

Original Article

Changes in hepatitis B virus DNA levels and liver function after transcatheter arterial chemoembolization of hepatocellular carcinoma

Xiang-Ming Lao,^{1,2*} Dian Wang,^{1*} Ming Shi,¹ Guipeng Liu,³ Shengping Li,¹ Rongping Guo,¹ Yunfei Yuan,¹ Minshan Chen,¹ Jinqing Li,¹ Yaqi Zhang¹ and Xiaojun Lin¹

¹Department of Hepatobiliary Oncology, Sun Yat-sen University Cancer Center and State Key Laboratory of Southern China, Guangzhou, China; ²Department of Surgical Oncology of University of Michigan, and ³University of Michigan, Ann Arbor, Michigan, USA

Aim: Reports concerning changes in hepatitis B virus (HBV) status and liver function in hepatocellular carcinoma (HCC) during or after transcatheter arterial chemoembolization (TACE) have been rare and the results inconsistent. The objective of this retrospective study was to evaluate these parameters in a large cohort of HBV-related HCC patients.

Methods: One hundred and seventy-two hepatitis B surface antigen positive HCC patients with Child–Pugh grade A or B liver disease who underwent 228 sessions of TACE were enrolled, and related clinical and laboratory data were analyzed.

Results: In total, HBV reactivated in 33 (14.5%), remained stable in 152 (66.7%) and decreased in 43 (18.8%) sessions. Univariate analysis revealed that sex and HBV DNA levels correlated with changes in HBV DNA status after TACE, while hepatitis B e-antigen (HBeAg), prothrombin time and chemotherapeutic agents were marginally significant factors.

Multivariate analysis demonstrated that the major factors that influenced the HBV DNA status were baseline HBV DNA levels ($P = 0.0002$) and HBeAg ($P = 0.0387$). A comparison of the post-TACE (30–90 days) liver function to the baseline revealed no significant differences. The reactivation group has the highest rate of exacerbation (12.1%) compared with the stable group (5.9%) and downregulation group (4.7%).

Conclusion: HBV DNA changes after TACE included reactivated, decreased and stable HBV DNA levels. Although HBV reactivation did not necessarily result in exacerbation of liver damage and most HCC patients with Child–Pugh grade A and B tolerated TACE well, careful post-procedure monitoring and managing is needed.

Key words: hepatitis B virus reactivation, hepatitis B virus DNA, hepatocellular carcinoma, liver function, transcatheter arterial chemoembolization

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is the sixth most common cancer and the third most common cause of cancer death worldwide.¹ In highly endemic areas, hepatitis B virus (HBV) infection plays a primary role in the etiology of HCC and is frequently observed in HCC patients.² While curative therapies

including hepatectomy, transplantation and ablation can be applied to some early stage HCC, transcatheter arterial chemoembolization (TACE) has become an important therapeutic modality improving survival in some carefully selected patients with unresectable HCC.^{3,4}

It is well known that in HBV-infected patient treated with systemic chemotherapy for hematological and some solid tumors HBV can reactivate, and such flare-ups of HBV have led to fatal results.⁵ Accordingly, society guidelines now recommend that hepatitis B surface antigen (HBsAg) positive patients receive pre-emptive nucleoside/nucleotide analog (NUC) administration during therapy (regardless of HBV DNA levels) and for 12 months after cessation of chemotherapy.⁶

Correspondence: Dr Yaqi Zhang and Dr Xiaojun Lin, Department of Hepatobiliary Oncology, Sun Yat-sen University Cancer Center, 651 Dongfeng Road East, Guangzhou 510060, China. Email: laoxming@mail.sysu.edu.cn; laoxm@sysucc.org.cn

*These authors contributed equally to this study.

Received 31 August 2010; revision 10 February 2011; accepted 16 February 2011.

However, although HCC has the highest infection rate of HBV compared to other malignancies⁷ and the complication of HBV reactivation is expected to be more frequently observed with more severe clinical course, reports concerning HBV status of HCC patients during or after TACE are rare, and the results have been controversial. While some studies have demonstrated HBV reactivation after TACE,^{8–10} some have not,^{11–13} and others have shown decreased HBV DNA levels after TACE.¹⁴

The objective of the current retrospective study was to evaluate changes in HBV DNA levels and liver function before and after a single session of TACE in HBsAg positive Child–Pugh grade A and B HCC.

METHODS

Patients

BETWEEN JULY 2005 and March 2007, patients who were diagnosed with HBsAg positive HCC and received TACE at the Hepatobiliary Department of Sun Yat-sen University Cancer Center were retrospectively recruited in this investigation. Demographic data of patients were recorded.

Baseline examinations before TACE included serum HBV DNA quantification, detection of HBsAg, hepatitis B surface antibody (HBsAb), hepatitis B core antibody (HBcAb), hepatitis B e-antigen (HBeAg), hepatitis B e antibody (HBeAb) and anti-hepatitis C virus (HCV) antibody, serum liver tests (alanine aminotransferase, ALT; aspartate aminotransferase, AST; alkaline phosphatase; total bilirubin, T-bil; and albumin, ALB), creatinine levels, prothrombin time (PT), α -fetoprotein (AFP) levels, complete blood counts and chest radiography. Serum hepatitis B markers and HCV antibody were tested by commercial enzyme immunoassays (Auszyme MC Dynamic; Abbott Laboratories, North Chicago, IL, USA). Serum HBV viral loads were measured by a quantitative HBV DNA fluorescence polymerase chain reaction detection kit (DaAn Gene, Guangzhou, China) with a lower detection limit of 100 IU/mL. Tumor characteristics and International Union Against Cancer Tumor–Node–Metastasis stage were evaluated by imaging. Within 1 week of baseline examinations, patients received TACE consisting of chemotherapeutic agents (including epirubicin [60 mg/session] and/or mitomycin [6 mg/session], carboplatin [300 mg/session], lobaplatin [50 mg/session], floxuridine [500 mg/session]), lipiodol and/or gelfoam embolization.

