

Usefulness of serum des- γ -carboxy prothrombin in detection of hepatocellular carcinoma

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Abstract

AIM: To evaluate whether DCP is better than AFP for differentiating HCC from nonmalignant liver disease and further evaluate the usefulness of DCP in early diagnosis of small HCC.

METHODS: Serum DCP and AFP levels were determined in 127 patients. Among these patients, 32 were with non-cirrhotic chronic hepatitis, 34 were with compensated cirrhosis, and 61 were with HCC. The cut-off value for the DCP and AFP were set as 40 mAU/mL and 20 ng/mL, respectively. To compare the diagnostic value of DCP and AFP in distinguishing HCC from nonmalignant chronic liver disease, receiver operating characteristic (ROC) curves were constructed for each assay.

RESULTS: The accuracy, sensitivity, and specificity of DCP were higher than AFP in detecting HCC (81.9%, 77%, and 86.4% vs 68.5%, 59%, and 77.3%, respectively). The area under the ROC (AUROC) curves revealed that DCP had a better accuracy than AFP in diagnosis of HCC (0.85 [95%CI, 0.78-0.91] vs 0.73 [95%CI, 0.65-0.81], $P = 0.013$). In 39 patients with solitary HCC, the positive rates of DCP were 100% in patients with tumor size larger than 3 cm, 66.7% in patients with tumor size 2-3 cm and 50% in patients with tumor size less than 2 cm. The positive rates of AFP in patients with tumor size larger than 3 cm, 2-3 cm and less than 2 cm were 55.6%, 50%, and 33.3%, respectively. The median level of DCP in HCC patients with tumor size larger than 3 cm was significantly higher than those with tumor size 2-3 cm and those with the size of less than 2 cm.

CONCLUSION: Our study indicates that DCP has a better diagnostic value than AFP in differentiating HCC from

nonmalignant chronic liver disease. DCP has not only a stronger correlation with HCC than AFP in tumor size but also more effectiveness than AFP in detecting small size of HCC.

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Key words: Des- γ -carboxy prothrombin; α -Fetoprotein; Hepatocellular carcinoma

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INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers in the world^[1]. The major causes of HCC are through chronic infection with HBV or hepatitis C virus (HCV)^[2], cirrhosis and finally culminating into HCC^[3,4]. HCC tends to occur in a definable population, so-called high risk population. Periodic screening among the high risk population would benefit in detecting HCC in an early curable stage and yield a long-term survival^[5,6]. However, the worldwide 5-year survival rate of HCC only slightly increased from 2% to 5% over the past two decades^[7]. This is partially due to the poor performance of currently available tumor markers, including α -fetoprotein (AFP), which cause the delay in diagnosis^[8]. There is a need to develop an additional sensitive serum marker to improve the early detection of small HCC.

In 1984, Liebman *et al.*, firstly reported the increasing levels of DCP in patients with HCC^[9]. Since then, numerous studies indicated that DCP would be a useful marker in detecting HCC^[10-14]. The conventional enzyme immunoassay (EIA) for DCP can detect advanced HCC with a high specificity. But the conventional EIA was limited in detecting small HCC^[14-17]. A new method, revised EIA (Eitest PIVKA-II, Eisai, Tokyo, Japan), had been developed in recent years. The new sensitive DCP kit can measure the low concentration of serum DCP in normal persons. Thereafter, the diagnostic sensitivity of DCP for HCC had been much improved. It had been reported to be more sensitive and specific than AFP for the diagnosis of HCC^[18,19]. However, the efficacy of DCP in diagnosis of small HCC still remains unclear.

Thus, we conducted a cross-sectional case control study to evaluate whether DCP is better than AFP for differentiating HCC from nonmalignant liver disease and further evaluate the usefulness of DCP in early diagnosis of small HCC.

