RESEARCH ARTICLE

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Diagnostic and Prognostic Values of Microfibrillar-Associated Protein 5 and Heat Shock Protein 90 Alpha in Patients with HCV-Induced Hepatocellular Carcinoma Undergoing Locoregional Therapy

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Abstract

Objectives: This study aimed to assess the role of HSP90 alpha and MFAP5 as potential diagnostic and prognostic markers for HCC patients after treatment. **Methods:** A prospective interventional study included 75 patients of which 50 patients had HCC and 25 were cirrhotic. The control group included 15 age and sex matched healthy subjects. Serum levels of alpha-fetoprotein (AFP), HSP90α and MFAP5 were measured. Patients with HCC received therapeutic interventions (MWA or TACE) and were monitored for one year. **Results:** AFP, HSP90α, and MFAP5 levels were significantly higher in HCC patients than cirrhotic patients and controls, and in cirrhotic patients than controls. Patients with tumor diameter more than 5cm, multifocal lesions, and BCLC B staging, higher HSP90α and MFAP5 were significantly more frequent in non-successful group after locoregional therapy compared to successful group. Overall survival in HCC patients was longer when HSP90α was < 243.55 ng/ml (96% and 88% at 6 and 12 months), and when MFAP5 was < 5509.3 pg /ml (96% and 92% at 6 and 12 months). **Conclusions:** HSP90α and MFAP5 can be used as diagnostic and prognostic markers for HCC patients after therapy.

Keywords: Hepatocellular carcinoma- Alpha Feto Protein- Heat Shock Protein 90 Alpha

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Introduction

Hepatocellular carcinoma (HCC) ranks as the sixth most prevalent malignancy and the third foremost cause of cancer-related deaths, constituting around 7% of all cancer fatalities globally [1, 2]. HCC is among the most aggressive malignancies globally, it accounts for over 906,000 new cases and 600,000 deaths every year [3].

Alpha-fetoprotein (AFP) is still used as the principal biomarker for HCC screening. Owing to its low sensitivity and specificity, the need has been raised to validate new markers with higher sensitivity and specificity, particularly in the early diagnosis and prognosis of HCC [1, 4].

Microfibrillar-associated proteins (MFAPs) are extracellular matrix (ECM) glycoproteins which play role in microfibril assembly, elastiogenesis, tissue homeostasis, cell survival and tumor progression [5]. MFAP5 has been reported as a potential biomarker for diagnosis and predicting prognosis of different cancers such as ovarian cancer, cervical cancer, tongue carcinoma and breast cancer but its role in diagnosis and prognosis of HCC remain unclear [6].

Heat Shock Proteins (HSP) are molecular chaperones that play a crucial role in maintaining cellular proteostasis. Many malignancies exhibit overexpression of HSP90 alpha, which is believed to promote tumor proliferation, survival, and resistance to treatment [7, 8]. Recent studies investigated HSP90 alpha as a promising biomarker for early cancer diagnosis and prognosis [9].

Locoregional therapy (LRT) such as percutaneous ablation either (thermal or chemical), transarterial chemoembolization (TACE) and radioembolization (TARE) has been shown to have promising role in HCC management. It is the preferred option for unresectable Barcelona Clinic Liver Cancer (BCLC) A and B disease [10].

There are still no sufficient prospective studies evaluating biomarkers that are potentially predictive of response, recurrence and survival after locoregional therapy for HCC.

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This study aims to evaluate the role of MFAP5 and HSP90α as diagnostic and prognostic biomarkers for patients with HCV associated hepatocellular carcinoma undergoing locoregional therapy.

Materials and Methods

Patients and methods

This prospective study was carried out on 75 cirrhotic patients on top of chronic HCV infection with or without HCC [all patients had achieved sustained virological response (SVR)] recruited from Tropical Medicine Department, Mansoura university hospital between October 2022 and October 2024. Fifteen healthy subjects were also included in this work.

Group I: 50 HCC patients on top of HCV-induced liver cirrhosis.

Group II: 25 liver cirrhosis patients on top of chronic hepatitis C.

Group III: 15 healthy subjects.

