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Long non-coding RNAs (CASC2 and TUG1) in hepatocellular carcinoma: Clinical significance

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Abstract

Background: The biology of hepatocellular carcinoma remains poorly understood. LncRNAs have been proved to be key regulators of most cell processes and cancer. lncRNA CASC2 was originally identified as a downregulated gene in endometrial cancer and acted as a tumor suppressor. lncRNA TUG1 has been shown to play an oncogenic role in various cancers. However, the relative expression of CASC2 and TUG1 in HCC on top of HCV and the relationship between both remains unclear. The aim of the current study was to evaluate both Lnc-RNA CASC2 and TUG1 relative gene expression in whole blood of HCC/HCV patients in relation to HCV and healthy subjects and to relate them to each other and to different clinicopathological factors.

Methods: The relative expression of CASC2 and TUG1 was estimated by quantitative RT-PCR in 30 HCC/HCV patients and were compared to 20 cases of HCV patients and 20 controls.

Results: CASC2 was down regulated in HCC/HCV patients while TUG1 was overexpressed in relation to HCV and control group indicating their antagonistic effect. This can suggest their role in the pathogenesis of HCC on top of HCV. Their expression was correlated to BCLC stage and serum AFP level.

Conclusion: CASC2 and TUG1 could be new potential biomarkers with a valid non-invasive technique.

1. Introduction

Hepatocellular carcinoma (HCC) represents the sixth most common neoplasm in humans. Its frequency is expanding, going somewhere in the range of 3% and 9% yearly.[1]

Generally, hepatocarcinogenesis is a multistep process involving several genetic or epigenetic alterations. The 5-year overall survival rate of HCC remains very poor and its biology remains poorly understood. Subsequently, the recognition of new biomarkers for HCC and an understanding of the molecular mechanisms underlying its pathogenesis will supply an arm for improving diagnosis and management of human HCC.[2]

More than 90 % of the genomic DNA could transcript into noncoding RNAs (ncRNAs) [3]. They play an important role in tumorigenesis, and their mis-expression confers tumor initiation, cancer cell growth and metastasis [4].

lncRNA could regulate tumor progression via MAPK pathway[5]. High expression levels of MAPK, phosphorylated MAPK and phosphorylated MEK1/2 were found in tumors of HCC patients.[6]

Interestingly, Overexpression of cancer susceptibility candidate 2 (CACS2) led to the inactivation of the ERK1/2 and JNK/MAPK pathway in gastric cancer[7]. Additionally, CASC2, was identified as a downregulated gene in endometrial cancer as it acted as a tumor suppressor[8]. Inactivation of this tumor suppressor gene occurs through either genetic mutation or epigenetic/regulative modifications in most cases[9].

It was also reported that CASC2 suppressed malignancy in human gliomas by miR-21[10] and was down regulated in non-small cell lung carcinoma as well [11]. In addition, low expression of long noncoding RNA CASC2 regulated cell proliferation in renal cell carcinoma cells [12] and indicated a poor prognosis in thyroid carcinoma [13].

Recently, it was found that the up regulation of CASC2 liver expression inhibited hepatoma cell proliferation, migration and invasion and induced apoptosis in vitro[14].

Taurine Up-regulated Gene 1 (TUG1), was first identified in a screen for genes upregulated by developing retinal cells in response to taurine. It was originally identified as a contributor in the formation of photoreceptors and in retinal development in mice[15]. Recently, **Huang et al.** [16] found that lncRNA TUG1 expression was upregulated in HCC tissues. Moreover, silencing of lncRNA TUG1 expression inhibited HCC cell proliferation, tumorigenicity and induced apoptosis in HCC cell lines.

Interestingly, in human non-small cell lung cancer, TUG1-mediated growth regulation was due to activation of AKT and MAPK pathways through specific modulation of HOXB7 expression[17].

