RESEARCH ARTICLE

Des-Gamma-Carboxyprothrombin for Early Identification and Prognosis of Hepatocellular Carcinoma - A Case Control Study from Western Nepal

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Abstract

Objective: To assess the diagnostic and prognostic value of AFP and des-gamma-carboxyprothrombin (DCP) in combination and alone for hepatocellular carcinoma. Materials and Methods: A case control study carried out in the Department of Biochemistry of Manipal College of Medical Sciences, Pokhara, Nepal between 1st January 2010 and 31st December 2011. The variables collected were age, gender, BMI, total proteins, albumin, AST, ALT, total bilirubin, DCP, AFP. Approval for the study was obtained from the institutional research ethical committee. Estimation of AFP was performed by ELISA reader for all cases. Analysis was done using descriptive statistics and confidence interval (CI). The data was analyzed using Excel 2003, R 2.8.0 Statistical Package for the Social Sciences (SPSS) for Windows Version 16.0 (SPSS Inc; Chicago, IL, USA) and the EPI Info 3.5.1 Windows Version. Results: The mean age of HCC cases was 53.6 ± 14.93 yrs. The percentage of females was less than males in both cases (23%) and controls (29%). The specificity of DCP reached 100% when its values was equal or greater than 150 (MAU/ml) for 0, 3, 6, 9, 12 months preceding the diagnosis of HCC. Similarly, the specificity for AFP was also nearly 100% when its value was equal or greater than 200 ng/ml 0, 3, 6, 9, 12 months earlier to the finding of HCC. The specificity of DCP (≥40MAU/mL) and AFP(≥20 ng/mL) in combination was 93%, 97%, 95%, 96%, 97% in respect to 0, 3, 6, 9, 12 months prior to the diagnosis of HCC. Conclusion: The combination of both DCP and AFP will improve the finding of initial HCC and the sensitivity of these markers was utmost at the time of HCC identification and noticeably lesser at former time points.

Keywords: HCC - diagnosis - AFP - des-gamma-carboxyprothrombin - Nepal

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Introduction

Hepatocellular carcinoma (HCC) is one of the most frequent malignant tumors and holds a meagerendurance rate. The management of patients at risk for developing HCC remains exigent (Bridges et al., 2011). An augmented perception of cancer biology and technological progress have facilitated identification of a large number of pathological, genetic, and molecular proceedings that drive hepatocarcinogenesis leading to discovery of copiousprospective biomarkers in this disease (Saelee et al., 2012). They are at present being belligerently appraised to set up their significance in early identification, optimization of treatment, plummeting the appearance of new tumors, and avert the reappearancesubsequent to surgical resection or liver transplantation. These indicators not only assist in forecast of diagnosis or recurrence but may also help out in make a decisionof suitable modality of treatment and may embodynewpossibletarget for therapeutic intercession (Ye et al., 2012).

Serum AFP levels are often high in early phase of HCC and then plunge or even normalize previous to rising again as disease succession transpires (Xu et al., 2012). Moreover, AFP rise has also been acknowledged in the occurrence of acute and chronic viral hepatitis in addition to in patients with cirrhosis caused by hepatitis C (Mittal et al., 2011).The low sensitivity and specificity of AFP and ultrasound in perceiving early HCC led to the need of more trustworthy biomarkers. Des-gamma-carboxyprothrombin (DCP) is an abnormal prothrombin deprived of carboxylation of 10 glutamic acid residues at its N-terminus. Due to the deficiency of carboxylation of the carbon atom at the γ-position, it is called des-γ-carboxyprothrombinand is devoid of coagulation activity. Des-gamma-carboxy (Abnormal) prothrombin (DCP) is formed by the malignant hepatocyte. Because AFP and

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DCP act individually in serum, they can counterpart each other for the finding of HCC. Hence, the combined use of AFP and DCP increases sensitivity as well as specificity for the diagnosis of HCC. Therefore, the main objective of our study was to assess the diagnostic and prognostic value of AFP and DCP in combination and alone for hepatocellular carcinoma.

Materials and Methods

It was a case control study carried out in the Department of Biochemistry of Manipal College of Medical Sciences, Pokhara, Nepal between 1st January 2010 and 31st December 2011. The variables collected were age, gender, BMI, total proteins, albumin, AST, ALT, total bilirubin, DCP, AFP. Approval for the study was obtained from the institutional research ethical committee. Estimation of AFP was performed by ELISA reader for all cases. The standard procedure was followed as per manufacturer's instructions for ELISA (Sell, 1990). DCP was measured by the chemiluminescent immunoassay using a sensitive anti-DCP antibody and threshold values were set to 40, 100, 200, and 400 MAU/mL for determining the presence or absence of a positive reaction (Fujikawa et al., 2009). All these laboratory parameters were analyzed using Human reagent kits and with the help of ELISA and semi autoanalyser (Humalyser 3500, Germany). Analysis was done using descriptive statistics and Confidence Interval (CI). The data was analyzed using Excel 2003, R 2.8.0 Statistical Package for the Social Sciences (SPSS) for Windows Version 16.0 (SPSS Inc; Chicago, IL, USA) and the EPI Info 3.5.1 Windows Version.

