Egypt. J. Chem. **Vol. 67**, No. 11 pp. 71 - 81 (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Assessing TGF β1 Gene Expression as a Prognostic Marker for Hepatocellular Carcinoma Development in HCV patients receiving Direct-Acting Antiviral (DAAs) Therapy

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Abstract

Hepatocellular carcinoma (HCC) is a complex and multifactorial disease. There is a considerable risk of developing HCC among HCV patients treated with direct acting antiviral therapies (DAAs). This study attempts to evaluate the role of tumor growth factor beta (TGF-β1) gene expression as a prognostic marker for the development of post-DAAs HCC. The study contained 220 participants distributed into four groups: de-novo HCC (Group 1, n=70), HCC after DAAs treatment (Group 2, n=50), HCV patients treated with DAAs without complications (Group 3, n=60), and a control group (Group 4, n=40). TGF-β1 gene-expression by Real Time PCR, routine investigations and clinical assessment were assessed for all participants. HCC de-novo exhibited significantly higher TGF-β1 gene expression than the other groups (P value <0.001). Hong Kong Classification (HKLC) staging showed significant differences between HCC groups (G1 & G2) (p= 0.024). Among the clinical parameters, the number of focal lesions showed a statistically significant association with TGF-β1 expression level in group 2 (p=0.021). Other parameters, such as Child-Pugh stage, lymph node involvement, metastasis, and BCLC, all had p>0.05. ROC analysis for the ability of TGF-β1 to differentiate de-novo from post-DAAs HCC revealed a sensitivity of 87.14% and specificity of 82% at cut-off values determined to be >1.92. Overall survival analysis showed no significant association between TGF expression in either de novo or post-DAAs developed HCC. In conclusion, serum TGF-β1 emerges as a promising marker for the occurrence of post-DAAs Hepatocellular carcinoma, exhibiting high sensitivity and specificity. Regular monitoring of TGF-β1 levels in hepatitis C virus cases following DAAs treatment can serve as a potential marker for HCC development.

Keywords: HCV infection; HCC; DAAs; TGF-β1

1. Introduction

Hepatocellular carcinoma is the main death related etiology all over the world and accounts for 70–85% of all causes of primary hepatic malignancies [1, 2]. Globally, HCC grads fifth in cancer incidence and second in cancer mortality [3]. In Egypt, the principal predisposing factor for HCC is hepatitis C viral infection (HCV), particularly noteworthy with the introduction of effective direct-acting antivirals (DAAs) for HCV clearance [4, 5].

Tumor growth factor beta (TGF-β) signaling is essential to homeostasis of epithelial and immune cells in addition to stromal compartments, in the gastrointestinal system, liver and pancreas [6]. TGF-β pathway plays crucial roles in gastrointestinal, hepatic, and pancreatic diseases. Notably, in chronic

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EJCHEM use only: Received date 05 March 2024; revised date 29 April 2024; accepted date 05 May 2024
DOI: 10.21608/ejchem.2024.273216.9395

inflammatory states and the transition from a noncancerous to cancer states [7,8.9].

TGF-β1 is a pleiotropic cytokine orchestrating different cellular processes. It has a crucial task in growth-regulation, cellular differentiation, and metastasis. Its influence spans critical cellular functions including; cell growth, apoptosis, extracellular matrix production, and immunomodulation [10, 11]. TGF-β1 assumes a pivotal effect in the pathogenesis of multiple hepatic diseases including the incidence of HCC [12, 13].

TGF-β1 sensitize epithelium for proliferation, loss of adherence and cellular invasion. It stimulates angiogenesis and suppress immunity for tumor paving for distant metastasis [14]. It inhibits cell proliferation in early stages of various malignancies, however, in late stages the opposite occurs. Also, high TGF-β1 levels, from the cancer itself or from the tumor microenvironment, was found to stimulate the progression of cancer [15, 16]. Numerous tumors gain a metastatic phenotype during progression, through an Epithelial-mesenchymal transition (EMT)-dependent process. In parallel, TGF-β1 was linked to poor prognosis and highly invasive HCC [17, 18].

