounts for 25%-45% of disease-related premature deaths in HH[64]. In HH, the primary risk factor for the development of HCC is the presence of cirrhosis. Studies that have assessed the association of risk factors for development of HCC in HH are listed in Tables 1 and 2. Some studies have indicated that the risk of HCC in cirrhotic HH patients is higher than in patients with cirrhosis from other causes. In a meta-analysis assessment of eight studies which included follow up of cirrhotic patients, the annual incidence of HCC was 1.20% per year[65]. One other study showed that, once cirrhosis has been established in HH patients, the annual incidence of HCC is approximately 4%[66]. Earlier studies have revealed that risk of HCC developing in cirrhotic HH patients was 200-fold higher than non-cirrhotic control groups[18,67]. These studies may have suffered referral bias and lacked *HFE* genetic testing with the diagnosis of HH based on clinical features and biochemical tests[18,67]. Recent studies utilised a combination of *HFE* genotyping, clinical examination and abnormal iron indices including transferrin saturation, serum ferritin, and iron deposition in liver biopsies to confirm diagnosis of HH[42,44]. These studies have revealed that risk of HCC developing in cirrhotic HH patients was 20-fold higher than non-cirrhotic control groups[42,44,48]. It is worth noting that HCC has occasionally been found to occur in HH patients with no cirrhosis[64,68,69]. In these patients, hepatic iron accumulation has been suggested to be directly involved in HCC development independently of cirrhosis[64,69,70]. An increased risk of HCC with cirrhosis among individuals heterozygous for *HFE* gene mutations has also been reported and is discussed below[42,51].

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Table 1. Association of liver cirrhosis as a risk factors for development of HCC in HH

Author	Risk factor	Study population/country	No. of cases	HFE mutation analysis	Comments
Elmberg *et al.* [42]	Cirrhosis	Population study, Sweden	1847 (HH) 5973 (first-degree relatives)	*C282Y* (+/+), *C282Y* (+/–), compound heterozygotes (*C282Y/H63D*), *H63D* (+/+), *H63D* (+/–)	HH were at a 20-fold risk of developing HCC. Overall cancer risk in first-degree relatives was not increased
Cauza *et al.* [48]	Cirrhosis	Case-control study, Austria	162	*C282Y* (+/+), *H63D* (+/+), compound heterozygotes (*C282Y/H63D*)	*C282Y* homozygotes had a 20-fold increased risk to develop HCC in patients with cirrhosis
Allen *et al.* [23]	Cirrhosis	Population study, Australia	31,192	*C282Y* (+/+), *C282Y* (+/–) compound heterozygotes (*C282Y/H63D*)	Homozygotes for the *C282Y* mutation developed HCC
Asberg *et al.* [62]	Cirrhosis	Population study, Norway	65,238	*C282Y* (+/+), compound heterozygotes (*C282Y/H63D*)	Low prevalence of cirrhosis, 3.7% in men and none in women homozygous for the *C282Y* mutation
Nowak *et al.* [73]	Cirrhosis	Case-control study, Swiss	147	*C282Y* (+/+), *H63D* (+/–), compound heterozygotes (*C282Y/H63D*)	9% of *C282Y* homozygotes develop HCC and majority of the individuals had liver cirrhosis
Fargion *et al.* [40]	Cirrhosis	Case-control study, Italy	81 (HCC), 128 (control)	*C282Y* (+/–) and *H63D* (+/–)	*C282Y* and *H63D* heterozygotes with cirrhosis have a high risk of HCC
Lauret *et al.* [52]	Cirrhosis	Case-control study, Spain	554(cirrhosis), 159 (control)	*C282Y* (+/–) and *H63D* (+/–)	20.9% patients with alcoholic cirrhosis and HCC were heterozygous for the *C282Y* mutation
Nahon *et al.* [53]	Cirrhosis	Case-control study, France	301	*C282Y* (+/–), compound heterozygotes (*C282Y/H63D*)	*C282Y* heterozygotes increased risk of developing HCC in patients with alcoholic but not with HCV-related cirrhosis
Blanc *et al.* [68]	Non-cirrhotic livers	Case-control study, France	35	*C282Y* (+/+), *H63D* (+/+), compound heterozygotes (*C282Y/H63D*)	50% of HH patients developed HCC in non-cirrhotic livers
Hiatt *et al.* [69]	Non-cirrhotic livers	USA	1	*C282Y* (+/+)	*C282Y* mutation increased the risk of HCC development in HH without cirrhosis

