Precore/basal core promoter mutants and hepatitis B viral DNA levels as predictors for liver deaths and hepatocellular carcinoma

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Abstract

AIM: To conduct a retrospective study in 400 chronic hepatitis B patients in order to identify hepatitis B viral factors associated with complications of liver disease or development of hepatocellular carcinoma.

METHODS: The mean follow-up time was 83.6 ± 39.6 mo. Alpha-fetoprotein test and abdominal ultrasound were used for cancer surveillance. Hepatitis B basal core promoter mutants, precore mutants, genotypes, hepatitis B viral DNA (HBV DNA) level and hepatitis B e antigen (HBeAg) were measured. Univariate analysis and logistic regression were used to assess odds ratios for viral factors related to liver deaths and hepatocellular carcinoma development.

RESULTS: During follow-up, 38 patients had liver deaths not related to hepatocellular carcinoma. On multivariate analysis, older age (odds ratio: 95.74 (12.13-891.31); \( P < 0.0001 \)), male sex (odds ratio: 7.61 (2.20-47.95); \( P = 0.006 \)), and higher log_{10} HBV DNA (odds ratio: 4.69 (1.16-20.43); \( P < 0.0001 \)) were independently predictive for these liver related deaths. Also, 31 patients developed hepatocellular carcinoma. Multivariate analysis showed that older age (odds ratio: 26.51 (2.36-381.47); \( P = 0.007 \)), presence of precore mutants (odds ratio: 4.23 (1.53-19.58); \( P = 0.02 \)) and presence of basal core promoter mutants (odds ratio: 2.93 (1.24-7.57); \( P = 0.02 \)) were independent predictors for progression to hepatocellular carcinoma.

CONCLUSION: Our results show that high levels of baseline serum HBV DNA are associated with non-hepatocellular carcinoma-related deaths of liver failure, while genetic mutations in the basal core promoter and precore regions are predictive for development of hepatocellular carcinoma.

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Key words: Basal core promoter mutants; Precore mutants; Hepatitis B viral genotypes; Hepatitis B viral DNA; Hepatitis B e antigen; Liver failure; Hepatocellular carcinoma

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INTRODUCTION

Infection with hepatitis B virus (HBV) results in different clinical outcomes which are related to age of exposure. Previous studies have shown that up to 90% of infants born to hepatitis B surface antigen (HBsAg) positive, hepatitis B e antigen (HBeAg) positive mothers become chronic carriers, while acute HBV infection in adults leads to chronicity in 5% to 10% of individuals\(^\text{[1,2]}\). Thereafter, 15% to 40% of patients with chronic hepatitis B will progress to cirrhosis and hepatocellular carcinoma (HCC)\(^\text{[3]}\). In a previous report, we showed that 9.5% of our HBsAg positive patients died of non-HCC related liver complications and another 7.8% developed HCC over a mean seven year follow-up period\(^\text{[4]}\). Significant clinical and biochemical factors which predicted all deaths of HBV infection in this group of patients include decreased serum albumin, low platelet levels and presence of cirrhosis. However, the reasons for the differences in these clinical outcomes remain unknown.

Hepatitis B viral factors such as HBeAg, HBV DNA, HBV genotype, basal core promoter (BCP) mutants and precore (PC) mutants may either alone or in combination play a major role in determining the progression of disease...
in patients with chronic hepatitis B. A report from Taiwan indicates that the relative risks for HCC is 9.6 among men who are positive for HBsAg alone but rise to 60 in those who are positive for both HBsAg and HBeAg. Also, the risk for HCC is increased in patients with high circulating levels of serum hepatitis B viral DNA (HBV DNA). Other studies from Asia indicate that HBV genotypes B and C are associated with an increased risk for HCC. A dual mutation in the BCP region of the HBV genome involving an A to T substitution at nucleotide 1762 and a G to A substitution at nucleotide 1764 has been associated with more severe liver damage and HCC development. In addition, a mutation in the PC region of the HBV genome involving a G to A change at nucleotide 1896 has been described in patients with HBeAg negative chronic hepatitis. However, its role in the pathogenesis of severe liver disease and HCC is less clear.

