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Article

Serum long non-coding ribonucleic acids urothelial carcinoma-associated 1 and highly upregulated in liver cancer in hepatocellular carcinoma in Egyptians: a case control study

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Abstract. *Background*: Combination of serum alpha-fetoprotein(AFP) and ultrasound abdomen are the strategy used for screening of HCC nodules in cirrhotic patients, but AFP has a low sensitivity and ultrasound is operator dependent . Long non-coding ribonucleic acids (lncRNAs) may act as "oncogenes" or "tumor suppressors" for cancers. Among lncRNAs, urothelial carcinoma-associated (UCA1) and highly upregulated in liver cancer (HULC) have a relationship with malignancies. This study aims at assessing serum expression of UCA1 and HULC in HCC patients.

Methods: Serum samples were collected from, forty cirrhotic patients with HCC classified into , twenty patients with early stages of HCC (stage A and B) and twenty patients with late stages(stage C and D) according to Barcelona-Clinic Liver Cancer staging system. In addition to twenty patients with liver cirrhosis without HCC and twenty age and sex matched healthy subjects. Total ribonucleic acids was extracted and following reverse transcription, relative expression of UCA1 and HULC were assessed by quantitative real time PCR. And their variation in expression between the studied groups was calculated using relative quantification method ($2^{-\Delta\Delta Ct}$).

Results: The median serum UCA1 and HULC relative expressions were significantly higher in patients with HCC than cirrhotic patients. and UCA1 was significantly higher in patients with early stages of HCC than cirrhotic patients. The receiver operating characteristic curve demonstrated that relative expressions of both UCA1 and HULC can highly discriminate between

early stages of HCC and cirrhotic group. And there was a significant positive correlation between their relative expressions and HCC stags.

Conclusion: Relative expressions of serum UCA1 and HULC can significantly discriminate between patients with HCC and cirrhotic patients. And UCA1 can significantly discriminate between early stages of HCC and cirrhotic patients, in additions both UCA1 and HULC have a positive correlation with HCC stages.

Keywords: lncRNAs, UCA1, HULC , Hepatocellular carcinoma, diagnosis, prognosis.

Introduction

Hepatocellular carcinoma (HCC) ranks the fifth most prevalent cancer and the third predominant cause of cancer- related deaths in the world.(1) In Egypt HCC constitutes 70.48% of all liver tumors among Egyptians.(2) Most HCC arise on top of chronic liver disease, usually in association with cirrhosis.(3) Despite recent progress in clinical management, the 5-year survival rate in HCC patients is still far from being satisfactory, largely because of delayed diagnosis, frequent cancer metastasis, and high recurrence rates. (4) However the 5-year survival rate exceeds 70% if patients with HCC are diagnosed at an early stage. (5)

At present, the combination of serum alpha-fetoprotein (AFP) and ultrasound abdomen is the most widely used strategy for screening of suspicious HCC nodules in cirrhotic patients. While the diagnosis of HCC is confirmed by an imaging technique such as triphasic computed tomography (CT) or magnetic resonance imaging (MRI) . AFP has a low sensitivity particularly in diagnosis of early-stage HCC and the serum level is raised in cases with chronic liver disease.(6) Ultrasound is widely available, but sensitivity and specificity for small nodules are limited, it is also dependent on the operator.(7) Therefore, there is an urgent need to detect novel biomarkers with high efficacy for early detection of HCC.

A great effort has been directed to understand the molecular mechanisms of HCC. Beside the genome that code for the protein in liver tumors, there has been a considerable attention to study the non-coding ribonucleic acid (ncRNA).The non-coding region in the genome are transcribed into two major classes: small non-coding RNAs and long non-coding ribonucleic acids (lncRNAs), the latter are defined as those >200 nucleotides in length. LncRNAs are transcribed from the "noisy region" of the genome and act as novel biomarkers that could describe disease recurrence and progression. Some lncRNAs, as protein-coding genes, may act as "oncogenes" or "tumor suppressors" for cancers and are beneficial in cancer diagnosis and prognosis, among the large number of screened lncRNAs, lncRNAs urothelial carcinoma-associated 1 (UCA1) and highly upregulated in liver cancer (HULC) have been paid special attention due to their relationship with malignancies.(8)