Results

Table 1 illustrates that mean ageof HCC cases was 53.58 ± 14.93 yrs. the percentage of females was less than males in both cases (23%) and controls (29%).the mean value of total proteins (6.72 ± 0.85 g/dL) and albumin (3.56 ± 0.53 g/dL) was less in HCC cases when compared to controls. the mean values of AST (85.12 ± 58.24 IU/L) and ALT (131.5 ± 94.46 IU/L) was markedly increased in HCC cases when compared to controls. The mean values of AFP (32.7 ± 42.9 ng/mL) and DCP (58.8 ± 64.9 MAU/mL) was grossly elevated in HCC cases in comparison to controls. Table 2 depicts that the specificity of DCP reached upto 100% when its values was equal or greater than 150 (MAU/ml) for 0, 3, 6, 9, 12 months preceding to the diagnosis of HCC. Similarly, the specificity for (AFP was also nearly 100% when its value was equal or greater than 200 ng/ml for 0, 3, 6, 9, 12 months earlier to the finding of HCC. The specificity of DCP (≥ 40 MAU/mL) and AFP (≥ 20 ng/mL) in combination was 93%, 97%, 95%, 96%, 97% in respect to 0, 3, 6, 9, 12 months prior to the diagnosis of HCC.

Table 1. Baseline Characteristics of HCC Cases and Controls

Variables	HCC cases(30)	Controls(30)
Age, years	53.58 ± 14.93	42.65 ± 20.80
Gender, % female	23	29
BMI kg/m^2	27.8 ± 4.3	29.4 ± 4.7
Total proteins	6.72 ± 0.85	6.96 ± 0.69
Albumin, g/dL	3.56 ± 0.53	3.81 ± 0.44
AST, IU/L	85.12 ± 58.24	26.6 ± 10.67
ALT, IU/L	131.5 ± 94.46	27.07 ± 12.21
Total bilirubin, mg/dL	0.9 ± 0.3	0.8 ± 0.2
DCP, mAU/mL	58.8 ± 64.9	24.9 ± 11.0
AFP, ng/mL	32.7 ± 42.9	18.4 ± 39.3

Table 2. Sensitivityand Specificity of DCP and AFP Alone and in Combination

	DCP (MAU/mL)			
	≥40 (MAU/mL)		≥150 (MAU/mL)	
	Sensitivity	Specificity	Sensitivity	Specificity
Months from HCC diagnosis				
0	71%	90%	46%	100%
-3	61%	81%	37%	100%
-6	61%	84%	14%	100%
-9	55%	83%	9%	100%
-12	46%	91%	5%	100%
	AFP (ng/mL)			
	≥20(ng/mL)		≥200(ng/mL)	
	Sensitivity	Specificity	Sensitivity	Specificity
0	64%	84%	25%	100%
-3	54%	83%	16%	98%
-6	55%	71%	6%	100%
-9	42%	73%	5%	100%
-12	45%	79%	4%	100%
	DCP(MAU/mL) and/or AFP(ng/mL)			
	DCP ≥40(MAU/mL) or AFP ≥20(ng/mL)		DCP ≥40(MAU/mL) and AFP ≥20(ng/mL)	
0	89%	71%	41%	93%
-3	82%	72%	40%	97%
-6	86%	69%	34%	95%
-9	80%	69%	18%	96%
-12	70%	74%	19%	97%

Discussion

DCP is an abnormal prothrombin that is produced by under-carboxylation of normal prothrombin. DCP applies a mitogenic out come on hepatic carcinoma cells via a Met-Janus kinase1-STAT3 signaling alleyway (Bertino et al., 2010). On the other hand, it has also been validated that the antiproliferative effect of vitamin K on hepatic carcinoma is not owing to a low production of DCP, but moderately instigated by protein kinase A and these findings concurred with the report of Suzuki et al (Suzuki et al., 2005). An important goal in cancer surveillance is the detection of preclinical tumors. Therefore, optimizing sensitivity is critical. At the higher cutoff values of DCP and AFP, sensitivities of these markers at month -12 were minimal: 5%and 4%, respectively. Improvement of sensitivities to 46% and 45% could be achieved by further lowering of DCP 40 MAU/mL and AFP 20 ng/ml cut off values (Weitz et al., 1993). This analysis took advantage to compare the accuracy of AFP and DCP alone and in combination in the early detection of HCC. The availability samples before the diagnosis of HCC