Cirrhosis was diagnosed on a clinical basis, by laboratory tests, endoscopic evidence, and sonography findings. We performed pelviabdominal US as a screening method for focal hepatic lesions. HCC diagnosis was confirmed by triphasic CT and/or dynamic MRI if needed utilizing noninvasive criteria of EASL guidelines 2004 [11]. Staging and determining appropriate line of treatment was chosen according to BCLC guidelines 2022 [12]. Patients were divided into three groups: HCC patients undergoing locoregional therapy group, cirrhotic group and control group. HCC group were subdivided into MWA subgroup and TACE subgroup. Only HCC patients with BCLC class A and B were included. HCC Patients with BCLC class C and D, portal vein thrombosis and metastasis were excluded. Additionally, patients with any of the following were excluded from this study: malignancies other than HCC, non-HCV related HCC, non-HCV related liver cirrhosis, autoimmune diseases, active infection at time of blood sampling, severe cardiac disease, severe renal impairment. (GFR < 10 ml /min), acute esophageal or gastric variceal bleeding. All patients included in this study were subjected to the following: full history taking, full clinical examination, routine laboratory tests including virology (HBS Ag, HCV Ab), PCR for HCV RNA, complete blood count, liver function tests (ALT, AST, albumin, bilirubin, prothrombin time) serum creatinine, tumor markers (Alpha feto protein).

Sampling: 5 ml of blood were collected one day before intervention from subjects of all groups by venipuncture in a tube. This tube was left standing at room temperature for 15-30 minutes and then centrifuged at 3000 round per minute (rpm) for 10 minutes to obtain serum and stored at -80°C.

Detection of MFAP5 and HSP90α

MFAP5 and HSP90α were measured by Sandwich-ELISA technique (Innova Biotech CO, LTD). Standards or samples were inserted into wells of Micro-ELISA strip plates precoated with an antibody specific to these proteins. Then a Horseradish Peroxidase (HRP)-conjugated antibody also specific for HSP90a and MFAP5 was added, being incubated 30 min at 37°C after sealed with closure plate membrane. Free components were washed away. The TMB (Tetra methyl benzidine) substrate solution was added to each well. Only those wells that contain the 2 targeted proteins and their HRP-conjugated forms appeared blue in color and then turned yellow after the addition of the stop solution. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm. Using standard curves, the concentrations were plotted on the x-axis, and the OD signal on the y-axis, with concentrations being determined from the corresponding signal. Finally, the original concentrations were calculated by multiplying the result by dilution factor.

HCC treatment and follow up: Patients with HCC received therapeutic interventions in the form of Transarterial chemoembolization (TACE) or Microwave ablation (MWA) based on BCLC criteria and were monitored every 3 months for one year, using triphasic CT or dynamic magnetic resonance imaging (MRI) and serum AFP. Assessment of response to interventional procedure was according to mRECIST criteria [13]

Ethical considerations

Every individual who was involved in the study signed a written informed consent form after approval from the Mansoura Faculty of Medicine's Institutional Research Board (IRB) Committee (code number: MD.21.09.525). The research was conducted in accordance with the basics of the Helsinki declaration.

Statistical analysis

The data was analyzed using version 21 of the Statistical Package for Social Sciences (SPSS). We used the Kolmogorov-Smirnov test to assess the normality of distribution. Continuous data was listed as mean ± SD or median (min-max). One-way ANOVA test was used for continuous normal data, Mann-Whitney U test/ Kruskal -Wallis H tests were used for continuous non normally distributed data and Chi-square test/ Fisher's exact test were used for categorical data. Univariate and multivariate Cox regression analysis were done to unveil the independent variables predicting overall survival in HCC group. A receiver operating characteristic curve (ROC) was done, and the best cutoff values for each marker to predict HCC were calculated. Significance was defined when the P value was ≤ 0.05.

Results

Table 1 shows demographic and laboratory data among the studied groups. Both controls, cirrhotic and HCC patients were matched as regard age and gender (p= 0.296, 0.137 respectively). There was a significant increase in INR and a significant reduction of hemoglobin concentration, platelet count and albumin level in both cirrhotic and HCC groups compared to control group (P for all ≤0.001). Additionally, there was a significant elevation of ALT, AST, bilirubin, AFP, HSP90α and MFAP5 in both cirrhotic and HCC patients when compared to controls and in HCC group when compared to cirrhotic group (P for all ≤0.001 except p1 for bilirubin=0.18, P3 for AFP= 0.03).

