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Combined estimation of serum alpha-fetoprotein and des-gama-carboxy prothrombin improves the diagnostic efficacy for hepato-cellular carcinoma in cirrhotic and chronic hepatitis Saudi patients

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ABSTRACT

This study aimed to evaluate the predictability of serum Des-γ-carboxyprothrombin (DCP) in cirrhotic and chronic hepatitis C (CHC) Saudi patients for the development of hepatocellular carcinoma (HCC) in comparison to alpha-fetoprotein (AFP). The study included 80 patients (30 with CHC, 30 with compensated liver cirrhosis and 20 with HCC) and 10 controls. Patients with CHC and cirrhosis were followed up for development of HCC. Serum levels of AFP and DCP were estimated for all patients and controls. The results showed that mean serum levels of AFP and DCP were significantly elevated in HCC patients compared to CHC and cirrhosis groups. Throughout follow-up period, 4 (13.3%) cirrhotic patients developed HCC. ROC curve analysis showed that serum DCP was significantly more specific predictor for HCC than AFP. Serum DCP and AFP levels at 38mAU/ml and 16μg/ml were defined as specific cutoff point for prediction of HCC with specificity rate of 88.7% and 73.6%, accuracy rate of 86.7% and 70%, and sensitivity rate of 71.4% and 42.9%, respectively.

KEYWORDS

Hepatocellular carcinoma;
Alpha-fetoprotein;
Des-γ-carboxyprothrombin;
Chronic hepatitis C.

ABBREVIATIONS

HCC (Hepatocellular carcinoma)
AFP (Alpha-fetoprotein)
DCP (Des-γ-carboxyprothrombin)
CHC (Chronic hepatitis C)
AUC (area under curve)
Std Error (standard error)

INTRODUCTION

Des-γ-carboxyprothrombin (DCP) also known as a protein induced by vitamin K absence or antagonist II (PIVKA-II), is an abnormal prothrombin that lacks γ-carboxylation of its glutamine residue. DCP is unable to bind calcium ion that is essential for its conformational transition and functional activity. Because the γ-carboxylation is vitamin K dependent, the DCP protein

TABLE 1 : Scoring Necro-inflammatory activity of chronic hepatitis

Grade	Portal/periportal activity	Lobular activity
0	None or minimal	None
1	Portal inflammation	Inflammation but no necrosis
2	Mild piecemeal necrosis (mild chronic active hepatitis; CAH)	Focal necrosis or acidophilic bodies
3	Moderate piecemeal necrosis (moderate CAH)	Severe focal cell damage
4	Severe piecemeal necrosis (severe CAH)	Damage includes bridging necrosis

TABLE 2 : Scoring fibrosis and cirrhosis

Stage	Criteria
0	None
1	Enlarged, fibrotic portal tracts
2	Periportal or portal-portal septa but intact architecture
3	Fibrosis with architectural distortion but no obvious cirrhosis
4	Probable or definite cirrhosis

appears when a patient is in a vitamin K-deficient state[1].

Prothrombin precursor has 10 glutamic acid (Glu) residues in the N terminus that are converted into γ-carboxyl-glutamic acid (Gla) residues by vitamin K-dependent γ-glutamyl carboxylase. All of these Glu residues need to be converted into Gla residues before prothrombin can obtain coagulation activity. In DCP, not all of the 10 Glu residues are transformed to Gla; some remain as Glu residues[2].

Liebman et al.[3] reported a relatively high incidence of DCP in HCC patients and suggested that it might be a useful tumor marker of HCC. The exact biochemical defect in HCC has never been clearly identified, although it is thought that vitamin K cannot act as a carboxylase cofactor, leading to a decrease in mature prothrombin production and an increase in DCP by the HCC. This defect may be a result of a vitamin K transport defect[4] or partly dependent on the vitamin K dose available, since in human hepatoma cell lines in culture, this defect could be corrected by addition of exogenous vitamin K[5]. The same correction was found after administration of pharmacological doses of vitamin K_1 to HCC patients[6]. Yoshiji et al.[7] reported that vitamin K_1 could weakly inhibit cell growth in vitro; however, the mechanism(s) for the weak growth inhibitory actions of natural K vitamins have not been identified. Since the major physiological function of K vitamins is to act as a cofactor for carboxylation of prothrombin (factor II) and other K vitamin dependent coagulation factors (VII, IX and X) as well as proteins C and S, prothrombin itself might have growth regulatory activity[1].

