Expression of hepatitis B surface antigen in liver tissues can serve as a predictor of prognosis for hepatitis B virus-related hepatocellular carcinoma patients after liver resection

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Background: Hepatitis B surface antigen (HBsAg) is a detectable index after hepatitis B virus (HBV) infection, which is a risk factor of hepatocellular carcinoma (HCC). However, few studies have focused on the expression of HBsAg in HCC patients’ liver tissues. This study aimed to explore the potential utility of using HBsAg protein expression in normal liver tissues as a prognostic factor for HCC patients who underwent liver resection.

Study design: The study enrolled 100 HCC patients with seropositivity for HBsAg. The liver tissues were collected, and tissue microarrays were constructed. The expression of HBsAg in liver tissues were measured by immunohistochemistry (IHC). Relevant clinical data and follow-up records were collected for analysis.

Results: HBsAg expressions was detected in 29 patients (positive group) and was unable to be detected in the remaining 71 patients (negative group). The patients in the positive group had higher HBV DNA levels ($P < 0.05$) than the patients in the negative group. The overall survival (OS) rate of the positive group was worse than the OS rate of the negative group ($P = 0.013$). The OS rates after resection at 1 and 2 years in negative group were 90.1% and 85.7%, respectively, while the value in the positive group were 79.3% and 65.5%, respectively. Multivariate analysis showed that HBsAg expression in liver tissues, ascites and alpha-fetoprotein levels were independent factors influencing OS. Similarly, after propensity score matching (PSM), the OS was worse in the positive group than in the negative group, and HBsAg expression could also serve as a predictor for OS ($P = 0.039$). The OS rates after resection and PSM at 1 and 2 years were 93.2% and 85.9% in the negative group, while the value in the positive group were 79.3% and 65.5%.

Conclusion: As determined according to grouping based on immunohistochemistry staining results for HBsAg, this study indicated that HBsAg expression in liver tissues could predict the OS of HBV-related HCC patients after liver resection. Eur J Gastroenterol Hepatol 33: 76–82

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Keywords: hepatitis B surface antigen, hepatocellular carcinoma, immunohistochemistry, liver resection, liver tissues, prognosis

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Introduction

Liver cancer is predicted to be the sixth most commonly diagnosed cancer and the fourth leading cause of cancer death worldwide in 2018, with approximately 841,000 new cases and 782,000 deaths annually. Among them, hepatocellular carcinoma (HCC) accounted for 75–85% of those cases [1]. China is the most high-risk area for HCC, and a key influence factor is chronic hepatitis B virus (HBV) infection. The natural history of chronic HBV infection indicates that adverse outcomes of HBV infection are HCC and decompensated cirrhosis [2–4]. Commonly, HBV is thought to be related to HCC recurrence [5]. Seropositivity for the hepatitis B surface antigen (HBsAg) is one of the most important risk factors for HCC [6]. Clinically, there are multiple staging systems for predicting overall survival (OS), such as the Barcelona Clinic Liver Cancer [7], the American Joint Committee on Cancer eighth edition criteria [8] and the Chinese University Prognostic Index [9]. However, few studies or staging systems have focused on HBV-related immunohistochemistry (IHC) results before. Previous studies have shown that HBV DNA levels are associated with early recurrence, whereas HBsAg levels are associated with late recurrence after curative resection in HBV-related HCC [10]. To date, a large number of studies have focus on serological HBV-related indicators, but no research has explored the relationship between pathologic HBV-related indicators and prognosis.