Recently, a significant rise in the occurrence of early hepatocellular carcinoma has been detected among patients with HCV infection who have undergone treatment with direct-acting antivirals [19, 20]. However, these findings have not been consistently supported by other studies, leading to considerable debate regarding the risk of HCC development following the treatment with DAAs [21–22]. It is worth noting that many early studies primarily focused on achieving high rates of HCV clearance in chronic hepatitis C (CHC) patients, rather than on detecting or predicting associated HCC [23]. Consequently, it is imperative to identify individuals at risk of developing HCC among HCV patients treated with DAAs, given that reaching a sustained virological response (SVR) does not eliminate this risk [24].

This study aims to evaluate the prognostic significance of TGF-β1 gene expression in HCV patients who received DAAs treatment and either developed post-DAA HCC or gained sustained virologic response, in comparison to HCV-complicated HCC patients (HCC cases with no history of receiving antiviral therapy), and healthy control group.

2. Subjects and Methods:

This study involved 220 participants, categorized into four groups: G1 comprised 70 patients with de-novo (HCC) on top of (HCV) without viral treatment; G2 consisted of 50 patients with HCC on top of HCV treatment (using Direct-Acting Antivirals, DAAs); G3 included 60 patients with HCV who were treated with DAAs and did not develop HCC (DAAs treated HCV). The DAA regimens used in groups 2 and 3 were as follows: a) Easy-to-treat group (new primary patients), received 12 weeks of Sofosbuvir/Daclatasvir, and b) Difficult-to-treat group received 12 weeks of Sofosbuvir/Daclatasvir + Ribavirin (RBV). Additionally, G4 comprised 40 apparently healthy subjects, matched in age and sex with patients, serving as the control group.

Patients were enlisted from the Hepatology unit at the National Liver Institute (NLI)-Menoufia University between September 2018 and October 2019, specifically from the cirrhotic and HCC patient's clinic. The follow-up period was 36 months. The diagnosis of chronic HCV patients was according to the American Association for the Study of Liver Diseases (AASLD) based on clinical and laboratory criteria, including the detection of anti-HCV RNA and imaging studies confirming the presence of chronic liver cirrhosis. The HCC patient groups included in the study were diagnosed based on imaging studies. Diagnosis relied on imaging criteria, which involved identifying single or multiple focal hepatic lesions associated with elevated serum AFP levels (> 400 ng/ml). Additionally, the study utilized the standard Child-Pugh classification and the Barcelona Clinic Liver Cancer (BCLC) staging system to stage HCC.

Detailed consent from patients and ethical approval by the institution's committee were obtained (Institutional Review Board of NLI, Menoufia University) in accordance with the Helsinki Declaration.

Inclusion criteria for all diseased groups involved Hepatitis C viral infection, while exclusion criteria encompassed Hepatitis B viral infection, other hepatic coinfections, autoimmune diseases, other malignancies, and HCC recurrence.

The following were done for all included subjects:

2.1. Clinical and Radiological Evaluation

All patients were subjected to history taking and clinical examination. liver cirrhosis, HCC focal lesions, lymph nodes, ascites and splenomegaly were diagnosed by ultrasonography and computed tomography (CT).

2.2. Laboratory Investigations

Blood samples were a septically taken and analysis of tested biochemical parameters and HCV-RNA (minimum detection <10 IU/m) was performed as previously shown [5]. Follow up by liver function test; CBC and HCV-RNA were done during and after end of treatment.

2.3. Molecular Analysis

2.3.1 RNA extraction and reverse transcription:

Total RNA was isolated from 200 microliters (µL) of fresh whole venous blood using RNeasy Kit (Qiagen, Germany). RNA concentration was assessed by NanoDrop 2000 spectrophotometer (Thermo Scientific, Delaware, USA). RNA reverse transcription was then fulfilled by applied biosystems TaqMan™ MicroRNA reverse transcription kit (California, USA). Briefly, reaction volume was 20µL: 10µL of universal master mix II Kit, 1µL TaqMan assay, 5µL RNase free water and 4µL of RNA template. The sample was then incubated in A 2720 thermal cycler, which was adjusted as follows for one cycle: 10 min at 42ºC, followed by 5 min at 95ºC and finally 5 min at 4ºC (Applied Bio systems-Singapore). The produced cDNA was saved at −20ºC.