Little is known about CASC2 and HCC, and its relationship with TUG1 remains unclear[16]**.** The aim of the current study was to evaluate both LncRNA CASC2 and TUG1 relative gene expression in whole blood of HCC/HCV patients, as well as HCV patients and normal healthy subjects aiming to explore their clinical significance in the pathogenesis of HCC/ HCV. The results could provide a simple and a non-invasive method for early detection of HCC.

2. Materials and methods

This study was conducted in collaboration between Medical Biochemistry and Molecular Biology department and Tropical Medicine department, Faculty of Medicine, Ain Shams University. It included 30 newly diagnosed HCC (CHILD class A with no extrahepatic spread or LN involvement) on top of HCV patients according to AASLD Practice Guidelines[18], 20 HCV patients diagnosed on clinical, laboratory and/or ultrasonographic basis after exclusion of malignancy by abdominal ultrasound and AFP and 20 normal healthy subjects.

Subjects were recruited from the outpatient hepatoma clinic and inpatients at Tropical

Medicine department at Ain Shams University Hospitals in the period from February 2016 to March 2017. Informed consent was taken from each subject, in accordance with the guidelines of institutional review board of the Faculty of Medicine Ethical Committee, Ain Shams University. None of the patients was below 18 years or had a history of cancer within the last five years other than HCC. All the patients had no previous radiotherapy or systemic chemotherapy or underwent former liver transplantation.

2.1. Sample collection and processing

A volume of 3 ml of peripheral blood was collected from each subject into EDTA-containing labeled tubes then diluted with an equal volume of phosphate-buffered saline (PBS). A volume of 1 ml diluted blood was layered carefully down the side of a tube containing 750 µl of Ficoll-Hypaque solution (Sigma-Aldrich, USA) and the tube was then centrifuged.

At the interface between the plasma (upper layer) and Ficoll–Hypaque (lower layer), mononuclear cells were found and were carefully removed from the interface and suspended in 3 volumes of PBS, gently mixed by pipetting then centrifuged at 1000 rpm at room temperature for 10 min. The supernatant was removed, and the leukocytes pellet was washed once with PBS then centrifuged. The supernatant was discarded, and the packed leukocytes were suspended in 1 ml of PBS and stored at -80°C until analyzed.

2.2. Extraction and Purification of total RNA from polymorphonuclear leucocytes (PMN)

The total RNA was isolated from polymorphonuclear leucocytes according to the initial silica extraction method originally described by[19]using the RNeasy Mini Kit, cat no: 217004, (Qiagen, Germany) and QIAzol® Lysis Reagent following the manufacturer's protocol.

2.3. Reverse transcription of RNA

Total RNAs were reversibly transcribed using a QuantiTect II RT kit (50) (Qiagen, Hilden, Germany) (Cat. no. 205311) according to the manufacturer's instructions.

2.4. Quantitative real time polymerase chain reaction (qRT-PCR) of lncRNA (CASC2) and (TUG1)

CASC2 and TUG1 relative gene expression levels were amplified using RT^2 LncRNA PCR primer assay; [Lnc-CASC2 primer assay (cat no:330701, ID: LPH01409A), Lnc-TUG1 Primer assay (cat no:330701, ID: LPH18394A) (Qiagen, Germany)], and RT^2 SYBR Green PCR Kit (cat no: 330520, Qiagen, Germany). Hs_GAPDH_1_SG Primer Assay (cat no: 249900, ID: QT00079247) was used as a housekeeping gene.

All samples were analyzed using the 5 plex Rotor Gene PCR Analyzer (Qiagen, Germany). The relative expression level of lncRNA CASC2 and TUG1 was normalized against that of the GAPDH. The relative quantitative value was expressed by the $2^{-\Delta\Delta Ct}$ method [20].

The PCR program for SYBR green based QPCR was as follows: at first, initial activation step at 95°C for 10 min followed by 40 cycles: denaturation for 15 sec at 95°C; then annealing for 30 sec at 55°C; lastly, extension for 30 sec at 72°C.

