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• *BRIEF REPORTS* •

Quantitative analysis of plasma HBV DNA for early evaluation of the response to transcatheter arterial embolization for HBV-related hepatocellular carcinoma

Ying-Wen Su, Yu-Wen Huang, Sheng-Hsuan Chen, Chin-Yuan Tzen

Ying-Wen Su, Division of Hematology-Oncology, Department of Internal Medicine, Mackay Memorial Hospital, Taipei, Taiwan, China
Yu-Wen Huang, Department of Medical Research, Mackay Memorial Hospital, Taipei, Taiwan, China
Sheng-Hsuan Chen, Department of Gastroenterology, Taipei Medical University Hospital, Taipei, Taiwan, China
Chin-Yuan Tzen, Department of Medical Research, Department of Pathology, Mackay Memorial Hospital, Taipei, Taiwan, China
Supported by the grant NSC91-2314-B-195-026 from National Science Council, Taiwan, China
Co-first-authors: Ying-Wen Su
Correspondence to: Dr. Chin-Yuan Tzen, MD, PhD, Department of Pathology, Mackay Memorial Hospital, 45 Minsheng Road, Tamshui, Taipei, Taiwan, China. jeffrey@ms2.mmh.org.tw
Telephone: +886-2-28094661-2491 Fax: +886-2-28093385
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Abstract

AIM: To assesse changes in plasma HBV DNA after TAE in HBV-related HCC and correlate the levels with the pattern of lipiodol accumulation on CT.

METHODS: Between April and June 2001, 14 patients with HBV-associated HCC who underwent TAE for inoperable or recurrent tumor were studied. Levels of plasma HBV DNA were measured by real-time quantitative PCR daily for five consecutive days after TAE. More than twofold elevation of circulating HBV DNA was considered as a definite elevation. Abdominal CT was performed 1-2 mo after TAE for the measurement of lipiodol retention.

RESULTS: Circulating HBV DNA in 10 out of 13 patients was elevated after TAE, except for one patient whose plasma HBV DNA was undetectable before and after TAE. In group I patients (n = 6), the HBV DNA elevation persisted for more than 2 d, while in group II (n = 7), the HBV DNA elevation only appeared for 1 d or did not reach a definite elevation. There were no significant differences in age or tumor size between the two groups. Patients in group I had significantly better lipiodol retention (79.31±28.79%) on subsequent abdominal CT than group II (18.43±10.61%) (P = 0.02).

CONCLUSION: Patients with durable HBV DNA elevation for more than 2 d correlated with good lipiodol retention measured 1 mo later, while others associated with poor lipiodol retention. Thus, circulating HBV DNA may be an early indicator of the success or failure of TAE.

Key words: Hepatocellular carcinoma; Transcatheter arterial embolization; HBV DNA

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INTRODUCTION

In patients with advanced hepatocellular carcinoma (HCC), transcatheter arterial embolization (TAE) has been widely used in Asian countries[1,2]. After TAE, tumor necrosis appears to be complete by 2 d[3]. Circulating plasma DNA is increased after traumatic injury and has been postulated as a prognostic marker for such condition[4]. We wondered if there were other circulating markers that would allow an earlier assessment of response to treatment. Although HCC lacks universal oncogene, HBV is closely linked to HCC. Integrated HBV fragments are frequently detected in the tumors of patients with positive hepatitis B surface antigen (HBsAg)[5]. We therefore studied patients with HBV-related HCC to determine how their circulating plasma and HBV DNA changed after TAE and how those measurements correlated with lipiodol retention on computed tomography (CT).

MATERIALS AND METHODS

Patients

HBsAg-positive patients with advanced HCC between April and June 2001 at the Mackay Memorial Hospital, Taipei, Taiwan were considered for enrollment. Only those who had inoperable tumors that were also not suitable for percutaneous ethanol injection were included. Diagnosis of HCC was made by cytology or by elevated α-fetoprotein (AFP) and typical imaging results (CT and celiac angiography). Patients with contraindications for TAE (such as uncorrectable bleeding disorder, severe thrombocytopenia [platelet count <50 000], a history of radiocontrast allergy, Child's grade C liver cirrhosis, elevated serum creatinine [>2.0 mg/dL], or tumor thrombus in the main portal trunk) were excluded. All patients studied gave informed consent. Patients who could not complete five consecutive days of study were excluded from the final analysis.

