Heliyon 5 (2019) e02798

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.heliyon.com

Research article

A pilot study of *Livin* gene and *Yes-associated protein 1* expression in hepatocellular carcinoma patients

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ARTICLE INFO

Keywords:
Cancer research
Oncology
Livin
YAP1
HCC and HCV

ABSTRACT

Background: Livin gene and *Yes-Associated Protein 1* (YAP*1* (play a pivotal role in organ size control and tumorigenesis.
Aim: In the present pilot study, we investigate the expression of Livin gene and YAP1 in hepatitis C virus (HCV) associated hepatocellular carcinoma (HCC) compared to other HCV patients and controls.
Methods: the studied patients were divided into three groups 30 patients in each group in addition to 30 healthy subjects as a control group. Relative quantification of *Livin* gene and YAP-1 were assessed by quantitative Real Time RT-PCR (qPCR) in all studied patients and healthy controls. other laboratory investigations were done including complete blood count (CBC),international normalized ratio (INR) as well as liver function tests and tumor markers.
Results: Significant overexpression of *Livin* gene and YAP-1 was detected in HCC group followed by Hepatitis C Virus (HCV) untreated group then HCV treated group. The relative quantitation (RQ) of both genes showed positive correlation to the carcinoembryonic antigen (CEA) level and a significant relation was found between higher level of *Livin* and *YAP1* genes and tumor size. The overall survival rate was low in those patients with high levels of *Livin* and *YAP 1* genes so they were considered as indicators of a bad prognosis.
Conclusion: There is overexpression of *Livin* gene and *YAP1* in hepatocellular carcinoma patients. They can be used as indicators of bad prognosis of the disease pathway together with low survival rate.

1. Introduction

Hepatocellular carcinoma (HCC) is the sixth most common malignancy and is the leading cause of mortality in patients (Alazawi et al., 2010). An estimated half million new cases are diagnosed each year world-wide with disease burden highest in developing countries (85% of all cases) (American Cancer Society, 2014).

It was reported that 40%–80% of HCC cases have a positive HCV infection (Mittal and El-Serag., 2013) and its highest incidence in the world was found in Egypt (Khattab et al., 2010).

A livin protein is a member of a large family of a related protein associated with tumor occurrence and development, called The inhibitors of apoptosis proteins (IAPs) which consists of 8 members, termed baculoviral IAP repeat containing (BIRC) 1–8. A BIRC7, also known as *Livin* (Vucic et al., 2000).

The *Livin* gene spans 4.6 kb on chromosome 20 at band q13. It is composed of six introns and seven exons. *Livin* protects cells from various pro-apoptotic stimuli by inhibiting the activity of caspase -3, -7 and -9, and it plays an important role in tumorigenesis and chemo resistance (Kasof et al., 2001).

Livin is rarely detected in normal adult tissues but highly expressed in cancerous tissues. It is thought that *Livin* protein expression may be an early event in the occurrence of HCC (Lazar et al., 2012).

Yes-associated protein 1 (YAP1) is a major downstream target of the Hippo-signaling pathway (Lian et al., 2010). Regulation of the Hippo-signaling pathway is known to be mediated by phosphorylation and subcellular localization of *YAP1*. Activation of the Hippo-signaling pathway induces phosphorylation of *YAP1*, which prevents the

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https://doi.org/10.1016/j.heliyon.2019.e02798
Received 10 April 2019; Received in revised form 16 August 2019; Accepted 31 October 2019
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translocation to the nucleus. When the Hippo-signaling pathway is inactivated, dephosphorylated *YAP1* is translocated to the nucleus where it interacts with transcription factors, eventually leading to the proliferation of cells to various organ systems (Liu et al., 2010).

The aims of this study were to evaluate the expression levels of *Livin* gene and *YAP1* in HCV associated HCC patients and their association to other laboratory parameters as well as the correlation of their expression levels with the overall survival rate in the HCC patients.

2. Subjects and methods

This pilot study is a case-control study. It was done by cooperation of Biochemistry department, Faculty of Science, Menoufia University, Medical Biochemistry & Molecular Biology and Microbiology departments, Faculty of Medicine, Menoufia University between December 2017 and June 2018 and included 90 patients and 30 healthy controls.

After taking informed written consent from all subjects and approval of the Ethical Committee of Medical Research- Menoufia Faculty of Medicine, all patients were subjected to the following: Full history taking, General and clinical examination, Ultrasound (US) and C.T, laboratory investigations included: Complete liver function tests, Hepatitis markers for Hepatitis A Virus (HAV): IgM for recent infection. IgG for old infection, for Hepatitis B Virus (HBV): HBsAg, HBsAb, HB core Ab,HBeAg, HBe Ab. for Hepatitis C Virus: HCV-Ab, PCR. kidney function tests, tumor markers including Alpha fetoprotein (AFP) and carcinoembryonic antigen (CEA), Detection of gene expression by real time PCR. Patients with other types of hepatitis were excluded from the study after making Hepatitis markers mentioned before from medical reports. Subjects were classified into four groups: Group I: patients with Hepatocellular carcinoma on the top of chronic Hepatitis C untreated (30 patients). Group II: patients with chronic Hepatitis C untreated after investigation include + HCV Ab,CT, tumor markers (30 patients). Group III: patients with HCV who received treatment (30 patients) in the form of Sofosbuvir 400 mg and Daclatasvir 60 mg daily for 12 weeks. During treatment, they were closely monitored at week 2, week 4, week 8, and week 12 by laboratory studies including CBC, creatinine, AST, ALT, and total bilirubin.Group IV: apparently healthy control subjects (a blood bank donors) (30 subjects).

