RESEARCH ARTICLE

CYP2C19 Genotype Could be a Predictive Factor for Aggressive Manifestations of Hepatocellular Carcinoma Related with Chronic Hepatitis B Infection in Thailand

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Abstract

Background: Chronic hepatitis B virus (HBV) infection related hepatocellular carcinoma (HCC) is a major health problem in the Asia-Pacific region including Thailand. Several factors have been proposed as contributing to hepatocarcinogenesis. This study was aimed to investigate the impact of CYP2C19 genotypic polymorphism in HCC related to chronic HBV infection in Thailand. Materials and Methods: A cross-sectional study was performed between April 2014 and January 2015. Chronic HBV patients with HCC (n=50) and without HCC (n=50) were included. Clinical information and blood samples of all patients were collected. The CYP2C19 genotype was determined by polymerase chain reaction-restriction fragment length polymorphism method, and was classified as rapid metabolizer (RM), intermediate metabolizer (IM) or poor metabolizer (PM). Results: The CYP2C19 genotype frequencies of RM, IM and PM in HBV patients were found to be 19/50 (38%), 25/50 (50%) and 6/50 (12%), respectively. The CYP2C19 genotype frequencies of RM, IM and PM in HBV with HCC patients were 21/50 (42%), 25/50 (50%) and 4/50 (8%), respectively. The distribution of CYP2C19 genotype was not different between patients with and without HCC. Interestingly, among HBV with HCC patients, the RM genotype of CYP2C19 tended to increase risk of aggressive manifestation (OR=2.89, 95%CI=0.76-11.25, P-value = 0.07), compared with non RM genotype carriers. Conclusions: CYP2C19 genotype IM was the most common genotype in Thai patients with chronic HBV infection. In addition, genotype RM could be an associated factor for aggressive presentation in HCC related to chronic HBV infection.

Keywords: Hepatocellular carcinoma - hepatitis B virus - CYP2C19 genotype

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Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer, and is responsible for approximately 1 million deaths yearly (Murray and Lopez, 1997; Fan et al., 2013; Abdelaziz et al., 2014). The incidence of HCC is increasing in Western countries, and remains high in the East. In general, HCC almost invariably arises in the setting of chronic liver diseases, in particular, chronic hepatitis B virus (HBV) and chronic hepatitis C virus (HCV) infection (Colombo, 1992; Okuda; 1992). Presently, chronic HCV infection is the major cause of chronic liver disease among western countries, and is associated with over 80% of HCC cases (De Bac et al., 1994). Whereas, chronic HBV infection accounts for the major cause of chronic liver disease in Africa and Asia, including Thailand (Norsa'adah and Nurhazalini-Zayani, 2013; Yeo et al., 2013; Sangmala et al., 2014; Somboon et al., 2014; Khan and Hashim, 2015).

Hepatocarcinogenesis is a multi-step process, reflecting genetic alterations that drive a progressive transformation of normal human cells into malignant cells. Many factors have been documented to be associated with HCC development, including host genetic polymorphisms of carcinogen metabolizing enzymes.

Cytochrome (CYP) P450 2C19 is an important member of the CYP450 family. Generally, CYP2C19 polymorphisms are genetically categorized into three genotypes: rapid metabolizer (RM), intermediate metabolizer (IM), and poor metabolizer (PM). CYP2C19 is known to be involved in the detoxification of potential carcinogen, and the bioactivation of some environmental procarcinogen(s) to reactive DNA binding metabolites (Fujita and Kamataki, 2001; Kappers et al., 2001; Sugimoto et al., 2004; Yamazaki et al., 2004). Several investigators have shown that this allele is possibly associated with HCC susceptibility in patients with chronic HCV infection (Zhou et al., 2013). The proposed

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Table 1. Baseline Characteristics of Chronic HBV Infected Patients with HCC and without HCC

General characteristics	HBV (n=50)	HBV with HCC (n=50)	P-value
Age	48.7±13.1	56.4±12.8	0.004
Male sex (n, %)	34 (68%)	37 (74%)	0.508
Family history of HCC (n,%)	8 (16%)	8 (16%)	1
Alcoholic drinking * (n,%)	22 (44%)	34 (68%)	0.02
Platelet (×1000/ul)	211.4±93.2	164±70	0.005
Albumin (g/dl)	3.8±0.5	2.9±0.7	<0.001
Total bilirubin (mg/dl)	0.7±0.5	1.3±0.9	<0.001
ALT (IU/L)	57±37	68±48	<0.17
HBeAg positive (n,%)	10 (20%)	10 (20%)	1

