Prognostic Significance of Simultaneous Measurement of Three Tumor Markers in Patients With Hepatocellular Carcinoma

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Background & Aims: **We conducted a prospective study to evaluate the significance of simultaneous measurement of 3 currently used tumor markers in the evaluation of tumor progression and prognosis of patients with hepatocellular carcinoma (HCC).** ***Methods:*** **Three tumor markers for HCC, alpha-fetoprotein (AFP),** ***Lens culinaris*** **agglutinin A–reactive fraction of AFP (AFP-L3), and des-gamma-carboxy prothrombin (DCP), were measured in the same serum samples obtained from 685 patients at the time of initial diagnosis of HCC. Positivity for AFP >20 ng/dL, AFP-L3 >10% of total AFP, and/or DCP >40 mAU/mL was determined. In addition, tumor markers were measured after treatment of HCC.** ***Results:*** **Of the 685 patients, 337 (55.8%) were positive for AFP, 206 (34.1%) were positive for AFP-L3, and 371 (54.2%) were positive for DCP. In a comparison of patients positive for only 1 tumor marker, patients positive for AFP-L3 alone had a greater number of tumors, whereas patients positive for DCP alone had larger tumors and a higher prevalence of portal vein invasion. When patients were compared according to the number of tumor markers present, the number of markers present clearly reflected the extent of HCC and patient outcomes. The number of markers present significantly decreased after treatment.** ***Conclusions:*** **Tumor markers AFP-L3 and DCP appear to represent different features of tumor progression in patients with HCC. The number of tumor markers present could be useful for the evaluation of tumor progression, prediction of patient outcome, and treatment efficacy.**

Hepatocellular carcinoma (HCC) is one of the most common malignancies, especially in southern and eastern Asia. Currently in Japan, HCC is the third leading cause of death from cancer. The development of various scanning techniques and the identification of sensitive and specific tumor markers for HCC have contributed not only to detection of HCC but also to evaluation of progression of HCC and determination of patient prognosis.

Three tumor markers specific for HCC are currently used clinically in Japan: alpha-fetoprotein (AFP), *Lens culinaris* agglutinin A–reactive fraction of AFP (AFP-L3), and des-gamma carboxy prothrombin (DCP), which is also called protein induced by vitamin K absence-II (PIVKA-II). Usefulness of the measurement of each of these tumor markers for detection and diagnosis of HCC, for evaluation of tumor progression, and for determination of patient prognosis has been reported.[1–4] However, these 3 tumor markers have not been evaluated together for evaluation of the progression of HCC and prediction of patient outcome.

In the present study, we measured levels of these 3 tumor markers simultaneously at the time of HCC diagnosis, and we analyzed them with respect to tumor progression and patient survival.

Patients and Methods

Patients

A total of 689 patients were diagnosed as having initial HCC (not recurrence) and treated at Ogaki Municipal Hospital between 1995 and 2004. Of these patients, 685 were enrolled in this prospective study; the remaining 4 patients were excluded because they were taking warfarin or vitamin K, which would influence the serum DCP level. The study group comprised 497 men and 188 women, with a mean age of 66.7 ± 8.7 years (median, 67 years; range, 31–93 years). Characteristics of the patients are shown in Table 1. HBV infection was detected in 104 patients, HCV in 508 patients, and both HBV and HCV in 13 patients; no hepatitis virus was detected in the remaining 60 patients. Of the 685 patients, 193 underwent

Abbreviations used in this paper: AFP, alpha-fetoprotein; AFP-L3, *Lens culinaris* agglutinin A–reactive fraction of AFP; DCP, des-gamma-carboxy prothrombin; HCC, hepatocellular carcinoma; LAT, locoregional ablative therapy; PIVKA-II, protein induced by vitamin K absence-II; TACE, transcatheter arterial chemoembolization.

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Table 1. Clinical Characteristics of the Study Patients (n = 685)

Age (y)	66.7 ± 8.7
Sex (female/male)	188 (27.4)/497 (72.6)[a]
Etiology of underlying liver disease (HBV/HCV/HBV, HCV/non-HBV, non-HCV)	104 (15.2)/508 (74.2)/13 (1.9)/60 (8.7)[a]
Child-Pugh class (A/B/C)	444 (64.8)/194 (28.3)/47 (6.9)[a]
Albumin (g/dL)	3.44 ± 0.60
Total bilirubin (mg/dL)	1.13 ± 1.44
Prothrombin time (%)	82.9 ± 17.6
15-minute retention of ICG (%)	22.9 ± 17.0
Size of largest tumor (cm)	3.58 ± 3.02
Number of tumors	1.86 ± 1.77
Portal vein thrombosis (−/+)	593 (86.6)/92 (13.4)[a]
AFP (ng/dL)	27.0 (0.8–2,402,000)[b]
AFP-L3 (%)	0.5 (0–92.1)[b]
DCP (mAU/mL)	52.0 (10.0–75,000)[b]
Treatment	
No treatment	90 (13.1)[a]
Hepatectomy	193 (28.2)[a]
LAT	166 (24.2)[a]
TACE	196 (28.6)[a]
Others	40 (5.9)[a]

