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TOPIC HIGHLIGHT

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Significance of viral status on occurrence of hepatitis B-related hepatocellular carcinoma

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Core tip: There are conflicting data on the relationship between hepatitis B virus infection risk factors and hepatocarcinogenesis. In this article, we reviewed the risk of hepatitis B surface antigen level, seropositivity of hepatitis B e antigen, high viral load, viral genotype, and specific viral sequence mutations, separately.

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Abstract

Hepatitis B virus (HBV) infection remains a challenging global health problem, with more than 350 million people chronically infected and at risk of developing hepatocellular carcinoma (HCC). Interactions that occur among host, environmental, and viral factors determine the natural course and predict the prognosis of patients with chronic HBV infection. In the past decades, several important viral factors of predictive of HCC have been identified, such as high hepatitis B surface antigen level, seropositivity of hepatitis B e antigen, high viral load, viral genotype, and specific viral sequence mutations. Identification of certain viral risk factors for HCC development and stratification of patient risk are very important to perform future surveillance programs. In this article, we thus reviewed the risk of viral factors involved in hepatocarcinogenesis.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a global health problem, as it is the fifth most common neoplasm and the third leading cause of cancer-related death[1]. Etiologically, HCC mainly develops in carriers of chronic hepatitis B virus (HBV), particularly those in East Asia and sub-Saharan Africa, where HBV is hyperendemic[2]. Worldwide, 350 million subjects with chronic HBV infection have a 15%-25% risk of dying from HBV-related advanced liver diseases, which includes HCC and decompensated cirrhosis[3]. At present, more than 500000 new HCC cases are diagnosed each year, with an age-adjusted incidence of 5.5-14.9 per 100000 population worldwide[1]. It has been reported that 75%-80% of global HCC patients were attributable to chronic viral infection with either HBV (50%-55%) or hepatitis C virus (HCV; 25%-30%)[4]. Compared with non-carriers, patients with chronic HBV infection have a greater than 100-fold increased risk of developing HCC[5]. The HBV genome comprises a partially double-stranded circular DNA molecule of ap-

proximately 3200 base pairs. This DNA strand encodes four overlapping open reading frames: S, for the surface/envelope protein; C, for the nucleocapsid; X, for the X protein; and P, for the DNA polymerase[6]. The regions upstream to S and C genes are termed as pre-S and pre-C, respectively[7]. HCC pathogenesis in chronic HBV-infected patients has been studied extensively, and several important viral risk factors have been identified, such as high hepatitis B surface antigen (HBsAg) level, seropositivity of hepatitis B e antigen (HBeAg), high viral load, genotype C, and specific viral sequence mutations. Identification of certain viral risk factors for HCC development and stratification of patient risk are very important to perform future surveillance programs. In this review, we focused on the effect of viral status on the occurrence of HBV-related HCC.