2.3.2. Gene expression analysis:

TGF-β1 gene expression was done by Real Time Polymerase Chain Reaction (RT-PCR) (Applied Biosystems ABI 7500). Amplification conditions were adjusted along these lines: denaturation at 95ºC (10 min), 40 cycles at 95ºC (15 sec) and finally at 60ºC (60 sec). The following Primer sequences were used: TGF-β1- F, 5'CAAGCAGAGTACACACAGCAT-3' and TGF-β1- R, 5'-TGCTCCACTTTTAACTTGAGCC-3'; GAPDH- F, 5′-GTCAGCCGCATCTTCTTT-3′; and GAPDH- R, 5′-CGCCCAATACGACCAAAT-3′. TGF-β1 expression levels with referral to GAPDH, housekeeping control gene, were estimated using the 2−ΔΔCT method. Relative expression = 2^- ΔΔCT, where CT is the threshold cycle for each sample. Δ Δ CT = Δ CT (tested sample) − Δ CT (normal sample). Δ CT (tested sample) = CT of target gene – CT of housekeeping gene of the same sample.

2.4. Statistical Analysis:

Data analysis involved SPSS 22.0 (IBM/SPSS Inc., Chicago, IL), multivariable logistic regression, Kaplan-Meier survival and Roc curve creation. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for independent risk factors were estimated. Variables with a P-value of 0.1 were included, while highly correlated ones were omitted to prevent multi-collinearity. Significance was set at $P < 0.05$.

3. Results

3.1. Demographic, laboratory and Clinical parameters

The demographic analysis revealed no significant differences in terms of sex and age among the studied groups (Table 1). A comprehensive assessment of laboratory results across the four groups revealed parameters associated with HCC de-novo (G1), including AST, ALT, and AFP, exhibited marked elevation ($p<0.001$) (Supplementary table 1).

Table 1. The baseline demographic characteristics of the enrolled population

	De-novo HCC (n = 70)		Post-DAAs HCC (n = 50)		DAAs treated HCV (n = 60)		Control (n = 40)		Test of sig.	p
	No.	%	No.	%	No.	%	No.	%		
Sex										
Male	60	85.7	40	80.0	45	75.0	26	65.0	χ^2= 6.601	0.081
Female	10	14.3	10	20.0	15	25.0	14	35.0		
Age (years)										
Min. – Max.	40.0 – 81.0		48.0 – 70.0		40.0 – 83.0		30.0 – 70.0			
Mean ± SD.	65.19 ± 7.70		61.04 ± 6.13		63.05 ± 11.60		62.45 ± 9.32		F= 2.233	0.086
Median (IQR)	65.0 (60.0 – 70.0)		63.0 (57.0 – 65.0)		64.0 (54.0 – 70.0)		64.50 (57.50 – 70.0)			

SD: Standard deviation IQR: Inter quartile range χ^2: Chi square test F: F for ANOVA test P: P value

Clinical data analysis revealed highly insignificant differences among the groups regarding, liver cirrhosis, lymph node involvement, portal vein (PV) invasion, splenomegaly, hypertension (HTN), relapse, and ascites ($p<0.05$). While, insignificant changes were found concerning other clinical parameters (Table 2). Evaluation by Hong Kong Classification (HKLC) staging system disclosed notable discrepancies between the HCC groups (G1 and G2) ($p= 0.024$). However, no significant variations were detected in relation to other staging parameters (Table 3).

Table 2. Comparison between different patient groups regarding clinical parameters

	De-novo HCC (n = 70)		Post-DAAs HCC (n = 50)		DAAs treated HCV (n = 60)		χ^2	P value
	No.	%	No.	%	No.	%		
Focal lesion								
Single	20	28.6	16	32.0	–	–	χ^2= 0.163	0.686
Multiple	50	71.4	34	68.0	–	–		
Size of hepatoma (mm)								
Min – Max	15.0 – 90.0		15.0 – 98.0		–			
Mean ± SD	43.56 ± 18.31		43.88 ± 20.41		–		U= 1665.0	0.650
Median (IQR)	44.0 (30.0 – 53.0)		40.0 (30.0 – 50.0)		–			
Cirrhosis	70	100.0	50	100.0	22	36.7	96.388*	<0.001*
Lymph node	8	11.4	16	32.0	0	0.0	24.527*	<0.001*
PV invasion	12	17.1	16	32.0	0	0.0	21.480*	<0.001*
Splenomegaly	54	77.1	42	84.0	0	0.0	124.037*	<0.001*
Metastasis	0	0.0	0	0.0	0	0.0	–	–
Ascites	6	8.6	8	16.0	0	0.0	10.929*	MCp= 0.033*
DM	32	45.7	16	32.0	–	–	2.286	0.131
HTN	32	45.7	14	28.0	–	–	3.872*	0.049*
Cynical history of decompensation	62	88.6	48	96.0	–	–	2.107	FEp=0.191
Relapse	–	–	18	36.0	0	0.0	25.826*	<0.001*

