Research Article

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HBV viral load and tumor and non-tumor factors in patients with HBV-associated HCC

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Abstract

Background and Aim: Several tumor and non-tumor factors affect the prognosis of hepatocellular carcinoma (HCC) patients. This study aimed to investigate the effects of hepatitis B virus (HBV) viral load on tumor and non-tumor factors in patients with HBV-associated HCC.

Materials and Methods: Patients with hepatitis B and HCC who presented to the HCC council at the Faculty of Medicine, Marmara University Liver Transplantation Institute, were included in our study. Patients were divided into two groups according to the presence or absence of HBV-DNA, and it was determined whether there were differences between these two groups with respect to tumor and non-tumor parameters.

Results: Comparison of serum alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), hepatitis B surface antigen (HBsAg), and C-reactive protein (CRP) levels between HBV-DNA negative and positive patients showed significant differences (respectively $p<0.01$, $p<0.01$, $p<0.05$, and $p<0.05$). A major finding was a very significant difference between the two patient groups in terms of portal vein invasion (PVI) and venous invasion ($p<0.001$ and $p<0.01$, respectively). However, there was no significant difference in metastasis or lymph node involvement between HBV-DNA negative and positive patients.

Conclusion: Our findings suggest that HBV viral load plays an important role in PVI in HCC patients, and there is a significant relationship between HBV viral load and inflammation.

Keywords: Hepatitis B; hepatocellular carcinoma; HBV-DNA.

Introduction

Hepatitis B virus (HBV) infection is a global public health problem that causes advanced liver diseases, such as cirrhosis and hepatocellular carcinoma (HCC). In chronic HBV, persistent viral replication is the most important risk factor for progression to cirrhosis and the development of HCC.[1] International guidelines, such as those from the American Association for the Study of Liver Diseases,[2] the European Association for the Study of the Liver,[3] and the Asia-Pacific Association for the Study of the Liver,[4] recommend suppressing HBV-DNA with interferon and nucleos(t)ide analogues (NAs) to prevent HCC. Previous studies have reported that a high HBV viral load is closely associated with a high risk of HCC recurrence and metastasis after liver resection.[5] Several meta-analyses have indicated that NAs, which inhibit HBV replication, can reduce the incidence of early recurrence and improve overall survival.[6,7]

Several tumor and non-tumor factors affect the prognosis of HCC patients. The presence of macroscopic portal vein invasion (PVI) in HCC is considered a poor prognostic factor,[8] and studies have reported a relationship between HBV replication and the development of vascular invasion in HCC patients.[9,10]

The aim of this study was to investigate the effects of HBV viral load on tumor and non-tumor factors in patients with HBV-associated HCC.

Materials and Methods

Patients with HBV and HCC who were presented to the HCC council at the Faculty of Medicine, Marmara University Liver Transplantation Institute, were included in our study. Serum Alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), C-reactive protein (CRP), alpha-fetoprotein (AFP), and HBV surface antigen (HBsAg) levels of the patients were recorded from their files. To evaluate the HBV replication level of the patients, the HBV-DNA levels obtained by the polymerase chain reaction method at the time of admission were determined. HBsAg levels were measured using a quantitative method. At the baseline clinical evaluation, it was determined whether the patients had cirrhosis, their Barcelona Clinic Liver Cancer (BCLC) stage, some characteristics of the tumor, including maximum tumor diameter (MTD), number of tumor nodules, portal venous invasion (PVI), presence of metastasis or lymph nodes. For this purpose, especially vascular invasions, baseline dynamic liver tomography, magnetic resonance imaging (MRI), and PET-CTs were reviewed in detail. Patients were then divided into two groups according to the presence or absence of HBV-DNA at the time of admission with the first diagnosis of HCC, and it was determined whether there were differences between these two groups with respect to tumor and non-tumor parameters.

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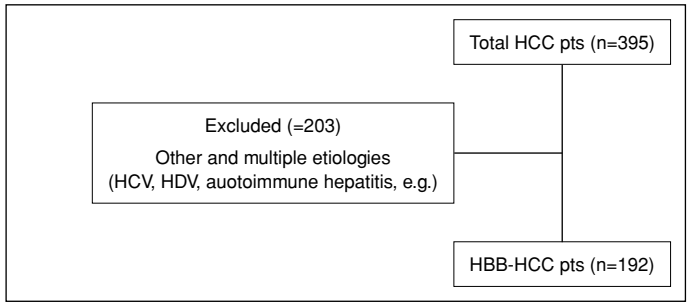

Figure 1. A flowchart detailing the exclusion criteria for patients in the study.
HCV: Hepatitis C virus; HDV: Hepatitis D virus.