Several studies revealed lncRNA UCA1 overexpression and its role as an oncogene in many malignancies, such as prostate, breast, colorectal, and pancreatic cancers. It was recently found to be significantly upregulated in the serum/ plasma of patients with osteosarcoma, lung cancer, and

gastric cancer and might be used for discrimination between patients with cancer and healthy controls. However, the potential significance of serum UCA1 in HCC remains elusive. (9) In addition, HULC is specifically expressed in hepatocytes and is associated with the molecular pathogenesis of HCC. (10) Thus, the present study was designed to evaluate the possible role of serum lncRNAs UCA1 and HULC as biomarkers in cirrhotic patients with HCC.

Material and methods

To achieve this goal, 60 patients with liver cirrhosis were included in the study. Patients were categorized into 2 groups: group I 40 cirrhotic patients with HCC divided into group IA(early stages of HCC), including 20 patients (stage A and B) and group IB(late stages of HCC), including 20 patients (stage C and D) according to Barcelona-Clinic Liver Cancer (BCLC) staging system . Group II: 20 cirrhotic patients without HCC . The diagnosis of HCC was based on serum levels of AFP, ultrasonography, triphasic CT and or MRI. The diagnosis of cirrhosis was determined by clinical, biochemical and ultrasonographic feature of cirrhosis and if any patient have high level of AFP, focal hepatic lesion was excluded by triphasic CT and or MRI . All patients of group I and II were selected from patients admitted to the Hepatobiliary Unit of the Main Alexandria University Hospital. Twenty age and sex matched healthy volunteers with no evidence of liver disease were enrolled as a control group (Group III).

Sample size was calculated using epi info software for case control study, considering power 80% and confidence level 95%.

Written informed consent of patients and volunteers was obtained following a detailed explanation of the procedures that they may undergo. The study was approved by the Ethics Review Board of the Faculty of Medicine, Alexandria University and the ethics committee approval number was 021023.

All patients and healthy subjects were evaluated clinically and ten ml of venous blood was withdrawn from them. Each blood sample was then divided into three aliquots; an ethylenediamine tetraacetic acid (EDTA) tube, a citrated tube and a plain tube. In the latter, blood was allowed to clot, then centrifuged at 1200 xg for 10 minutes to separate serum samples, which were kept frozen at -80°C until use. Complete blood picture, liver test profile and hepatitis virus markers (hepatitis C virus antibody and hepatitis B surface antigen) were assayed . Serum AFP levels were measured using enzyme-linked immunosorbant assay (ELISA) provided by Diagnostic Automation /Cortez Diagnostics (Catalog No. 5101Z).(11) The severity of liver disease was graded according to Child-Pugh classification.(12) The HCC stage was assessed according to BCLC staging system. (13)

Total RNA isolation from serum samples was performed using Qiagen® miRNeasy Mini Kit. (Cat. No. 217004). The concentration and purity of RNA were measured using nanodrop then complementary deoxy ribonucleic acids (cDNA) was synthesized using High Capacity cDNA

Reverse Transcription Kit (Applied Biosystems, USA,Cat. No. Archive).(9, 14) Each reaction comprised approximately 10 μg RNA extract, 2 μl of reverse transciptase Buffer, 0.8 μl of deoxy nucleotide triphosphate (dNTP), 1 μl of reverse transciptase, 1μl RNase Inhibitor, 2 μl RT Random Primers, then the total volume was completed to 20 μl using nuclease-free water. The thermal cycle was programmed at 10 min hold at temperature 25 °C, 120 min hold at temperature 37 °C, 5 min hold at temperature 85 °C, then lowering the temperature to 4 °C and stopping the run.