Because of the comprehensive tumor heterogeneity in HCC [11], this study aimed to explore the role of HBsAg expression in liver tissues as a predictor of prognosis for HBV-related HCC patients after liver resection.
Patients and methods

Study patients

This was a retrospective study. This study was approved by the Institutional Review Board of Sun Yat-sen University Cancer Center and conducted in accordance with approved guidelines. It was approved by the Institutional Ethics Committee. All patients were fully informed that their data were to be used for research, and related consent was signed. From June 2014 to January 2016, we consecutively collected 211 patients who had diagnosed as HCC based on the criteria of the European Association for the Study of the Liver and undergone liver resection by our group at Sun Yat-sen University Cancer Center. Among them, 100 patients meeting the following criteria were finally enrolled in our research. Inclusion criteria: (1) pathological diagnosis of HCC after liver resection; (2) receiving liver resection as the initial treatment for HCC; (3) positive serum HBsAg, as performed by the Elecsys® (Roche Diagnostics, Shanghai, China) system; (4) no other type of tumor combined; (5) available liver tissue samples for IHC test and complete electronic medical record and follow-up data. A total of 111 patients were excluded because seven patients were not pathological diagnosis of HCC, 42 cases had received other treatment before liver resection, serum HBsAg negative in 51 patients, eight patients combined with other type of tumors, three patients’ records and follow-up data were incompletely.

Material preparation

We collected the adjacent liver specimens of the 100 eligible HCC patients, which were embedded as paraffin samples. First, we performed sectioning and HE staining to identify normal liver tissues. Then, the liver tissues were sampled in an area approximately 2 cm from the tumor, and tissue microarrays were made. Finally, the following were used to prepare the reagents for IHC: Ms mAb to HBV surface antigen (prediluted, ab859; Abcam, Shanghai, China), ethanol (100%, 95%, 85%, and 70% solutions were diluted in distilled water), 3% hydrogen peroxide (20 ml 30% hydrogen peroxide mixed with 180 ml distilled water), PBS buffer, EDTA pH 8.0 diluted in distilled water to 1 mM, BSA (Sigma-Aldrich, Shanghai, China), the DaKo REAL EnVision Detection System, deionized water, xylene, and hematoxylin.

Immunohistochemistry

After deparaffinization, rehydration and reduction of endogenous peroxidase activity, antigen retrieval of tissue microarrays was performed via heating in a microwave oven at 100°C for 5 min and in a slide container at 50–60°C. Tissue microarrays were incubated with freshly prepared HRP rabbit/mouse reagent (DaKo REAL EnVisionTM Detection System) for 30 min at 37°C. Immunohistochemical staining was not performed with DAB (DaKo REAL EnVision Detection System) until the desired stain intensity was observed. For counterstaining, tissue microarrays were stained in hematoxylin for 15 s. Tissue microarrays were rinsed with tap water for at least 5 min. Finally, tissue microarrays were air-dried and sealed.

Immunohistochemical staining of hepatitis B surface antigen in liver tissue microarrays and observations

Since these cases were serum HBsAg positive, IHC for HBsAg was performed in liver tissues after liver resection in 100 HCC cases. Under the guidance of two skilled pathologists, in the calculation of an histochemistry score [12], the liver tissues were observed in tissue microarrays and judged as positive or negatives. In the positive samples, small or large amounts of brown staining areas with different degrees of staining could be observed, while the negative samples had no staining. Seventy-one cases were characterized into the IHC (HBsAg)-negative group (Fig. 1a), while 29 cases were characterized into the positive group (Fig. 1b–c). The positive group included weakly positive (+), positive (++), and strongly positive (+++) reactions, which were classified by the summation of the percentage of area stained at each intensity level multiplied by the weighted intensity [12]. For example, 0 is negative (−), one is weakly positive (+), two is positive (++), three is strongly positive (+++).

The samples were matched with the patient after recording the results, and patients were divided into an IHC (HBsAg)-negative group and an IHC (HBsAg)-positive group.

Data collection

Through the electronic medical record system of Sun Yat-sen University Cancer Center, we recorded relevant information, including basic information, laboratory examinations before HCC resection, surgical records, pathological diagnosis and follow-up data. The follow-up information was collected through regular reexamination and telephone contact.