TAE through selective hepatic arterial catheterization

was performed via a femoral artery by radiologists at Mackay Memorial Hospital. Under fluoroscopic guidance, 3-10 mL of iodized oil (Laboratoire Guerbet, France) was injected slowly followed by gelatin-sponge pieces (Gelfoam, Johnson and Johnson Med Ltd) and 1 g of cephazolin. Gelfoam was used to activate intravascular thrombosis and cephazolin was used to prevent bacterial infection. After embolization, angiography was performed to determine the extent of vascular occlusion and to assess blood flow in other arteries. After the procedure, patients received intravenous hydration, antiemetic medication, and acetaminophen for pain control. They were discharged from the hospital when oral intake was adequate.

Blood tests and follow-up studies

Blood samples for HBV DNA were taken before TAE and after the procedure every 18-24 h for five consecutive days. Liver function was evaluated before and at 1 wk and 1 mo after TAE to assess the degree of toxicity. Enhanced CT scans of the abdomen using a 10-mm thick slice were performed on a GE HiSpeed scanner (GE Medical Systems) 1-2 mo after TAE. The tumor volume and volume of tumor retaining lipiodol were calculated with GE HiSpeed CT/i system software by reconstructing and viewing the data on a GE Advantage Windows Workstation. The morphologic therapeutic response was assessed by calculation of lipiodol-retaining tumor volume as a percentage whole tumor volume: Percentage of lipiodol retention = (lipiodol-retained lesion volume)/(whole tumor volume)×100%.

Real-time quantitative PCR for HBV DNA

Real-time quantitative PCR analysis was performed on an ABI Prism 7700 Sequence detection system (PE Applied Biosystems) by using SYBR green I as a double-strand DNA-specific binding dye. Briefly, DNA was extracted from a 400 μL plasma sample by using a QIAamp Blood kit (Qiagen Ltd) according to the manufacturer's instruction. The primers for HBV DNA were 184F 5'-GGA CCC CTG CTC GTG TTA CA-3' and 273R 5'-GAG AGA AGT CCA CCM CGA GTC TAG A-3' according to Pas *et al*.[6]. A product of 89 bp from a region of the HBV pre-S gene was amplified. The region was chosen because the pre-S to S open reading frame is generally conserved whereas the core gene is frequently deleted and the X ORF is often truncated[7]. Each reaction mixture contained 5 μL of 10× SYBR Green PCR buffer, 25 mmol/L $MgCl_2$, 200 nmol/L of each deoxynucleoside triphosphate (dATP, dCTP, dTTP, and dGTP), Taq Gold DNA polymerase 0.25 μL, AmpErase uracil *N*-glycosylase (UNG) 0.5 μL, 100 nmol/L of forward and reverse primers and 5 μL of the DNA extract from the plasma sample. The final volume of each PCR reaction mixture was 50 μL. Each sample was assayed in triplicate. Multiple negative water blanks were included in each run as a negative control. Thermal cycling was performed under the following conditions: stage I: at 50 ℃ 2 min, during which UNG could inactivate possible contaminating amplicons, and at 95 ℃ for 10 min, to allow Taq Gold polymerase to activate and inactivate the UNG; stage II: 40 cycles at 95 ℃ for 15 s and 60 ℃ for 1 min, and then stage III: 95 ℃ for 15 s, followed by 60 ℃ for 2 min. After real-time PCR, the amplified products were gradually heated to 95 ℃ for about 20 min. The instrument automatically measured the changing level of fluorescence continuously. This analysis gave the melting temperature. HBV standards were prepared from the serum of two patients with recently reactivated hepatitis B. Amplified PCR products were cloned into pGEM®-T easy vector (Promega Co.) and transformed into JM 109 competent cells. Plasmid DNA was isolated using a plasmid DNA miniprep system (Viogene, USA). Plasmid preparations were quantified spectrophotometrically and standards were prepared by serial dilution from 4×10^1 to 4×10^8 copies/mL.