Blood samples: after overnight fasting ten ml of venous blood was obtained from each participantand divided into three parts. First part two ml was put in citrated tube for use in detection of prothrombin time and international normalized ratio (INR). The second part two ml was put in EDTA tubes for complete blood count and total RNA extraction to be used in determination of *Livin* gene and *YAP-1* gene expression. The remaining part was put in plain tube and left to stand for 10 min then centrifuged for 10 min at 4000 RPM. The supernatant serum was put into several aliquots and stored at -80 to until used for determination of liver function tests and tumor markers including AFP and CEA measurement by enzyme linked immunosorbent assay (ELISA - DRG International Inc., USA.) and detection of HCV-RNA presence by real-time polymerase chain reaction using COBAS TaqMan HCV quantitative test, version 2.0 (Roche Molecular Systems, Inc., Branchburg, NJ, USA) (Ghany et al., 2009).

2.1. RNA extraction and quantitative real time PCR assay of Livin and YAP1 genes

Total RNA was isolated from whole blood using (The Invitrogen PureLink RNA Mini Kit), according to the manufacturer's' protocol. RNA quantification was conducted by Gene Quant II (Pharmacia Biotech) at 260 nm. Total RNA was stored at -80 °C until molecular investigation was performed. 1 μg of total RNA from each sample was used for cDNA generation in a final reaction volume of 20 μl with High Capacity cDNA Archive Kit (Applied Biosystems).

The cDNA amplification by real-time PCR: The cDNA was used in SYBR green based quantitative real-time PCR for quantification of *YAP1* and *Livin* gene expression by (SensiFAST TM SYBR Lo-ROX Kit, Bioline), using the designed primers (Midland,TX). As shown in TableA PCR was conducted under the following conditions: 95 °C for 10 min, then 40 cycles; denaturation at 94 C for 15 s, annealing at 60 C for 30 s and extension at 72 C for 30 s. Data analysis with Applied Biosystems 7500 software version 2.0.1 was carried out. The relative quantification (RQ) of gene expression completed using comparative ΔΔCt method where the amount of the target *Livin* gene and YAP 1 gene, are normalized to an endogenous reference gene (*GAPDH)* and relative to a control. Each run was completed using melting curve analysis to confirm specificity of the amplification and absence of primer dimers (Liu et al., 2013).Fig. 1a shows the amplification plot and melting curve of *Livin* gene expression. While Fig. 1b shows the amplification plot and melting curve of *YAP1* gene expression.

Table A
Primers used for detection of *YAP1* gene and *Livin* gene.

gene	Primer	Accession number
YAP1 gene	Forward TAGCCCTGCGTAGCCAGTTA Reverse TCATGCTTAGTCCACTGTCTGT	NM_001130145.3
Livin gene	Forward TGAGGAGTTGCGTCTGG Reverse GCACGGCACAAAGACGAT	NM_139317.3
GAPDH	Forward TGCACCACCAACTGCTTAGC Reverse GGCATGGACTGTGGTCATGAG	NM_002046.7

2.2. Statistical methods

Data collected was analyzed using SPSS version 23 computer statistical software package. The results were expressed as mean ± SD. The **ANOVA** F test was used to determine significant difference between test and control subjects.**Kruskal Wallis test,** Pairwise comparison between each 2 groups was done using Post Hoc Test (Dunn's test) for multiple comparisons test). Spearman coefficient was done for correlation between different studied parameters in each subject group. Cox regression of overall survival in HCC group was done for determination of hazard ratio. Statistical significance level was considered when $p < 0.05$.

