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Serum clusterin as a promising diagnostic and prognostic marker for hepatocellular carcinoma after locoregional treatment

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Abstract

Hepatocellular carcinoma (HCC) is one of the most common aggressive tumors, with a rising prevalence in Egypt. Clusterin is a secretory heterodimeric glycoprotein linked to cancer development and progression. This study was conducted to evaluate the diagnostic and prognostic role of serum clusterin as a possible biomarker of HCC and correlate its level with the mRECIST scoring system. This study included 45 patients with liver cirrhosis and HCC eligible for locoregional treatment and 20 patients with liver cirrhosis without HCC as controls. All patients underwent standard laboratory tests and abdominal ultrasound. For HCC patients, a triphasic CT scan, alpha-fetoprotein (AFP), and clusterin levels were measured at baseline and one month after intervention. HCC patients had a substantially higher baseline clusterin level than cirrhotic patients (122.291 ± 61.898 vs. 74.015 ± 41.571, *P* = 0.002). Five patients in the HCC group were not eligible for intervention because they had evidence of portal vein invasion. At one month follow-up after HCC treatment, serum clusterin levels declined significantly from baseline (from 122.291 ± 61.898 to 81.125 ± 62.321, *P* = <0.001). According to the mRECIST scoring, baseline clusterin levels were significantly higher among patients with progressive disease than those with partial response than those with complete response (180.722 ± 55.908, 161.310 ± 56.339, 84.810± 41.389, respectively, overall *P* = <0.001). Clusterin was a useful marker in detecting HCC with 73.33% sensitivity and 75% specificity at a cutoff of ≥ 86.6 mg/L, and it also had 95.24% sensitivity and 77.78% specificity in detecting tumor progression at a cutoff of ≥ 146.6 mg/L, according to the mRECIST scoring system. In conclusion, clusterin may be a helpful diagnostic and prognostic marker for HCC after locoregional treatment, as its baseline level is useful in predicting response and progression of HCC in correlation with the mRECIST scoring system.

Keywords: Clusterin, Hepatocellular carcinoma, mRECIST.

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Introduction

Hepatocellular carcinoma (HCC) is the world's sixth most frequent cancer and the third leading cause of cancer-related death.[1] The main cause of HCC is liver cirrhosis resulting from chronic viral hepatitis, as well as metabolic disorders. So, biomarker surveillance in high-risk patients is important for early diagnosis and better outcomes. While several molecular biomarkers have been linked to HCC, only a few have been shown to have clinical significance.[2]

Abdominal ultrasound and serum alpha-fetoprotein (AFP) are commonly used to screen for HCC. Although AFP level detection is simple and inexpensive, it has limited sensitivity because elevated AFP levels are common in patients with chronic liver disease, pregnancy, and germ cell tumors. Thus, new markers with higher sensitivity and specificity are required.[3]

Clusterin (CLU) is a 449 amino acid heterodimeric glycoprotein that is thought to play a role in tumor cell regeneration, migration, and anti-apoptosis.[4] It encodes two isoforms, nuclear clusterin (nCLU) and secretory clusterin (sCLU), which have a wide distribution in tissues and body fluids and are involved in various biological functions such as lipid transport, senescence, complement cascade, membrane recycling, cell adhesion, and programmed cell death.[5]

The nCLU is found in the nucleus and is thought to play a role in cell death. On the other hand, the sCLU is found primarily in the cytoplasm and always plays an anti-apoptotic role, and its abnormality has been linked to various tumors' development and progression by contributing to angiogenesis, chemo-resistance, cell survival, or metastasis.[6]

Clusterin is overexpressed in a variety of metastatic cancer cells, including colon, bladder, HCC, and renal cell carcinoma, according to emerging data. HCC has been linked to abnormalities in sCLU levels.[7]

Clinical studies revealed that sCLU expression performed exceptionally well for HCC diagnosis, and it was also suggested as a prospective survival indicator for HCC prognosis.[6] The aim of this study was to evaluate the diagnostic and prognostic role of serum clusterin as a biomarker of HCC among Egyptian patients before and after locoregional treatment.

Subjects and Methods

This study was carried out in the Hepatology and Gastroenterology Unit, Department of Internal Medicine, Ain Shams University during the period between August 2020 and March 2021.