Data analysis

After amplification, data were captured onto a Macintosh computer, analysis was done with Sequence Detection software (PE Applied Biosystems). Data were collected at the annealing step (60 ℃) of every cycle, and the threshold cycle (Ct) for each sample was calculated by determining the point at which the fluorescence exceeded the threshold limit. A standard curve was calculated automatically by plotting the Ct values against standards of known concentration and calculation of the linear regression line of this curve. Calculation of the correlation coefficient (r^2) was done for each run, using a minimal value of 0.95. Sample copy numbers were calculated by interpolation of the experimentally determined standard curve. In this study, the percent coefficient of variation (%CV) of threshold cycle was 1.25% (range: 0-4.73%) for HBV DNA, similar to that of a previous report 19.

Changes in plasma HBV DNA and statistical analysis

Patient's plasma HBV DNA with twofold elevation was considered as a definite elevation. We classified the changes in plasma HBV DNA into two groups. Group I patients had an elevated HBV DNA level post-TAE for more than 2 d. In group II patients, the HBV DNA elevation only appeared for 1 d or did not reach the definite elevation before d 5. Data were reported as the mean±SD. Clinical characteristics and lipiodol retention of the two groups were compared by using Student *t* test and Fisher test. A P value <0.05 was considered statistically significant.

RESULTS

Fourteen HBsAg seropositive patients with HCC (12 men and 2 women) were studied. The average age was 62 years, ranging between 39 and 81 years. Thirteen had Child Class A and one had Child Class B cirrhosis. Five patients received TAE for the first time and the others were being treated again for recurrent disease. The post-embolization symptoms (fever, abdominal pain, and vomiting) in all patients were mild and subsided within one week. Liver function recovered completely in all patients within one week, and no subsequent liver dysfunction was noted over the course of the study.

Plasma HBV DNA changes after TAE

Except for one patient with undetectable plasma HBV DNA before and after TAE, 10 out of 13 patients had increased plasma HBV DNA after embolization. Six patients had HBV DNA rising for more than 2 d (group I). Among seven

patients in group II, three patients did not reach definite HBV elevation and the other four had temporal HBV DNA elevation within 3 d after TAE. The mean diameter of the embolized tumor in group I was 5.92±3.98 and 5.00±2.20 cm in group II, a non-significant difference (P = 0.61). The lipiodol retention in the embolized tumors in group I was 79.31±28.79% and in group II, 18.43±10.61%, a statistically significant difference (P = 0.02). No significant differences were observed between the two groups regarding age or tumor size (Table 1).

Table 1 Basic characteristics before TAE and lipiodol retention percentage after TAE of group I and group II patients

	Group I (n = 6)	Group II (n = 7)	P
Age, yr (range)	57.6 (39-79)	65.6 (38-81)	0.34
Gender (male/female)	6/0	5/2	-
Pretreatment AFP (ng/mL)	707±1 536	3 024±5 307	0.32
Tumor size (cm)	5.92±3.98	5.00±2.20	0.61
Disease status at entry			-
For the first time TAE (n)	3	2	
For recurrence (n)	3	5	
Lipiodol retention (%)	79.31±28.79	18.43±10.61	0.02[a]

n: patient number. Statistical data are expressed as mean±SD. [a]P<0.05, with statistical significance.

DISCUSSION

Our study showed a significant correlation between durable elevation of plasma HBV DNA concentration after TAE and lipiodol retention one month later. Group I patients with an persistently elevated HBV DNA level apparently sustained more tumor damage after TAE as evidenced by good lipiodol retention, while group II patients with temporary or no elevation in HBV DNA probably had unsuccessful TAE, as indicated by poor lipiodol retention.

The anticancer effects caused by TAE are due to selective blockade of the tumor blood supply. Integrated HBV DNA can be detected in both tumor and nontumor liver tissues of HBV carriers[8]; so the immediate elevation of plasma HBV DNA after TAE might come from damages to the two parts of the same arterial territory. Both integrated HBV DNA and non-integrated episomal HBV DNA are included. Although it has been recently published that HBV may be reactivated by transarterial chemoembolization (TACE), the reported time to reactivation after the last TACE was more than 25 d[9]. In our study, none of our patients had persistently abnormal or deteriorating liver dysfunction after TAE, suggesting that HBV reactivation was not likely to occur within the short period.