Genotypes: +/+ indicates homozygotes, +/– indicates heterozygotes. HCC: hepatocellular carcinoma; HH: hereditary hemochromatosis; HCV: hepatitis C virus

Table 2. Association of HFE mutations with other risk factors of HCC

Author	Risk factor	Study population/country	No. of cases	HFE mutation analysis	Comments
Elmberg *et al.* [42]	Gender	Population study, Sweden	1847 (HH), 5973 (first-degree relatives)	*C282Y* (+/+), *C282Y* (+/–), compound heterozygotes (*C282Y/H63D*), *H63D* (+/+), *H63D* (+/–)	The risk of developing HCC in HH patients was 30-fold among men and 7-fold among women
Haddow *et al.* [44]	Gender	Population study, USA	1,000,000	*C282Y* (+/+)	The relative risk for this cancer in *C282Y* homozygotes is 23
Ezzikouri *et al.* [49]	Gender	Case-control study, Morocco	222 (control), 96 (HCC)	*H63D* (+/+), *H63D* (+/–), *C282Y* (+/–)	Men carrying the *H63D* mutation had a greater risk of HCC
Willis *et al.* [71]	Gender	Population study, UK	144	*C282Y* (+/+)	The penetrance of *C282Y* homozygous genotype in HH with HCC was 1.31%-2.1% for males and zero for females
Allen *et al.* [23]	Gender	Population study, Australia	31,192	*C282Y* (+/+), compound heterozygotes (*C282Y/H63D*)	In *C282Y* homozygotes, HCC developed in a substantial proportion of men but in a small proportion of women
Shi *et al.* [56]	Chronic hepatitis B	Case-control study, China	56 (HCC), 60 (control)	*C282Y* (+/+), *H63D* (+/+)	*C282Y* mutation is associated with susceptibility to HCC after chronic hepatitis B
Fracanzani *et al.* [72]	Chronic hepatitis B and Gender	Case-control study, Italy	303	*H63D* (+/–), *C282Y* (+/–)	*C282Y* heterozygous males were 3.8-fold more likely to be HBV positive in HCC patients
Nowak *et al.* [73]	Age	Case-control study, Swiss	147	*C282Y* (+/+), *H63D* (+/–), compound heterozygotes (*C282Y/H63D*)	Higher age at diagnosis showed the strongest association with the occurrence of HCC
Elmberg *et al.* [42]	Age	Population study, Sweden	1847 (HH), 5973 (first-degree relatives)	*C282Y* (+/+), *C282Y* (+/–), compound heterozygotes (*C282Y/H63D*), *H63D* (+/+), *H63D* (+/–)	The risk of developing HCC was not associated with age

Genotypes: +/+ indicates homozygotes, +/– indicates heterozygotes. HCC: hepatocellular carcinoma; HH: hereditary hemochromatosis; HBV: hepatitis B virus

Other risk factors that may synergise with cirrhosis include chronic viral hepatitis, alcohol abuse, diabetes, age and gender[47,71] [Table 2]. Patients with HCC and diabetes mellitus were 82 times more likely to have HH[47]. The risk of HCC was higher in males who were *C282Y* homozygotes when compared to *C282Y* homozygous females - reflecting in part the higher iron burden in men[44,49,71]. A study reported 1.3%-2.1% penetrance of the *C282Y* homozygous genotype in HH patients with HCC for males and zero for females[71]. Another study found penetrance of the *C282Y* homozygous genotype in male HH patients with HCC was 5.56%[23]. Studies have also found an unequivocal relationship between risk of HCC and *C282Y* mutation in patients with chronic hepatitis B and male gender[56,72]. Another study identified increased age at diagnosis as a strong predictor for the development of HCC in HH patients and the authors suggested that this is a surrogate marker of duration of exposure to iron[73]. This latter finding has not been substantiated in other studies. Serum ferritin level of above 1000 mg/L at diagnosis confirming high iron overload was a risk factor for HCC in the study by Nowak *et al.*[73]. A serum ferritin concentration of over 1000 mg/L is also associated with a high risk of cirrhosis, which is the likely explanation for that association. In contrast to other studies, this study found no association between alcohol consumption and HCC development[73]. However, the level of alcohol consumption defined as "considerable" was greater than 10 g/day for women and 20 g/day for men and this may below the oncogenic threshold.

