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Effects of transarterial chemoembolization combined with antiviral therapy on HBV reactivation and liver function in HBV-related hepatocellular carcinoma patients with HBV-DNA negative

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Abstract

Background: The aim of this study was to investigate the reactivation of the hepatitis B virus (HBV) following transarterial chemoembolization (TACE) in primary hepatocellular carcinoma (HCC) patients with HBV-DNA negative and to evaluate the effects of TACE combined with antiviral therapy.

Methods: This prospective study involved 98 patients with HBV-related and HBV-DNA negative HCC (HBV DNA $< 10^3$ copies/mL) underwent TACE procedures with serial HBV DNA tests. Patients were divided into the antiviral treatment group and the no-antiviral group. The antiviral group received entecavir antiviral therapy, and the other group received no antiviral therapy. Two groups of patients were compared in rate of HBV reactivation and liver function before and after only 1 session of TACE in average 1-month follow-up after operation. $P < .05$ indicated differences with a statistical significance.

Results: HBV reactivation occurred in 11 patients in the nonantiviral group (11/47, 23.4%) but only 3 patients in the antiviral group (3/51, 5.9%, $P < .05$). On multivariate analysis, HBeAg-positive status, number of tumors more than 3, and absence of antiviral therapy were the independent risk predictor of HBV reactivation. Liver function indicators did not differ significantly between the antiviral group and the nonantiviral group in 5 days after TACE. However, the level of alanine aminotransferase and bilirubin were raised and albumin was reduced at the HBV reactivation group compared with no HBV reactivation group ($P < .05$). At 1 month after TACE, liver function indicators did not differ significantly between the HBV reactivation group and without HBV reactivation group.

Conclusion: HCC patients with HBV DNA negative still remain associated with risk of HBV reactivation after TACE. HBeAg-positive, number of tumors more than 3, and absence of antiviral therapy in HCC patients after TACE have a higher risk of HBV reactivation. Antiviral therapy can reduce the risk of reactivation, helping improve liver function after TACE.

Abbreviations: AFP = the serum alpha-fetoprotein, ALT = alanine aminotransferase, BCLC = Barcelona Clinic Liver Cancer, HBeAg = hepatitis B envelope antigen, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, TACE = transarterial chemoembolization.

Keywords: hepatitis B, hepatocellular carcinoma, reactivation, transarterial chemoembolization

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer-related death. [1] Approximately 85% of patients with HCC in China are infected with chronic hepatitis B virus (HBV).[2,3] Most patients with HCC are diagnosed in an advanced stage. Consequently, less than 20% of patients are suitable for partial hepatectomy. Transarterial chemoembolization (TACE) is currently the standard nonsurgical treatment for patients with intermediate-stage or advanced-stage HCC.[4,5] It is well known that in HBV-infected patient treated with systemic chemotherapy for hematological and some solid tumors HBV can reactivate.[6] Moreover, HBV reactivation can occur following TACE and radiofrequency ablation (RFA) in HCC patients.[7,8] HBV reactivation can lead to fatal results, including liver failure. Although it has been observed that HBV reactivation occurs after the end of TACE in HBV-related HCC patients in previous studies, these studies were not analyzed for patients with HBV DNA negative ($<10^3$ copies/mL), and the incidence of reactivation of HBV was still unclear, which caused an ignorance of antiviral treatment. Meanwhile, questions such as when does TACE cause HBV reactivation, and what are the risk factors of HBV reactivation, are still unanswered. It is also unclear whether antiviral therapy can reduce risk of post-TACE HBV reactivation in patients who are negative for HBV DNA. The purpose of this

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prospective study was to investigate the incidence of HBV reactivation and associated factors in HBV-related HCC patients with HBV-DNA negative, and to evaluate the effects of antiviral therapy combined with TACE.