With gelfoam embolization, it can produce temporary or permanent occlusion of the targeted artery[10]. From our results, plasma HBV DNA elevated within short-term follow-ups in 10 out of 13 patients. They can be considered as consequences of ischemic injury to liver although the effects may be temporary or persistent. Persistence of HBV DNA in our patients indicates that the occlusion of tumor feeding artery by TAE was complete. It correlated with good lipiodol retention, a factor known to predict a good response. Among patients without persistent elevation of circulating DNA (group II), they tend to have less successful TAE, which may be due to technical or tumor factors (such as incomplete arterial occlusion, presence of collateral blood supply, or tumors with resistance to reperfusion injury)[11-15]. In such patients, other treatment modalities should be considered early to achieve better tumor control.

Monitoring the outcome of TAE is important because it is an important prognostic factor influencing management decisions, such as retreating, incorporation of various non-surgical procedures (e.g., percutaneous ethanol injection, intra-arterial chemotherapy, or local ablation techniques), or deciding to withhold further anti-tumor therapy[16]. In order to evaluate response to TAE, clinicians commonly use tumor regression, AFP levels, and the degree of lipiodol accumulation on CT. All these methods have limitations. Tumor regression is seldom recognized before 4 wks after TAE, and some tumors fail to shrink despite complete necrosis found on pathology of resected lesions[17,18]. Although a good correlation has been found between histologically proven tumor necrosis and reduction in AFP, this correlation has only been demonstrated in patients with a pre-TAE AFP level $\geqslant$200 ng/mL[19]. This marker is therefore of little use in patients who do not have markedly elevated AFP before TAE. The degree of lipiodol retention also correlates well with the degree of necrosis[18]. Compact lipiodol uptake has also been correlated with a better chance of survival than less compact uptake, indicating its usefulness as a post treatment prognostic marker. However, the need to wait 4 wk after treatment for the agent to be cleared from the uninvolved hepatic parenchyma[20] limits its usefulness. In some cases, hepatic washout is particularly slow, making interpretation of the CT image difficult[21].

Recent data indicate that human plasma DNA possesses a short half-life in the circulation[22]. The rapid kinetics suggests that circulating DNA analysis may be useful in monitoring clinical progress. One potential limitation of its clinical application is that serial blood sampling after TAE may be inconvenient for patients. If, as implied by this preliminary study, circulating plasma DNA persistently correlates with lipiodol retention, it may prove to be a useful tool for early evaluation of the response to TAE. If further studies support our findings, circulating levels of HBV DNA after TAE may be an early indicator for which patients require additional treatment.

REFERENCES

1 **Liu CL**, Fan ST. Nonresectional therapies for hepatocellular carcinoma. *Am J Surg* 1997; **173**: 358-365
2 **Acunas B**, Rozanes I. Hepatocellular carcinoma: treatment with transcatheter arterial chemoembolization. *Eur J Radiol* 1999; **32**: 86-89
3 **Yoshida T**, Sakon M, Umeshita K, Kanai T, Miyamoto A, Takeda T, Gotoh M, Nakamura H, Wakasa K, Monden M. Appraisal of transarterial immunoembolization for hepatocellular carcinoma: a clinicopathologic study. *J Clin Gastroenterol* 2001; **32**: 59-65
4 **Lo YM**, Rainer TH, Chan LY, Hjelm NM, Cocks RA. Plasma DNA as a prognostic marker in trauma patients. *Clin Chem* 2000; **46**: 319-323
5 **Lai MY**, Chen DS, Chen PJ, Lee SC, Sheu JC, Huang GT, Wei TC, Lee CS, Yu SC, Hsu HC. Status of hepatitis B virus DNA

in hepatocellular carcinoma: a study based on paired tumor and nontumor liver tissues. *J Med Virol* 1988; **25**: 249-258

6 **Pas SD**, Fries E, De Man RA, Osterhaus AD, Niesters HG. Development of a quantitative real-time detection assay for hepatitis B virus DNA and comparison with two commercial assays. *J Clin Microbiol* 2000; **38**: 2897-2901

7 **Brechot C**. Pathogenesis of hepatitis B virus-related hepatocellular carcinoma: old and new paradigms. *Gastroenterology* 2004; **127**(5 Suppl 1): S56-S61

8 **Shafritz DA**, Shouval D, Sherman HI, Hadziyannis SJ, Kew MC. Integration of hepatitis B virus DNA into the genome of liver cells in chronic liver disease and hepatocellular carcinoma. Studies in percutaneous liver biopsies and post-mortem tissue specimens. *N Engl J Med* 1981; **305**: 1067-1073