3. Results

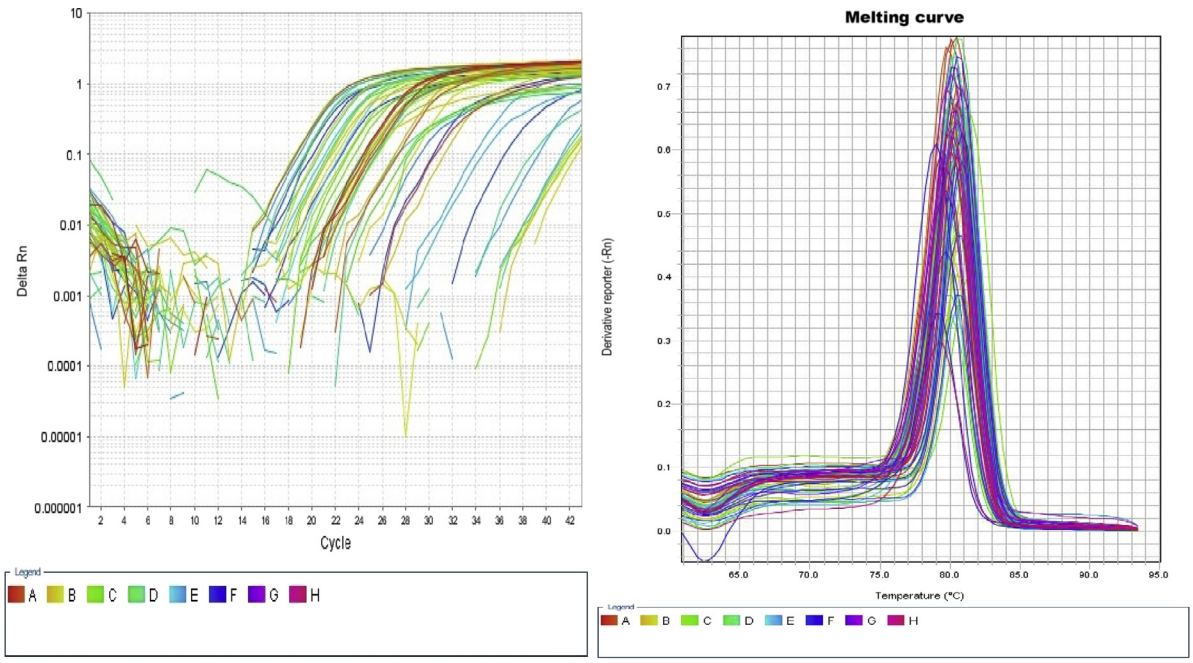
120 subjects were included in these study 90 patients and 30 healthy controls with 30 subjects in each of the studied group. No statistical significant difference was detected between different studied groups regarding demographic data or risk factors (Table 1).

A high statistical significant difference was detected between the three studied groups and between all studied groups and control group regarding relative quantitation (RQ) of *Livin* and *YAP1* genes expression levels with highest level was in HCC group followed by HCV untreated group then HCV treated group and control with median (8.76, 4.33, 0.78 and 0.78) of *Livin* gene and median of (9.42, 4.62, 4.70 and 0.55) in YAP 1 gene (Table 2 & Fig. 2).

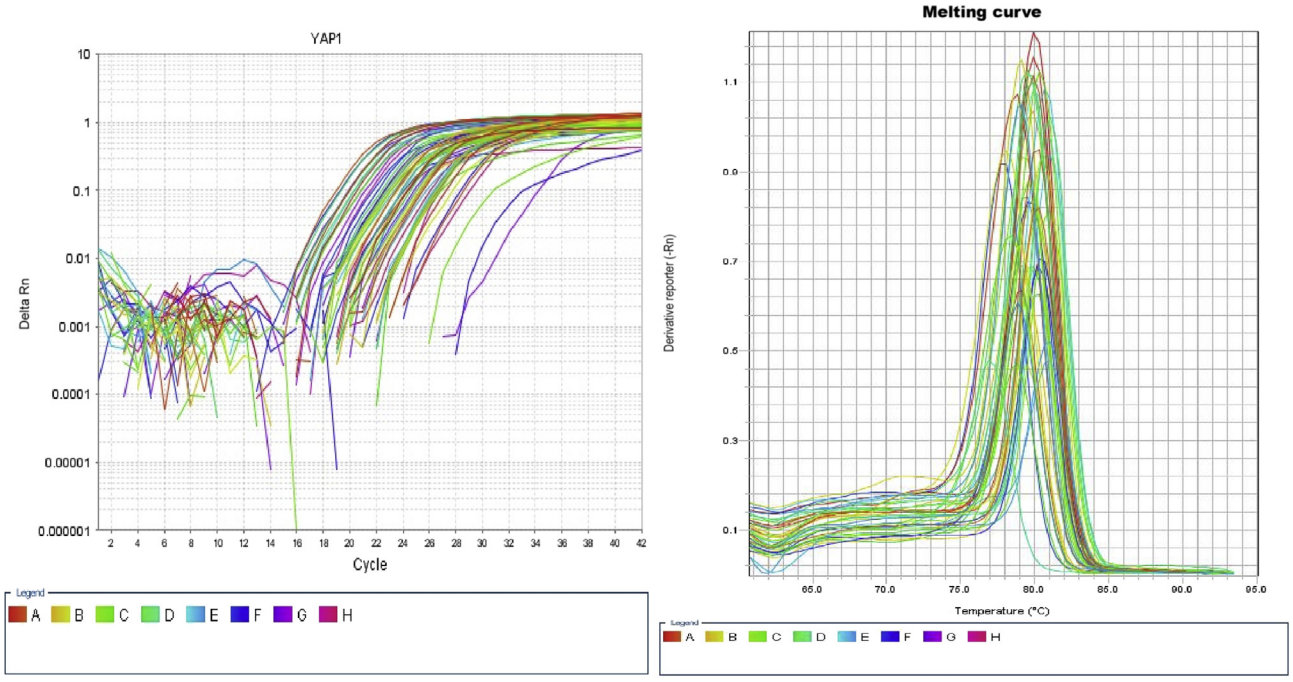
Correlation between RQ of *Livin* gene expression and laboratory investigations in each group was estimated using Spearman coefficient method and the following were concluded from the results: there was a significant positive coefficient correlation between RQ of *Livin* gene expression with RQ of *YAP1* gene expression, serum CEA and albumin levels in HCC group, while in HCV untreated group there was a significant coefficient negative association between RQ of *Livin* gene expression with serum creatinine level, Blood Urea Nitrogen (BUN)and INR level, also positive coefficient association was found between RQ of *Livin* gene expression and AFP serum level in HCV with treatment group ($p < 0.05$) (Table 3).

