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EVALUATION OF ACTIVATED LEUCOCYTE CELL ADHESION MOLECULE IN HEPATOCELLULAR CARCINOMA PATIENTS

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ABSTRACT

Background: Besides, cost/effectiveness and efficacy of surveillance and screening of patients with cirrhotic liver for the diagnosis of hepatocellular carcinoma (HCC) is still debated. Search for suitable biomarkers of HCC is very intense. Roles of activated leucocyte cell adhesion molecule (ALCAM) in HCC diagnosis are largely unknown. This study aimed to investigate clinical diagnostic power of serum ALCAM to discriminate HCC patients from non-malignant cirrhotic patients. **Methods:** Activated leucocyte cell adhesion molecule was tested for 50 HCC patients, 30 cirrhotic patients and 25 healthy individuals by enzyme-linked immunosorbent assay (ELISA). Data from all participants were retrospectively analyzed and receiver operating characteristic (ROC) curve was established to evaluate ALCAM value as HCC biomarker. **Results:** Hepatocellular carcinoma patients were associated with significantly (P<0.001) higher ALCAM serum levels than cirrhotic and healthy controls. Serum ALCAM has high HCC diagnostic power with AUC of 0.963, sensitivity 90%, specificity 91%, positive predictive value 90%, negative predictive value 90.9% and accuracy 90.5%. Elevated ALCAM levels were associated with multiple nodes and large tumors (>3cm). Also, ALCAM levels were Significantly correlated with ALT, AST, albumin, total bilirubin, AFP and also with oxidative stress parameters include catalase (CAT), total antioxidant capacity (TAC) and malondialdehyde (MDA). **Conclusions:** Our results indicate that serum ALCAM might be served as a potential biomarker with high diagnostic value for HCC screening.

KEYWORDS: Hepatocellular carcinoma, Diagnosis, Biomarker, ALCAM, Oxidative stress.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy and the sixth most common cancer worldwide.[1] HCC constitutes worthy public health problem facing health authorities in Egypt.[2] This had been strongly related to hepatitis C virus (HCV) epidemic. Egypt was reported as the highest prevalence of HCV in the world as during the last 3 decades HCV affected around 10-15% of the Egyptian population.[3] From preexisting conditions including hepatitis viruses, nonalcoholic and alcoholic cirrhosis, most HCC cases obtains after a history of chronic hepatitis-related cirrhosis in which there is persistent hepatocytes injury and regeneration.[4] After discovery, HCC has a poor prognosis which is usually at a late stage of disease.[5]

Traditional methods for HCC diagnosis include imaging and serological tests are with low sensitivity and specificity.[6] Outcomes of imaging methods including ultrasound (US) and computed tomography (CT) depend on the radiologist experience. Also, they are associated with high false results rate, high costs, and potential barrier related to contrast-related injury and radiation exposure.[7] There are other limitations when imaging cirrhotic or obese patients.[8] From another hand, however, there has been a plethora of studies regarding the diagnostic utility of alpha-fetoprotein (AFP) and other HCC biomarkers which often generate contradicting findings. These biomarkers are unsatisfactory because of their low diagnostic value in HCC detection particularly in early tumor stages.[9] So, more specific and sensitive biomarkers may aid in HCC prognosis and early diagnosis.

Activated leucocyte cell adhesion molecule (ALCAM/CD166) is a member of the immunoglobulin superfamily that is involved in maintenance of immune responses, tissue architecture, and tumor progression.[10] Former studies have reported that ALCAM expression is correlated with aggressiveness in different tumors, including melanoma[11], colorectal[12], bladder[13], breast[14], prostate[15], ovarian[16] and other cancers and has been used as a prognostic marker.[10] In HCC, ALCAM is an upstream regulator of Yes-associated Protein (YAP) and was reported to enhance YAP function to exert a carcinogenetic role.[17] However, limited data was found regarding the use of ALCAM as valuable HCC serum biomarker.

In the present study, we aimed to determine the efficacy of ALCAM serum levels in HCC prediction in high risk chronic hepatitis C (CHC) patients. In such patients, oxidative stress is included in hepatocarcinogenesis through viral proteins direct effects or secondarily to

chronic inflammation.[18] Thus, we also evaluated the relation between ALCAM and some classical oxidative stress markers related to cancer include catalase (CAT), total antioxidant capacity (TAC) and malondialdehyde (MDA).

