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ORIGINAL ARTICLE

Role of Some Cancer Stem Cell Markers in Hepatitis C Virus-Associated Liver Disease

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ABSTRACT

Background: *Chronic infection with hepatitis C virus is one of major risk factors in the development of the hepatocellular carcinoma. Cancer stem cells (CSCs) particularly with biomarkers (CD133, CD90 and CK19) show higher ability for self-renewal, differentiation and also tumorigenesis.* ***Objectives:*** *to evaluate the role of CD133, CD90 and CK19 in detection and prognosis of HCC on top of HCV infection.* ***Methodology:*** *This study enrolled 75 participants; 30 HCC patients secondary to HCV infection, 15 HCV patients before receiving the antiviral drug treatment, 15 HCV patients who completed the course of antiviral therapy for 3 months and 15 age and sex-matched apparently healthy volunteers. HCV was detected and quantitated by Quantitative Real-Time PCR and the expression level of CD133, CD90 and CK19 on PBMC was determined by Quantitative Real-Time reverse transcription PCR.* ***Results:*** *this study showed high significant increase in mean expression values of CD133, CD90 & CK19 was in patients with HCC than other studied groups. Also, they were highly significantly increased in HCV patients before than those after receiving treatment and their expression showed high significant positive correlation with HCV load.* ***Conclusion:*** *There were higher expressions of CSC biomarkers (CD133, CD90 and Ck19) in patients with HCC in comparison with those with hepatitis C infection. Their expressions had good diagnostic and prognostic values for HCC.*

INTRODUCTION

Hepatitis C virus (HCV) is single stranded, positive sense RNA virus belongs to family *Flaviviridae*. HCV infection is one of the main risk factors for liver diseases[1]. Natural pathogenesis of hepatitis viruses usually involves a sequentially damaging process. Pathogenesis starts with cell mediated immune response causing endoplasmic reticulum stress, destruction of DNA, mitochondrial dysfunction, finally leads to liver fibrosis, cirrhosis ending in HCC. Despite advances in preventable techniques and new updates in diagnosis and treatment, HCC incidence and related mortality still rising[2].

Viral hepatitis can have an important role in the switch of the cells through stimulating the appearance of CSC[3]. Cancer stem cells, small subset of tumor cells, have the ability to self-renew and differentiate into different lines of cancer cells, showing 'stem cell-like' characteristics. The CSCs are recognized in a wide range of epithelial and other solid organ malignancies[4]. CSCs are unique, and can be a source for the maintenance and growth of the tumor. The bulk of tumors are composed of non-tumorigenic cells that have little capacity to be responsible for cancer progression[3]. The commonly-reported liver cancer stem cell (LCSC) surface markers are EpCAM, CD133, CD90, CD44, and CD13. Other surface markers, including OV6, K19, c-kit, member 2 of ATP binding cassette subfamily G and aldehyde dehydrogenase[5].

CD133 is a glycoprotein composed of five transmembrane domains and two large extracellular glycosylation chains, found in hematopoietic and nervous stem cells. CD133 is expressed on the cell surface in many solid tumors, including liver, colon, brain, lung and prostate. HCC patients with high CD133 expression in their tumors have poor prognosis and increased recurrence[5].

CD90 is a surface marker found on human HCC cells, also tissues and blood of patients having HCC, indicating more tumorigenic capacity and indefinite ability to proliferate than the CD90- cells, so that CD90+ cells could be a 'hepatocellular stem cell. Poor prognosis is highly correlated to CD90 expression[6,7].CD90 can up regulate the expression of

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CD133, and this abnormal expression can promote tumor progression[8].

CK19 is one of low molecular cytokeratins and has particular cell form and immunophenotype. When the oval cell transform to normal liver cell, the expression of CK19 was restrained, and when oval cell transform to tumorous cell, it would express CK19 again. HCC which express CK19 has some specific properties, such as higher hyperplasia capacity, higher aggressiveness, higher malignant level and worse prognosis[9].

So, this study aimed for evaluation of the specific expression of CSCs biomarkers (CD133, CD90 and CK19) in HCC on top of HCV, to assess the role of CSCs biomarkers and to detect their sensitivity in prediction of the HCC prognosis.

