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Clinical and paraclinical features and their correlation with genotype and subgenotype c of hepatitis B virus in patients with hepatocellular carcinoma

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Abstract

Objective: *To describe the clinical and paraclinical characteristics and analyze their association with hepatitis B virus (HBV) genotypes and subgenotypes in patients with hepatocellular carcinoma (HCC).* ***Subject and method:*** *A cross-sectional study with prospective data collection was conducted on 129 patients diagnosed with HCC by histopathology, having pure HBV infection, and with complete HBV genome sequencing from January 2022 to December 2024.* ***Result:*** *Among 129 HBV-related HCC patients, genotype B (55.8%) was more prevalent than genotype C (44.2%). Genotype C was associated with older age (61.3 vs. 55.8 years, p<0.05), higher cirrhosis prevalence (78.9% vs. 55.6%, p<0.05), elevated viral load (6.8 vs. 5.1 log10 IU/mL, p<0.05), and more severe liver dysfunction. Subgenotype C1 (24.8%) demonstrated more aggressive tumor features, including larger tumor size (68.8% >5cm) and vascular invasion (43.8%) compared to B2. Multivariate analysis identified cirrhosis (OR=3.8) and high HBV DNA (>6 log10 IU/mL, OR=4.5) as independent predictors for genotype C.* ***Conclusion:*** *Genotype B (55.8%) predominates over genotype C (44.2%) in HBV-related HCC patients, with genotype C showing more severe clinical manifestations and poorer prognosis. Cirrhosis and high viral load serve as independent predictors for genotype C.*

Keywords: *Hepatocellular carcinoma, Hepatitis B, Genotype, Subgenotype.*

I. Introduction

Liver cancer, primarily hepatocellular carcinoma (HCC), is the sixth most common malignancy and the third leading cause of cancer-related death worldwide. In 2020, approximately 906,000 new cases of liver cancer were reported, and by 2022, the global incidence had reached 866,136 new cases [1]. Chronic hepatitis B virus (HBV) infection remains the predominant global cause of hepatocellular carcinoma. Individuals with chronic HBV infection have a more than 100-fold increased risk of developing HCC compared to the general population [2].

Several international studies have investigated the genotypic diversity of HBV and its association with HCC. Genotypes B and C—particularly subgenotypes Ba, C1, and C2—are prevalent in Asian populations [3]. Among these, genotype C, especially subgenotype Ce, has been associated with a higher risk of developing HCC compared to genotype B [4]. In Vietnam, studies utilizing whole-genome sequencing (WGS) of HBV in patients with histologically confirmed HCC remain limited. Therefore, we conducted this study titled: "Clinical and Laboratory Characteristics and Their Association with HBV

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Genotype and Subgenotype in Patients with Hepatocellular Carcinoma", with the following objectives: To describe the clinical and laboratory characteristics of patients with hepatocellular carcinoma. To analyze the association between HBV genotype/subgenotype and clinical features in patients with hepatocellular carcinoma.

II. Subjects and Methods

2.1. Subjects

The study included 129 patients with histologically confirmed hepatocellular carcinoma (HCC) and chronic HBV monoinfection, for whom whole-genome HBV sequencing was successfully performed between January 2022 and December 2024.

Inclusion Criteria

Underwent liver resection surgery with histopathological confirmation of HCC.

Positive HBV DNA detected in blood or non-tumorous liver tissue, and successful whole-genome HBV sequencing.

Age above 16 years.

Provided informed consent to participate in the study.

Exclusion Criteria

HCC patients with HCV co-infection, a history of alcohol abuse, or presence of other malignancies.

Declined to participate in the study.

2.2. Methods

Study Design:

A cross-sectional descriptive study with prospective analytical components.

Sample Size (n):

$$n = \frac{Z_{(1-a/2)}\, p_{(1-p)}}{d^2}$$

The required sample size was calculated using the formula for estimating a population proportion with specified absolute precision. Z(1–α/2) = 1.96 (corresponding to a 95% confidence level), d = 0.06 (desired margin of error), p = estimated prevalence of HBV genotypes in HCC patients, based on Orito E. (Japan): genotype B = 13%, genotype C = 86%. Based on these parameters, the calculated sample size was 129 patients.

Sampling Method:

A convenience sampling approach was used. All patients diagnosed with histologically confirmed hepatocellular carcinoma (HCC), BV monoinfection, and successfully sequenced for the full HBV genome between January 2022 and December 2024, and who met the inclusion and exclusion criteria, were included in the study. A total of 129 patients were enrolled.