χ^2: Chi square test FE: Fisher Exact
p: p value between the studied groups *: Statistically Significant

Table 3. Comparison between De-novo HCC and Post-DAAs HCC groups according to clinical staging

	De-novo HCC (n = 70)		Post-DAAs HCC (n = 50)		Test of sig.	P value
	No.	%	No.	%		
Child score						
A	62	88.6	38	76.0		
B	4	5.7	8	16.0	χ2= 3.846	MCp= 0.145
C	4	5.7	4	8.0		
Min – Max	5.0 – 11.0		5.0 – 10.0			
Mean ± SD	5.77 ± 1.43		6.12 ± 1.75		U= 1670.0	0.628
Median (IQR)	5.0 (5.0 – 6.0)		5.0 (5.0 – 6.0)			
BCLC staging	14	20.0	10	20.0		
A					4.571	0.102
B	40	57.1	20	40.0		
C+D	16	22.9	20	400		
HKLC staging						
I	12	17.1	6	12.0		
II A	0	0.0	2	4.0		
II B	0	0.0	2	4.0		
III A	28	40.0	14	28.0	χ2= 14.594*	MCp= 0.024*
III B	16	22.9	6	12.0		
IV A	8	11.4	12	24.0		
IV B	2	2.9	6	12.0		
V B	4	5.7	2	4.0		

χ^2: Chi square test MC: Monte Carlo U: Mann Whitney test
p: p value between the studied groups *: Statistically Significant

3.2. TGF-β1 gene expression

Table 4 underscores a substantial increase in TGF-β1 gene expression in de-novo HCC, demonstrating a highly significant difference compared to other groups (p-value <0.001). Similarly, post-DAAs HCC (G2) and DAAs treated HCV group (G3) displayed a noteworthy elevation in TGF-β1 gene expression, significantly differing from the control group (p-value <0.001). Although (G2) exhibited heightened TGF-β1 gene expression compared to (G3), no significant change was detected between the two groups (G2 and G3).

Table 4: Comparison between the different groups regarding TGF-β1

TGF-β1 gene expression	De-novo HCC (n = 70)	Post-DAAs HCC (n = 50)	DAAs treated HCV (n = 60)	Control (n = 40)	H	P value
Min. – Max.	1.50 – 30.0	1.0 – 4.0	1.0 – 3.0	0.83 – 1.30		
Mean ± SD.	12.91 ± 11.52	1.82 ± 0.48	1.66 ± 0.64	1.03 ± 0.13	146.455*	<0.001*
Median (IQR)	3.40 (1.95 – 24.0)	1.79 (1.66 – 1.83)	1.39 (1.23 – 1.90)	1.0 (0.90 – 1.17)		
P_1	<0.001*	<0.001*	<0.001*			
Sig. bet. groups	p_2<0.001*, p_3<0.001*, p_4=0.386					

SD: Standard deviation R: Inter quartile range H: H for Kruskal Wallis test
p: p value between the studied groups
p_1: between control and each other groups
p_2: between de-novo HCC and post-DAAs HCC (G1 & G2)
p_3: between de-novo HCC and DAAs treated HCV (G1 & G3)
p_4: between post-DAAs HCC and DAAs treated HCV (G2 & G3) *: Statistically significant

Table 5 showed a significant difference between post-DAAs HCC and DAAs treated HCV groups (G2 and G3) with respect to the type of drug used. The regimen predominantly employed in the post-DAAs HCC (G2) was SOF/DAC/RBV. However, this regimen course does not associate with specific TGF-β1 gene expression pattern in HCC.

