Original Article

Clinical and pathological comparison of Budd-chiari syndrome associated hepatocellular carcinoma and HBV-associated hepatocellular carcinoma

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Abstract: Objective: This study was to compare the pathological changes and postoperative survival time between patients with Budd-Chiari syndrome (B-CS) associated hepatocellular carcinoma (HCC) and those with hepatitis B virus (HBV) associated HCC. Methods: 15 B-CS-associated HCC and 30 HBV-associated HCC patients undergoing operation were analyzed. Results: The average Ki67 index of B-CS-associated HCC group (19.7±14.2) was significantly lower than that of HBV-associated HCC group (31.2±18.0) (P=0.037). However, regarding the positive expression rate of CD34 and Glypican-3, our study did not find any significant difference between the two groups (73.3% vs 83.3%, P=0.693; 86.7% vs 76.7%, P=0.693). There were also no significant differences in the pathological grade and clinical stage between both groups. Moreover, One-year, two-year and three-year survival rates of B-CS associated HCC patients were 93.3%, 80.0% and 41.6% respectively after surgery, compared with 86.3%, 75.5% and 20.7% in those HBV associated HCC patients (P<0.05). Conclusions: The malignant degree of B-CS-associated HCC may not be lower than that of HBV-associated HCC. However, the survival time of the former is significantly longer than that of the latter after resection.

Keywords: Budd-Chiari syndrome (B-CS), hepatitis B virus infection, hepatocellular carcinoma (HCC), malignant degree

Introduction

Budd-Chiari syndrome (B-CS) is a posthepatic portal hypertension and/or inferior vena caval hypertension resulted from hepatic venous outflow obstruction, which may occur at any level from the small hepatic veins to the junction of the inferior vena cava and the right atrium [1-3]. It is a global disease with a low incidence and features by obvious regional difference, which mainly manifests as hepatic vein thrombosis in the western countries and membranous obstruction of inferior vena cava (MOVC) in China, Japan, India and other Asian countries [1, 3]. Studies have suggested that the cause of B-CS may be related to dysplasia, thrombosis, myeloproliferative disorders, mutations and others, but the detailed pathogenesis is unclear [3-8]. The condition of B-CS patients is complex and has a variety of clinical manifestations depending on different vascular obstruction locations and lesion severities, such as lower limb edema, splenomegaly, ascites, varicose veins of the thoracic and abdominal wall, upper gastrointestinal hemorrhage, etc. Hepatocellular carcinoma (HCC) is a more severe complication of B-CS and may shorten greatly the survival time of the patients. At present, B-CS has become a clear risk factor for HCC. B-CS accounts for 0.7% of all cases of HCC [9], and it is found that the prevalence of HCC in B-CS patients are highly variable, ranging from 2.0% to 51.6%, and the pooled prevalence of HCC is 17.6% in B-CS patients [10].

Hepatitis B virus (HBV) is a major risk factor for human health worldwide. Replication and proliferation of HBV can cause repeated injuries and liver cell regeneration, thus resulting in posthepatic cirrhosis and intrahepatic portal hypertension complicated by HCC. There are many similarities between B-CS and HBV-
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Nevertheless, many scholars pointed out that, compared with HBV-associated HCC, B-CS-associated HCC shows a lower malignant degree, and would bring a longer survival time and a better long-term efficacy to the patients after comprehensive treatment [11-13]. In this study, the clinical and pathological data of some B-CS-associated HCC and HBV-associated HCC patients who underwent surgical treatment in our hospital were reviewed, and the malignant degree as well as the postoperative survival time between them were compared. Now the details are reported below.

Materials and methods

Ethics

The study had been approved by the Medical Ethics Committee of The First Affiliated Hospital of Zhengzhou University and conducted after getting the informed consent of all patients.

Selection of patients

Patients who were diagnosed as B-CS-associated HCC or HBV-associated HCC and received excision from January 2010 to December 2013 in our hospital were selected randomly. A total of 45 patients were enrolled in the study, including 15 B-CS-associated HCC patients and 30 HBV-associated HCC patients.

