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Plasma CD24 level as a promising prognostic biomarker of hepatocellular carcinoma

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Abstract

Background Hepatocellular carcinoma constitutes the most common primary hepatic cancer and remains a major medical burden in both developing and developed world. It ranks fifth in terms of global cases and second in terms of deaths for males.CD24 is known as a heavily glycosylated cell surface molecule that is highly expressed in a wide variety of human malignancies. It plays an important role in self-renewal, proliferation, migration, invasion, and drug resistance. The aim of this work was to evaluate the potential role of serum CD24 in the diagnosis and prediction of response to interventional therapy among hepatocellular carcinomas.

Methods This study included 40 adult Egyptian patients who had liver cirrhosis and hepatocellular carcinoma (HCC group). Another group of 20 patients with liver cirrhosis only served as controls (Cirrhosis group). All patients underwent standard laboratory tests and abdominal ultrasound. For HCC patients, a triphasic CT scan, alpha-fetoprotein was done. CD24 levels were measured in all patients, and in HCC patients at baseline and one month after intervention.

Results Baseline CD24 was significantly higher among HCC group in comparison to cirrhosis group (19.463 ± 8.573 vs. 0.725 ± 0.125 mg/L) with an overall p value < 0.001. Serum CD24 levels significantly declined after locoregional treatment from 19.463 ± 8.573 mg/L to 3.569 ± 1.248 mg/L (p < 0.001). Baseline CD24 was a useful marker in eligibility for HCC intervention with 80% sensitivity and 74.29% specificity at a cutoff of ≤ 23 mg/L, and it also had 62.96% sensitivity and 100% specificity in prediction of cure after locoregional treatment at a cutoff of ≤ 19.5 mg/L.

Conclusion CD24 could be a helpful diagnostic and prognostic marker for HCC, as its baseline level is useful in predicting both eligibility for intervention and cure after locoregional treatment.

Keywords CD24, Hepatocellular carcinoma, Locoregional treatment

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer all over the world and the third leading cause of cancer-related fatalities. This necessitates surveillance in high-risk patients for early diagnosis and better quality of life. While several molecular biomarkers have been linked to HCC, only few had clinical significance [1].

Abdominal ultrasonography and serum Alpha fetoprotein (AFP) are typically used to detect HCC. AFP level detection is affordable and simple to use, however its sensitivity is limited. More sensitive and specific markers are required since patients with germ cell malignancies, pregnancy, and chronic liver disease frequently have elevated AFP levels [2].

Cluster of differentiation 24 (CD24) is a mucin-like cell surface glycoprotein that is glycophosphatidylinositol (GPI)-anchored and encoded on chromosome 6. It regulates signals for proliferation and differentiation

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on mature B cells and granulocytes. According to the mounting data, aberrant over-expression of this protein is a prognostic factor in wide range of cancers, resulting in cell growth, proliferation, and metastasis [3].

Moreover, p53 inactivation and tumor cell proliferation may be influenced by the cytoplasmic accumulation of CD24. In addition to primary central nervous system and hematologic malignancies, overexpression of CD24 has been reported in a number of cancers, including lung, breast, hepatic, colorectal, ovarian, pancreatic, urothelial, prostate, and head and neck cancer [4].

Prior studies have indicated that CD24 plays a crucial role in drug resistance, invasion, migration, self-renewal, and proliferation of HCC [5]. On the other hand, not much is understood regarding CD24 expression and function in HCC. Consequently, the aim of this study was to evaluate the function that serum CD24 play in both the diagnostic and predictive aspects of HCC in Egyptian patients, before and after locoregional treatment.

Methods

This study was conducted in the Hepatology and Gastroenterology Unit, Department of Internal Medicine at Ain Shams University, in the period between June 2020 and March 2021.

Based on the characteristic vascular enhancement in triphasic CT abdomen, sixty adult Egyptian patients were enrolled and divided into two groups: one for cirrhosis, comprising 20 patients with liver cirrhosis without HCC, and another for HCC, comprising 40 patients with liver cirrhosis and HCC. In accordance with 2011 AASLD guidelines [6]. The study excluded patients with extrahepatic metastases, other malignancies, platelet counts less than 50×10^9 /L, and prothrombin activities less than 50%.

The study protocol was evaluated and approved by the Ain Shams University Faculty of Medicine's Research Ethics Committee (FMASU M S 56/2019). Written informed consent was acquired by each participant prior to their inclusion in the study.

Thorough medical histories and comprehensive clinical examinations were acquired from all subjects. Tests included a full liver profile (AST, ALT, ALP, GGT, total and direct bilirubin, albumin, and INR), a complete blood count, serum creatinine, HCV Ab, and HBs Ag. This study used the Child–Pugh score, the Barcelona Clinic Liver Cancer (BCLC) staging system, and the Model of End-Stage Liver Disease (MELD).

Testing for serum AFP was done in both groups. employing a human AFP enzyme immunoassay (EIA) kit (lot. REF 600–10 produced by CanAg Diagnostics AB, Gothenburg, Sweden) before and one month after intervention for HCC patients.

