Original Research Article

Serum calcium channel alpha2 delta1 $\alpha_2\delta1$ subunit as a diagnostic and a predictive marker of therapeutic ablation outcome for hepatocellular carcinoma in cirrhotic hepatitis C patients.

Abstract

Background: Hepatocellular carcinoma is the 7th common cancer worldwide. Its diagnosis depends mainly on abdominal ultrasound and serum alpha-fetoprotein. The alpha2 delta1 (α2δ1) subunit is excessively presented in Tumor Initiating Cells (TICs) of HCC, and is mandatory in calcium transport, which is important to maintain TICs properties. The aim was to assess serum α2δ1 subunit as a diagnostic marker and for follow up of hepatocellular carcinoma patients after ablation in comparison to alpha-fetoprotein.

Methods: This study was performed on 60 subjects. They were divided into three groups; Group I: included 20 patients with hepatocellular carcinoma, Group II: included 20 patients without hepatocellular carcinoma, and Group III: included 20 healthy individuals as control ones. Serum α2δ1 subunit was measured in all subjects and followed up in group I 3 months after ablation.

Results: Serum α2δ1 subunit was significantly higher in group I than either group II or group III with a cut off value >100 ng/mL (sensitivity 80%, specificity 100%), and was decreasing after ablation in well ablated cases in the period of follow up. Conclusion: α2δ1 subunit is a useful marker in diagnosis of hepatocellular carcinoma, also in follow up after ablation.

Keywords: Hepatocellular carcinoma, α2δ1 subunit, Tumor Initiating Cells

Introduction

Primary liver cancer is one of the most common malignancies over the world and represents the seventh-most frequently occurring cancer [1]. Globally, hepatocellular carcinoma (HCC) is the main type of liver cancer which accounts more than 75% of all liver cancers [2].

Curative treatments are only available at an early stage, where the 5-year survival is 50–70%. In contrast, patients presenting with advanced HCC are only eligible for palliative treatments and have a poor outcome with a median survival of less than 1 year [3]. Therefore, early detection of HCC is crucial in increasing survival [4].

The early screening of HCC depends on imaging techniques including mainly ultrasonography and laboratory tests involving mainly serum alpha-fetoprotein (AFP) [5].

Although ultrasound has improved in recent years, it has visualization limitations in patients with obesity, steatosis and advanced fibrosis or cirrhosis, also it is an operator-dependent procedure in addition, it fails to detect small tumors [6]. As well, the accuracy of AFP as a diagnostic biomarker for the screening of HCC patients at the early stage is modest with the sensitivity of 60% to 80% and with the specificity of 70% to 90% [7].

The roles of ion channels in various cell functions, including mitogenesis, cell proliferation, differentiation, apoptosis and metastasis are now well recognized [8]. Voltage-gated calcium channels (VGCC) exist throughout the body and perform many physiological functions. In cancer cells, VGCCs are involved in several of the cancer hallmarks as sustaining proliferative signaling, evading growth suppressors, resisting cell death, metastasis and evading immune destruction [9].

The alpha 2 delta (α2δ) proteins are auxiliary subunits of voltage-gated calcium channels, and were found to be encoded by many different genes [10]. The overexpression of $\alpha_2\delta 1$subunit was attributed to maintain properties of the liver Tumor Initiating Cells (TICs) through regulation of Ca2+ influx [11].

The aim of the present work was to study serum calcium channel alpha2 delta1 subunit as a diagnostic and a predictive marker of therapeutic ablation outcome for hepatocellular carcinoma in cirrhotic hepatitis C patients.

Patients and methods

This prospective cohort study was carried out on 60 subjects from the outpatient clinic and inpatient of Tropical Medicine and Infectious Diseases Department at Tanta University Hospitals from December 2018 to February 2021.Written consents were obtained from all individuals and the study was approved by the ethical committee.