Sixty-five Egyptian patients were recruited and divided as follows: Cirrhosis Group included 20 patients with liver cirrhosis without HCC as a control group. Exclusion of HCC was based on the absence of any hepatic focal lesions on triphasic CT abdomen. HCC Group included 45 patients with liver cirrhosis and HCC, HCC was diagnosed based on the development of characteristic vascular enhancement in triphasic abdominal CT scan, according to 2011 AASLD guidelines.[8]

Patients who had platelet count less than $50x10^9$/L, prothrombin activity less than 50 %, Child-Pugh class C, other malignancies, or extrahepatic metastases, were excluded from the study.

The study protocol was reviewed and approved by the Research Ethics Committee at the Faculty of Medicine, Ain Shams University (FMASU M S 387/2020). Participants submitted their informed written consent before being included in the study.

All patients had their medical histories taken in detail. A thorough clinical examination was performed. Laboratory investigations included complete blood count using a Coulter Counter (Beckman Coulter, California 92821, USA), serum creatinine, full hepatic profile (AST, ALT, total and direct bilirubin, albumin, and INR) using Clinical Chemistry Analyzer (Beckman Coulter, California 92821, USA), HCVAb, and HBsAg. In this study, Child-Pugh score (a universal method of stratification of cirrhotic patients into three classes A, B, and C), Model of End-Stage Liver Disease (MELD), and Barcelona Clinic Liver Cancer (BCLC) staging were employed.

In patients and controls, serum AFP was tested. In HCC patients twice: before and one month after the intervention using a human AFP enzyme immunoassay (EIA) kit (lot. REF 600-10 manufactured by CanAg Diagnostics AB, Majnabble Terminal SE-414, 55 Gothenburg, Sweden), according to the manufacturer's instructions.

Serum clusterin was quantified using a double antibody sandwich ELISA kit (Biovendor-Laboratorni Medicina a.s., Cat. No. RD194034200R), according to the manufacturer's instructions. For HCC patients, it was measured before intervention and reassessed one month after.

Abdominal ultrasonography was used to detect liver cirrhosis, ascites, and hepatic focal lesions. A three-phase contrast enhancement abdominal CT scan was performed. It was performed on all patients with suspected focal lesion in abdominal ultrasound, and patients with HCC had a follow-up triphasic CT one month following the intervention.

Patients with (BCLC-0) or (BCLC-A) HCC who were ineligible for surgical resection or transplantation and had tumor diameters of less than 5 cm or less than three nodules with a maximum diameter of 3 cm were treated with radiofrequency ablation (RF). By introducing an electrode into the lesion and creating a thermal destruction zone encircling the tumor.[9]

Transarterial chemoembolization (TACE) was used in patients with intermediate BCLC stages who had Child A-B, large or multifocal HCC and were not candidates for resection or RF. To accomplish the synergistic effect of medication cytotoxicity and ischemia, lipiodol emulsion was first injected into the tumor feeding artery, followed by embolization.[10]

The modified Response Evaluation Criteria in Solid Tumors (mRECIST) criteria were developed in 2010 as a mechanism to adapt the RECIST criteria to the unique characteristics of HCC. Since its adoption or inclusion in clinical practice guidelines for the management of HCC, mRECIST has served its function; it has also been useful for measuring response and time-to-event endpoints in various studies. In the early/intermediate stages of HCC, mRECIST has become the standard method for measuring radiological endpoints.[11]

The (mRECIST) scoring system was used to compare tumor shrinkage before and after locoregional therapy.[11] Complete response (CR) was defined as the disappearance of any intra-tumoral arterial enhancement in all typical intrahepatic target lesions and disappearance of all atypical intrahepatic target lesions and extrahepatic target lesions.[11] A Partial response (PR), was defined as a decrease of at least 30% in the sum of the target lesions' diameters (including viable tumor diameters for typical intrahepatic target lesions), using the baseline sum of the longest diameters as a reference.[11] Progressive disease (PD) was defined as at least a 20% rise and an absolute increase of at least 5 mm in the sum of diameters of the target lesions (including viable tumor diameters for typical intrahepatic target lesions), with the nadir sum of diameters recorded since baseline as a reference.[11] Moreover, Stable disease (SD) was neither sufficient decrease to qualify for PR nor sufficient increase to qualify for PD.[11]

Statistical analysis

IBM SPSS V22 was used to evaluate the data. For quantitative data, descriptive statistics such as mean and standard deviation were calculated. Quantitative data was analyzed using independent t-tests, paired t-tests, ANOVA, and post hoc Tukey testing. For qualitative data, the chi-square and Fisher's exact tests were applied. A *P* value of <0.05 was considered statistically significant. Sensitivity, specificity, positive and negative predictive values, and accuracy were calculated. The overall diagnostic performance was assessed by receiver-operating characteristics (ROC) curve analysis.