METHODOLOGY

Study Population

The study was carried out at Microbiology and Immunology Department in collaboration with Internal Medicine Department, National Liver Institute, Menoufia University, Egypt throughout the span from July 2017 to October 2018 enrolling 75 participants. Participants were classified into four groups: group I; 30 HCC patients secondary to HCV infection, they were previously diagnosed by computed tomography, and liver biopsy and classified into different stages according to both Barcelona-Clinic Liver Cancer (BCLC) and histolopathological grading. Group II; 15 HCV patients before receiving the antiviral drug treatment and they were diagnosed clinically, radiologically and by laboratory investigations. Group III; 15 HCV patients who completed the course of antiviral therapy (sofosbuvair, and daclatasvir) for 3 months. Group IV (control group); 15 age and sex-matched apparently healthy volunteers had no serologic evidence for HCV infection and their laboratory investigations were normal. All participants gave their written informed consents before the study. The Ethical Committee of Menoufia University approved the study protocol.

Methods:

All participants were subjected to history taking and full clinical examination. Laboratory data regarding complete blood picture, prothrombin concentration, INR, function tests for liver and kidney were obtained from each patient's file. Blood samples were collected from all participants for:

Measurement of serum Alpha fetoprotein (AFP) level:

Serum AFP was determined by two-step sandwich solid phase enzyme immunoassay based on electro-chemiluminescence immunoassay "ECLIA" on cobas e411 immunoassay analyzer.

Detection of HCV-RNA by Real-time PCR:

HCV RNA was extracted by the use of QIAamp® DSP Virus Spin Kit (QAIGEN GmbH, Germany) according to manufacturer's instructions then reverse transcription and amplification of HCV RNA were performed using one-step methodology with TaqMan Probes. Forward primer: 5'-GTC TAG CCA TGGCGT TAG TA-3', Reverse primer: 5'-CTC CCG GGGCAC TCG CAA GC- 3' and TaqMan Probe: 5'-CCGATCAGCCATAGTGGTCTGCGGAAGAT C GG-3. Total volume of the reaction was 50µl containing 30µL reaction mixture; TaqMan Universal PCR Master Mix containing 12µl Master A and 18µl Master B and 20 µl sample. The protocol described by Qiagen (Germany); reverse transcriptase (RT) step (cDNA synthesis) at 50°C for 10 minutes, initial denaturation /enzyme activation at 95°C for 1 second for 1 cycle each then denaturing at 95°C for 60 seconds, annealing step for 20 seconds at 55°C finally, elongation step at for 20 seconds 72°C. Forty five cycles were performed on Rotor-Gene Q (Corbett Research, Australia). Data analysis and quantitative real-time RT-PCR curves were done using Rotor-Gene 3000 software version 6.0.23.

Detection of markers of Cancer Stem Cell (CD90, CD133, and CK19) by real time PCR:

Messenger RNA was extracted from separated peripheral blood mononuclear cells using Direct-zol™ RNA MiniPrep, ZYMO RESEARCH CORP following manufacturer's instructions and used for synthesis of complementary DNA (cDNA) using QuantiTect® Reverse Transcription, QIAGEN following manufacturer's instructions. Detection of the markers was done using QuantiTect SYBR Green PCR Kit (Qiagen, Germany) and Applied Biosystems 7500 fast real-time PCR, USA. Primers for CD133; Forward: CTGGGGCTGCTGT TTATTATTCTG and reverse: ACGCCTTGTCCTTGGTAGTGTTG. Primers for CD90; Forward: TCAGGAAATGGCTTTTCCCA and reverse: TCCTCAATGAGATGCCATAAGCT. Primers for CK19; Forward: TCGACAACGCCCGTCTG and reverse: CCACGCTCATGCGCAG. Primers for GAPDH (internal control); Forward AAG GTC GGA GTC AAC GGA TTTGGT and reverse: AGT GAT GGC ATG GAC TGT GGTCAT. The final volume of RT-PCR reaction was 25µl containing; 12.5 µl of QuantiTect SYBR Green PCR Master Mix, 2.5 µl of forward and 2.5 µl of reverse primers, 5 µl of cDNA, and 2.5µl of RNase-free water, according to the manufacturer's instruction. The PCR assay involved an activation step for 15 minutes at 95°C followed by annealing step for 30 seconds at 55°C and a final extension step at 70°C for 30 sec. A total of 40 cycles were performed using a Light Cycler (7500 Fast Real time PCR system, Germany). Quantitative real-time RT-PCR curves were analyzed by Light Cycler (Roche Diagnostics).

