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ORIGINAL ARTICLE

High Hepatitis B Viral Load Predicts Recurrence of Small Hepatocellular Carcinoma after Curative Resection

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Abstract A retrospective cohort study was conducted to identify risk factors for recurrence of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) after curative resection. A total of 317 patients who had received curative resection of pathologically proven small HCC (≤3 cm in diameter) were analyzed to ascertain the factors affecting recurrence. The median follow-up period was 33.7 months. Cumulative recurrence rates at 1, 3, and 5 years after resection were 23.5%, 49.5%, and 65.5%, respectively. Male sex, alpha-fetoprotein (AFP) ≥400 ng/mL, HBV DNA level ≥4 $\log_{10}$ copies/mL, prolonged prothrombin time, tumor size ≥2 cm, microvascular invasion, absence of capsular formation, moderate/poor tumor differentiation, and absence of postoperative interferon-alpha (IFN-α) treatment were associated with increased cumulative risk of HCC recurrence. By multivariate analysis, HBV DNA level ≥4 $\log_{10}$ copies/mL ($P<0.001$, hazard ratio (HR) 2.110), AFP ≥400 ng/mL ($P=0.011$, HR 1.574), microvascular invasion ($P<0.001$, HR 1.767), and postoperative IFN-α treatment ($P=0.022$, HR 0.562) remained to be independently associated with HCC recurrence. Those contributing to late recurrence (>2 years) were older age and HBV DNA level ≥4 $\log_{10}$ copies/mL. Patients with persistent HBV DNA level ≥4 $\log_{10}$ copies/mL at resection and follow-up had the highest recurrence risk ($P<0.001$, HR 4.129). HBV DNA level ≥4 $\log_{10}$ copies/mL at the time of resection was the most important risk factor for recurrence. Postoperative IFN-α treatment significantly decreased the recurrence risk after resection.

Keywords Hepatocellular carcinoma · Recurrence · Hepatitis B viral load

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third most common cause of cancer-related death in the world.[1] Etiologically, majority of HCC develops in chronic hepatitis B virus (HBV) carriers, especially in East Asia and sub-Saharan Africa, where HBV is endemic. During the past decades, with periodic serum alpha-fetoprotein (AFP) assays and the development of modern imaging systems, such as ultrasonography (US), computed tomography (CT), and magnetic resonance imaging (MRI), more and more small HCCs of diameter ≤3 cm can be detected and diagnosed early. For these patients, curative resection is considered the most effective treatment and the prognosis of HCC was greatly improved.[2,3] However, high possibility of intrahepatic recurrence remains one major obstacle for further improving the survival and prognosis of HCC patients after curative resection.[4] For patients who undergo tumor resection for hepatitis B-related HCC, the cumulative recurrence rate at 3 years after surgery is estimated to be as high as 50%.[5,6] It has been reported that tumor size, macroscopic vascular invasion, and intrahepatic metastasis were related significantly to HCC recurrence.[7–10] However, recurrence is also common in cases with small HCC having neither macroscopic vascular invasion nor intrahepatic metastasis.

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Recently, a significant association between high hepatitis B viral load and increased risk of HCC and liver cirrhosis was observed in several studies.[11,12] But only a few studies have evaluated the viral replicative status of subjects as a predictor of postoperative recurrence of HCC. In two case series studies on the recurrence of HCC after surgical resection, patients with high serum HBV DNA level at study entry had a significantly higher risk of HCC recurrence than those with low level.[13,14] However, in previous studies, the relation between hepatitis B viral load and the recurrence of HCC after resection may be confounded by other major risk factors for recurrence, such as macroscopic vascular invasion or noncurative resection. And these were limited in that most investigators evaluated the serum HBV DNA level at one time only (usually at the time of surgery) as a risk factor. To our knowledge, no reports published to date have demonstrated a relation between fluctuated hepatitis B viral load and recurrence risk in small HCC patients after curative resection. The goal of the present study was to assess the significance of hepatitis B viral load with other demographic, biochemical, tumor factors in the recurrence of small HCC patients after curative resection.

