

Hepatitis B Virus Double Mutations, is There any Role in Pathogenesis of Hepatocellular Carcinoma in Young Patients?

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ABSTRACT

Background: The incidence of hepatocellular carcinoma (HCC) below age 40 years old in our institution were relatively high compared with other institutions in Asia. Hepatitis B virus (HBV) basal core promoter (BCP) double mutations correspond with increasing age. The aim of this study was to know if there was any role of HBV double mutations in young HCC patients.

Method: A descriptive study was performed on HBV related HCC patients in Cipto Mangunkusumo Hospital in May 2006–November 2008. Patient were recruited consecutively and divided in to two groups, below 40 and above 40 years old. The genotypes were examined by polymerase chain reaction (PCR) method. The alpha feto protein (AFP) values were diagnosed based on ELISA method. The BCP A1762T/G1764A double mutations were examined by direct sequencing.

Results: There were 49 HBV related HCC samples consist of 14 (28.5%) samples with age below 40 years old and 35 (71.5%) samples with age above 40 years old. We only found two genotype, genotype B was dominant in patients with HBV related HCC compare to genotype C, 43 (88%) and 6 (12%) respectively. The increasing of AFP level above 400 ng/mL was only found in about half of the samples, 7 (50%) < 40 years old, 19 (54%) > 40 years old. Double mutations of A1762T/G1764A in BCP occurred in 5 (36%) < 40 years old, 15 (43%) > 40 years old.

Conclusion: The incidence of HBV related HCC in young patients were relatively high. The proportion of patients with AFP level < 400 ng/mL in patients below 40 years old were higher compared to patients above 40 years.

Keywords: hepatocellular carcinoma, BCP double mutation, HBV genotype

INTRODUCTION

The chronic hepatitis B virus (HBV) increases the risk of hepatocellular carcinoma (HCC) by more than 100-fold.¹ However, the molecular mechanism of this process is not well understood. In Asia, genotypes B and C are the predominant genotypes of HBV infections. In a longitudinal follow-up study of

426 chronic hepatitis B patients for up to 5 years in Hong Kong, genotype C HBV was found to be associated with higher risk of HCC than genotype B HBV.² However, the unpublished study held in Division of Hepatology, Faculty of Medicine University of Indonesia showed that incident of genotype B was predominant than genotype C. The HBV genomic mutations occur due to a spontaneous error rate of viral reverse transcriptase and evolving of HBV genome under the antiviral pressure of host immune response. Specific mutations may affect the translation of hepatitis B e antigen (HBeAg), as well as the replication of HBV and thus may modify

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the clinical outcome of HBV infection and contribute to HCC development.³ Recently, several studies have shown that A1762T and G1764A mutations were associated with the aggressive progression of liver disease, in which inactive carriers have developed active hepatitis, and eventually liver cirrhosis and HCC.⁴ Mutations in the basal core promoter (BCP) at nucleotide 1762 (A1762T) and nucleotide 1764 (G1764A) have been described elsewhere.⁵ These mutations resulted in coding changes at codons 130 and 131 in the open reading frame x (ORF x) protein changing lysine to methionin and valine to isoleucine, respectively. The incidence of HCC below age 40 years old in our institution were relatively high compared with other institutions in Asia. The HBV BCP double mutations correspond with increasing age. The aim of this study was to know if there iwas any role of HBV double mutations in young HCC patients.

METHOD

A descriptive study was performed on the HBV related HCC patients in Cipto Mangunkusumo hospital in May 2006 – November 2008. Patient were recruited consecutively and divided in to two groups, below 40 and above 40 years old.

HBV genotyping; the existence of genotypes was examined by polymerase chain reaction (PCR) method. While the alpha feto protein (AFP) values were based on ELISA method. The BCP A1762T/G1764A double mutations were examined by direct sequencing.

RESULTS

Age-related Distribution of HBV Genotype in Patients with HCC

In order to investigate the age-related distribution of HBV genotypes in HCC patients, they were classified into two age groups (< 40 and ≥ 40 years). There were 49 HBV related HCC samples consist of 14 (28.5%) samples with age below 40 years old and 35 (71.5%) samples with age above 40 years old.

Table 1. Baseline characteristics of the study (n = 49)

Variable	n (%)
Sex	
Male	36 (73%)
Female	13 (27%)
Age (year)	
< 40	14 (29%)
≥ 40	35 (71%)
Genotype	
B	43 (88%)
C	6 (12%)
AFP level (ng/mL)	
< 400	23 (43%)
> 400	26 (53%)
Double mutation	
Yes	20 (41%)
No	29 (59%)

The gender of the samples was dominated by male 36 (73.5%) overall, and if we breakdown according to age group consist of 12 (86%) < 40 years old, 24 (71%) > 40 years old, respectively. We only found two genotype, genotype B was dominant in patients with HBV related HCC compare to genotype C, 43 (88%) and 6 (12%) respectively, consist of 12 (86%) < 40 years old, 31 (91%) > 40 years old, respectively. The increasing of AFP level above 400 ng/mL was only found in about half of the samples, 7 (50%) < 40 years old 19 (54%) > 40 years old. Double mutations of A1762T/G1764A in BCP occurred in 5 (36%) < 40 years old, 15 (43%) > 40 years old.