Table 5. Comparison between (G2) & (G3) groups regarding the drug type

Type of drug	Post-DAAs HCC (n = 50)		DAAs treated HCV (n = 60)		χ^2	P value
	No.	%	No.	%		
SOF/ DAC	8	16.0	48	80.0	44.698*	<0.001*
SOF/ DAC/ RBV	42	84.0	12	20.0		

χ^2: Chi square test
p: p value between the studied groups *: Statistically significant

3.3. Association of TGF-β1 gene expression and different HCC parameters

No significant differences in TGF-β1 gene expression and all parameters within HCC de-novo (G1) (Table 6). However, highly significant differences were noted in TGF-β1 and focal lesions number within HCC on top of DAAs therapy (G2) (Table 7). Additionally, significant differences were identified in TGF-β1 and ALT between de-novo HCC and post-DAAs HCC (G1 and G2), with both acting as independent risk factors for HCC. Notably, the chances of HCC incidence increased by 3.7 times in instances of high TGF-β1 expression values (Table 8).

Table 6: Relation between TGF-β1 expression and different parameters in De-novo HCC group (n = 70)

	N	TGF gene expression			Test of Sig.	P value
		Min. – Max.	Mean ± SD.	Median		
Focal lesion						
Single	20	1.80 – 30.0	12.48 ± 11.98	3.40	U= 462.50	0.623
Multiple	50	1.50 – 30.0	13.09 ± 11.45	11.45		
Cirrhosis	70	1.50 – 30.0	12.91 ± 11.52	3.40	–	–
Lymph node	8	1.80 – 30.0	11.67 ± 12.78	3.40	U=233.5	0.787
PV invasion	12	1.80 – 30.0	13.80 ± 12.26	11.95	U=294.0	0.397
Splenomegaly	54	1.50 – 30.0	13.03 ± 11.34	11.95	U=412.5	0.784
Metastasis	0	–	–	–	–	–
Ascites	6	1.80 – 29.0	18.13 ± 13.0	24.0	U=153.5	0.429
DM	32	1.80 – 30.0	13.01 ± 11.47	11.45	U=607.0	0.991
HTN	32	1.80 – 30.0	16.86 ± 11.30	20.0		
Cynical history of decompensation	62	1.50 – 30.0	12.58 ± 11.59	2.90	U= 191.50	0.294
Child score						
A	62	1.50 – 30.0	12.18 ± 11.46	2.90		
B	4	1.80 – 20.0	10.94 ± 10.46	10.98	H=5.662	0.059
C	4	20.0 – 29.0	26.25 ± 4.19	28.0		
BCLC staging						
A	14	1.95 – 30.0	13.50 ± 12.27	11.45		
B	40	1.50 – 28.0	11.11 ± 10.90	2.0	H=5.781	0.056
C+D	16	1.80 – 30.0	16.91 ± 12.03	20.0		
HKLC staging						
I	12	1.95 – 30.0	13.06 ± 11.89	11.45		
III	44	1.50 – 30.0	11.97 ± 11.28	2.55	H= 5.800	0.122
IV	10	1.80 – 30.0	11.56 ± 12.03	3.40		
V	4	20.0 – 29.0	26.25 ± 4.19	28.0		
Alive or dead						
Alive	20	1.90 – 30.0	15.83 ± 12.08	20.0	U=392.0	0.157
Died	50	1.50 – 30.0	11.75 ± 11.20	2.90		

H: H for Kruskal Wallis test U: Mann Whitney test p: p value between different parameters

Table 7: Relation between TGF-β1 expression and different parameters in post-DAAs HCC group (n = 50)