allowed for the comparison of the accuracy of AFP and DCP in differentiating HCC cases from matched controls before clinical diagnosis, an important feature in HCC surveillance. DCP had greater accuracy than AFP at all time points between month -12 and time of diagnosis but the differences were not statistically significant (Fujiyama et al., 1991). DCP testing alone had a sensitivity of 71% and 46% and a specificity of 90%; 100% in differentiating HCC cases and controls at the time of diagnosis using a cut off value of 40; 150 MAU/mL respectively (Okuda et al., 2002). In comparison, AFP testing alone had a sensitivity of 64%; 25% and a specificity of 84%;100%. Using a cut off value of 20; 200 ng/mL respectively (El-Attar et al., 2010). In last comparison either using a cut off value of 40 MAU/mL for DCP or using a cut off value of 20 ng/ml for AFP, the sensitivity and specificity was equal to 89% and 71% respectively at the time of diagnosis of HCC. Further, when AFP and DCP were combined using a cut off value of 20 ng/ml and 40 MAU/mL respectively, the sensitivity and specificity were 41% and 93% at the time of diagnosis of HCC (Yamamoto et al., 2010).

In conclusion, the conjoining both DCP and AFP will improve the finding of initial HCC and the sensitivity of these markers was utmost at the time of HCC identification and noticeably lesser at former time points.

References

Bertino G, Ardiri AM, Calvagno GS, et al (2010). Prognostic and diagnostic value of des-γ-carboxy prothrombin in liver cancer. *Drug News Perspect*, **23**, 498-508.

Bridges JF, Joy SM, Gallego G, et al (2011). Needs for hepatocellular carcinoma control policy in the Asia-Pacific region. *Asian Pac J Cancer Prev*, **12**, 2585-91.

El-Attar HA, Kandil MH, El-Kerm YM, et al (2010). Comparison of serum survivin and alpha fetoprotein in Egyptian patients with hepatocellular carcinoma associated with hepatitis C viral infection. *Asian Pac J Cancer Prev*, **11**, 897-903.

Fujikawa T, Shiraha H, Yamamoto K (2009). Significance of des-gamma-carboxyprothrombin production in hepatocellular carcinoma. *Acta Med Okayama*, **63**, 299-304.

Fujiyama S, Izuno K, Gohshi K, et al (1991). Clinical usefulness of des-gamma-carboxy prothrombin assay in early diagnosis of hepatocellular carcinoma. *Dig Dis Sci*, **36**, 1787-92.

Mittal A, Sathian B, Chandrashekharan N, et al (2011). Diagnostic significance of alpha fetoprotein in carcinomas of liver and biliary tract - a comparative study from the western region of Nepal. *Asian Pac J Cancer Prev*, **12**, 3475-8.

Okuda H, Nakanishi T, Takatsu K, et al (2002). Clinicopathologic features of patients with hepatocellular carcinoma seropositive for alpha-fetoprotein-L3 and seronegative for des-gamma-carboxy prothrombin in comparison with those seropositive for des-gamma-carboxy prothrombin alone. *J Gastroenterol Hepatol*, **17**, 772-8.

Saelee P, Chuensumran U, Wongkham S, et al (2012). Hypermethylation of suppressor of cytokine signaling 1 in hepatocellular carcinoma patients. *Asian Pac J Cancer Prev*, **13**, 3489-93.

Sell LS (1990). Cancer markers of the 1990s. *Clin Lab Med*, **10**, 1-37.

Suzuki M, Shiraha H, Fujikawa T, et al (???). Des-gammacarboxyprothrombin is a potential autologous growth factorfor hepatocellular carcinoma. *J Biol Chem*, **280**, 6409-15.

Weitz IC, Liebman HA (1993). Des-gamma-carboxy (abnormal) prothrombin and hepatocellular carcinoma: a critical review. *Hepatology*, **18**, 990-7.

Xu J, Liu C, Zhou L, et al (2012). Distinctions between clinicopathological factors and prognosis of alpha-fetoprotein negative and positive hepatocelluar carcinoma patients. *Asian Pac J Cancer Prev*, **13**, 559-62.

Yamamoto K, Imamura H, Matsuyama Y, et al (2010). AFP, AFP-L3, DCP, and GP73 as markers for monitoring treatment response and recurrence and as surrogate markers of clinicopathological variables of HCC. *J Gastroenterol* , **45**, 1272-82.

Ye JZ, Miao ZG, Wu FX, et al (2012). Recurrence after anatomic resection versus nonanatomic resection for hepatocellular carcinoma: a meta-analysis. A*sian Pac J Cancer Prev*, **13**, 1771-7.