Results

This study included 65 HCV infected patients. Of these, 45 patients were with liver cirrhosis-related HCC (mean age 57.5±7.2 years; 31 males (68.9 %) and 14 females (31.1%)) and 20 patients, as a control group, with liver cirrhosis without HCC (mean age 53.7±8.4 years; 12

males (60%) and 8 females (40%)). Of the total 65 study subjects, 26 patients were Child-Pugh A class (21 in the HCC group and 5 in the cirrhosis control group), 29 patients were Child-Pugh B (19 in the HCC group and 10 in the cirrhosis group), and 10 patients were Child-Pugh C (5 patients in each group). Demographic and laboratory data are shown in Table 1.

Table 1. Demographic and laboratory parameters of the studied groups.

		Group		*P* value
		Cirrhosis	HCC	
Age (years)	Range	42 - 67	39 -70	NS
	Mean ±SD	53.700 ±8.442	57.533 ±7.229	
Gender (n, %)	Male	12 (60%)	31 (68.89%)	NS
	Female	8 (40%)	14 (31.11%)	
Child score (n, %)	Grade A	5 (25%)	21 (46.7%)	NS
	Grade B	10 (50%)	19 (42.2%)	
	Grade C	5 (25%)	5 (11.1%)	
MELD score	Range	8 - 25	4 -34	NS
	Mean ±SD	17.800 ± 5.187	15.622 ±N6.318	
Hemoglobin (g\dl)	Range	7.3 - 11.5	6.5 - 12	NS
	Mean ±SD	9.445 ± 1.296	9.302 ± 1.285	
TLC (x10^3 /mL)	Range	2.5 - 10.1	3 - 11.4	NS
	Mean ±SD	8.590 ± 4.845	9.716 ± 5.398	
Platelets (x10^3 /mL)	Range	55 - 269	50 - 315	NS
	Mean ±SD	137.000 ± 62.284	146.844 ± 65.851	
AST(IU/L)	Range	14 - 329	19 - 251	NS
	Mean ±SD	70.000 ± 83.281	103.444 ± 64.615	
ALT (IU/L)	Range	19 - 342	29 - 329	NS
	Mean ±SD	69.700 ± 77.873	108.356 ± 71.429	
Total bilirubin (mg/dL)	Range	0.6 - 7.8	0.5 - 5.8	NS
	Mean ±SD	2.425 ± 1.622	1.949 ± 1.090	
Direct bilirubin (mg/dL)	Range	0.3 - 6	0.2 - 4.9	NS
	Mean ±SD	1.465 ± 1.272	1.204 ± 0.903	
Albumin (g/dL)	Range	1.9 - 4.1	1.5 - 4.5	NS
	Mean ±SD	3.150 ± 0.550	3.158 ± 0.717	
INR	Range	1 - 2.4	0.9 - 2.3	NS
	Mean ±SD	1.570 ± 0.444	1.418 ± 0.389	

P value>0.05 is not significant (NS).

The current study revealed that the baseline clusterin level was significantly higher among HCC patients compared to cirrhotic control patients (122.291 ± 61.898 vs. 74.015± 41.571, *P* = 0.002). Also, the HCC group had a higher AFP as shown in Table 2.

Table 2. Comparison between the two studied groups regarding baseline clusterin and AFP.

		Group		*P value
		Cirrhosis	HCC	
Baseline clusterin (µg/l)	Range	11.1-177.8	12.1-276.9	0.002
	Mean ±SD	74.015±41.571	122.291±61.898	
Baseline AFP (ng/ml)	Range	5-366	182-1326	<0.001
	Mean ±SD	82.250±115.530	764.511±338.192	

**P* value<0.05 is significant

Regarding triphasic CT findings among the HCC group, patients with multiple hepatic focal lesions, larger size, and evidence of portal vein invasion had a statistically significantly higher AFP as shown in Table 3.

Table 3. Comparison between baseline AFP and other studied parameters among the HCC group.