Collectively, these data illustrate that the presence of cirrhosis is the primary risk factor for the development of HCC in patients with HH. Other risk factors, as discussed above, seem to amplify the oncogenic potential of cirrhosis. Importantly, some of these other risks can be reduced by lifestyle modifications and/or therapy of other liver diseases, particularly chronic viral hepatitis.

HFE heterozygotes and the risk of HCC in patients with cirrhosis of other causes

This area is controversial and there are conflicting data on the role of heterozygosity for *HFE* mutations in the development of HCC in patients with cirrhosis from other causes. For example, Hellerbrand *et al.*[51] indicated that HCC patients with cirrhosis were more likely to be *C282Y* heterozygotes compared to cirrhotic patients without HCC or normal controls. Additionally, elevated levels of transferrin saturation, serum ferritin and liver iron deposition were reported in HCC patients harbouring the heterozygous *C282Y* mutation compared to those lacking this mutation, suggesting that altered hepatic iron metabolism played a pathogenic role[51]. The prevalence of the heterozygous *C282Y* and *H63D* mutation was also observed to be higher in 81 Italian patients with cirrhosis and HCC than in 128 normal controls (8.6% *vs.* 1.6%)[50]. Similarly, Lauret *et al.*[52] found a 20.9% prevalence of the C282Y heterozygous mutation in 43 Spanish HCC patients. In 301 cirrhotic French patients prospectively followed up for six months, hepatic iron overload and the heterozygous *C282Y* mutation were associated with an increase in the incidence of HCC in cirrhotic patients with alcohol-related problems but not in patients with hepatitis C viral infection[53].

In contrast, a large prospective multicentre French study compared the prevalence of *HFE* mutations in 133 cirrhotic patients with HCC and 100 cirrhotic patients without HCC with a follow up of 2.5 years[74]. This study concluded that *C282Y* mutation is not linked to an increased risk of HCC in cirrhotic patients[74]. Similarly, in another study of 162 patients with HCC and cirrhosis, the frequency of the C282Y mutation did not differ between the patients with cirrhosis or healthy controls[48].

Initially, it was proposed that the *H63D* mutation has no direct association with HH[19,33]. In line with this, several studies reported no association between the prevalence of the *H63D* mutation and the risk of developing HCC[75]. Conversely, other studies have implicated occurrence of *H63D* mutation with an increased risk of HCC in HH patients[55,61,63]. In a study of 196 HCC patients and 181 healthy controls, the *H63D* mutation was associated with an increased risk of HCC[55]. HCC developed in HH patients exhibiting *H63D* mutations along with predisposing factors such as liver cirrhosis due to chronic hepatitis C virus infection and/or ethanol abuse and chronic hepatitis B virus-infection[55]. Another large study

involving 5758 cases and 14,741 controls demonstrated that *H63D* mutation was more likely to be involved in susceptibility to HCC without cirrhosis in the African population[61]. A positive association between compound heterozygosity for *C282Y*/*H63D* and the risk of HCC was also observed[61]. Conversely, no cases of HCC were identified among the 44 compound heterozygotes examined in another study[59]. In an Egyptian cohort study, patients with the *H63D* mutation had a higher risk of developing HCC[63]. Additionally, the role of *S65C* in HCC remains to be elucidated. A number of other studies demonstrated that individuals harbouring *C282Y* or *H63D* mutation did not develop HCC, suggesting there was no association between HFE mutation and HCC[74,76-78]. Thus, whether there is a link between these *HFE* mutations and the HCC risk remains somewhat uncertain with significant variation between different populations groups, and different underlying diseases. More studies are needed to definitively assess the influence of the *HFE* mutations on the development of HCC in HH patients.