Patients and Methods

Patients

Between 2002 and 2005, 1,462 patients with hepatitis B-related HCC underwent tumor resection in the Department of Liver Surgery, Zhongshan Hospital, Fudan University. Of these, 354 patients who had received curative resection of pathologically proven small HCC (≤3 cm) were retrieved from a prospectively collected database. A total of 317 were finally entered into the analyses and 37 patients were excluded for the following reasons; seven patients died in hospital due to postoperative hepatic failure, 11 patients had early recurrence within 3 months after surgery (suggesting preexisting metastases before HCC resection), and data were lacking for 19 patients. No patients received antiviral drugs or adjuvant anti-tumor therapy before surgery. All patients had confirmed HCC in the surgical specimen from tumor resection. Curative resection was defined as (1) complete resection of all tumor nodules and the surgical free margin of more than 5 mm by pathological examination; (2) no cancerous thrombus found in the portal vein (main trunk or two major branches), hepatic veins, or bile duct; (3) the number of tumor nodules not exceeding three; and (4) no extrahepatic metastasis found. Histological grade proposed by Edmondson and Steiner with little modification,[15] maximal tumor size, nodule number, capsular formation around the tumor, microvascular invasion, and liver cirrhosis were also determined. Various surgical procedures were classified as wedge resection, segmentectomy, and two or more segmentectomies. This study was approved by the research ethics committee at Zhongshan Hospital, Fudan University, Shanghai, China.

Follow-up and End Point

After surgery, 36 patients received interferon-alpha1b (Sinogen, Kexing Bioproducts Co., Shenzhen, P. R. China) treatment, which was started at a pilot dose of 3 million units (mu) two times a week by intramuscular injection for 2 weeks, then 5 mu three times a week for 18 months. The interferon-alpha (IFN-α) treatment was terminated when recurrence was confirmed. No further anti-tumor treatment was given to 317 patients until recurrence was confirmed. All patients were followed up by determination of monthly AFP and US, as well as three monthly CT or MRI scan for 1 year. Then, all patients were screened by AFP and US every 3 months and helical CT or MRI every 6 months thereafter, and hepatic angiography when recurrence was suspected. The diagnosis of intrahepatic recurrence was based on histopathologic findings of tumor tissue in 47 patients undergoing repeat hepatic resection and on the characteristic appearance on US, CT, MRI, and hepatic angiography in 136 patients. The primary end point was tumor recurrence. Time to recurrence was defined as the period between surgery and the diagnosis of recurrence. If recurrence was not diagnosed at the time of study, the cases were censored on the date of death or the last date of follow-up. All follow-up data were summarized as of the end of October 2007.

Statistical Analysis

Virological data were analyzed with conventional clinical variables at the time of resection to identify factors that influenced recurrence via the Cox proportional hazards model. Risk factors contributing to late recurrence (>2 years) were investigated by stratified Cox regression analysis. Cumulative recurrence rate was calculated by the Kaplan-Meier method and differences were compared by the logrank test. Multivariate analysis was performed by the Cox proportional hazards regression model. Statistical significance was defined by a P value of less than 0.05. Statistical analyses were performed using the Statistical Program for Social Sciences (SPSS 11.5 for Windows; SPSS, Inc., Chicago, IL, USA).

Result

During the observation period (3-66.5 months), intrahepatic recurrence was detected in 183 patients (57.7%). The

cumulative recurrence rates at 1, 2, 3, 4, and 5 years after curative resection were 23.5%, 39.4%, 49.5%, 55.1%, and 65.5%, respectively. The baseline demographic, biochemical, tumor, and viral factors of the whole study population were depicted in Table 1.

Demographic Profile and HCC Recurrence

Male patients had a higher cumulative risk of developing HCC recurrence after resection when compared to female patients (P=0.034). Age at the time of curative resection did not have a significant effect on HCC recurrence (P=0.429; Table 2).

Prognostic Effect of Clinical Factors and HCC Recurrence

High serum AFP level and prolonged prothrombin time (PT) at the time of resection were the significant risk factors for recurrence in univariate analyses. AFP ≥400 ng/mL was associated with a higher cumulative risk of developing HCC recurrence after resection (Fig. 1). Among 183 recurrence patients, 141 (77.0%) had HBV DNA level ≥4 $\log_{10}$ copies/mL at the time of tumor resection. While, 70 (52.2%) of 134 nonrecurrence patients had HBV DNA level ≥4 $\log_{10}$ copies/mL (P<0.001). A significant biological gradient of recurrence risk by HBV DNA level from less than 4-6 $\log_{10}$ copies/mL or greater was observed. In