SERUM HBV DNA LEVELS AND HCC

For the progress of technology in highly sensitive testing methods, the measurement of serum HBV DNA levels are available recently. The presence of circulating virus is a biomarker of active chronic HBV infection and indicates the potential for chronic damage to the liver. Recently, the importance of serum viral load as a predictor of HCC development was extensively studied. Many case-control and cohort studies have reported a significant relationship between high viral load and increased HCC risk. A community-based, nested case-control study from a prospective cohort of chronic HBV carriers in Taiwan revealed that the association between serum HBV DNA levels at study recruitment and HCC risk was significant. Compared with patients who had HBV DNA level < 2.5 pg/mL, the adjusted odds ratio was 2.3 and 6.0 for patients with HBV DNA level of 2.5-13.0 pg/mL and > 13.0 pg/mL at study entry, respectively[8]. A hospital-based cohort study with a follow-up period of more than 7 years of 1006 patients from Hong Kong demonstrated that serum HBV DNA levels at study entry were significantly associated with subsequent development of HCC in a dose-response relationship[9]. The Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-HBV study (REVEAL-HBV study) that included 4155 HBsAg-seropositive adults aged from 30 to 64 years at recruitment in 1991-1992 and followed through 2004 reported a significant biological gradient of HCC risk by HBV DNA levels. The incidence of new-onset HCC increased with the levels of serum HBV DNA (copies/mL) at study entry, ranging from 108 (less than 300), 111 (300 to 9.9×10^3), 297 (1.0×10^4 to 9.9×10^4), 962 (1.0×10^5 to 9.9×10^5), to 1152 (more than 1×10^6) per 100000 person-years, respectively. Multivariate Cox regression analyses have shown that a high HBV DNA level was the strongest independent risk factor for HCC development after cirrhosis[10]. Similar to previous study outcomes from Taiwan, Hong Kong, and Japan, a significant relationship was observed in a community-based nested case-control study conducted in Qidong which enrolled 170 newly diagnosed HCC patients and 276 chronic HBV carrier controls with normal alanine aminotransferase levels at baseline[11]. However, the actual relationship between viral replication and HCC progression during HBV infection remain unknown. Theoretically, persistent HBV replication may lead to chronic hepatic inflammation and fibrosis, mediate alterations in the production of alpha 2-macroglobulin and transforming growth factor-beta 1, and finally cause carcinogenesis[12,13]. A large meta-analysis, which incorporated 26 studies, demonstrated a significant association between histological grades and serum HBV viral loads at enrollment and the end of treatment. A strong correlation between the improvement of histological grade and decreased serum HBV DNA levels was also observed[14]. In addition, as a dynamic parameter during chronic HBV infection, HBV DNA levels always vary over time in most patients with chronic HBV infection. The measurement of HBV DNA from only one time point may be insufficient to depict the degree of liver damage. We believe that serial examination of viral load during chronic infection can provide more opportunity to differentiate immune-tolerant individuals with quiescent disease from subjects with more active immune responses and persistent liver injury.

HBSAG LEVELS AND HCC

The examination of HBsAg is usually used to identify HBV infection. The level of serum HBsAg reflects the transcriptional activity of covalently closed circular DNA (cccDNA). The association between serum HBsAg and intrahepatic markers of HBV infection (integrated HVB DNA and cccDNA) has been demonstrated previously[15]. Different HBsAg levels during chronic HBV infection reveal cccDNA distribution during the respective disease phases[16,17]. HBsAg is a major biomarker that indicates active HBV infection as well as helps predict the prognosis of chronic HBV infected subjects[18]. Several studies have suggested the effect of quantitative serum HBsAg on predicting virological response to antiviral drugs, such as in patients who received pegylated interferon therapy[19]. HBsAg seems useful to recognize non-responders as early as 12-24 wk after the start of pegylated interferon therapy and tailor therapy duration in responders[19]. Meanwhile, the measurement of quantitative HBsAg is an easy and reproducible method and can be used concomitantly with the examination of serum HBV DNA levels to categorize patients during the natural history of infection and to evaluate the effect of antiviral therapy in clinical practice. A lower level of HBsAg was found to be correlated with a higher opportunity of HBsAg clearance and a lower risk of liver disease activity in genotype B or C infection[20,21]. Recently, an HBsAg level more than 1000 IU/mL was reported as an independent risk factor for the development of HCC in patients with low hepatitis B viral load (< 2000 IU/mL), suggesting that HBsAg level might complement HBV DNA level in predicting the progression of HCC, particularly in the lowly viremic

HBV carriers[22].

HBEAG AND HCC

HBeAg is an alternative protein product of the core region sequence, which is one of the four overlapping open reading frames of HBV genome. The translation of HBeAg is started from the precore gene containing a leader sequence that directs the protein to the secretory pathway[23]. HBeAg has an immunomodulatory role, and HBeAg positivity usually demonstrates active HBV replication. The presence of HBeAg was often used as a criterion for treatment before the introduction of HBV DNA examination[24]. The effect of HBeAg positivity on predicting HCC risk remains debatable. The results of several previous studies showed that HBeAg prevalence was lowest, highest, and intermediate among patients with HCC, chronic HBV infection, and liver cirrhosis, respectively[25,26]. However, HBeAg prevalence among HBsAg positive HCC patients was reported to be significantly higher than that among matched HBsAg positive controls in other studies[27-29]. In a large prospective study conducted in Taiwan, HBeAg positivity at recruitment was related to an significant increased HCC risk[8]. Although this result proposed that the role of HBeAg may be to contribute to the development of HCC, this study was limited because HBV DNA levels were not tested in all HBV carriers from this cohort regardless of the status of HBeAg. With the progress of technology, examination of HBV DNA has become a routine test for chronic HBV carriers. In the REVEAL-HBV study, high serum HBV DNA level (≥ 10000 copies/mL) was confirmed to be a strong HCC risk predictor independent of HBeAg status[10]. Consistent with the above-mentioned Taiwanese study, our previous nested case-control study from Qidong also revealed that positivity of HBeAg was not an independent risk factor of HCC[30]. The results from several prospective studies indicated that HBeAg was most likely a marker of active HBV replication correlated with an increased HCC risk.