9 **Jang JW**, Choi JY, Bae SH, Kim CW, Yoon SK, Cho SH, Yang JM, Ahn BM, Lee CD, LeeYS, Chung KW, Sun HS. Transarterial chemo-lipiodolization can reactivate hepatitis B virus replication in patients with hepatocellular carcinoma. *J Hepatol* 2004; **41**: 427-435

10 **Huang MS**, Lin Q, Jiang ZB, Zhu KS, Guan SH, Li ZR, Shan H. Comparison of long-term effects between intra-arterially delivered ethanol and Gelfoam for the treatment of severe arterioportal shunt in patients with hepatocellular carcinoma. *World J Gastroenterol* 2004; **10**: 825-829

11 **Liou TC**, Shih SC, Kao CR, Chou SY, Lin SC, Wang HY. Pulmonary metastasis of hepatocellular carcinoma associated with transarterial chemoembolization. *J Hepatol* **23**: 563-568

12 **Savastano S**, Miotto D, Casarrubea G, Teso S, Chiesura-Corona M, Feltrin GP. Transcatheter arterial chemoembolization for hepatocellular carcinoma in patients with Child's grade A or B cirrhosis: a multivariate analysis of prognostic factors. *J Clin Gastroenterol* 1999; **28**: 334-340

13 **Sonomura T**, Yamada R, Kishi K, Nishida N, Yang RJ, Sato M. Dependency of tissue necrosis on gelatin sponge particle size after canine hepatic artery embolization. *Cardiovasc Intervent Radiol* 1997; **20**: 50-53

14 **Yamakado K**, Nakatsuka A, Tanaka N, Matsumura K, Takase K, Takeda K. Long-term follow-up arterial chemoembolization combined with transportal ethanol injection used to treat hepatocellular carcinoma. *J Vasc Interv Radiol* 1999; **10**: 641-647

15 **Selzner M**, Rudiger HA, Selzner N, Thomas DW, Clavien PA. Transgenic mice overexpressing human Bcl-2 are resistant to hepatic ischemia and reperfusion. *J Hepatol* 2002; **36**: 218-225

16 **Catalano O**, Esposito M, Lobianco R, Cusati B, Altei F, Siani A. Hepatocellular carcinoma treated with chemoembolization: assessment with contrast-enhanced doppler ultrasonography. *Cardiovasc Intervent Radiol* 1999; **22**: 486-492

17 **Solomon B**, Soulen MC, Baum RA, Haskal ZJ, Shlansky-Goldberg RD, Cope C. Chemoembolization of hepatocellular carcinoma with cisplatin, doxorubicin, mitomycin-C, ethiodol, and polyvinyl alcohol: prospective evaluation of response and survival in a U.S. population. *J Vasc Interv Radiol* 1999; **10**: 793-798

18 **Takayasu K**, Arii S, Matsuo N, Yoshikawa M, Ryu M, Takasaki K, Sato M, Yamanaka N, Shimamura Y, Ohto M. Comparison of CT findings with resected specimens after chemoembolization with iodized oil for hepatocellular carcinoma. *Am J Roentgenol* 2000; **175**: 699-704

19 **Okusaka T**, Okada S, Ueno H, Ikeda M, Yoshimori M, Shimada K, Yamamoto J, Kosuge T, Yamasaki S, Iwata R, Furukawa H, Moriyama N, Sakamoto M, Hirohashi S. Evaluation of the therapeutic effect of transcatheter arterial embolization for hepatocellular carcinoma. *Oncology* 2000; **58**: 293-299

20 **Choi BI**, Kim HC, Han JK, Park JH, Kim YI, Kim ST, Lee HS, Kim CY, Han MC. Therapeutic effect of transcatheter oily chemoembolization therapy for encapsulated nodular hepatocellular carcinoma: CT and pathologic findings. *Radiology* 1992; **182**: 709-713

21 **Lencioni R**, Pinto F, Armillotta N, Di Giulio M, Gaeta P, Di Candio G, Marchi S, Bartolozzi C. Intrahepatic metastatic nodules of hepatocellular carcinoma detected at lipiodol-CT: imaging-pathologic correlation. *Abdom Imaging* 1997; **22**: 253-258

22 **Lo YM**, Zhang J, Leung TN, Lau TK, Chang AM, Hjelm NM. Rapid clearance of fetal DNA from maternal plasma. *Am J Hum Genet* 1999; **64**: 218-224

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