ICG, indocyanine green test.
[a]Percentages are shown in parentheses.
[b]Ranges are shown in parentheses.

hepatectomy, 166 were treated by locoregional ablative therapy (LAT) including ethanol injection, microwave thermocoagulation, or radiofrequency ablation, and 196 were treated by transcatheter arterial chemoembolization (TACE).

The study protocol was approved by the hospital ethics committee and was in compliance with the Helsinki Declaration. Written informed consent was obtained from all patients before the study for use of the pathology and laboratory data.

Measurement of the Three Tumor Markers

AFP, AFP-L3, and DCP were measured in the same serum sample at the time of HCC diagnosis. In patients who underwent hepatectomy, LAT, or TACE as a treatment for HCC, they were also measured approximately 1 month after the end of therapy. The serum AFP level was determined by enzyme-linked immunosorbent assay with a commercially available kit (ELISA-AFP; International Reagents, Kobe, Japan). A cutoff value of 20 ng/mL AFP was used to establish positivity for AFP, as proposed by Oka et al[5] and Koda et al.[6] Serum AFP-L3 was measured by lectin-affinity electrophoresis coupled with antibody-affinity blotting (AFP Differentiation Kit L; Wako Pure Chemical Industries, Ltd, Osaka, Japan) and was finally expressed as the percentage of AFP-L3 (AFP-L3 level/total AFP level × 100).[7,8] The cutoff value used to establish positivity for AFP-L3 was AFP-L3 10%, as proposed by Shimizu et al.[9] The serum DCP level was determined by sensitive enzyme immunoassay (Eitest PIVKA-II kit; Eisai Laboratory, Tokyo, Japan) according to the manufacturer's instructions.[10–12] The cutoff value used to establish positivity for DCP was 40 mili arbitrary unit (mAU)/mL, as proposed by Okuda et al.[13]

Characteristics of HCC such as size of tumor, number of tumors, presence of portal vein thrombosis, and tumor stage according to American Joint Committee on Cancer (AJCC) and patient survival rates were compared according to tumor markers. In patients who underwent hepatectomy, LAT, or TACE, the changes in the status of tumor markers before and after treatment were analyzed.

Statistical Analyses

Data are expressed as mean ± standard deviation values or the median and range unless otherwise specified. Differences in proportions of number of patients between groups were analyzed by χ^2 test. Differences in quantitative values were analyzed by Student t test if the data were normally distributed; otherwise, differences were analyzed by Mann–Whitney U test. The date of HCC diagnosis was defined as time zero for calculations of patient survival. Surviving patients and patients who died of causes other than liver disease were censored. Patients who died of HCC-related causes or liver failure were not censored. The Kaplan–Meier method[14] was used to calculate survival rates, and the log-rank test[15] was used to analyze differences in survival.

The Cox proportional hazards model[16] was used for multivariate analysis for factors that influenced patient survival. The variables analyzed were age, sex, Child-Pugh class (A vs B, C), initial treatment (no treatment vs hepatectomy, LAT, TACE, or other treatment), and the number of tumor markers present (none, 1, 2, or 3). The JMP statistical software package, version 4.0 (SAS Institute, Cary, NC), was used for all statistical analyses. All P values were derived from two-tailed tests, and $P < .05$ was accepted as statistically significant.

Results

Serum AFP was above the cutoff level in 387 of the 685 patients (56.5%), serum AFP-L3 was above the cutoff level in 227 patients (33.1%), and serum DCP was above the cutoff level in 371 patients (54.2%). No tumor markers were above the cutoff level in 159 patients (23.2%). Only 1 of 3 tumor markers was above the cutoff level in 220 patients (32.1%; only AFP in 96 patients, only AFP-L3 in 14 patients, and only DCP in 110 patients). Two of 3 tumor markers were above the cutoff level in 153 patients (22.3%; AFP and AFP-L3 in 44 patients, AFP and DCP in 72 patients, and AFP-L3 and DCP in 15 patients). All 3 tumor markers were above the cutoff level in the remaining 153 patients (22.3%) (Figure 1).