Statistical Analysis

The normality of the quantitative variables was assessed by the Shapiro-Wilk test. Median, minimum, and maximum values were used as descriptive statistics for quantitative data. In comparisons, for two independent groups the Mann-Whitney U test, for more than two independent groups the Kruskal-Wallis test and the Conover pairwise comparison method were used. The distribution of qualitative variables was presented by count and percentage. Comparisons were performed using Pearson's chi-square test, continuity-corrected chi-square test, or Fisher's exact test, where appropriate. The Bonferroni correction was used for pairwise comparisons between categories for multinomial variables. In all analyses, a two-sided significance level was considered as $p<0.05$. IBM SPSS Statistics for Windows version 22.0 (Armonk, NY: IBM Corp.) was used for the statistical analyses.

Results

Patient Demographics

In this study, there were 192 patients, of which 92.18% were male. Figure 1 provides a detailed flowchart describing the exclusion of patients from the study. The median age was 59 years. The median values for serum ALT, GGT, CRP, and AFP levels, as well as the median MTD, PVI, and frequencies of multinodularity among the patients, are presented in Table 1.

HBV Status in Relation to Liver Function

A comparison of serum ALT, GGT, HBsAg, and CRP levels between HBV-DNA negative and positive patients showed significant differences (Table 2), with p-values of <0.01, <0.01, <0.05, and <0.05 respectively. A majority of the patients included in the study had cirrhosis (82%), but there was no significant difference between HBV-DNA negative and positive patients in terms of the presence of cirrhosis.

HBV Status in Relation to Tumor Parameters

Table 3 compares tumor parameters between HBV-DNA negative and positive patients. While there was no significant difference between the two groups in terms of multinodularity, significant differences were observed in MTD ($p<0.05$, Table 3), serum AFP levels, and BCLC stages ($p<0.05$ and $p<0.01$, respectively). A major finding was the very significant difference between the two patient groups in terms of PVI and venous invasion ($p<0.001$ and $p<0.01$, respectively). However, no significant difference was found between HBV-DNA negative and positive patients regarding metastasis or lymph node involvement.

Table 1. Demographic and clinical characteristics of the patients included in the study

	Frequency n (%)
Male	177 (92.18%)
	Median (Min–Max)
Age	59 (19–84)
MTD (cm)	5 (1–20)
AFP (ng/mL)	40 (1–54000)
ALT (U/L)	43 (8–672)
GGT (IU/L)	112 (16–1784)
CRP (mg/dL)	0.3 (0–22)

	No n (%)	**Yes n (%)**
Portal invasion	100 (52.1)	92 (47.9)
Multinodular	106 (55.2)	86 (44.8)

MTD: Maximum tumor diameter; CRP: C-reactive protein; AFP: Alpha-fetoprotein; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase.

Table 2. Non-tumoral factors in HBV-DNA negative and positive patients

	HBV-DNA Negative n (%)	**HBV-DNA Positive n (%)**	**p**
ALT			**0.002**
≤30	41 (38)	9 (14.3)	
>30	67 (62)	54 (85.7)	
GGT			**0.003**
≤100	64 (59.3)	22 (34.4)	
>100	44 (40.7)	42 (65.6)	
HBsAg level	3200 (0–7857)	3538.5 (77–5918)	**0.022**
Cirrhosis			
No	7 (6.5)	2 (3.3)	0.492
Yes	100 (93.5)	58 (96.7)	
CRP			**0.025**
<5	97 (93.3)	44 (80)	
≥5	7 (6.7)	11 (20)	

HBV: Hepatitis B virus; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase; CRP: C-reactive protein.

Discussion

The most striking finding of this study was the significantly different frequency of PVI between HBV DNA-negative and positive HCC patients. Another notable observation was the substantial variation in serum CRP levels between these groups.