Statistical analysis

Statistical analysis was performed with SPSS 24.0 software (SPSS, Chicago, Illinois, USA) and R 3.4.3 (https://www.r-project.org/). Student’s t-test was applied to compare continuous variables when the data were normally distributed. The Mann–Whitney U test was used to compare skewed data. The chi-squared or Fisher’s exact test was used for categorical variables. Spearman’s bivariate correlation test was applied to test the correlation between the clinical and laboratory parameters. For some indicators, we converted continuous variables into categorical variables for analysis. OS was calculated via the Kaplan–Meier method, and the curves were compared via the log-rank test. Univariate and multivariate Cox proportional hazards models were applied to evaluate risk factors for prognosis after HCC resection. To confirm the results of the two groups, propensity score matching (PSM) at a 1:2 ratio with the nearest available match was established by the ‘MatchIt’ package of R. The following factors were selected for PSM: tumor size, tumor number, microvascular invasion (MVI) and macrovascular invasion. The P-value of the result was less than 0.05 (P < 0.05) and was considered statistically significant.
Result

Baseline characteristics of patients

In this study, 100 patients were enrolled, including 90 males (90%) and 10 females (10%). The median ages were 50.5 years. The maximum tumor size at resection was 64.0 ± 41.5 mm. There were 74 patients (74%) who had a single HCC tumor and 26 patients (26%) who had multiple tumors. Macro vascular invasion presented in nine patients (9%).

These 100 patients were divided into two groups based on the results of IHC for HBsAg in liver tissues: 71 patients were in the IHC (HBsAg)-negative group, and 29 patients in the IHC (HBsAg)-positive group. Table 1 shows the baseline characteristics of patients before PSM. There was no significant difference in sex, age, number or maximum diameter of tumors, or MVI, but there was a significant difference in HBV DNA levels (</=10 000 IU/ml) (P = 0.006).

After PSM, there were 73 cases in total, including 44 in the IHC (HBsAg)‐negative group and 29 in the IHC (HBsAg)‐positive group. The baseline characteristics of patients after PSM are shown in Table 2. Similar to Table 1, the index of HBV DNA (</=10 000 IU/ml) (P = 0.031) was significantly different between the groups.

Table 1. Baseline characteristics of patients (pre-PSM)

| Characteristics                              | Negative group | Positive group | P value |
|----------------------------------------------|----------------|----------------|---------|
| No. of cases                                 | 71             | 29             |         |
| Sex (male/female)                            | 65/6           | 25/4           | 0.419   |
| Age (years, <65/≥65)                         | 64/7           | 28/1           | 0.284   |
| Child-Pugh class (A/B/C)                     | 67/4/0         | 28/1/0         | 0.649   |
| MELD score (range)                           | 4.48 (2.59 to 11.07) | 3.16 (4.63 to 9.33) | 0.453   |
| Number of tumors (single/multiple)           | 54/17          | 20/9           | 0.463   |
| Pathological differentiation (well/moderately/poorly) | 1/32/38        | 0/8/21         | 0.199   |
| Maximum tumor diameter (<3/3–5/>5 cm)        | 18/26/29       | 2/8/19         | 0.052   |
| Tumor capsule (no/incomplete/complete)       | 26/19/26       | 7/10/12        | 0.468   |
| MaVI (Y/N)                                   | 7/64           | 2/27           | 0.639   |
| MVI (Y/N)                                   | 31/40          | 18/11          | 0.095   |
| Cirrhosis (Y/N)                              | 54/17          | 22/7           | 0.984   |
| Ascites (Y/N)                                | 17/54          | 5/24           | 0.463   |
| White blood cells (x10^9/L)                  | 6.25 (2.9–12.8) | 6.50 (3.26–11.55) | 0.566   |
| Platelet count (x10^9/L)                     | 184.6 (80–401) | 197.8 (53.7–373.9) | 0.402   |
| Hemoglobin (g/L)                             | 115 (100–180)  | 149 (122–174)  | 0.144   |
| Serum ALT (U/L)                              | 44.6 (13.2–274.0) | 54.4 (18.7–327.2) | 0.340   |
| Serum AST (U/L)                              | 45.0 (17.2–231.9) | 48.7 (16.5–185.8) | 0.619   |
| Total bilirubin (μmol/L)                     | 14.8 (4.8–61.9) | 12.2 (6.1–20.2) | 0.097   |
| Albumin (g/L)                                | 43.0 (29.5–55.7) | 43.9 (32.4–51.0) | 0.272   |
| Prothrombin time (s)                         | 11.57 (9.9–14.8) | 11.46 (9.7–15.5) | 0.582   |
| Antiviral therapy before (Y/N)               | 18/53          | 8/21           | 0.817   |
| HBV DNA (</≥10 000 IU/ml)                    | 36/35          | 6/23           | 0.006<sup>a</sup> |
| AFP (ng/mL, </≥400)                           | 43/28          | 22/7           | 0.146   |