MATERIALS AND METHODS

Patients

A total of 127 patients who were regularly followed up at Ren-Ai Branch, Taipei City Hospital were consecutively enrolled. Among the 127 patients, 32 had chronic hepatitis with higher serum alanine aminotransferase (ALT) levels than normal (upper limit of normal: 40 U/L) for at least 6 mo before enrollment, 34 had compensated cirrhosis (Child-Pugh score < 7) (20) and 61 had HCC. Cirrhosis was defined by clinical development of esophageal varices, thrombocytopenia (platelet count less than 100 000/mm³), splenomegaly or small liver size with irregular liver surface to be noted by imaging studies at enrollment. Among all patients with chronic hepatitis and cirrhosis, HCC must be ruled out on the basis of imaging examinations including sonography and/or computed tomography (CT) performed on a regular examination. Also, cirrhotic patients who developed HCC within 6 mo after getting serum were excluded. The diagnosis of HCC was made on 47 (77%) histologically confirmed patients. The remaining 14 (23%) patients, who had advanced HCC with tumor size larger than 3 cm or patients with portal vein invasion, were confirmed by various combination of imaging studies, such as ultrasonography, enhanced CT, magnetic resonance imaging and/or angiography. Tumor size was estimated by using ultrasonography. Blood samples from HCC patients were drawn before initial treatment. The rest of the data obtained at enrollment included hepatitis B surface antigen (HBsAg), antibody to HCV (anti-HCV), ALT, albumin, total bilirubin, platelet count, prothrombin time, and serum level of tumor markers, AFP and DCP. Patients who had a history of alcohol consumption in excess of 80 g/ethanol per day for more than 5 years, serum total bilirubin level of more than 20 mg/L or under vitamin K medication were excluded. All subjects had given informed consent for the participation in the study. Serum samples were collected and stored at -70 °C until examined.

Biochemical and serological testing

The biochemical tests were measured by using routine automated methods. The HBsAg, anti-HCV were assayed by commercial kits (General Biological HBsAg RIA, General Biological Cooperation, Taiwan; HCV EIA II, Abbott Laboratories, North Chicago, IL, USA).

AFP assay

AFP was tested by using commercially available immunometric assay (Architect AFP assay, Abbott Laboratories, North Chicago, IL, USA). The cut-off value of AFP for HCC was set at 20 ng/mL, the most commonly set value^[21-24].

DCP assay

DCP level was measured by using an ELISA (Eitest PIVKA-II, Eisai Co., Tokyo, Japan), according to the manufacturer's

instructions. The detection limit is 10 mAU/mL. The cut-off value is determined as 40 mAU/mL for differentiation of HCC and nonmalignant liver disease based on previous studies^[19].

Histological grading of HCC

Based on the criteria proposed by the Liver Cancer Study Group of Japan^[25], each HCC was histologically graded into well differentiated, moderately differentiated, or poorly differentiated. Patients with multiple tumors consisted of more than two grades of histological differentiation; therefore, the most dedifferentiated grade was used.

Statistical analysis

Values were presented as mean ± SD and median:range. Data were analyzed by χ^2 test, Fisher's exact test, Student's *t*-test, Kruskal-Wallis ANOVA median test, and Pearson correlation where appropriate. All of the tests of significance were two-tailed and a *P* value of less than 0.05 was considered statistically significant. To compare the accuracy of DCP and AFP in the diagnosis of HCC, receiver operating characteristic (ROC) curves were constructed by using all possible cut-offs for each assay. The area under the ROC (AUROC) curves were calculated and compared by using a computer program of the MedCalc software version 7.5 (Mariakerke, Belgium).

RESULTS

Patient characteristics

Serum DCP and AFP levels were determined in 32 patients with chronic hepatitis, 34 patients with cirrhosis and 61 patients with HCC (their clinical characteristics were summarized in Table 1). The three groups were comparable in terms of gender, prevalence of HBsAg and anti-HCV. The mean age of HCC patients was significantly higher than those of chronic hepatitis patients and cirrhotic patients (63 ± 13 years vs 52 ± 10 years and 57 ± 12 years respectively, *P* < 0.001).