	N	TGF-β1 gene expression			Test of Sig.	P value
		Min. – Max.	Mean ± SD.	Median		
Focal lesion						
Single	16	1.0 – 2.0	1.38 ± 0.31	1.26	U=161.50*	0.021*
Multiple	34	1.0 – 4.0	1.80 ± 0.71	1.53		
Cirrhosis	50	1.0 – 4.0	1.66 ± 0.64	1.39	–	
Lymph node	16	1.20 – 2.80	1.45 ± 0.41	1.30	U=187.0	0.077
PV invasion	16	1.20 – 2.80	1.45 ± 0.41	1.30	U=187.0	0.077
Splenomegaly	42	1.0 – 4.0	1.69 ± 0.69	1.39	U=151.50	0.668
Metastasis	0	–	–	–	–	
Ascites	8	1.23 – 1.80	1.39 ± 0.20	1.30	U=132.0	0.354
DM	16	1.0 – 4.0	1.76 ± 0.77	1.40	U=242.50	0.539
HTN	14	1.27 – 4.0	2.03 ± 0.89	1.83		
Cynical history of decompensation	48	1.0 – 4.0	1.68 ± 0.65	1.40	U=32.0	0.472
Child score						
A	38	1.0 – 4.0	1.71 ± 0.72	1.38		
B	8	1.23 – 2.0	1.52 ± 0.33	1.35	H=0.097	0.953
C	4	1.30 – 1.80	1.52 ± 0.21	1.50		
BCLC staging						
A	10	1.0 – 2.0	1.45 ± 0.37	1.34		
B	20	1.0 – 4.0	1.93 ± 0.85	1.86	H=3.140	0.208
C+D	20	1.20 – 2.80	1.50 ± 0.39	1.34		
HKLC staging						
I	6	1.0 – 2.0	1.41 ± 0.43	1.22		
II	4	1.34 – 2.0	1.52 ± 0.32	1.37		
III	20	1.0 – 4.0	1.93 ± 0.85	1.86	H= 5.009	0.286
IV	18	1.20 – 2.80	1.50 ± 0.41	1.30		
V	2	1.49 – 1.50	1.50 ± 0.01	1.50		
Alive or dead						
Alive	8	1.21 – 2.40	1.66 ± 0.44	1.68	U=156.0	0.765
Died	42	1.0 – 4.0	1.66 ± 0.68	1.38		
Type of drug						
SOF/ DAC	8	1.38 – 2.0	1.62 ± 0.26	1.50	U=117.50	0.185
SOF/ DAC/ RBV	42	1.0 – 4.0	1.67 ± 0.69	1.32		

H: H for Kruskal Wallis test U: Mann Whitney test p: p value between different parameters

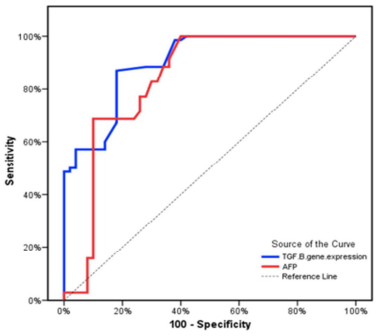
Table 8: Univariate and multivariate Logistic regression analysis between de-novo HCC patients (n = 70) and post-DAAs HCC (n= 50)

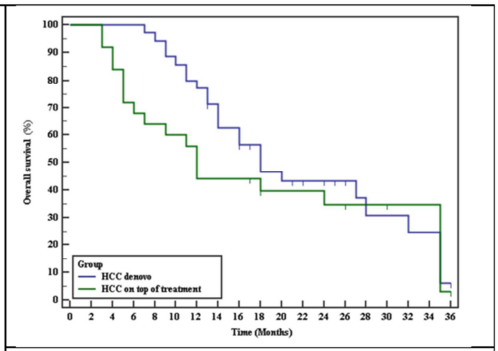
	Uni-variate		#Multi-variate	
	P. value	OR (95% C I)	P. value	OR (95% C I)
TGF-β1 gene expression	0.003*	4.215 (1.649 – 10.771)	0.004*	3.791 (1.526 – 9.4150)
AFP	0.524	1.0 (1.0 – 1.0)		
ALT	<0.001*	1.060 (1.034 – 1.087)	<0.001*	1.055 (1.025 – 1.086)
ALB	0.417	1.293 (0.695 – 2.407)		
Cirrhosis	0.999	–		
Splenomegaly	0.357	0.643 (0.251 – 1.6450		
Relapse	–	–		

OR: Odd`s Ratio C I: Confidence Interval *: Statistically significant
#: P <0.05 variables were only enlisted in the multivariate

3.4. Prognostic Value of TGF-β1 and AFP in HCC

To distinguish de-novo HCC patients (G1) from post-DAAs HCC (G2), a Receiver Operating Characteristic (ROC) curve analysis was conducted. It revealed a sensitivity of 87.14% & 68.57%, specificity of 82% & 90%, and positive predictive value of 87.14% & 90.6%, with a negative predictive value of 82% & 67.2%. The optimal cut-off values were determined to be >1.92 and 20 ng/dl for TGF-β1 and AFP, respectively (Figure 1A). Furthermore, comparison of overall survival between the two HCC subgroups (de-novo HCC and post-DAAs HCC), showed unimportant differences in overall survival between these two groups (Figure 1B).