Table 1 Patient Characteristics

Characteristics	No. (%)	Values
No. of patients	317 (100)	
Median age, years (range)		51 (26-82)
Male/female ratio	270:47 (85.2:14.8)	
HBeAg seropositivity	106 (33.4)	
HBV DNA level ≥4 $\log_{10}$ copies/mL	211 (66.6)	
Alpha-fetoprotein ≥400 ng/mL	60 (18.9)	
Presence of cirrhosis	244 (77.0)	
Co-existing HCV infection	9 (2.8)	
Median baseline biochemistry and hematology (range)		
Total bilirubilin, μM		15.9 (5.3-40.1)
Albumin, g/L		42 (27-54)
Aminotransferase, IU/L		42 (10-398)
Prothrombin time, s		11.6 (9.2-19.6)
Tumor size (<2 cm: ≥2 cm)	132:185 (41.6:58.4)	
Tumor number (Single/multiple)	272:45 (85.8:14.2)	
Microvascular invasion	80 (25.2)	
Capsular formation	165 (52.1)	
Differentiation of tumor		
Well-differentiated	95 (30.0)	
Moderate	177 (55.8)	
Poor	45 (14.2)	
Child-Turcotte-Pugh grade		
A	304 (95.9)	
B	13 (4.1)	
Okuda stage		
I	301 (95.0)	
II	16 (5.0)	
Type of surgical procedure		
Wedge resection	225 (71.0)	
Segmentectomy	71 (22.4)	
Two or more segmentectomies	21 (6.6)	
Postoperative IFN-α treatment	36 (11.4)	
Median follow-up time (months)		33.7 (3-66.5)
Median time of recurrence (months)		16 (3-65)

Table 2 Factors Identified on Univariate Cox Regression Analysis that Influenced Recurrence in Small HCC Patients Undergoing Curative Resection

Factors	*P* value	Hazard ratio	95% CI
Sex (male vs. female)	0.034	1.655	1.040-2.633
Age, years	0.429	1.006	0.992-1.020
HBeAg seropositivity	0.293	1.177	0.869-1.593
HBV DNA level 4-5.99 $\log_{10}$ copies/mL	<0.001	1.981	1.366-2.872
≥6 $\log_{10}$ copies/mL	<0.001	3.086	2.054-4.634
Alpha-fetoprotein ≥400 ng/mL	0.005	1.626	1.155-2.291
Co-existing HCV infection	0.422	1.363	0.640-2.902
Total bilirubilin	0.690	0.996	0.976-1.016
Albumin	0.260	0.981	0.947-1.015
Aminotransferase	0.521	1.001	0.998-1.004
Prothrombin time	0.032	1.113	1.009-1.228
Presence of cirrhosis	0.138	1.318	0.915-1.899
Tumor size (≥2 cm vs. <2 cm)	0.011	1.487	1.097-2.015
Tumor number (multiple vs. single)	0.386	1.192	0.801-1.773
Microvascular invasion	<0.001	2.017	1.473-2.762
Capsular formation	0.030	0.724	0.541-0.970
Differentiation (moderate or poor differentiated vs. well differentiated)	0.038	1.424	1.020-1.989
Child-Turcotte-Pugh grade (A vs. B)	0.172	1.698	0.794-3.631
Okuda stage (I vs. II)	0.816	1.088	0.535-2.214
Postoperative IFN-α treatment	0.044	0.606	0.373-0.987

Fig. 2, there was a stepwise increase in the cumulative risk of recurrence with increasing hepatitis viral load starting from HBV DNA level ≥4 $\log_{10}$ copies/mL. The relationship between pathological factors and recurrence was also demonstrated by the univariate Cox regression analyses. Tumor size ≥2 cm, moderate/poor tumor differentiation, presence of microvascular invasion, and absence of capsular formation were significantly associated with intrahepatic recurrence. Figure 3 depicted the presence of microvascular invasion was associated with a significantly higher cumulative risk of tumor recurrence. Other clinical factors including serum albumin, total bilirubilin, aminotransferase, HBeAg statue, co-existing hepatitis C virus (HCV) infection, presence of cirrhosis, tumor number,