The correlation of RQ of livin and YAP1 gene expression and viral load: there was a non significant correlation between viral load and both livin and yap1 gene expression in all groups (not shown).

The Correlation between RQ of *YAP1* gene expression and laboratory investigations in each group was estimated with the following results:



(a)



(b)

Fig. 1. (a): Amplification plot and melting curve of *Livin* gene expression. (b): Amplification plot and melting curve of *YAP1* gene expression.

there was a significant positive coefficient correlation between RQ of *YAP1* gene expression with serum CEA level in HCC group and negative association in HCV untreated group which demonstrated also a coefficient negative association between RQ of *YAP1* gene expression with platelet count and positive association with prothrombin time. No association was found between RQ of YAP 1 gene expression with any of the studied parameters in HCV with treatment group and control group (Table 4 & Fig. 3).

There was a significant difference between the RQ of *Livin* and *YAP1* genes expression levels with different tumor size detected by ultrasound (US) in HCC group with the highest levels in multifocal lesion, followed by tumor of diameter larger than 5 cm (Table 5 & Fig. 4).

The CEA level, RQ of *Livin* and *YAP1* genes expression levels can be considered as a bad sign for overall survival in HCC patients by univariate

Table 1
Comparison between the different studied groups according to demographics data and risk factors.

	HCC (n = 30)		HCV no treatment (n = 30)		HCV with treatment (n = 30)		Control (n = 30)		Test of Sig.	p
	No.	%	No.	%	No.	%	No.	%		
Gender										
Male	26	86.7	20	66.7	24	80.0	18	60.0	$\chi^2 = 6.818$	0.078
Female	4	13.3	10	33.3	6	20.0	12	40.0		
Age (years)										
Min. – Max.	35.0–65.0		19.0–70.0		38.0–68.0		30.0–68.0		F = 2.459	0.066
Mean ± SD.	51.03 ± 7.64		49.77 ± 16.34		56.87 ± 7.83		49.47 ± 14.0			
Median	53.0		55.0		56.0		45.0			
Risk factors										
Smoking	13	43.3	12	40.0	7	23.3	6	20.0	5.700	0.127
Diabetes	14	46.7	7	23.3	8	26.7	14	46.7	6.198	0.102
Hypertension	10	33.3	5	16.7	5	16.7	8	26.7	3.354	0.340

χ^2: **Chi square test**F: **F for ANOVA test**p: p value for comparing between the studied groups.

Table 2
Comparison between the different studied groups according to RQ.

	HCC (n = 30)	HCV no ttt (n = 30)	HCV w ttt (n = 30)	Control (n = 30)	H	p
RQ of *Livin* gene						
Min. – Max.	3.54–25.67	1.89–8.20	0.43–4.25	0.43–4.25	95.201*	<0.001*
Mean ± SD.	9.52 ± 4.48	4.30 ± 1.45	0.94 ± 0.72	1.04 ± 0.93		
Median	8.76	4.35	0.78	0.78		
p_1	<0.001*	<0.001*	0.873			
Sig. bet. Grps	$p_2 = 0.002$*,$p_3 < 0.001$*,$p_4 < 0.001$*					
RQ of *YAP1* gene						
Min. – Max.	4.75–40.68	0.04–15.70	1.28–10.50	0.02–1.10	77.131	<0.001*
Mean ± SD.	14.06 ± 10.84	3.98 ± 3.85	5.16 ± 2.44	0.51 ± 0.29		
Median	9.42	4.64	4.70	0.55		
p_1	<0.001*	<0.001*	<0.001*			
Sig. bet. Grps	$p_2 < 0.001$*,$p_3 = 0.001$*,$p_4 = 0.112$					

H: H for **Kruskal Wallis test,** Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Dunn's for multiple comparisons test)**.
p: p value for comparing between the studied groups.
p_1: p value for comparing between control and each other groups.
p_2: p value for comparing between HCC group and HCV no ttt group.
p_3: p value for comparing between HCC group and HCV w ttt group.
p_4: p value for comparing between HCV no treatment group and HCV with treatment.
* Statistically significant at $p \leq 0.05$.