To be included in this retrospective study, enrolled patients were required to have at least 3 months of follow up. Post-procedure, the same laboratory and clinical data as obtained at baseline were collected 30–90 days after TACE. The inclusion criteria also included HBsAg positivity, adequate baseline liver function (Child–Pugh grade A or B) and adequate renal function (serum creatinine <124 μ mol/L).

Patients with any of the following were excluded: a history of antiviral therapy prior to the designated post-TACE tests as mentioned above (30–90 days after TACE); negative test for HBsAg; evidence of superinfection with other hepatotropic viruses or HIV; intrahepatic arteriovenous shunts; loss to follow up within 3 months after TACE; obstructive jaundice; hepatic encephalopathy; Child–Pugh grade C; any other malignancy; concurrent non-malignant severe illness; history of hepatotoxic medication within 8 weeks prior to current TACE session; history of corticosteroid administration or any treatment for HCC within 12 weeks prior to the study; and sessions with poor data integrity. In total, 172 HBsAg positive HCC patients undergoing 228 sessions of TACE were enrolled in this study.

TACE procedure

Transcatheter arterial chemoembolization was performed as we previously described.¹⁵ When the catheter tip was advanced to the tumor feeding arteries, one or several chemotherapeutic agents with mixed lipiodol were then slowly injected. Gelatin sponge particles were injected in some sessions if the chemoembolized artery territory did not show stagnant flow. The selection of different combinations of anticancer agents, lipiodol emulsion dosage and use of gelfoam were made based on a case-by-case basis.

Definitions^{14,16–18}

Hepatitis B virus DNA status changes after TACE for all patients were grouped into three categories: (i) HBV reactivation; (ii) HBV downregulation; and (iii) stable status. HBV reactivation was defined as a 10-fold or more increase in HBV DNA level compared to baseline or the appearance of HBV DNA from an undetectable level at baseline, and post-TACE HBV DNA of more than 200 IU/mL. HBV downregulation was defined as a 10-fold or more decrease in HBV DNA level compared to baseline or the disappearance of HBV DNA from a detectable level at baseline, and the baseline HBV DNA before TACE were all more than 200 IU/mL. Those patients that did not fall into either of these categories were defined as having a “stable status”. Hepatitis was

defined as a threefold or more increase in serum ALT levels above the upper limit of normal (normal <40 IU/L) or an absolute increase of ALT to more than 100 IU/L. Icteric hepatitis was defined by a serum T-bil level that exceeded twice the reference range (normal <20.5 $\mu\text{mol/L}$) in the presence of hepatitis. Exacerbation of liver damage included both hepatitis and icteric hepatitis as defined above.

Statistical analysis

Demographic data (mean, standard deviation, sample size and percentage) were calculated. Analyses were conducted using the independent Student's *t*-test, ANOVA, Student's paired *t*-test, χ^2 -test and Fisher's exact test when appropriate. Univariate and multivariate analyses (backward elimination multinomial logistic regression) were performed to evaluate the possible factors that related to the changes of HBV DNA and exacerbation of liver damage after TACE. The following variables that might correlate with the HBV DNA status and hepatic exacerbation were evaluated: age, sex, TACE before, ALT, T-bil, ALB, HBeAg, HBV DNA, AFP, PT, chemotherapeutic agents and Child–Pugh grade (Tables 2,3). Data were analyzed using SAS ver. 9.1.

RESULTS

Study population

OF THE 228 sessions of TACE, 218 (95.6%) were for males and 10 (4.4%) were for females with an overall mean age of 49.0 ± 11.6 years. The frequency of chronic HBV infection (confirmed by HBsAg or HBV DNA positivity prior to the current study) was 88.2%. One hundred and twenty-seven (55.7%) sessions comprised the initial treatment of the HCC. Previous treatments prior to the current TACE session included TACE, hepatectomy and local thermal ablation. The baseline HBV DNA values ranged from undetectable (<100 IU/mL) to 1.8×10^7 IU/mL. When \log_{10} transformed, the HBV DNA values satisfied conditions for normal distribution with a mean value of $4.0 \pm 2.2 \log_{10}$ IU/mL. The clinical and laboratory features at entry are shown in Table 1.

Clinical characteristics during the 228 sessions of TACE procedures

Digital subtraction angiography (DSA) showed that there were 23 (10.1%) sessions with single hepatic tumor lesions, and 205 (89.9%) sessions with multiple hepatic lesions. The infused lipiodol dose ranged

Table 1 Baseline characteristics of all 228 sessions of TACE

Baseline characteristics	Description
History of HBV infection	
Yes	201 (88.2%)
No	27 (11.8%)
Duration of HBV infection (<i>n</i> = 201)	
1–5 years	21 (10.4%)
5–10 years	34 (16.9%)
10–20 years	77 (38.3%)
20–30 years	47 (23.4%)
>30 years	22 (11.0%)
Initial treatment	
Yes	127 (55.7%)
No	101 (44.3%)
TACE before the current session	
Yes	81 (35.5%)
No	147 (64.5%)
Hepatectomy before the current session	
Yes	38 (16.7%)
No	190 (83.3%)
Local ablation before the current session	
Yes	18 (7.9%)
No	210 (92.1%)
WBC ($10^9/\text{L}$)	6.7 ± 2.4
HGB (g/L)	136.0 ± 21.0
PLT ($10^9/\text{L}$)	177.3 ± 95.5
AST (u/L)	66.2 ± 60.6
ALT (u/L)	54.2 ± 37.4
ALB (g/L)	40.6 ± 4.5
T-bil ($\mu\text{mol/L}$)	14.9 ± 6.1
AFP (ng/mL)	
≥ 200	118 (51.8%)
<200	110 (48.2%)
PT (s)	12.5 ± 1.7
APTT (s)	29.1 ± 5.0
HBV DNA (\log_{10} IU/mL)	4.0 ± 2.2
HBeAg	
Positive	52 (22.8%)
Negative	176 (77.2%)
Child staging	
A	221 (96.9%)
B	7 (3.1%)
UICC TNM stage	
IIIA	148 (64.9%)
IIIB	31 (13.6%)
IIIC	35 (15.4%)
IV	14 (6.1%)