MATERIALS AND METHODS

Patients

In this study, a total of 105 consecutive Egyptian individuals were enrolled. They were classified into 3 groups: group (1): 50 patients with CHC and HCC, group (2): 30 patients with CHC and liver cirrhosis and group (3): 25 healthy controls. Patients were referred from Mansoura University Hospitals, Mansoura, Egypt. Eligible patients were positive for serum HCV antibody and HCV-RNA and had histopathological confirmation of chronic hepatitis. Diagnosis of HCC was by at least two imaging tests (US and CT) showing an arterial enhancing lesion or in few cases by histopathology. Patients with any other tumors were excluded. The study has been approved by the Ethics and scientific committees of Mansoura University Hospitals, Mansoura, Egypt.

Blood collection and laboratory assays

After fasting for at least 6 hours, venous blood (5 ml) was withdrawn from all patients. Without an anticoagulant, serum samples were collected from one part of the blood. Serum samples were freshly tested for biochemical parameters on an automated biochemistry analyzer (Hitachi 902; Roche Diagnostics) including alanine and aspartate transaminases (ALT, AST), total bilirubin, albumin and creatinine. Another blood part in tube with citrate solution was used to measure prothrombin-international normalized ratio (INR). For complete blood count, the final blood part in K-EDTA tubes were used and analysed on an automated hematology analyzer (Sysmes Corporation, Kobe, Japan). Serum AFP levels were measured by chemiluminescence, with an Immulite AFP (1000) kit (Diagnostic Products Corporation, Los Angeles, CA, USA). Serum CAT (Chongqing Biospes Co., Ltd., Chongqing, China, Catalog # BYEK2178) and ALCAM (Boster Biological Technology, Pleasanton CA, USA, Catalog # EK0995) were determined according to the manufacturer's instructions of human commercial ELISA kits. Serum TAC and MDA levels were determined by colorimetric methods (BIODIAGNOSTIC, Dokki, Giza, Egypt).

Statistical analysis

For all statistical analyses, GraphPad Prism package and SPSS (SPSS Inc., Chicago, IL) were used. Quantitative results were expressed as the mean±standard deviation (SD), whereas

qualitative results were expressed as numbers. Differences between independent two groups were compared with unpaired Student's *t* test or Mann–Whitney (U) test. ANOVA or Kruskal-Wallis tests were used to examine the differences between more than two groups. Receiver operating characteristic (ROC) curve was performed to evaluate the independent discriminative value of ALCAM for HCC detection. ALCAM cutoff value was selected according to the point on the curve closest to the (0, 1) point (the minimal $(1-\text{sensitivity})^2 + (1-\text{specificity})^2$).[19] ALCAM diagnostic performances were derived from a 2×2 contingency table. Correlation evaluations obtained by Pearson's correlation coefficient.

RESULTS

Baseline characteristics of patients and controls

Clinical and demographic features of patients and controls are presented in (**Table 1)**. HCC patients were associated with older age, higher AFP, total bilirubin and liver enzyme activities and lower albumin levels. Also, patients with HCC had lower levels of CAT and TAC and higher MDA levels in comparison with cirrhosis and healthy controls.

Hepatocellular carcinoma was associated with high ALCAM serum levels

Using an anti-ALCAM antibody and ELISA assay, HCC patients were associated with significantly (P<0.001) higher serum levels (75.1±17.2 ng/mL) of ALCAM than cirrhotic patients (50.0±7.3 ng/mL) and healthy controls (23.7±7.4 ng/mL) "**Fig. 1A**". In contrast to AFP, the ROC analysis findings showed that the use of serum ALCAM per se gave at cutoff 50 ng/mL an AUC=0.963 **"Fig. 1B"** with sensitivity, specificity, positive predictive value, negative predictive value and accuracy of 90%, 91%, 90%, 90.9% and 90.5%, respectively. These values increased when HCC discriminate from healthy controls only "**Fig. 1C**, **Table 2**".