DCP and AFP in patients with chronic hepatitis, cirrhosis, and HCC

Both DCP and AFP levels increased progressively from nonmalignant chronic liver disease to HCC (Table 1). The mean values of DCP in patients with HCC was significantly higher than patients with chronic hepatitis and cirrhosis (2 808 ± 6 216 vs 21 ± 9 and 33 ± 14 mAU/mL, respectively, *P* < 0.001). In all, 2 of 32 (6.3%) patients with chronic hepatitis, 7 of 34 (20.6%) patients with cirrhosis, and 47 of 61 (77%) with HCC had DCP levels above the cut-off value of 40 mAU/mL.

The mean values of AFP were comparable among the three groups of patients (54 174 ± 296 329 vs 15 ± 24 and 13 ± 14 ng/mL, respectively *P* > 0.05). The AFP level above 20 ng/mL was found in 36 of 61 (59%) HCC, in 9 of 34 (26.5%) cirrhosis and in 6 of 32 (18.8%) chronic hepatitis patients. The overall accuracy, sensitivity, specificity, and positive and negative predictive values for the usefulness in the diagnosis of patients with HCC are shown in Table 2. ROC curves were plotted to compare the accuracy of DCP and AFP in the diagnosis of HCC, AUROC curves indicated

a better accuracy for DCP than AFP in diagnosis of HCC (0.85 [95%CI, 0.78-0.91] *vs* 0.73 [95%CI, 0.65-0.81], $P = 0.013$, Figure 1).

Relation between serum levels of AFP and DCP

Both DCP and AFP levels above cut-off value were found in 32 (52.5%) of 61 patients with HCC. Fifteen (60%) of twenty-five patients with AFP level less than 20 ng/mL had elevated concentrations of DCP. We failed to find any correlation between serum levels of AFP and DCP in 61 HCC patients (Figure 2, $r = 0.12$, $P = 0.36$).

Positive rates of DCP and AFP in patients with HCC

The positive rates of DCP and AFP in patients with HCC were detailed as follows: (1) When the largest size of HCC was more than 3 cm ($n = 22$), between 2 and 3 cm ($n = 16$) and less than 2 cm ($n = 23$), the rates were 100%, 75%, and 56.5% for DCP, 77.3%, 56.3%, and 43.5% for AFP;

(2) When HCC was moderately to poorly differentiated ($n = 39$) and well differentiated ($n = 8$), the rates were 76.9% and 37.5% for DCP, 53.8% and 12.5% for AFP; (3) When the number of HCC were more than 2 ($n = 22$) and single ($n = 39$), the rates were 95.5% and 66.7% for DCP, 86.4% and 43.6% for AFP (Table 3).

The relation between the tumor size of HCC and levels of DCP and AFP were further analyzed. In the 39 patients with solitary HCC, 9 more than 3 cm, 12 between 2 and 3 cm, and 18 less than 2 cm, the positive rates were 100%, 66.7%, and 50% for DCP, 55.6%, 50%, and 33.3% for AFP respectively. DCP also had a better correlation with tumor size in comparison with AFP. The median (range) levels of DCP in patients with a size of HCC more than 3 cm was significantly higher than those with 2-3 cm and less than 2 cm [6 729 (40.4-20 000) *vs* 159 (12-655) and 89 (7-348) mAU/mL, respectively, $P = 0.01$] (Figure 3).

Table 1 Clinical characteristics of 127 patients with different stages of liver disease

	Chronic hepatitis ($n = 32$)	Cirrhosis ($n = 34$)	HCC ($n = 61$)	P
Age (yr/o)	52±10	57±12	63±13	<0.001 ^a
Male/female	2.2	2.8	3.4	NS
Positive for HBsAg	17	18	28	NS
Positive for anti-HCV	15	13	24	NS
Positive for both HBsAg and anti-HCV	0	1	4	
Negative for both HBsAg and anti-HCV	0	2	4	
DCP level (mAu/mL), mean±SD	21±9	33±14	2 808±6 216	<0.001 ^a
AFP level (ng/mL), mean±SD	15±24	13±14	54 174±296 329	NS

^a $P < 0.05$, HCC *vs* chronic hepatitis and cirrhosis. HBsAg: hepatitis B surface antigen; anti-HCV: antibody to hepatitis C virus; DCP: des- γ -carboxy prothrombin; AFP: α -fetoprotein.