In the present report, we describe the baseline hepatitis B vireo profiles of 400 HBsAg positive patients who were followed up in our liver center. The clinical endpoints in this study were either death of non-HCC related liver disease or of progression to HCC. Odds ratios were used to identify significant hepatitis B viral factors associated with these serious and lethal complications.

MATERIALS AND METHODS

Patients
From January 1989 to March 1998, we followed up 400 HBsAg positive patients who presented to our clinic. Patients who were hepatitis C antibody positive, human immunodeficiency virus antibody positive, and had a history of chronic alcoholism with other chronic liver diseases were excluded. In this report, patients who received liver transplantation for non-HCC hepatic decompensation or for HCC were considered as deaths. The baseline demographic information, laboratory tests and outcomes have been reported recently.

Laboratory tests
At baseline, HBeAg and hepatitis B e antibody (anti-HBe) were measured with commercially available kits (Abbott Laboratories, North Chicago, Ill). Serum was also collected at the initial visit and stored at -70°C. In 2004, we collaborated with the Hepatitis Research Center at National Taiwan University Hospital in Taipei, Taiwan, and serum samples were sent for analysis of HBV DNA, HBV genotypes, PC and BCP mutants.

Serum HBV DNA was quantified by a real-time polymerase chain reaction assay in the linear range from 10^2 to 10^11 copies/mL. For reporting purposes, the HBV DNA values were log_{10} transformed. The identification of HBV genotypes was performed by melting curve analysis, and supplemented by direct sequencing of the pre-S amplicon and phylogenetic analysis by comparing the nucleotide sequence with 33 reference HBV strains. Precore and BCP mutants and HBV DNA predict liver deaths and HCC.

RESULTS

Patients
One hundred and thirty-nine of 400 HBsAg positive patients had liver biopsy confirmed cirrhosis and were classified in the Child-Pugh Class A category. The remaining 261 were non-cirrhotic patients with chronic hepatitis B. Seventy-eight percent of our patients were of Asian descent. The mean follow-up time was 83.6 ± 39.6 mo.

Baseline virologic characteristics

Basal core promoter 1762/1764 mutants: Of 280 patients who had detectable BCP sequences, 124 (44.3%) had BCP 1762/1764 mutants and 156 (55.7%) had BCP 1762/1764 wild type sequences (Table 1). Patients with BCP 1762/1764 mutants were older than those with BCP 1762/1764 wild type sequences (P = 0.01). Seventy-four percent of patients with BCP 1762/1764 mutants had genotype C (P < 0.0001). Patients with BCP 1762/1764 wild type sequences had higher mean serum HBV DNA levels than those with BCP 1762/1764 mutants (P = 0.003). No concordance was noted between patients with BCP 1762/1764 mutants and PC 1896 mutants (P = 0.8, Kappa = 0.001). Also, there was no significant difference in the HBeAg status among patients with BCP 1762/1764 mutants.

Precore 1896 mutants: Of 382 patients who had detec-
Non-HCC related deaths
During follow-up, 38 patients died of non-HCC related deaths. Twenty-seven died of liver failure, seven of bleeding esophageal varices, and four of sepsis. Four other patients died of non-liver related disease. Univariate analysis showed that older age, male sex, presence of cirrhosis and high baseline serum HBV DNA levels were associated with non-HCC related liver deaths (Table 5). On multivariate analysis, old age (OR: 95.74; 95% CI: 12.13-891.31; P < 0.0001), male sex (OR: 7.61; 95% CI: 2.20-47.95; P = 0.006), and high baseline HBV DNA (OR: 4.69; 95% CI: 1.16-20.43; P = 0.03) were independently predictive of non-HCC related liver deaths. HBeAg, HBV genotype, PC 1896 mutants and BCP 1762/1764 mutants were not associated with non-HCC related liver deaths.