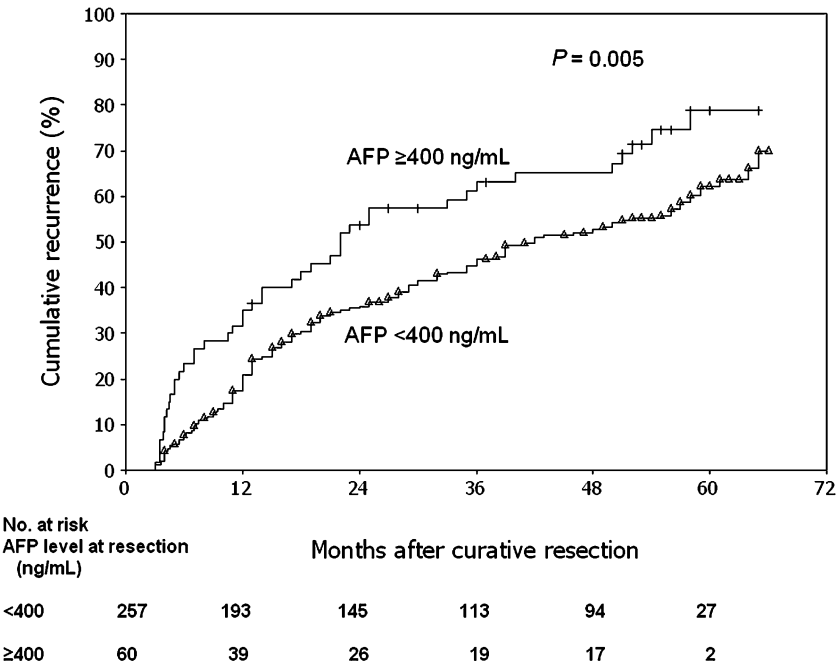

Figure 1 Cumulative HCC recurrence related to AFP level at the time of tumor resection (=0.005, logrank test).

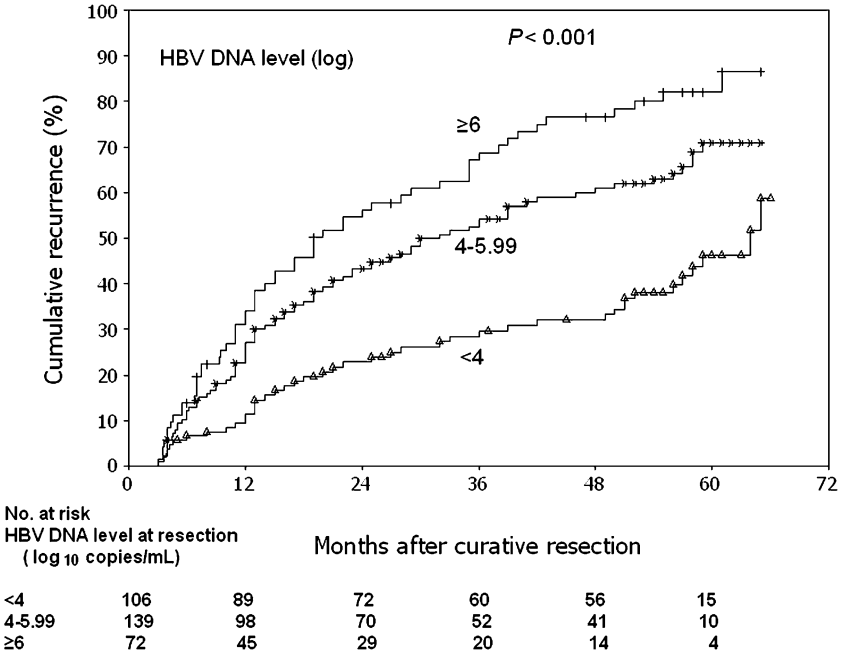

Figure 2 Cumulative HCC recurrence related to serum HBV DNA level at the time of tumor resection ($P<0.001$, logrank test).

Child-Turcotte-Pugh grade, and Okuda stage were not associated with HCC recurrence (Table 2).

Postoperative IFN-α Treatment and HCC Recurrence

A total of 36 patients received IFN-α treatment after curative resection. No other anti-tumor treatment was given to 317 patients until the recurrence was confirmed. Treatment with IFN-α after tumor resection was associated with significantly lower cumulative risk of recurrence compared to patients without IFN-α treatment (Fig. 4).

Multivariate Analysis of Risk Factors for HCC Recurrence

Univariate analysis revealed that the following factors had a significant effect on recurrence: male sex, AFP ≥400 ng/mL, HBV DNA level ≥4 $\log_{10}$ copies/mL, prolonged PT, tumor size ≥2 cm, microvascular invasion, absence of capsular formation, moderate/poor tumor differentiation, and patients without postoperative IFN-α treatment. All these variables were entered into the multivariate analysis by the Cox proportional hazards regression model. AFP ≥400 ng/mL, HBV DNA level ≥4 $\log_{10}$ copies/mL, microvascular