Table 1. Comparison of Patients' Characteristics, Laboratory Parameters, Tumor Markers among Studied Groups

Parameter		Control N=15	Cirrhotic N=25	HCC N=50	P	P1	P2	P3
Age (years)		57.2 ± 6.9	57.5 ± 7.8	59.7 ± 6.5	0.296			
Gender	M	11 (73.3%)	14 (56%)	39 (78%)	0.137			
	F	4 (26.7%)	11 (44%)	11 (22%)				
Hb g/dl		12.6 ± 1.05	10.5 ± 1.41	10.3 ± 1.04	≤0.001	≤0.001	≤0.001	1
WBCS $\times 10^9$ /L		6.8	5.7	4.8	0.222			
		(4.5-9.5)	(2-10.3)	(2.1-10.4)				
PLT $\times 10^9$ /L		236	116	112	≤0.001	≤0.001	≤0.001	0.884
		(155-400)	(64-252)	(55-239)				
INR		1	1.2	1.3	≤0.001	≤0.001	≤0.001	0.518
		(1-1.1)	(1.1-1.6)	(1-1.7)				
ALT U/L		18	49	78	≤0.001	≤0.001	≤0.001	0.001
		(14-32)	(17-107)	(27-209)				
AST U/L		23	50	88	≤0.001	≤0.001	≤0.001	0.042
		(16-39)	(18-204)	(27-324)				
Bilirubin mg/dl		0.9	1	1.6	≤0.001	0.018	≤0.001	0.015
		(0.6-1.1)	(0.8-4)	(0.8-4.9)				
Albumin g/dl		4.1 ± 0.4	3.2 ± 0.5	3.1 ± 0.6	≤0.001	≤0.001	≤0.001	0.553
Creatinine mg/dl		0.9	1	1	0.126	0.051	0.145	0.319
		(0.6-1)	(0.6-1.6)	(0.6-1.7)				
AFP (ng/ml)		6.4	46	65	≤0.001	≤0.001	≤0.001	0.03
		(2.5-13)	(6.5-107)	(10.9-1871)				
HSP90α (ng/ml)		11.8	69.6	243.6	≤0.001	≤0.001	≤0.001	≤0.001
		(6.9-38.9)	(19.3-137)	(143.8-2001)				
MFAP5 (pg/ml)		213.7	3806	5509.3	≤0.001	≤0.001	≤0.001	≤0.001
		(161.2-1376.6)	(1958.3-5169.4)	(4009.3-18601.9)				

P, between 3 groups; P1, between control and cirrhotic; P2, between control and HCC; P3, between cirrhotic and HCC. Significance level when P value < 0.05.

A ROC analysis in Table 2 and Figure 1 was conducted to discriminate cirrhotic patients from control. AFP, HSP90α and MFAP5 best cut-off values for prediction of cirrhosis were 13.2, 39.4, 1667.4 with areas under the curve 0.896, 0.984, 1 respectively (P for all ≤0.001). Another ROC analysis was conducted to discriminate HCC patients from control. AFP, HSP90α and MFAP5 best cut-off values for prediction of HCC were 13.4, 91.4, 2693 with areas under the curve 0.996, 1, 1 respectively (P for all ≤ 0.001). The third ROC analysis was conducted to discriminate HCC patients from those with cirrhosis. AFP, HSP90α and MFAP5 best cut-off values for prediction of HCC were 52.2, 140.4, 4426.4 with areas under the curve 0.655 (P= 0.03), 1.00 (P≤ 0.001), 0.943 (P≤ 0.001) respectively. These AUCs increase when combining AFP with HSP90α (AUC= 1, p=0.03), MFAP5 (AUC= 0.943, p≤ 0.001) or both (AUC= 1, p=0.03) as shown in Table 3. Table 4 revealed that higher levels of HSP90α and MFAP5, tumor diameter more than 5cm, multifocal lesions BCLC B staging, Child classification class B, TACE locoregional therapy were significantly associated with unsuccessful response to locoregional therapy (p= 0.001, 0.014, 0.016, 0.03, 0.038, ≤0.001 respectively). However, recurrence of HCC was not significantly related to AFP, HSP90α and MFAP5 levels (p= 0.48, 0.3, 0.6). COX regression analysis for HCC patients as shown in Table 5 and Figure 2 revealed that the significant independent factors for shorter OS in in univariate analysis are BCLC B, higher AFP, HSP90α, MFAP5 and unsuccessful response to locoregional therapy (p= 0.005, 0.002, 0.001, 0.001 respectively). However, multivariate analysis did not reveal any significant factors for shorter OS.