AFP, α -fetoprotein; ALB, serum albumin; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; HBeAg, hepatitis B e-antigen; HGB, hemoglobin; HBV, hepatitis B virus; PLT, platelets; PT, prothrombin time; TACE, transcatheter arterial chemoembolization; T-bil, total bilirubin; UICC TNM, International Union Against Cancer Tumor–Node–Metastasis; WBC, white blood cells.

Table 2 Univariate analyses for the possible factors correlating to the changes of HBV DNA status after TACE

Variables	Stable (<i>n</i> = 152)	Reactivation (<i>n</i> = 33)	Downregulation (<i>n</i> = 43)	<i>P</i> -value
Age (year)	48.6 ± 11.6	50.4 ± 11.3	49.2 ± 11.9	0.7142
Sex				0.0460*
Male	148	32	38	
Female	4	1	5	
TACE before				0.3327
Yes	59	10	12	
No	93	23	31	
ALT (U/L)	51.9 ± 30.8	53.8 ± 59.8	62.4 ± 36.4	0.2671
T-bil (μmol/L)	14.6 ± 5.9	15.0 ± 5.9	15.9 ± 7.0	0.4631
ALB (g/L)	40.9 ± 4.6	40.6 ± 3.9	39.6 ± 4.4	0.2434
HBeAg				0.0863
Positive	40	3	9	
Negative	112	30	34	
HBV DNA (log ₁₀ IU/mL)	3.7 ± 2.3	3.6 ± 1.7	5.3 ± 1.4	<0.0001**
AFP (ng/mL)	12 379.2 ± 30 367.7	11 953.3 ± 30 587.6	12 553.1 ± 31 270.8	0.9963
PT (s)	12.3 ± 1.6	13.1 ± 2.0	12.8 ± 2.0	0.0501
Chemotherapeutic agents				0.0527
Epirubicin only	35	6	17	
>2 agents	117	27	26	
Child–Pugh grade				0.7299
A	148	32	41	
B	4	1	2	

*Significant differences between stable group and downregulation group.

**Significant differences between stable group and downregulation group, as well as reactivation group and downregulation group. AFP, α-fetoprotein; ALB, serum albumin; ALT, alanine aminotransferase; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus; PT, prothrombin time; TACE, transcatheter arterial chemoembolization; T-bil, total bilirubin.

5–30 mL with a mean of 15.8 ± 8.8 mL. As for the chemotherapeutic regimens, 58 (25.4%) sessions used a single agent (epirubicin) while the other 170 (74.6%) sessions used combinations of chemotherapeutic agents. Specifically, 94 (41.2%) sessions applied epirubicin, mitomycin and lobaplatin; and 76 (33.4%) sessions applied epirubicin, floxuridine and carboplatin. Sixty-one sessions (26.8%) applied gelfoam emboliza-

tion while the other 167 sessions (73.2%) did not. All the recruited TACE sessions were performed without severe complications during the peri-treatment period.

Changes of HBV DNA status after TACE

As described in the methods, HBV DNA values and the related liver parameters were evaluated during the designated 30–90 days (mean: 51.8 ± 15.3 days) after

Table 3 Multinomial logistic regression analyses for the possible factors correlating to the changes of HBV DNA status after TACE

	HBV DNA (log ₁₀ IU/mL)		HBeAg (negative vs positive)†	
	OR	95% CI	OR	95% CI
Downregulation vs stable	1.611	1.275–2.035	2.409	0.963–6.029
Reactivation vs stable	1.027	0.853–1.236	3.589	0.991–12.994

Backward elimination multinomial logistic regression was conducted to select variables related to hepatitis B virus (HBV) DNA status changes with the “stable” status group as a reference. The removal significance level was 0.05. The analyses included HBV DNA status changes as a dependent factor, while age, sex, transcatheter arterial chemoembolization (TACE) before, alanine aminotransferase, total bilirubin, albumin, hepatitis B e-antigen, HBV DNA, α-fetoprotein, prothrombin time, chemotherapeutic agents and Child–Pugh grade were included as independent factors.

†The “positive” category was the reference group.

CI, confidence interval; HBeAg, hepatitis B e-antigen; OR, odds ratio.

TACE. In total, HBV DNA reactivation occurred after 33 sessions (14.5%), maintained a stable status after 152 (66.7%) sessions and HBV DNA downregulation occurred after 43 (18.8%) sessions.

