Clinical analysis of the risk factors for recurrence of HCC and its relationship with HBV

Di-Peng Ou, Lian-Yue Yang, Geng-Wen Huang, Yi-Ming Tao, Xiang Ding, Zhi-Gang Chang

Abstract

AIM: To comprehend the risk factors of recurrence of hepatocellular carcinoma (HCC) and its relationship with the infection patterns of hepatitis B virus (HBV).

METHODS: All materials of 270 cases of postoperative HCC were statistically analyzed by SPSS software. Recurrence and metastasis were classified into early (<2 years) and late phase (>2 years). Risk factors for recurrence and metastasis after surgery in each group were analyzed.

RESULTS: Out of 270 cases of HCC, 162 cases were followed up in which recurrence and metastasis occurred in 136 cases. There were a lot of risk factors related to recurrence and metastasis of HCC; risk factors contributing to early phase recurrence were serum AFP level, vascular invasion, incisal margin and operative transfusion, gross tumor classification and number of intrahepatic node to late phase recurrence. The HBV infective rate of recurrent HCC was 94.1%, in which "HbsAg, HBeAb, HbcAb" positive pattern reached 45.6%. The proportion of HBV infection in solitary large hepatocellular carcinoma (SLHCC) evidently decreased compared to nodular hepatocellular carcinoma (NHCC) (P<0.05).

CONCLUSION: The early and late recurrence and metastasis after hepatectomy of HCC were associated with different risk factors. The early recurrence may be mediated by vascular invasion and remnant lesion, the late recurrence by tumor’s clinical pathology propert, as multicentric carcinogenesis or intrahepatic carcinoma de novo. HBV replication takes a great role in this process. From this study, we found that SLHCC has more satisfactory neoplasm biological behavior than NHCC.

Key words: Hepatocellular carcinoma; Recurrence and metastasis; Risk factor; Hepatitis B virus

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INTRODUCTION

Because of the advances in surgical techniques and perioperative management, hepatectomy of hepatocellular carcinoma (HCC) has become a safe operation with low operative mortality. However, the long-term prognosis remains poor due to a high incidence of recurrence and metastasis of HCC, ranging from 50% to 80%[4,5]. The effect of HCC’s therapy is also unpleasant; the high rate of recurrence and metastasis after HCC resection has always disturbed clinical surgeon nowadays. There are lots of reports based on the fact that intrahepatic recurrence or multicentric carcinogenesis de novo were found after resection of HCC[5,6]. Because different risk factors for recurrence and metastasis have been identified in previous articles[5], it is a controversy regarding the way of recurrence and metastasis, which need us continue to disclose its mechanism.

Hepatitis B virus (HBV) is a hepatavirus, discussed worldwide as resulting in viral hepatitis in humans. HBV gets distributed at various ages, the infectious rate of HBV has reached 10% in China. Once it infects the human body, the HBV gets replicated and proliferated into hepatocytes, induces hepatocyte lesion and develops into chronic hepatitis. It has been reported that HBV is the prime cause for the development of HCC. In order to discuss the relationship between HBV and the recurrence and metastasis of HCC, we studied the clinical data of postoperative HCC and its follow-up.

MATERIALS AND METHODS

Population

The base population consisted of 270 consecutive patients who had undergone initial and curative hepatic resection for HCCs at Central South University Xiangya Hospital from 1992 to 2002. The indication of surgical resection and operative procedure was determined according to the decision criteria based on the liver function test including ascites, serum bilirubin level, and image data. Systematic resection of Couinaud’s segment was a preferred operative
tumor invasion was observed at the surgical cut surface. Resection with 1 cm was defined as curative so long as no cancer-related factors were censored at the time of death. All patients who died for reasons not related to HCC were censored at the time of death. All patients (n = 270) Recurrent patients (n = 136)