Treatment

Among 15 B-CS-associated HCC patients, 4 MOVC patients underwent balloon dilatation of inferior vena cava obstruction to restore blood flow of the inferior vena cava and then laparotomy to resect the tumor tissues; 9 patients with mixed obstruction of inferior vena cava and hepatic vein also underwent the same operation; and 2 patients with hepatic venous obstruction only received tumor resection. In 30 HBV-associated HCC patients, tumor resection plus splenectomy was performed in 5 patients due to severe esophageal and gastric varices or history of upper gastrointestinal hemorrhage.

Transhepatic arterial chemotherapy and embolization (TACE) or medication treatment (liver protectants, antitumor drug, etc.) was given to patients who were found to have relapse of HCC during the follow-up visit.

Detection method of Ki67, CD34 and Glypican-3 and judgment standard

Ki67 index, CD34 and Glypican-3 were detected using SP immunohistochemical method by the technicians from the pathology department in The First Affiliated Hospital of Zhengzhou University. Ki67-positive cells were determined based on the presence of brown-yellow nucleus, and the mean Ki67 index was represented by the percentage of the positive cells in five randomized high-power fields. CD34-positive staining was considered in case that a single or clustered vascular endothelial cell(s) were brown, and Glypican-3-positive staining was judged in case that brown particles uniformly distributed in the cytoplasm and the membrane of HCC cells.

Histological grading of HCC was analyzed based on an international common Edmondson-Steiner grading system by an associate chief physician specialized in pathological diagnosis [14, 15]. The clinical staging of HCC for all the patients was performed based on Staging Standard of Hepatoma in China [16] by an associate chief physician from imaging depart-

<table>
<thead>
<tr>
<th>Features</th>
<th>B-CS group</th>
<th>HBV group</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Ki67</td>
<td>19.7±14.2</td>
<td>31.2±18.0</td>
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<tr>
<td>CD34</td>
<td>4.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>11 (73.3%)</td>
<td>25 (83.3%)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>4 (26.7%)</td>
<td>5 (16.7%)</td>
<td></td>
</tr>
<tr>
<td>Glypican-3</td>
<td>0.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>13 (86.7%)</td>
<td>23 (76.7%)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>2 (13.3%)</td>
<td>7 (23.3%)</td>
<td></td>
</tr>
<tr>
<td>Pathological grade</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>3 (20%)</td>
<td>5 (16.7%)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>11 (73.3%)</td>
<td>13 (43.3%)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>1 (6.7%)</td>
<td>12 (40%)</td>
<td></td>
</tr>
<tr>
<td>Clinical stage</td>
<td>0.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I a</td>
<td>3 (20%)</td>
<td>4 (13.3%)</td>
<td></td>
</tr>
<tr>
<td>I b</td>
<td>5 (33.3%)</td>
<td>8 (26.7%)</td>
<td></td>
</tr>
<tr>
<td>II a</td>
<td>0 (0%)</td>
<td>10 (33.3%)</td>
<td></td>
</tr>
<tr>
<td>II b</td>
<td>7 (46.7%)</td>
<td>8 (26.7%)</td>
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</table>
Budd-Chiari syndrome associated HCC vs. HBV-associated HCC

Figure 1. Expression of Ki67 in B-CS-associated HCC tissues (A) and HBV-associated HCC (B) (positive result: nucleus was brown-yellow (×400)).

Figure 2. Expression of CD34 of B-CS-associated HCC tissues (A) and HBV-associated HCC (B) (positive result: vascular endothelial cell was brown (×200)).

Postoperative follow-up

The follow-up was performed by outpatient service or telephone survey. Color Doppler ultrasound scan of the abdomen, serum AFP detection and others for reexamination were performed in all patients every one to two months after surgery, and enhanced CT or MRI was performed if necessary to make a more precise diagnosis, thereby determining whether relapse or metastasis of the tumor existed.

Data analysis

SPSS 17.0 was adopted for data analysis. Count data and measurement data were expressed as a ratio and mean ± standard deviation, respectively. t test was employed for comparison of quantitative data, and chi-square test was adopted for comparison of qualitative data, and Mann-Whitney U test was used for comparison of ranked data. Kaplan-Meier was used for survival analysis, and Log-rank test was adopted for comparison of the difference in the survival rate. P<0.05 was considered statistically significant.