2. Methods

2.1. Study design and population

From June 2012 to December 2016, 119 patients with HBV-DNA negative (HBV DNA $< 10^3$ copies/mL) and positive for serum HBsAg underwent TACE procedure for unresectable HBV-related HCC at our hospital. Among these patients, 21 patients were excluded for the following reasons: the significant use of antiviral therapy for at least 3 months before TACE (n = 16), and with another cancer (n = 3) or autoimmune disease (n = 2). The remaining 98 patients were finally enrolled into this study. Fifty-one patients were included in the antiviral group and 47 in the nonantiviral group with random (Table 1). The study was carried out after obtaining patient consent and under protocols approved by the institutional review boards. The diagnosis of HCC was made according to the updated standards for the diagnosis and treatment of primary liver cancer from the Chinese Society of Liver Cancer/Chinese Society of Clinical Oncology/Chinese Medical Association branch of Hepatology.[9]

Clinical parameters of enrolled patients before TACE were assessed as follows: age, gender, platelet count, prothrombin time, total bilirubin, albumin, alanine aminotransferase (ALT), the serum alpha-fetoprotein (AFP), hepatitis B serology, serum HBV DNA level, Child–Pugh classification. Serum HBV DNA level was using an HBV quantitative Kit (Shanghai kehua Bio-engineering Company Limited, Shanghai, People's Republic of China) with a detection limit of 10^3 copies/mL.

2.2. TACE procedure

TACE was performed according to traditional method. The chemotherapeutic regimens used for TACE were determined by the interventional radiologist. Pharmaceutical agents including raltitrexed (2 mg) and oxaliplatin (50 mg) were infused into the feeding arteries of tumor, and then 5 to 20 mL Lipiodol mixed with oxaliplatin (50 mg) and pirarubicin (40 mg) was infused into feeding arteries until stasis flow in tumor vascularity was achieved. The need for polyvinyl alcohol particles was determined by the interventional radiologist.

Table 1

Baseline patient characteristics (N = 98).

Variable	Antiviral group (n = 51)	Nonantiviral group (n = 47)	*P*
Sex (M/F)	42/9	40/7	.713
Age, y	57.35 ± 8.26	58.68 ± 8.33	.430
BCLC stage (A/B/C)	28/18/5	27/16/4	1.000
Tumor number (<3/≥ 3)	37/14	35/12	.830
Tumor size (> 5/≤ 5 cm)	29/22	27/20	.953
Liver cirrhosis (present/absent)	36/15	32/15	.788
AFP (≥ 400/ < ng/mL)	40/11	35/12	.644
Prothrombin time, s	12.27 ± 0.38	12.17 ± 0.36	.207
Platelet count, x10^9/L	127.25 ± 24.90	125.06 ± 21.75	.645
Total bilirubin, μmol/L	13.39 ± 2.47	13.68 ± 2.82	.590
Albumin, g/L	42.17 ± 3.31	43.03 ± 2.85	.171
ALT, U/L	32.73 ± 11.04	30.78 ± 11.25	.390
Ascites (present/absent)	2/49	2/45	1.000
Child–Pugh class (A/B)	37/14	35/12	.830
HBeAg (Positive /Negative)	11/40	10/37	.972

AFP = serum alpha-fetoprotein, ALT = alanine aminotransferase, BCLC = Barcelona Clinic Liver Cancer, HBeAg = hepatitis B envelope antigen.

2.3. Antiviral treatment

These patients who were assigned as the antiviral group would receive entecavir (0.5 mg/day; Zhengda Tianqing, Lianyungang, China) starting 3 days before TACE and for at least 1 month afterwards. In the nonantiviral group, patients did not receive any antiviral therapy.

2.4. Clinical follow-up

On postoperative days 1 to 5, all patients received drug therapy (Magnesium Isoglycyrrhizinate for Injection, 150 mg/day; Zhengda Tianqing, Lianyungang, China) to protect liver function. HBV DNA levels, liver functioning (using indices alanine aminotransferase, total bilirubin, albumin), and prothrombin time were measured before TACE and on days 1, 5, 14, and 30 after TACE. HBV reactivation was defined as reappearance or an increase more than 10-fold in serum HBV DNA compared with the baseline level during follow-up.[6] In this study, HBV reactivation means serum HBV DNA rises to $>10^3$ copies/mL. In the nonantiviral group, when HBV reactivation occurred, patients would be administered entecavir at a dose of 0.5 mg daily as a therapeutic measure.

2.5. Data analysis

All data analyses were performed using SPSS 18.0 (IBM, Chicago, IL) and with $P < .05$ as the threshold of statistical significance. Categorical variables were analyzed using Chi-squared test and Fisher exact test when appropriate. Continues variables were analyzed using Student *t* test. Binary logistic regression analysis was performed to identify the independent predictors of HBV reactivation.