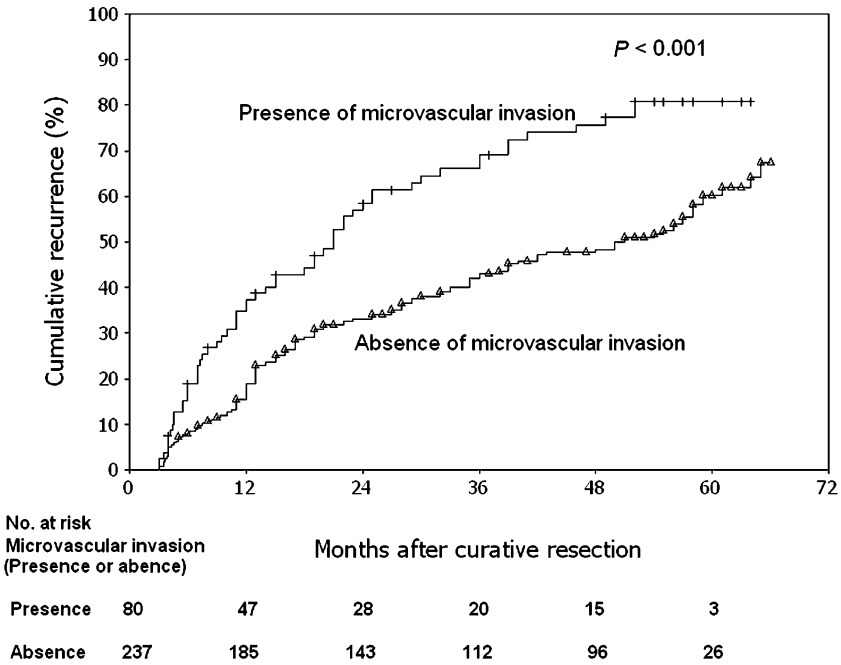

Figure 3 Cumulative HCC recurrence related to the presence of microvascular invasion in the resected tumor ($P<0.001$, logrank test).

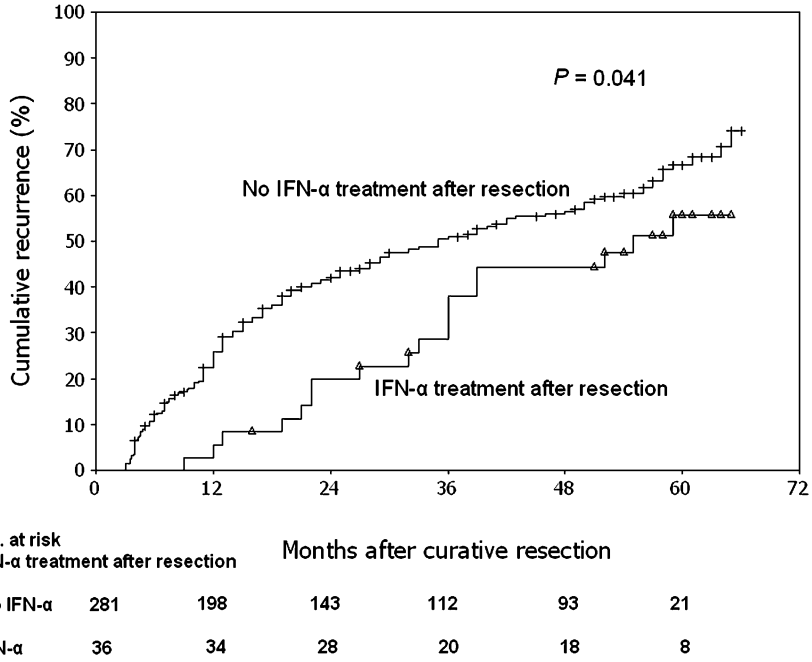

Figure 4 Cumulative HCC recurrence related to the IFN-α treatment after curative resection (P=0.041, logrank test).

invasion, and absence of postoperative IFN-α treatment were independent risk factors of HCC recurrence after curative resection (Table 3).

Factors Contributing to Late Recurrence (>2 years)

Factors related to late recurrence (>2 years) were investigated in 171 patients who were recurrence-free at first 2 years, as suggested by Imamura's study.[16] Recurrence was detected in 63 patients within follow-up. Stratified Cox regression analysis identified three factors contributing to late recurrence: male sex (P=0.043; hazard ratio [HR] 2.565, 95%CI 1.028-6.396), older age (P=0.028; HR 1.028, 95% CI 1.003-1.054), and HBV DNA level ≥4 $\log_{10}$ copies/mL (P=0.009; HR 2.047, 95% CI 1.193-3.512). By multivariate analysis, older age and HBV DNA level≥4 $\log_{10}$ copies/mL were independently associated with risk of late recurrence (Table 4). Patients with HBV DNA level ≥4 $\log_{10}$ copies/mL at resection had a significantly higher cumulative late recurrence rate than those with HBV DNA level <4 $\log_{10}$ copies/mL (Fig. 5).