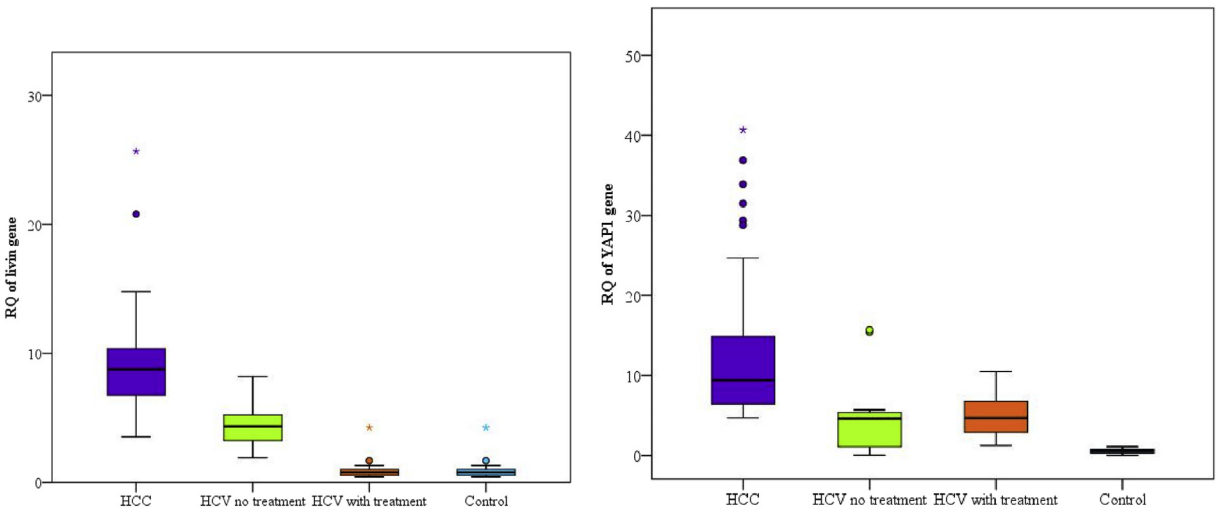


Fig. 2. Comparison between the different studied groups according to RQ of *Livin* and *YAP1* genes.

analysis, while by multivariate analysis only RQ of *YAP1* gene expression can be considered as bad sign for overall survival in HCC patients (Table 6).

4. Discussion

Despite great advances in diagnosis and treatment of HCC, the mortality rate is still high, especially in advanced stage. This indicates that a great effort is needed to identify novel prognostic markers and to develop new therapeutic strategies (Yin et al., 2008).

Table 3
Correlation between RQ of *Livin* gene expression and laboratory investigation & RQ of *YAP1* gene expression in each group.

	RQ of *Livin* gene expression							
	HCC		HCV no ttt		HCV w ttt		Control	
	r_s	p	r_s	p	r_s	p	r_s	p
TLC (x103/ul)	-0.259	0.168	0.118	0.535	0.123	0.516	0.052	0.784
Platelets (x10^3/ul)	-0.003	0.986	-0.140	0.459	-0.019	0.921	-0.037	0.848
Prothrombin time percent	-0.146	0.443	0.148	0.435	0.126	0.508	0.069	0.718
INR	0.085	0.653	-0.482*	0.007*	-0.281	0.133	-0.026	0.891
ALT (IU/L)	0.217	0.248	-0.345	0.062	0.011	0.954	0.212	0.260
AST (IU/L)	0.059	0.757	-0.195	0.302	-0.215	0.253	0.073	0.701
ALP (IU/L)	0.138	0.468	-0.067	0.724	-0.131	0.491	-0.060	0.755
GGT (IU/L)	-0.366	0.046	-0.103	0.589	-0.090	0.636	-0.110	0.563
AFP (ng/ml)	0.358	0.052	0.163	0.391	0.449*	0.013*	-0.211	0.264
CEA (mg/dl)	0.392*	0.032*	-0.281	0.133	-0.357	0.053	0.093	0.625
Albumin (gm/dl)	0.451*	0.012*	0.287	0.124	-0.226	0.231	0.073	0.701
Total bilirubin (mg/dl)	-0.119	0.531	-0.115	0.544	0.063	0.740	0.184	0.330
Direct bilirubin (mg/dl)	0.170	0.370	-0.273	0.145	0.161	0.395	-0.095	0.618
BUN (mg/dl)	-0.053	0.779	-0.503*	0.005*	0.305	0.101	-0.011	0.955
Creatinine (mg/dl)	-0.320	0.084	-0.438*	0.016*	0.021	0.913	0.222	0.237
RQ of *YAP1* gene expression	0.680	0.001	0.0246*	0.897	0.1415	0.4557	0.196	0.299

r_s: Spearman coefficient.
* Statistically significant at $p \leq 0.05$.