2.3. Data Collection and Analysis

Study Procedure

Step 1: Patient enrollment was conducted at the Hepatobiliary Surgery Department, Cho Ray Hospital, including individuals diagnosed with HBV infection and hepatocellular carcinoma (HCC) who underwent curative surgery, according to the Ministry of Health guidelines (2020).

Step 2: Immediately after surgery, two fresh liver tissue samples were collected per patient-one from the tumor margin and one from a site of non-tumorous liver tissue located at least 5 cm away from the tumor edge. These samples were rapidly cryopreserved at -80°C at the Biotek Andrology Center.

Step 3: Histopathological confirmation of HCC was performed at the Department of Pathology, Cho Ray Hospital, in accordance with the 2019 WHO classification.

Step 4: Whole-genome sequencing of HBV was conducted to determine the viral genotype, subgenotype, and relevant HBV gene mutations.

Study Parameters

Demographic and clinical characteristics: age, sex, AFP levels.

Distribution of HBV genotypes and subgenotypes.

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Data Analysis

Data were analyzed using SPSS version 22.0. Quantitative variables were expressed as mean ± standard deviation, while qualitative variables were presented as percentages. The Student's t-test was used to compare means between two groups, with a p-value < 0.05 considered statistically significant. In addition, multivariate logistic regression was performed to identify factors associated with HBV genotype.

III. Results

Table 1. Demographic and Clinical Characteristics (n = 129)

Characteristic	Total (n = 129)	Genotype B (n = 72)	Genotype C (n = 57)	p
Age (years), mean ± SD	58,5 ± 10,2	55,8 ± 9,5	61,3 ± 10,1	0,003
Male sex, n (%)	92 (71,3%)	50 (69,4%)	42 (73,7%)	0,59
Cirrhosis, n (%)	85 (65,9%)	40 (55,6%)	45 (78,9%)	0,006
ALT (U/L), median (IQR)	45 (30-78)	38 (25-65)	58 (40-95)	0,001
AFP ≥ 400ng/mL, n (%)	47 (36,4%)	20 (27,8%)	27 (47,4%)	0,02

Comment: Among patients with hepatocellular carcinoma, genotype B accounted for 55.8%, while genotype C represented 44.2%. There was a statistically significant difference in mean age between the two groups, with genotype C patients being older (61.3 years vs. 55.8 years, p=0.003). Cirrhosis was more prevalent in the genotype C group (78.9% vs. 55.6%, p=0.006). Median ALT levels were also significantly higher in genotype C (58 U/L vs. 38 U/L, p=0.001). Furthermore, the proportion of patients with AFP ≥ 400ng/mL was greater in genotype C (47.4% vs. 27.8%, p = 0.02), suggesting more advanced disease in this subgroup.

Table 2. Distribution of HBV Genotypes and Subgenotypes

Genotype/Subgenotype	Frequency, n (%)	Associated Clinical Features
Genotype B	72 (55,8%)	Lower mean age, less cirrhosis
- B2	50 (38,8%)	Lower AFP, better prognosis
Genotype C	57 (44,2%)	Higher viral load, more advanced cirrhosis
- C1	32 (24,8%)	Associated with multifocal tumors and vascular invasion
Hỗn hợp/Khác	0 (0%)	Not observed

Comment: Among subgenotypes, B2 accounted for 38.8%, while C1 represented 24.8% of cases. No mixed or rare genotypes were detected. The mean HBV DNA level in genotype C patients was significantly higher (6.8 $\log_{10}$ IU/mL) compared to genotype B (5.1 $\log_{10}$ IU/mL, p<0.001).

Table 3. Laboratory Findings by HBV Genotype

Parameter	Genotype B (n = 72)	Genotype C (n = 57)	p-value
HBV DNA ($\log_{10}$ IU/mL)	5,1 ± 1,6	6,8 ± 2,0	<0,001
Albumin (g/dL)	3,6 ± 0,5	3,0 ± 0,6	<0,001
Bilirubin (mg/dL)	1,7 ± 0,8	2,5 ± 1,1	<0,001
Platelets (×10³/μL)	130 ± 55	95 ± 40	<0,001

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Comment: There were marked differences in liver function parameters between genotypes. Albumin levels were significantly lower in genotype C patients (3.0 ± 0.6g/dL) compared to genotype B (3.6 ± 0.5g/dL, p<0.001). Bilirubin levels were higher (2.5 ± 1.1mg/dL vs. 1.7 ± 0.8mg/dL, p <0.001), and platelet counts were lower (95 ± 40 ×10³/μL vs. 130 ± 55 ×10³/μL, p<0.001), indicating more severe liver dysfunction in genotype C.