Following reverse transcription, cDNA was stored at −20 °C to be used in real time quantitative polymerase chain reaction experiments (RT-qPCR). RT-qPCR was performed on Applied Biosystems Step-one Real-time using Thermo Scientific Maxima SYBR Green qPCR Master Mix (2X) (Thermo Scientific, Cat. No. K0251), and specific primers for UCA1 with a forward primer 5′-TTCCTTATTATCTCTTCTG-3′ and a reverse primer 5′-TCCATCATACGAATAGTA-3′, HULC with a forward primer 5′-ATCTGCAAGCCAGG-AAGAGTC-3′ and a reverse primer 5′-CTTGCTTGATGCTTTGGTCTGT-3′ and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an endogenous control with a forward primer 5′-GAAGGTGAAGGTCGGAGTCAAC-3′ and a reverse primer5′-CAGAGTTAAAA-GCAGCCCTGGT -3′. Each reaction contained 12.5 μl Maxima SYBR Green qPCR Master Mix (2X), 1 μl forward Primer (50 pmol), 1 μl reverse primer (50 pmol), 0.1 μl ROX Solution, 7.4 μl nuclease free water and 3 μl cDNA. Samples were assayed in duplicates. A no template control (NTC) was performed in each assay . RT-qPCR was programmed as follows: an initial cycle of 95 °C, 10 minutes ; followed by 3 –step cycling: (40 cycles) Denaturation 95 °C, 15 seconds ; Annealing 53°C for UCA1, 63°C for HULC and 65°C for GAPDH gene for 30 seconds and finally Extension step 72 °C for 30 seconds. Melting curve was performed to verify specificity and identity of the PCR products. The fold change between a sample and a normal control for UCA1 and HULC was calculated with the relative quantification method (RQ=2–ΔΔCT). Results were analyzed using The StepOne™ Software.

Statistical analysis (15)

Statistical analyses was performed using IBM Statistical Package for Social Sciences (SPSS) Statistics version 20.0 software. Comparison between different groups regarding categorical variables was tested using Chi-square test. When more than 20% of the cells have expected count less than 5, correction for chi-square was conducted using Monte Carlo correction. The distributions of quantitative variables were tested for normality. For normally distributed data, comparison between more than two population were analyzed F-test analysis of variance (ANOVA) to be used and Post Hoc test (Tukey. For abnormally distributed data), Kruskal Wallis test was used to compare between different groups and pair wise comparison was assessed using Mann-Whitney test. Significance of the obtained results was judged at the 5% level (p value ≤ 0.05). The Pearson's correlation coefficient was used to calculate correlations. The diagnostic value for differentiating between HCC patients and the control was shown by receiver operating characteristic (ROC) curve, calculated as area under the curve (AUC).

Results

The studied groups were matched as regard age and sex, furthermore demographic and clinical data were done but not shown .

The serum level of AFP in late stages of HCC (IB) ranged between 4.6 and 1253 ng/ml with a mean of 480.4 ± 481.7 ng/ml, while in early stages of HCC (IA), it ranged between 13.5 and 180 ng/ml with a mean of 41.49 ± 48.84 ng/ml. In cirrhotic group (II), it varied from 4.5 to 170 ng/ml with a mean of 30.66 ± 40.96 ng/ml. However, in healthy subjects (group III), the range of serum AFP level was between 4 and 5.6 ng/ml with a mean value of 4.76 ± 0.40 ng/ml. The mean of AFP level was significantly higher in patients with late stages of HCC, early stages of HCC and cirrhosis compared to healthy subjects ($p < 0.001$). AFP was also significantly higher in patients with late stages of HCC than patients with cirrhosis ($p = 0.004$) . but no significant difference between patients with early stages of HCC and cirrhosis ($p = 0.278$) .

The mean of UCA1 expression in serum was significantly high in patients with late stages, early stages HCC and cirrhosis compared to healthy subjects ($p < 0.001$). Although the mean of UCA1 expression showed no significant difference between patients with late stages and early stages of HCC ($p= 0.403$), it was significantly higher in patients with late stages of HCC than patients with cirrhosis($p =0.001$) and also UCA1 was significantly higher in patients with early stages HCC than cirrhotic patients ($p =0.017$) . On the other hand, the mean relative expression of HULC in serum was significantly high in patients with late stages, early stages HCC and cirrhosis compared to healthy subjects ($p < 0.001$). The mean of relative expression of HULC was significantly higher in patients with late stages of HCC than cirrhotic patients ($p2<0.001$), but there were no significant difference between patients with late stages and early stages of HCC and also no significant difference between patients with early stages of HCC and cirrhotic patients ($p =0.069$ and $p =0.090$ respectively) (**Table 1**) .