The threshold cycle (C_t) value of each sample was calculated using StepOnePlus™ software v2.2.2 (Applied Biosystems). Amplification plots and Tm values were analyzed to affirm the specificities of the amplicons for SYBR Green -based PCR amplification.

2.5. Statistical analysis

Statistical analysis was done using the Statistical Package for the Social Sciences (SPSS software version 20, Chicago, Illinois) on a personal computer. Kruskal-Wallis test was performed to evaluate the difference in lncRNA TUG1 and CASC2 relative expression between HCC/HCV and the other two groups.

The relationships between lncRNA CASC2 and TUG1 expression and different clinicopathological characteristics were evaluated using the Mann-whitney-U test.

Spearman's correlation test was used to assess the statistical significance correlation between two variables. Receiver Operating Characteristics curve (ROC) was used to determine the threshold value for optimal sensitivity and specificity. A "P" value of ≤0.05 was considered statistically significant.

3. Results

The present study included (70) subjects stratified into (3) groups: Group 1: HCC/HCV patients (n=30, mean age 60.13±6.8years, ranging from 50-75years), group 2: HCV patients (n=20, mean age 57.35±7.5 years, ranging from 37-68 years) and control group: normal healthy subjects (n=20, mean age 58.65±7.3 years, ranging from 49-76 years). The demographic and clinicopathological variables of the different study groups are shown in table (1).

There was a non-significant statistical difference between subjects of the three groups in terms of age and sex ($p>0.05$), as shown in table (1).

There was a high significant difference between the three groups as regards the gene expression (RQ of Lnc-TUG1 and CASC2, $p< 0.01$) as well as the different laboratory parameters; total bilirubin, albumin, INR, serum AST, ALT and AFP ($p< 0.01$) as shown (Table 2).

Both HCC/HCV patients and HCV group showed a significant decrease in Lnc-CASC-2 expression than the control group, ($p<0.01$).

On the other hand, expression of Lnc-TUG1 was higher in HCC/HCV group in relation to HCV and control group ($p<0.01$, $p<0.01$ respectively). Moreover, there was statistically significant difference between HCV patients and control group with higher expression in HCV group ($p<0.01$).

RQ values of Lnc-TUG1 and CASC2 in relation to different demographic and clinicopathological variables in HCC/HCV group showed no significant difference (P>0.05) except for serum AFP and BCLC score (P<0.05), table (3).

ROC analysis was performed to determine a cut off value for each marker and their performance characteristics were documented in (table 4, fig 1). The positivity rates of Lnc-CASC2 and TUG1 in the different study groups showed a high significant difference (p<0.01), table 5.

There was a highly significant positive correlation between Lnc-TUG1 RQ values, BCLC scores and serum AFP, table (6).

On the other hand, there was a highly significant negative correlation between Lnc-TUG1 and CASC2 RQ. Moreover, there was a highly significant negative correlation between Lnc-CASC2 RQ values, BCLC scores and AFP, table (6).

4. Discussion

HCC represents (85-90%) of primary liver cancers[21]. Lack of early diagnostic markers and poor prognosis of HCC attributes to its high morbidity. Consequently, exploration of new tools in HCC diagnosis and therapeutics is highly recommended [22].

AFP is the most widely used tumor biomarker currently available for HCC diagnosis. However, it misses one third of early stages HCC. This clarifies the need for other reliable and minimal invasive methods for early detection of HCC [23].

It has been previously demonstrated that most of the human genome can be transcribed into non-coding RNAs [24]. The role of long non-coding RNAs (lncRNAs) in the regulation of target genes and their oncogenic or tumor silencer jobs in tumorigenesis was uncovered [25]. The intracellular ncRNAs that were significantly altered in HCV-induced HCC can be used as potential molecular targets for therapy [26].