*Persons drinking alcohol > 40 gram per day for over 5 years

Table 2. Distribution of CYP2C19 Genotypes in Chronic HBV Infected Patients with and without HCC (p=0.807)

CYP2C19 genotype	HBV (n=50)	HBV with HCC (n=50)
RM	19 (38%)	21 (42%)
IM	25 (50%)	25 (50%)
PM	6 (12%)	4 (8%)

Table 3. Distribution of *CYP2C19* genotypes in Chronic HBV and HCC Patients with and without Aggressive presentations* (p=0.1)

CYP2C19 genotype	HCC without aggressive presentation (n=31)	HCC with aggressive presentation (n=19)
RM	10 (32.3%)	11 (57.9%)
IM	19 (61.3%)	6 (31.6%)
PM	2 (6.4%)	2 (10.5%)

*Large HCC > 10 cm, rupture or metastasis at presentation

mechanism is that PM genotype may diminish enzymatic expression that effect the metabolism and elimination of some carcinogen and endogenous compounds.

So far, there is not much information regarding the role of CYP2C19 genetic polymorphisms and the susceptibility of developing HCC in HBV patients. The present study was conducted to determine the association between CYP2C19 genetic polymorphisms and the development of HCC in Thai patients with chronic HBV infection.

Materials and Methods

Subjects

This study was conducted in Thammasat University Hospital, Pathumthani, Thailand between April 2014 and January 2015. Fifty patients with chronic HBV with HCC, and 50 patients with chronic HBV without HCC were included. The diagnosis of HCC was based on the diagnostic criteria proposed by the American association study of liver disease (AASLD), which established based on histological or imaging criteria (Bruix and Sherman, 2011).

Serum hepatitis B surface antigen (HBsAg), hepatitis B e-antigen (HBeAg) and hepatitis B surface antibody (anti-HBs) were tested. Serum HBV DNA was measured by quantitative polymerase chain reaction. Serum HCV antibody as well as other biochemical and serological tests were done to exclude other causes of liver disease. All subjects were provided informed consent prior to their participation.

CYP2C19 genotypic testing

From all patients, 3 ml venous blood sample were drawn and collected in ethylenediaminetetraacetic acid vials, and then deoxyribonucleic acid was extracted from peripheral leukocytes using an extraction kit. The genotype analysis of the polymorphic CYP2C19 genes was performed by a polymerase chain reaction-restriction fragment length polymorphism method as described by de Morais et al (De Morais et al., 1994). The investigators who carried out the genotypic testing were blinded from the clinical data of all patients. The wild type CYP2C19*1 gene and the two mutated alleles, CYP2C19*2, and CYP2C19*3, were identified. Cases that were homozygous for either the CYP2C19*2 or CYP2C19*3 mutation (*2/*2 or *3/*3) and heterozygous for CYP2C19*2 and CYP2C19*3 (*2/*3) were categorized as PM. Cases that were heterozygous for the wild type and mutation (*1/*2 or *1/*3) were categorized as IM, and those homozygous for the wild type (*1/*1) were categorized as RM.

Statistical analysis

The clinical characteristics of the patients with and without HCC were compared by independent t-test or Chi-square test. The data of blood chemistry were presented as mean and 95% confidence interval. The frequencies of each phenotypic status in chronic HBV patients with and without HCC were compared using Chi-square test. The P-value <0.05 was considered to be statistically significant. All statistic analyses were performed using SPSS for Windows Version 19.0 (IBM Corp., Armonk, NY). The study was conducted according to the good clinical practice guideline as well as the Declaration of Helsinki and was approved by our local ethics committee.

Results

General characteristics of the participants

Table 1 shows the baseline characteristics of chronic HBV patients with and without HCC (N=50 in each group). As shown, there was no significant difference in sex, family history of HCC, HBeAg status and serum ALT between two groups. However, the chronic HBV with HCC group were older and had a higher proportion of significant alcohol drinking. Moreover, as shown, the HCC group had poorer baseline liver biochemical test

compared with those without HCC.