Results

General data

A total of 15 patients in B-CS-associated HCC group included 7 males and 8 females who were 33-66 years old with a mean age of 52.7±10.1 years old. 30 patients in HBV-associated HCC group included 16 males and...
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14 females who were 31-72 years old with a mean age of 56.3±10.8 years old. There were no significant differences in gender and age between the two groups (P>0.05). The follow-up was conducted until June 2014, during which, loss to follow-up occurred in 7 patients in B-CS-associated HCC group and 3 patients in HBV-associated HCC group respectively.

Pathological features and clinical stage

The average Ki67 index of B-CS-associated HCC group (19.7±14.2) was significantly lower than that of HBV-associated HCC group (31.2±18.0) (P=0.037). There were no significant difference in CD34-positive rate (73.3% (11/15) vs 83.3% (25/30)) and Glypican-3-positive rate (86.7% (13/15) vs. 76.7% (23/30)) between the two groups.

According to Edmondson-Steiner grading standard, in B-CS-associated HCC group, the grade I, II and III HCC patients accounted for 20% (3/15), 73.3% (11/15) and 6.7% (1/15), respectively, while the percentages in HBV-associated HCC group were 16.7% (5/30), 43.3% (13/30) and 40% (12/30); and no grade IV HCC patient was found in both groups. Hence, there was no significant difference in the pathological grade between the two groups (P=0.075). Moreover, based on the clinical stage standard of HCC in China, stage Ia, Ib, Ila and IIb patients accounted for 20% (3/15), 33.3% (5/15), 0% (0/15) and 46.7% (7/15) of B-CS-associated HCC group, while the percentages in HBV-associated HCC group were 13.3% (4/30), 26.7% (8/30), 33.3% (10/30) and 26.7% (8/30); and there was no stage IIIa and IIIb HCC patient in both groups. No significant difference was found in

Figure 3. Expression of Glypican-3 of B-CS-associated HCC tissues (A) and HBV-associated HCC (B) (positive result: brown particles appear in the cytoplasm and membrane of HCC cells (×400)).

Figure 4. HCC tissues in patients with B-CS-associated HCC (A) and those with HBV-associated HCC (B) showed enhanced nucleus, increased mitosis and disordered cell arrangement (×400).
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the clinical stage between the two groups (P=0.940).

The pathological results and clinical stages were shown in Table 1. The expression and differentiation of Ki67, CD34 and Glypican-3 were seen in Figures 1-4.

Postoperative survival time

In B-CS-associated HCC patients, one-year, two-year and three-year survival rates were 93.3%, 80.0% and 41.6%, respectively, with a median survival time of 36 months (95% CI: 32.8-39.2); while in HBV-associated HCC patients, the rates were 86.3%, 75.5% and 20.7% with a median survival time of 28 months (95% CI: 26.1-29.9). There was significant difference in the postoperative survival time between the two groups (P=0.034). See Figure 5 for Kaplan-Meier survival curve.

Discussion

Cause for B-CS-associated HCC

B-CS has become a clear risk factor for HCC, and it is found that the prevalence of HCC in B-CS patients is highly variable, ranging from 2.0% to 51.6%, and the pooled prevalence of HCC is 17.6% in B-CS patients [10]. However, the exact pathogenesis is not clear and is considered to be related to the following factors in the previous studies. 1) Hepatic congestion-cirrhosis. Hepatic venous outflow obstruction may cause hepatic congestion and anoxia, along with repeated necrosis and regeneration of hepatic cells to result in liver fibrosis and cirrhosis, thereby increasing the ratio of canceration. Studies have indicated that the incidence of HCC in patients with obstruction of the inferior vena cava showed an increasing trend with time [12, 17], and the histological examination of such patients showed that the parenchyma around most of HCC lesions manifested as fibrosis and cirrhosis [17, 18]. This reflects the secondary fibrosis and cirrhosis due to hepatic congestion plays an important role in progression of HCC. 2) Obstruction of the inferior vena cava. According to several studies, MOVC patients have a significantly higher incidence of HCC compared with those with obstruction of hepatic vein [17-19]. The possible cause is that the condition of the latter is generally acute or subacute, and even may lead to acute hepatic failure in the short term, while the former mainly manifest as the chronic injury. 3) Hepatitis B virus. Many scholars have pointed out that HBV may not be the main factor for B-CS-associated HCC because the infection rate of HBV in B-CS-associated HCC patients is significantly lower than that in the overall HCC patients [4, 20]. However, some scholars also believe that HBV might play an additional role in development of HCC of B-CS patients [21]. 4) Gender. B-CS-associated HCC usually occurs in females; hence, being a female is an independent risk factor for B-CS-associated HCC, which may be due to the fact that estrogen accelerated the progression of HCC [11, 12].