| Variable factors                        | All patients (n = 270) | Recurrent patients (n = 136) | Early phase (n = 107) | Late phase (n = 29) |
|----------------------------------------|------------------------|-------------------------------|-----------------------|--------------------|
| Age (yr)                               | 46.9±13.0              | 4.0±11.5                      | 44.2±11.1             | 47.7±12.9          |
| Sex (male/female)                      | 247/23                 | 126/10                        | 99/8                  | 27/2               |
| Gross tumor classification             | 66/168/36              | 35/77/24                      | 23/63/21              | 12/14/3            |
| Edmondson classification               | 128/142                | 65/71                         | 46/61                 | 19/10              |
| Tumor capsule (present/absent)         | 55/215                 | 23/113                        | 13/94                 | 10/19              |
| Vascular invasion (present/absent)     | 145/125                | 79/57                         | 62/45                 | 7/12               |
| Alpha-fetoprotein (positive/negative)  | 141/129                | 85/51                         | 75/32                 | 10/19              |
| Hepatocirrhosis (positive/negative)    | 200/70                 | 105/31                        | 82/25                 | 23/6               |
| Type of liver resection                | 149/121                | 78/58                         | 59/48                 | 19/10              |
| Incisal margin (≤1 cm/>1 cm)           | 22/258                 | 15/131                        | 14/93                 | 1/28               |
| HBV infection (yes/no)                 | 239/31                 | 128/8                         | 102/5                 | 26/3               |
| Blood transfusion (positive/negative)  | 199/71                 | 100/36                        | 75/32                 | 25/4               |
| Survival time (d)                      | –                      | 575.9±899.6                   | 384.7±313.1           | 1 248.5±450.9      |
| Recurrent time (d)                     | –                      | 435.6±417.9                   | 23.1±199.6            | 1 072.2±396.9      |

Note: Small HCC (SHCC), solitary large HCC (SLHCC), nodular HCC (NHCC).

Follow-up and end point
After discharge, monthly follow-up by tumor marker (alpha-fetoprotein, AFP) and ultrasound (US) as well as helical computed tomographic (CT) scan for every 4 mo were conducted for 1 year. Then, we screened patients by tumor marker measurement and US for every 2 mo and helical CT for every 6 mo thereafter, and hepatic angiography when recurrence was suspected. Recurrence was diagnosed based on the combined findings of these clinical examinations. The end point of this study was time-to-recurrence, which was defined as the period between surgery and the diagnosis of recurrence and metastasis. Patients who died for reasons not related to HCC were censored at the time of death. All follow-up data were summarized by the end of April 2004.

Variables analyzed
Discrete variables were computed directly, whereas continuous variables were classified into binary or polytomous categorical data. Cut-off points for AFP were 20 μg/L, and maximal tumor diameter were determined according to operative records, which gave the best discrimination between time-to-recurrence curves among several stratifications.

The studied variables could be classified as host-, cancer-, or surgery-related (Table 1). The host-related factors were age, sex, viral markers (HBsAg-, HBeAg-, HBsAb-, HBeAb-, HBcAb-). HBV infection pattern was graded according to the difference of viral markers positive expression. Fibrosis was assessed using non-cancerous parts of the resected specimens. The stage of liver fibrosis was classified into four categories (no, mild, moderate, and cirrhosis) to represent the severity of liver disease. Cancer-related factors were gross classification of the tumor, tumor multiplicity, maximum diameter of tumor, microscopically assessed vascular invasion, intrahepatic metastasis, microscopically assessed presence and/or invasion of a tumor capsule, tumor cell...
differentiation, serum AFP level. Multiple tumor nodules and intrahepatic metastasis were differentiated based on macro- and microscopical findings. In brief, multiple tumors were classified into intrahepatic metastases if they were (a) tumors apparently growing from portal venous tumor thrombi, or (b) multiple satellite nodules surrounding a main tumor with similar or poorer degree of cell differentiation. Surgery-related factors included type of resection (anatomical vs non-anatomical) and surgical free margin (>1 cm vs <1 cm).