Univariate analysis (Table 2) revealed that sex and baseline HBV DNA may correlate with the changes of HBV DNA status after TACE, while HBeAg, PT and chemotherapeutic agents showed marginally significant associations. Specifically, the proportion of females in the downregulation group was 11.6% (5/43), significantly higher than in the stable group (2.6%, 4/152). HBV DNA values were significantly higher in down-regulated group ($5.3 \pm 1.4 \log_{10}$ IU/mL) compared to reactivated and stable groups (3.6 ± 1.7 and $3.7 \pm 2.3 \log_{10}$ IU/mL, respectively). The frequency of HBeAg seropositivity in the reactivated group was 9% (3/33), lower (marginally significantly) than that of the stable status group (40/152, 26.3%). The PT was longer in the reactivation group (13.1 ± 2.0 s) than in the stable group (12.3 ± 1.6 s). Reactivation was more frequent (marginally significantly) in patients that received combinations of chemotherapeutic agents (27/33, 81.8%) compared to those patients that had down-regulation of HBV levels (26/43, 60.5%). Specifically, Figure 1 shows the correlation between HBV DNA status and the different combinations of chemotherapeutic agents. There were no significant differences among the frequencies of reactivation, stable and downregulation status of group A (pure epirubicin), group B (epirubicin, mitomycin and lobaplatin) and group C (epirubicin, floxuridine and carboplatin). The results of multinomial logistic regression analysis are presented in Table 3. The HBV DNA status' changes were overall significantly correlated with baseline HBV DNA levels ($P=0.0002$). For one unit (\log_{10} transformed) increase in HBV DNA level, the risk of downregulation increased significantly (odds ratio [OR] = 1.611; 95% confidence interval [CI] = 1.275–2.035; $P < 0.0001$), but HBV DNA level was not correlated with reactivation (OR = 1.027; 95% CI = 0.853–1.236; $P = 0.7793$). The changes of HBV DNA status were also overall significantly correlated with HBeAg ($P = 0.0387$). Compared to the HBeAg positive patients, the HBeAg negative patients were marginally significantly correlated with the increased risks of reactivation (OR = 3.589; 95% CI = 0.991–12.994; $P = 0.0516$) or downregulation (OR = 2.409; 95% CI = 0.963–6.029; $P = 0.0603$). Age, sex, TACE before, ALT, T-bil, ALB, AFP, PT, chemotherapeutic agents and Child–Pugh grade were not significantly correlated with the changes of HBV DNA after TACE in the current model.

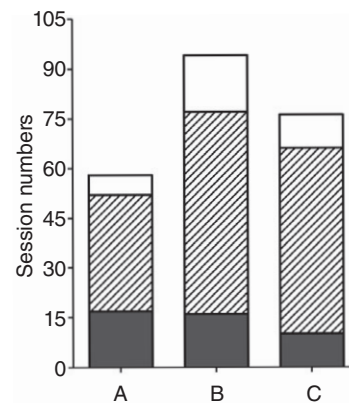


Figure 1 Various combinations of chemotherapeutic agents and changes in hepatitis B virus (HBV) DNA status after transcatheter arterial chemoembolization (TACE). (A) Epirubicin only (reactivation, 6 cases; stable, 35 cases; downregulation, 17 cases). (B) Epirubicin, floxuridine and carboplatin (reactivation, 17 cases; stable, 61 cases; downregulation, 16 cases). (C) Epirubicin, floxuridine and lobaplatin (reactivation, 10 cases; stable, 56 cases; downregulation, 10 cases). (Dosages administrated: epirubicin 60 mg; mitomycin 6 mg; carboplatin 300 mg; lobaplatin 50 mg; floxuridine 500 mg.). There were no significant differences among groups (A–C) concerning changes of HBV DNA status. (□) Reactivation, (▨) stable status, and (■) downregulation.

Changes in the liver tests after TACE as a function of change in HBV DNA levels are shown in Table 4. There were no significant differences concerning AST, ALT, ALB, T-bil, AFP, PT, activated partial thromboplastin time and Child–Pugh grade. The occurrence rate of exacerbation of liver damage was highest in the reactivation group (4/33, 12.1%), although there were no significant differences compared to the other two groups (stable 9/152, 5.9%; downregulated 2/43, 4.7%). The post-TACE HBV DNA value in the reactivated group ($5.6 \pm 1.3 \log_{10}$ IU/mL) was significantly higher than the other two groups (stable $3.7 \pm 2.3 \log_{10}$ IU/mL; down-regulated $3.3 \pm 1.8 \log_{10}$ IU/mL).

Comparisons between pre- and post-TACE clinical and biological characteristics

In addition to testing in the designated time period (30–90 days after TACE), liver testing was also done shortly after TACE administration (within 7 days). The results are listed in Table 5. Compared to the baseline (Table 1), the mean values of AST, ALT and T-bil increased significantly soon after TACE (within 7 days). However, they decreased to levels close to baseline at the subsequent designated time point (30–90 days after

Table 4 Comparison of liver tests after TACE among the three groups based on HBV DNA changes

Serum liver tests	Stable (<i>n</i> = 152)	Reactivation (<i>n</i> = 33)	Downregulation (<i>n</i> = 43)	<i>P</i> -value
AST (u/L)	65.1 ± 61.1	70.9 ± 45.3	76.5 ± 55.0	NS
ALT (u/L)	52.4 ± 40.3	64.7 ± 67.4	61.6 ± 50.7	NS
ALB (g/L)	39.9 ± 4.4	40.0 ± 5.2	38.9 ± 3.9	NS
T-bil (μmol/L)	14.4 ± 9.2	14.4 ± 12.1	15.6 ± 9.9	NS
AFP (ng/mL)	12 839.8 ± 30 815.6	14 897.8 ± 33 941.6	16 116.9 ± 35 197.5	NS
PT (s)	12.4 ± 1.6	12.4 ± 1.6	12.7 ± 1.5	NS
APTT (s)	29.2 ± 5.3	29.9 ± 4.8	30.3 ± 4.4	NS
HBV DNA (log ₁₀ IU/mL)	3.7 ± 2.3	5.6 ± 1.3	3.3 ± 1.8	<0.0001*
Child–Pugh grade (A : B)	148:4	31:2	40:3	NS
Exacerbation of liver damage	9 (5.9%)	4 (12.1%)	2 (4.7%)	NS

*Significant differences between reactivation group and stable status group, as well as between reactivation group and downregulation group.

AFP, α-fetoprotein; ALB, serum albumin; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; HBV, hepatitis B virus; PT, prothrombin time; TACE, transcatheter arterial chemoembolization; T-bil, total bilirubin.