3. Results

3.1. Incidence and risk factors of HBV reactivation

Fourteen of 98 (14.3%) developed HBV reactivation during the follow-up period. HBV reactivation occurred in 11 of 47 patients in the nonantiviral group (23.4%) but only in 3 of 51 patients in the antiviral group (5.9%, $P < .05$). Most reactivation events occurred within day 5 after TACE: 1 patient experienced reactivation on postoperative day 3, 9 patients on day 5 (including the 2 patients in the antiviral group), 3 patients on day 14, and 1 patient on day 30 (Table 2). Univariate analysis identified the following risk factors of HBV reactivation (all $P < .05$; Table 3): cirrhosis, number of tumors more than 3, hepatitis B envelope antigen (HBeAg)-positive status, and absence of antiviral therapy. Nevertheless, on multivariate analysis, HBeAg-positive status (hazard ratio, 13.393; $P = .029$), number of tumors more than 3 (hazard ratio = 11.459; $P = .001$), and absence of antiviral therapy (hazard ratio = 8.124; $P = .009$) were the independent risk predictors of HBV reactivation ($P < .05$; Table 4).

3.2. Comparison of liver function

At 5 days after TACE, liver function indicators did not differ significantly between the antiviral group and the nonantiviral

Table 2

HBV DNA levels (10^3copies/mL) in patients in the antiviral and nonantiviral groups who experienced HBV reactivation within 1 month of TACE.

Day patient	D3	D5	D14	D30
1	1.3	1.6	1.4	<1
2		1.8	1.9	<1
3		5.1	2.5	1.3
4		3.2	1.8	<1
5		11	6	1.5
6		7.5	5.2	<1
7*		2.9	2.2	1.2
8*		3.4	1.6	<1
9		21	13	2.9
10		33	20	3.4
11			3.9	2.1
12			1.8	1.1
13*			1.4	<1
14				1.7

* Patients in the antiviral group who experienced HBV reactivation.

group. However, the level of ALT was significantly higher and albuminin was significantly lower at the HBV reactivation group than no HBV reactivation group ($P < .05$). One month after TACE, liver function indicators did not differ significantly between the HBV reactivation group and without HBV reactivation group.

4. Discussion

TACE causes high intratumoral concentrations of drugs and cuts off blood supply to the tumor resulting in ischemic necrosis. However, chemotherapy administered to patients harboring HBV can result in reactivation of HBV, which is caused by those chemotherapeutic agents suppressing the immune system.[10] The goal of TACE is to deliver highly concentrated doses of chemotherapeutic agents to the tumor, to prolong the contact time between the anticancer drugs and the tumor, and to minimize systemic toxicity. A study reported that TACE can lower the peak concentration of chemotherapeutic agents in

Table 3

Univariate to identify factors related to HBV reactivation.

Variable	HBV reactivation (n = 14)	No HBV reactivation (n = 84)	P
Sex (M/F)	11/3	71/13	.577
Age, y	54.68 ± 11.74	55.37 ± 12.18	.235
BCLC stage (A/B/C)	8/5/1	47/29/8	1.000
Tumor number (< 3/≥3)	5/9	67/17	.001
Tumor size (>5/≤5 cm)	9/5	49/37	.560
Liver cirrhosis (present/absent)	13/1	55/29	.058
AFP (≥400 /<400 ng/mL)	8/6	67/17	.088
Prothrombin time, s	12.24 ± 0.41	12.22 ± 0.36	.877
Platelet count, x10^9/L	126 .14 ± 25.58	126.21 ± 23.12	.992
Total bilirubin, μmol/L	13.94 ± 2.92	13.46 ± 2.59	.537
Albumin, g/L	43.14 ± 3.02	42.49 ± 3.14	.475
ALT, U/L	29.15 ± 11.00	32.23 ± 11.15	.340
Ascites (present/absent)	2/12	2/82	.097
Child–Pugh class (A/B)	10/4	62/22	1.000
HBeAg (Positive /Negative)	7/7	14/70	.010
Antiviral therapy (yes/no)	3/11	48/36	.013

AFP = the serum alpha-fetoprotein, ALT = alanine aminotransferase, BCLC = Barcelona Clinic Liver Cancer, HBeAg = hepatitis B envelope antigen, HBV = hepatitis B virus.