		Baseline AFP		*P-value
		N	Mean±SD	
Gender	Male	31	840.968±334.496	0.022
	Female	14	595.214±290.007	
Child score	Grade A	21	659.588±331.604	NS
	Grade B	19	767.278±339.234	
	Grade C	5	937.900±303.459	
Liver Size	Shrunken	20	886.050±334.646	NS
	Average	11	710.455±358.532	
	Enlarged	14	633.357±284.313	
Spleen Size	Average	21	654.762±322.493	0.040
	Enlarged	24	860.542±328.246	
Ascites	No	21	656.238±334.786	NS
	Mild	10	863.700±341.147	
	Moderate	10	768.100±319.024	
	Tense	4	1076.000±177.576	
Number of focal lesions	Solitary	14	525.000±327.165	0.001
	Multiple	31	872.677±287.237	
Size of focal lesions	<3 cm	16	561.938±309.805	<0.001
	3-5 cm	14	668.643±269.285	
	>5 cm	15	1070.067±182.125	
Portal vein invasion	No	40	721.500±330.442	0.014
	Yes	5	1108.600±164.295	
BCLC	Stage 0	4	326.500±74.719	<0.001
	Stage A	16	606.688±309.311	
	Stage B	20	892.350±270.267	
	Stage C	5	1108.600±164.295	
Intervention	None	5	1108.600±164.295	<0.001
	TACE	20	892.350±270.267	
	RF	20	550.650±299.391	

**P* value>0.05 is not significant (NS).

Patients with multiple hepatic focal lesions had higher serum clusterin levels than those with a single lesion, however, the difference did not reach statistical significance (*P* = 0.191). Also, serum clusterin level was not different among subjects with different size of hepatic focal lesions and portal vein invasion. When using Pearson multivariate correlation, portal vein diameter was the only parameter that was significantly correlated with baseline clusterin level among the cirrhosis group (Tables 4 and 5).

Table 4. Comparison between baseline clusterin and other studied parameters among the HCC group.

		N	Baseline clusterin Mean±SD	*P value
Gender	Male	31	133.448±56.536	NS
	Female	14	97.586±68.099	
Child Score	Grade A	21	127.271±43.703	NS
	Grade B	19	111.361±81.193	
	Grade C	5	111.200±49.585	
Liver Size	Shrunken	20	117.665±64.123	NS
	Average	11	127.473±68.081	
	Enlarged	14	124.829±57.710	
Spleen Size	Average	21	124.376±66.889	NS
	Enlarged	24	120.467±58.581	
Ascites	No	21	128.338±52.544	NS
	Mild	10	106.250±82.728	
	Moderate	10	141.300±64.208	
	Tense	4	83.125±22.911	
Number of focal lesions	Solitary	14	104.186±59.506	NS
	Multiple	31	130.468±62.152	
Size of focal lesions	<3 cm	16	129.444±59.487	NS
	3-5 cm	14	117.979±66.387	
	>5 cm	15	118.687±63.771	
Portal vein invasion	No	40	125.515±64.624	NS
	Yes	5	96.500±22.185	
BCLC	Stage 0	4	91.550±75.397	NS
	Stage A	16	134.469±50.211	
	Stage B	20	125.145±73.306	
	Stage C	5	96.500±22.185	
Intervention	None	5	96.500±22.185	NS
	TACE	20	125.145±73.306	
	RF	20	125.885±56.553	

**P* value>0.05 is not significant (NS).

Table 5. Correlations of Baseline clusterin with other parameters among the studied groups.

Baseline Parameters	Correlation			
	Cirrhosis		HCC	
	Baseline clusterin			
	r	*P* value	r	**P* value
AFP (ng/ml)	0.262	NS	0.113	NS
Age (years)	0.134	NS	0.078	NS
Portal vein diameter (cm)	0.456	0.043	0.137	NS
MELD	-0.060	NS	0.028	NS
Liver Size (cm)	-0.013	NS	0.124	NS
Spleen Size (cm)	-0.216	NS	-0.010	NS
Serum Creatinine (mg/dL)	-0.009	NS	0.035	NS
Hemoglobin (g\dL)	0.133	NS	0.012	NS
TLC (x10^3 /mL)	-0.172	NS	0.150	NS
Platelets (x10^3 /mL)	0.247	NS	0.032	NS
AST (IU/L)	-0.429	NS	0.175	NS
ALT (IU/L)	-0.340	NS	0.179	NS
Total bilirubin (mg/dL)	-0.315	NS	0.024	NS
Direct bilirubin (mg/dL)	-0.355	NS	0.008	NS
Albumin (g/dL)	0.181	NS	0.103	NS
INR	-0.006	NS	-0.020	NS

**P* value>0.05 is not significant (NS).

Among the HCC group, 5 patients were excluded from intervention as they had evidence of portal vein invasion by triphasic CT, while the remaining 40 patients underwent locoregional treatment according to the approved selection criteria, where 20 patients were candidates for RF and 20 for TACE.