Table 2 Diagnostic values of DCP and AFP for the detection of HCC

Groups	Case number	DCP		AFP		DCP/AFP	
		40 mAU/mL		20 ng/mL		DCP 40 mAU/mL or AFP 20 ng/mL	
		Positive	Negative	Positive	Negative	Positive	Negative
Nonmalignant liver disease							
Chronic hepatitis	32	2	30	6	26	8	24
Cirrhosis	34	7	27	9	25	13	21
HCC	61	47	14	36	25	51	10
Overall accuracy (%)		81.9		68.5		75.6	
Sensitivity		77.0		59.0		83.6	
Specificity		86.4		77.3		68.2	
Positive prediction rate		83.9		70.6		70.8	
Negative prediction rate		80.3		67.1		81.8	

DCP: des- γ -carboxy prothrombin; AFP: α -fetoprotein; HCC: hepatocellular carcinoma.

Table 3 Positive rates for serum concentrations of DCP and AFP in relation to largest size, histologic differentiation, and number of HCC

Total HCC	Largest size of HCC			Histologic differentiation of HCCz		Number of HCC		
	2 cm	Between 2 and 3 cm	≥3 cm	Well	Moderately and poorly	1	≥2	
2Case number	61	23	16	22	8	39	39	22
DCP (%)	77.0	56.5	75.0	100	37.5	76.9	66.7	95.5
AFP (%)	59.0	43.5	56.3	77.3	12.5	53.8	43.6	86.4

DCP: des- γ -carboxy prothrombin; AFP: α -fetoprotein; HCC: hepatocellular carcinoma.

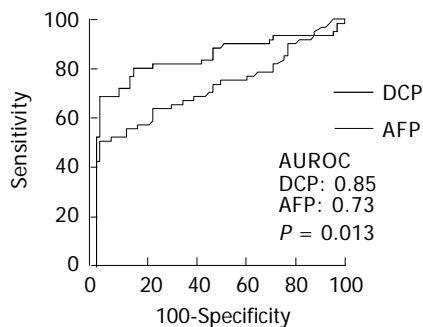


Figure 1 AUROC curves comparing DCP and AFP in patients with HCC and those with nonmalignant liver disease.

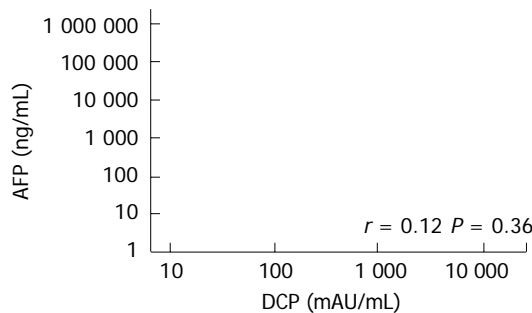


Figure 2 Relationship between DCP and AFP levels in 61 patients with HCC.

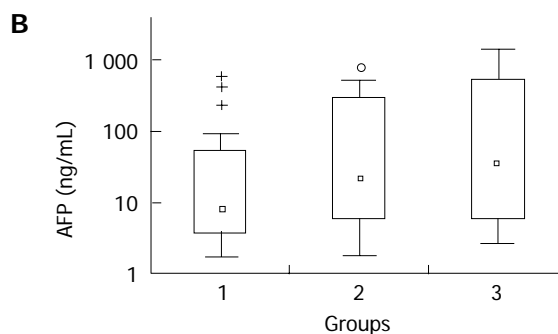
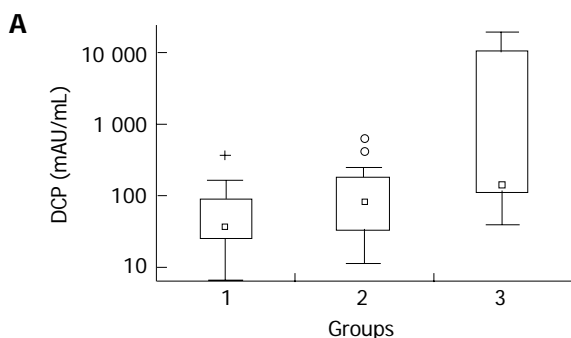


