Original Article

Diagnostic Utility of Alpha-fetoprotein and Des-gamma-carboxyprothrombin in Nigerians with Hepatocellular Carcinoma

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ABSTRACT

Background: Alpha-fetoprotein (AFP) and Des-gamma-carboxyprothrombin (DCP) have been extensively studied as biomarkers for the diagnosis of and prognostication in hepatocellular carcinoma (HCC). However there are only few reports on the clinical characteristics of hepatocellular carcinoma in relation to the combination of the two tumor markers in hepatitis B virus-related HCC. **Aim:** The aim of this study was to investigate the clinical characteristics of HBV-related HCC in relation to different sets of AFP and DCP values. **Methods:** Sixty-two patients with untreated HCC were studied. The positive value of AFP was set at 20 1U/L while DCP positive value was set at 150 mAU/ml. Patients were divided into three groups: Group 1(n=36) with AFP ≥ 20 IU/L and DCP ≥ 150 mAU/ml. Group 2(n=24) with AFP <20 1U/L and DCP ≥ 150 mAU/ml. Group 3 (n=2) with AFP < 20 1U/L and DCP < 150 mAU/ml. There were no patients in group 4 meant for those with AFP ≥ 20 1U/L and DCP < 150 mAU/ml. Clinical and laboratory variables were compared among the groups. **Results:** Clinical and laboratory variables were comparable among the groups with the exception of gender and values of serum alanine aminotransferase (ALT). Males were significantly more than females among the groups (p<0.03). ALT values were significantly different among the groups (p<0.006). Paired comparisons between the groups showed the mean values of serum ALT were significantly higher in group 2 than in group 1(p<0.003). The mean serum ALT values were also higher in group 2 than in group 3 (p <0.014). There was no significant difference between group 1 and group 3 (*P* = 0.124). **Conclusion:** HCC patients who are sero-positive for DCP and sero-negative for AFP have significantly higher levels of serum ALT; serum ALT levels may be of diagnostic importance in AFP-negative, HBV-related HCC patients.

Keywords: *Alanine aminotransferase, alpha-fetoprotein, des-gamma-carboxyprothrombin, hepatitis B virus, hepatocellular carcinoma*

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Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancy and the third most common cause of cancer-related deaths worldwide.[1] It is a disease with a dismal outcome. The poor prognosis worldwide is due to late detection with more than two-thirds of patients diagnosed at advanced stages of the disease.[2]

A major problem with HCC diagnosis is the lack of reliable tumor markers. Alpha-fetoprotein (AFP) has been used as a marker of HCC since 1970, but about 40% of HCC do not secrete AFP.[3,4]

Moreover, AFP can be secreted by regenerative liver cells in patients with benign liver diseases such as liver cirrhosis, chronic hepatitis, and acute hepatitis which make it poorly specific for HCC.[4]

Des-gamma-carboxyprothrombin (DCP) is an abnormal prothrombin (coagulation factor II) induced by Vitamin K

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absence or antagonist, and its level increases specifically in HCC.[5] It was first detected in the serum of patients with HCC in 1984 by Liebman *et al*.[5] DCP has been reported to be of better prognostic significance than AFP. It was found to be the most useful predisposing clinical parameter for the development of portal vein invasion and for tumor recurrence.[6] High DCP levels also reflect poor prognosis after radiofrequency ablation, transarterial chemoablation of HCC, and surgical resection.[7-9] Patients with high levels of DCP and low levels of AFP have been reported to have more advanced HCC compared with patients with low levels of DCP.[10,11]

Multiple reports abound in the literature regarding the use of these two complementary tumor markers in the diagnosis of HCC. However, only limited amount of data is available at present regarding the clinical characteristics of HCC in relation to the combination of AFP and DCP. Among the few reports using the combination of both biomarkers, most reports involve HCC with hepatitis C virus etiological background.

The aim of this study therefore was to investigate the clinical and laboratory characteristics of HCC patients (with predominant hepatitis B virus etiological background), in relation to the combination of both tumor markers.

Materials and Methods

The design of this study has been described previously and results from the data had been partially used.[12] Patients were drawn from referrals to the gastroenterology unit of our center between April 2011 and March 2012. Sixty-two consecutive patients with HCC were diagnosed using the European Association for the Study of the Liver, and the American Association for the Study of Liver Diseases criteria were enrolled.[13,14] Ethical clearance was obtained from the hospital ethical committee (Obafemi Awolowo University Teaching Hospitals Complex registration number: NHREC/27/02/2009a), and informed consent was obtained from all the patients before enrolling them for the study. A full history and detailed clinical examination were carried out on all the patients. The tests included hepatitis B surface antigen (HBsAg), hepatitis C antibody (anti-HCV), and liver function tests carried out by conventional methods. HCC diameter was measured by ultrasound and/or computed tomography scan. Patients with a history of the use of warfarin or other dicoumarols or total bilirubin level above 20 mg/dl (340 μmol/l) were excluded from the study. All the patients used for this study were native Nigerians.