It is believed that the elevation of the serum DCP level correlates with the presence of vascular invasion or intrahepatic metastases[8]. Furthermore, DCP has been reported to be an independent prognostic factor for recurrence and survival after transarterial chemoembolization treatment[9], hepatic resection[10], ablation treatment[11] and liver transplantation[12].

Hepatocellular carcinoma is the most common primary malignancy arising within the liver and almost always in the setting of cirrhosis[13]. Approximately, 75-80% of primary liver cancers are attributable to persistent viral infections with either hepatitis B virus (HBV) or hepatitis C virus (HCV)[14].

Only a minority of patients with cirrhosis diagnosed with HCC have tumor amenable to potential curative therapy. All therapies have best results with single nodules ≤3 cm in size[15], including liver transplantation[16]. This clinical scenario has important implications for screening and diagnosing HCC at an early stage[17].

The current study was conducted to evaluate the predictability of estimation of serum DCP in cirrhotic and CHC patients for the development of de novo HCC in comparison to AFP.

EXPERIMENTAL

The present prospective comparative study was conducted at Clinical Biochemistry Department, Faculty of Medicine, King AbdulAziz University and the center for infectious diseases control at preventive medicine department, PHC, Jeddah. The study was conducted since June 2006 till August 2009 to allow a minimum follow-up period of 6 month for the last enrolled patient.

This two-arm study included 30 patients with chronic hepatitis C (CHC group) infection persisting for longer than 6 months with HCV antibody positive and increased serum ALT values and radiological assessment excluded the presence of any evidence of cancer. After complete history taking and full clinical examination, all patients were subjected to the following investigations: urine & stool analysis, complete blood

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TABLE 3 : Mean ±SD Scheuer scores according to histopathological examination of liver biopsies

	CHC (n=30) Mean ±SD (range)	Cirrhosis (n=30) Mean ±SD (range)
Scheuer-portal score	2.23 ±1 (1-4)	2 ±0.87 (1-4)
Scheuer-lobular score	1.13 ±0.73 (0-3)	1.67 ±0.76 (1-3) †
Scheuer-fibrosis score	2.23 ±1.33 (0-4)	2.83 ±0.83 (2-4) †
Total Scheuer score	5.6 ± 2.37 (2-10)	6.5 ±1.48 (4-9)

†: significant difference versus CHC group

TABLE 4 : Mean (±SD) serum levels of AFP and DCP

	AFP (μg/ml)	DCP (mAU/ml)
Control group	5±2.6	12.7±1.2
CHC group	12±5.9*	22.3±7.3*
Cirrhosis group	15±5.2*†	33.3±12.7*†
HCC group	61051±26935*†‡	1680.3±500.8*†‡

*: significant difference versus control group, †: significant difference versus CHC group, ‡: significant difference versus cirrhosis group

TABLE 5 : Correlation coefficient of serum AFP and DCP and histopathological grading

	AFP		DCP	
	"r"	p	"r"	p
CHC group	0.200	>0.05	0.315	>0.05
Cirrhosis group	0.235	>0.05	0.460	= 0.011
HCC group	0.327	>0.05	0.597	= 0.005

TABLE 6 : AUC for serum DCP and AFP levels as specific predictor for HCC

	AUC	Std. error	P value	95% confidence interval	
				Lower	Upper
DCP	0.892	0.057	0.023	0.781	1.003
AFP	0.608	0.198	>0.05	0.220	0.996

picture, ultrasonographic scanning for the abdomen and ELISA test for hepatitis C virus antibodies (HCVab)[18] for confirmation of inclusion criteria.

The second group (HCC group) included 20 patients with HCC that was histologically confirmed. Tumor size was estimated by using ultrasonography and patients who had advanced HCC with tumor size >3 cm or patients with portal vein invasion were identified. Blood samples from HCC patients were drawn before initial treatment. All HCC patients underwent determination of hepatitis B surface antigen (HBsAg), antibody to HCV (anti-HCV) and serum levels of ALT, albumin, total bilirubin, and platelet count and prothrombin time.