HBV GENOTYPE AND HCC

Eight different HBV genotypes (A-H) are identified on the basis of 8% or more nucleotide divergence in the complete HBV sequence and each genotype has a specific ethnic and geographic distribution[31]. Genotypes A and D prevail in India, Europe, and Africa; genotype E only in West Africa only; genotype F in Central and South America; and genotypes B and C dominate the HBV types in East Asia[32]. Of special note, increasing evidence suggests that clinical and therapeutic outcomes may be affected by HBV genotypes. In the Asia-Pacific regions where chronic HBV infection is endemic and genotypes B and C prevail, the differences of prognosis between genotypes B and C infection have been studied extensively. Moreover, in western countries with genotypes A and D prevailing, the prognosis of different genotypes infection has also been recognized increasingly. However, because of distinct distribution of HBV genotypes in western and Asian countries, the clinical outcomes of different genotypes infection could only be reliably compared between genotypes A and D or genotypes B and C[33]. In Asia, compared with genotype B, genotype C was found to be more commonly correlated with advanced liver diseases, such as liver cirrhosis and HCC[34-36]. However, a interesting finding was the association of genotype B HBV with the development of HCC in patients under the age of 50 years in Taiwan[37]. Serologically, compared to patients with genotype B, genotype C patients had significant higher HBV DNA levels and higher incidence of HBeAg positivity. Furthermore, those with genotype C exhibit a delayed HBeAg seroconversion during the phase of immune clearance. The data on the clinical course of chronic HBV infection with genotypes other than B and C was limited. In Western countries, genotype D was reported to be correlated with progressive liver disease and a higher risk of HCC development than genotype A[38]. Nevertheless, in another study, Livingston *et al*[39] found a preponderance of genotype F in HCC patients and genotype C was not related to a higher likelihood of HCC development than other genotypes[39]. The relationship between genotype E and its clinical relevance was seldom reported. Until now, no previous study has aimed to evaluate the risk of HCC development among patients infected with all eight HBV genotypes. Such studies are lacking because of the preponderance of one or two HBV genotypes in most of the geographical regions. Taken together, previous findings thus suggest that the long-term prognosis of chronic HBV infection also differs among HBV genotypes, and further study will help elucidate differences in disease status caused by various HBV genotypes worldwide.

SPECIFIC MUTATIONS AND HCC

Among all HBV-produced proteins, the X protein can modify host transcription and degradation of protein, regulate signal transduction pathways and cell responses to genotoxic stress[40]. Several mutations in the X region sequence are frequently observed in HCC patients, indicating that these mutations may play a potential role in hepatocarcinogenesis. The clinical implications of the mutations in the enhancer Ⅱ (Enh Ⅱ) and basal core promoter (BCP) regions have been reported in many studies. The most common naturally occurring mutations in BCP region are T1762 and A1764 double mutations. BCP double mutations were confirmed to be related to HCC occurrence by two large prospective cohort studies[41,42]. Apart from these double mutations, other mutations in core promoter (CP) region have become increasingly identified to be associated with the occurrence of HCC. For example, several reports have identified an significant association between HCC development and T1653 mutation in box α of Enh Ⅱ, and T1766, A1768, and V1753 mutations in BCP region[43-48]. The proper