At one month follow-up, serum clusterin levels were significantly lower than at baseline (from 122.291± 61.898 to 81.125 ± 62.321, overall *P* = <0.001). Also, follow up of AFP level showed significant reduction (from 764.511 ± 338.192 to 643.575 ± 303.589, overall *P* = <0.001) (Table 6).

Table 6. Comparison between serum clusterin and AFP levels before and one month after intervention among HCC group.

		Time		Differences	**P*-value
		Before	One month After	Mean±SD	
AFP (ng/ml)	Range	182-1326	160-1175	77.925±74.808	<0.001
	Mean ±SD	764.511±338.192	643.575±303.589		
clusterin (μg/L)	Range	12.1-276.9	9-274	44.390±39.922	<0.001
	Mean ±SD	122.291±61.898	81.125±62.321		

**P* value<0.05 is significant

On applying the mRECIST scoring system for follow up of HCC patients after locoregional treatment, 21 patients showed complete response (CR) and 10 patients showed partial response (PR), while the remaining 9 patients showed progressive disease (PD).

According to the mRECIST scoring, baseline serum clusterin levels were significantly higher in patients with progressive disease than in those with partial response or complete response (180.722 ± 55.908, 161.310 ± 56.339 and 84.810 ± 41.389, respectively overall *P* = <0.001). AFP showed the same pattern of difference; however, the difference did not reach statistical significance (*P* = 0.129) (Table 7).

Table 7. Relation between baseline AFP, clusterin and mRECIST grade among HCC group.

	mRECIST			*P-value
	CR	PR	PD	
	Mean±SD	Mean±SD	Mean±SD	
Baseline AFP (ng/ml)	645.429±300.036	710.600±408.764	911.111±251.989	NS
Baseline clusterin (μg/L)	84.810±41.389	161.310±56.339	180.722±55.908	<0.001

**P* value>0.05 is not significant (NS).

Furthermore, at one month follow-up clusterin levels were significantly higher in patients with progressive disease than in those with partial response than those with complete response (168.889 ± 65.982, 76.800 ± 33.967, and 45.571 ± 22.589, respectively overall *P* = <0.001). AFP showed the same pattern of difference; however, the difference did not reach statistical significance (*P* = 0.093) (Table 8).

Table 8. Relation between follow-up AFP, clusterin and mRECIST grade among HCC group.

	mRECIST			*P-value
	CR	PR	PD	
	Mean±SD	Mean±SD	Mean±SD	
Follow-up AFP (ng/ml)	578.238±259.441	608.100±387.757	835.444±240.191	NS
Follow-up clusterin (μg/L	45.571±22.589	76.800±33.967	168.889±65.982	<0.001

P value>0.05 is not significant (NS).

A ROC curve was used to reveal the diagnostic effect of clusterin and AFP levels and to determine the appropriate cutoff value for distinguishing the HCC group from the cirrhosis group. (Table 9 and Figure 1).

Table 9. Diagnostic performance of baseline clusterin and AFP levels in differentiating HCC group from Liver Cirrhosis group.

	ROC curve between cirrhosis and HCC					
	Cutoff	Sens.	Spec.	PPV	NPV	Accuracy
Baseline clusterin	>86.6	73.33	75.00	86.8	55.6	74.7%
Baseline AFP	>291	93.33	95.00	97.7	86.4	98.7%

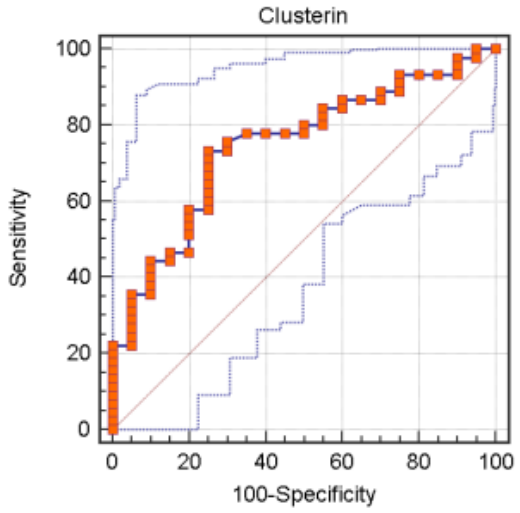

Figure 1. ROC curve for baseline clusterin in diagnosis of HCC. A cutoff of >86.6 was selected.