Viral infections may deregulate cellular lncRNAs and may express viral lncRNAs or chimeric lncRNAs [2] in response to viral replication or by the antiviral pathways induced by infection [27].

To the best of our knowledge, this study is the first study to evaluate the role of Lnc-TUG1 and CASC2 in blood of HCC/HCV patients aiming to suggest a potential diagnostic biomarker with a valid non-invasive technique. Lnc-TUG1 and CASC2 levels based on RQ values were detected in the different investigated groups to evaluate their clinical significance in HCC on top of HCV and to relate between them and to different clinicopathological factors.

lncRNA, cancer susceptibility candidate 2 (CASC2), was identified as a tumor suppressor gene downregulated in endometrial cancer [8]. CASC2 expression was significantly downregulated in HCC tissues, especially in aggressive and recurrent cases[28]. Additionally, it was found that the upregulation of CASC2 expression inhibited hepatoma cell proliferation, migration and invasion and induced apoptosis in vitro[14].

This was consistent with the current results which reported the significant down expression of LncRNA-CASC2 RQ in HCC/HCV patients when compared to HCV patients and normal healthy control individuals (P< 0.01). lncRNA-CASC2 threshold could be used to discriminate HCC/HCV patients from control group as well as to discriminate HCV patients from control group. Correlations of lncRNA CASC2 expression with clinicopathologic variables of patients with HCC/HCV were statistically analyzed and observed negative correlation with advanced BCLC stages.

BCLC B/C patients often have a larger tumor size, satellite nodes, or portal/hepatic vein tumor thrombosis and this could justify that CASC2 expression was markedly downregulated in aggressive HCC tissues compared with non-aggressive HCCs ($P < 0.01$)[28]. Moreover, another study demonstrated that the expression of CASC2 was negatively correlated with greater tumor size and advanced TNM stage in non-small cell lung cancer[29].

Additionally, in the current study, there was a highly significant negative correlation between Lnc-CASC2 and AFP. Elevated AFP levels were found to be associated with higher pathological grade in HCC[30] and was proven to be negatively related to clinical stage and

tumor grade[31].

Taurine Up-regulated Gene 1 (TUG1) was aberrantly expressed and involved in carcinogenesis and progression. LncRNA TUG1 was found to be upregulated in urothelial carcinoma of the bladder [32], osteosarcoma [33] and Esophageal Squamous Cell Carcinoma [34].

In the present study, lncRNA TUG1 relative expression showed highly significant increase in HCC/HCV patients compared with HCV group and normal subjects (P< 0.01). lncRNA- TUG1 threshold could be used to discriminate HCC/HCV from HCV patients and to discriminate HCC/HCV from control group as well as between HCV patients from control group (P< 0.01). This suggests the role of TUG1 in HCV infection and its use in monitoring the course of disease.

This was in consistence with **Huang et al.**[16]**,** who found that lncRNA TUG1 expression was upregulated in HCC tissues. Moreover, silencing of lncRNA TUG1 expression inhibited HCC cell proliferation, tumorigenicity and induced apoptosis in HCC cell lines [35].

The correlations of lncRNA TUG1 expression with clinicopathologic features of patients with HCC/HCV were statistically analyzed. High lncRNA TUG1 expression level was observed to be highly correlated ($P < 0.01$) with advanced BCLC stage in HCC and this agreed with **Huang et al.**[16] Additionally, the high expression level of lncRNA TUG1 in gastric cancer patients was positively correlated with invasion depth and TNM stage suggesting its oncogenic role and its potential usage as biomarker or therapeutic target [36].

Moreover, there was a highly significant positive correlation between Lnc-TUG1 and AFP in the current study. Previous studies found that elevated AFP levels were associated with higher pathological grade [30], more advanced Barcelona Clinic Liver Cancer stage [37], TNM stage and larger tumors [38]. This also comes in agreement with **Fujioka et al.** [39] who stated that HCC patients with a high AFP concentration tended to have greater tumor size, bi-lobar involvement, massive or diffuse types, portal vein thrombosis, and a lower median survival

rate. As regards the pathological grade, this wasn't applicable in the current study as it only included HCC/HCV patients Child Pugh (class A).