Mechanisms of iron toxicity in HH leading to HCC

Iron is ubiquitously present in cells and a physiological optimal balance of iron is critical for the normal functioning of cells[79,80]. Iron is essential for several important processes including the transfer of oxygen throughout the body by haemoglobin, the mitochondrial electron transport chain and as a cofactor in enzymatic reactions. However, excess iron can be very toxic to the cell due to its redox reactivity that promotes oxidative stress[81,82]. Homeostasis of iron in the body is maintained by four major cell types: duodenal enterocytes (dietary iron absorption), erythroid precursors (iron utilisation), reticuloendothelial macrophages (iron storage and recycling) and hepatocytes (iron storage and endocrine regulation)[83]. Duodenal enterocytes absorb dietary iron and store it in the form of ferritin. Enterocytes release iron into the circulation through the basolateral iron exporter, ferroportin. In the blood stream, iron binds to the plasma iron transport protein transferrin[82,84]. The majority of iron in the body is found in the oxygen-carrying haemoglobin of erythrocytes. Iron is also stored in the form of ferritin in hepatocytes and reticuloendothelial macrophages. The macrophages phagocytose the senescent erythrocytes and the iron from haemoglobin is loaded onto transferrin for iron recycling[83]. Importantly, in humans, there are no active mechanisms to eliminate excess iron from the body[15,42].

Transferrin is highly saturated during iron overload and additional iron released into the circulation binds to low-molecular-weight compounds and is termed non-transferrin bound iron (NTBI). Excess iron in circulation enters into hepatocytes by binding the transmembrane TFR1 and TFR2 on hepatocytes[17,83]. While both TFR1 and TFR2 are capable of iron uptake, TFR1 has a higher iron binding affinity than TFR2. TFR2 is an iron sensor that regulates body iron uptake and is sensitive to changes in transferrin saturation in the blood[82,85]. Hepatocytes have a significant role in iron homeostasis as they also produce the hormone hepcidin, an important regulator of iron balance[86]. Hepcidin binds ferroportin and stimulates the internalisation and subsequent degradation of ferroportin, thus decreasing the absorption of iron from the gut and release of iron into the circulation[84]. HFE works in conjunction with multiple proteins including TFR2 and Hemojuvelin to induce hepcidin expression[87]. In HH patients harbouring the *HFE* mutations, the hepcidin protein is not properly expressed, which leads to uncontrolled iron absorption, resulting in iron overload[17]. In addition, *HFE* mutation also leads to a loss of transferrin sensitivity, suggesting that TFR2 and HFE complex may be involved in iron-sensing[88]. The hepcidin-mediated increased iron absorption from the gut leads to preferential iron loading of the hepatocytes. It has been hypothesised that this in turn causes injury and subsequent malignant transformation of hepatocytes[79,89]. The mechanisms responsible for a direct hepatocarcinogenic effect of iron have yet to be fully elucidated[79,89].

Increase in iron absorption over time leads to iron accumulation in hepatocytes, leading to injury and subsequent malignant transformation of hepatocytes[79,89]. The role of iron in hepatocarcinogenesis has been suggested from epidemiologic studies, animal models and *in vitro* studies[90-93]. The carcinogenic effect of iron has been related to its ability to form mutagenic hydroxyl radicals, enhance lipid peroxidation, promote immune escape or facilitate chronic inflammation leading to cirrhosis.