The optimal cut off value for baseline clusterin for discriminating progressive HCC from complete and partial response to treatment was identified using ROC curve (Table 10 and Figure 2). Another ROC curve was performed for baseline clusterin and AFP for discriminating progressive HCC from complete response to treatment (Table 11, Figure 3).

Table 10. Diagnostic performance of baseline clusterin level in diagnosing progression of HCC after intervention according to mRECIST Grading

	ROC curve between PD and CR+PR					
	Cutoff	Sens.	Spec.	PPV	NPV	Accuracy
clusterin	≥133.8	64.52	88.89	95.2	42.1	80.3%

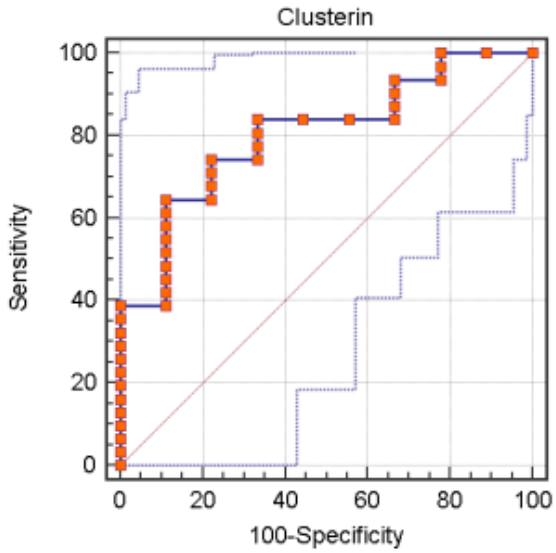

Figure 2. ROC Curve for clusterin performance in differentiating between progressive disease (PD) and complete response (CR) plus partial response (PR). A cutoff of ≥133.8 was selected.

Table 11. Diagnostic performance of baseline AFP and clusterin level in differentiating progressive disease (PD) from complete response (CR) after intervention of HCC

	ROC curve between PD and CR					
	Cutoff	Sens.	Spec.	PPV	NPV	Accuracy
Baseline clusterin	≥146.6	95.24	77.78	90.9	87.5	92.1%
Baseline AFP	≥723	71.43	88.89	93.7	57.1	76.2%

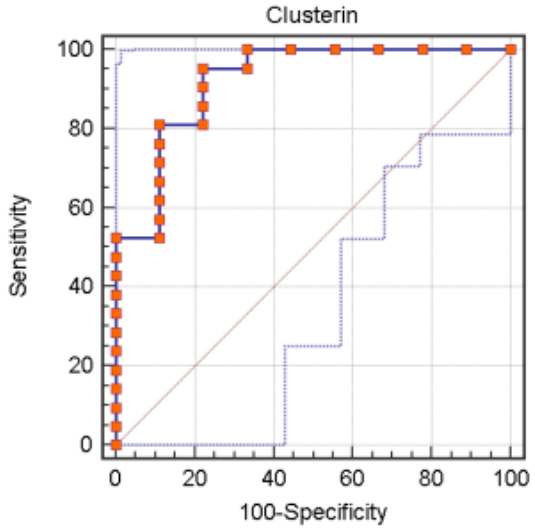

Figure 3. ROC curve for clusterin level performance in differentiating between progressive disease (PD) and complete response (CR). A cutoff of ≥146.6 was selected.

Discussion

The aim of this study was to evaluate serum clusterin as a potential diagnostic biomarker for HCC in cirrhotic patients and its role in the prediction of response to locoregional treatment. Serum Clusterin is a 70-80 kD heterodimeric sulphated glycoprotein transcribed from a single-copy gene on 8p21. It is involved in lipid transport, senescence, complement cascade, membrane recycling, cell adhesion, and programmed cell death and other biological processes. It is also related to HCC dynamics, due to its role in anti-apoptosis, angiogenesis, and pro-metastasis. Given its outstanding diagnostic and prognostic capabilities, Clusterin has been proposed as a biomarker for HCC.[12]