Table 3 Multivariate Analysis of Independent Risk Factors Associated with Recurrence

Factors	P value	Hazard ratio	95% CI
HBV DNA level ≥4 $\log_{10}$ copies/mL	<0.001	2.110	1.483-3.002
Alpha-fetoprotein ≥400 ng/mL	0.011	1.574	1.109-2.234
Microvascular invasion	<0.001	1.767	1.286-2.429
Postoperative IFN-α treatment	0.022	0.562	0.343-0.921

Recurrence Risk by HBV DNA Level at the Time of Resection and Follow-up in Combination

We further examined the association between recurrence risk and persistently elevated serum HBV DNA level at resection and follow-up. Among 317 patients, 224 (70.7%) had received at least once time HBV DNA examination during follow-up (before the detection of recurrence). The median interval between the time of resection and last HBV DNA examination was 23 months (range from 6-55 months). We then evaluated the recurrence risk with HBV DNA level at the time of resection and follow-up in combination. Compared with subjects who had HBV DNA level <4 $\log_{10}$ copies/mL both at the time of resection and follow-up, the HR was 2.233 (95%CI 1.051-4.743) for subjects with HBV DNA level <4 $\log_{10}$ copies/mL at resection and ≥4 $\log_{10}$ copies/mL at follow-up. Subjects who had persistent HBV DNA level ≥4 $\log_{10}$ copies/mL both at the time of resection and follow-up were expected to have the highest recurrence risk (Table 5). Cumulative recurrence rate of respective groups are shown in Fig. 6.

Table 4 Multivariate Analysis of Independent Risk Factors Associated with Late Recurrence (>2 years) in 171 HCC Patients

Factors	P value	Hazard ratio	95% CI
Older age	0.017	1.031	1.005-1.057
HBV DNA level ≥4 $\log_{10}$ copies/mL	0.009	2.053	1.192-3.534

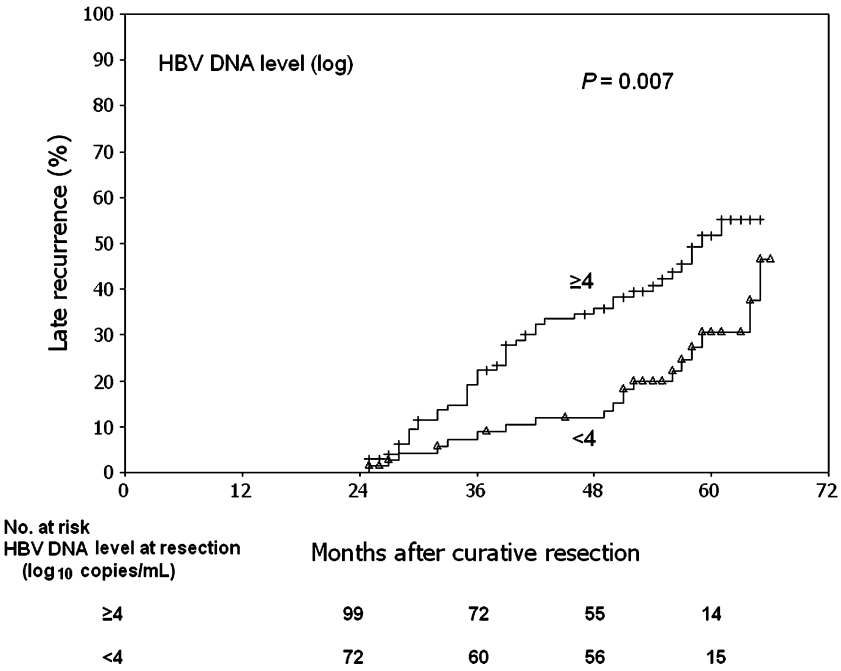

Figure 5 Cumulative late recurrence (>2 years) related to the initial HBV DNA level at the time of tumor resection (P=0.007, logrank test).

Discussion

The prognosis of HCC remains unsatisfactory although it has been improved much in the past decades. Even after curative resection, recurrence of hepatitis B-related HCC is extremely high.[17] Previous studies have shown that tumor size, nodule number, vascular invasion, high AFP level, a positive surgical margin, and Edmondson's grade are prognostic factors predicting recurrence.[6,7,9,13,18–20] The current study focused primarily on the correlation between hepatitis B viral load and recurrence of small HCC after curative resection.