Table 4. Tumor Characteristics by HBV Subgenotype

Characteristic	B2 (n = 50)	C1 (n = 32)	Others (n = 47)	p-value
Tumor size > 5 cm, n (%)	15 (30%)	22 (68,8%)	10 (21,3%)	<0,001
Multifocal tumors, n (%)	10 (20%)	18 (56,3%)	8 (17,0%)	<0,001
Vascular invasion, n (%)	6 (12%)	14 (43,8%)	7 (14,9%)	0,001

Comment: Tumor characteristics varied significantly by subgenotype. Patients with subgenotype C1 exhibited a markedly higher prevalence of tumors > 5cm (68.8% vs. 30.0% in B2, p<0.001), multifocal tumors (56.3% vs. 20.0%, p<0.001), and vascular invasion (43.8% vs. 12.0%, p=0.001). These findings highlight the more aggressive tumor behavior associated with subgenotype C1.

Table 5. Logistic Regression Analysis of Factors Associated with HBV Genotype C

Factor	OR (95% CI)	p-value
Age > 60 tuổi	2,3 (1,2-4,5)	0,01
Cirrhosis	3,8 (1,9-7,6)	<0,001
HBV DNA > 6 log10	4,5 (2,2-9,3)	<0,001
AFP ≥400 ng/mL	1,7 (0,8-3,5)	0,15

Comment: Logistic regression analysis identified cirrhosis (OR = 3.8; 95% CI: 1.9-7.6) and HBV DNA > 6 $\log_{10}$ IU/mL (OR = 4.5; 95% CI: 2.2-9.3) as the strongest predictors of genotype C infection (p < 0.001). Age > 60 years was also significantly associated but to a lesser extent (OR = 2.3; 95% CI: 1.2-4.5; p=0.01). In contrast, AFP ≥ 400ng/mL was not statistically significant (p=0.15), suggesting it is not a reliable marker for genotype C.

IV. Discussion

Our study demonstrated the distribution of HBV genotypes among patients with hepatocellular carcinoma (HCC), with genotype B being more prevalent (55.8%) than genotype C (44.2%). This finding is consistent with Kao et al. (2000) in Taiwan, which also reported genotype B as the most common strain in Asia [5]. However, a study by Chan et al. (2004) in Hong Kong showed a higher prevalence of genotype C (58.3%) in HCC patients [4]. This discrepancy may reflect regional epidemiologic differences in HBV and variability in patient selection criteria across studies.

Clinically, we observed significant differences between the two genotypes. Patients with genotype C were older (mean age: 61.3 ± 10.1 vs. 55.8 ± 9.5, p=0.003) and had a significantly higher rate of cirrhosis (78.9% vs. 55.6%, p=0.006). These results align with the findings of Yu et al. (2005) in China [6], but differ from those of Sumi et al. (2003) in Japan, who reported no age difference in HCC onset between genotypes [7]. Such variations may be influenced by environmental factors and lifestyle habits.

In terms of laboratory characteristics, genotype C patients exhibited significantly higher

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HBV DNA levels (6.8 ± 2.0 vs. 5.1 ± 1.6 log_{10}IU/mL, p<0.001). This supports the hypothesis of more active viral replication in genotype C, as described by Chen et al. (2006) [8]. Notably, subgenotype C1 (24.8% in our cohort) was strongly associated with more malignant tumor features, consistent with Liu et al. (2006), who linked Pre-S2 mutations with HCC progression [9]. However, in contrast to the study by Yang et al. (2008), which reported differences in HBeAg status between genotypes [10], our study found no significant difference in HBeAg positivity across genotypes.

Logistic regression analysis revealed that cirrhosis (OR = 3.8; 95% CI: 1.9-7.6; p<0.001) and HBV DNA > 6 log_{10} IU/mL (OR = 4.5; 95% CI: 2.2-9.3; p<0.001) were strongly associated with genotype C infection, indicating a significantly higher risk of genotype C in patients with cirrhosis or high viral load. Age over 60 was also statistically significant but with a weaker association (OR = 2.3; 95% CI: 1.2-4.5; p=0.01). In contrast, AFP ≥ 400 ng/mL was not significantly associated with genotype C (OR = 1.7; 95% CI: 0.8–3.5; p = 0.15), suggesting that AFP is not a genotype-specific marker.

V. Conclusion

The study identified significant differences in clinical and paraclinical characteristics among HBV genotypes in patients with hepatocellular carcinoma (HCC), with genotype C particularly subgenotype C1 being associated with poorer prognosis, more aggressive tumor features, and more severe liver dysfunction. These findings underscore the importance of HBV genotyping in risk stratification and clinical management of liver cancer patients, and suggest the need for further research into genotype-specific pathogenic mechanisms.

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