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Update on the Risk of Hepatocellular Carcinoma in Chronic Hepatitis B Virus Infection

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Abstract Chronic hepatitis B virus infection is an important cause of liver-related morbidity and mortality, with hepatocellular carcinoma being the most life-threatening complication. Because of the highly variable clinical course of the disease, enormous research efforts have been made with the aim of revealing the factors in the natural history that are relevant to hepatocarcinogenesis. These include epidemiological studies of predisposing risk groups, viral studies of mutations within the hepatitis B viral genome, and clinical correlation of these risk factors in predicting the likelihood of development of hepatocellular cancer in susceptible hosts. This update addresses these risks, with emphasis on the latest research relevant to hepatocarcinogenesis.

Keywords Hepatitis B virus · Hepatocellular carcinoma · Risk factors · Mutation · Risk prediction

Introduction

Chronic hepatitis B (CHB) infection is a global problem affecting nearly 400 million individuals worldwide. The majority of those infected are ethnically of Asian and African origin. Asians and Africans account for more than 80% of hepatitis B-related mortality, from hepatocellular carcinoma (HCC) and cirrhosis. As the natural history of

CHB infection unfolds with ongoing clinical research, we strive to elucidate the pathogenic mechanisms and carcinogenesis behind hepatitis B infection and its chronic sequelae. An exciting area of hepatitis B research focuses on determining the risk factors of development of HCC. This review focuses on the latest basic and clinical research on various risk factors that have been identified in CHB-infected individuals who develop HCC. A summary of studies—mainly population studies, case-controls, cohort studies, and genetic studies—are highlighted, with the emphasis on the themes and mechanisms of hepatocellular carcinogenesis.

Hepatitis B Virus and Carcinogenesis

Hepatitis B virus (HBV) infection has a highly variable clinical presentation ranging from asymptomatic carrier state to fulminant hepatic failure. CHB infection can lead to cirrhosis, resulting in liver failure and HCC. There are inherent barriers to studying the hepatitis B virology because of the inability to culture HBV for in vitro studies. The multifactorial and complex processes—from initial infection to viral persistence, chronic hepatitis, and ultimately to HCC—have yet to be fully unraveled. One of the mechanistic pathways of HBV carcinogenesis includes HBV integration into the host genome. This may lead to chromosomal translocations, duplications, deletions, and amplification of cellular DNA [1]. Subsequent intracellular events regulated and mediated by the hepatitis B X gene (HBx) products include regulatory proteins that affect transcription and transactivation of proto-oncogenes with modulation of cell signaling pathways. Similarly, proteins from the pre-S2/S region have been implicated in HBV carcinogenesis because HBx or pre-S2/S viral proteins have

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been consistently found in a large proportion of tumor cells. Furthermore, inhibition of DNA repair mechanisms may culminate in mutations in the pre-core and core promoter regions of the HBV viral genome, which have been shown in various studies to correlate with a greater chance of developing HCC. Because 80–85% of patients with HBV-related HCC have underlying cirrhosis, chronic infection with persistent and rapid regeneration of hepatocytes can lead to accumulation of mutations that predispose the hepatocytes to malignant transformation. In HBV-related HCC, chromosomal mutations are more common than in non-HBV-related HCC [2]. Likewise, a variety of genetic alterations and marked heterogeneity of gene expression profiles have been documented in HCC cases. The close connection between cirrhosis and the development of HCC is similar to alcohol- and hepatitis C-related HCC. However, the absence of cirrhosis in 15–20% of HBV-related HCC highlights the direct oncogenic properties of the virus itself.

Host Risk Factors

Host and demographic factors associated with a higher risk of developing HCC include age greater than 40 years, male gender, Asian race, and various other lifestyle or modifiable environmental risk factors such as smoking, alcohol consumption, ingestion of aflatoxin B₁, and family history of HCC. A strong association between individuals greater than 40 years old and HBV-related HCC has been demonstrated by various cohort studies [3]. Compared to the 30 to 39 years age group, the relative risk of HCC development in the 40 to 49 years group is 3.6 to 5.4 and in the 60 years or older group is 8.3 to 17.7 [3]. Male gender is also associated with an increased risk of HCC compared to women in two large Taiwanese studies [4, 5]. Asian race is associated with a high prevalence of HBV-related HCC. More than 70% of newly diagnosed HCC cases occur in Asia, where 75% of CHB-infected patients reside. (An exception is Japan, where HCV is the etiology in 80% of HCC cases [6]). In North America, ethnic Asians and African-Americans are more likely to develop HCC from hepatitis B infection when compared to Caucasians [7]. In another large Taiwanese population study, first-degree relatives, particularly siblings, are more likely to develop HCC, with a multivariate-adjusted rate ratio of 2.4 compared to non-HCC controls [8]. Other modifiable demographic factors that predispose HBV-infected individuals to have a higher chance of developing HCC include heavy regular alcohol consumption [9], consumption or exposure to aflatoxins [10], and diabetes mellitus, which may be related to steatohepatitis [11].