Table (1): Relative expression($2^{-\Delta\Delta Ct}$) of serum UCA1 and HULC in cirrhotic patients with and without HCC and healthy subjects.

$2^{-\Delta\Delta Ct}$ UCA1	HCC		Cirrhosis(II) (n = 20)	Healthy subjects (III) (n = 20)	H	p
	Late(IB) (n = 20)	**Early(IA) (n = 20)**				
Min. – Max.	2.59 – 69.56	2.95 – 41.51	1.18 – 13.12	0.22 – 4.63	52.172*	<0.001*
Mean ± SD.	31.44 ± 24.34	14.23 ± 9.25	5.69 ± 2.74	1.33 ± 1.06		
Median	23.24	12.20	5.34	1.05		
U ($p_{control}$)	<0.001*	<0.001*	<0.001*			
Sig. bet. grps.	p_1=0.403,p_2=0.001*,p_3=0.017*					
$2^{-\Delta\Delta Ct}$ HULC						
Min. – Max.	2.03 – 83.57	1.48 – 50.57	1.52 – 16.46	0.27 – 2.65	51.186*	<0.001*
Mean ± SD.	31.99 ± 27.07	13.59 ± 13.78	4.73 ± 3.62	1.22 ± 0.70		
Median	23.92	8.76	3.39	1.23		
U ($p_{control}$)	<0.001*	<0.001*	<0.001*			
Sig. bet. grps.	p_1=0.069,	p_2<0.001*,	p_3=0.090			

UCA1: urothelial carcinoma-associated1

HULC: highly upregulated in liver cancer

HCC: hepatocellular carcinoma

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 different groups

$p_{control}$: p value for comparing between control and each other group

p_1: p value for comparing between late and early stages of HCC

p_2: p value for comparing between late stages of HCC and cirrhosis

p_3: p value for comparing between early stages of HCC and cirrhosis

*: Statistically significant at $p \leq 0.05$

$2^{-\Delta\Delta Ct}$: relative expression

Furthermore the median serum UCA1 and HULC expression was significantly higher in patients with HCC than cirrhotic patients (p <0.001) and also AFP was significantly higher in patients with HCC than cirrhotic patients (p = 0.003) (**Table 2**)

Table (2): Relative expression($2^{-\Delta\Delta Ct}$) of serum UCA1 and HULC and serum AFP in cirrhotic patients with and without HCC.

	HCC (n = 40)	Cirrhosis (n = 20)	U	p
$2^{-\Delta\Delta Ct}$ UCA1				
Min. – Max.	2.59 – 69.56	1.18 – 13.12	133.0*	<0.001*
Mean ± SD.	22.83 ± 20.16	5.69 ± 2.74		
Median	15.03	5.34		
$2^{-\Delta\Delta Ct}$ HULC				
Min. – Max.	1.48 – 83.57	1.52 – 16.46	154.0*	<0.001*
Mean ± SD.	22.79 ± 23.16	4.73 ± 3.62		
Median	13.38	3.39		
AFP (ng/ml)				
Min. – Max.	4.60 – 1253.0	4.50 – 170.0	210.5*	0.003*
Mean ± SD.	260.97 ± 404.5	30.66 ± 40.96		
Median	34.15	19.50		

$2^{-\Delta\Delta Ct}$ **UCA1**: relative expression urothelial carcinoma-associated1

$2^{-\Delta\Delta Ct}$ **HULC**: relative expression highly upregulated in liver cancer

HCC: hepatocellular carcinoma

AFP: alpha-fetoprotein

U: **Mann Whitney test**

p: p value for comparing between the studied groups

*: Statistically significant at $p \leq 0.05$

The diagnostic performance of serum UCA1 and HULC expression levels to detect cirrhosis was done using ROC curve and it showed that UCA1 had a sensitivity of 90% and a specificity of 95% at the cut-off value more than 3.08, while HULC had a sensitivity of 90 % and a specificity of 85% at the cut-off value more than 1.92 and the AUC were 0.960 and 0.945 respectively.