Activated leukocyte cell adhesion molecule and some clinical features

Patients with HCC were classified according to lesions of tumor (single/multiple), tumor size (<3cm/>3cm) and AFP levels (<200/>200 U/L) **"Fig. 2"**. Serum ALCAM levels (ng/mL) increase in patients with multiple nodels (85.9±10.6 ng/mL), large tumors (88.9±10.4 ng/mL) and AFP >200 U/L (77.0±4.5 ng/mL) than patients with single nodels (74.6±11.7 ng/mL), small tumors (75.5±11.2 ng/mL) and AFP <200 U/L (74.0±3.5 ng/mL), respectively **"Fig. 3"**. Also, ALCAM serum levels were significantly correlated with activities of ALT and AST, albumin, total bilirubin and AFP serum levels and also with CAT, TAC and MDA (**Table 3**).

DISCUSSION

Alpha-fetoprotein (AFP) has been used for a long time for HCC screen. Nevertheless in patients with active hepatitis, AFP has high rate of false positive results and this represent one major disadvantage of using AFP in HCC detection.[20] In this study we found that ALCAM was significantly elevated in sera of HCC than non-malignant hepatitis-related cirrhosis patients.

A cell surface immunoglobulin ALCAM is over-expressed in many epithelial tumors.[11-16] In hepatic tumor cells, ALCAM overexpression was induced by the activation of anti-apoptotic canonical NF-κB signaling after serum deprivation "a condition which inhibits cell growth and caused apoptosis".[21] One of oncoproteins that have serious roles in transformative phenotype maintenance in liver cancer cells is YAP.[22] Ma et al. investigated the mechanism of ALCAM anti-apoptotic role in hepatic tumor cells and reported that the ALCAM function on liver tumorigenesis relied on YAP.[17] Our results are consistent with the results of Ma et al. who found that ALCAM was detected in sera of HCC patients compared with very low concentrations in healthy controls, patients with hepatitis B or C and patients with cancers other than HCC like colon, lung breast and gastric cancers.[23]

Meanwhile, this study indicated that serum ALCAM level was significantly correlated with the bulk of diseased tissue in HCC. As ALAM elevated levels were significantly associated with tumor size and number of lesions. Thus, it is likely that there is a meaningful association between ALCAM and HCC tumor stage. However, further studies are needed to characterize this association.

Liver damage induced by tumor previously reported and indicated by impaired liver function.[24] Here, we investigated the relationships between ALCAM serum levels and activities of liver enzymes and albumin serum levels. Our findings revealed that serum ALCAM reflects liver damage extent in HCC. Serum ALCAM was significantly correlated with elevated ALT and AST activities and total bilirubin but negatively correlated with albumin levels.

Biomarkers that closely correlate with disease pathophysiological process are the most promising ones.[25] Oxidative stress is included in hepatocarcinogenesis through viral proteins direct effects or secondarily to chronic inflammation.[18] Significant decrease of antioxidants was reported in HCV-related HCC patients compared to healthy controls.[26] Here we also

determined the correlation between ALCAM and CAT, TAC and MDA. We found significant correlation between ALCAM serum levels and these oxidative stress markers.

As well, ALCAM serum levels enabled the correct detection of HCC patients from all non-HCC individuals with 0.963 AUC. This diagnostic power is superior to those of imaging methods and other well established HCC biomarkers. US, CT, and MRI sensitivities in HCC detection were 84, 79, and 77%, respectively.[27] The sensitivity and specificity of AFP were 61 and 71%, of AFP-L3 were 37 and 92%, of DCP were 39 and 90%, of osteopontin were 74 and 66% and of dickkopf-1 (DKK1) were 69.1 and 90.6%, respectively.[28-30]

CONCLUSIONS

In conclusion, these results suggested that ALCAM could be a potential marker for identifying HCC in patients with chronic liver disease like cirrhosis and may aim to overcome AFP insufficiency. However, this study was limited by the sample size and further multicenter studies including a greater number of patients are needed.

Table 1: Baseline clinical and demographic characteristics of HCC patients and controls.