**Statistical analysis**
The overall hazard function for recurrence and metastasis was also estimated to visually depict chronological change in the recurrence rate after hepatectomy. We investigated factors contributing to early and late phase recurrence, separately, setting 2 years as the cut-off between the early and late phases[7]. For this purpose, we first conducted a multiple regression analysis by setting the different baseline hazard functions for respective institutions and by censoring patients without recurrence at 2 years after surgery at this time point. Then, we performed a landmark analysis on recurrence-free status at 2 years after surgery. Finally, the relationship between the viral infection patterns was investigated using the Mantel-Haenszel trend test on data from patients showing only one of two hepatitis markers. As additional sets of analyses, we performed the same analyses exclusively in patients with solitary tumor. Likewise, we also performed analyses dividing patients with and without liver cirrhosis. All statistical analyses in the present study were performed using the Statistical Package for Social Sciences (SPSS11.0 software).

**RESULTS**

**Analysis of the recurrence and metastasis of HCC**
Among the 162 cases of follow-up, the median follow-up time was 1 742 d (range 7-4 200 d). Recurrence and metastasis were observed in 136 patients. There was a patient who survived more than 11 years. Overall cumulative recurrence and metastasis rate curves for all patients are shown in Figure 1, and Figure 2 depicts the overall recurrence and metastasis at each year after operation, which peaked at the first year postoperatively, then gradually decreased year by year. The overall cumulative recurrence and metastasis rate were 32.7% (200 d), 59.2% (400 d), 64.5% (600 d), 69.8% (800 d), 73.5% (1 000 d), 80.3% (1 200 d), 84.1% (1 400 d); there were 46.3% (1st year), 36.8% (2nd year), 32.7% (3rd year), 24.3% (4th year) and 12.4% (5th year) respectively from 1st to 5th year.

**Factors contributing to early (≤2 years) phase recurrence and metastasis**
Recurrence and metastasis before 2 years was observed in 107 patients. Multiple regression analysis identified four variables as contributing to early phase recurrence and metastasis: Serum AFP (alpha-fetoprotein) level, vascular invasion, incisal margin and operative transfusion (Table 2). As a result, the causes of recurrence and metastasis for early phase were the factors correlated to surgery.

**Factors contributing to late (>2 years) phase recurrence and metastasis**
In the same way, recurrence and metastasis was observed in 29 patients. Factors related to late phase recurrence and metastasis were gross tumor classification and number of intrahepatic node (Table 2). NHCC had higher recurrence and metastasis rate than SLHCC (P = 0.026). It was coincident to the results of our prophase study on the SLHCC characterization at basic and clinical[8].

**Relationship on the HBV infection with recurrence and metastasis of HCC**
It has been confirmed by lots of epidemiological survey that HCC genesis is closely related to the HBV infection. Our further study disclosed that the HBV infection has related to the recurrence and metastasis of HCC. In this paper, the infective rate was 88.5% on 270 cases of HCC, but 94.1% on 136 cases of recurrence and metastasis of HCC. Among the 162 cases of follow-up, there were 128 recurrences with HBV infection (147 cases) and only 8 recurrences without HBV infection (15 cases). Statistic comparison had high significant difference (χ2 = 11.501, P = 0.001); it prompted that the HBV infection was an important cause of recurrence and metastasis of HCC.

The HBV infective pattern was different in various clinical classifications of HCC. Contrast analysis of the HBV infective pattern among 270 HCC patients that accepted operation with 136 HCC patients showed that recurrence and metastasis occurred; we found that HBV infection not only related to
Table 2: Multiple regression analysis of recurrence and metastasis of 136 HCC patients