In addition, the diagnostic performance of serum UCA1 and HULC relative expression levels to diagnose early HCC from cirrhosis were performed by ROC curve that showed that the sensitivity of serum UCA1 in detecting early HCC has been estimated to be 85% while its specificity has been shown to be 80% at the cut-off value of > 6.55($p < 0.001$). While HULC had a sensitivity of 60% and a specificity of 70% at the cut-off value of >5.03($p = 0.019$).When combined together, they had a sensitivity of 70% and a specificity of 85% with the AUC was equal to 0.913 ($p < 0.001$). (**Fig.1**)

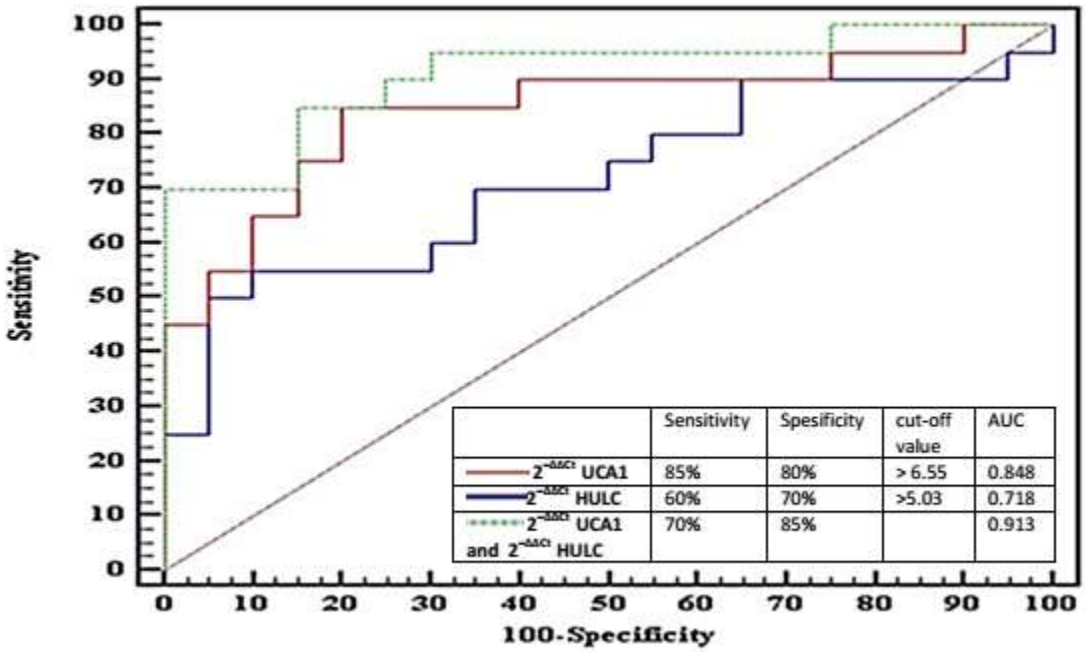

Figure (1): ROC curve for UCA1 and HULC to detect Early HCC cases from cirrhotic cases

Furthermore, ROC curve showed that relative expressions of serum UCA1and HULC can significantly differentiate between HCC and cirrhotic patients. At cut-off value of >6.6, UCA1 showed sensitivity of 82.5% and specificity of 80% with the AUC was 0.834 (p <0.001).While the sensitivity and specificity of HULC were 77.5% and 65% respectively at cut-off value of >4.2 and the AUC was 0.808 (p <0.001).Whereas AFP showed sensitivity of 65% and specificity of 60% with the AUC was 0.737 (p =0.003) Figure (2). While combined UCA1 and HULC the sensitivity and specificity were 85% and 75% respectively and the AUC was 0.915 in addition, combined UCA1and AFP the sensitivity and specificity were 90% and 75% respectively with AUC was 0.900, while HULC and AFP the sensitivity and specificity were 75% and 60 % respectively with AUC was 0.821.