The results of the current study revealed a highly significant negative correlation as regard lncRNA-TUG1 and lncRNA-CASC2 RQ indicating their antagonistic effect, where TUG1 was upregulated and CASC2 was down regulated. This can emphasize their role in the pathogenesis of HCC on top of HCV via MAPK pathway.

The MAPK/ERK pathway communicates a signal from a receptor on the surface of the cell to the DNA in the nucleus of the cell [40]. Many cancers develop when one of the proteins in the pathway is mutated as it becomes stuck in the "on" or "off" position.[41] Therefore, many compounds which inhibit steps in the MAP/ERK pathway are potential drugs for treating cancer [42].

Recent studies indicated that lncRNA could regulate tumor progression via MAPK pathway. For example, **Huang et al.** [43] suggested that HBx-related long non-coding RNA DBH-AS1 could promote cell proliferation and survival by activating MAPK signaling in hepatocellular carcinoma, as well as high expression levels of MAPK, phosphorylated MAPK and phosphorylated MEK1/2 were found in tumors of HCC patients [6].

In human non-small cell lung cancer, TUG1 could epigenetically modulate homeobox B7 (HOXB7) [17]. HOXB7 promotes cell proliferation through activating AKT and MAPK pathways [44].

Additionally, Overexpression of CACS2 led to the inactivation of the ERK1/2 and JNK/MAPK pathway in gastric cancer. Moreover, combination treatment of CASC2 overexpression and suppression of ERK1/2 or JNK produced synergistic inhibitory effects in vitro, indicating that

overexpression of lncRNA CASC2 inhibited GC cell proliferation by regulating MAPK pathway inactivation [7].

Additionally, after HepG2 and HuH7 cells were transfected with the CASC2 plasmid for 48 hr., western blot analyses were done and confirmed that CASC2 overexpression significantly decreased ERK and JNK phosphorylation compared with that in blank cells and negative cells suggesting that CASC2 could inactivate the MAPK signaling pathway [14].

So, this pathway could be a suggested common mechanism between both TUG1 and CASC2 in HCC pathogenesis for further studies.

Conclusion

lncRNA- TUG1 and CASC2 detected by quantitative RT-PCR could be used as promising potential biomarkers for early detection of HCC developing on top of HCV. Further multicentric studies including larger sample size must be conducted for better evaluation of lncRNA TUG1 and CASC2 expression and their role in HCC.

Conflict of interest: none

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Table 1: Demographic and clinicopathological variables of different study groups

Demographic and clinicopathological variables	HCC/HCV N (%)	HCV N (%)	Control N (%)	P [(a)]
Age				
≤60	16 (53.3)	14 (70)	14 (70)	0.36
>60	14 (46.7)	6 (30)	6 (30)	
Gender				
Male	19 (63.3)	11 (55)	11(55)	0.78
Female	11 (36.6)	9 (45)	9 (45)	
PVT		……..	……	……..
Positive	4 (13)			
Negative	26 (87)			
Liver Size		……	…….	……
Average	17 (56.6)			
Hepatomegaly	13 (43.3)			
HFL site		…….	…….	……
Unilateral	27 (90)			
Bilateral	3 (10)			
HFL size (cm)		……..	……	……..
Small≤4 cm	17 (56.6)			
Large>4 cm	13 (43.3)			
BCLC score		…….	……	…….
A	15 (50)			
B	10 (33)			
C	5 (17)			

PVT: portal venous thrombosis, HFL: hepatic focal lesion, BCLC: Barcelona clinic liver cancer.