| Variable                  | Results on early phase | Results on late phase |
|---------------------------|------------------------|-----------------------|
|                          | Regression coefficient | t         | P     | Regression coefficient | t         | P     |
| AFP level                 | -0.328                 | -3.693               | 0.000 | -0.280                  | -1.306                | 0.208 |
| Hepatocirrhosis          | 0.024                  | 0.274                | 0.785 | 0.077                   | 1.530                | 0.143 |
| Type of liver resection  | 0.023                  | 0.260                | 0.795 | 0.245                   | 1.235                | 0.233 |
| Incisal margin            | 0.217                  | 2.485                | 0.015 | 0.106                   | 0.420                | 0.679 |
| Blood transfusion         | -0.223                 | 2.553                | 0.012 | 0.268                   | 1.266                | 0.222 |
| Gross tumor classification| -0.051                 | -0.583               | 0.561 | -0.518                  | -2.433               | 0.026 |
| Vascular invasion         | 0.266                  | 3.013                | 0.003 | -0.361                  | -1.156               | 0.281 |
| Tumor capsule             | 0.023                  | 0.259                | 0.797 | -0.281                  | -1.111               | 0.281 |
| Edmondson classification  | 0.126                  | 1.444                | 0.152 | 0.065                   | 0.292                | 0.774 |

Table 3: Statistics of the HBV infective pattern

| Serum markers          | All patients (n = 270) | Recurrent patients (n = 136) |
|------------------------|------------------------|-----------------------------|
|                        | Positive number | Ratio (%) | Positive number | Ratio (%) |
| HBsAg+HBeAg+HbcAb+     | 20          | 7.4      | 14              | 10.3      |
| HBsAg+HBeAb+HbcAb+     | 118         | 43.7     | 62              | 45.6      |
| HBsAg+HbcAb+           | 57          | 21.1     | 32              | 23.5      |
| HbcAb+                 | 9           | 3.3      | 6               | 4.4       |
| HbcAb                  | 2           | 0.7      | 2               | 1.5       |
| HbcAb+HbcAb+           | 7           | 2.6      | 1               | 0.7       |
| HbsAg+                 | 16          | 5.9      | 9               | 6.6       |
| HbcAg+HbcAb+           | 2           | 0.7      | 0               | 0.0       |
| HbsAg+HbcAb+           | 6           | 2.2      | 1               | 0.7       |
| HBsAg+HbcAb+           | 2           | 0.7      | 1               | 0.7       |
| HBV negative           | 31          | 11.5     | 8               | 5.9       |

Note: Dependent variable was the time of recurrence and metastasis (d).

DISCUSSION

Recurrence and metastasis of HCC could divide into intrahepatic recurrence and extrahepatic metastasis. The HCC genesis and recurrence, but also that their infective pattern varied (t = 2.407, P = 0.057, Table 3). Further comparison of the infective pattern of recurrent SLHCC and recurrent NHCC, results showed the HBV infection pattern of recurrent NHCC, which was obviously different compared with SLHCC, and the proportion of recurrent NHCC with HBV infection was obviously step-up than recurrent SLHCC (P<0.05, Table 4).
increased in whom accepted transfusion compared to non-
transfusion \((P = 0.012)\), indicated operative transfusion is
auxo-action for recurrence and metastasis at the early phase
of postoperation. The mechanism maybe: Thrombosis is
the advantage of cancer cell implantation, growth factors
are released to reinforce cancer cell growth directly or indirectly,
inhibit the NK cell’s activity and result in the function of
NK immunocytes decreased, to accelerate the growth of
remnant cancer cells. Therefore surgeon should weigh the
merits and demerits while performing HCC hepatectomy,
adopt acrobatic hepatectomy technique, reduced operative
hepatic bleeding and blood transfusion as far as possible, to
adopt self-transfusion when necessary. Preoperative vascular
invasion or carcinoma thrombosis are fundamental cause
for postoperative recurrence and metastasis, leading to
decrease of postoperative survival rate. Above all, the micro
vascular invasion under microscopy could not be judged,
which directly lead to recurrence and metastasis at early phase
after hepatectomy\(^9\).