Statistical analysis

The data were interpreted using version 17.0 of SPSS (SPSS Inc, Chicago, Illinois, USA) using Student t-test, Mann–Whitney U-test, $\chi 2$-test, Kruskal–Wallis test, Tukey's post-hoc test and Correlation analysis. P value of >0.05 was considered statistically non-significant, <0.05 was statistically significant and <0.001 statistically highly significant[10].

RESULTS

Seventy five participants; 30 had HCC, 30 had HCV infections and 15 as controls. CSC markers were detected by real time PCR in all groups as shown in figure (1&2).

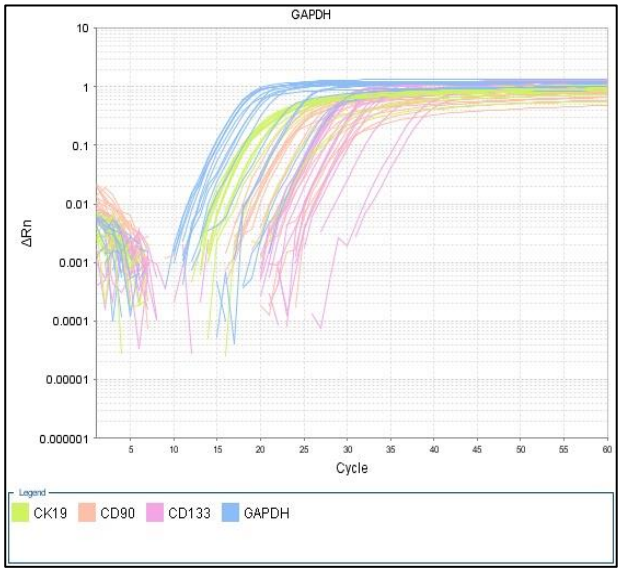
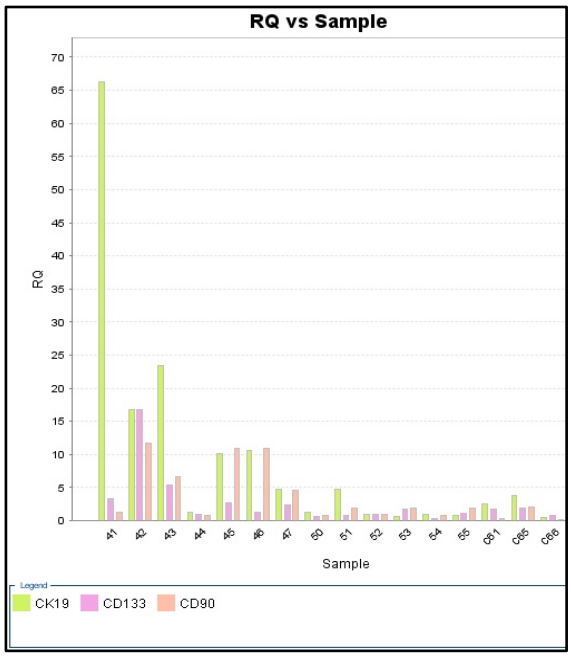


Fig. 1: Expression curve of CSC markers and housekeeping gene (GADH) by real time PCR (Case no. 42)



41, 42, 45, 46, 55: HCC cases
47, 51: HCV patients before receiving treatment
44, 50, 52, 53, 54: HCV patients after receiving treatment
C61, C65, C66: controls

Fig. 2: Expression of CSC markers among the studied participants by real time PCR

The mean values of AFP, CD133, CD90 and CK19 were highly significantly increased in HCC patients in comparison with HCV patients before and after receiving treatment and controls. Also, they were highly significantly increased in HCV patients before than those after receiving treatment. While, there was no significant difference between HCV patients after receiving treatment and controls (table 1).