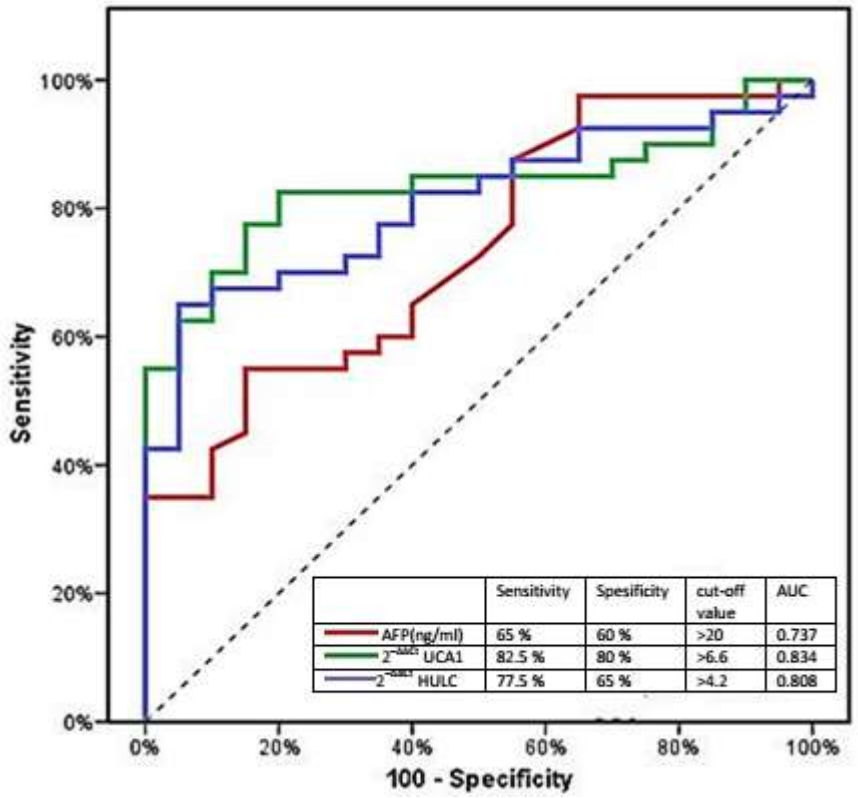

Figure (2): ROC curve for AFP, UCA1 and HULC to discriminate HCC patients from cirrhotic patients

In addition, There was a significant positive correlation between both serum IncRNA UCA1,HULC relative expression and stages of HCC (p =0.031,P=0.016 respectively) **Figure (3).**

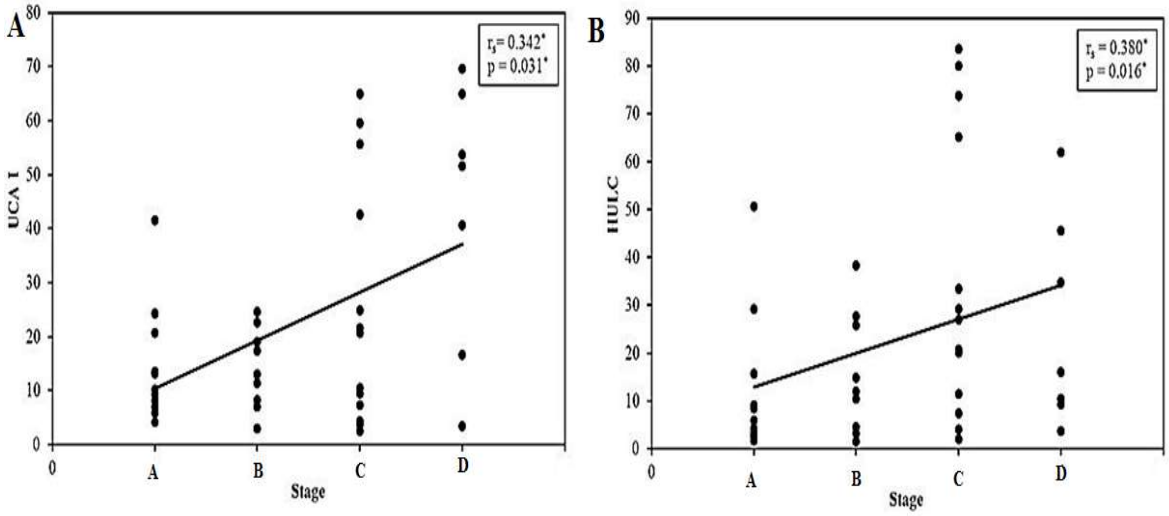

Figure (3): Statistical correlation of relative expression of serum UCA1 and HULC with stages of HCC