Five milliliters of venous blood was collected from each patient and sera were stored at −20°C until they were analyzed for the tumor markers. The analysis for both tumor markers (DCP and AFP) was carried out at a national research institute: Nigerian Institute for Medical Research, Lagos, Nigeria.

AFP and DCP levels were determined as previously described.[12] Essentially, the DC*P* values were obtained using "Haicatch PIVKA-II" enzyme Immunoassay test kit (Sanko Junyaku Co. Ltd., Tokyo, Japan). AFP was tested using commercially available immunoenzymometric assay kit manufactured by INTECO Diagnostics, UK Ltd, London. Both tests were carried out as per manufacturers' instructions.

To determine the characteristics of HCC in relation to DCP and AFP, the positive value for AFP was fixed at 20 IU/ml which is the globally accepted standard while the positive value for DCP was set at 150 mAU/ml. This DCP value is higher than the 40 mAU/ml used in Japan and other Asian countries but reports elsewhere and also from our earlier findings support this cutoff point for use in our center.[12,15]

Patients were initially classified into four groups according to their levels of positivity for DCP and AFP. Group I (n = 36) were those with positive levels of both AFP and DCP. Group 2 (n = 24) were those with positive DCP levels and negative AFP levels. Group 3 (n = 2) were those with low levels of both AFP and DCP. Group 4 (n = 0) were those with low levels of DCP and high levels of AFP. Due to the lack of patients in Group 4 (n = 0), it was excluded from the tabulations and analysis. Clinicopathologic variables were then compared among the first three groups.

The control group consisted of 57 patients with benign liver diseases including liver cirrhosis, chronic hepatitis, and nonalcoholic fatty liver disease. There were 38 males and 19 females. The mean age of the controls was 43.2 (±12.2) years.

Now, DC*P* value of 400 mAU/ml has been reported to be of great prognostic significance.[16-18] Hakamada *et al*.[16] reported the DC*P* value ≥400 mAU/ml, in addition to vascular invasion, to be independent prognostic factors for tumor of all sizes. Similarly, the Kyoto criteria for liver transplantation in HCC patients beyond Milan included DCP ≤400 mAU/ml, ≤10 nodules all ≤5 cm.[18] In arriving at the Kyoto criteria, the authors found that in predicting recurrence, the area under the curve was much higher for DCP than for AFP (0.84 vs. 0.69). We therefore decided to identify independent variables that would predict the elevation of serum DCP level ≥400 mAU/ml.

Statistical analysis

Data generated from the study were entered into Microsoft Excel and then transferred to STATA 10 software (Statacorp, Texas, USA) for data management and analysis. Data were expressed as means ± standard deviations. Multiple comparisons were made using the nonparametric Kruskal–Wallis test followed by

the Dunn procedure for group to group comparisons. Pearson's Chi-square test was used for comparison of qualitative variables. Both univariate and multivariate logistics analyses were used to determine the risk factors for elevated serum DCP. In both univariate and multivariate analyses, the odds ratios were calculated using the multiple logistic regression models. $P < 0.05$ was considered statistically significant.

Results

The baseline clinical and laboratory characteristics of the HCC patients used in this study are shown in Table 1. There were 48 males and 14 females (ratio 3.4:1) and the mean age of the patients was 46.3 (±15.6) years. The median value for AFP was 39 IU/ml while that of DCP was 2000 mAU/ml.

Table 1: Baseline Clinical and Laboratory Characteristics of HCC Patients

Variables	Mean±SD or *N* (%)
Gender M: F	48:14
Age	46.3±12.2
AST	87.8±45.2
ALT	42.0±36.2
AST/ALT ratio	2.8±1.6
ALP	228.3±195.1
Albumin	31.5±5.5
Total Bilirubin	99.2±113.0
AFP	122.8±148.2
DCP	1657.6±687.1
HBs Ag Positivity	41 (66.1%)
Anti-HCV Positivity	0
Nodule Sizes	7.2±3.5
Number of Nodules (Multiple: Single)	59:3

ALT=Alanine aminotransferase; AST=Aspartate aminotransferase; ALP=Alkaline phosphatase; HBsAg=Hepatitis B surface antigen; Anti – HCV=Hepatitis C antibody; AFP=Alpha-fetoprotein; DCP=Des-gamma-carboxyprothrombin

Clinical and laboratory variables were compared among the three groups as shown in Table 2. Apart from gender and serum alanine transaminase (ALT), other variables were not significantly different among the three groups implying similar clinical background for the groups. There were significantly more males than females among the groups ($P < 0.03$). The mean serum ALT levels were significantly different among the three groups ($P < 0.006$).