TACE), and the only significant change concerning liver tests was a decline in ALB from 40.6 ± 4.5 g/L at baseline to 39.8 ± 4.4 g/L after TACE.

Clinical features

The clinical features of the 15 patients with exacerbation of liver damage after TACE are shown in Table 6. Among them there were five sessions/patients (patients 11–15) with icteric hepatitis.

The baseline liver tests of these 15 patients were fairly favorable, mostly Child–Pugh grade A, except for patient 6. However, there were six (patients 10–15)

patients that developed into grade B after TACE, and most of them experienced post-TACE icteric hepatitis.

Hepatitis B e-antigen was positive in three sessions, and negative in 12 sessions at baseline. Most of these remained unchanged except patient 7 who changed from HBeAg negative at baseline to positive after TACE.

The HBV DNA values at baseline ranged from undetectable (<100 IU/mL) to 1.2 × 10⁶ IU/mL with a median of 2.0 × 10³ IU/mL. HBV DNA reactivated after four sessions, downregulated after two and remained stable after nine sessions. HBV DNA values were more than 200 IU/mL in most (11/15, 73.3%) sessions

Table 5 Related laboratory features after TACE (*n* = 228)

Variables	Post-TACE (within 7 days)	<i>P</i> *	Post-TACE (30–90 days)	<i>P</i> *
WBC (10 ⁹ /L)	8.6 ± 3.3	<0.05	6.0 ± 2.0	<0.05
HGB (g/L)	126.8 ± 20.1	<0.05	129.0 ± 18.9	<0.05
PLT (10 ⁹ /L)	124.8 ± 75.3	<0.05	163.1 ± 82.0	<0.05
AST (u/L)	184.7 ± 218.3	<0.05	68.1 ± 57.9	NS
ALT (u/L)	183.3 ± 181.0	<0.05	55.9 ± 47.2	NS
ALB (g/L)	37.3 ± 4.7	<0.05	39.8 ± 4.4	<0.05
T-bil (μmol/L)	30.7 ± 18.5	<0.05	14.6 ± 9.7	NS
AFP (ng/mL)	NA	NA	13 755.4 ± 32 009.3	NS
PT (s)	NA	NA	12.5 ± 1.6	NS
APTT (s)	NA	NA	29.5 ± 5.1	NS
HBV DNA (log ₁₀ IU/mL)	NA	NA	3.9 ± 2.2	NS
HBeAg positive rate	NA	NA	24.1% (55/228)	NS
Child staging (A/B)	NA	NA	219/9	NS

*Compared to baseline.

AFP, α-fetoprotein; ALB, serum albumin; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; HBeAg, hepatitis B e-antigen; HGB, hemoglobin; HBV, hepatitis B virus; NA, non-applicable; NS, no significance; PLT, platelets; PT, prothrombin time; TACE, transcatheter arterial chemoembolization; T-bil, total bilirubin; WBC, white blood cells.

Table 6 Clinical features of 15 patients with exacerbation of liver damage after TACE

Pt. NO	Baseline characteristics										TACE					After TACE (within 60–90 days)				
	Sex/Age (year)	ALT (u/L)	ALB (g/L)	T-bil (μmol/L)	HBsAg	DNA value	Child grade	Lipiodol (mL)	Agents	ALT (u/L)	ALB (g/L)	T-bil (μmol/L)	HBsAg	DNA level	DC	Child grade	ST			
1	M/69	14.4	30.9	11.3	-	<100	A	10	1	261.4	32.8	26.7	-	<100	1	A	A*			
2	M/32	21.2	39.2	15.1	+	<100	A	10	1	100.4	44.9	9.8	+	<100	1	A	T*			
3	M/43	68	49.2	26	-	2.0 × 10 ³	A	20	2	240.9	40.9	14.8	-	1.6 × 10 ⁴	1	A	A*			
4	M/32	32.7	43	15	-	3.4 × 10 ³	A	22	2	104	35.9	11.3	-	2.4 × 10 ⁴	1	A	T*			
5	M/68	33.4	38.5	9.4	-	3.4 × 10 ³	A	6	1	117.1	42.7	17.2	-	1.2 × 10 ⁴	1	A	T*			
6	F/53	31.6	26.7	8.7	+	7.6 × 10 ⁵	B	15	2	115.3	31.4	10.9	+	3.8 × 10 ⁵	1	A	A*			
7	M/52	53	46.5	16	-	<100	A	5	2	341.2	44.5	16.1	+	2.4 × 10 ⁵	2	A	T*			
8	M/51	26.2	41.1	13.3	-	<100	A	25	2	191	43.3	11.2	-	3.2 × 10 ⁴	2	A	S*			
9	M/50	24	35.1	14.9	-	<100	A	20	2	129.5	32	10.9	-	2.4 × 10 ²	2	A	T*			
10	M/64	100	33.7	18.9	-	1.2 × 10 ⁶	A	10	1	246	29.2	10	-	8.4 × 10 ⁴	3	B	N*			
11	M/53	50	40.7	12.3	-	2.8 × 10 ⁴	A	10	2	172.2	40.8	65.4	-	3.0 × 10 ⁴	1	B	N*			
12	M/49	41	44.9	13.9	-	4.6 × 10 ³	A	8	2	219.7	40	58.4	-	<100	3	B	N*			
13	M/50	47	34.7	28.4	-	<100	A	8	2	77	38.4	79.6	-	<100	1	B	N†			
14	M/35	74.9	38.7	6.2	+	8.0 × 10 ⁵	A	20	2	52.9	32.2	52.7	+	2.4 × 10 ⁵	1	B	N‡			
15	M/72	67.6	42.5	30.7	-	3.8 × 10 ²	A	8	1	86.3	34	78.7	-	4.2 × 10 ³	2	B	N*			

*Still alive at 4 months follow up after TACE.

†Died 96 days after TACE.