The current study found that the cirrhotic group had a mean age of 53.7 years with a male to female (M: F) ratio of 3:2 and the HCC group had a mean age of 57.5 years with a M: F ratio of 2:1. These observations are in agreement with findings of a study by Nafee et al., (2012),[13] who found that the liver cirrhosis group had a mean age of 54.7 with a M: F ratio of 2:1 and the HCC group had a mean age of 55.5 years and a M: F ratio of 4:1. Such data clearly indicate that HCC is sex-biased. It is well recognized that patterns of gene expression vary between sexes across different tissues. These variances could be causing etiological disparities between female and male cancers. Yuan et al., (2016)[14] reported extensive sex-biased signatures in gene expression in HCC. Another reason for gender disparity could be the difference in sample size, sex hormones, and risk factor exposure. Hepatocarcinogenesis may be influenced by sex hormones, iron deposition, and ethnic differences, which could explain why HCC is more common in men.[15]

The present work found no significant differences in Child and MELD scores between cirrhotic and HCC patients. Furthermore, most of the HCC patients were found to be Child A and B, which agreed with data of a study by D'Amico et al., (2006)[16] asserting that HCC can occur at any stage of cirrhosis.

In the current study, clusterin levels were significantly higher in the HCC group (122.291

±61.898 µg/l) than in the cirrhosis group (74.015 ± 41.571 µg/l, *P*=0.002), denoting its role in carcinogenesis. Similar findings were also reported in a study by Hamed et al., (2019)[17] who found that clusterin levels in HCC and cirrhosis patients were 128.5 µg/l and 88.3 µg/l, respectively. They also agree with Nafee et al., (2012)[13] study in which clusterin levels were 198.5 µg/l and 44.5 µg/l in the HCC and cirrhosis groups, respectively. These findings may lead to the hypothesis that clusterin is secreted by tumor cells of HCC and its level is reflected in the serum. This is further supported by the notion that clusterin exists as both an intracellular form and an extracellular secreted glycoprotein.[17]

In the HCC group, clusterin levels were also higher in Child A patients compared to Child C patients, but this was statistically insignificant. This may suggest a possible preventive role of clusterin against liver cell fibrogenesis, which eventually results in cirrhosis. Jung et al., (2012)[18] proposed a similar hypothesis in renal fibrosis, claiming that upregulation of clusterin during renal injury has a protective effect against the development of renal fibrosis in a mouse model. Likewise, Hogasen et al., (1996)[19] and Wang et al., (2010)[20] reported a reduction in serum clusterin in alcoholic liver cirrhosis and HBV related cirrhosis, respectively.

According to the current study, patients with multiple hepatic focal lesions showed no difference in serum clusterin level from those with a solitary lesion. These findings were in concordance with those of Lau et al., (2006)[21], who stated that overexpression of clusterin in hepatoma cells transfected with clusterin genes boosted cell migration by twofold in vitro and the development of metastatic tumor nodules in the liver by eightfold *in vivo*.

Clusterin's relationship to HCC progression was similarly inconsistent among different studies. In the present study, we found no link between serum clusterin levels and tumor size or extrahepatic dissemination in the form of portal vein invasion. These findings were supported by data of Wang et al., (2010)[20] and Nafaa et al., (2012)[13], who observed no significant variation in serum clusterin levels between different tumor sizes. However, Chen et al., (2012)[22], provided evidence that clusterin increases MMP-2 expression and decreases E-cadherin expression, implying that clusterin plays an essential role in HCC invasiveness.

Regarding AFP, baseline levels were significantly higher in HCC patients with multiple and large focal lesions as well as those with portal vein invasion, these findings were consistent with those of El-Shenawy et al., (2012)[23], who found a positive correlation between serum AFP levels with both portal vein invasion and tumor size. The accuracy of AFP in detecting HCC varies depending on the cutoff parameters employed. According to the present data, at a cutoff of ≥ 291 ng/ml the sensitivity was 93.33%, specificity was 95%. These results were in accordance with Isaac et al., (2021)[15], who stated that AFP at a cutoff of ≥ 220ng/ml, the sensitivity and specificity, were 86.7 % and 90%, respectively.

Regarding serum clusterin performance in HCC detection, at a cutoff of 86.8 µg/l, it showed a sensitivity and specificity of 73.33% and 75%, respectively. These results agreed with those of Hamed et al., (2019)[17], who stated that serum clusterin at a cutoff value of 135µg/l had 72.5% sensitivity and 92 % specificity. Furthermore, Nafee et al., (2012)[13] observed that serum clusterin was overexpressed in HCC patients compared to cirrhotic patients and, at a cutoff value of 128 µg/mL, it had 90% sensitivity and 87% specificity for HCC detection.