Figure 3 Box plot for DCP and AFP values in relation to size of tumor in 39 patients with solitary HCC (group 1, tumor less than 2 cm; group 2, tumor

between 2 and 3 cm; and group 3, tumor more than 3 cm). The data were presented as the median and the range from the 25th-75th percentile(A and B).

DISCUSSION

Almost no symptoms would be noticed in patients with small HCC. The symptomatic HCC was usually incurable and lethal, and most previous studies reported that the median survival was only 3-6 mo after the onset of symptoms^[26-28]. Early diagnosis is the most important issue in improving the long-term survival rate of HCC. Up to now, periodic screening among high risk population of HCC is the only way to detect the small HCC^[5,6]. AFP is the most commonly used marker in diagnosing HCC. However, in 35-45% of HCC patients, the AFP level may be normal^[21-24], particularly in patients with small HCC^[29]. On the other hand, patients with cirrhosis or chronic hepatitis, the elevated AFP level may be observed as well^[30]. The dilemma is the reason why some cases of HCC could not be correctly diagnosed by AFP alone.

Based on our results, AUROC curves indicated a better accuracy for DCP than AFP in diagnosis of HCC (0.85 [95%CI, 0.78-0.91] vs 0.73 [95%CI, 0.65-0.81], *P* = 0.013). The cut-off value of DCP and AFP for HCC was set at 40 mAU/mL and 20 ng/mL, respectively. These values yielded a sensitivity and specificity for DCP of 77% and 86.4%, and for AFP of 59% and 77.3%, respectively. The sensitivity, specificity, and accuracy of DCP were significantly higher than APP in diagnosis of HCC (Table 2). Meanwhile, 25 of 61 (41%) patients with HCC had AFP level lower than 20 ng/mL. On the other hand, 15 of 66 (22.7%) patients with nonmalignant chronic liver disease had serum AFP level higher than 20 ng/mL. Taken together, DCP

would be a better marker than AFP for HCC. Our results are also in accordance with recent studies that showed the unsatisfactory performance of AFP in diagnosis of HCC^[8].

We did not find any correlation between the DCP and AFP in HCC patients (Figure 2). AFP secretion in HCC resulted from re-expression in the tumor of fetal origin^[31]. DCP was produced by the malignant hepatocyte that resulted from an acquired post-translational defect in the vitamin K-dependent carboxylase system^[32]. Because no correlation was observed between DCP and AFP, the simultaneous determination of both markers might be more effective in the diagnosis of HCC^[33]. In our data, the increase in sensitivity from 77% to 83.6% (Table 2) in detecting HCC was found by the complementary use of the two markers.

We further analyzed 39 patients with solitary HCC in our study. We found that DCP levels had a better correlation with tumor size when compared with AFP levels (Figure 3). DCP also had a higher diagnostic sensitivity than AFP in patients with tumor size less than 2 cm in diameter. Particularly, for five patients with well-differentiated HCC less than 2 cm, the positive detection rate of DCP was 40%, which was much higher than 0% of AFP. It seems that DCP might be a better marker than AFP in detecting early-stage HCC. Because of the limited number of early-stage HCC in our report, further study is necessary to determine whether DCP plays an important role in early diagnosis of HCC.

In conclusion, our study indicates that DCP has a better diagnostic value than AFP in differentiating HCC from

nonmalignant chronic liver disease. DCP has not only a stronger correlation with HCC than AFP in tumor size but also more effectiveness than AFP in detecting small size of HCC.

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