The sandwich ELISA kit (Cat. No. KT-10663) was used to quantify serum CD24 in accordance with the manufacturer's instructions. The sandwich ELISA principle serves as the foundation for the assay. An immobilized polyclonal antibody that recognizes CD24 is placed on the microtiter plate surface. Another biotinylated monoclonal antibody that recognizes CD24 is added after the sample or recombinant CD24 standard has been incubated. By including streptavidin–horseradish peroxidase (HRP), the latter was detected. Using TMB as substrate for the enzyme HRP, the amount of sCD24 protein could be quantified. With the exception of reagent F (TMB), which should be stored at 4 °C, all buffers should be allowed to reach room temperature (20–25 °C) before to use. Before usage, demineralized water is used to dilute the washing buffer (reagent D) and dilution buffer (reagent E) ten times. For HCC patients, it was measured before intervention and reassessed one month after.

Each participant had an abdominal ultrasound to check for ascites, hepatic focal lesions, and liver cirrhosis. Patients with suspected focal lesion in abdominal ultrasound underwent a three-phase contrast enhancement abdominal CT scan; and patients with HCC had another follow-up triphasic CT one month after intervention.

Radiofrequency ablation (RF) was used to treat patients with (BCLC-0) or (BCLC-A) HCC who were not eligible for surgical resection or transplantation and had tumor sizes of less than 5 cm or less than three nodules with a maximum diameter of 3 cm. by inserting an electrode into the lesion and enclosing the tumor with a zone of thermal destruction [7].

Patients with Child A-B, large or multifocal HCC and intermediate BCLC stages who were not candidates for resection or RF underwent trans-arterial chemoembolization (TACE). In order to achieve the combined effects of drug cytotoxicity and ischemia, the tumor feeding artery was first filled with lipiodol emulsion, followed by embolization [8].

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 intratumoral arterial enhancement in all typical intrahepatic target lesions and disappearance of all atypical intrahepatic target lesions and extrahepatic target lesions. 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 [8].

Statistical analysis

IBM SPSS statistics software version 20 was used to tabulate and analyze the gathered data. The mean ± SD was used to express quantitative data. ANOVA tests, paired t-tests, and independent t-tests were applied to quantitative data. The Chi-Square test was used to assess the qualitative data. The Pearson correlation coefficient test was used to conduct correlations. To assess how well various tests performed and to distinguish between the groups that were included, a ROC curve was created. A *P* value of less than 0.05 was considered statistically significant.

Results

This study was conducted on 60 patients with liver cirrhosis: 40 patients with HCC on top (HCC group), and 20 patients without HCC (Cirrhosis group). Demographic and laboratory data are shown in Table 1.

Table 1 Demographic and laboratory parameters of the studied groups

		Group				T-Test	
		Cirrhosis		HCC		t	*P*-value
Age	**Range**	30-72		48-79		-1.811	0.075
	Mean ± SD	57.750 ± 10.548		61.900 ± 7.067			
		N%		**N%**		**Chi-Square (X2)**	***P*-value**
Sex	**Male**	18	90.00	34	85.00	0.288	0.591
	Female	2	10.00	6	15.00		
		N	**%**	**N**	**%**	**Chi-Square (X2)**	***P*-value**
Child Score	**Child A**	20	100.00	10	25.00	30.000	< 0.001*
	Child B	0	0.00	25	62.50		
	Child C	0	0.00	5	12.50		
MELD	**Range**	8-17		10-28		-5.453	< 0.001*
	Mean ± SD	10.850 ± 2.412		17.175 ± 4.883			
Hb (g/dl)	**Range**	9.8-15.9		9.6-14.9		3.647	0.001*
	Mean ± SD	13.845 ± 1.510		12.470 ± 1.307			
WBC x 10^3/uL	**Range**	4.3-10.4		3.1-16.5		0.309	0.758
	Mean ± SD	7.135 ± 1.847		6.908 ± 3.011			
PLT x 10^3/uL	**Range**	90-490		57-284		2.928	0.005*
	Mean ± SD	216.250 ± 90.361		159.900 ± 58.027			
AST (IU/L)	**Range**	13-46		22-224		-5.690	< 0.001*
	Mean ± SD	28.550 ± 9.622		87.250 ± 45.446			
ALT(IU/L)	**Range**	7-44		16-154		-4.629	< 0.001*
	Mean ± SD	23.200 ± 9.345		55.675 ± 30.555			
Total bilirubin (mg/dL)	**Range**	0.36-1.91		0.5-4.9		-3.402	0.001*
	Mean ± SD	0.834 ± 0.441		1.740 ± 1.146			
Direct bilirubin (mg/dL)	**Range**	0.1-0.5		0.1-1.65		-3.020	0.004*
	Mean ± SD	0.211 ± 0.111		0.487 ± 0.400			
ALP (IU/L)	**Range**	40-96		90-250		-11.840	< 0.001*
	Mean ± SD	63.300 ± 18.012		152.050 ± 30.919			
GGT (IU/L)	**Range**	12-45		30-120		-9.694	< 0.001*
	Mean ± SD	26.150 ± 10.499		69.650 ± 18.589			
Albumin (g/dL)	**Range**	3.4-4.7		2.1-4.8		7.553	< 0.001*
	Mean ± SD	4.200 ± 0.376		3.197 ± 0.530			
INR	**Range**	0.81-1.28		1-2		-4.354	< 0.001*
	Mean ± SD	0.988 ± 0.105		1.192 ± 0.195			
PT (seconds)	**Range**	10.2-16.5		11.5-21		-2.829	0.006*
	Mean ± SD	12.645 ± 1.406		14.173 ± 2.195			
PTT (seconds)	**Range**	16.6-36		28-65		-4.843	< 0.001*
	Mean ± SD	28.755 ± 3.939		37.743 ± 7.794			