The third group (Cirrhosis group) included thirty compensated cirrhosis (modified Child-Pugh score <7) patients. Cirrhosis was defined by clinical development of esophageal varices, thrombocytopenia (platelet count less than 100 000/mm^3), 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 was ruled out depending on sonography and/or CT performed on a regular examination through out the follow-up period till end of the study and at least for 6 months. The study also included 10 volunteers to donate blood samples as Control group.

Patients on chronic use of non-steroidal anti-inflammatory drugs, aspirin, and anticoagulants or had concomitant other non-gastroenterologic diseases, in particular, rheumatoid arthritis or other connective tissue inflammatory diseases were excluded from the study. Patients with prothrombin concentration <60% of the control, serum total bilirubin level >20 mg/l, decompensated liver disease or on vitamin K therapy were also excluded from the study.

Liver biopsies were done by means of the Biopty gun (Biopter) MBD-Multiple Biopsy Device, US, Biopsy, Franklin. Histopathological inflammatory activity (Grading, 0-4 scale, TABLE 1) and fibrosis stage (Staging, 0-4 Scale, TABLE 2) were evaluated according to Scheuer classification[19]. HCC was histopathologically graded according to the criteria proposed by the Liver Cancer Study Group of Japan[20] into well differentiated, moderately differentiated, or poorly differentiated.

All patients and controls gave a fasting blood sample (5ml) prior to initiation of therapy. Blood samples were collected in plain tube and allowed to clot and centrifuged at 5000 rpm for 10 minutes and serum was collected and stored at -80°C till assayed for estimation of serum levels of:

1. AFP using the commercially available immunometric assay (Architect AFP assay, Abbott Laboratories, North Chicago, IL, USA). The cut-off value of AFP for HCC was set at 20μg/ml; the most commonly set value[21].
2. DCP level was measured by using an ELISA (Eitest PIVKAII, Eisai Co., Tokyo, Japan), according to the manufacturer's instructions. The detection limit is 10 mAU/ml, with value of 40 mAU/ml as cutoff point for differentiation of HCC and non-malignant liver disease based on previous studies[22].

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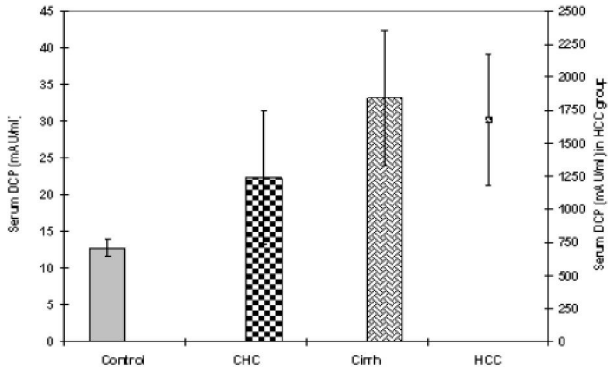

Figure 1 : Mean (±SD) level of DCP estimated in studied patients' groups

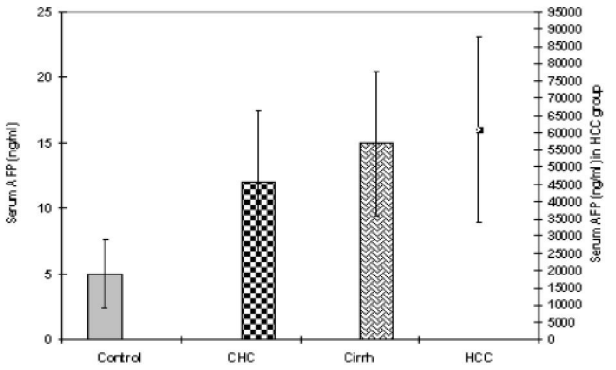

Figure 2 : Mean (±SD) level of AFP estimated in studied patients' groups

This was done in Clinical Biochemistry Department, Faculty of Medicine, King AbdulAziz University, Jeddah, KSA.

Statistical analysis

Obtained data were presented as mean±SD, ranges, numbers and ratios. Results were analyzed using paired t-test and Chi-square test. Possible relationships were investigated using Pearson linear regression. Regression analysis using Stepwise method was used to test the predictability of estimated parameters for de novo HCC. Test validity characters and cutoff points were evaluated using the receiver operating characteristic (ROC) curve analysis judged by the area under the curve (AUC). Statistical analysis was conducted using the SPSS (Version 10, 2002) for Windows statistical package. P value <0.05 was considered statistically significant.