Table 4

Multivariate analysis with logistic regression to identify factors related to perioperative HBV reactivation.

Variable	HR	95% CI	P
HBeAg (Positive)	13.393	1.304–137.513	.029
Tumor number (≥3)	11.459	2.789–47.077	.001
Antiviral therapy	8.124	1.677–39.345	.009

CI = confidence interval, HBeAg = hepatitis B envelope antigen, HBV = hepatitis B virus, HR = hazards ratios.

serum and increase the intratumoral concentration.[11] Thus, the dosage of chemotherapeutic agents was lower than systemic chemotherapy, but still remain associated with risk of viral reactivation after TACE.

HBV reactivation may be followed by the increase in serum transaminase levels and active hepatitis even can cause life-threatening hepatic failure.[12]

It is well-known that TACE can reactivate HBV replication in HBV-related HCC patients with high baseline HBV DNA level. However, few reports concerned the HBV reactivation in HBV-DNA negative patients with HCC following TACE. A previous study reported HCC patients with low serum HBV DNA level still remain associated with risk of viral reactivation after TACE in China, and HBeAg-positive HCC patients have a higher risk than patients with HBeAg-negative status.[13]

In the present study, 11 of 47 (11/47, 23.4%) developed HBV reactivation in the nonantiviral group during the follow-up period. The result shows the incidence of HBV reactivation in HBV-related HCC patients with HBV-DNA negative after TACE is as frequent as in HBV-DNA positive patients. This implies that HBV reactivation should not be overlooked in patients with HBV-DNA negative. According to the results, HBeAg-positive status, number of tumors more than 3, and absence of antiviral therapy were found to be the independent risk predictors of HBV reactivation. Earlier studies have shown that HBeAg-positive status was the independent predictor of HBV reactivation in TACE therapy,[13] which were coincident with our study. Furthermore, number of tumors more than 3 was a risk factor of HBV reactivation. How this situation can reactivate viral replication is poorly understood. We attribute this association to the chemotherapeutic agents infused into multiple lesions in the liver. This may increase the risk of HBV replication after TACE.

For patients with HBV-related HCC who are positive for HBV DNA, official guidelines recommend entecavir and tenofovir as first-line antiviral therapy during chemotherapy or immunosuppressive therapy to suppress viral replication and thereby help improve liver function, decrease tumor recurrence.[14,15] However, there are no clinical guidelines for managing TACE-caused HBV reactivation in HBV-related HCC patients.

For HCC patients who are HBsAg positive but negative for HBV DNA negative, how HBV reactivation occurs remains unclear. We believe that the chemotherapeutic agents may play key roles because of their distinct influences on the extent of immunosuppression. HBV covalently closed circular DNA can integrate into liver cells and persist for long periods.[16] In addition, TACE may induce immunosuppression and trigger genome amplification, leading in turn to HBV reactivation.[17] In this study, patients who received antiviral therapy with entecavir showed a significantly lower incidence of HBV reactivation than the nonantiviral therapy group (5.9% vs 23.4%, $P < .05$), and the rate of deterioration of liver function was higher in the HBV reactivation group than in the nonreactivation group. This

demonstrates that prophylactic antiviral therapy with entecavir can reduce HBV reactivation and improve liver function.

This study was limited by the amount of patients and the observation time was short. HBV reactivation was evaluated after only a single session of TACE and patients in the antiviral therapy group received entecavir only. In addition, our study was based on a small sample, and our detection limit of 1000 copies/mL of HBV DNA may not be sufficiently sensitive to detect low levels of viral replication. Future study with larger numbers of patients and longer follow-up periods is necessary to elucidate these findings.

In conclusion, our results demonstrated that HBV reactivation rates also appear to be relatively high for patients with HBV-DNA negative during TACE therapy. Initiation of antiviral therapy for patients before TACE significantly reduced the risk of HBV reactivation and protected liver function. Thus, perioperative antiviral therapy should be administered to HCC patients who are positive for HBsAg and negative for HBV DNA, especially for HBeAg-positive status and number of tumors more than 3 patients. Our results should be confirmed and extended in larger studies with longer follow-up.

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