Host Response to HBV infection

The various stages of human infection, immunotolerance, immunoclearance, and late/residual phases are characterized by changes in hepatitis B early antigen (HBeAg), alanine aminotransferase (ALT), and HBV DNA levels. The host immune response is mainly cytotoxic T-lymphocyte-mediated and drives the events that may lead to ultimate viral clearance. The immunotolerance phase is associated with HBeAg-positive state, normal ALT, and high viral load. The immunoclearance phase occurs with fluctuating ALT levels and often correlates with HBeAg seroconversion. At this time, the HBV DNA may also be reduced to lower levels, and less frequently, to undetectable levels. In the chronic late/residual phase, mild ALT fluctuations associated with viremia are often seen, with a significant proportion of patients progressing to established cirrhosis. The levels of ALT have been shown to be poor predictors of progression of liver disease to cirrhosis and HCC. In a Korean study of individuals with no known liver diseases, high “normal” ALT values were associated with increased liver-related mortality [12]. In a study of 3,233 Chinese patients with CHB, it was shown that ALT levels of 0.5 to 1.0 times the upper limit of normal (ULN) and 1 to 2 times the ULN were associated with increased risk of developing cirrhosis complications and HCC compared with patients with ALT levels less than 0.5 times the ULN ($P < 0.0001$ for both groups) [13]. Cirrhosis is another virus-mediated, host-driven response. Cirrhosis has been consistently shown to predict the development of HCC, as exemplified by cross-sectional or case-cohort studies [13, 14].

HBV Risk Factors

Many potential virological factors may be associated with a higher risk of developing HCC. HBeAg, HBV DNA load, viral genotype, and pre-core and core promoter region mutations have all been studied. HBeAg positivity reflects active viral replication and has been associated with higher incidence of HCC [15]. However, it should be noted that two thirds of patients with HCC are already positive for antibody to HBeAg [13]. In the landmark Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-Hepatitis B (REVEAL-HBV) study, more than 3,500 HBsAg-positive adults aged 30 to 64 years were followed from 1991 to 2004 for the incidence of cirrhosis and HCC. This study documents that the higher the viral load, the more likely the chance of progression to cirrhosis and HCC, independent of HBeAg status and ALT levels [16]. In another recent study conducted in Japan, in patients over the age of 40 years, HBV DNA viral load again was confirmed to be an important risk factor [17].

In addition, several studies have shown that mutations in the HBV genome are associated with a higher risk of HCC development. The HBV genome consists of four overlapping open reading frames (ORFs) encoding the surface, core, X, and polymerase proteins. The HBV core promoter region includes the basal core promoter (nucleotides 1751–1769) and the enhancer II (nucleotides 1636–1744), which overlaps with the X ORF (nucleotides 1374–1835). The core promoter is regulated by enhancer II (and to some extent, enhancer I) and controls the transcription of pre-core mRNA and pre-genomic RNA. In the basal core promoter region, A1762T/G1764A nucleotide substitutions, particularly when occurring as a double mutation, are shown to be associated with a higher chance of HCC, in genotypes B and C [18]. Furthermore, these mutations can be detected in the plasma up to 8 years before HCC diagnosis, signifying a possible role as serum biomarkers for predicting HCC occurrence [19]. However, in a recent cross-sectional study from Qidong, China, 852 hepatitis B-infected subjects were followed (for up to 7 years) with annual blood tests for HBV gene sequencing. Fifty-eight subjects who developed histologically proven HCC were compared with 71 subjects with chronic hepatitis who did not develop HCC. Both T1762/A1764 mutations were frequently seen across both groups; there was no difference in the prevalence of these mutations between the two groups ($P>0.05$). However, the adjacent nucleotide mutations C1766T and T1768A were significantly higher in HCC patients when compared to hepatitis B controls [20]. Of the 58 cases of HCC with identified HBV sequences, 22.4% possessed triple mutations compared to only 7% in the hepatitis B controls ($P<0.05$). T1766 and A1768 mutations occurred more frequently at a later time point before HCC detection, which may indicate a crucial role in carcinogenesis.