Discussion

Because of late diagnosis and limited lines of effective treatment, HCC is considered a poor prognostic carcinoma, therefore the challenge is to develop novel biomarkers for early diagnosis, adequate treatment, selection of patients and post treatment prognosis [4]. This study aimed to evaluate the role of MFAP5 and HSP90α in diagnosis and prognosis of HCC patients after locoregional therapy by TACE or MWA.

This study included 90 individuals classified into; 50 HCV associated HCC patients, 25 liver cirrhosis patients on top of chronic hepatitis C and 15 healthy controls. There was a highly statistically significant difference among the studied groups as regard HSP90α (p≤ 0.001). It was higher

Table 2. Diagnostic Performance of AFP, HSP90α and MFAP5 for Discrimination between Cirrhosis Patients from Control, HCC Patients from Control, and HCC Patients from Cirrhosis Patients

		AFP	HSP90α	MFAP5
Discrimination between cirrhosis patients from control	AUC	0.896	0.984	1
	95% CI	0.800-0.992	0.955-1	1-Jan
	P value	≤0.001	≤0.001	≤0.001
	Cut off	13.2	39.4	1667.4
	Sensitivity (%)	76%	88%	100%
	Specificity (%)	100%	100%	100%
	PPV	100%	100%	100%
	NPV	71.40%	83.30%	100%
Discrimination between HCC patients from control	AUC	0.996	1	1
	95% CI	0.987-1	1-Jan	1-Jan
	P value	≤0.001	≤0.001	≤0.001
	Cut off	13.4	91.4	2693
	Sensitivity (%)	96%	100%	100%
	Specificity (%)	100%	100%	100%
	PPV	100%	100%	100%
	NPV	88.20%	100%	100%
Discrimination between HCC patients from cirrhosis	AUC	0.655	1	0.934
	95% CI	0.528-0.782	1-1	0.883-0.986
	P value	0.03	≤0.001	≤0.001
	Cut off	52.5	140.4	4426.4
	Sensitivity (%)	58%	100%	90%
	Specificity (%)	64%	100%	80%
	PPV	76.30%	100%	90%
	NPV	43.20%	100%	80%

AUC, Area Under curve; CI, Confidence Interval; PPV, Positive Predictive Value; NPV, Negative Predictive Value

Table 3. Diagnostic Performance of Combined AFP + HSP90α, AFP + MFAP5 and AFP+ HSP90α +MFAP5 for Discrimination between HCC Patients from Cirrhosis

	AFP+ HSP90α	AFP+ MFAP5	AFP+ HSP90α +MFAP5
AUC	1	0.943	1
95% CI	1.00-1.00	0.896-0.990	1.00-1.00
P value	0.03	≤0.001	≤0.001

AUC, Area Under curve; C, Confidence Interval

in HCC patients compared to cirrhotic patients and healthy controls. In agreement with this result, Han et al.,2021 reported that serum levels of HSP90α were significantly higher in HCC patients (200.58 ± 143.64 ng/ml) than in cirrhotic patients (110.64 ± 90.06 ng/ml) and healthy controls (35.07 ± 15.42 ng/ml) (p≤ 0.001) [14]. Another