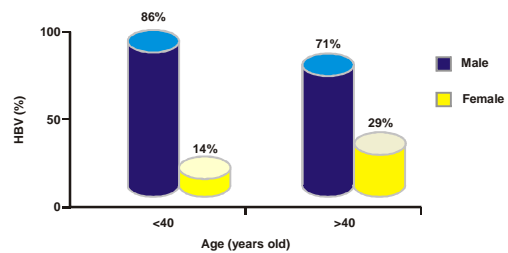


Figure 1. HBV related hepatocellular carcinoma

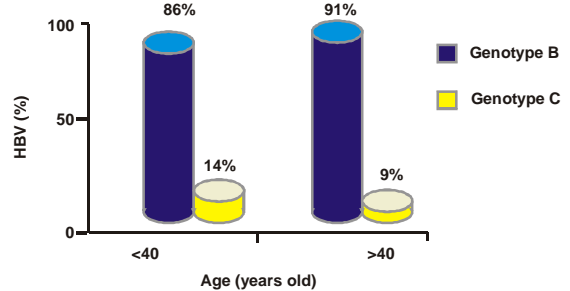


Figure 2. HBV related hepatocellular, genotype

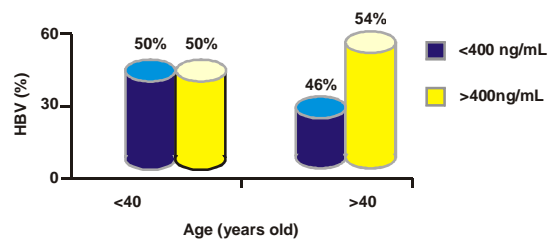


Figure 3. HBV related hepatocellular carcinoma, AFP level

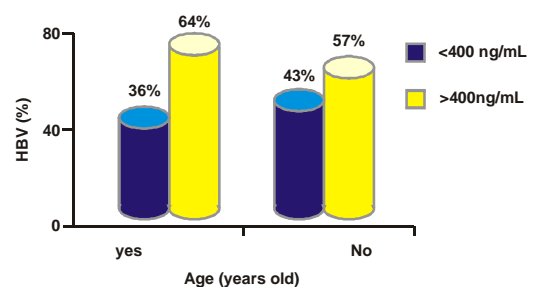


Figure 4. HBV related hepatocellular carcinoma double mutation

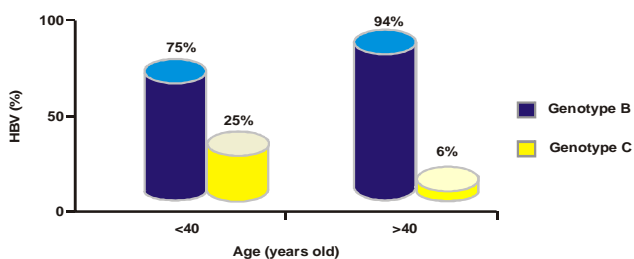


Figure 5. HBV related hepatocellular carcinoma double mutation and genotype

DISCUSSION

It has been reported that genotype C HBV is more aggressive for the development of liver cirrhosis and HCC.⁶ Interestingly, in our study the mutation rate of A1762T/G1764A of genotype B is dominant compare to genotype C. Based on the analysis, mutation rate of A1762T/G1764A in genotype B is much higher than genotype C. This result is different from another country in Asia. The high frequency of A1762T/G1764A mutations in genotype B correlates with an increased risk of HCC. Recent studies have shown that genotype B with A1762T/G1764A mutations is also associated with an increased risk of HCC development.⁷ These results provide further support for the important role played by A1762T/G1764A mutations in the progression of HBV-associated diseases, including HCC.

The contribution of HBV to the pathogenesis of liver cancer is multi factorial and complicated by the identification of mutant variants of HBV that modulate the carcinogenesis process.⁸ Clinical studies have shown that HBV patients with A1762T/G1764A mutations have low levels of HBeAg and are often associated with HCC.

The role of HBV genotype and mutants in disease progression continue to be of great interest. Several mechanisms of hepatocarcinogenesis related to the BCP A1762T/G1764A mutant have been hypothesized. It has been proposed that the BCP mutation may enhance HBV virulence by increasing host immune response, increasing viral replication or altering the coding region for the X antigen. The BCP mutations appears to enhance the efficiency of viral replication either by modulating the relative level of the precore and core RNA's or by creating

a hepatocyte nuclear factor 1 transcription factor binding site. The incidence of HBV double mutations increased corresponds with the age. It is needed to be proven that BCP plays an important role in young age HBV related HCC. The other interesting finding was the AFP level in patients below age 40 was relatively high (50%) compared with patients above age 40 (46%), and also didn't consistent with previous reports.

CONCLUSION

The incidence of HBV related HCC in young patients (< 40 years old) were relatively high (up to 29%). The proportion of patients with AFP level < 400 ng/mL in patients below 40 years old were higher compared to patients above 40. These findings should emphasize more detail study needed to reveal the pathogenesis of HCC in our region.

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