The possible carcinogenic roles of other HBV mutations have been studied. Present evidence does not suggest that the precore mutations, a G1896A mutation abolishing the production of HBeAg, play an important role in hepatocarcinogenesis [21•]. However, a recent meta-analysis showed that C1653T in the enhancer II region and T1753V of the X region are linked with an increased risk of HCC [22]. Another recent study from China analyzed 196 HCC patients (with 323 controls) and showed that pre-S deletion, and T1762/A1764 double mutations, are independent risk factors for HCC [23]. A novel F141L mutation in the pre-S2 region is also identified as a risk factor for HCC in a Korean study of genotype C patients [24]. A study performed in Thailand comparing the HBV DNA sequences of HCC versus non-HCC hepatitis B patients showed that A1762T/G1764A and G1899A mutations were independently associated with a higher risk of HCC after multiple logistic regression analysis [25].

Although G1899A mutations have not been consistently demonstrated in HCC cohorts in other studies [26], this mutation and its association with other, better known, mutations may play a synergistic role in hepatocarcinogenesis. In addition to identifying mutations in the HBV genome that predispose patients to HCC occurrence, a recently published study showed that HBV DNA level ($>3.0\times 10^7$ copies/g) and basal core promoter A1762T/G1764A mutation are both independently predictive of postoperative increased survival in surgically operated HCC patients, whereas a small pre-S deletion between codons 107 and 144 heralds a poorer postoperative prognosis [27•]. The identification of pre-S2 deletion and its significance is gaining in importance. In a Taiwanese study involving pediatric patients with HCC, the sera of HCC patients had a much higher rate of pre-S deletion (nearly all were in S2 region and more commonly seen in genotype C) than non-HCC controls. The odds ratio is 36.69 ($P=0.015$), highlighting the fact that even in pediatric age groups, pre-S mutations are potentially carcinogenic [28]. Despite the identification of multiple nucleotide substitutions, these mutations and their mechanism of carcinogenesis remain largely unknown. [29].

HBx Protein

The HBx regulatory protein is encoded by the X gene with multiple functions. This small protein is a transcriptional activator and is essential for HBV viral replications and infection. HBx has been shown to promote tumor formation in transgenic mice [30]. In humans, HBx has pleiotropic activities on cell cycle regulation, signaling pathways, and DNA repair. Its transactivation function may play a crucial role in hepatic carcinogenesis because a large number of signaling pathways, cellular genes, and inflammatory and regulatory mediators are involved. Studies have shown that X gene mutations are linked with increased risk of hepatocellular carcinoma [31, 32]. The impact of basal core promoter mutations has an indirect effect on the HBx protein. A three-amino acid substitution (lysine to methionine, valine to isoleucine, and phenylalanine to tyrosine at aa 130–132) can lead to reduced growth-inhibitory effects of HBx [20]. In a recent review published by Neuveut et al. [33•], HBx and its regulation of transcription indirectly via cellular signaling pathways (eg, nuclear factor- κ B, mitogen-activated protein kinase, and janus kinase/signal transducer and activator of transcription pathways) are elegantly outlined. However, the HBx regulatory protein and its mechanisms of tumorigenesis in humans remain largely understudied, and further research is needed to define its oncogenic role in HCC development.

HBV Genotypes

Currently, there are eight major HBV genotypes (A to H) as determined by a genomic sequence divergence of greater than 8%. Different genotypes are found in various parts of the world. Because genotypes B and C are most commonly seen in Asia, these two genotypes have been studied in greater detail. Different HBV genotypes have distinct patterns of viral mutations in the preS and the enhancer II/basal core promoter/precore regions. The preS deletion mutations and core promoter mutations are more frequently found in genotype C than in genotype B patients [34, 35••]. It is also generally known that genotype C carries a higher chance of cirrhosis and subsequently HCC. A recent study showed that genotype C, viral load greater than 10^4 copies/mL, A2962G, preS2 start codon mutation, T105C, T1753V, and A1762T/G1764A are independently associated with increased risk of HCC [36]. However, genotype B has been linked to a younger HCC profile, in which cirrhosis was less commonly seen [37]. Yet other studies have shown that genotype B and C do not differ in the risk of development of HCC [38, 39]. The apparent differences may be due to the higher prevalence of core promoter mutations (which is an independent risk factor for HCC) seen in genotype C patients. Because of the smaller number of individuals with the other major HBV genotypes (A, D–H), cohort studies defining the mutational phenotypes are still scarce. Table 1 summarizes the known host and viral risk factors for HCC development in patients with CHB.