Paired comparisons between the groups showed that the mean ALT levels in Group 2 were significantly higher than in Group 1 ($P < 0.003$). The mean ALT level in Group 2 was also significantly higher than in Group 3 ($P < 0.014$). There was no significant difference in the mean ALT values between Group 1 and Group 3 ($P = 0.124$).

As shown in Table 3, univariate analysis of nine clinical and laboratory parameters indicated that elevation of DCP to a level ≥400 mAU/ml was significantly associated with gender, HBsAg positivity, and elevated serum AST level. Elevation of DCP to a level ≥400 mAU/ml was not associated significantly with age, serum ALT, alkaline phosphatase, albumin, total bilirubin, or nodule sizes.

Based on the results of the univariate analysis, three variables were used for multivariate analysis to identify the variables that can independently predict the elevation of DCP to values ≥400 mAU/ml [Table 4]. The variables include gender, serum AST, and HBsAg positivity that initially showed significant association on univariate analysis. After adjustment for gender, serum AST and HBsAg positivity remained independently associated with elevation of DC*P* values ≥400 mAU/ml [Table 4].

Table 2: Comparison of Clinico-pathologic Variables among the Groups

Variables	Group 1 AFP ≥20 IU/ml DCP ≥150 mAU/ml (mean±SD) *n*=36	Group 2 AFP <20 IU/ml DCP ≥150 mAU/ml (mean±SD) *n*=24	Group 3 AFP <20 IU/ml DCP <150 mAU/ml (mean±SD) *n*=2	Statistical Significance (*P*)
Gender (M: F)	29:7	19:5	0:2	*P*<0.031
Age (years)	46.7±11.9	45.3±13.1	51±1.4	NS
HBsAg (Positive/Negative)	26: 10	16:8	0:2	NS
AST (IU/L)	87.7±53.3	94.5±25.4	16±0	NS
ALT (IU/L)	36.2±38.9	52.6±31.4	15.0±4.2	*P*<0.006
AST/ALT ratio	3.17±1.7	2.35±1.4	1.11±0.3	NS
ALP (IU/L)	241.4±168.2	212.1±275.5	158.0±22.6	NS
Albumin	31.6±6.2	31.1±4.7	33.0±0	NS
Total Bilirubin	105.3±118.0	95.3±110.1	16.0±0	NS
Nodule size (cm)	10.4±3.9	6.7±3.2	7.1±0	NS
Number of Nodules (Multinodular/Solitary)	34:2	23:1	2:0	NS

HBs Ag=Hepatitis B surface antigen; AST=Aspartate amino transferase; ALT=Alanine amino transferase; ALP=Alkaline Phosphatase; NS=Not Statistically Significant

Table 3: Univariate Analysis of Variables Elevating DCP ≥400 mAU/ml in HCC Patients

Variables	OR (95% CI)	*P*
Gender	11.25 (2.32-54.44)	0.003
Age	0.95 (0.89-1.01)	0.120
AST	1.03 (1.01-1.05)	0.006
ALT	1.04 (1.00-1.09)	0.066
ALP	1.00 (1.00-1.01)	0.293
Albumin	0.88 (0.75-1.03)	0.120
Total Bilirubin	1.04 (0.98-1.09)	0.216
HBs Ag Positivity	29.09 (3.28-258.20)	0.002
Nodule Sizes	1.43 (0.95-2.13)	0.083

OR=Odds Ratio; AST=Aspartate aminotransferase; ALT=Alanine aminotransferase; ALP=Alkaline Phosphatase; CT=Confidence Interval; HBsAg=Hepatitis B Surface Antigen

Table 4: Multivariate Analysis of Variables Elevating DCP ≥400 mAU/ml in HCC Patients

Variables	OR 95% CI	*P*
Gender	1.08 0.11-10.41	0.950
AST	1.03 1.00-1.06	0.030
HBsAg Positivity	28.68 1.55-528.60	0.024

OR=Odds Ratio; CI=Confidence Interval; AST=Aspirate Aminotransferase; HBsAg=Hepatitis B Surface Antigen

Discussion

AFP and DCP have been extensively studied as biomarkers for the diagnosis of and prognostication in HCC. However, there are only few reports on the clinical characteristics of HCC in relation to the combination of the two tumor markers in HCC. Among the few reports using the combination of both biomarkers, most reports involve HCC with hepatitis C virus etiological background. This study therefore set out to investigate the clinical characteristics of HBV-related HCC in relation to different sets of AFP and DCP.