Table 4
Correlation between RQ of *YAP1* gene and laboratory investigation in each group.

	RQ of *YAP1* gene							
	HCC		HCV no ttt		HCV w ttt		Control	
	r_s	p	r_s	p	r_s	p	r_s	p
TLC (x10^3/ul)	-0.137	0.471	-0.024	0.898	0.135	0.478	-0.359	0.051
Platelets (x10^3/ul)	0.130	0.493	-0.528*	0.003*	0.302	0.105	-0.269	0.151
Prothrombin time percent	0.035	0.856	0.561*	0.001*	-0.184	0.331	-0.134	0.480
INR	0.012	0.949	-0.350	0.058	0.202	0.286	0.045	0.813
ALT (IU/L)	0.341	0.065	-0.069	0.716	-0.028	0.883	-0.004	0.984
AST (IU/L)	0.244	0.194	-0.085	0.656	0.030	0.877	-0.263	0.160
ALP (IU/L)	0.380	0.038	0.017	0.930	0.142	0.453	-0.348	0.060
GGT (IU/L)	-0.331	0.074	0.066	0.728	-0.248	0.186	0.190	0.314
AFP (ng/ml)	0.308	0.097	0.290	0.120	-0.182	0.335	-0.026	0.892
CEA (mg/dl)	0.711*	<0.001*	-0.606*	<0.001*	0.329	0.076	0.133	0.483
Albumin (gm/dl)	0.402	0.028	-0.120	0.529	-0.030	0.877	-0.263	0.160
Total bilirubin (mg/dl)	-0.171	0.367	0.199	0.291	0.195	0.302	-0.202	0.284
Direct bilirubin (mg/dl)	0.059	0.758	-0.031	0.870	0.100	0.598	0.206	0.275
BUN (mg/dl)	-0.087	0.646	0.030	0.874	-0.200	0.290	0.253	0.177
Creatinine (mg/dl)	-0.099	0.604	-0.035	0.856	-0.171	0.365	-0.026	0.892

r_s: Spearman coefficient.
* Statistically significant at $p \leq 0.05$.

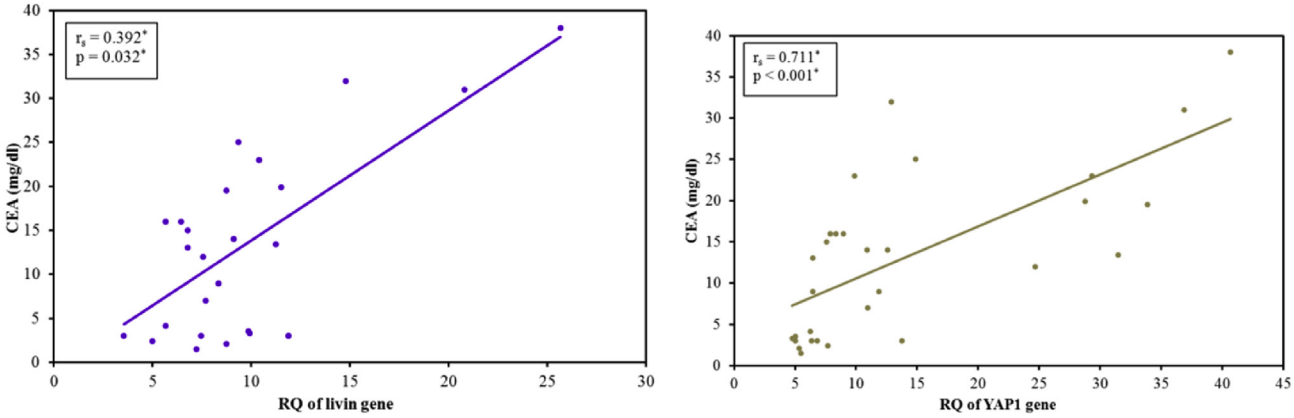


Fig. 3. Correlation between RQ of *Livin* and *YAP1* genes and CEA (mg/dl) in HCC group.

Table 5
Relation between RQ of *Livin* gene and RQ of *YAP1* gene with tumor size by US in HCC group (n = 30).

Tumor size by US	N	RQ of *Livin* gene			H	p
		Min. – Max.	Mean ± SD.	Median		
≤5	14	3.54–9.93	7.13 ± 1.83	7.01	11.644*	0.003*
>5	9	5.67–20.80	11.37 ± 4.45	11.53		
Multifocal	7	7.56–25.67	11.92 ± 6.18	10.39		
Tumor size by US	N	RQ of *YAP1* gene			H	p
		Min. – Max.	Mean ± SD.	Median		
≤5	14	4.75–11.89	6.94 ± 1.96	6.45	15.622*	<0.001*
>5	**9**	6.25–36.89	18.35 ± 11.68	13.78		
Multifocal	**7**	9.89–40.68	22.79 ± 11.94	24.67		

H: H for Kruskal Wallis test.
p: p value for comparing between the different categories.
* Statistically significant at p ≤ 0.05.

