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

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Abbreviations: HBV, Hepatitis B virus; HCC, hepatocellular carcinoma; NA, nucleos(t)ide analogue; PVI, portal vein invasion; ALT, Alanine aminotransferase; GGT, gamma-glutamyl transferase; CRP, C-reactive protein; AFP, alpha-fetoprotein; Hepatitis B surface antigen, PCR, polymerase chain reaction; BCLC, Barcelona Clinic Liver Cancer; MTD, maximum tumor diameter; MRI, Magnetic resonance imaging.
Abstract

**Background:** Several tumor and non-tumor factors affect the prognosis of HCC patients. 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 hepatitis B and HCC who were presented to the HCC council at aculty of Medicine, Marmara University liver transplantation institute were included in our study. Patients were then divided into two groups according to 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 ALT, GGT, HBsAg and CRP levels between HBV-DNA negative and positive patients, showed that there were significant differences between them (respectively p<0.01, p<0.01, p<0.05 and p<0.05). A major finding was a very significant difference between the 2 patient groups in terms of portal vein invasion and venous invasion (p<0.001 and p<0.01, respectively), but no significant difference between HBV-DNA negative and positive patients in regard to metastasis or lymph node involvement.

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

**Keywords:** Hepatitis B, hepatocellular carcinoma, HBV-DNA.
Introduction

Hepatitis B (HBV) infection is a global public health problem that causes advanced liver diseases such as cirrhosis and hepatocellular carcinoma (HCC). In chronic HBV, persistant viral replication is the most important risk factor for progression to cirrhosis and HCC development (1). International guidelines such as AASLD (2), EASL (3) and APASL (4) recommend suppression of HBV-DNA with interferon and nucleotide analogues (NA) for the prevention of HCC. Previous studies have reported that high HBV viral load is closely associated with a high risk of HCC recurrence and metastasis after liver resection (5). Several meta-analyses have reported that NAs that 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 there are studies reporting 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 hepatitis B and HCC who were presented to the HCC council at 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 Hepatitis B surface antigen (HBsAg) levels of the patients were recorded from their files. In order to evaluate the HBV replication level of the patients, the HBV-DNA levels obtained by the polymerase chain reaction (PCR) method at the time of admission were determined. HBsAg levels were measured with quantitative method. At 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 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 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 between these two groups with respect to tumor and non-tumor parameters.

**Statistical methods**

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

Results

Patient demographics

There were 192 patients in the study and 92.18% were male. Figure 1 shows a flow-chart describing in detail how patients were excluded in the study. Median age was 59 years. The median values for serum ALT, GGT, CRP and AFP levels as well as the median MTD, PVI and multinodularity frequencies of the patients are shown in Table 1.

HBV status in relation to Liver Function

Comparison of serum ALT, GGT, HBsAg and CRP levels between HBV-DNA negative and positive patients, showed (Table 2) that there were significant differences between them (respectively p<0.01, p<0.01, p<0.05 and p<0.05). Most 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 shows a comparison of tumor parameters between HBV-DNA negative and positive patients. While there was no significant difference between HBV-DNA negative and positive patients for multinodularity, there was a significant difference between the patients in terms of MTD (p<0.05, Table 3), serum AFP levels and BCLC stages (p<0.05 and p<0.01, respectively). A major finding was a very significant difference between the 2 patient groups in terms of portal vein invasion and venous invasion (p<0.001 and p<0.01, respectively), but no significant difference between HBV-DNA negative and positive patients in regard to metastasis or lymph node involvement.
Discussion

The most striking finding of this study was that the frequency of PVI was significantly different between HBV-DNA negative and positive HCC patients. Another remarkable finding was that serum CRP levels were also significantly different between the two groups.

HCC is a disease with a high tendency for PVI, a major adverse prognostic factor. Although different rates have been reported in various studies, approximately 30-62% of advanced HCC cases have macroscopic PVI (8). Patients with PVI often have an aggressive disease course, limited treatment options, higher relapse rates after treatment, and worse overall survival (11). Prolonged hepatitis B infection is one of the most important risk factors for portal vein invasion in patients with HCC (12). There are studies reporting that active HBV replication is associated with vascular invasion in HCC patients (9, 10). In one study, the incidence of PVI was reported to be higher (79.0% vs. 18.1%) in patients who did not receive NA therapy than in those who did (13). Wang Z et al. reported that preoperative antiviral therapy can reduce the relative risk of microvascular invasion by 40% in patients with HBV-related HCC (14). In our study, we determined that there is a significant relationship between HBV replication and PVI. Our finding is consistent with the above related publications and suggests that HBV viral load plays an important role in portal vein invasion.

Although there are many publications on the role of hepatitis B virus in the pathogenesis of HCC, information on how HBV viral load affects PVI is negligible. Liu K et al. reported that pERK was activated in HCC patients without antiviral treatment, but not in the group with antiviral treatment, and therefore, antiviral treatment in patients with HBV may reduce microvascular invasion formation by affecting the activation of MAPK/ERK signaling pathway.
Investigating the effects of HBV viral load on MAPK/ERK signaling pathway activation may lead to understanding and prevention of the pathogenesis of PVI in HBV-HCC patients.

In our study, serum C-reactive protein (CRP) levels were significantly different between HBV-DNA negative and positive patients. Chronic inflammation has been demonstrated to be an important factor in the initiation, promotion, and progression of hepatocellular carcinoma (HCC) (16). CRP is an acute-phase reactant that is synthesized by hepatocytes in response to inflammation and is helpful for detecting or predicting outcomes of inflammation. There are studies reporting that CRP levels are a strong indicator of prognosis in HCC patients (17,18). Although there are studies reporting that serum CRP levels are correlated with HBV-DNA levels in chronic hepatitis B patients (19, 20), no study has been found to evaluate HBV-DNA levels and CRP levels in HCC patients. Our study results are in line with the literature reporting that CRP is an important prognostic biomarker in HCC and support a significant relationship between HBV viral load and inflammation in HCC patients due to hepatitis B.

The majority of the patients in our study were male. In most countries, HCC incidence rates among men are two to four-fold higher than rates among women (21). Males also have a greater incidence, prevalence, and mortality from HCC than females across geographic location and age; studies have reported a 2 to 3 times increased risk of developing HCC in males compared to females (22, 23). Although the causes are multiple and complex, factors that have been suggested include behavioural ones (such as greater smoking and drug use in males), differences in metabolism (shown for aflatoxin B1) and role of androgens, and lower alcohol consumption in females (21, 24). Our study was about HCC patients with HBV. Chronic HBV has also a greater prevalence in males than females across all geographic regions (25). Our findings, in line with the literature, support that male gender is an important risk factor for HCC disease with HBV.

In conclusion, our findings suggest that HBV viral load plays an important role in PVI in HCC patients and that there is a significant relationship between HBV viral load and inflammation. These results support the idea that HBV therapy in patients with HCC might contribute to HCC therapy and possibly survival.
Table Legends.
Table 1. Demographic and clinical characteristics of the patients included in the study.
Table 2. Non-tumoral factors in HBV-DNA negative and positive patients.
Table 3. Tumor factors in HBV-DNA negative and positive patients.

Figure Legend.
Figure 1. A flow-chart describing in detail how patients were excluded in the study. (HCV: Hepatitis C virus, HDV: Hepatitis D virus)

References.