HCC development
During follow-up, HCC developed in 31 (7.8%) patients. Twenty-two of 139 (15.8%) patients with cirrhosis and nine of 261 (3.4%) patients without cirrhosis progressed to HCC. Baseline tests in these 31 patients showed that 12 (38.7%) were HBeAg positive, 18 (58.1%) were anti-HBe positive, and one HCC patient was positive for both. In comparing the 31 patients who developed HCC to those who did not, univariate analysis of baseline variables showed that older age, male sex, cirrhosis, presence of PC 1896 mutants and BCP 1762/1764 mutants were independent predictors for HCC development. Other patients died of non-liver related disease. Univariate analysis showed that older age, male sex, presence of cirrhosis and high baseline serum HBV DNA levels were associated with non-HCC related liver deaths (Table 5). On multivariate analysis, old age (OR: 95.74; 95% CI: 12.13-891.31; P < 0.0001), male sex (OR: 7.61; 95% CI: 2.20-47.95; P = 0.006), and high baseline HBV DNA (OR: 4.69; 95% CI: 1.16-20.43; P = 0.03) were independently predictive of non-HCC related liver deaths. HBeAg, HBV genotype, PC 1896 mutants and BCP 1762/1764 mutants were not associated with non-HCC related liver deaths.

DISCUSSION
Our findings of death of non-HCC liver complications and development of HCC in hepatitis B patients in the United States are in accordance with natural history studies.
from Asia and Europe. The highest complication rate occurred in the 139 patients who presented with cirrhosis. During follow-up, 38 (27.3%) patients died either of liver failure, bleeding esophageal varices or of sepsis. Our annual rate of non-HCC related liver deaths was 3.9% per year and is similar to 2.4%-4% reported elsewhere. Also, 9 (3.4%) of 261 patients without cirrhosis and 22 (15.8%) of 139 patients with cirrhosis developed HCC, resulting in annual rates of 0.5% and 2.3%, respectively. These also are similar to 1.5%-3.8% in Europe[13] and 0.7%-2.2% in Asia[14].

In the present report, BCP 1762/1764 mutants were detected in 44% of our HBsAg positive patients. The BCP 1762/1764 mutants were most often detected in our patients with genotype C or B patients (59%, 30% and 35%, respectively). A recent report from Taiwan showed that genotype C has a significantly higher prevalence of BCP 1762/1764 mutants than genotype B. Also, BCP 1762/1764 mutants are more frequently detectable in patients with HCC than in chronic carriers or patients with chronic hepatitis[7,8,10,31]. In one report from Guangxi Zhuang Autonomous Region in China, BCP 1762/1764 mutants were detectable in all 11 HCC tissues tested[15]. Other studies indicated that BCP 1762/1764 mutants were most often present in patients who had more progressive liver disease and hepatic decompensation than in those with stable liver disease[15,16]. In the present study, the presence of BCP 1762/1764 mutants was closely associated with non-HCC liver deaths as well as HCC development.

The significance of PC 1896 mutants is less clear. A study from Taiwan showed that the frequency of PC 1896 mutants was similar in both HCC patients and inactive carriers[14]. Also, no difference has been found in the prevalence of PC 1896 mutants between Chinese patients with or without cirrhosis related complications[15]. Another report from Japan suggested that acquisition of mutation in the PC 1896 region may contribute to inactivation of chronic liver disease[13]. In our study, 29.3% of patients had the PC 1896 mutants, which were primarily HBeAg negative, but 30% were HBeAg positive. One study from China also showed that PC mutants were already present in HBeAg-positive patients who had not yet experienced seroconversion to anti-HBe[10]. These authors suggested that development of HCC may not be related to PC 1896 mutations but probably due to the persistence of significant viremia after HBeAg seroconversion. The low incidence of PC 1896 mutants in our genotype A patients is in accordance with other reports since genotype A patients have a C at nucleotide 1858 which destabilizes the stem loop region and precludes emergence of the PC 1896 mutants[10,32]. In our report, 42% of the patients with PC 1896 mutants had cirrhosis, and 45.2% of patients who developed HCC had PC 1896 mutants. The role of PC 1896 mutants in disease progression requires further investigation.