One of the mechanisms by which iron accumulation in the liver may promote malignant transformation of hepatocytes is directly by the mechanism of oxidative stress[79]. It has been proposed that the formation of free radicals by Fenton reaction causes oxidative stress, leading to the malignant transformation of hepatocytes[94]. Although the Fenton reaction has been implicated in carcinogenic effect of iron, there is relatively little direct or experimental data to support this claim. The excess ferrous iron (Fe^{2+}) accumulates in hepatocytes and undergoes a Fenton reaction by interacting with hydrogen peroxide to form Fe^{3+} and highly reactive oxygen free radicals (ROS)[95]. Generation of ROS causes hepatocyte injury by inducing peroxidation of membrane fatty acids followed by the production of toxic by-products that disrupt DNA and protein synthesis[79,94,96]. In addition, ROS causes DNA damage and mutagenesis, which may lead to neoplastic transformation over time[97-99]. Iron-generated ROS can induce mutations in *p53*, an important tumour suppressor gene[100]. Iron-generated ROS also contributes to the production of a mutagenic and cytotoxic oxidatively DNA-damaged product, 8-hydroxy-2'-deoxyguanosine (8-OHdG)[101,102]. 8-OHdG causes G:C to T:A transversions, DNA unwinding and strand breaks[101,103,104]. A study has shown correlation of 8-OHdG levels with iron levels in serum in HCC patients[101]. In liver tissue, the rate of DNA unwinding and strand breaks have been associated with 8-OHdG levels[90]. Another study has highlighted the link between DNA unwinding and the risk of HCC in HH patients[18]. An abnormal form of NTBI, called labile plasma iron or reactive plasma iron, also contributes to oxidative stress and the subsequent liver damage during HH[105]. Overall, several studies support the role of iron-induced ROS formation as the main mechanism of development of HCC in HH[32,80,99,106,107].

Iron accumulation can also lead to cirrhosis and the subsequent development of HCC, indirectly through the induction of chronic inflammation[79]. Excess hepatic iron promotes the activation of hepatic stellate cells in HH[92]. This can promote fibrogenesis. Iron has also been shown to induce transforming growth factor-beta, which plays an important role in the development of liver fibrosis[108]. The combination of elevated iron levels with environmental and acquired factors such as excessive alcohol consumption, viral hepatitis and steatosis may act synergistically to precipitate the development of HCC[109]. Iron has a direct effect on tumour growth by promoting cellular proliferation. In human HCC cell lines, iron enhances proliferation and iron deprivation leads to cell cycle arrest and increased apoptosis[110]. It has been reported that increased iron concentration in HCC cells was associated with enhanced migration, invasion, high metastasis rate and recurrence[111].

In addition, iron reduces immune surveillance for malignant transformation by impairing T-cell proliferation and inhibiting tumoricidal activity of macrophages[79,103,104,112,113]. Epigenetic alterations due to iron overload have also been implicated in hepatocarcinogenesis. Epigenetic defects such as increased DNA methylation commonly occur in HCC[114]. Lehmann *et al.*[115] found 84% of the non-cancerous liver biopsies derived from HH patients exhibited hypermethylation of genes that are often hypermethylated in HCC. DNA hypermethylation was independent of age, cirrhosis or hepatitis infection. Several studies support the role of iron in the development of HCC in HH.

Diagnosis and treatment of HCC in HH patients

Prior to the identification of *HFE*, the diagnosis of HH was based on parameters including clinical features, increased ferritin levels, high serum transferrin saturation and characteristic findings on liver biopsy[17]. After the discovery of the *HFE* mutations, genetic screening became the preferred diagnostic test for HH. *HFE* genetic testing together with measurements of serum transferrin saturation and ferritin levels have gained traction as the diagnostic test of choice for HH[9-11]. A serum ferritin concentration of > 1000 μg/L in patients with HH has been associated with an increased risk of cirrhosis and HCC[11]. Magnetic resonance imaging (MRI) has recently been applied as an imaging modality for the detection and quantification of hepatic iron in those patients where there is diagnostic uncertainty. Additionally, MRI can be utilised to evaluate HCC in HH patients[17].

Excess iron should be removed by venesection (phlebotomy) therapy and this should eliminate the risk of progression to cirrhosis and the development of HCC in non-cirrhotic individuals. Early diagnosis and iron depletion therapy has the potential of improving the survival rate of patients[9]. HH is readily treated by venesection therapy, which is very efficient in removing excess iron and involves two successive treatment phases[116]. In the initial induction phase, the excess iron present at the time of diagnosis is removed by 1-2 weekly venesections (7.5 mL/kg body weight per venesection). After the removal of excess iron, maintenance therapy, the second treatment phase, prevents recurrent iron overload. Maintenance therapy involves removal of 2-4 units/year[117].