[(a)]Chi-square test, N (%): number of case (percentage), P>0.05= non-significant, *P≤0.05=significant, **P≤0.01= highly significant

Table 2: Relative quantification (RQ) of CASC2 and Lnc-TUG1 and laboratory parameters among different study groups

Variable	HCC/HCV N= 30	HCV N=20	Control group N=20	
		Median (IQR)		P value[(a)]
Lnc-CASC2 (Log^{10})	0.76 (0.01-7.6)	1.049 (0.67-1.55)	16.45 (6.45-79.8)	P<0.01**
Lnc-TUG (Log^{10})	41.38 (5.35-99)	10 (5.4-18.4)	0.94 (0.55-1.49)	P<0.01**
Total Bilirubin (mg/dL)	1.1 (0.7-1.6)	0.5 (0.3-0.67)	0.4 (0.3-0.475)	P<0.01**
Albumin (g/dL)	3.6 (3.175-4)	4 (3.5-4.3)	5 (4.13-5.375)	P<0.01**
INR	1.2 (1.1-1.3)	1.02 (1-1.1)	1 (1-1.04)	P<0.01**
AST (U/L)	66 (40.7-87.25)	55 (45.5-70)	14 (12.2-19.75)	P<0.01**
ALT (U/L)	47 (28-68.5)	41 (35.5-49.5)	12 (11-14)	P<0.01**
AFP (ng/mL)	131.8 (29.7-323.25)	3.75 (2.12-4.27)	3.7 (2-4)	P<0.01**

IQR: interquartile range, [a] Kruskal-Wallis test, **P<0.01= highly significant

Table 3: Comparison between the different HCC subgroups in HCC/HCV group for the relative expression of Lnc-CASC2 and Lnc-TUG 1 gene.

Variable	N (%)	Lnc-TUG1 relative expression		Lnc-CASC2 relative expression	
		Median (IQR)	P value	Median (IQR)	P value
Demographic data					
Age					
≤60	16 (53.3)	43.4 (7.36-99)	0.57[(a)]	0.72 (0.2-1)	0.24[(a)]
>60	14 (46.7)	40.9 (5.35-91.77)		0.91 (0.3-2.6)	
Gender					
Male	19(63)	38.05 (5.35-99)	0.25[(a)]	0.75 (0.3-1)	0.3[(a)]
Female	11 (37)	46.8 (25.46-89.26)		0.99 (0.3-1.75)	
Residency					
Urban	7 (23)	54.9 (46.9-65)	0.76[(a)]	0.5 (0.05-2.5)	0.57[(a)]
Rural	23 (77)	40 (32.9-77.7)		0.8 (0.35-1.4)	
clinicopathological data					
Jaundice					
Positive	2 (7)	46.8 (23.26-70.52)	0.8[(a)]	0.169 (0.05-0.2)	0.056[(a)]
Negative	28 (93)	41.38 (5.35-99)		0.79 (0.37-7)	
Bleeding					
Positive	19 (63.3)	42.8 (23.26-99)	0.5[(a)]	0.81 (0.4-1.5)	0.35[(a)]
Negative	11(36.7)	39.67 (5.35-79.34)		0.54 (0.2-1.3)	
Splenomegaly					
Yes	21(70)	39.6 (7.36-99)	0.42[(a)]	0.69 (0.3-1.2)	0.42[(a)]
No	9 (30)	47.5 (5.35-79)		1.19 (0.2-2.7)	
AFP (ng/ml)					
≤132	15 (50)	38 (7.36-88.65)	P<0.05* [(a)]	0.89 (0.7-2.5)	P<0.01**[(a)]
>132	15 (50)	59.7 (5.35-99)		0.4 (0.2-0.99)	
PVT					
Positive	4 (13)	50.6 (25.46-99.04)	0.71[(a)]	0.9 (0.1-3.6)	0.4[(a)]
Negative	26 (87)	41.38 (5.35-91.77)		0.76 (0.3-1.3)	
Liver size					
Average	17 (56.6)	39.1 (7.36-91.77)	0.23[(a)]	0.75 (0.3-1.4)	0.738[(a)]
Hepatomegaly	13 (43.3)	51.27 (5.35-99)		0.78 (0.2-1.6)	
HFL site					
Unilateral	27 (90)	39.9 (5.35-99)	0.51[(a)]	0.78 (0.35-1.4)	0.8[(a)]
Bilateral	3 (10)	70.5 (28-79.34)		0.19 (0.05-3)	
HFL size (cm)					
Small≤4 cm	17 (56.6)	40 (22.94-99)	0.93[(a)]	0.78 (0.3-1.4)	0.4[(a)]
Large>4 cm	13 (43.3)	46.85 (5.35-91.77)		0.75 (0.3-1.7)	
BCLC score					
A	15 (50)	39.12 (22.94-77,71)	P<0.01** [(b)]	1.3 (0.7-2.5)	P<0.01**[(b)]
B	10 (33)	36.52 (5.35-91.77)		0.7 (0.3-0.9)	
C	5 (17)	79.34 (39.6-99.07)		0.3 (0.2-0.4)	