Patients were classified into three groups: group I included 20 HCC hepatitis C cirrhotic patients before and three months after ablation, group II included 20 hepatitis C cirrhotic patients without HCC and group III included 20 healthy individuals as a control group. We included patients with the following criteria; Age >18 years, performance status (PS) ≤ 2 according to Eastern Cooperative Oncology Group, in addition to Child–Pugh classification A, HCV positive cirrhotic patients, Liver cirrhosis diagnosed by physical examination, laboratory investigations and imaging evidence of cirrhosis.

Ultrasonography was used for the initial detection of hepatic focal lesions and triphasic computed tomography (CT) scans or dynamic contrast-enhanced magnetic resonance imaging (MRI) was used to confirm the diagnosis of HCC. Staging of HCC was evaluated according to BCLC. The first group underwent loco regional therapy by either microwave ablation (MWA) or radiofrequency ablation (RFA) and followed up over a period of three months by triphasic CT scan, AFP & α 2δ1 subunit.

The patients with the following conditions were excluded; Metastatic tumors of the liver, other malignancies anywhere in the body, patients not candidate for loco regional therapy were ruled out from first group and HCC causes other than HCV.

Methodology

All cases were subjected to complete history taking and abdominal examination. Full laboratory investigations were done in all cases including; CBC, serum ALT, AST, INR and prothrombin time, serum albumin, serum bilirubin, HCV Ab, HBs Ag or Anti HBc (total) for HBV by ELISA technique and AFP.

Determination of serum level of α 2δ1 subunit

Serum calcium channel alpha2 delta1 (α 2δ1) subunit was estimated from peripheral blood samples by ELISA kits (catalog No. 201126958 China) in all groups and three months after ablation in group I.To perform the assessment, 3ml of venous blood was collected from all patients by clean venipuncture using plastic disposable syringes. Centrifuge was at 2000–3000 rpm for 20 min., the samples were frozen and stored at - 80 °C until use.

Loco-regional ablation of HCC

The procedure was done in a special sterilized unit containing the ultrasound machine (Siemens, Toshiba).

First of all, patients were fasting 6 hours. Sterilization of skin was made using betadine and alcohol. Local anesthesia was performed by 10 ml of 2% xylocaine. It was along the needle track from the entry site on the skin to the liver capsule.

RFA was made using The RITA ® Model 1500x RF," produced by Angio-Dynamics, USA". As an RFA session began, a hyperechoic focus developed around the uninsulated portion of the electrode. This was attributed to tissue vaporization and cavitations. The area of echogenicity was round; most often progressively increased in size over the course of ablation and generally enveloped the entire tumor with variable extensions in the surrounding liver by the end of the treatment. When the time was over, the generator automatically went into cool down mode for 30 seconds (5minutes on the generator display), when the Cool Down was complete, the temperatures from all leads had to be above 70°C. In all cases, tract ablation was done before removal of the needle.

As regards MWA the microwave needle (AMICA™ - MW Ablation System) was inserted deep in the lesion avoiding big vessels and surrounding viscera. Ablation was done using 80 Watt for 10 minutes. When ablation was completed needle track ablation was done to avoid post procedural bleeding.

Strong IV analgesics were given as pethidine hydrochloride 50 mg or tramadol, intravenous antiemetic was given if needed and prophylactic antibiotic was given as, amoxicillin-clavulanic acid. All patients were observed clinically for 2-3 hours.

In this study 12 patients were well ablated following loco regional therapy by either RFA or microwave and no recurrence or de novo lesions appeared till three months of follow up with cure rate 60%. Recurrent and de novo lesions appeared in 8 patients and required second cession of ablation.

Statistical analysis

Data were analyzed using SPSS 22 and SigmaStat 4.0. Mean comparisons were done using analysis of variance (ANOVA) test followed by Post Hoc test. For comparison of data, the Chi square test was performed. Spearman's rank correlation test was used to detect associations between different variables. Receiver Operating Characteristics (ROC) curve was plotted and cut off values of both α 2δ1subunit, and AFP was calculated with sensitivity, specificity, positive predictive value, and negative predictive value. For all testes, P value ≤ 0.05 are considered significant.