The distribution of genotypes A, B, C, and D (16%, 27%, 49%, and 3% respectively) in our patients is consistent with the results of a recent survey of HBV genotypes in patients with hepatitis B from the western part of the United States where there is a predominant Asian population (18%, 34%, 41%, and 5% respectively)[30]. In our study, 63% of the patients have genotype A. HBV genotypes A and D appear to be more common in Caucasians, while HBV genotypes B and C are predominantly found in Asians[7,10,31]. However, no HBV genotypes were found to be predictive for either non-HCC liver deaths or progression to HCC in our study. Studies from Asia showed that the prevalence of genotypes B and C is similar in patients with cirrhosis and HCC, but progression to cirrhosis might be slower in genotype B patients[18]. Another report indicated that genotype B may be responsible for HCC development in children and young non-cirrhotic adult males in Taiwan[11,32]. These conflicting findings indicate that the role of genotypes in the natural history of HBV is still unclear.

Recently, the role of HBV DNA in predicting

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**Table 3** Demographic and virologic characteristics of HBV genotypes

| Characteristics | Genotype A | Genotype B | Genotype C | \( P \) |
|-----------------|------------|------------|------------|------|
| sex             | n=56       | n=92       | n=166      |      |
| Age (yr) (mean ± SD) | 51.0 ± 15.8 | 46.3 ± 17.7 | 47.4 ± 14.7 | 0.2  |
| Male/Female     | 47/9       | 64/28      | 117/49     | 0.02 |
| Asian/Non-Asian | 16/40      | 84/8       | 157/9      | < 0.0001 |
| HBV-DNA (mean ± SD, copies/mL) | 6.68 ± 2.53 Log10 | 6.12 ± 2.08 Log10 | 6.94 ± 1.81 Log10/L | 0.003 |
| HBeAg, n (%)    | + 37/55 (67.3%) | 35/91 (38.5%) | 104/165 (63.0%) | 0.0002 |
|                | - 18/55 (32.7%) | 56/91 (61.5%) | 61/165 (37.0%) |      |

**Table 4** Demographic and virologic characteristics of baseline serum HBV-DNA Levels (mean ± SD, copies/mL)

| Characteristics | Mean HBV-DNA levels |
|-----------------|---------------------|
| Sex             |                     |
| Male            | 6.39 ± 2.30 Log10   |
| Female          | 5.52 ± 2.14 Log10   |
| Race            |                     |
| Asian           | 6.05 ± 2.21 Log10   |
| Non-Asian       | 6.39 ± 2.57 Log10   |
| HBeAg           |                     |
| + (n=194)       | 7.42 ± 1.97 Log10   |
| = (n=191)       | 4.86 ± 1.81 Log10   |

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progression to cirrhosis and HCC has been reported from Taiwan\textsuperscript{[6,33]}. These authors showed that elevated levels of baseline HBV DNA were associated with increased deaths of cirrhosis\textsuperscript{[6,33]}. Also, levels of HBV DNA $\geq 10,000$ copies/mL at baseline and at follow-up were a strong predictor of HCC development, which was independent of HBeAg, serum ALT and cirrhosis\textsuperscript{[6]}. In our report herein, patients who died of non-HCC related liver complications had significantly higher baseline HBV DNA levels than those who remained alive. Thus, chronic inflammation caused by the host immune response to active viral replication appears to be more responsible for the non-HCC related liver deaths than mutations in the HBV genome\textsuperscript{[34]}. However, the baseline levels of HBV DNA in our patients who developed HCC during follow-up were similar to patients who did not progress to HCC. The role of HBeAg in predicting progression of liver disease is unclear. A recent study from Taiwan showed that the presence of HBeAg positivity significantly increased the risk of developing HCC. Also, another study from Europe showed that HBeAg positivity was associated with worse survival and that the risk of death decreased after HBeAg seroconversion. However, in our report 53\% of our cirrhosis patients were HBeAg positive on presentation, and 47\% were HBeAg negative, indicating that progression to cirrhosis occurs regardless of HBeAg status. Also, we noted that HBeAg positive patients had higher baseline HBV DNA levels than HBeAg negative patients, and our HBeAg negative patients had more PC 1896 mutants than HBeAg positive patients. However, during our analysis of deaths of non-HCC liver complications or development of HCC, HBeAg did not appear to play a predictive role in predicting either complication.