mechanisms of hepatocarcinogenesis relating to these above mentioned mutations remain unclear. Many trans-regulating nuclear factors bind several HBV sites overlap with the X region sequence (such as nucleotide 1653, 1753, 1762, and1764), including hepatocyte nuclear factor 4, CCAAT/enhancer binding protein α, and the ubiquitous transcription factor Sp1. T1653, V1753, T1762, A1764, and A1768 mutations are mis-sense mutations that convert amino acid of X coding region (aa 94 H to Y, aa 127 I to T/N/S, aa 130 K to M, aa 131 V to I, and aa 132 F to Y), which could contribute to hepatocarcinogenesis[35,49-51]. These alterations in the X protein might cause transactivation of oncogenes responsible for HCC development, or transactivators encoded by some oncogenes may select certain viral mutations during the process of multiple steps in carcinogenesis[52,53].

The G1896A mutation in the precore gene, which was the first important common HBV mutation to be identified[54], creates a premature stop codon leading to abolishment of "e" antigen synthesis[55]. The effect of the G1896A mutation in the development of HCC has been widely studied and remains controversial[56-60]. In a study by Tong *et al*[46], the prevalence of the G1896A mutation was significantly higher in HCC patients than chronic carriers. They speculated that A1762/T1764 double mutations may be the chief driving force for the HCC development and the G1896A mutation also played a synergistic, albeit lesser, role in the progression to HCC. Recently, a large prospective cohort study from Taiwan has demonstrated that the presence of G1896A mutation was correlated with a decreased HCC risk[41].

Because the pre-S regions contain several epitopes specific to T or B cells, these regions are essential in the interaction with the immune responses[61,62]. The presence of pre-S deletions have been found to cause decreasing the expression of hepatitis B surface proteins, leading to intracellular accumulation of HBV envelope proteins and formation of ground glass appearing hepatocytes, resulting in significant endoplasmic reticulum stress and genomic instability, and finally hepatocarcinogenesis[35,63,64]. It is biologically rational that pre-S deletion mutations could contribute to the progression of HCC. Recent case-control and longitudinal cohort studies have also confirmed the risk of pre-S deletions on HCC development[43,65,66].

In addition, majority of previous studies have mainly aimed to the relationship between specific single point mutation and the risk of HCC, thus, it is unknown whether these factors are confounding or a certain mutation combination profile is related to the progression of HCC. A longitudinal study has revealed that the mutations in BCP gene (involving 1762/1764/1766/1768) were gradually accumulated during the development of HCC[67]. Recently, a significant biological gradient of HCC risk by amount of mutations in Enh Ⅱ/BCP regions was observed in the study of Bai *et al*[68]. Our previous longitudinal observation also showed a sequential accumulation of T1762/A1764 double mutations, T1766/A1768 mutations, and pre-S deletions during the progression of HCC[69]. These studies have indicated that the combination of viral complex mutations may have a sequential and synergistic role in the progression of HCC. This relationship between combined mutations and HCC risk was confirmed by experimental investigations, in which the combined mutations (A1753, T1762/A1764, and A1768), but not single or double mutations in CP, accelerated the degradation of p21(WAF1/CIP1) through up-regulated expression of cyclin E and S-phase kinase-associated protein 2 in HepG2 cells and primary hepatocytes, leading to the combined mutations accelerating cell cycle progression[70]. A combined examination of these mutations might help to speculate the clinical prognosis of individuals with chronic HBV infection more precisely, thus helping those high HCC risk patients to benefit from early diagnoses and treatment.

CONCLUSION

In conclusion, we systemically reviewed the associations between HBV DNA levels, status of HBsAg and HBeAg, viral genotypes, and specific viral sequence mutations and the risk of HCC in this study. There is sufficient and powerful evidence connecting elevated serum HBV DNA levels with the risk of developing HCC in patients with chronic HBV infection. An elevated serum HBV DNA level is a major risk factor for the progression of liver disease as well as most suitable to intervention. Meanwhile, the significance of viral genotypes and the long-term emergence of specific sequence mutations in the development of HCC remain controversial and still need following studies. Furthermore, since host genomic background also contributes to final pathogenic outcome, future studies should focus on interactions of genetic factors of both the viral and host genomes, thus helping chronic HBV carriers at high HCC risk to benefit from early disease detection and intervention.

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