Ethics: All participating subjects provided written informed consents. The study was approved in November 2018 by the Ethics Committee of the faculty of medicine, Tanta University code 32693/11/18.

Results

Group I comprised 20 patients with HCC (15 males and 5 females; mean age, 51.40 ± 7.089 years old). Before ablation 100% of patients were Child A and in three months follow up period 90% were Child A and 10% were Child B.

Group II comprised 20 patients with HCV cirrhosis (8 males and 12 females; mean age, 57.75 ± 11.23 years old).

Group III comprised 20 apparently healthy volunteers (16 males and 4 females, mean age, 48.25 ± 14.41 years old). All participants were sero-negative for HCV and HBV antibodies, with normal clinical; laboratory, and radiological findings. Demographic data was demonstrated in the Table 1.

As regards laboratory data, there were statistically significant differences between group I and group II as regards to HB, serum albumin, AST and total bilirubin while

there were no statistically significant differences between the two groups as regards to ALT and platelet. Table 1

Detection of serum α2δ1 subunit for diagnosis and prediction of ablation outcome of HCC

α2δ1 subunit level was significantly higher in group I (mean level was; 182.3) than group II (mean level was; 61.3) and higher in group I than group III (mean level was; 14.7) (P <0.001).α2δ1 subunit level was higher in group II than group III however there was no statistical significant difference in-between the two groups (P =0.076). Table 3. In group I, a significant positive correlation was detected between AFP and α2δ1 subunit (r= 0.640) (P = 0.002). In addition; both AFP and α2δ1 subunit were positively correlated with serum creatinine (r= 0.663) (P = 0.001), (r= 0.487) (P = 0.029) respectively. Table 2.

In Assessment of prognostic value of AFP and α2δ1subunit in follow up after ablation, AFP level was significantly decreasing in the follow up period after ablation in well ablated cases. Mean baseline of α2δ1subunit, was significantly decreasing in the follow up periods after ablation in well ablated cases , but not significantly decreasing in the follow up period in de novo and residual cases (P =0.028 & 0.758 respectively). α2δ1 subunit mean baseline in well ablated cases was (130.333 ± 48.944), after three months it was (93.367 ± 43.186) (P = 0.028) ,while its baseline level in de novo and residual cases was (260.388 ± 136.842), after three months it was (238.046 ± 131.555) (P = 0.758)

Table 4.

Receiver operating characteristic (ROC) curve was plotted to discriminate between HCC group and cirrhotic liver group. Cut – off values for AFP and α2δ1 subunit were calculated (with sensitivity, specificity, positive predictive value and negative predictive value). α2δ1 subunit: (cut off value 100 ng/mL, sensitivity 80%, specificity 100%, positive predictive value 100% and negative predictive value 83.3%), and AFP level: (cut off value 70 ng/mL, sensitivity 45%, specificity 100%, positive predictive value 100% and negative predictive value 63.1%) (Table 5 and Figure 1)