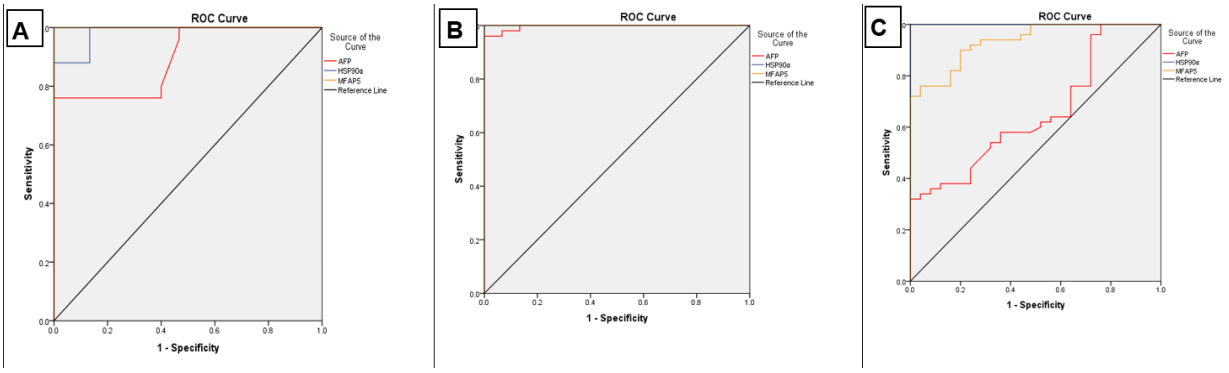

Figure 1. Roc Curves of AFP, HSP90α and MFAP5 for Discrimination between Cirrhosis Patients from Control (A) HCC patients from control (B), and HCC patients from cirrhosis patients (C).

Table 4. Comparison of Patients with Successful Response to Locoregional Therapy versus Those with No- Successful Response and Patients with Non Recurrent HCC versus Those with Recurrent HCC after Successful Response

		Successful N=27	Non-successful N=23	P	Non- recurrent N=16	Recurrent N=11	P
Age **		59.5 ± 6.53	59.8 ± 6.68	0.883	58.3 ± 6.74	60.2 ± 6.62	0.475
Gender	M	20 (74.1%)	19 (82.6%)	0.52	12 (75%)	7 (63.6%)	0.675
	F	7 (25.9%)	4 (17.4%)		4 (25%)	4 (36.4%)	
Tumor diameter	< 5 cm	27 (100%)	18 (78.3%)	0.016*	16 (100%)	11 (100%)	------
	> 5 cm	0 (0%)	5 (21.7%)		-----	------	
Number of focal lesions	Unifocal	25 (92.6%)	15 (65.2%)	0.03*	16 (100%)	9 (81.8%)	0.157
	Multi focal	2 (7.4%)	8 (34.8%)		0 (0%)	2 (18.2%)	
BCLC	A	26 (96.3%)	12 (52.2%)	≤0.001*	16 (100.0%)	10 (90.9%)	0.407
	B	1 (3.7%)	11 (47.8%)		0 (0%)	1 (9.1%)	
Child	A	27 (100%)	19 (82.6%)	0.038*	16 (100%)	11 (100%)	------
	B	0 (0%)	4 (17.4%)		-----	------	
PLT $\times 10^9$ /L*		114 (55-239)	111 (64-221)	0.74	121 (70-236)	105 (55-239)	0.456
INR*		1.3 (1-1.6)	1.3 (1-1.7)	0.12	1 (1-1.5)	1.1 (1-1.7)	0.211
ALT U/L*		77 (27-208)	79 (29-209)	0.82	67 (27-92)	87 (45-208)	0.075
AST U/L*		87 (27-294)	89 (29-324)	0.55	87 (27-92)	92 (45-294)	0.05
Bilirubin mg/dl*		1.4 (0.9-3.9)	2.3 (0.9-4.9)	0.19	1.3 (0.9-3)	1.6 (0.9-3.9)	0.15
Albumin g/dl**		3.1 ± 0.649	3.0 ± 0.628	0.58	3.12 ± 0.619	2.91 ± 0.628	0.397
Creatinine mg/dl*		1 (0.6-1.7)	1 (0.6-1.7)	0.88	1 (0.6-1.5)	1 (0.7-1.7)	0.72
AFP (ng/ml) *		46 (10.9-1426)	68 (13.8-1871)	0.47	36.2 (10.9-547.0)	54 (14.3-1426)	0.48
HSP90α (ng/ml) *		235.1 (143.8-563.6)	305.2 (178-2001)	0.001*	231.7 (143.8-563.6)	241.7 (150.9-407)	0.3
MFAP5 (pg/ml) *		5411.2 (4009.3-6000)	5775.7 (4177.5-18601.9)	0.014*	5327.1 (4037.4-6000)	5439.2 (4009.3-5887.8)	0.6
Type of locoregional therapy	MWA	18 (66.7%)	7 (30.4%)	0.011*	13 (81.2%)	5 (45.5%)	0.097
	TACE	9 (33.3%)	16 (69.6%)		3 (18.8%)	6 (54.5%)	