RESULTS

The study included 90 patients; 55 males (61.1%) and 35 females (38.9%) and 10 controls; 7 males and 3 females with a non-significant difference between patients and controls as regards sex distribution, (X^2 = 0.146, p>0.05). Mean age of studied patients was 47.5±8.7; range: 26-64 years, while mean age of controls was 49.7±12.5; range: 29-66 years, with a non-significant (p>0.05) difference between both groups.

Regarding HCC patients, histopatholgical grading showed 12 patients with well differentiated HCC, 4 patients with moderately differentiated and 4 patients had poorly differentiated.

Mean serum levels of AFP and DCP were significantly elevated in HCC group compared to cirrhosis, CHC and control groups as shown in (TABLE 4, Figure 1, 2).

Correlation between serum levels of AFP and DCP in patients' groups and histopathological grading showed a positive correlation between serum levels of both markers and histopathological grading in all groups. This positive correlation was significant only between serum DCP and total Scheuer score in cirrhotic group and pathological grading in HCC group (TABLE 5).

Regression analysis of serum levels of DCP and AFP defined DCP as the significant predictor for pathological grading of HCC (F = 6.216, p = 0.009) but the combined use of both markers improved the predictability of AFP (F = 9.950, p = 0.005).

Throughout the study period, follow-up investigations detected 4 (13.3%) cirrhotic patients developed HCC. Using ROC curve analysis, with a null hypothesis that the true area under curve = 0.5, showed that estimation of serum DCP was significantly (p = 0.023) more specific predictor for the presence of HCC, irrespective of the underlying pathology, with AUC = 0.892, while specificity of AFP as a predictor showed non-significant (p>0.05) difference compared to the true area with AUC = 0.608, (TABLE 6, Figure 3).

Also using ROC analysis for verification of serum DCP and AFP cutoff points above which malignant lesion could be predicted defined serum DCP level at 38mAU/ml as more specific cutoff point with AUC = 0.698, sensitivity rate of 71.4%, specificity rate of 88.7% and accuracy rate of 86.7%, while DCP levels at cutoff point of 37mAU/ml showed AUC = 0.614, sensitivity rate of 85.4%, specificity rate of 84.9% and accuracy rate of 85%. For AFP serum cutoff point at 16μg/ml

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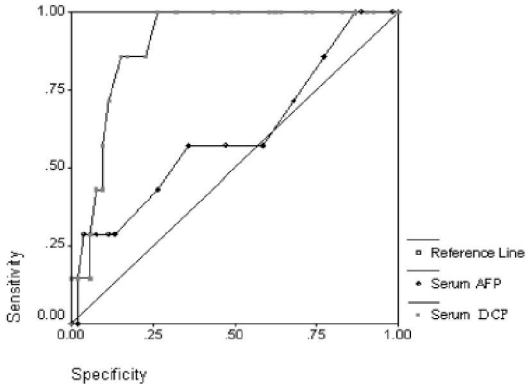

Figure 3 : ROC curve analysis of serum DCP and AFP as predictor for development of malignant hepatic lesions

was the most specific cutoff point with AUC = 0.565, specificity rate of 73.6% and accuracy rate of 70% but showed sensitivity rate of 42.9%, while cutoff point at 12μg/ml showed higher sensitivity rate (71.4%) with low specificity and accuracy rates, 32.1% and 36.7%, respectively and AUC = 0.472, (TABLE 7).

TABLE 7 : AUC and sensitivity, specificity and accuracy rates for probable cutoff points of serum DCP and AFP levels for discrimination between HCC and non-HCC hepatic lesions

	Cutoff point	AUC	Sensitivity	Specificity	Accuracy
Serum DCP	37 mAU/ml	0.614	85.7%	84.9%	85%
	38 mAU/ml	0.698	71.4%	88.7%	86.7%
Serum AFP	12 ng/ml	0.472	71.4%	32.1%	36.7%
	16 ng/ml	0.565	42.9%	73.6%	70%

DISCUSSION

The present study was based as two-arm survey including healthy volunteers as negative control group and patients with histologically documented HCC as positive control group to evaluate the usefulness of serum AFP and DCP in prediction of HCC. Both markers showed significantly higher serum levels in cirrhotic and CHC patients compared to control group with significantly higher levels in cirrhosis patients compared to hepatitis patients. Also, AFP and DCP levels were significantly higher in HCC group compared to other groups. These finding illustrate the impact of lesion type and progression of disease status on the serum levels of both markers and indicated the applicability of both parameters as markers for liver endangerment. Similarly, Durazo et al.[23] found levels of both DCP and AFP were significantly higher in patients with HCC than in those without HCC.