AFP, alpha-fetoprotein; HBV, hepatitis B virus; MaVI, macro vascular invasion; MVI, microvascular invasion.<sup>a</sup>Statistically significant.

Fig. 1. Representative pictures of immunohistochemistry staining for HBsAg in liver tissues. (a) IHC (HBsAg) negative (−); (b) IHC (HBsAg) weakly positive (+); (c) IHC (HBsAg) positive (++); (d) IHC (HBsAg) strongly positive (+++). IHC, immunohistochemistry.
Overall survival and recurrence-free survival with hepatocellular carcinoma resection

The median follow-up duration in the negative and positive groups was 39.1 and 38.9 months, respectively. The cumulative OS rates at 1 and 2 years were 90.1% and 85.7%, respectively, in the negative group, and 79.3% and 65.5% in the positive group before PSM. After PSM, the cumulative OS rates at 1 and 2 years were 93.2% and 85.9%, respectively, in the negative group and 79.3% and 65.5%, respectively, in the positive group. The OS with HCC resection is shown in Fig. 2, including pre-PSM (P = 0.013) and post-PSM (P = 0.039).

The recurrence-free survival (RFS) with HCC resection is shown in Fig. 3. There was no significant difference between the two groups (pre-PSM, P = 0.89; post-PSM, P = 0.57).

A predictor of overall survival after liver resection for hepatocellular carcinoma

Before PSM, there were several risk factors affecting OS after HCC resection, including IHC (HBsAg, −/+). The multivariate analysis showed that the risk factors for OS after HCC resection were IHC (HBsAg, −/+), MVI (HR = 3.837, P = 0.001), MVI (HR = 3.837, P = 0.002), ascites (HR = 2.841, P = 0.021), C-reactive protein (HR = 2.841, P = 0.021), and maximum tumor diameter, MVI and C-reactive protein did not qualify.

After PSM, we found that the risk factors for OS in univariate analysis were IHC (HBsAg, −/+), MVI (HR = 3.837, P = 0.001), MVI (HR = 3.837, P = 0.002), ascites (HR = 2.841, P = 0.021), C-reactive protein (HR = 2.841, P = 0.021), and maximum tumor diameter, MVI and C-reactive protein did not qualify.

In multivariate analysis, the risk factors for OS after HCC resection were IHC (HBsAg, −/+), MVI (HR = 3.837, P = 0.001), MVI (HR = 3.837, P = 0.002), ascites (HR = 2.841, P = 0.021), and AFP (HR = 4.777, P = 0.001), the same as pre-PSM.

Given the results of the above analysis, we suggest that the IHC results of HBsAg in liver tissues could be used as a predictor of OS after liver resection for HCC patients in addition to AFP and ascites.

Discussion

In this study, we investigated whether the expression of HBsAg in liver tissues could serve as a predictor of prognosis for patients with HCC. Our study indicated that the results of IHC staining for HBsAg in liver tissues were associated with OS after liver resection in HCC patients.