Overall survival	Mean	%	Log rank	
De-novo HCC	21.691	6.2	χ^2	p
Post-DAAs HCC	18.059	3.2	1.720	0.190

Figure 1A. ROC curve analysis of TGF-β1 and AFP

Figure 1B. Kaplan-Meier survival curve for overall survival

	A.U.C	P	95% CI	Cut off	Sensitivity	Specificity	PPV	NPV
TGF-β1 gene expression	0.900	<0.001*	0.846 – 0.954	>1.92	87.14	82	87.14	82
AFP	0.837	<0.001*	0.753 – 0.920	>20	68.57	90.0	90.6	67.2

Figure 1. ROC and Kaplan Meier survival curves between HCC subgroups; de-novo HCC and post-DAAs HCC

4. Discussion

Hepatocellular carcinoma is one of the major causes of cancer-related deaths worldwide. Tumor Growth Factor-beta1 (TGF-β1) was shown to impede growth of normal cells and stimulates proliferation of malignant cells via increasing life span, invasion and distant metastasis [25]. The intricate role of TGF-β1 in influencing normal and malignant cell behavior prompted our exploration into its potential impact on HCC incidence and aggressiveness. Additionally, the prevalence of HCV complicated by HCC in Egypt prompted our investigation into the post-DAA era's implications on HCC development.

In the current study, the majority of HCC and HCV patients were males, 80.56% were males and 19.44% were females; age range from forty to eighty years. The age distribution and gender proportions were consistent with previous studies conducted in similar populations [26, 27].

Laboratory parameters exhibited significant alterations in HCC patients, with increased liver enzymes, bilirubin, and serum creatinine, and decreased serum albumin. These changes align with established patterns in liver disease progression and underscore the clinical impact of HCC on hepatic function [28]. In agreement with our finding, Bishoy et al., 2018 [29] detected that prothrombin was significantly decreased in chronic liver disease and HCC groups and this was explained by deterioration of synthetic functions of liver. Additionally, the observed increase in Alpha-Fetoprotein (AFP) levels further supports its role as a diagnostic marker for HCC, consistent with prior research [30, 31].

Clinical correlates, including Cirrhosis, Lymph node involvement, Portal Vein invasion, Splenomegaly, Ascites, and Hypertension (HTN), displayed significant differences between HCC groups (G1 and G2) and DAAs treated HCV patients without complications (G3). In the same line, Rinaldi et al., 2019 [32] showed significant differences in age, sex, DM, hypertension, and cirrhosis between HCC and HCV. These clinical manifestations highlight the multifaceted nature of HCC and its impact on various organ systems.

The association between TGF-β1 expression and advanced stages of Hong Kong Classification (HKLC staging) in HCC groups further underscores the potential prognostic value of TGF-β1 in predicting disease progression. On the other hand, the lack of significant differences in other staging parameters suggests the specificity of TGF-β1 in capturing specific aspects of HCC advancement. Previous studies detected a similar prognostic correlation between TGF-β1 expression and HKLC staging in addition to poor prognosis of HCC [33, 34].

Our data suggested an association between HCC occurrence and Sofosbuvir-related treatment with Ribavirin. In accordance of this finding, Ghanem et al., 2021 [5] and 2023 [35] assured increased incidence of HCC with Sofosbuvir-based therapy with Ribavirin. However, in contrast, Rinaldi et al., 2019 [32] reported that Ribavirin addition to treatment not to be blamed in HCC incidence. This emphasizes the need for further studies with extended follow-up periods to elucidate the nuanced effects of different treatment regimens.

Our findings revealed a significant up-regulation of TGF-β1 gene expression in de-novo HCC patients compared to other studied groups whether post-DAAs treated or control group. The association between high TGF-β1 expression and HCC was in line with previous research, emphasizing the potential prognostic relevance of this molecular marker [36]. In parallel, high TGF-β1 expression in HCC post-DAAs treated patients was associated with multiple focal lesions. This is in accordance with Kohla et al., 2017 [37] who found that high expression levels of TGF-β1 in HCC patients was related to more advanced stages and aggressive invasion.