‡Died 102 days after TACE.

DNA level: HBV DNA viral load value (IU/ml).

Agents: 1, epirubin only; 2, combination of more than two chemotherapeutic agents.

DNA changes (DC): 1, stable status; 2, reactivation; 3, downregulation.

A, focal ablation; ALB, serum albumin; ALT, alanine aminotransferase; N, no subsequent treatment; Pt, patient; S, sorafenib; ST, subsequent treatment; T, TACE; T-bil, total bilirubin.

(patients 3–11 and 14–15) after TACE, and lamivudine or entecavir was initiated in these patients early after the diagnosis of exacerbation of liver damage.

After supportive and/or antiviral treatment, most of the liver tests returned to relatively normal levels. Some of the patients received subsequent treatment (such as focal ablation, TACE or sorafenib) for HCC. However, the liver tests of patients 13 and 14 remained elevated and they died 96 and 102 days after the current TACE, respectively. Their HBV DNA status remained stable. The causes of death in these two patients were likely deterioration in liver function and progression of the liver tumors.

To determine factors related to cases of exacerbation of liver damage, 12 variables listed in Table 2 and HBV DNA status changes were analyzed. None of these variables showed a statistically significant relationship to exacerbation as determined by univariate or multivariate analysis (data not shown).

DISCUSSION

AS TO THE changes of HBV DNA status after TACE, some studies demonstrated that reactivation did occur, similar to systemic chemotherapy for hematological malignancies. Jang *et al.* demonstrated a 33.7% rate of HBV reactivation in HCC patients undergoing repeated transarterial chemo-lipiodolization (with a mean cycle number of >5).⁸ Some case reports also showed that TACE represents a major risk factor for HBV reactivation in HBV-related HCC patients.^{10,19,20} However, different or even controversial results were observed in other reports.^{11–14} A prospective study showed that a single session of TACE had little effect on the incidence of HBV reactivation, and usually the HBV reactivated patients recovered spontaneously.¹¹ Another study¹³ demonstrated that there were no significant changes of HBV DNA levels between the pre-TACE and post-TACE stage either in patients with or without exacerbation of liver damage. In a Chinese HBV-related HCC population,¹⁴ the positivity rate of HBV DNA decreased significantly from 71.6% (pre-TACE) to 55.6% (post-TACE). The controversies of the results may be due to the differences in study designs, HBV DNA assays, definitions of HBV reactivation or downregulation, regimens of chemotherapy and patient populations.

To the best of our knowledge, the current report describes the first large study to evaluate the possible changes in HBV DNA (including reactivation, downregulation and stable status simultaneously) after TACE. HBV DNA levels remained stable in the majority

(66.7%) of TACE sessions. The reactivation frequency was 14.5% in our study, lower than that in the treatment of hematological tumor reported previously.¹⁷ As to the reasons for this discrepancy, we believe that chemotherapeutic agents and being corticosteroid-free may play key roles because of their distinct influences on the extent of immunosuppression. It has been reported that immune suppression of greater intensity carried a higher risk for HBV reactivation, while mild chemotherapy carried a lower risk.^{21,22} The dosages of the chemotherapeutic agents we used were less than that of systemic chemotherapy for hematological malignancies. More importantly, no monoclonal antibodies such as rituximab (anti-CD20), alemtuzumab (anti-CD52) and infliximab (anti-tumor necrosis factor) were administered in our current study. These agents can cause strong and long-lasting immunosuppression, and contribute to a high risk of HBV reactivation, and subsequent fulminant hepatitis.^{5,17} Moreover, no corticosteroids were administered in the recruited patients in our current study, while “steroid-free” chemotherapy has been proposed to minimize the risk of HBV reactivation.^{5,23} Therefore, the agents used in our TACE are likely to be considered as low intensity chemotherapy with mild immune suppression, which may account for a relatively lower rate of HBV reactivation.

In our current study, 81.8% (27/33) of the reactivation cases administered combinations of chemotherapeutic agents, while 76.9% (117/152) of the stable cases and 60.5% (26/43) of the downregulation cases received combinations of chemotherapy (Table 2). But this difference was not statistically significant, and Figure 1 also demonstrates that there were no significant differences among the frequencies of reactivation, stable and downregulation status of the different groups of combinations of chemotherapeutic agents. This is probably due to the relatively low intensity chemotherapy with mild immune suppression in the current study discussed above.

Contrary to the results of some studies,^{8,18} the absence of HBeAg positivity appeared to be a risk factor for HBV reactivation compared to the HBV stability by multivariate analyses in the current study. The reason for the discrepancy is not clear, which may be correlated with the different study designs, regimens of chemotherapy, and patient populations and selection bias. In Yeo *et al.*'s study¹⁸ of cancer patients undergoing systemic cytotoxic chemotherapy, the recruited cancer type included breast, gastrointestinal, lung, lymphoma, head and neck, and gynecological cancer, while HCC was excluded. The risk factors for HBV reactivation of these

non-HCC cancer patients with HBV infection included male sex, younger age, HBeAg seropositivity and the diagnosis of lymphoma. But in another Yeo *et al.* study¹⁶ of HCC patients undergoing systemic cytotoxic chemotherapy, the only identifiable associated risk factor for HBV reactivation was elevated pretreatment ALT while HBeAg showed no influences. In Jang *et al.*'s study,⁸ HCC patients undergoing repeated transarterial chemolipiodolization (with a mean cycle number of >5), there were higher proportions (22.9%) of Child B/C patients, which are different in our current study (single TACE session and only 3.1% patients with Child B). Another possible reason may be attributed to the presence of the precore/core promoter HBV mutants.²⁴ One more possibility is that fluctuation of HBV DNA might easily develop among HBeAg negative patients, as compared with HBeAg positive patients. However, this needs to be further investigated. In addition, viral load is more likely to be closely related to HBeAg status because HBeAg is a marker of active viral replication, and HBeAg negativity and low baseline HBV DNA were correlated with reactivation in the present study. Therefore, caution should be exercised in HBV-related HCC patients regardless of the HBeAg status.