Discussion

The prognosis of HCC is mostly poor, due to detection at late stages. Potentially curative therapy is limited only for cases with early stages of HCC.(16) Unfortunately the sensitivity of AFP is low particularly in detection of early-stage HCC.(6) For this reason more effective surveillance strategies should be used for early detection of HCC targeted to the population at risk. lncRNAs have emerged recently as a potential useful player regarding this respect. Cumulative evidence suggests that lncRNAs play crucial roles in tumorigenesis and metastasis. Among the large number of screened lncRNAs, UCA1 and HULC have been paid special attention due to their relationship with malignancies.(8)

Urothelial carcinoma-associated 1 proposed to induce cell proliferation and migration and confer drug resistance. Subsequent researches revealed UCA1 overexpression and its role as an oncogene in various cancers, such as prostate cancer, gastric cancer, breast cancer, colorectal cancer, pancreatic cancer, and osteosarcoma.(17) On the other hand HULC is specifically expressed in hepatocytes, and aberrantly up-regulated in a various human cancers as osteosarcoma, pancreatic cancer, gastric cancer and hepatic metastasis of colorectal cancer.(18) The present study was designed to evaluate the possible significance of serum UCA1 and HULC as biomarkers for diagnosis of HCC.

In the current study the mean of relative expression of UCA1 in serum was significantly higher in patients with late stages and, early stages HCC than healthy subject. It was also significantly higher in patients with HCC than cirrhotic patients. Indeed, There was also a significant positive correlation between relative expression of UCA1 and stages of HCC. Numerous researches suggest that UCA1 play a fundamental role in some cancers development and progression. Wang et al.(2015)(19) found that up-regulation of UCA1 contributed to progression of HCC by means of binding and inactivation of micro RNA-216b and activation of fibroblast growth factor receptor 1 (FGFR1)/extracellular signal–regulated protein kinase (ERK) signaling pathway. In addition, Zheng et al (2018)(9) showed that increased serum UCA1 expression is correlated with high tumor grade and advanced TNM stage and may be useful as a diagnostic and prognostic factor for HCC. UCA1 can cause tumor progression through multiple mechanisms in diverse types of cancer. UCA1 may act as a sponge that bind and inhibit several tumor suppressor microRNAs, such as miR-204-5p, miR-184, and miR-182. Some signaling pathways that are linked to tumor progression, as AKT/mTOR and p27Kip1/CDK2 signaling pathways have also been identified.

Xiao et al. (2017)(20) also reported that over expression of UCA1 increased epithelial–mesenchymal transition (EMT) in HCC via sponging to miR-203 and as a result activating the up regulation of transcription factor Snail2. In contrast to the present study Li et al (2015)(21) demonstrated that no significant difference of UCA1 level was observed between HCC patients and the control group.

In the present study the mean of relative expression of HULC in serum was significantly higher in patients with late stages and early stages HCC than healthy subjects. It was also

significantly higher in patients with HCC than cirrhotic patients, There was also a significant positive correlation between relative expression of HULC and stages of HCC. In agreement with the current study, Xie et al.(2013)(14) revealed that plasma level of HULC could be a promising new biomarker for diagnosing HCC after it was detected by qRT-PCR at a higher frequency in the plasma of HCC patients than the healthy controls . Li et al (2015) (21) demonstrated that the plasma levels of HULC and Long intergenic non-coding RNA 00152(Linc00152) increased dramatically in HCC patients more than the healthy control group . Both HULC and Linc00152 plasma levels indicate a significant predication of tumor growth and metastasis of HCC, the combination of HULC, Linc00152 and AFP would obtain better accuracy in diagnosis.