The clinical classification of HCC was the independence
related risk factor during the late phase of postoperation.
On the basement of previous abundant basic and clinical
research, we divided HCC into small HCC (SHCC), solitary
large HCC (SLHCC) and nodular HCC (NHCC) according
to its clinical pathology property. Analyzed 29 cases of
recurrent data at late phase in contrast with 107 cases at
early phase, we found the proportion of SLHCC decreased
obviously than NHCC \((58.9\% vs 48.3\%)\), there was a
significant difference \((\chi^2 = 3.929, P = 0.047)\). It indicated
SLHCC has more satisfactory neoplasm biological behavior
than NHCC, which coincided with the results of our
prophase research\(^9\).

Chronic HBV infection is the main cause of HCC genesis
in China. We statistically analyzed the materials of in-patients
accepted hepatectomy at our hospital, the HBV infective
rate reached \(90\\%\). The closed relation between HBV
infection and HCC could manifest in the concordance on
their epidemiologic character, such as local distribution, sex,
age and family history\(^9\). The main HBV infective pattern
of 270 patients was positive “HBsAg, HBeAb, HBcAb”
 viral markers. Positive HBsAg shows those patients existing
with HBV infection, positive HBeAb and HBcAb shows
HBV replicated in hepatocytes at lower level. If HBeAb
presented long-term positive, the HBV-DNA has been
integrated with host hepatocyte chromosome DNA, kept
latentiation ever since\(^20-21\). Our data on 136 cases of recurrence
and metastasis shows that the pattern of positive HBeAb
was distinctly enhanced. We could explain that the HCC
patients with HBV infection have lower immunological
function, with decreased resistant ability, HBV promoted
recurrence and metastasis by various kinds of pathway as
activating HBx function, integrating host cell DNA,
destroying apoptosis of normal hepatocyte, inactivating P53
gene, facilitating extracellular matrix degradation\(^22-24\).

Among the recurrent HCC, the HBV infective pattern
of NHCC was evidently different from that of SLHCC,
furthermore, the proportion of NHCC with HBV infection
was evidently increased \((P<0.05)\). It is interpreted that NHCC
more easily combines with HBV reproduction than SLHCC
in the course of recurrence and metastasis. So the prognosis
that NHCC is worse than SLHCC could be elucidated from
another point of view.

In conclusion, this study provides an epidemiological
evidence that intrahepatic recurrence and metastasis of HCC
after hepatic resection has two obvious etiologies. The early
recurrence may be mediated by vascular invasion and remnant
lesion, the late recurrence by tumor’s clinical pathology
proport, as multicentric carcinogenesis or intrahepatic
carcinoma de novo. So we could prevent recurrence and
metastasis of HCC in the following way: to adopt limited
hepatectomy and acrobatic hepatectomy technique, to
reduce operative hepatic bleeding and blood transfusion as
far as possible, to control HBV infection in HCC patients.
In this way, we can increase postoperative survival rate of
HCC on whole.

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**Table 4** Compare the HBV infective pattern between SLHCC and NHCC

| Serum markers | All patients \((n = 270)\) | Recurrent patients \((n = 136)\) | \(\chi^2\) | \(P\) |
|---------------|--------------------------|-------------------------------|----------|-----|
|               | SLHCC ratio (%) | NHCC ratio (%) | SLHCC ratio (%) | NHCC ratio (%) |       |
| HBsAg+HBcAb+  | 40.0          | 13.3            | 33.3          | 25.0          | 4.244 | 0.059 |
| HBsAg+HBeAb+  | 56.3          | 12.5            | 53.1          | 12.2          | 0.003 | 0.955 |
| HBsAg+HBeAb+  | 70.2          | 12.8            | 73.1          | 11.5          | 0.079 | 0.778 |
| HBsAg+        | 71.4          | 14.3            | 60.0          | 20.0          | 1.833 | 0.176 |
| HBeAb+HBcAb+  | 75.0          | 12.5            | 0.0           | 100.0         | 4.876 | 0.000 |
| HBeAg+        | 63.2          | 5.3             | 50.0          | 12.5          | 4.867 | 0.027 |
| HBV negative  | 69.6          | 4.3             | 87.5          | 0.0           | 4.877 | 0.027 |
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