Table 1. Comparison between demographic and laboratory data of the three groups

	Groups			Chi-Square	TUKEY'S Test
	Group I	Group II	Group III		

		N		%	N		%	N		%	X^2	P-value	I&II	I&III	II&III
Sex	**Male**	15		75.00	8		40.00	16		80.00	8.352	0.015*	-	-	-
	Female	5		25.00	12		60.00	4		20.00					
ANOVA											**F**	**P-value**	**I&II**	**I&III**	**II&III**
Age	**Range**	41	-	62	40	-	80	23	-	70	3.697	0.031*	0.184	0.652	0.026*
	Mean ±SD	51.400	±	7.089	57.750	±	11.050	48.250	±	14.411					
Hb%	**Range**	10.7	-	16	7	-	12	10.8	-	15	55.458	<0.001*	<0.001*	0.979	<0.001*
	Mean ±SD	13.610	±	1.442	9.600	±	1.364	13.525	±	1.319					
TLC	**Range**	2.8	-	11	1.4	-	11	4	-	12	2.010	0.143			
	Mean ±SD	5.520	±	1.892	4.391	±	2.477	5.570	±	1.888					
Platelets	**Range**	48	-	228	31	-	212	132	-	280	57.645	<0.001*	0.066	<0.001*	<0.001*
	Mean ±SD	117.550	±	38.042	89.650	±	41.345	214.450	±	36.183					
S. Albumin	**Range**	3.5	-	4.5	2.2	-	4.1	3.5	-	5	50.578	<0.001*	<0.001*	0.039*	<0.001*
	Mean ±SD	3.915	±	0.300	2.845	±	0.572	4.290	±	0.500					
ALT	**Range**	15	-	66	19	-	63	12	-	30	17.740	<0.001*	0.420	<0.001*	<0.001*
	Mean ±SD	40.350	±	11.758	35.650	±	15.832	19.300	±	4.900					
AST	**Range**	25	-	87	16	-	68	19	-	37	14.739	<0.001*	0.009*	<0.001*	0.059
	Mean ±SD	49.600	±	19.047	35.850	±	13.819	25.400	±	6.778					
T.B.	**Range**	0.7	-	1.5	0.8	-	4.3	0.7	-	1.1	12.860	<0.001*	<0.001*	0.918	<0.001*
	Mean ±SD	1.030	±	0.178	1.890	±	1.106	0.949	±	0.114					
D.B	**Range**	0.11	-	0.5	0.1	-	1.8	0.1	-	0.23	10.931	<0.001*	0.001*	0.901	<0.001*
	Mean ±SD	0.202	±	0.089	0.617	±	0.588	0.155	±	0.044					
INR	**Range**	1	-	1.5	1	-	2.4	1	-	1.29	16.886	<0.001*	<0.001*	0.365	<0.001*
	Mean ±SD	1.196	±	0.179	1.503	±	0.347	1.097	±	0.083					
S. Cr.	**Range**	0.7	-	1.4	0.7	-	1.3	0.7	-	1.3	2.162	0.124			
	Mean ±SD	0.893	±	0.190	0.980	±	0.177	1.009	±	0.185					
S. Urea	**Range**	18	-	78	13	-	40	13	-	40	1.483	0.235			
	Mean ±SD	28.350	±	14.232	25.850	±	7.066	23.000	±	6.113					

Table 2. Pearson correlation between α2δ1 subunit, Alpha-fetoprotein levels and other parameters

Correlations								
	Group I				**Group II**			
	α2δ1		**AFP**		**α2δ1**		**AFP**	
	R	**P-value**	**R**	**P-value**	**R**	**P-value**	**R**	**P-value**
AFP	0.640	0.002*			0.279	0.234		
Age	0.248	0.292	-0.172	0.468	0.007	0.976	-0.068	0.776
Hb%	0.028	0.906	0.153	0.519	0.076	0.749	-0.133	0.576
TLC	0.221	0.348	0.397	0.083	0.307	0.188	0.541	0.014*
Platelets	0.116	0.627	0.241	0.306	0.114	0.632	0.109	0.648
S. Albumin	-0.128	0.590	0.150	0.529	0.226	0.337	-0.299	0.201
ALT	0.177	0.455	0.139	0.560	-0.403	0.078	-0.027	0.911
AST	0.421	0.065	0.593	0.006*	-0.360	0.118	-0.427	0.060
T.B.	-0.172	0.468	-0.138	0.563	0.128	0.591	0.353	0.127
D.B	-0.175	0.460	-0.135	0.572	0.021	0.929	0.342	0.139
INR	0.309	0.184	-0.111	0.640	-0.157	0.508	0.218	0.355
S. Cr.	0.663	0.001*	0.487	0.029*	-0.081	0.733	-0.358	0.121
MELD	0.404	0.078	-0.051	0.830	-0.041	0.865	0.207	0.380
FL Size CT	0.045	0.850	-0.037	0.877	-	-	-	-

Table 3. Comparison between the three studied groups as regards AFP and α2δ1 subunit.