Baseline CD24 was significantly higher among HCC group in comparison to cirrhosis group (19.463 ± 8.573 vs. 0.725 ± 0.125 mg/L) with *p*-value < 0.001, and similar values were seen for AFP (Table 2).

Regarding triphasic CT findings among the HCC group, male patients, and patients with larger hepatic focal lesions, and those with advanced BCLC stages had a statistically significantly higher AFP as shown in Table 3.

Serum CD24 level showed insignificant difference regarding numbers or sizes of hepatic focal lesions, However, patients with multiple lesions, and lesions larger than 5 cm experienced the highest levels of CD24, as well as patients with BCLC-C. Yet this was of no statistical significance (Table 4).

When using Pearson multivariate correlation test, none of the studied parameters was significantly correlated with baseline CD24 level among either of the studied groups (Table 5).

Among the HCC group, 5 patients were excluded from intervention treatment as they had evidence of portal vein invasion by triphasic CT, while the remaining 35 patients underwent locoregional treatment according to the approved selection criteria, where 15 patients were candidates for RF and 20 for TACE. At one month follow-up, serum CD24 levels significantly declined from 19.463 ± 8.573 mg/L to 3.569 ± 1.248 mg/L with an overall *p*-value of < 0.001. Also, follow up AFP showed a significant reduction after locoregional treatment (Table 6).

Table 2 Comparison between the studied groups regarding baseline CD24 and AFP

		Group		T-Test	
		Cirrhosis	HCC	T	*P*-value
Baseline CD24	**Range**	0.5-0.9	2.5-32	-9.732	< 0.001*
	Mean ± SD	0.725 ± 0.125	19.463 ± 8.573		
Baseline AFP	**Range**	1-196	50-1522	-4.832	< 0.001*
	Mean ± SD	26.69 ± 44.24	540.750 ± 494.594		

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

Group			Baseline AFP		T-Test or ANOVA	
			N	Mean ± SD	T or F	*P*-value
HCC	**Sex**	**Male**	34	607.676 ± 507.508	2.127	0.040*
		Female	6	161.500 ± 71.285		
	Disease	**HCV**	31	509.742 ± 483.827	-0.731	0.469
		HBV	9	647.556 ± 546.030		
	Types of intervention	**TACE**	20	563.250 ± 543.815	0.554	0.579
		RF	15	452.800 ± 430.923		
		Palliative treatment	5	714.600 ± 510.970		
	Number of Lesions	**Single focal lesion**	14	465.357 ± 461.640	1.216	0.308
		Two focal lesions	6	647.333 ± 584.350		
		Multiple focal lesions	20	786.000 ± 546.037		
	Size of lesions	**< 3 cm**	11	429.545 ± 431.095	4.854	0.013*
		3–5 cm	17	373.882 ± 401.642		
		> 5 cm	12	879.083 ± 532.387		
	BCLC	**Stage A**	15	442.194 ± 444.272	3.860	0.030*
		Stage B	20	1087.250 ± 556.099		
		Stage C	5	714.600 ± 510.970		
	Ascites	**No**	15	481.600 ± 476.022	1.453	0.244
		Mild	14	455.286 ± 425.844		
		Moderate	9	828.778 ± 604.810		
		Severe	2	286.500 ± 231.224		

Table 4 Comparison between baseline CD24 and other studied parameters among HCC group