three studies reported that the serum levels of HSP90α was significantly higher in HCC patients compared to healthy controls [15-17]. Several oncoproteins need high levels of HSPs to preserve their function, and increased

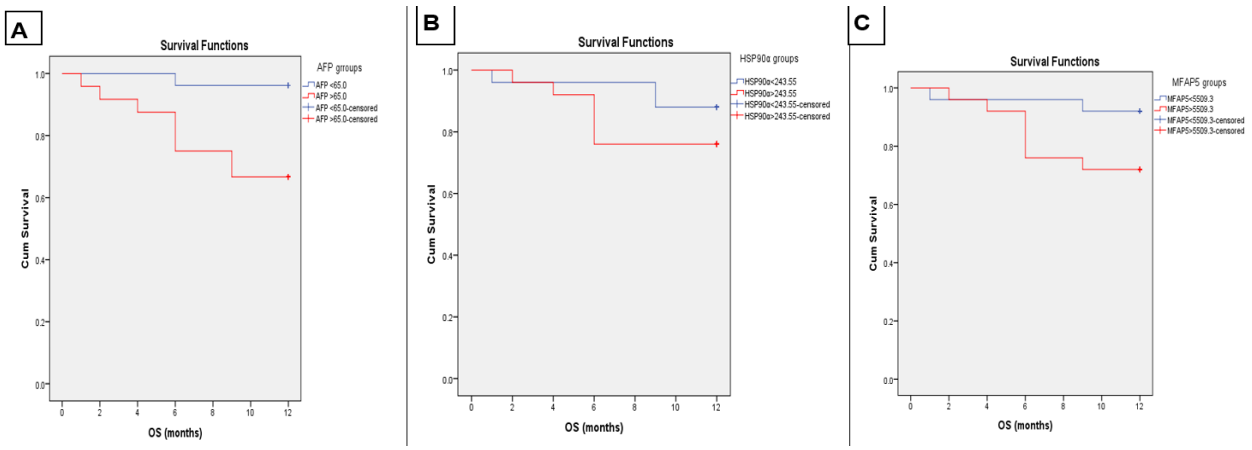

Figure 2. Cox Regression Analysis to Predict the Hazardous Factor that could Predict OS in HCC Patients A) AFP level among studied groups, B) HSP90α level among studied groups and C) MFAP5 level among studied groups.

Table 5. Cox Regression Analysis to Predict the Hazardous Factor that could Predict OS in HCC Patients

Parameter	Univariable			Multivariable		
	P	HR	95 % CI	P value	HR	95 % CI
Age	0.12	1.017	0.822-1.223			
Gender	0.967	0.89	0.415-1.979			
Tumor diameter (>5cm vs <5cm)	0.013	5.868	1.450-23.747			
Number of focal lesions (multi vs uni)	0.063	3.485	0.935-12.997			
Child classification (B vs A)	0.073	4.231	0.874-20.471			
BCLC classification (B vs A)	0.005	7.226	1.796-29.070	0.419	2.198	0.325-14.865
Ascites	0.161	3.082	0.639-14.871			
Splenomegaly	0.386	29.264	0.014-60.997			
Hemoglobin	0.659	0.862	0.445-1.668			
Platelets count	0.264	0.992	0.979-1.006			
ALT	0.93	1.011	0.982-1.037			
AST	0.425	1.003	0.995-1.012			
Albumin	0.975	0.984	0.348-2.783			
Bilirubin	0.425	1.345	0.649-2.785			
AFP	0.002	1.002	1.001-1.003	0.183	1.001	0.999-1.003
HSP90α	0.001	1.011	1.001-1.029	0.728	1.002	0.996-1.002
MFAP5	0.001	1.01	1.001-1.030	0.323	1.001	0.997-1.011
Type of regional therapy (MWA vs TACE)	0.095	3.816	0.792-18.388			
Response (not successful vs successful)	0.024	11.058	1.381-88.553	0.454	2.163	0.288-16.255
Recurrence (recurrent vs not recurrent)	0.451	3.449	0.691-6.265			