Livin is a member of the inhibitors of apoptosis proteins family, it plays a vital role in the regulation of apoptosis with subsequent modulation of cell cycle and cell proliferation. *Livin* is over-expressed in several cancer types, its anti-apoptotic activity is mediated mostly by the direct inhibition of caspase 3, 7 and 9 (Altieri et al., 2017). Our study proved that *Livin* gene was found to be significantly overexpressed in hepatocellular carcinoma patients, similar results were demonstrated by other authors (Cho et al., 2015). *Livin* gene expression was also reported by many authors to be elevated in a number of other tumors like adrenocortical tumors, colorectal tumors (Myung et al., 2013)& (Wang et al., 2014)superficial bladder cancer tumors (Gazzaniga et al., 2003)neuroblastoma (Kim et al., 2005)acute lymphoblastic leukemia (Choi et al., 2007) and melanoma as well as many other types of tumors (Lazar et al., 2012).

Many studies concluded that IAP members as *Livin* gene represent attractive molecular targets for the design of new classes of anticancer drugs which can give promising results for treatment of many cancer patients (Wang et al., 2008) (Sbiera et al., 2013). However, the expression of this protein in many normal tissues may represent a challenge for its role in cancer therapy and represent a lot of side effects like nephrotoxicity, infertility and gastrointestinal disorders (Ding et al., 2013).

*YAP1*is a well-known oncogenic protein in human cancer (Harvey et al., 2013). It is a transcriptional regulator and it plays a pivotal role in organ size control (Zanconato et al., 2016). In HCC, *YAP1* was found to be overexpressed and could promote the growth and metastasis of HCC cells (Farazi and DePinho, 2006).

In our study significant overexpression of YAP-1 was detected in HCC patients as well as patients with HCV confirming the results which were reported by many other previous studies (Xu et al., 2009) (Xu et al., 2013) (Zhang et al., 2012).

Correlation between RQ of both *Livin* gene and *YAP1* expression and laboratory investigations in each group was estimated using Spearman coefficient method and there was a significant positive coefficient correlation between both RQ of *Livin* and YAP1 gene expression, RQ of *Livin* gene expression with serum CEA in HCC group also between RQ of *YAP1* gene expression with serum CEA level in HCC group, this finding when added to the result that There was a significant difference between the RQ of *Livin* and *YAP1* genes expression levels with different tumor size detected by US in HCC group with the highest levels in multifocal lesion, followed by tumor of diameter larger than 5 cm indicating that *Livin* and *YAP1* genes are associated with HCC and indicating bad prognosis of the disease. Previous findings were reported by other studies like Fan et al.who concluded that an increased expression of YAP-1 within PBMCs could serve as a bad indicator for the prognosis of HCC patients as that study reported high level of *YAP1* in mononuclear cells and showed positive linear correlation to Treg percentage which is immunosuppressant cells (Fan et al., 2017*)*.

The CEA level, RQ of *Livin* and *YAP1* genes expression levels can be considered as bad signs for overall survival in HCC patients by univariate Cox regression analysis while by multivariate analysis only RQ of *YAP1* gene expression can be considered as a bad sign for overall survival in HCC patients. This finding confirms what obtained by Zhang et al. (2017*)* who demonstrated that high level of YAP 1 together with low level of miRNA-345 was associated with low survival rate. Otherstudy also reported that YAP-1 is associated with increased) TGF-β(within HCC and hyperplasia of oval cells together with activation of inflammatory cell infiltration and fibrosis (Nishio et al., 2016).

IHC studies on human HCC samples showed that elevated expression of YAP correlates with poor tumor differentiation and is prognostic of bad outcome (Guo et al., 2015). YAP protein levels are upregulated starting from precancerous lesions, but overt nuclear localization of YAP can be found only in fully developed HCC and CC (Perra et al., 2014).

Livin gene expression was also reported as a bad prognostic marker by Augello et al.,2009 who concluded that *Livin* overexpression in HCC patients imply that its level could be used as a marker of cancer tissue and more importantly, could be related with patients' survival.

Also, the study of Hua Guo et al., 2013 found that Livin protein expression was significantly higher in HCC tissues than that in normal hepatic tissues and hepatitis/hepatic cirrhosis tissues, with no significant