Parameter [a]	Healthy N=25	Cirrhosis N=30	HCC N=50	*P* value
Male/female	16/9	25/5	40/10	
Age (years)	54.8±6.5	55.0±5.5	56.3±4.7	0.50
ALT (U/L)	25.7±5.2	59.0±13.7	65.8±14.3	<0.001
AST (U/L)	28.4±4.4	66.4±14.2	70.9±15.3	<0.001
Albumin (g/dL)	4.1±0.5	3.15±0.5	3.18±0.4	<0.001
Total bilirubin (mg/dL)	0.74±0.1	2.4±0.8	2.1±0.7	<0.001
INR	1.2±0.2	1.6±0.6	1.2±0.3	<0.001
Creatinine (mg/dL)	1.02±0.2	0.81±0.28	0.96±0.49	0.05
AFP (ng/ml)	6 (3-9)	13.8 (2.8-300)	75.5 (5.1-2104)	<0.001
Platelet count ($\times10^3$/µL)	221±59.3	80.6±35.7	133.0±79.6	<0.001
WBCs ($\times10^3$/µL)	7.6±2.1	4.43±1.5	6.9±4.5	0.006
RBCs ($\times10^6$/µL)	4.5±0.77	3.7±0.9	4.33±0.6	0.001
Heamoglobin	13.1±1.9	11.9±2.1	12.5±1.5	0.75
Catalase (ng/ml)	9.2±0.7	6.5±0.53	3.8±1.13	<0.001
TAC (mM/L)	2.7±0.3	1.3±0.24	1.0±0.2	<0.001
Malondialdehyde (nmol/mL)	8.9±1.1	15.9±1.1	23.4±3.5	<0.001

Variables were expressed as mean±SD except AFP as median (range). Abbreviations: alanine aminotransferase (ALT), aspartate aminotransferase (AST), international normalized ratio (INR), alpha-fetoprotein (AFP), white blood cells (WBCs), red blood cells (RBCs) and total antioxidant capacity (TAC).

Table (2): Diagnostic performances of ALCAM against AFP to discriminate HCC patients from non-HCC individuals.

	Marker	AUC (95% CI)	*P* value	Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
HCC *vs.* all non-HCC	AFP (ng/mL)	0.850 0.77-0.93	<0.001	55	58	91	85.3	70.4	75.2
	ALCAM (ng/mL)	0.963 0.92-0.99	<0.001	50	90	91	90	90.9	90.5
	Marker	AUC (95% CI)	*P* value	Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
HCC *vs.* Healthy	AFP (ng/Ml)	0.941 0.90-0.99	<0.001	55	58	100	100	54.3	72
	ALCAM (ng/mL)	0.992 0.98-1.0	<0.001	50	90	96	97.8	82.8	92

Abbreviations

AFP = Alpha-fetoprotein; ALCAM= Activated Leukocyte Cell Adhesion Molecule; PPV= Positive predictive value; NPV= Negative predictive value; All non HCC= cirrhotic patients + healthy individuals.

Table 3: Correlation between serum levels of ALCAM and age, liver related and oxidative stress parameters.

Factor correlated with ALCAM	Pearson correlation coefficient (*r*)	*P* value
Age	0.280	0.080
ALT	0.669	<0.001
AST	0.638	<0.001
Albumin	-0.478	<0.001
Total bilirubin	0.434	<0.001
AFP	0.347	0.001
Catalase	-0.409	<0.001
Total antioxidant capacity	-0.512	<0.001
Malondialdehyde	0.405	<0.001

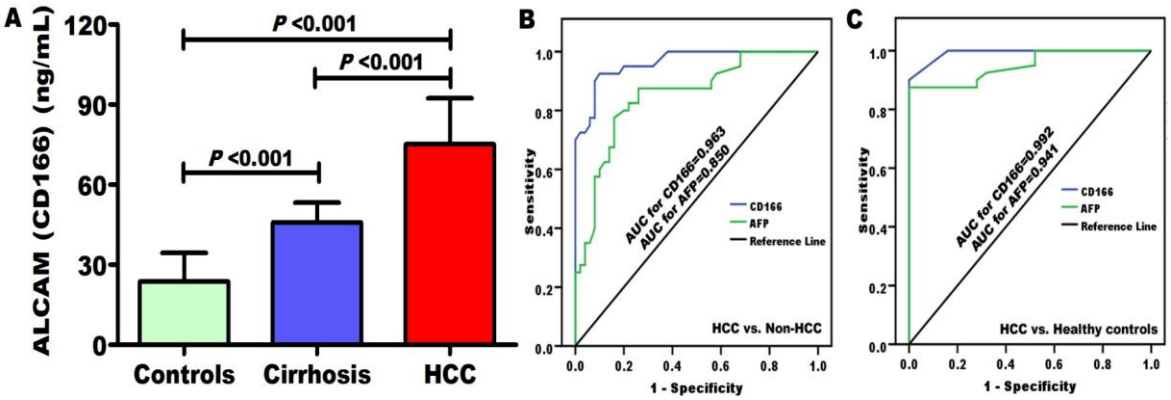

Fig. 1. ALCAM levels increase with HCC development. (A) As measured by ELISA, serum ALCAM was significantly elevated in HCC compared with cirrhotic patients and