It is clinically meaningful to predict OS after liver resection in HCC patients as soon as possible, thus, IHC results were chosen as a predictor. Because of tumor heterogeneity in HCC, IHC staining was performed in liver tissues with an Ms mAb to HBV surface antigen (prediluted, ab859; Abcam). According to the different degrees of staining, samples were divided into negative, weakly positive, positive, and strongly positive ratings.

In previous studies, HBV infection was the key determinant or risk factor for HCC. Quantification of HBsAg could be used as a marker to manage or monitor patients with chronic hepatitis B [15,16]. Some studies have shown that serum hepatitis B surface antigen (HBsAg) levels can predict disease progression and serve as a risk factor for HCC in patients with low HBV loads [17,18]. In other previous reports, a high HBV DNA load was associated with the recurrence of HBV-related HCC [19,20]. The baseline HBV DNA level (<2×10⁴ IU/ml in our two groups was significantly different (pre-PSM, P = 0.006; post-PSM, P

### Table 2. Baseline characteristics of patients (post-PSM)

| Characteristics                        | Negative group | Positive group | P value |
|----------------------------------------|----------------|----------------|---------|
| No. of cases                           | 44             | 29             |         |
| Sex (male/female)                      | 41/3           | 25/4           | 0.322   |
| Age (years, <65/65)                    | 42/2           | 28/1           | 0.817   |
| Child-Pugh class (A/B/C)               | 41/3/0         | 28/1/0         | 0.536   |
| MELD score (range)                     | 4.38 (2.59–9.70) | 3.16 (4.63 to 9.33) | 0.061   |
| Number of tumors (single/multiple)     | 30/14          | 20/9           | 0.944   |
| Pathological differentiation (well/moderately/poorly) | 1/20/33     | 5/8/19         | 0.777   |
| Maximum tumor diameter (<2–5 cm)       | 5/13/6         | 2/8/19         |         |
| Tumor capsule (no/incomplete/complete) | 15/16/33       | 7/10/12        | 0.520   |
| MaVI (Y/N)                             | 4/40           | 2/27           | 0.738   |
| MVI (Y/N)                              | 27/17          | 18/11          | 0.952   |
| Ascites (Y/N)                          | 11/33          | 5/24           | 0.433   |
| Cirrhosis (Y/N)                        | 33/11          | 22/7           | 0.933   |
| White blood cells (<10⁹/L)             | 6.35 (2.9–12.8) | 6.50 (5.26–11.55) | 0.757   |
| Platelet count (<10⁹/L)                | 195.7 (80–401) | 197.8 (53.7–733.9) | 0.906   |
| Hemoglobin (g/L)                       | 143 (110–180)  | 149 (122–174)  | 0.085   |
| Serum ALT (U/L)                        | 39.2 (13.2–104.0) | 54.4 (18.7–327.2) | 0.187   |
| Serum AST (U/L)                        | 42.5 (17.2–139.1) | 48.7 (18.5–185.8) | 0.360   |
| Total bilirubin (μmol/L)               | 14.2 (4.6–36.6) | 12.2 (6.1–20.2) | 0.119   |
| Albumin (g/L)                          | 42.3 (29.5–48.2) | 43.9 (32.4–51.0) | 0.074   |
| Prothrombin time (s)                   | 11.6 (10.1–14.8) | 11.46 (9.7–15.5) | 0.655   |
| Antiviral therapy before(Y/N)          | 5/39           | 6/21           | 0.076   |
| HBV DNA (<2×10⁴ IU/ml)                 | 20/24          | 8/23           | 0.031*  |
| AFP (ng/ml; ≤400/>400)                 | 27/17          | 22/7           | 0.197   |

AFP, alpha-fetoprotein; HBV, hepatitis B virus; MaVI, macro vascular invasion; MVI, microvascular invasion.