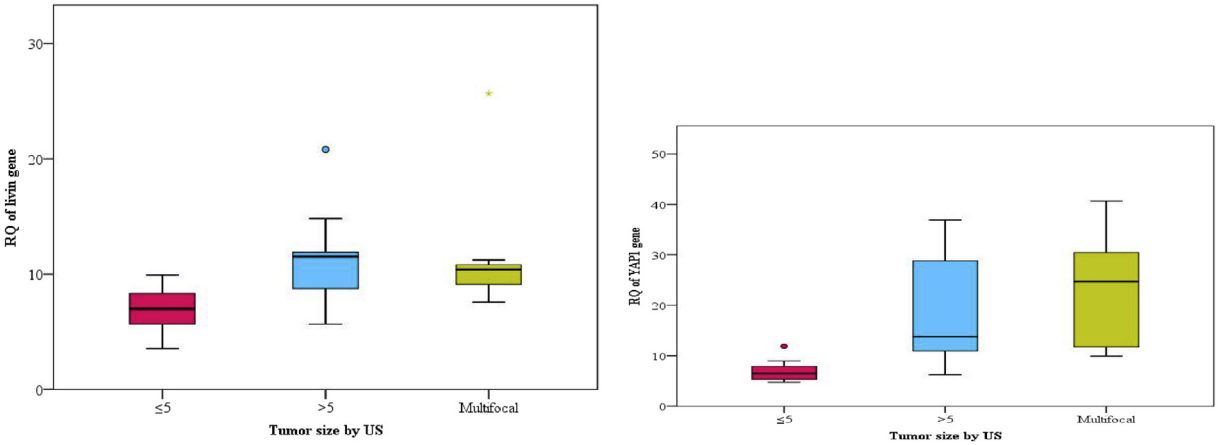


Fig. 4. Relation between RQ of *Livin* and YAP1 genes and tumor size in HCC group.

Table 6
Cox regression of overall survival in HCC group for determination of hazard ratio.

	Univariate		#Multivariate	
	p	HR (95%C.I)	p	HR (95%C.I)
Gender (female)	0.442	1.887 (0.400–8.892)		
Age (years)	0.960	0.998 (0.923–1.079)		
Smokin	0.712	0.788 (0.222–2.796)		
Diabetes	0.616	0.724 (0.204–2.564)		
Hypertension	0.463	1.607 (0.453–5.701)		
Hb level (gm/dl)	0.383	1.158 (0.833–1.608)		
TLC (x103/ul)	0.929	0.984 (0.685–1.412)		
Platelets (x103/ul)	0.687	1.002 (0.992–1.012)		
Prothrombin time percent	0.875	1.004 (0.960–1.049)		
INR	0.951	0.908 (0.044–18.963)		
ALT (IU/L)	0.610	1.007 (0.980–1.035)		
AST (IU/L)	0.845	1.003 (0.973–1.033)		
ALP (IU/L)	0.948	1.000 (0.991–1.008)		
Albumin (gm/dl)	0.113	2.133 (0.837–5.437)		
GGT (IU/L)	0.274	0.996 (0.988–1.004)		
AFP (ng/ml)	0.883	1.000 (0.999–1.001)		
CEA (mg/dl)	0.023*	1.074 (1.010–1.142)	0.629	0.964 (0.829–1.120)
Total bilirubin (mg/dl)	0.094	0.149 (0.016–1.383)		
Direct bilirubin (mg/dl)	0.218	0.067 (0.001–4.954)		
BUN (mg/dl)	0.969	1.002 (0.928–1.081)		
Creatinine (mg/dl)	0.230	0.294 (0.040–2.168)		
PCR $x10^5$	0.088	1.002 (1.000–1.004)		
RQ of *Livin* gene	0.008*	1.168 (1.402–1.308)	0.438	0.914 (0.728–1.147)
RQ of *YAP1* gene	<0.001*	1.124 (1.065–1.186)	0.001*	1.196 (1.078–1.328)

OR: Odd's ratio, C.I: Confidence interval.
All variables with $p < 0.05$ was included in the multivariate.
* Statistically significant at $p \leq 0.05$.

difference between HCC tissues and pericarcinoma tissues.

YAP can also induce the expression of several negative regulators of apoptosis such as the inhibitors of apoptosis proteins (IAP) family members baculoviral IAP repeat containing *5 (BIRC5)*/survivin and *BIRC2*/cIAP1 and the B-cell lymphoma-2 (BCL2) family gene myeloid cell leukemia-1 (*MCL1*). Thus, YAP can act as potent inhibitor of apoptosis in the regulation of organ size (Smolewski and Robak, 2011).

5. Conclusions

Based on the findings in our study, we concluded that there is over-expression of *Livin* and YAP 1 genes in hepatocellular carcinoma patients and HCV patients. So they can be used as indicators of bad prognosis of the disease pathway together with low survival rate in HCC patients. Future studies should focus on their patho -physiological role in progress of HCC as well as in other cancer types in order to develop new therapeutic choices. However, there are some limitations in the current study; limited sample sizes together with that serum samples were not correlated with expressed levels of investigated gene expression, also IHC staining of livin and yap1 in the patient samples could help. a HCC group without HCV infection can be used as a reference group to confirm that the change of livin and YAP1 expression is due to HCC cancer alone or HCV or both.

Declarations

Author contribution statement

Eman Badr: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Ibrahim El Tantawy: Conceived and designed the experiments; Wrote the paper.

Mohamed Assar, Sahar Ali: Analyzed and interpreted the data; Wrote the paper.

Nehal Ibrahim: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

This pilot study acknowledges the central laboratory unit, faculty of Medicine, Menoufia University for providing us with the necessary instruments for completion of the study.

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