In 18.8% of sessions, there was HBV DNA downregulation after TACE. As far as we know, only one other study¹⁴ reported HBV DNA downregulation after TACE. In other studies, the terminology "non-reactivation" would pertain to both the "downregulation" and "stable" groups described in our study. Univariate (Table 2) and multivariate analyses (Table 3) showed that a high HBV viral load at baseline was a predictive factor for downregulation, a result similar to that of Xu *et al.* which demonstrated that patients with HBV DNA of more than 10^5 copies/mL had greater decreases in HBV viral load. That study also showed that the HBV DNA decrease was correlated with decreases in AFP. The mechanism by which downregulation of HBV occurs is unclear. Explanations include a natural course of chronic hepatitis B even without exposure to TACE,²⁵ natural spontaneous fluctuation in HBV DNA levels and possible tumor shrinkage after TACE.

Interestingly, sex also correlated with the HBV DNA downregulation in the univariate analysis. In the downregulation group, 11.6% of them were female (5/43), while only 2.6% were female in the stable group (4/152). In Yeo and Lok *et al.*'s studies,^{18,26} sex appeared to be a risk factor for HBV DNA reactivation after chemotherapy for non-HCC malignancies. The mechanism concerning the relationship between sex and HBV DNA changes remains unclear,

The laboratory parameters concerning the liver (ALT, AST and T-bil) were transiently increased within 1 week post-TACE (Table 5), which may reflect the effects of TACE on hepatic vasculature. The transiently increased indices returned to baseline levels within a few weeks or months, similar to the report of Ahmad *et al.*¹² Caturelli *et al.*²⁷ reported that tumor necrosis did not cause worsening of liver function after TACE in patients with Child A or B liver disease. A prospective study in Hong Kong also demonstrated that in the majority of their patients, liver tests after TACE returned to pretreatment levels with time, and only a minority of patients eventually developed irreversible liver failure.²⁸ Usually, most patients with favorable liver function have been able to tolerate more than one session of TACE.^{4,28} Of the 15 patients with exacerbation of liver damage in our study, in only two patients did liver function not recover after TACE (Table 6) and HBV DNA status remained stable in both cases. However, although the occurrence of exacerbation of liver damage was not significantly different among the three groups (Table 4) and HBV DNA status changes did not show a statistically significant relationship with it, the reactivation group had the highest rate of exacerbation (12.1%) compared with the stable group (5.9%) and downregulation group (4.7%). These data indicated that careful post-TACE monitoring is needed, especially in those with HBV DNA reactivation.

Another possible factor leading to liver damage is the direct effect of TACE (usually ischemia). Huo *et al.*²⁹ described a 45% incidence of ischemic hepatitis after TACE. Although ischemic hepatitis usually occurs immediately after embolization and persists for a variable period of time, it is difficult to judge the relationship in hepatitis that is attributable to viral reactivation. In Huo *et al.*'s opinion,³⁰ overt hepatitis may be due to either ischemia or viral reactivation, depending on the time point of serum biochemistry measurement, and both components may occur simultaneously in a substantial proportion of patients. To our best knowledge, there is no clear cut-off time point to divide ischemic hepatitis and non-ischemic hepatitis. As Huo *et al.*³⁰ demonstrated, the cause of overt hepatitis after TACE might be multifactorial in the usual clinical setting.

One more concern is that HBV DNA values after TACE were more than 200 IU/mL in 73.3% of cases with exacerbation of liver damage although its occurrence was not significantly related to HBV DNA status changes. These patients were treated with nucleoside analogs, and most of them recovered (Table 6). As to the antiviral therapy in HBV-related HCC patients undergoing TACE, there have been few reports concerning these

specific populations. Several studies and case reports demonstrated the efficacy of NUC in the prophylaxis and treatment of reactivation of HBV hepatitis.

In summary, HBV DNA levels after TACE included reactivation, downregulation and stable groups. In the present study, HBeAg negativity and low baseline HBV DNA were correlated with reactivation. Although HBV reactivation did not necessarily result in deterioration of hepatic functions, careful monitoring and management are needed in case of the potential occurrence. Conversely, those with exacerbation of liver function did not always have signs of HBV reactivation. All these data would raise caution to physicians involved in the treatment of HCC. One drawback of the current retrospective study is that serum HBV DNA and liver function could not be monitored over time or serially. Additional studies with longer and serial follow up concerning the correlation between viral kinetics and host immunity are needed. However, this study did reveal that most HBV-related HCC patients with Child–Pugh grade A and B are able to tolerate TACE with careful management.

ACKNOWLEDGMENTS

THE AUTHORS THANK Professors George Y. Wu and Ting-Tsung Chang for their insightful and valuable comments during the preparation of this manuscript. The authors thank Professor Qing Liu for his support in statistical analysis. This work was partly supported by the Eleventh Five-Year Key Plan of the China National Science and Technique Foundation (2006BAI02A04).