*Statistically significant.
= 0.031), while the HBV DNA level was not a risk factor for OS (pre-PSM, \( P = 0.186 \); post-PSM, \( P = 0.320 \)). The detection of HBsAg in liver tissues might improve the OS prediction of surgical patients with HBV-related HCC. To the best of our knowledge, this is the first study to use IHC for HBsAg in liver tissues as a prognostic factor after liver resection for patients with HCC. The patients in our study were divided into negative (pre-PSM, 71 cases; post-PSM, 44 cases) and positive groups (pre-PSM or post-PSM, both 29 cases) based on IHC results, and there were statistically significant differences in OS between the groups (pre-PSM, \( P = 0.013 \); post-PSM, \( P = 0.039 \)). There was no significant difference in RFS (pre-PSM, \( P = 0.89 \); post-PSM, \( P = 0.57 \)). HBsAg results could provide another method for predicting the prognosis of patients who have undergone liver resection for HCC.

Few studies have reported whether HBsAg will continue to exist in normal liver tissues in HBV-related patients or whether HCC is more likely to progress in HBV-related patients. In our current study, we found that the OS was worse in HBV-related patients who had positive HBsAg IHC staining. This phenomenon is worthy of further study.

![Fig. 2. OS of the IHC (HBsAg)-negative group and the IHC (HBsAg)-positive group. (a) OS after HCC surgical resection between the IHC (HBsAg)-negative group and the IHC (HBsAg)-positive group before PSM; (b) OS after HCC surgical resection between the IHC (HBsAg)-negative group and the IHC (HBsAg)-positive group after PSM. HCC, hepatocellular carcinoma; IHC, immunohistochemistry; OS, overall survival; PSM, propensity score matching.](image)

![Fig. 3. RFS of the IHC (HBsAg)-negative group and the IHC (HBsAg)-positive group. (a) RFS after HCC surgical resection between the IHC (HBsAg)-negative group and the IHC (HBsAg)-positive group before PSM; (b) RFS after HCC surgical resection between the IHC (HBsAg)-negative group and the IHC (HBsAg)-positive group after PSM. HCC, hepatocellular carcinoma; IHC, immunohistochemistry; PSM, propensity score matching; RFS, recurrence-free survival.](image)

### Table 3. Univariate and multivariate analysis of variables affecting overall survival (pre-PSM)

| Variable | Univariate | Multivariate |
|----------|------------|--------------|
|          | \( P \) value | HR   | 95% CI | \( P \) value | HR   | 95% CI |
| Sex      | 0.801      | 0.830 | 0.195–3.533 | 0.194 | 1.837 | 0.734–4.597 |
| Maximum tumor diameter (<3/3–5/>5 cm) | 0.002* | 3.670 | 1.630–8.264 | 0.383 | 1.756 | 0.522–5.912 |
| Number of tumors (single/multiple) | 0.052 | 2.236 | 0.992–5.043 | 0.021* | 2.841 | 1.169–6.905 |
| MVI      | 0.004* | 3.837 | 1.521–9.680 | 0.111 | 1.861 | 0.542–6.018 |
| Cirrhosis | 0.058 | 4.073 | 0.956–17.356 | 0.023* | 2.841 | 1.169–6.905 |
| IHC (HBsAg, −/+ | 0.017* | 2.574 | 1.194–5.922 | 0.021* | 2.841 | 1.169–6.905 |
| C-reactive protein | 0.002* | 4.125 | 1.707–9.968 | 0.023* | 2.841 | 1.169–6.905 |
| HBV DNA level, 10 000 IU/ml | 0.186 | 1.812 | 0.752–4.371 | 0.264 | 1.172 | 0.642–2.162 |
| Ascites | 0.028 | 2.524 | 1.102–5.778 | 0.009* | 3.346 | 1.360–8.233 |
| AFP (ng/ml, ≤400/>400) | 0.009* | 2.967 | 1.317–6.868 | 0.006* | 3.331 | 1.199–7.525 |

AFP, alpha-fetoprotein; CI, confidence interval; HBV, hepatitis B virus; HR, hazard ratio; MVI, microvascular invasion.