Group			Baseline CD24		T-Test or ANOVA	
			N	Mean ± SD	T or F	*P*-value
HCC	**Sex**	**Male**	34	607.676 ± 507.508	-2.127	0.0.040*
		Female	6	161.500 ± 71.285		
	Disease	**HCV**	31	19.048 ± 8.873	-0.562	0.577
		HBV	9	20.889 ± 7.753		
	Type of intervention	**TACE**	20	19.850 ± 8.541	0.039	0.962
		RF	15	19.100 ± 8.881		
		Palliative treatment	5	19.000 ± 9.618		
	Number of lesions	**Single focal lesion**	14	15.375 ± 8.213	0.795	0.459
		Two focal lesions	6	15.500 ± 9.274		
		Multiple focal lesions	20	19.167 ± 9.968		
	Size of lesions	**< 3 cm**	11	19.364 ± 7.801	0.210	0.812
		3–5 cm	17	19.618 ± 9.635		
		> 5 cm	12	20.750 ± 8.203		
	BCLC	**Stage A**	15	19.000 ± 9.618	0.511	0.604
		Stage B	20	19.000 ± 8.791		
		Stage C	5	23.625 ± 5.793		
	Ascites	**No**	15	18.767 ± 8.113	0.189	0.903
		Mild	14	20.286 ± 9.589		
		Moderate	9	20.111 ± 7.607		
		Severe	2	16.000 ± 15.556		

On sorting patients according to offered treatment modalities, there was a statistically significant decline in CD24 after RF or TACE with *P* value < 0.001 (Table 7).

On sorting patients according to offered treatment modalities, follow up of response after TACE treatment according mRECIST scoring showed a statistically significant decline in CD24 among both patients with complete response [in 14 patients] and those with partial response [in 6 patients] with *P* value < 0.001, with higher baseline values among complete responders than those with partial response (Table 8).

Serum CD24 levels showed a statistically significant decline one month after locoregional treatment among patients with single focal lesion and those with multiple lesions ($p < 0.001$ and $p = 0.01$, respectively) (Table 9).

At Baseline and at one month follow up, CD24 levels showed no significant difference between both studied groups as regards the tumor sizes ($p = 0.812$ and 0.762, respectively). However, its level showed a statistically significant decline one month after treatment among different tumor sizes (Table 10).

A ROC curve was used to compare the diagnostic effect of CD24 and AFP level in HCC patients. (Table 11).

The diagnostic accuracy of baseline CD24 and AFP was assessed for the discrimination between HCC patients with and those without portal vein thrombosis, and thus their eligibility for treatment. A ROC curve was applied, showing a best cutoff value of 23 mg/L with sensitivity 80%, specificity 74.29% and diagnostic accuracy 65.1% for CD24 while AFP at cutoff 421 ng/l showed lower accuracy 62.3.1% (Table 12 and Figs. 1 and 2).

Another ROC curve was performed for baseline CD24 and AFP in prediction of cure after HCC intervention. The best cutoff value was 19.5 mg/L with sensitivity 62.96%, specificity 100% and diagnostic accuracy 87.3% for CD24 while AFP at cutoff 190 ng/l showed lower accuracy 54.2% (Table 13, Figs. 3 and 4).

Discussion

Over 90% of all primary liver tumors are hepatocellular carcinomas, which affect about 85% of patients with liver cirrhosis. Estimates for HCC's five-year survival places it second to pancreatic cancer at 18%. In Egypt HCC is regarded as the second cause of cancer mortality, while it is the fourth most common type among all cancers [9].

Over the past few decades, a great deal of research has been done on the diagnostic and prognostic accuracy of many HCC biomarkers. As of right now, AFP is the serum biomarker most frequently employed for early diagnosis and surveillance. However, up until now, there has been much debate on its accuracy, with reports indicating that its sensitivity and specificity are roughly 60%

Table 5 Correlations of Baseline CD24 with other parameters among the studied groups

Correlations				
	Cirrhosis		HCC	
	Baseline CD24			
	r	*P*-value	r	*P*-value
Baseline AFP	-0.109	0.648	0.208	0.198
Age	0.153	0.521	-0.262	0.103
PT	-0.114	0.631	0.029	0.858
PTT	0.178	0.454	-0.009	0.955
Creatinine	-0.039	0.870	0.128	0.431
MELD	-0.318	0.172	-0.017	0.919
Child Score	0.144	0.546	0.022	0.893
Focal lesion size in CT (cm)	-	-	0.087	0.594
Hemoglobin	-0.148	0.533	0.181	0.264
Total leucocytic count	0.124	0.604	0.162	0.319
Platelet count	0.041	0.863	0.197	0.224
AST	-0.178	0.452	0.119	0.464
ALT	-0.099	0.678	-0.012	0.944
Total bilirubin	0.059	0.804	-0.040	0.804
Direct bilirubin	-0.001	0.997	0.065	0.688
ALP	0.326	0.161	-0.007	0.965
GGT	-0.091	0.702	-0.076	0.640
Albumin	-0.045	0.851	-0.165	0.310
INR	-0.168	0.479	0.141	0.386

and 80%, respectively. As a result, a number of additional biomarkers, such as glypican 3, serum Golgi protein 73, and des-gamma carboxyprothrombin, have been studied as potential substitutes; nevertheless, further studies have failed to reach superior results to those of AFP [10].

A variety of human cancers have high expression levels of CD24, a highly glycosylated glycoprotein that is found on the surface of most B cells [11]. CD24 may be a key factor in the development, spread, and possibly metastasis of tumors. Overexpressing CD24 makes cancer cells extremely vulnerable to the development of the epithelial-mesenchymal transition, which is an important step in promoting the invasion and metastasis of cancer cells [12].