Although venesection is the treatment of choice in hemochromatosis, other iron depletion therapies have also been tested in HH patients[118,119]. Another iron depletion therapy involves the application of iron chelation therapy to facilitate iron mobilisation and excretion. The iron chelating drugs desferioxamine, deferiprone and deferasirox have been tested in HH patients[118,119]. A phase I/II clinical trial for deferasirox has shown it to reduce iron burden in HH patients homozygous for the *C282Y* mutation[118]. Desferioxamine is administered either by intravenous or subcutaneous route, while deferiprone and deferasirox are oral iron chelators. These iron chelators have several side effects including skin rashes, gastrointestinal disturbances and occasionally abnormal liver function tests and should only be considered in patients in whom venesection is not a possibility[118]. Of interest, iron chelators with antitumor properties and favourable toxicity profiles have emerged[120]. Several iron chelators with effective antitumor activities have been identified [Triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone), DpT (di-2-pyridylketone thiosemicarbazone) and PKIH (di-2-pyridylketone isonicotinoyl hydrazone) analogues] but these are not routinely used in clinical practice[120-122]. Both venesection and iron chelation therapies do not target the biological mechanisms involved in iron metabolism. HH is characterised by low hepcidin synthesis and clinical trials evaluating the role of therapeutic hepcidin by subcutaneous administration are currently underway. Patients with cirrhosis should undergo six monthly surveillance by ultrasound (with or without alpha-fetoprotein measurement) to detect HCC at an early stage when curative therapy is more likely to be successful. Of interest, liver transplantation remains an option for some patients with HCC within appropriate criteria and this procedure will also normalise hepcidin synthesis and prevent iron overload (provided the donor does not have HH)[117,123].

DISCUSSION

Early diagnosis and treatment of HH by preventing the development of cirrhosis may reduce the incidence of HCC in the future. The American Association for the Study of Liver Diseases guidelines recommend regular surveillance for HCC in cirrhotic patients only[9]. It has been recommended that screening for HCC be continued throughout life of the HH patients as HCC may develop years after the depletion of iron has been achieved. Whilst controversial, some recommend iron depletion therapy in patients with even minor increases in iron stores, when non-alcoholic fatty liver disease, hepatitis B or C coexist, in an attempt to reduce the risk of progressive fibrosis and subsequent HCC[72]. It is also recommended that family screening for HH mutations and iron overload in all first-degree relatives of HH patients be performed. As *HFE* gene mutation often synergises with other risk factors of HCC, HH patients with known HCC risk factors should be regularly counselled to avoid environmental or toxic injury to the liver.

Besides HH, there are many other causes of iron overloading that result in excessive iron accumulation in the liver and other organs. It has been reported that patients with high total body iron have a higher risk of developing HCC in the absence of HH[124-126]. As iron overload is not a benign condition, it is recommended that HCC surveillance be undertaken in patients with excess body iron, particularly in patients with cirrhosis[127].

Further studies to identify genetic or environmental factors that could act in concert with *HFE* mutations to increase the risk of developing HCC are warranted. Investigations are underway to determine the role

of iron-regulatory proteins in abnormal iron uptake in HCC. In-depth understanding of the intricate pathways involved in HH-associated HCC needs attention and future research needs to be focused on the prevention of HCC in these patients.

CONCLUSION

Despite the controversies in the field regarding the degree of penetrance of *HFE* mutations in different patient populations and their role in hepatic iron overload, HH patients with cirrhosis are at a high risk of developing HCC. Further study in this field is needed to better understand the pathogenic process toward HCC and to prevent HCC development in HH patients, considering that there are currently no effective therapies for HCC. Furthermore, an in-depth understanding of the metabolic iron regulatory pathways in HFE-related HCC in HH patients will allow the discovery of novel druggable targets for effective therapeutic approaches.

DECLARATIONS

Authors' contributions

Contributed to conception and design of the study and manuscript writing: Jayachandran A, Shrestha R, Bridle KR, Crawford DHG

Final approval of manuscripts: Jayachandran A, Shrestha R, Bridle KR, Crawford DHG

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Ethical approval and consent to participate

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