	Groups	**ANOVA**	**TUKEY'S Test**

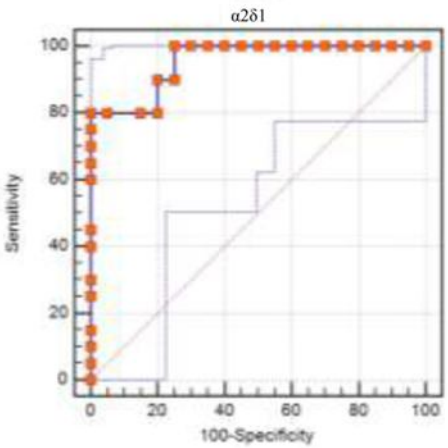
		Group I	Group II	Group III	F	P-value	I&II	I&III	II&III
AFP	Range	3.6 - 2400	1.5 - 70	1 - 10	9.983	<0.001*	0.001*	0.001*	0.993
	Mean ±SD	698.870 ± 969.544	23.800 ± 18.766	4.155 ± 2.645					
α2δ1	Range	78.7 - 477	22.9 - 100	1.5 - 33	34.097	<0.001*	<0.001*	<0.001*	0.076
	Mean ±SD	182.355 ± 112.066	61.305 ± 23.779	14.775 ± 6.658					

Table 4. Assessment of prognostic value of α2δ1subunit in follow up after ablation.

α2δ1		Triphasic CT findings		T-Test	
		Well ablated lesions	Residuals	t	P-value
Before	Range	78.7 - 210	143.5 - 477	-3.047	0.007*
	Mean ±SD	130.333 ± 48.944	260.388 ± 136.842		
After	Range	40 - 145	71.8 - 393.09	-3.573	0.002*
	Mean ±SD	93.367 ± 43.186	238.046 ± 131.555		
Differences	Mean ±SD	36.967 ± 50.687	22.341 ± 197.474		
Paired Test	P-value	0.028*	0.758		

Table 5. Diagnostic performance of α2δ1subunit and Alpha-fetoprotein for discrimination of HCC from chronic liver disease cases.

ROC curve between Group I and Group II						
	Cutoff	Sens.	Spec.	PPV	NPV	Accuracy
α2δ1	>100	80.0	100.0	100.0	83.3	95.5%
AFP	>70	45.0	100.0	100.0	64.5	63.1%



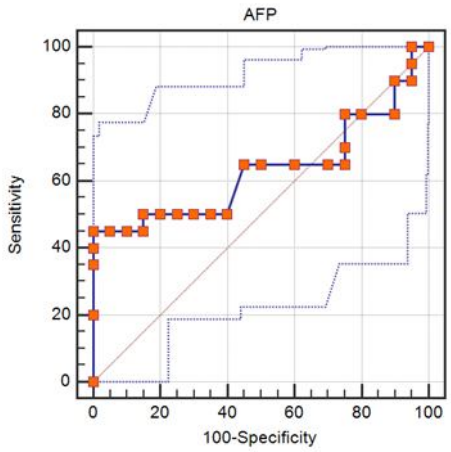


Figure 1. Receiver operating characteristic curves of α2δ1subunit and alpha-fetoprotein for the diagnosis of hepatocellular carcinoma

Discussion

Hepatocellular carcinoma (HCC) is nowadays one of the most frequent malignancies and a leading cancer-related death worldwide [12]. It is estimated that, by 2025, >1 million individuals will be affected by liver cancer annually [13]. Over 90% of HCC cases occur in the setting of chronic liver disease. Cirrhosis from any aetiology is the strongest risk factor for HCC [14]. The transition from chronic liver disease to cirrhosis involves inflammation and activation of hepatic stellate cells with ensuing fibrogenesis and angiogenesis [15]. Surveillance for HCC is recommended in all patients with cirrhosis and has been associated with improved early detection and survival [16].