AFP has been reported in America to be of low sensitivity among various racial groups but worst among the African Americans.[19] A Nigerian study reported a sensitivity of 62.9% at an optimum cutoff point of 18 IU/L.[12] About 40% of HCC do not secrete AFP.[4] Due to its low sensitivity, it is usually puzzling, to observe patients with clinical features of advanced HCC but with AFP below the standard cutoff point of 20 IU/L. It would be of interest to explore the clinical characteristics of this group of AFP-negative HCC patients.

Our findings in this study showed that the mean values of all the variables were higher in the group with higher values of both AFP and DCP (Group 1) with the exception of the mean values of ALT and AST. ALT was significantly higher in Group 2 than in Group 1 ($P < 0.003$). The mean AST value was also higher in Group 2 than in Group 1 but it fell short of statistical significance. Both variables (ALT and AST) are known markers of liver cell inflammation and necrosis.

The molecular mechanisms of HCC pathogenesis remain largely unknown. However, oxidative stress and chronic inflammation from viral and endogenous chemical processes have been linked to an increased risk of liver cancer.[20,21] Hepatitis B virus is known to be carcinogenic through multiple mechanisms which include, among others, indirect mechanisms of inflammation leading to genetic damage over time.[22] Therefore, it would not be surprising if levels of markers of inflammation and necrosis are increased in HCC patients. Indeed, it has been reported that C-reactive protein is positive in a large proportion of patients with HBV-related HCC and that C-reactive protein has significant diagnostic power in AFP-negative, HBV-related HCC.[23]

The finding in our study of significantly elevated serum ALT levels in patients with AFP-negative but DCP-positive HCC may indicate that in patients with HCC who present with negative AFP levels, it may be useful to carefully assess the ALT values as an aid to diagnosis of HCC. This view is supported by previous studies.[24-28] In a large trial involving 76,347 HCV-infected patients, it was observed that in the presence of HCC, the AFP and ALT levels were nearly independent of each other.[24] This dissociation between AFP and ALT levels in the presence of HCC was noted in the study to be in sharp contrast with the strong positive correlation between AFP and ALT at all levels in the absence of HCC.[24] Tarao *et al*.[25] also noted that the serum ALT levels are associated more closely with the development of HCC than the serum AFP. In their report, 71.4% of patients having HCV-associated cirrhosis with persistently high ALT (>2 times the upper limit of normal) developed HCC within 5 years, compared to 37.5% of cirrhotic patients with persistently high AFP (≥30 ng/ml) within the same period. Moreover, serum AFP levels have been reported to be reduced at the height of intense inflammation as evidenced by increasing jaundice, in patients with acute liver failure from chronic HBV infection.[26] Adachi *et al*.[27] also reported serum ALT to be an independent risk factor associated with intrahepatic tumor recurrence. Furthermore, a large study involving 6831 HCC patients in Taiwan noted a high ALT and a high AST/ALT ratio to be independent poor prognostic factors.[28]

In our study, no patient was found in the group with low DCP and high AFP (Group 4). This may be explained by the late presentation of our patients which is a common occurrence in resource-poor countries.[29,30] AFP is reported to be more sensitive for early stages HCC

while DCP is more sensitive for late stages tumors.[31] Thus, patients with high AFP and low DCP levels would be expected more in the early stages of HCC.

Multivariate analysis of variables that predispose to elevation of DCP ≥400 mAU/ml showed that HBsAg positivity and AST were the only independent predictors of DCP elevation to a level ≥400 mAU/ml after adjusting for gender. High AST and high AST/ALT ratio have been implicated in various reports as predictors of poor prognosis in HCC patients.[32-35] Similarly, HBV infection is an established poor prognostic factor in HCC patients.[33,36]

The main limitation of our study is the small sample size. Further studies using larger sample sizes which may also include early stage HCC patients would be required to confirm our findings.

Conclusion

Based on our findings, we conclude that HCC patients who are seropositive for DCP and seronegative for AFP have significantly high levels of ALT and that high ALT levels may have significant diagnostic power in AFP-negative, HBV-related HCC patients. We found HBsAg positivity and serum AST to be independent predictors for the elevation of DCP.

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Conflicts of interest

There are no conflicts of interest.

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