HCC developed in 31 of our patients during follow-up. In comparing patients who developed HCC to those who did not, both PC 1896 mutants and BCP 1762/1764 mutants were independent predictors for development of HCC. Our findings confirm studies from Asia which have

\begin{table}
\centering
\caption{Univariate analysis of factors associated with non-HCC related liver deaths}
\begin{tabular}{|l|l|l|l|}
\hline
Factor & Alive & Expired & $P$ \\
\hline
n & 326 & 43 & - \\
Age (yr) (mean ± SD) & 45.5 ± 14.7 & 57.2 ± 12.2 & $<0.0001$ \\
Male/Female & 213/113 & 41/2 & $<0.0001$ \\
Asian/Non-Asian & 264/62 & 24/19 & 0.0005 \\
Cirrhosis & 77/139 & 38/139 & $<0.0001$ \\
Yes & 257/261 & 4/261 & 0.02 \\
No\textsuperscript{4} & 6.0 ± 2.38 Log\textsubscript{10} copies/mL & 6.92 ± 1.96 Log\textsubscript{10} copies/mL & 0.07 \\
Baseline HBV-DNA level (mean ± SD) & Yes & 86 & 20 \\
No & 132 & 16 & 0.78 \\
PC 1896 mutant & Yes & 86 & 12 \\
No & 223 & 30 & 0.43 \\
Genotype & A & 46 & 8 \\
B & 76 & 8 & - \\
C & 126 & 19 & 0.17 \\
HBeAg & + & 159 & 26 \\
− & 163 & 17 & \textsuperscript{4}Four other patients without cirrhosis died of non-liver related deaths.
\hline
\end{tabular}
\end{table}

\begin{table}
\centering
\caption{Univariate analysis of factors associated with development of HCC}
\begin{tabular}{|l|l|l|l|}
\hline
Factor & HCC & No HCC & $P$ \\
\hline
n & 31 & 369 & - \\
Age (yr) (mean ± SD) & 57.4 ± 2.7 & 46.9 ± 0.8 & 0.0002 \\
Male/Female & 28/3 & 254/115 & 0.006 \\
Asian/Non-Asian & 26/5 & 288/81 & 0.4 \\
Cirrhosis & Yes & 22/139 & 117/139 & $<0.0001$ \\
No & 9/261 & 252/261 & 0.007 \\
BCP 1762/1764 mutant & Yes & 18 & 106 & 0.05 \\
No & 6 & 148 & 0.2 \\
PC 1896 mutant & Yes & 14 & 98 & 0.3 \\
No & 17 & 253 & 0.001 \\
Genotype & A & 2 & 54 \\
B & 8 & 84 \\
C & 19 & 147 \\
HBeAg & + & 12 & 165 \\
− & 18 & 180 & 0.6 \\
Baseline HBV-DNA level (mean ± SD, copies/mL) & 6.3 ± 1.4 Log\textsubscript{10} & 6.1 ± 2.3 Log\textsubscript{10} & 0.001 \\
\hline
\end{tabular}
\end{table}
implicated BCP 1762/1764 mutants and HCC. In addition, our observations indicate that patients with PC 1896 mutations also have an increased risk of developing HCC. Since the majority of our HBsAg positive patients are from Asian countries, the risk of developing HCC persists in those with pre-existing BCP 1762/1764 mutants and PC 1896 mutants, even after years of immigration from their native countries to the USA.

There were the limitations in our study. The analyses of HBV genotypes and BCP 1762/1764 mutations were not possible in some of our patients because of low or undetectable levels of HBV DNA, which did not permit adequate amplifications of the serum samples. In addition, this study only described analysis of laboratory tests performed at the time of presentation, and no longitudinal analyses were completed. It is well known that patients with chronic hepatitis B experience exacerbations and remissions which are usually accompanied with abnormal liver tests and varying levels of HBV DNA. However, our primary endpoints in this study were either death of liver disease or development of HCC, and our aim was to identify hepatitis B viral factors at presentation which may predict these serious outcomes.

In summary, the presence of high baseline serum HBV DNA levels can predict non-HCC related liver deaths, while mutations in the BCP and PC regions of the HBV genome are more predictive for HCC development. These findings will assist in the planning of treatment strategies to prevent these serious and lethal complications which occur in patients with chronic hepatitis B infection.

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