Alpha-fetoprotein (AFP) is the most commonly used biomarker for HCC surveillance [17]. Despite the disadvantage of low sensitivity, low specificity and also limited accuracy in HCC early diagnosis, AFP has still been recommended as a serum biomarker for diagnosis of HCC in clinical practice [18]. There is a clinical need to discover novel biomarkers for HCC that fully correlate with the tumor stage; can be detected in early HCC; and allow for tumor surveillance and prognosis [19].

Voltage-gated calcium channels (VGCC) exist throughout the body and are required for many key functions [20]. The expression of ion channel transcripts has been highlighted as a potential biomarker of certain types of cancer, including prostate cancer, HCC and breast cancer [21]. The alpha2 delta1 (α2/δ1) subunit is a trans-membrane

protein that contains glycosyl-phosphatidylinositol (GPI) anchored protein, which affects the calcium channel function [22].

The VGCC α2/δ1 subunit is a member of the α2/δ subunit family, and acts as a marker of tumor-initiating cells and controls calcium influx into liver tumor-initiating cells [23].

In this study on comparing the three studied groups as regards to AFP, its level was significantly higher in group I than group II and higher in group I than group III , while no significant difference as regards AFP level was found between group II&III. Di Bisceglie, et al. [24] declared that AFP in serum is currently available diagnostic marker for HCC and for patients with chronic liver disease, a sustained increase in AFP serum level was shown to be one of the risk factors of HCC development.

On the other hand Singal, et al. [25] demonstrated that the addition of AFP to ultrasound does not substantially improve the sensitivity of diagnosis of HCC, independent of the cut-off level used.

In our study AFP was found normal in 11 cases of group I in the pretreatment period, meanwhile α2/δ1 subunit was high. This explains the usefulness of α2/δ1 subunit assay in AFP negative HCC, this was in agreement with Sherman. [26] who stated that AFP is not elevated in the majority of patients with early HCC.

Our results indicated that α2/δ1 subunit level was significantly higher in group I than group II ($P<0.001$) and higher in group I than group III ($P<0.001$), also it was elevated in cirrhotic non HCC group (group II) than control group, however this did not pose statistical significance ($P=0.07$). This was supported Badr, et al. [27] who found that the serum level of the α2/δ1 subunit was significantly higher with patients in group I (mean = 20.12 ng/mL) compared with the patients in group II (mean = 10.41 ng/mL) and group III.

In agreement of our results the study done by Ahmed, et al. [28] which reported that the serum levels of α2δ1 subunit were significantly different across all 3 groups ($P < 0.001$) with the highest value in HCC group (mean = 19.53 ± 6.87 ng/ dl), then the

cirrhotic group (mean = 6.24 ± 2.64 ng/dl) and the least value in control group (mean = 0.67 ± 0.48 ng/dl).

The most direct explanation of increase level of α2/δ1 subunit in HCC patients is the study done by Ali et al. [29] who found that changes in intracellular free Ca2+ concentrations play a central role in the hormonal regulation of liver metabolism and of the pathways which regulate hepatocyte proliferation and apoptosis.

Also Zhang, et al. [30] confirmed that numerous mutations in genes encoding Ca2+-signaling proteins have been identified from HCC liver tissue, and a number of Ca2+-signaling proteins are over expressed in HCC. Zhao, et al. [16] identified Ca2+-signaling proteins which appear to play important roles on the initiation and progression of HCC and which could be potential therapeutic targets. They used monoclonal antibody to identify cells related to HCC recurrence and to investigate the nature of these cells. 1B50-1 bound to an antigen on the Hep-12 cell membrane, while it recognized few Hep-11 cells. They noticed that Hep-12 cells which is 1B50- 1+ HCC specimens expressed high levels of α2/δ1 protein, while it was undetectable in Hep-11 cells.