Although TGF-β1 in the post-DAAs HCC group was significantly less than that detected in de-novo HCC group, it did not differ significantly from that in the HCV group who went into DAAs induced SVR without developing HCC. The relatively low levels of TGF-β1 in post-DAAs-HCC patients may be attributed to the reducing effect of DAAs therapy on TGF-β levels, which in turn reduces the pro-fibrotic process in these patients [38]. This reduction may potentially hamper the transition from a noncancerous condition to a cancerous state and impact the expression of certain cytokines such as TGF-β1 in host cells [6]. Therefore, caution is advised when interpreting TGF-β1 levels in post-DAAs HCC cases, and regular monitoring during and after DAAs therapy is recommended.

Comparisons between HCC groups (de-novo and on top of treatment) regarding overall survival revealed no significant differences, consistent with existing literature. Ghanem et al 2023 [35] and Kamp et al., 2019 [39] assured no prominent difference or the same overall survival between different HCC groups. The absence of pronounced variations in survival rates between these groups that aligns with previous findings, emphasizing the complex interplay of factors influencing HCC outcomes.

We suggest that regular monitoring of TGF-β1 levels following DAAs treatment could help identify individuals at risk of HCC development. This proposal has significant translational implications, as it could potentially impact clinical decision-making and patient outcomes. However, it is essential to further elaborate on the practical implications of these findings in the context of clinical practice. For example, how would the incorporation of TGF-β1 monitoring into routine clinical care affect patient management strategies? Would it lead to earlier detection of HCC, thereby enabling timely intervention and improved prognosis? Additionally, are there any challenges or limitations associated with implementing TGF-β1 monitoring in real-world clinical settings, such as cost-effectiveness, accessibility of testing, or interpretation of results?

Finally, we acknowledge some limitations of this study as that HCC patient groups were diagnosed based on imaging studies without definitive biopsy confirmation according to AASLD 2018 [40]. While imaging studies provided valuable diagnostic information, histological confirmation through biopsy was not available for the included patients. The invasive nature of biopsy and the potential for serious complications, especially in compromised patients, precluded its routine use in this study. Consequently, the histological grade of HCC, which typically requires biopsy for accurate assessment, may not be available in cases where diagnosis is based mainly on imaging studies.

Other limitations include the presence of potential for confounding variables that influence TGF-β1 expression. Despite efforts to control various factors such as age, gender, comorbidities, and lifestyle factors, there may still be unaccounted variables that could impact the results. Furthermore, the relatively small sample size and inclusion of patients from a single institution may limit the generalizability of the findings. Addressing these limitations by conducting larger, prospective studies with diverse patient populations and comprehensive control for confounding variables would strengthen the validity and reliability of the results.

5. Conclusion

The current study provides valuable insights into the molecular, clinical, and prognostic aspects of HCC in the context of HCV and its treatment. TGF-β1 emerges as a potential prognostic marker in distinguishing between Hepatocellular Carcinoma and patients with chronic hepatic conditions. Further research and longer-term studies are warranted to enhance our comprehension of HCC pathogenesis and treatment outcomes in the evolving landscape of antiviral therapies.

6. Abbreviations:

AASLD: American Association for the Study of Liver Diseases, AFP: Alpha fetoprotein, ALB: Albumin, ALT: Alanine transaminase, AST: Aspartate transaminase, AUC: Area under the curve, BCLC: Barcelona Clinic Liver Cancer, CHC: chronic hepatitis C, DAAs: Direct acting antiviral therapies, EMT: epithelial-mesenchymal transition, HCC: Hepatocellular carcinoma, HCV: Hepatitis C virus, HKLC: Hong Kong Classification, HTN: hypertension, INR: international normalized ratio, NLI: National liver institute, NPV: Negative predictive value, PPV: Positive predictive value, PV: Portal Vein, ROC: Receiver Operating Characteristic, RBV: Ribavirin, RT-PCR: Real Time Polymerase Chain Reaction, SD: Standard deviation, TGF-β: tumor growth factor beta, χ^2: Chi square test.

7. Declaration of conflicting interests: The authors declare no conflict of interest.

8. Funding: This work did not receive any grants.

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