Distribution of the CYP2C19 genotypes

In all subjects, the CYP2C19 genotype frequencies of RM, IM and PM were found to be 40/100 (40%), 50/100 (50%) and 10/100 (10%), respectively. As shown in Table 2, there was no significant difference in the frequency of CYP2C19 genotypes between chronic HBV with and without HCC patients.

Association of CYP2C19 RM genotype with a risk of aggressive presentations of HCC

In chronic HBV infection and HCC group, 19/50 (38%) presented with aggressive manifestations, that defined as large HCC>10 cm, rupture or metastasis at presentation. The results of CYP2C19 genotype in these patients are listed in Table 3. Interestingly, HCC patients with aggressive presentations had a higher proportion of RM genotype, compared to those without aggressive presentations. However, this difference was not statically significant. By statistical analysis, the RM genotype of CYP2C19 tends to increase a risk of aggressive manifestations (OR=2.89, 95%CI=0.76-11.25, p=0.07) in chronic HBV patients with HCC, compared with non- RM genotype carriers.

Discussion

The Human Genome Project has provided a number of genetic information that helps us understanding the effects of xenobiotics on biological systems. It is well-known that cigarette smoking can cause lung cancer, but the fact is not every smoker suffers from such cancer (Shi and Chen, 2004). In the same way, some chronic HBV infected patient progress to HCC, while others never develop HCC.

It is recognized that hepatocarcinogenesis is a multistep process. One of the main contributing factors is the patient's capability to metabolize xenobiotics, because some xenobiotics play an important role in the carcinogenesis. Thus, genetic polymorphisms in the activating and detoxifying enzymes might be associated with an altered risk for HCC development. Previous studies on CYP2C19 polymorphism and its association with HCC carcinogenesis have shown self-contradictory results. Chau et al. reported that the PM genotype caused by the mutation of CYP2C19 gene in HCV related cirrhotic patients is associated with a high risk for developing HCC (Chau et al., 2000). The possible explanation is that an accumulation of unknown carcinogen(s) produced by HCV related chronic inflammation could not be detoxified due to a reduced enzymatic activity of CYP2C19. However, this association could not be demonstrated in the study by Tsuneoka et al., which also conducted in patients with chronic HCV infection (Tsuneoka et al., 1996)

In this study, we found that the CYP2C19 genetic polymorphism was not different between chronic HBV infected patients with and without HCC. Interestingly, the pattern of CYP2C19 genetic polymorphism in our chronic HBV patient was comparable to that previously studied in Thai subjects (Jainan and Vilaichone, 2014; Srinarong et al., 2014). Therefore, the aforementioned concept may not be applied to HBV related hepatocarcinogenesis. This distinctive finding could be explained by the fact that the risk of HCC development in chronic HBV patients mainly depends on the virus itself (Bartosch et al., 2009; Gearhart and Bouchard, 2010; Liang et al., 2013). Indeed, from the pathogenic perspective, the HBV gets integrated into the host genome, causing DNA rearrangement, mutagenesis and active inflammation, which eventually lead to the accumulation of genetic and epigenetic alterations. By contrast, HCV-related HCC solely occur on a background of cirrhosis, together with the pattern of genetic alterations in viral-related HCC was quite discretely different between these 2 viruses (Brechot, 2004; Munaf et al., 2014).

Interestingly, our study demonstrated that the RM genotype of CYP2C19 tends to increase a risk of aggressive manifestations compared with non- RM genotype carriers in chronic HBV patients with HCC. This finding suggested that CYP2C19 might be involved in the activation of some procarcinogens or the detoxification of some enzyme that involve in the carcinogenic process of HCC. However, this result should be interpreted with caution due to the fact that the liver is the main tissue localization of the enzymes that encoded by CYP2C19 gene. Therefore, the genotypic pattern of CYP2C19 may not provide an accurate picture of the enzyme activity in the setting of chronic liver diseases.

In conclusion, we postulate that the CYP2C19 genetic polymorphism was not different between HBV with HCC patients in comparison to that of HBV patients without HCC. However, the CYP2C19 RM genotype in HBV-related HCC patients might increase a risk of developing aggressive manifestations. However, our study had some limitations. First, the sample size was rather small. Second, some potential factors of HCC development, for example alcohol drinking, could not be controlled in our study. Therefore, our finding must be confirmed by larger prospective trials.

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