healthy controls. ALCAM serum levels at cutoff point of 50 ng/mL had good diagnostic power to discriminate HCC patients from (B) all non-HCC combined and (C) healthy controls only.

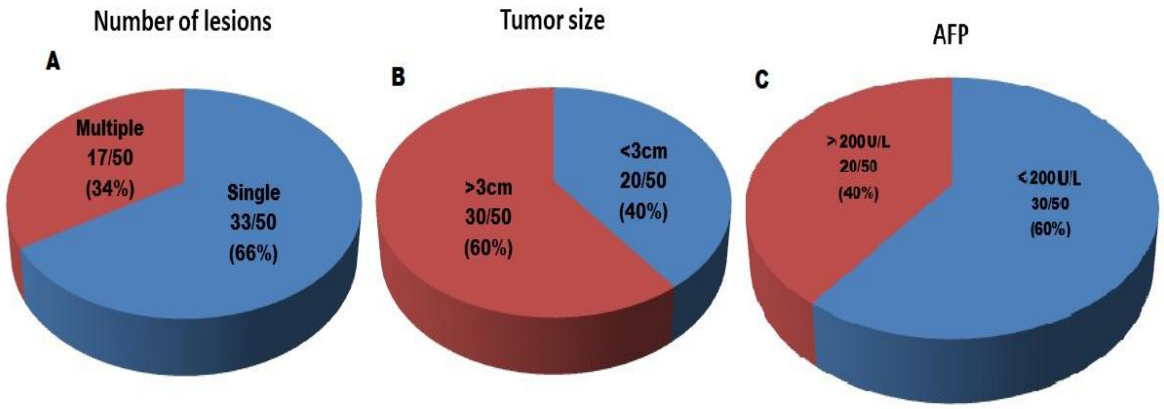

Fig. 2: HCC cases were classified according to (A) number of tumor lesions, (B) tumor size (cm) and (C) AFP levels (ng/ml).

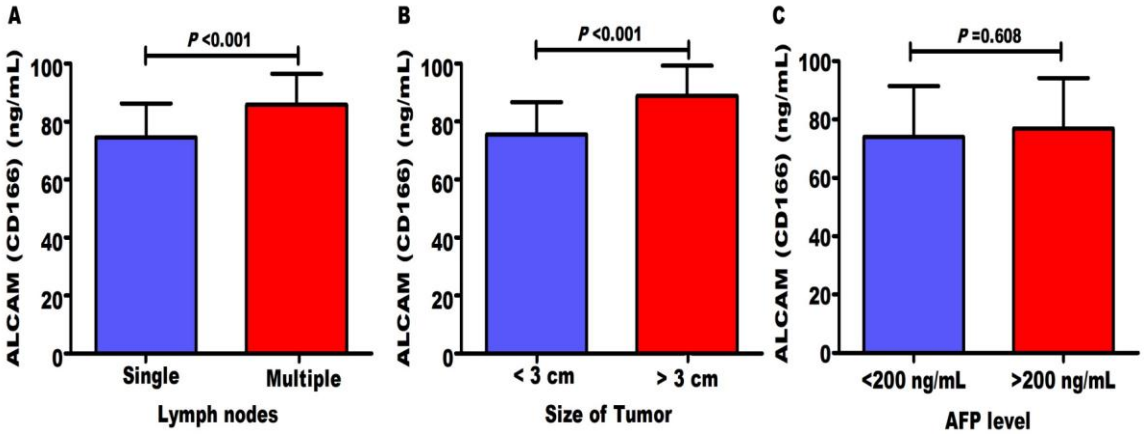

Fig. 3: Distributions of ALCAM serum levels values according to (A) number of lesions, (B) tumor size and (C) AFP low and high levels.

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