Table 1: Mean values of AFP and CSC markers among the studied groups

Studied variables	Group I HCC (No.=30)	Group II HCV before treatment (No.=15)	Group III HCV after treatment (No.=15)	Group IV Control (No.=15)	K P value	Post hoc test
AFP Mean ±SD	393.6±120	11.5±7.58	4.96±3.06	4.32±0.57	55.7 **0.001 (HS)**	**P1:0.001(HS) P2:0.001(HS) P3:0.001(HS) P4:0.017(S) P5:0.040(S)** P6:0.693(NS)
CD133 Mean ±SD	4.06±2.53	0.51±0.37	0.23±0.22	0.08±0.12	57.8 **0.001 (HS)**	**P1:0.001(HS) P2:0.001(HS) P3:0.001(HS) P4:0.020(S) P5:0.001(HS)** P6:0.309(NS)
CD90 Mean ±SD	6.70±3.97	1.17±1.01	0.32±0.25	0.15±0.12	59.7 **0.001 (HS)**	**P1:0.001(HS) P2:0.001(HS) P3:0.001(HS) P4:0.001(HS) P5:0.001(HS)** P6:0.141(NS)
CK19 Mean ±SD	10.2±6.31	0.93±0.58	0.46±0.43	0.42±0.28	56.1 **0.001 (HS)**	**P1:0.001(HS) P2:0.001(HS) P3:0.001(HS) P4:0.021(S) P5:0.007(HS)** P6:0.852(NS)

P1: Comparison between group I and group II ; P2: Comparison between group I and group III
P3: Comparison between group I and group IV; P4: Comparison between group II and group III
P5: Comparison between group II and group IV; P6: Comparison between group III and group IV

Expression of CSC markers was associated with deteriorated liver functions as there was a positive correlation between their expression levels and the serum level of AST, ALT, ALP, γGT, bilirubin (total and direct) and INR. While they were negatively correlated with prothrombin and hemoglobin concentrations and platelet count in all patient groups (table 2).

Table 2: Correlation between AFP and CSC markers and laboratory investigations among the studied patient groups:

Laboratory investigations	Group I HCC				Group II HCV before treatment				Group III HCV after treatment			
	AFP R	CD133 R	CD90 R	CK19 R	AFP R	CD133 R	CD90 R	CK19 R	AFP R	CD133 R	CD90 R	CK19 R
Liver function test												
– **AST**	0.204	0.029	0.009	0.094	0.193	0.291	0.095	0.043	0.077	0.121	0.182	0.357
– **ALT**	0.174	0.142	0.074	0.123	0.115	0.242	0.227	0.025	0.390	0.271	0.234	0.196
– **ALP**	0.234	0.103	0.041	0.023	0.029	0.055	0.048	0.188	0.540	0.301	0.172	0.250
– **γGT**	.200	0.139	0.201	0.104	0.298	0.266	0.068	0.239	0.127	0.181	0.118	0.420
– **Total bilirubin**	0.108	0.277	0.192	0.176	0.027	0.315	0.358	0.089	0.127	0.029	0.357	0.141
– **Direct bilirubin**	0.137	0.155	0.226	0.185	0.387	0.304	0.227	0.399	0.289	0.382	0.256	0.239
– **Albumin**	-0.086	-0.169	-0.068	-0.100	-0.365	0.238	-0.054	0.107	-0.126	-0.007	-0.261	-0.060
Coagulation profile												
– **Pc**	-0.245	-0.329	-0.425	-0.355	-0.288	-0.196	-0.130	-0.030	-0.007	-0.058	-0.292	-0.366
– **INR**	0.107	0.234	0.257	0.160	0.109	0.033	0.127	0.262	0.039	0.370	0.098	0.372
CBC parameters												
– **HB%**	-0.279	-0.138	-0.130	-0.161	-0.387	-0.278	-0.048	-0.114	-0.474	-0.088	-0.366	-0.036
– **TLC**	-0.150	0.160	-0.070	0.127	-0.256	0.475	-0.263	0.331	-0.223	0.245	-0.542	0.151
– **PLT**	-0.026	-0.235	-0.249	-0.231	-0.524	-0.055	-0.036	-0.424	-0.217	-0.328	-0.122	-0.444
Renal function tests												
– **BUN**	-0.184	-0.001	-0.033	-0.117	-0.069	-0.199	-0.052	-0.379	-0.051	-0.473	-0.188	-0.023
– **Creatinine**	-0.01	-0.181	-0.145	-0.216	-0.009	-0.166	-0.058	-0.310	-0.274	-0.070	-0.144	-0.219

(R) Correlation (-) Negative correlation

The viral load was significantly increased in HCV patients before receiving treatment in comparison to HCC patients as 100% of HCV patients before receiving treatment had moderate and high viral load vs. 76.6% of HCC patients. Also, it was high significantly increased in HCC and HCV patients before receiving treatment and in comparison to HCV patients after receiving treatment (table 3). There was significant positive correlation between the mean expression values of CD133, CD90 and CK19 and HCV virus load in HCC and HCV patients while there was non-significant positive correlation between AFP mean values and HCV virus load in HCC and HCV patients (table 4).