Although the pathogenesis of B-CS-associated HCC is not clear, many investigations have pointed that it is different from that of HBV-associated HCC, and patients of B-CS-associated HCC show a better prognosis, so we can suppose that there are existing differences between B-CS-associated HCC and HBV-associated HCC. Herein, we conduct a comparative study to investigate the pathology differences between the two HCCs.
Ki67 protein, a proliferating cell nuclear antigen (PCNA) involved in cell cycle and a reliable factor reflecting the proliferative activity of the diseased tissues, has a significant value in predicting tumor recurrence and metastasis [22]. It has been found that Ki67 is closely associated with the pathological features and the prognosis of HCC and can better reflect the proliferative activity and biological behavior of hepatoma cells [23, 24]. CD34, a specific marker of vascular endothelial cell, can reflect the proliferation of vascular endothelial cell in the tumor tissues; meanwhile, it can reflect invasion ability of hepatic cancer and the prognosis of the patients to some extent by promoting tumor angiogenesis and participate in the carcinogenesis of hepatic cells [25, 26]. As a member of Glypican family, Glypican-3 plays an important role in the formation and development stages of the tissues and organs at embryo and fetal period and can prevent excessive growth of the tissues and organs mainly through the mechanism of negative regulation [27]. It is indicated that abnormal expression of Glypican-3 in adults is associated with the occurrence and development and the malignant degree of hepatic cancer as Glypican-3 can accelerate hepatoma cells proliferation by activating integrin, Wnt and other signaling pathways and enhance their invasive ability [28, 29]. Many scholars pointed out that expression of Glypican-3 is closely related with metastasis and recurrence of HCC after surgery and is an independent risk factor for the prognosis of HCC patients [30, 31].

In order to assess the pathological differentiation of HCC, we adopted an international common Edmondson-Steiner grading system that is of great significance in assessing the malignant degree of HCC [14, 15]. Although there are different clinical staging standards for HCC at home and abroad at present, such as TNM staging, Okuda staging and Clip staging, etc. [32, 33], there is no optimal staging standard as each staging standard is suitable for the specific group. In the this study, Staging Standard of Hepatoma in China was used [16], which integrates hepatic function, tumor size, metastasis and others and has a good value in judging the prognosis of the patients [34].

Our results suggested that Ki67 of B-CS-associated HCC group was obviously lower than that of HBV-associated HCC (19.7±14.2 vs 31.2±18.0, P=0.037), which indicated slower growth of the tumor cells, good tissue differentiation and lower invasion in patients with B-CS-associated HCC. However, as in the positive expression rate of CD34 and Glypican-3, our study did not find any significant difference between the two groups (73.3% vs 83.3%, P=0.693; 86.7% vs 76.7%, P=0.693). Based on these two indicators, we cannot conclude that B-CS-associated HCC has a lower malignant degree.

For HCC differentiated degree, Gwon et al. [12] conducted histological examination of 8 B-CS-associated HCC patients and the results showed that all the patients had good differentiation degree of HCC. In addition, after analysis and comparison, Shin et al. pointed out that the differentiation degree of B-CS-associated HCC seemed to be higher than that of HBV-associated HCC [11]. Our study also found that there was no grade IV B-CS-associated HCC, and the degree of pathological differentiation in the majority of B-CS-associated HCC was higher than that in HBV-associated HCC patients, with obvious difference in the pathological grade (P=0.075) (Table 1). We believed that this might be associated with the lower number of B-CS-associated HCC patients undergoing histological examination in the study by Shin et al. (only 3 patients).