*Statistically significant.
Table 4. Univariate and multivariate analysis of variables affecting overall survival (post-PSM)

| Variable                    | Univariate |               | Multivariate |
|-----------------------------|------------|---------------|--------------|
|                             | P value    | HR            | 95% CI       | P value    | HR            | 95% CI       |
| Sex                         | 0.840      | 1.162         | 0.269–5.015  |            |               |              |
| Maximum tumor diameter (<3/3–5/>5 cm) | 0.064      | 2.326         | 0.953–5.676  |            |               |              |
| Number of tumors (single/multiple) | 0.135      | 1.963         | 0.811–4.750  |            |               |              |
| MVI                         | 0.133      | 2.174         | 0.789–5.989  |            |               |              |
| Cirrhosis                   | 0.046a     | 7.771         | 1.038–58.161 | 0.056      | 7.340         | 0.951–56.646 |
| IHC (HBsAg) (−/+), C-reactive protein | 0.031a     | 2.863         | 1.098–7.463  | 0.018a     | 3.579         | 1.240–10.333 |
| HBV DNA level, 10 000 IU/ml  | 0.320      | 1.671         | 0.607–4.600  | 0.466      | 1.482         | 0.514–4.270  |
| Ascites                     | 0.012a     | 3.183         | 1.295–7.822  | 0.004a     | 4.287         | 1.589–11.565 |
| AFP (ng/ml, ≤400/>400)      | 0.021a     | 2.817         | 1.166–6.807  | 0.001a     | 4.777         | 1.907–11.986 |

AFP, alpha-fetoprotein; CI, confidence interval; HBV, hepatitis B virus; HR, hazard ratio; MVI, microvascular invasion.

*Statistically significant.

Serum AFP is the most widely used method for screening and surveillance in HCC [21,22]. In this study, these patients were diagnosed with HCC; however, the baseline AFP levels (ng/ml, ≤400/>400) of the two groups were not significantly different (pre-PSM, P = 0.146; post-PSM, P = 0.197). In univariate analysis, AFP (pre-PSM, P = 0.009; post-PSM, P = 0.021) and IHC (HBsAg, −/+), C-reactive protein (pre-PSM, P = 0.017; post-PSM, P = 0.046) were variables that could affect OS. After multivariate analysis with other variables (pre-PSM with maximum tumor diameter, MVI, C-reactive protein and ascites; post-PSM with cirrhosis, C-reactive protein and ascites), we found that IHC (HBsAg, −/+), C-reactive protein and ascites, and post-PSM with cirrhosis was an independent risk factor for OS after HCC resection in addition to AFP (pre-PSM, P = 0.018; post-PSM, P = 0.001). The IHC results for HBsAg in liver tissues provides another effective factor for prognostication of HCC patients after liver resection, and it can be studied at an earlier time than current variables involved in predicting prognosis.

There were some limitations in this study. This was a retrospective analysis and was limited to cases in which only existing para-cancerous tissue samples could be collected. This predictor cannot be used in patients who have not undergone liver resection for HCC. As the study used the immunohistochemical results of HBsAg staining, non-HBV-related HCC patients were excluded. The small sample size was a limitation in this study, especially lacking in multicenter samples.

Interestingly, in the HBV-related HCC patients, the immunohistochemical results of HBsAg were positive in the para-cancerous tissues of some of the patients, and how HBV causes HCC has not been clear thus far. This phenomenon deserves further in-depth research.

In conclusion, for HBV-related HCC patients, immunohistochemical results for HBsAg staining in liver tissues can serve as a predictor of prognosis after liver resection for patients with HCC. Patients with positive results have worse OS, and patients with negative results have better OS. Clinically, IHC staining for HBsAg can be performed on liver tissues from HBV-related HCC patients after liver resection as a new method of predicting OS.

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Conflicts of interest

There are no conflicts of interest.

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