Table 3: HCV virus load by real time PCR among the studied patients:

Viral load by PCR	Group I HCC (No.=30)		Group II HCV before treatment (No.=15)		Group III HCV after treatment (No.=15)		X2	P value
	No.	%	No.	%	No.	%		
Negative	0	0.00	0	0.00	8	53.3		
Positive	30	100	15	100	7	46.7		
• **Low** ($2\text{-}12X10^4$)	7	23.3	0	0.00	5	33.3	8.21	P1:0.016(**S**)
• **Moderate** ($2X10^5$ - $2X10^6$)	19	63.3	8	53.3	0	0.00	18.5 23.7	**P2:0.001(HS)** **P3:0.001(HS)**
• **High** ($2X10^6$- $5X10^6$)	4	13.3	7	46.7	2	13.3		

P1: Comparison between group I and group II
P2: Comparison between group I and group III
P3: Comparison between group II and group III

Table 4: Correlation between mean values of AFP and CSC markers and viral load among the studied groups:

Viral load in the studied cases	AFP		CD133		CD90		CK19	
	R	P value	R	P value	R	P value	R	P value
Group I	0.020	0.918 (NS)	0.822	0.000 (HS)	0.853	0.000 (HS)	0.812	0.000 (HS)
Group II	0.234	0.400 (NS)	0.933	0.000 (HS)	0.630	0.012 (S)	0.655	0.008 (HS)
Group III	0.264	0.342 (NS)	0.916	0.000 (HS)	0.685	0.005 (HS)	0.568	0.049 (S)

The mean values of CD133, CD90 and CK19 expression had a tendency to increase with disease progression as expression of CD133 showed significant elevation in multiple focal lesions (6.41±5.21), poorly differentiated tumors (7.51±1.22), AJCC grade IV (5.46±0.01), terminal BCLC stage (16.1±4.36) and occurrence of metastasis (6.25±4.46). Regarding to expression of CD90, there was a high significant increase in poorly differentiated tumors (8.67±4.12), AJCC grade IV (9.21±3.33), terminal BCLC stage (14.1±1.74) and occurrence of metastasis (8.55±2.99). For CK19, there was a high significant increase in CK19 mean values in poorly differentiated tumors (13.3±6.22), AJCC grade IV (15.8±3.34), terminal BCLC stage (22.6±2.74) and occurrence of metastasis (13.1±4.63). Conversely, there was non-significant difference between AFP level and the studied tumor characters (the number of focal lesions, tumor size, tumor grade, AJCC grade, BCLC stage or occurrence of metastasis) (table 4).

Table 5: Relation between mean values of AFP and CSC markers and histopathological characters among HCC patients:

	AFP Mean±SD	Test of sig. P value	CD133 Mean±SD	Test of sig. P value	CD90 Mean±SD	Test of sig. P value	CK19 Mean±SD	Test of sig. P value
Number of focal lesions*		U=0.423				U=0.490		U=1.22
● Solitary	367.4±148.2	0.672	3.10±1.76	U=2.48	6.13±3.19	0.62	9.40±5.01	0.221
● Multiple	411.6±97.7	(NS)	6.41±5.21	**0.021 (S)**	7.09±4.46	(NS)	10.8±7.11	(NS)
Tumor size **								
● <5 cm	410.7±103.1	U=1.32	3.71±2.56	U=1.69	6.27±4.22	U=1.39	7.51±4.37	U=1.54
● >5 cm	494.4±14.3	0.185 (NS)	5.21±2.21	0.091 (NS)	8.14±2.80	0.16 (NS)	10.4±5.29	0.122 (NS)
Tumor grade***								
● Well differentiated	352.0±113.2	K=4.57	1.48±0.44	K=18.8	2.14±0.20	K=11.0	3.19±0.93	K=12.3
● Moderately differentiated	415.3±108.3	0.102 (NS)	2.77±0.76	**0.001 (HS)**	5.74±1.84	**0.004 (HS)**	8.73±4.18	**0.002 (HS)**
● Poorly differentiated	463.2±71.9		7.51±1.22		8.67±4.12		13.3±6.22	
● Un differentiated	-		-		-		-	
AJCC grade								
● Ia	362.7±101.5	K=8.50	1.51±0.31	K=18.8	1.93±0.39	K=19.4	2.69±0.69	K=20.6
● Ib	375.3±154.4	0.075	1.28±0.51	**0.001**	1.81±0.68	**0.001**	3.31±1.13	**0.001**
● II	358.0±117.1	(NS)	2.91±0.32	**(HS)**	5.33±2.01	**(HS)**	7.23±2.28	**(HS)**
● III	470.0±56.3		4.06±2.53		7.03±0.59		13.7±5.41	
● IV	510.5±7.77		5.46±0.01		9.21±3.33		15.8±3.34	
BCLC stage								
● Early	352.0±113.2	K=11.9	1.49±0.44	K=20.8	2.14±0.20	K	3.19±0.93	K=15.5
● Intermediate	421.7±101.5	0.095	3.35±1.52	**0.001**	6.29±2.79	14.8	9.55±4.75	**0.001**
● Advanced	459.4±70.1	(NS)	5.91±1.91	**(HS)**	7.36±3.16	**0.002**	11.1±4.13	**(HS)**
● Terminal	510.0±9.00		16.1±4.36		14.1±1.74	**(HS)**	22.6±2.74	
Metastasis		U=1.47		U=2.66		U=2.92		U=2.80
● Present	467.7±57.4	0.141	6.25±4.46	**0.008**	8.55±2.99	**0.003**	13.1±4.63	**0.005**
● Absent	392.8±114.8	(NS)	3.75±4.18	**(HS)**	4.86±4.04	**(HS)**	7.51±6.66	**(HS)**

*According to pathological records. ** According to Rozeik et al.,[15].
*** According to WHO classification of tumors of digestive system.

CD 133 had high sensitivity of 97% and specificity of 80% at a cut-off point of 0.90 in detection of HCC. At a cut-off point of 1.92 of CD 90, HCC could be detected with sensitivity of 93%, specificity of 93%. As CSC, CK 19 at a cut-off point of 1.28, HCC could be detected with sensitivity of 100%, specificity of 80% (table 5).

Table 6: Evaluation of CSC markers for detection of HCC

	AUC	Cutoff point	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
CD133	0.995	0.90	97%	80%	83%	96%	88%
CD90	0.972	1.92	93%	93%	90%	95%	93%
CK19	0.991	1.28	100%	80%	83%	100%	90%

PPV: Positive predictive value NPV: Negative predictive value

DISCUSSION

Hepatitis C virus is a major etiological factor of chronic hepatic diseases and HCC, with significant mortality and morbidity rates. HCC is the fifth most common tumor and second cause of cancer-related death worldwide[12]. The presence of liver cancer stem cells can cause marked recurrence and resistance to treatment thus hindering survival of HCC patients. LCSCs are group of cancer cells which are able to self-renew and also to differentiate[4]. During recent years, new developments lead to identification of specific surface markers for LCSCs that help us to explore potential biological functions, signaling pathways and therapeutic approaches. CD133, CD90 and CK19 are widely recognized as LCSCs surface markers[4].

In this study, AFP level was highly significantly increased in HCC patients in relation to HCV patients before and after receiving treatment and controls. This result was in agreement with Bahnassy et al.,[11] who stated that AFP was significantly higher in patients having HCC than CH and control groups. While some previous studies as Masuda et al.,[13] showed that AFP

had low specificity in detection of HCC so that the American Association for the Study of Liver Diseases-Practice Guidelines Committee recommended use of ultrasound examination alone without using AFP for HCC surveillance. But as the interpretation of ultrasound depends on the operator and can be difficult in patients who have underlying cirrhosis or obese patients. Therefore, other reliable biomarkers as CSC markers are needed for use with ultrasound for early detection and for proper diagnosis of HCC[11].