In this study, there was no statistically significant correlation found between the serum level of the α2/δ1 subunit and the clinicopathological features of patients except for serum creatinine, this is in agreement with Badr, et al. [27]. Our results revealed that there is a significant positive correlation between α2/δ1subunit and AFP (R= 0.640, P= 0. 002), also this was in agreement with Badr, et al. [27].

In the current study, there was a significant decrease in α2/δ1 subunit level in well ablated lesions after ablation, this was in agreement with Zhao, et al.[16] who concluded that α2δ1subunit and hence L- and N- type voltage-operated Ca2+ channels are expressed in tumor initiating cells of HCC, promote/maintain the self-renewal these cells. These findings contribute to the understanding of the role of α2δ1 subunit in HCC recurrence and prognosis.

Zhao, et al. [16] identified that targeted inhibition of α2δ1subunit could induce apoptosis in liver cancer stem cells and hence reduce the development of HCC and which could be potential therapeutic targets.

When the ROC curve was applied to select the cut-off value, the data revealed that the serum level of the α2/δ1 subunit at the cut-off value >100ng/mL had a sensitivity of 80 %, a specificity of 100%, a positive predictive value (PPV) of 100%, and a negative predictive value (NPV) of 83.3%. The AUC showed a high accuracy for the α2/δ1 subunit (95.5%).

Using 14.22 ng/ml as a cut-off value, Badr, et al. [27] concluded that the α2/δ1 subunit level had a sensitivity of 100 %, a specificity of 96%, a positive predictive value (PPV) of 98%, and a negative predictive value (NPV) of 100%. So the serum level of α2/δ1 subunit may be a novel diagnostic biomarker for HCC diagnosis.

Ahmed, et al.[28] found that serum α2/δ1subunit has a sensitivity of 95% and specificity of 80% at a level of ≥ 8.75 ng/dl with PPV of 82.6%, NPV of 94.1%, and accuracy of 87.5%, they demonstrated a higher level that gives more specificity of α2δ1 subunit was that at a cut-off of 12 ng/dl; sensitivity and specificity were 85% and 100%, respectively, with 100% PPV, 87% NPV, and 92.5% accuracy.

Limitations of the study

Limitations of the current study should be considered. Firstly, small sample size, short duration follow up, added to that, HCV infected patients only have been enrolled in the present study and locoregional ablation was the only included modality in our work.

Strengths of the study

α2/δ1 subunit is a novel biomarker for HCC diagnosis that correlated positively with the presence of HCC and its level was elevated in cases with normal AFP level in addition its level in cirrhotic patient was significantly different with HCC patients. α2/δ1 subunit level was significantly decreasing after intervention in well ablated HCC lesions which indicates its significance as a predictive marker of therapeutic outcome.

Conclusion

The present study suggests that serum α2δ1 subunit could serve as a potential diagnostic biomarker for HCC. α2δ1 subunit could be used as a predictive marker of therapeutic ablation outcome in hepatocellular carcinoma patients.

Abbreviations

AFP	Alfa fetoprotein
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
CBC	Complete Blood Picture
CSC	cancer stem cell
CT	Computed tomography
ELISA	Enzyme-linked immunosorbent assay
GP73	Golgi protein 7
GPC-3	Gypican-3
GSP	Glycosyl-phosphatidylinositol
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
INR	International normalized ratio
MRI	Magnetic resonance imaging
MWA	Microwave ablation
NPV	Negative predictive value
PPV	Positive predictive value
RFA	Radiofrequency ablation
ROC	Receiver Operating Characteristics
TICs	Tumor Initiating Cells
VGCC	Voltage-gated calcium channels
α2δ	Alpha 2 delta

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