The aim of this study was to evaluate the diagnostic value of serum level of CD24 as a possible noninvasive marker for HCC, and its predictive value after locoregional therapeutic modalities.

In accordance with El-Zayadi et al. [13], we found a higher prevalence of HCC in males 34 (85%) than in females 6 (15%). On the contrary, Tokushige et al. [14] have found different results. The variations in sample size, exposure to risk factors, and sex hormones could all contribute to this explanation. The increased incidence of HCC in males has been explained by the hormonal effects of androgens and estrogens, the degree of iron deposition, and ethnic differences, all of which have been proposed to affect hepato-carcinogenesis [15].

The age range of the HCC patients in the current study was 48–79 years old, with a mean age of

Table 6 Comparison between serum CD24 and AFP levels before and one month after intervention among HCC group

		Time		Differences		Paired Test	
		Baseline	One month after	Mean	SD	t	*P*-value
AFP	**Range**	50-1522	8-80	494.729	491.785	5.951	< 0.001*
	Mean ± SD	540.750 ± 494.594	21.186 ± 18.426				
CD24	**Range**	2.5-32	2-7	15.960	8.268	11.419	< 0.001*
	Mean ± SD	19.463 ± 8.573	3.569 ± 1.248				

Table 7 Comparison between baseline and post- interventional CD24 levels in relation to types of intervention

CD24		Types of intervention		T-Test	
		TACE	RF	T	*P*-value
Baseline	**Range**	4-32	2.5-32	0.253	0.802
	Mean ± SD	19.850 ± 8.541	19.100 ± 8.881		
One month after intervention	**Range**	2-6	2.5-7	-0.837	0.409
	Mean ± SD	3.415 ± 0.893	3.773 ± 1.619		
Differences	**Mean ± SD**	16.435 ± 8.343	15.327 ± 8.415	–	–
Paired Test	***P*-value**	< 0.001*	< 0.001*		

Table 8 Comparison between serum CD24 levels before and one month after TACE intervention according to response to treatment

Response to TACE		CD24		Differences		Paired Test	
		Before	After	Mean	SD	t	*P*-value
Complete response	**Range**	20-32	2.5-6	24.650	5.282	11.431	< 0.001*
	Mean ± SD	28.333 ± 5.715	3.683 ± 1.283				
Partial response	**Range**	4-32	2-4	12.914	6.819	7.086	< 0.001*
	Mean ± SD	16.214 ± 6.827	3.300 ± 0.696				

Table 9 Comparison between baseline and post- interventional CD24 levels in relation to number of focal lesions

CD24		Time		Differences		Paired Test	
		Baseline	One month after	Mean	SD	t	*P*-value
One focal lesion	**Range**	4-32	2-7	16.192	8.084	10.213	< 0.001*
	Mean ± SD	15.375 ± 8.213	2.712 ± 1.374				
Two focal lesions	**Range**	2.5-24	2.5-3.8	11.040	9.081	2.719	0.053*
	Mean ± SD	15.500 ± 9.274	2.760 ± 0.581				
Multiple focal lesions	**Range**	5-32	3-4	20.600	7.124	5.784	0.010*
	Mean ± SD	19.167 ± 9.968	3.650 ± 0.443				
ANOVA	**F**	0.795	1.246				
	***P*-value**	0.459	0.301				

Table 10 Comparison between baseline and post- interventional CD24 levels in relation to size of focal lesions

CD24		Time		Differences		Paired Test	
		Baseline	One month after	Mean	SD	t	*P*-value
< 3 cm	**Range**	5-32	2.5-6.5	15.582	7.232	7.146	< 0.001*
	Mean ± SD	19.364 ± 7.801	3.782 ± 1.499				
3–5 cm	**Range**	2.5-32	2-7	15.094	9.380	6.635	< 0.001*
	Mean ± SD	19.618 ± 9.635	3.524 ± 1.299				
> 5 cm	**Range**	5-32	2.5-4	18.657	7.398	6.672	0.001*
	Mean ± SD	20.750 ± 8.203	3.343 ± 0.658				
ANOVA	**F**	0.210	0.274				
	***P*-value**	0.812	0.762				

Table 11 Diagnostic performance of CD24 and AFP level in diagnosis of HCC

ROC curve of CD24 and AFP level in diagnosis of HCC						
	Cutoff	Sens	Spec	PPV	NPV	Accuracy
AFP	> 100	70.0	93.33	91.3	75.7	82%
CD24	> 0.9	100	100	100	100	100%

61.90 ± 7.06 years, which is likely related to how long the underlying liver disease has been present. The findings aligned with the findings of Konstantin et al. [16], who reported an average age of 63.79 ± 9.99 years in HCC patients, validating the incidence of HCC in the fifth and sixth decades of life. This is more than the mean age of patients with liver cirrhosis, which was 57.75 ± 10.54 years, with a range of 30 to 72 years.