REFERENCES

- 1 Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; 55: 74–108.
- 2 Beasley RP, Hwang LY, Lin CC, Chien CS. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22 707 men in Taiwan. *Lancet* 1981; 2: 1129–33.
- 3 Llovet JM, Real MI, Montana X *et al.* Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002; 359: 1734–9.
- 4 Yuen MF, Chan AO, Wong BC *et al.* Transarterial chemoembolization for inoperable, early stage hepatocellular carcinoma in patients with Child-Pugh grade A and B: results of a comparative study in 96 Chinese patients. *Am J Gastroenterol* 2003; 98: 1181–5.
- 5 Lalazar G, Rund D, Shouval D. Screening, prevention and treatment of viral hepatitis B reactivation in patients with haematological malignancies. *Br J Haematol* 2007; 136: 699–712.
- 6 European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of chronic hepatitis B. *J Hepatol* 2009; 50: 227–42.
- 7 Bruix J, Llovet JM. Hepatitis B virus and hepatocellular carcinoma. *J Hepatol* 2003; 39 (Suppl 1): S59–63.
- 8 Jang JW, Choi JY, Bae SH *et al.* Transarterial chemoembolization can reactivate hepatitis B virus replication in patients with hepatocellular carcinoma. *J Hepatol* 2004; 41: 427–35.
- 9 Jang JW, Choi JY, Bae SH *et al.* A randomized controlled study of preemptive lamivudine in patients receiving transarterial chemo-embolization. *Hepatology* 2006; 43: 233–40.
- 10 Vizzini GB, Luca A, Marino IR. Hepatitis B virus reactivation after a single session of transarterial chemoembolization in patients with hepatocellular carcinoma. *Ann Intern Med* 2003; 138: 691–2.
- 11 Park JW, Park KW, Cho SH *et al.* Risk of hepatitis B exacerbation is low after transcatheter arterial chemoembolization therapy for patients with HBV-related hepatocellular carcinoma: report of a prospective study. *Am J Gastroenterol* 2005; 100: 2194–200.
- 12 Ahmad J, Rhee J, Carr BI. The effects of hepatic artery chemotherapy on viral hepatitis in patients with hepatocellular carcinoma. *Dig Dis Sci* 2005; 50: 331–5.
- 13 Nagamatsu H, Kumashiro R, Itano S, Matsugaki S, Sata M. Investigation of associating factors in exacerbation of liver damage after chemotherapy in patients with HBV-related HCC. *Hepatol Res* 2003; 26: 293–301.
- 14 Xu J, Wang YH, Xia JL, Ge NL, Chen Y, Ye SL. [Effect of transcatheter arterial chemoembolization on HBV DNA level in primary liver cancer patients.]. *Chin J Cancer* 2009; 28: 520–3.
- 15 Shi M, Chen JA, Lin XJ *et al.* Transarterial chemoembolization as initial treatment for unresectable hepatocellular carcinoma in southern China. *World J Gastroenterol* 2010; 16: 264–9.
- 16 Yeo W, Lam KC, Zee B *et al.* Hepatitis B reactivation in patients with hepatocellular carcinoma undergoing systemic chemotherapy. *Ann Oncol* 2004; 15: 1661–6.
- 17 Yeo W, Johnson PJ. Diagnosis, prevention and management of hepatitis B virus reactivation during anticancer therapy. *Hepatology* 2006; 43: 209–20.
- 18 Yeo W, Chan PK, Zhong S *et al.* Frequency of hepatitis B virus reactivation in cancer patients undergoing cytotoxic chemotherapy: a prospective study of 626 patients with identification of risk factors. *J Med Virol* 2000; 62: 299–307.
- 19 Tamori A, Nishiguchi S, Tanaka M *et al.* Lamivudine therapy for hepatitis B virus reactivation in a patient receiving intra-arterial chemotherapy for advanced hepatocellular carcinoma. *Hepatol Res* 2003; 26: 77–80.
- 20 Hung HH, Su CW, Wu JC, Lee SD. Reactivation of hepatitis B virus after transarterial chemo-embolization for hepatocellular carcinoma in one patient with negative hepatitis B surface antigen. *J Hepatol* 2010; 52: 463–5.

- 21 Lu W, Li YH, Yu ZJ *et al.* A comparative study of damage to liver function after TACE with use of low-dose versus conventional-dose of anticancer drugs in hepatocellular carcinoma. *Hepatogastroenterology* 2007; 54: 1499–502.
- 22 Liaw YF. Hepatitis viruses under immunosuppressive agents. *J Gastroenterol Hepatol* 1998; 13: 14–20.
- 23 Cheng AL, Hsiung CA, Su IJ *et al.* Steroid-free chemotherapy decreases risk of hepatitis B virus (HBV) reactivation in HBV-carriers with lymphoma. *Hepatology* 2003; 37: 1320–8.
- 24 Carman WF, Fagan EA, Hadziyannis S *et al.* Association of a precore genomic variant of hepatitis B virus with fulminant hepatitis. *Hepatology* 1991; 14: 219–22.
- 25 Wu IC, Chow NH, Cheng PN *et al.* Characterization of viral kinetics in patients with hepatitis B e antigen-positive chronic hepatitis B. *J Med Virol* 2007; 79: 663–9.
- 26 Lok AS, Liang RH, Chiu EK, Wong KL, Chan TK, Todd D. Reactivation of hepatitis B virus replication in patients receiving cytotoxic therapy. Report of a prospective study. *Gastroenterology* 1991; 100: 182–8.
- 27 Caturelli E, Siena DA, Fusilli S *et al.* Transcatheter arterial chemoembolization for hepatocellular carcinoma in patients with cirrhosis: evaluation of damage to nontumorous liver tissue-long-term prospective study. *Radiology* 2000; 215: 123–8.
- 28 Chan AO, Yuen MF, Hui CK, Tso WK, Lai CL. A prospective study regarding the complications of transcatheter intraarterial lipiodol chemoembolization in patients with hepatocellular carcinoma. *Cancer* 2002; 94: 1747–52.
- 29 Huo TI, Wu JC, Lee PC, Chang FY, Lee SD. Incidence and risk factors for acute renal failure in patients with hepatocellular carcinoma undergoing transarterial chemoembolization: a prospective study. *Liver Int* 2004; 24: 210–15.
- 30 Huo TI, Lee SD, Wu JC. Hepatitis after arterial embolization for hepatocellular carcinoma: viral reactivation or ischemia? *Hepatology* 2006; 43: 1400–1.