For HCC, the only definitive therapeutic modalities that can lead to a cure are hepatic resection and transplantation. However, only a small percentage of patients (10% to 20%) are deemed fit for these modalities.[24] The remaining patients are mostly treated with the so-called liver-directed regional therapies.[24] In the present study, 44 % of HCC patients received TACE and 44% were treated with RF. The remaining patients had macrovascular invasion and could not be treated with these modalities.

The current study revealed that AFP levels were significantly decreased one month after treatment regardless of whether TACE or RF was employed. These findings were in accordance with those of Corey & Pratts, (2009)[25]

Although the prognostic significance of clusterin in a variety of malignances has been evaluated in previous studies, serum clusterin over-expression was reported in colon cancer cells, leading to the conclusion that it could be used as a diagnostic marker for colorectal cancer.[26] According to Lokamani et al., (2011)[27], clusterin may be used as a marker to distinguish cervical neoplasia with borderline morphological characteristics. Abnormal expression of clusterin has also been reported in patients with transitional bladder cell carcinoma, suggesting that it could be used as a diagnostic and prognostic biomarker for the disease.[28]

While serum clusterin's role in detecting HCC response to different treatment modalities was rarely reported, the current study found that clusterin levels were significantly declined one month after intervention (from 122.291 ± 61.898 to 81.125 ± 62.321, *P*<0.001). On analysis of published data dealing with the prognostic role of clusterin, the link between clusterin and HCC advancement was diverse in different reports. According to an in vitro study by Lau et al., (2006)[21], overexpression of clusterin increased cell migration and the formation of metastatic tumor nodules. According to Lin et al., (2015)[6], clusterin was also recommended as a prospective survival indicator predicting HCC prognosis. However, Wang et al., (2015)[29] found no significant difference in clusterin serum levels between different tumor sizes and stages.

In absence of sufficient data linking mRECIST to clusterin level, the current study assessed response to locoregional treatment using mRECIST criteria and observed that baseline and follow-up serum clusterin levels were significantly higher in patients with PD than those with PR and then CR patients (overall *P* <0.001). AFP showed the same pattern, but the difference was insignificant. These results matched those of Wang et al., (2020)[30], who investigated the prognostic value of clusterin in HCC patients treated with oxaliplatin and observed that patients with low clusterin mRNA expression were more frequently identified in the CR+PR group than in the SD+PD group. suggesting that clusterin mRNA levels were clearly associated with oxaliplatin treatment response in HCC patients (*P*= 0.001). Subjects with high expression exhibited high oxaliplatin resistance, whereas low ones showed a good response rate.

The present work found that serum clusterin levels at cut off of ≥146.6 could distinguish patients with PD from those with CR with sensitivity of 95.2%, specificity of 77.8%, and accuracy of 92.1%, making clusterin a promising prognostic marker for HCC following locoregional treatment. While AFP had a lower sensitivity (71.43%), specificity (88.89%), and accuracy (76.2 %) at a cut off ≥723.

All the above-mentioned findings agreed with those of Wang et al., (2020)[30], concluded that clusterin over-expression was observed in HCC patients' plasma, and its levels were linked to tumor stage and lymph node metastases. Assessment of clusterin level may also help in predicting the overall survival of HCC patients treated with chemotherapeutic agents, which could provide a different strategy to combat therapeutic resistance in cancer treatment.

In conclusion, AFP outperformed clusterin in aspects of the diagnostic performance of HCC. However, clusterin may prove to be a valuable marker for patient follow-up following locoregional treatment. It may also be a target for new therapy to halt tumor progression. Consequently, the combined parallel approach might improve overall sensitivity, which is required in diagnosis and follow-up after treatment.

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Author Contributions

In addition to being the corresponding author, HSR proposed the research idea. HAM made significant contributions to the authoring and critical revision. Data collection and paper revision were aided by ESM. ASM aided by revising the laboratory analysis and tabulating the data. AI assisted with manuscript review and drafting. The submitted manuscript was reviewed and approved by all authors.

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee at the Faculty of Medicine, Ain Shams University (FMASU M S 387/2020).

Informed consent

Participants submitted their informed written consent before being included in the study.

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