Table 12 Diagnostic performance of baseline CD24 and AFP level in prediction of portal vein thrombosis and ineligibility for locoregional treatment

ROC curve in prediction of portal vein thrombosis and ineligibility for locoregional treatment						
	Cutoff	**Sens**	**Spec**	**PPV**	**NPV**	**Accuracy**
Baseline CD24	≥ 23	80.00	74.29	30.8	96.3	65.1%
Baseline AFP	≤ 421	68.57	80.00	96.0	26.7	62.3%

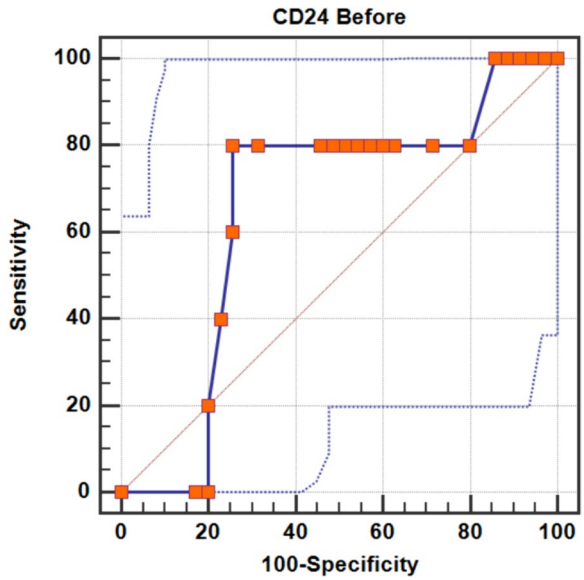

Fig. 1 ROC curve for baseline CD24 level in predicting ineligibility for locoregional treatment

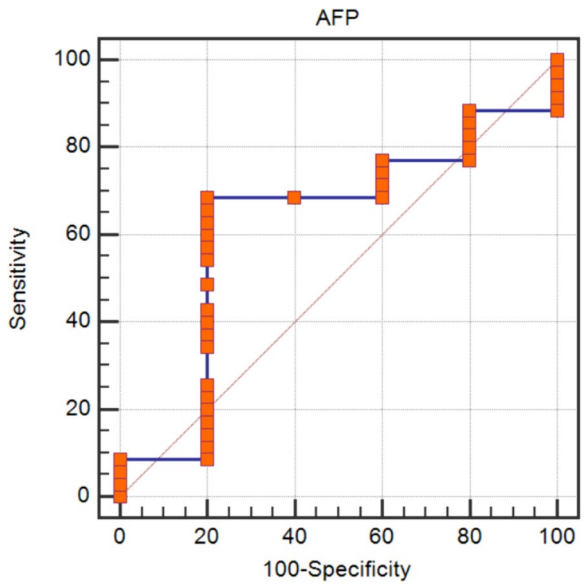

Fig. 2 ROC curve for baseline AFP level in predicting ineligibility for locoregional treatment

According to laboratory results of the groups under study, patients with HCC had statistically significantly lower levels of hemoglobin than patients with liver cirrhosis. This difference in hemoglobin levels could be caused by acute or chronic blood loss, hypersplenism, a folate deficiency that complicates the course of liver disease, or anemia from a chronic illness. This result is consistent with that of Radwan et al. [17], who found that patients with HCC had significantly lower hemoglobin levels than both patients with cirrhosis alone and healthy controls. It's interesting to note that most HCC patients are anemic, which is connected to various tumor consequences, despite the high serum erythropoietin levels that may be present in approximately 25% of HCC patients [18].

Additionally, we found a significantly lower count of platelets in HCC patients in comparison to cirrhotic patients. This could be explained by the existence of portal hypertension, which induces platelet sequestration in the congested spleen. Additionally, there is a drop in thrombopoietin levels, which are mostly generated by the liver. These findings are consistent with those of earlier research by Radwan et al. [17] and Zekri et al. [19], which showed that the HCC and liver disease groups had considerably lower platelets than the control group.

Furthermore, compared to the cirrhosis group, patients with HCC exhibited statistically significant higher levels of ALT, AST, total and direct bilirubin, and INR, according to our study. In contrast, HCC patients' serum albumin levels were statistically significantly lower, and these findings were consistent with research done by Bannaga et al. [20],

These findings may be explained by the findings of Xie et al. [21], who reported that elevated levels of ALT and other cholestatic enzymes predict the risk of fibrosis and cirrhosis, which may contribute to the disease's progression into HCC. Additionally, therapeutic interventions that improve abnormal cholestasis biomarkers may be a further component of the care provided to patients with chronic viral hepatitis in order to improve their future prognoses.