The present study demonstrated that, CD133, CD90 and CK19 expression was highly significantly increased in HCC patients than in HCV patients before and after receiving treatment and controls. These findings agree with Bahnassy et al.,[11] and Guo et al.,[14] who found that CK19 and CD90 were highly expressed in the HCC cases compared to CH or controls. Also, Rozeik et al.[15] found that the mean expression values of CD133 showed a significant increase with disease progression from 20.5% in non-cirrhotic hepatitis group, to 37.75% in the cirrhotic group, recording the highest value (76.7%) in the HCC group. Also, the mean expression values of CSC markers were increased in HCV patients before receiving treatment than those after receiving treatment and controls. This was in harmony with Tsamandas et al.[16] who proved that progenitor cells of liver were frequently detected in liver tissues of patients infected by hepatitis C and that they were increasing in number with disease progression to cirrhosis, an established risk factor for initiation of HCC. This might be explained by the ability of HCV infection to generate an epithelial-mesenchymal transition state and tumor-initiating cancer stem-like cells in human hepatocytes[17]. HCV infection also extends hepatocyte life span; these hepatocytes undergone sphere formation and survived for about 12 weeks. A number of CSC markers were expressed on cells displaying sphere formation[18].

In this study, there was positive correlation between mean expression values of CD133, CD90, CK19 and deteriorated liver functions. These results were in agreement with Rozeik et al.,[15] and Zahran et al.,[19] who reported that CSC markers expression was correlated with increased inflammatory activity of the liver and directly correlated with ALT, AST.

In this study, it was suggested that higher HCV virus load was correlated with elevated CD133, CD90 and CK19 expressions. This agreed with Ali et al.,[20] who observed that by HCV sub-genomic replicon insertion in cells in culture led to the development of CSC with enhanced expression of CD133, and CK19. Conversely, removal of the replicon from those cells suppressed markers expression.

Regarding to tumor criteria, CSC markers expression values were higher in tumors larger than 5cm in size in this study. These findings came in a line with Rozeik et al.,[15] who stated that CD133 expression was higher in tumors more than 5cm and CD90 expression was highly associated with higher histopathology grade and larger tumor size.

The current study and many studies; Akyol and Yilmaz,[23], Sukowati et al.,[3] and Zhang et al.,[24] stated that elevated CD133, CD90 and CK19 expression were associated with tumor differentiation grades and their expression exhibit strong correlation with metastasis, invasion and poor prognosis in HCC. These results suggest that CSC markers are involved in the onset and/or progression of HCC and they have a role in regulation of the invasion and migration of liver cancer[14,21]. Also, CSC (+) cells show a high proliferation rate and low apoptosis rate as compared to control cells[4]. El-Emshaty et al.,[22] reported that CK19+ cells in HCC displayed powerful correlation with invading, large, poorly differentiating, metastatic tumors, showing micro vascular invasion and it is a major predictive element for prognosis of patients, survival rate and recurrence of tumor.

Our results showed high sensitivity and specificity of CD133, CD90 and CK19. These agreed with Liu et al.,[25] who observed that HCC tissues alone significantly expressed CSC markers with the highest specificity (91.9%) and sensitivity of 46.6% with CD90 followed by CD133 with specificity of 40%. Also, Jun et al.,[26] reported high CD133 specificity and sensitivity (70% both) in detection of HCC. Lou et al.,[27] observed that the sensitivity for diagnosing HCC had increased from 54.2% when using GP3 alone to 90.6% with the combination of CK19 and GPC3. In the study done by Bahnassy et al.[11], detection of HCC by CK19 and CD90 displayed high sensitivity (87.1% and 82.5%) respectively and specificity (81.0% and 89.6%) respectively.

Exploration of possible targeted therapies towards LCSCs may provide a single way to overcome the bottleneck of treatment of HCC as no other treatment can prevent HCC recurrence and metastasis. Current HCC therapeutic strategies targeting LCSCs; inducing apoptosis, inhibiting proliferation of LCSC, inducing their differentiation thus improving response to radiochemotherapy, destruction of the LCSC microenvironment and direct targeting of LCSC surface markers, including CD133 and CD90[5].

CONCLUSION

The expression values of CD133, CD90 and CK19 was increased in HCC and HCV patients and hence they could detect HCC with high sensitivity. Their expression profiles could enhance understanding HCC progression, prognosis, metastasis and also helping in developing novel therapeutic agents targeting HCC. Further analyses of circulating liver CSCs are important to understand mechanisms underlying metastasis, their role in occurrence of recurrence and for the

establishment of new approaches directed against these cells.

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