In support of these data, the present study detected a positive significant correlation between serum levels of DCP and total Scheuer score in cirrhotic and pathological grading of HCC, but the correlation was non-significant in CHC groups. On contrary, these correlations were positive non-significant correlation with AFP. Serum DCP was found as a significant predictor for pathological grade, while AFP was non-significant, but the predictability of these markers for HCC grading was improved on using combination of these markers. These findings go in hand with Toyoda et al.[24] who evaluated the significance of simultaneous measurement of AFP, Lens culinaris agglutinin A-reactive fraction of AFP (AFP-L3), and DCP in the evaluation of tumor progression and prognosis of patients with HCC and found AFP-L3 and DCP appear to represent different features of tumor progression in patients with HCC and could be useful for the evaluation of tumor progression, prediction of patient outcome, and treatment efficacy. Also, Murakami et al.[25] found DCP is the most useful prognostic tumor marker in HCC patients and Tateishi et al.[26] found diagnostic accuracy of AFP in small HCC was substantially limited and surveillance including DCP with optimal cutoff value should be conducted to confirm the efficacy of the policy. Kim et al.[27] found serum PIVKA-II level, not serum AFP, was a valuable independent prognostic factor in HBV-related HCC.

Throughout follow-up, 4 cirrhotic patients (13.3%) developed HCC; such frequency of de novo HCC goes in hand with Sterling et al.[28] who followed-up 298 patients with hepatitis C virus-related cirrhosis for 2 years and reported that HCC developed in 34 of 298 (11.4%) who were free of HCC at entry and with Brady et al.[29], who reported a frequency rate of de novo HCC of 13.6%, in cirrhotic patients waiting for liver transplantation.

The ROC curve analysis showed that estimation of serum DCP was significantly (p = 0.023) more specific predictor for the presence of HCC, irrespective of the underlying pathology, with AUC = 0.892 than serum AFP that showed an AUC = 0.608. Moreover, for prediction of development of malignant lesion, serum DCP and AFP levels at ≥ 38mAU/ml and 16μg/ml, respectively, were the most specific cutoff points with AUC =

0.698 and 0.565, respectively. These data goes in hand with Okuwaki et al.[30] who found serum DCP level ≥ 40mAu/ml, local tumor progression, and ablative margin <5 mm were related to multiple intrahepatic distant recurrence and recommended careful follow-up to monitor any intrahepatic distant recurrence in HCC patients with high serum DCP.

Moreover, Sterling et al.[28] found that DCP levels have higher specificity and negative predictive value for HCC than total AFP and the combination of DCP and AFP-L3 could identify individuals with negative imaging results who would benefit from follow-up evaluation. Marrero et al.[31] tried to determine performance of DCP and AFP-L3% for the diagnosis of early HCC and what factors affect DCP, AFP-L3%, or AFP levels and found that AFP had the best sensitivity, but at the expense of specificity, followed by DCP and AFP-L3% for early stage HCC. Baek et al.[32] found PIVKA-II is a useful marker for detecting HCC, especially in small HCC and have correlations with known staging systems.

Despite the diagnostic and prognostic value of estimation of serum DCP in patients with HCC, the mechanisms for its production still a matter of debate; Bertino et al.[33] reported that DCP detectable serum levels are the result not only of vitamin K deficiency or selective defects of carboxylase, because probably alterations of membrane receptors or cytoplasmatic transfers, that are necessary for the function of vitamin K, are involved. On the other hand, Ma et al.[34] tried to examine in vivo the efficacy of vitamin K_2 on the production of DCP as well as tumor cell growth and invasion and found vitamin K_2 might suppress the growth and invasion of HCC cells via decrease of DCP.

However, Murata et al.[35] hypothesized that DCP might be produced from HCC cells with functional impairment of vitamin K uptake.

CONCLUSION

It could be concluded that estimation of serum DCP in cirrhotic and CHC patients free of HCC could predict the de novo development of HCC with high specificity but its diagnostic validity could be improved by combination with AFP serum level estimation. However, wider-scale studies were advocated for establishment of valid cutoff points.

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