Males and patients with BCLC B staging had considerably higher AFP levels. This finding was consistent with that of Indreswara et al. [22], who discovered

Table 13 Diagnostic performance of baseline CD24 and AFP level in prediction of cure after HCC intervention

ROC curve in prediction of cure after HCC intervention						
	Cutoff	**Sens**	**Spec**	**PPV**	**NPV**	**Accuracy**
Baseline CD24	≤ 19.5	62.96	100.00	100.0	44.4	87.3%
Baseline AFP	> 190	70.37	62.50	86.4	38.5	54.2%

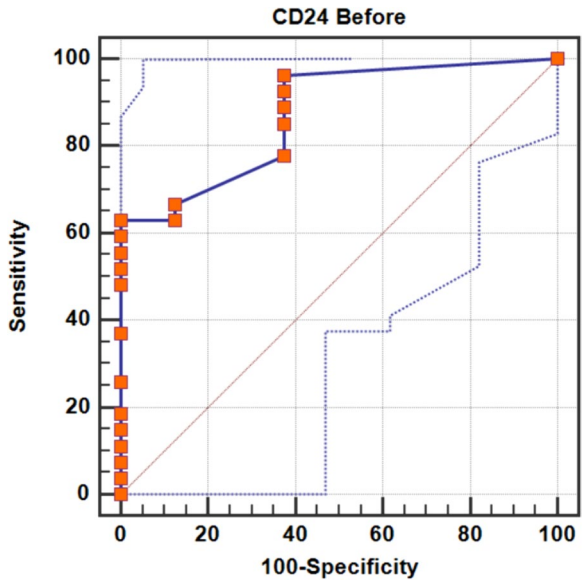

Fig. 3 ROC curve for baseline CD24 level in prediction of cure after HCC intervention

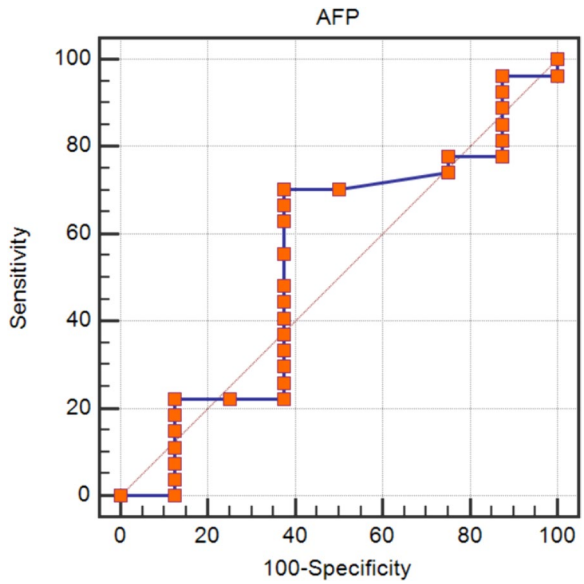

Fig. 4 ROC curve for baseline AFP level in prediction of cure after HCC intervention

that male patients with BCLC B staging had AFP levels above 400 ng/ml, whereas most female patients had normal AFP levels below 20 ng/ml. In contrast to these findings, Shata et al. [23] discovered no sex-related differences in AFP levels.

According to descriptive statistics, patients with single or multiple nodules had AFP levels > 400 ng/ml, hence there was no statistical difference in AFP levels based on the number of nodules. However, it revealed noticeably higher levels among larger tumor sizes, which was in line with the findings of Indreswara et al. [22].

These results were also supported by Rusie et al. [24] who observed that in patients with decompensated cirrhosis, where the mean HCC size was 5.8 cm, AFP levels were mostly over 300 ng/ml, with many values exceeding 500 ng/ml. On the other hand, both compensated cirrhosis and F3 hepatitis patients presented with significantly smaller tumors with mean HCC size being 3.5 and 3 cm, and had lower AFP levels, mostly between 100 and 300 ng/ml in accordance with their dimensions.

The high expression of HMGA1, a protein that promotes oncogenic transformation and progression of cancer that causes significant liver damage, was the source of the association between tumor size and AFP [25].

The present study showed a significant higher CD24 level among patients with HCC than those without HCC (19.463 ± 8.573 vs 0.725 ± 0,125 ng/ml) with overall p value < 0,001, This finding is consistent with the findings of Yang et al. [26], who reported that HCC tumor proliferation, high invasiveness and metastatic potential, and activation of the Wnt/β-catenin pathway were all linked to overexpression of CD24 in HCC. Also, after surgery CD24 may be a unique predictor of a poor prognosis for patients with HCC. Additionally, Maimaitiming et al. [27] showed that prior to intervention, the plasma CD24 level was considerably greater in HCC than in the cirrhotic.

While the exact role that CD24 plays in the development of liver carcinogenesis is unknown, it appears that Notch1 signaling activations and the epithelial-mesenchymal transition (EMT), which are both mediated by CD24, are plausible processes that promote the growth of HCC. Furthermore, the regulation of liver cancer stem-like cell self-renewal appears to be mostly dependent on the Twist2-CD24-STAT3-Nanog pathway [10].

In the present study, 20 HCC patients (50%) were eligible for TACE and 15 patients (37.5%) were treated with RF. The remaining 5 patients had macrovascular invasion and could not be treated with such modalities.