AFP: alpha-feto protein; PVT: portal vein thrombosis; HFL: hepatic focal lesion; BCLC: Barcelona scoring system for liver diseases. [a] Mann-Whitney Test, [b] Kruskal-Wallis test. P>0.05= non-significant, P<0.05= significant, P<0.01= highly significant.

Table 4: Performance characteristics of investigated biomarkers for detection of HCC/HCV from HCV and healthy control group

	Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
TUG1 RQ	20.6	93.3%	100%	100%	95.2%	97.1%
CASC2 RQ	1.018	67%	78%	68.9%	73.8%	72.8%
Combined TUG1 and CASC2 RQ		96.6%	72.5%	72.5%	96.6%	82.85%
AFP (ng/ml)	4.6	97%	90%	87.87%	97.3%	92.86%

PPV: Positive Predictive Value, NPV: Negative Predictive Value

Table 5: The positivity rate of investigated biomarkers among different study groups

Positivity rate	**HCC/HCV N (%)**	**HCV N (%)**	**control N (%)**	**P [(a)]**
Lnc-TUG1 relative expression				
N of cases ≥ 20.6 (%)	28 (93.3)	0 (0)	0 (0)	P<0.01**
Lnc-CASC2 relative expression				
N of cases ≤ 1.018 (%)	20 (66.7)	9 (45)	0 (0)	P<0.01**
Serum AFP				
N of cases ≥ 4.6 (ng/ml) (%)	29 (96.7)	4 (20)	0 (0)	P<0.01**

[a] Chi-square test, **P<0.01= highly significant

Table 6: Correlation between Lnc-TUG1, Lnc-CASC2 and different laboratory parameters and clinicopathological variables in HCC/HCV group

Variables	TUG1 relative expression		CASC2 relative expression	
	r	P	r	P
TUG1 (Log^{10})	-----	-----	-0.563	P<0.01**
BCLC score	0.549	P<0.01**	-0.568	P<0.01**
TLC ($x10^3$ /mm^2)	-0.18	0.33	0.133	0.48
AST (U/ml)	-0.24	0.2	0.049	0.79
ALT(U/ml)	-0.09	0.61	-0.12	0.514
Total bilirubin (mg/dl)	0.058	0.75	-0.25	0.168
Prothrombin time (seconds)	0.1	0.599	-0.23	0.219
INR (%)	0.355	0.054	-0.29	0.11
BUN (mg/dl)	-0.01	0.95	-0.12	0.5
Creatinine (mg/dl)	-0.24	0.198	0.16	0.39
AFP (ng/ml)	0.469	P<0.01**	-0.55	P<0.01**

r= correlation coefficient, *P<0.05= significant, P>0.05= non-significant, **P<0.01= highly significant