Furthermore, the results show there is no significant difference in the clinical stage between the two groups (P=0.940) according to Staging Standard of Hepatoma in China. The possible reason may be due to selection of patients in this study. All the patients enrolled in this study are those who can receive surgical treatment with a relatively early clinical stage. Those with multiple metastatic tumors and poor hepatic function were excluded.

Prognosis of B-CS-associated HCC

Most studies indicated that, compared with HBV-associated HCC patients, surgical resection, TACE and other treatments can achieve a better outcome and would cause obviously increased survival rate for B-CS-associated HCC patients, which may be put down to rare biliary tract and portal vein invasion in the lat-
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ter [11, 12, 35]. Shin et al. [11] thought that the invasion rate to hepatic duct in B-CS-associated HCC patients was significantly lower than that in HBV-associated HCC patients according to the findings: only one patient had portal vein invasion and no one was found to have biliary tract invasion among 15 B-CS-associated HCC patients; while 96 patients were found to have portal vein invasion and 47 patients were found to have biliary tract invasion among 211 HBV-associated HCC patients. Hence, they considered that the lower invasion of B-CS-associated HCC might be attributed to good prognosis of such patients.

Different from HBV-associated HCC, B-CS-associated HCC can be treated only both B-CS and HCC are taken into account simultaneously, that is, we need to not only treat the congestion of the liver, but also suspend the tumor growth by resection. Based on some clinical results, removal of the hepatic venous outflow obstruction can reduce the incidence of postoperative complications and prolong the survival time in such patients [13]. For treating B-CS-associated HCC patients, we advocate that the surgery should be performed as soon as possible after removing the obstruction since this would slow the progression of HCC and decrease amount of bleeding during resection. In 15 B-CS-associated HCC patients of this study, 4 MOVC patients were all treated with PTA; and 9 patients with mixed obstruction only underwent PTA for treating the obstruction of inferior vena cava as the hepatic congestion can be relieved to a certain extent after removing the obstruction of inferior vena cava and such patients had excessive intrahepatic and extrahepatic collateral circulations; 2 patients with obstruction of hepatic vein were only given resection of the tumors to avoid greater trauma to the body. In this study, one-year, two-year and three-year survival rates of B-CS-associated HCC patients after surgery were 93.3%, 80.0% and 41.6%, while those of HBV-associated HCC patients were 86.3%, 75.5% and 20.7%, respectively. Meanwhile, the survival time of the former was apparently longer than that of the latter (P=0.034). All the results of this study were consistent with those of the majority researches globally.

Conclusions

In a word, according to our study results, Ki67 index of B-CS-associated HCC tissues was significantly lower than that in HBV-associated HCC, but there were no significant differences in CD34, Glypican-3, Edmondson-Steiner grade expression and clinical stage between the two groups. Although it has not been confirmed in the pathological studies that the malignant degree of B-CS-associated HCC was lower than that of HBV-associated HCC, the survival time of the former after resection was significantly longer than that of the latter, for which the specific mechanisms remains in-depth study.

The deficiencies of this study are detailed as follows: 1) Patients who were not able to receive the surgery due to excessive size of the tumor, intrahepatic and extrahepatic metastasis and poor hepatic function were excluded. Therefore, the results reflect the clinical and pathological features of the patients with the indications for surgery and are not representative of the general features of all B-CS-associated HCC and HBV-associated HCC patients. 2) The clinical and pathological indexes selected may not perfectly reflect the malignant degree of HCC, and the expression of CD34 and Glypican-3 was only be analyzed qualitatively without quantitative data. 3) In this study, the number of the patients is limited, and some patients lose to the follow-up visit. In consequence, in order to further understand the prognosis, further study with large samples and complete clinical data is still required.

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Disclosure of conflict of interest

None.

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