HCC is a condition highly prone to PVI, a critical adverse prognostic indicator. While various studies report differing rates, approximately 30–62% of advanced HCC cases exhibit macroscopic PVI.[8] Patients with PVI often experience an aggressive disease course, limited treatment options, higher relapse rates

Table 3. Tumor factors in HBV-DNA negative and positive patients

	HBV DNA		
	Negative n (%)	Positive n (%)	p
MTD			**0.022**
≤5	64 (61)	26 (42.6)	
>5	41 (39)	35 (57.4)	
AFP			**0.012**
≤100	67 (64.4)	28 (44.4)	
>100	37 (35.6)	35 (55.6)	
Multinodular			
No	58 (54.7)	31 (47.7)	0.372
Yes	48 (45.3)	34 (52.3)	
Portal invasion			**<0.001**
No	73 (68.2)	24 (37.5)	
Yes	34 (31.8)	40 (62.5)	
Venous invasion			**0.005**
No	80 (75.5)	35 (54.7)	
Yes	26 (24.5)	29 (45.3)	
Met			0.675
No	85 (80.2)	48 (76.2)	
Yes	21 (19.8)	15 (23.8)	
N1			0.185
No	82 (78.1)	44 (67.7)	
Yes	23 (21.9)	21 (32.3)	
BCLC			**0.001**
0	4 (3.8)	1 (1.5)	
A*	32 (30.5)[a]	4 (6.2)[b]	
B	23 (21.9)	16 (24.6)	
C	26 (24.8)	19 (29.2)	
D*	20 (19)[a]	25 (38.5)[b]	

*: Column proportions differ based on these categories (Superscript letters a and b indicate the statistical significance). HBV DNA positive and negative groups only differ in terms of A and D for BCLC distribution. Venous invasion: Hepatic vein or vena cava invasion. HBV: Hepatitis B virus; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase; CRP: C-reactive protein.

post-treatment, and poorer overall survival.[11] Prolonged hepatitis B infection is among the most significant risk factors for PVI in HCC patients.[12] Several studies have indicated that active HBV replication correlates with vascular invasion in HCC.[9,10] In one study, the incidence of PVI was higher (79.0% vs. 18.1%) in patients who did not receive NA therapy compared to those who did.[13] Wang Z et al.[14] observed that preoperative antiviral therapy could reduce the relative risk of microvascular invasion by 40% in HBV-related HCC patients. In our study, we identified a significant association between HBV replication and PVI. This finding aligns with the previously mentioned studies and implies that HBV viral load is a critical factor in PVI.

Although numerous publications have discussed the role of the HBV in the pathogenesis of HCC, details on how HBV viral load impacts PVI remain scant. Liu K et al.[15] noted that the pERK was activated in HCC patients who did not receive antiviral treatment, whereas it was not in those who did. This suggests that antiviral treatment in patients with HBV might reduce microvascular invasion formation by influencing the activation of the MAPK/ERK signaling pathway. Investigating the effects of HBV viral load on the activation of the MAPK/ERK signaling pathway could enhance our understanding and prevention of PVI in HBV-HCC patients.

In our study, serum CRP levels varied significantly between HBV-DNA negative and positive patients. Chronic inflammation is a well-established factor in the initiation, promotion, and progression of HCC.[16] CRP, an acute-phase reactant synthesized by hepatocytes in response to inflammation, aids in detecting or predicting the outcomes of inflammation. Studies have shown that CRP levels are a robust indicator of prognosis in HCC patients.[17,18] While research has demonstrated a correlation between serum CRP and HBV-DNA levels in chronic hepatitis B patients,[19,20] no study has yet assessed this relationship in HCC patients. Our results align with literature identifying CRP as a vital prognostic biomarker in HCC and support a significant link between HBV viral load and inflammation in HCC patients due to hepatitis B.

The majority of participants in our study were male. Globally, HCC incidence rates are two to four times higher in men than in women.[21] Men also exhibit higher incidence, prevalence, and mortality from HCC than women across geographic locations and ages, with studies indicating a 2 to 3 times higher risk of developing HCC in males compared to females.[22,23] Multiple and complex causes include behavioral factors like higher rates of smoking and drug use in males, metabolic differences (e.g., in processing aflatoxin B1), the influence of androgens, and lower alcohol consumption in females.[21,24] Our study focused on HCC patients with HBV. Chronic HBV also shows a higher prevalence in males across all regions.[25] Our findings, consistent with the literature, suggest that male gender is a significant risk factor for HBV-associated HCC.

Conclusion

Our findings suggest that HBV viral load plays a significant role in PVI in HCC patients, and there is a notable relationship between HBV viral load and inflammation. These results bolster the notion that HBV therapy in patients with HCC might contribute positively to HCC treatment and potentially improve survival.

Ethics Committee Approval: The Inonu University Scientific Research Ethics Committee granted approval for this study (date: 20.06.2023, number: 4741).

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