HR, Hazard ratio; CI, Confidence interval

levels of HSPs have been reported to be significantly higher in malignant cells than that in normal cells [18]. The secretion of HSP90α in normal cells enhances tissue repair under stress, however its secretion in malignant cells boosts proliferation and correlates positively with the degree of malignancy and ability of metastasis [7, 19].

In this study, there was a highly statistically significant increase in MFAP5 in HCC patients compared to cirrhotic patients and healthy controls ($p \leq 0.001$). Up to our knowledge, this is the first study of MFAP5 on HCC but MFAP5 has been indicated as a potential biomarker for diagnosis and predicting prognosis of different cancers such as intrahepatic cholangiocarcinoma (ICC), ovarian cancer, cervical cancer, tongue carcinoma and breast cancer [6]. Li & Mao, 2013 revealed a significantly elevated MFAP5 serum level in ICC compared to healthy controls. MFAP5 facilitates the aggressiveness of ICC through activation of NOTCH1 signaling pathway (Neurogenic locus notch homolog protein 1) [20].

In the current study, the ROC curve of AFP to differentiate HCC patients from control revealed AUC of 0.996 with sensitivity 96%, specificity 100%, at a cut-off value of 13.4ng/ml. The ROC curve of AFP to differentiate HCC patients from cirrhotic patients revealed AUC of 0.655 with sensitivity 58%, specificity 64%, at a cut-off value of 52.5ng/ml. In two studies by Fu et al., 2017 and Wei et al., 2020 AUC for AFP in discriminating HCC from normal control was 0.922, 0.887 with sensitivity of 81%, 75 % and specificity of 94%, 92% at cutoff 5.38, 6.98 ng/ml respectively [15, 16]. For differentiating HCC from cirrhosis, Yagmur et al. 2007, Romeo et al. 2010, Lok et al. 2010 reported that sensitivity of AFP was 42%, 75 %, 61% and specificity was 84%, 90%, 81% at cut off value of 7.7, 20, 20 ng/ml respectively [21-23]. All these wide variations of AFP cutoff among large number of studies indicate that AFP is not a sufficient marker for diagnosis of HCC due to its poor sensitivity and specificity especially in cirrhotic patients.

In the current study, the ROC curve of HSP90α to differentiate HCC patients from control revealed AUC of 1.00 with sensitivity 100%, specificity 100%, at a cut-off value of 91.4 ng/ml ($p \leq 0.001$). The ROC curve of HSP90α to differentiate HCC patients from cirrhotic patients revealed AUC of 1.00 with sensitivity 100%, specificity 100%, at a cut-off value of 140.4 ng/ml ($p \leq 0.001$). To discriminate between HCC patients and healthy controls; Fu et al., 2017 and Wei et al., 2020 reported that HSP90 had sensitivity of 93%, 67% and specificity of 90%, 90% at cutoff 62.44, 69.1 ng/ml respectively [15, 16]. Despite differences between various studies in level and/or cutoff of HSP90α but all agree with our data in that level of HSP90α in HCC patients is higher than non-liver cancer controls which can be explained by difference in sample size taken by each study.

In the present study, the ROC curve of MFAP5 to differentiate HCC patients from normal controls revealed AUC of 1.00 with sensitivity 100%, specificity 100%, at a cut-off value of 2693 pg/ml. The ROC curve of MFAP5 to differentiate HCC patients from cirrhotic patients revealed AUC of 0.934 with sensitivity 90%, specificity 80%, at a cut-off value of 4426.4 pg/ml.