Numerous studies have proven AFP's capacity to predict survival and responsiveness to therapy. However, there is no consensus yet regarding the extent of the decline in AFP that defines AFP response [28]. In the current study we found that AFP levels declined significantly in all patients one month after treatment of HCC (from 540.750 ± 494.594 to 21. 186 ± 18. 426 ng/ml), while on sorting patients according to different treatment modalities they were subjected to, there was a significant decrease in AFP after RF (from 452.80 ± 430.923 to 17.333 ± 10.026 ng/ml) and after TACE (from 563.250 ± 543.815 to 24.075 ± 22.649 ng/ml) These results go along with Toro et al. [29] who stated that AFP significantly dropped in HCC patients who underwent either TACE or RF.

Transcript profiling research suggests that CD24 is significantly expressed in HCC and may be a useful biomarker for prognostic prediction. So far, there hasn't been a thorough examination of CD24's predictive significance in HCC, particularly in long-term studies or large patient populations at follow-up [30]. In our study, we found that CD24 is highly expressed in HCC and on follow up after different locoregional treatment modalities, CD24 showed significant decline (from 19.463 ± 8.573 to 3.569 ± 1.248 ng/ml) with overall p value < 0.001, with decline (from 19.10 ± 8.881 to 3,773 ± 1.619 ng/ml) post RF and (from 19.850 ± 8.541 to 3.415 ± 0.893 ng/ml) post TACE.

Patients with multiple hepatic focal lesions showed insignificantly higher CD24 levels in comparison to solitary lesions with a significant decline in its levels after treatment. On the other side, CD 24 levels showed insignificant increase with advancement of tumor sizes, and a statistically significant decline in its levels after different treatment modalities.

These findings were consistent with Maimaitiming et al. [27] finding that there was no correlation between plasma CD24 and clinicopathologic characteristics such as age, gender, tumor size, number, capsulation status, HBsAg status, stage of tumor node metastasis, ALT, AFP, and GGT level.

Also, the present study reveled that HCC patients with evidence of vascular invasion showed insignificantly higher serum CD24 levels. Whereas at cut off of ≥ 23 ng/ml baseline CD24 could distinguish patients with Portal vein invasion from those without, with sensitivity of 80%, specificity of 74.29%, and accuracy of 65.1% and in comparison, AFP has a lower accuracy 62.3%.

Although the role of serum CD24 in detecting HCC response to different treatment modalities has rarely been studied, the current study found that baseline CD24 levels at cut off of 19.5 ng/ml could predict cure after different locoregional treatment modalities with sensitivity of 62.96%, specificity of 100%, and accuracy of 87.3%, and in comparison, AFP has a lower accuracy 54.2% making CD24 a promising prognostic marker for HCC.

CD24 is a protein that is not yet fully understood, but it has become a promising target for diagnosing, treating, and predicting the prognosis of many types of tumors. Many tumors have been found to have high levels of CD24 and activating the CD24/Siglec-10 pathway has been shown to inhibit the function of cytotoxic T cells and phagocytosis mediated by macrophages, which promotes tumor immune evasion. CD24 is also involved in tumor cell migration and metastasis and has been identified as a prognostic marker for various types of cancers. Therefore, CD24 has attracted a lot of attention as a potential target for drug therapy against tumor cells or tumor stem cells. Several oncology clinical trials worldwide are currently testing the clinical efficacy of anti-CD24-based tumor therapy [31].

Finally, additional high-quality studies on various subgroups of tumor genesis, size and stages along with extended follow up intervals are required to assess the utility of CD24 for the identification of de novo and recurrent HCC.

Conclusion

CD24 may prove to be a valuable marker for diagnosis as well as eligibility for intervention and follow-up following locoregional treatment among HCC patients.

Abbreviations

HCC	Hepatocellular carcinoma
AFP	Alpha fetoprotein
CD24	Cluster of differentiation 24
GPI	Glycophosphatidylinositol, Child–Pugh score
MELD	Model of End-Stage Liver Disease
BCLC	Barcelona Clinic Liver Cancer
RF	Radiofrequency ablation
TACE	Trans-arterial chemoembolization

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Authors' contributions

HSR participated in the conceptualization, editing, methodology and publication. EAA took part in writing the study and revised and drafted the work. ASB assisted in data collection. ESM contributed significantly to the writing and critical revision of the paper. AI wrote a significant portion of the manuscript and critically revised it for significant intellectual content. SAI provided updating the laboratory analysis and tabulating the data. Each author has reviewed and approved the work in its submitted form.

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Availability of data and materials
The datasets that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Declarations

Ethics approval and consent to participate
This study was performed according to the ethical standards for human experimentation and in accordance with the ethical principles of the 1975 Declaration of Helsinki. Patients included in this study signed an informed written consent to participate and all the procedures were in accordance with the standards of the Research Ethics Committee (REC) of the Faculty of Medicine, Ain Shams University. (FWA 000017585)

Consent for publication
Consent was taken from each author for publication.

Competing interests
The authors declare that they have no competing interests.

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