Risk Factors and Predictive Scores

Predictive scores have been designed to give the clinician more quantitative measurements in guiding treatment and HCC surveillance. In a case-cohort study of 820 treatment-naïve patients with CHB, the 5-year prevalence of HCC was 4.4% and the 10-year prevalence was 6.3% [40]. Cox regression analysis showed that male gender ($P=0.025$, RR 2.98), increasing age ($P<0.001$, RR 1.07), higher HBV DNA levels ($P=0.02$, RR 1.28), core promoter mutations ($P=0.007$, RR 3.66), and presence of cirrhosis ($P<0.001$, RR 7.31) were independent risks for the development of HCC. A risk score was derived from the above factors: the Guide with Age, Gender, HBV DNA, Core promoter mutations, and Cirrhosis (GAG-HCC) score. The formula was $16 * \text{sex (male=1, female=0)} + \text{age (in years)} + 3 * \text{HBV DNA (copies/mL in log)} + 19 * \text{core promoter mutations (mutant=1, wild type=0)} + 30 * \text{cirrhosis (presence=1, absence=0)}$. The optimal cut-off of the HCC score for the prediction of 5- and 10- year development of HCC was 101. The sensitivity using this

Table 1 Risk factors for development of hepatocellular carcinoma in patients with chronic hepatitis B

Risk factor	Strength of association
Host	
Male	++
Age>40 y	++
Family history	+
Regular alcohol consumption	+/-
Host-viral interaction	
ALT levels	+
Cirrhosis	++
Viral	
High HBV DNA levels	++
Genotype C	+
Precore mutation	-
Core promoter mutations	++
Pre-S	+
HBx	+

–none, +/- possible association, + known association, ++ strong association, ALT alanine aminotransferase, HBV hepatitis B virus, HBx hepatitis B X gene

cut-off was 84.1% (at 5 years) and 88.0% (at 10 years). The specificity was 76.2% (at 5 years) and 78.7% (at 10 years). The inherent limitations of this predictive scoring model lie in its oversimplification and synergistic effects (rather than simply additive) of multiple and confounding risk factors. In addition, the core promoter mutation analysis may not be available in non-research-based laboratories. With recent research highlighting the importance of other newly identified mutations (eg, the pre-S deletion mutations), the score should be revised for more accurate risk prediction.

Other clinical scoring systems have been devised and validated using simple clinical parameters, without the need for determining HBV DNA mutations or HBV genotypes. For example, Yang et al. [41] derived a nomogram (from a study with more than 3,600 patients) using parameters of sex, age, family history of HCC, alcohol consumption habit, serum ALT level, HBeAg status, serum HBV DNA level, and HBV genotype to predict the risk of development of HCC at 5 and 10 years. Wong et al. [42] evaluated more than 1,000 patients and determined five clinical parameters that independently predicted HCC development: age, albumin, bilirubin, HBV DNA, and the presence of cirrhosis. A prediction scoring system was formulated and validated with another cohort with high accuracy and predictability. The application of these scoring systems using clinical and virologic parameters should play an important role in guiding clinicians' individual patient management.

Conclusions

This review highlights the main important risk factors of HCC development, with particular focus on demographics, host, and viral factors. The most susceptible host would be an Asian male aged older than 40 years with a long-standing history of hepatitis B infection. High levels of circulatory HBV DNA coupled with the presence of clinical cirrhosis are further disease-related risk factors. The importance of recognizing particular naturally occurring mutations such as pre-S deletions, core promoter mutations that may or may not overlap with the X region, and novel mutations that are being discovered cannot be overemphasized. Important mutations identified in cohort studies may serve as potential serum biomarkers in susceptible individuals. HBV genotype C remains an important association with HCC despite the conflicting data available. Scoring systems to predict HBV-related HCC have been developed and may be refined with further elucidation of the mechanisms of HCC carcinogenesis.

Disclosure Conflicts of interest: A. Hsu—none; C.L. Lai—grants from LG Life Sciences and FibroGen, speakers' bureaus for Bristol-Myers Squibb and Gilead Sciences Europe, and US Nonprovisional Patent Application and US Patent Cooperation Treaty Application; M.F. Yuen—grants from Research Grants Council, Hong Kong Research Fund for Clinical Infectious Diseases, speakers' bureaus for GlaxoSmithKline and Bristol-Myers Squibb, and US Nonprovisional Patent Application and US Patent Cooperation Treaty Application.

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