The results of the current study denoted that ROC curve

of combined AFP+HSP90α to differentiate HCC patients from cirrhotic patients revealed AUC of 1.00, ROC curve of combined AFP+MFAP5 revealed AUC of 0.943 and ROC curve of combined AFP+HSP90α+MFAP5 revealed AUC 1.00. This was in accordance with Fu et al., 2017, Wei et al., 2020 and Han et al., 2021 studies which reported that ROC curve of combined AFP+HSP90α revealed a sensitivity of 94%, 96%, 86% and a specificity of 94%, 98%, 98% respectively, which is higher than results of AFP and HSP90α separately.

The present study included 50 HCC patients, 25 patients underwent transarterial chemoembolization (TACE) and 25 patients underwent microwave ablation (MWA). Out of 50 patients, 27 patients showed successful results and 23 showed unsuccessful results after intervention. In the follow up period, 11 out of 27 patients became recurrent. Our study showed that patients with tumor diameter more than 5cm, multifocal lesion and BCLC B staging, higher HSP90α and MFAP5 were significantly more frequent in non-successful group compared to successful group. Hsp47 is a collagen-specific molecular chaperone that is vital for collagen maturation and secretion [24], which accumulates at the periphery of RFA zone in HCC and increases resistance to heat ablation and promotes tumor progression [25]. But among non-recurrent and recurrent patients, no significant difference was detected due to small sample size.

In our study, there was significant independent factors for short OS in HCC patients in univariate analysis which are BCLC B, high AFP, high HSP90α, high MFAP5 and unsuccessful response to locoregional therapy. However, there were no significant independent factors for shorter OS in HCC patients in multivariate analysis.

In the current study, OS was 96.2% at 6,12 months when AFP was <65 ng/ml which decreased to 75% and 66.7% at 6, 12 months respectively when AFP was >65 ng/ml. As regard HSP90α level, OS was longer when HSP90α was <243.55 ng/ml (96% and 88% at 6, 12 months respectively) compared to shorter OS (76% at 6, 12 months interval) when HSP90α was >243.55 ng/ml. This was in accordance with Su et al., 2022 who suggested that HSP90α <143.5mg/ml had longer OS (80%, 65% at 10, 20 months respectively). Furthermore, they showed that patient with AFP ≥400 ng/ml had higher HSP90α levels compared to patients with AFP <400 ng/ml. They confirmed that the cut off value (143.5 ng/ml) can be applied as a prognostic and predictive value in different treatment groups [26]. In the multivariate cox analysis of TACE group, the HSP90α ≥143.5mg/ml cutoff value was also an independent poor prognostic factor for OS [26]. Additionally, Xiang et al., 2023 showed that HSPs expressions were associated with poor prognosis in HCC patients confirmed that increased HSPs may serve as a potential prognostic predictor of HCC [17].

As regard median value of MFAP5 level, OS estimates 96.0% at 6 months interval and 92.0% at 12 months interval in MFAP5 <5509.3 group, also OS estimates 76.0% at 6 months interval and 72.0% at 12 months interval in MFAP5 >5509.3 group. In studies of MFAP5 in other types of tumors, Li et al., 2019 showed that MFAP5 expression was up regulated in ICC patients and associated with poor outcome [20]. Yang et al., 2016 showed that the OS was 65.785 ± 2.902 months in patients negative for MFAP5 and 50.929 ± 3.840 months in patients positive MFAP5 (p 0.047) [27].

Limitation

The limitation of this study is the small number of patients. Large multi-centric studies are needed.

In conclusion, HSP90α and MFAP5 levels were significantly higher in HCC patients than cirrhotic patients and controls and in cirrhotic patients than controls. The sensitivity and specificity of these markers increase dramatically when being combined with each other. Higher levels were associated with unsuccessful response to locoregional therapy and shorter overall survival indicating their valuable diagnostic and prognostic role in HCC and locoregional therapy era. MFAP5 needs further investigations to be considered as a new promising marker for diagnosis and prognosis of HCC.

Author Contribution Statement

All authors contributed equally in this study.

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Disclosure statement

The authors declare no conflict of interests related to this paper.

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