Multivariate analysis demonstrated that HBV DNA level ≥4 $\log_{10}$ copies/mL, AFP ≥400 ng/mL, microvascular invasion, and absence of IFN-α treatment after curative resection were four independent factors associated with higher cumulative risk of tumor recurrence. According to our statistical analysis, the study revealed that HBV DNA level ≥4 $\log_{10}$ copies/mL at resection was the most important risk factors for recurrence of small HCC after surgery. Hung et al. have reported a similar finding in a study of 72 hepatitis B-related HCC patients after surgery.[13] Previous studies hypothesized that early and late intrahepatic recurrence of HCC was attributable to two different mechanisms: intrahepatic metastasis and de novo multicentric carcinogenicity.[16,18] The latter is clonally independent from the primary tumor.[21] Imamura et al. proposed a convenient framework to clinically differentiate each type of recurrence as "early" or "late" recurrence based on a cutoff of 2 years after surgery.[16] With the successful implementation of HCC surveillance and curative treatment, more patients avoid the risk of early recurrence and thus survive longer enough to acquire late recurrence. But risk factors contributing to late recurrence after surgery had not been investigated on a comprehensive basis. As the impact of viral factors on the recurrence of HCC after resection may be overshadowed by tumor-related factors during the early recurrence, we then investigated factors possibly contributing to late recurrence separately. In a subgroup of 171 patients who were recurrence-free at first 2 years, HBV DNA level ≥4 $\log_{10}$ copies/mL and older age

Table 5 Recurrence Risk by HBV DNA Level at the Time of Resection and Follow-Up in Combination

HBV DNA Level ($\log_{10}$ Copies/mL) at Resection at Follow-up[a]	No. of Participants (n=224) (%)[b]	No. of Recurrence (n=117) (%)	Hazard Ratio (95% CI)	P value
<4 <4	55 (24.6)	16 (13.7)	1.0 (reference)	-
<4 ≥4	24 (10.7)	12 (10.3)	2.233 (1.051-4.743)	0.037
≥4 <4	49 (21.9)	21 (17.9)	1.749 (0.911-3.358)	0.093
≥4 ≥4	96 (42.9)	68 (58.1)	4.129 (2.364-7.211)	<0.001

[a] Data of last HBV DNA examination during follow-up before the detection of recurrence

[b] Because of rounding, percentages do not always total 100

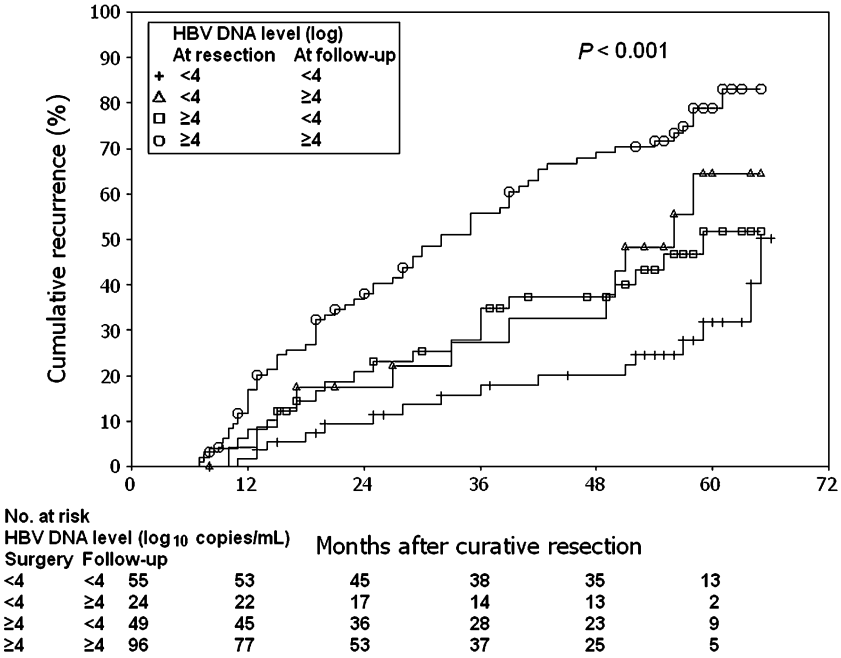

Figure 6 Cumulative HCC recurrence risk by HBV DNA level at the time of resection and follow-up in combination in 224 patients ($P<0.001$, logrank test).

were independent risk factors for late recurrence. However, tumor factors, such as AFP ≥400 ng/mL and microvascular invasion were not associated with late recurrence. Our finding supported that late recurrence was attributable to de novo HCC and the key role of high viral load on the development of late recurrence. Additionally, serum HBV DNA level may fluctuate during the course of chronic infection.[22] Previous studies were limited in that they measured only the high HBV DNA level at the time of resection as a risk factor. The fluctuation of HBV DNA level after resection has seldom been evaluated. In this study, we further examined the relationship between recurrence risk and serum HBV DNA level at resection and follow-up (before the detection of recurrence) in combination. Compared to those with instantaneous high HBV DNA level at the time of resection, patients who had persistent HBV DNA level ≥4 $\log_{10}$ copies/mL during follow-up were expected to have the highest risk of recurrence. It is likely that these patients who had ongoing active viral replication were more prone to recurrence. In theory, treating high viral load patients with antiviral drugs both pre- and post-operatively is reasonable.

Although the precise mechanism for recurrent carcinogenesis associated with HBV in the remaining liver in patients who have undergone curative resection is unclear, it is possible that sustained viremia and subsequent active viral replication may contribute to the carcinogenic process. Firstly, integration of subgenomic HBV DNA fragments into the host liver cell may activate cellular genes directly to allow selective growth advantage, while production of HBV X protein can act as a transactivator on various cellular genes for tumor development.[23] Secondly, continuing HBV replication can induce chronic liver inflammation and fibrosis and mediate alteration in transforming growth factor-beta1 and alpha2-macroglobulin production, thereby leading to carcinogenesis.[24,25] Thirdly, the upregulation of adhesion molecules on the cells lining the sinusoids may enhance tumor development and spread.[26]

IFNs are cytokines possessing a variety of biologic properties, including antiviral, immunomodulatory, antiproliferative, and antiangiogenic effects.[27,28] Many studies confirmed that postoperative IFN-α treatment can decrease HCC recurrence after resection. However, most trials included predominantly HCV infections. Whether such treatment will also benefit HBV-related HCC remains to be elucidated. In this study, multivariate analysis showed that IFN-α treatment decreased the recurrence rate: the HR of postoperative IFN-α treatment was 0.562 (95% CI, 0.343-0.921), indicating that such IFN-α treatment could decrease the hazard of HCC recurrence rate to approximately 44% of that with untreated patients. However, in a subgroup of 171 patients who were recurrence-free at first 2 years, both univariate and multivariate analysis did not show that initial 18 months IFN-α treatment after resection could decrease late recurrence. Based on this disaccording, we hypothesized that during the 18 months treatment with IFN-α, the recurrence rate was lower than that untreated patients, whereas, when IFN-α treatment stopped, the recurrence rate was similar between the two groups, which may imply that tumor growth was suppressed by IFN-α treatment, but became clinically evident after IFN-α treatment was stopped. Since the current study was a retrospective cohort study and the number of patients in the present study was too small to reach a firm conclusion, a prospective randomized controlled study should be conducted in the future.

Other independent risk factors associated with HCC recurrence after curative resection that were identified in this study included preoperative AFP ≥400 ng/mL and the presence of microvascular invasion. These findings were similar to that described previously.[13,29,30] Patients with high AFP level tended to have greater tumor size, bilobar involvement, massive or diffuse types, and tumor vascular invasion.[31] The presence of microvascular invasion was consistently reported as strongly predictive of intrahepatic metastasis.

There is strong evidence linking elevations in serum HBV DNA level and HCC progression in chronic hepatitis B.[12] In the present study, we further proved that HBV DNA level ≥4 $\log_{10}$ copies/mL was associated with a higher recurrence rate after tumor resection. At study entry, no patients received antiviral treatments with nucleoside analogs. During follow-up, seven patients received lamivudine treatment after resection. The median interval between resection and lamivudine treatment was 19 months (range from 11 to 37 months). Although we could not evaluate the effect of lamivudine treatment in preventing recurrence, postoperative IFN-α treatment showed a beneficial effect in reducing HCC recurrence. It might support the antiviral treatment in the prevention of recurrence after curative resection. The present data did not suggest that concurrent HBV and HCV infection have a deleterious effect on the prognosis of HCC patients, probably due to the relatively small number of patients with co-existing HCV infection.

Conclusion

In conclusion, HBV DNA level ≥4 $\log_{10}$ copies/mL, AFP ≥400 ng/mL, microvascular invasion, and postoperative IFN-α treatment were independently associated with HCC recurrence after surgery. Patients with persistent HBV DNA level ≥4 $\log_{10}$ copies/mL during follow-up were expected to have the highest recurrence risk. Elevations in serum HBV DNA level is not only a major risk factor for HCC recurrence, but the risk factor most amenable to modification. This may support prioritized use of anti-HBV treatment as adjuvant therapy after the resection of HCC for the patients with a high HBV DNA level to prevent recurrence. A potential limitation of the present study is that the data were based on a retrospective cohort of small HCC patients, large-scale prospective trials are necessary to elucidate the effects of sustained viremia on recurrence after surgery and the protective roles of antiviral treatment.

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Potential competing interests None.

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