Li et al (2016) (22) showed similar results that HULC expression in HCC tissues was significantly higher than that observed in normal liver tissues. They also demonstrated that HULC expression in HCC tissues was significantly correlated with clinical stage in HCC, as based upon clinicopathological analysis and the increase level of HULC may be linked to the development and progression of HCC in the majority of HCC patients. These results similar to other findings which reveal that HULC is correlated with tumor progression in pancreatic and gastric cancers.(23, 24) Gandhy et al reported that HULC which is regulated by specificity protein -transcription factor, was important for development of liver cancer, progression and EMT and they proposed that medication such as metformin that down regulate Sp and HULC may be beneficial in treating HCC patients.(25)

Li et al (2016)(22) also found that miR-200a-3p was over expressed in HULC low HCC tissues, while Zinc finger E-box-binding homeobox (ZEB1) messenger RNA (mRNA) was down regulated. And there was a negative correlation between HULC expression and miR-200a-3p in HCC tissues. In addition miR-200a-3p level was negatively correlated with ZEB1 mRNA expression in HCC samples. Therefore, they hypothesized that HULC might promote angiogenesis as related to changes in ZEB1 and miR-200a-3p. More over Sun et al (2015)(26) showed that increase HULC expression was linked to poor pathological and clinical consequence in pancreatic cancer, osteosarcoma and gastric cancer.

Hammerle et al (2013)(27) found that HULC was overexpressed in human HCCs than normal liver tissues. In consistent with the current study the up-regulation was most prominent in low-stage HCC and progressively decreased along advancing tumour stages . HULC up-regulation was also more noticeable in well-differentiated than poorly differentiated HCC . Also, Yang et al (2015)(28) revealed that high HULC expression was associated with less vascular invasion and better overall survival of HCC patients .

In the present study ROC curve analysis applied to assess the diagnostic performance of serum UCA1 and HULC expression levels to diagnose early HCC from cirrhosis; demonstrated that serum UCA1 had a sensitivity of 85% and a specificity of 80% at the cut-off value >6.55 for detection of early HCC. While HULC at the cut-off value >5.03 showed 60% sensitivity and 70% specificity in detecting early HCC. When combined together, the detection sensitivity and specificity were 70% and 85 % respectively and the AUC was equal to 0.913. Consistent with the present study, Kamel et al.(2016)(29) demonstrated that lncRNA-UCA1 level in the serum of

HCC patient was significantly over expressed in patients with HCC than chronic hepatitis C virus (HCV) patients and healthy controls. Serum and tissue levels of this gene were highly correlated, and combining lncRNA-UCA1 with serum AFP had 100% sensitivity. In agreement with the existing study, Li et al. (2015)(21) also revealed that circulating HULC was significantly over expressed in plasma samples from HCC patients using qRT-PCR, with AUC of 0.78.

In the current study the median of UCA1and HULC expression in serum was significantly higher in patients with cirrhosis than healthy subject and the diagnostic performance of serum UCA1 and HULC expression levels to detect cirrhosis was done using ROC curve and it showed that UCA1 had a sensitivity of 90% and a specificity of 95% at the cut-off value of 3.08, while HULC had a sensitivity of 90 % and a specificity of 85% at the cut-off value of 1.92 . Zhu et al (2019)(30) showed that decrease expression of UCA1 could inhibit liver injury in rats with liver cirrhosis. And they demonstrated also the role of HULC in liver cirrhosis. It is suggested that the expression of HULC is up-regulated in liver tissues of rats with liver cirrhosis; and down regulation of HULC reduced the contents of aspartate transaminase activity and alanine transaminase activity in serum of rats, inhibited liver tissue lesions and liver fibrosis in rats, and suppressed apoptosis of hepatocytes (lower expression of caspase-3 and Bax as well as higher Bcl-2 expression) in rats with liver cirrhosis. Also Shen et al (2019)(31) found that HULC up regulation is associated with progression of non-alcoholic fatty liver disease (NAFLD) and revealed that inhibition of lncRNA HULC ameliorate hepatic fibrosis and reduce hepatocyte apoptosis in rats with NAFLD by inhibiting the MAPK signaling pathway, suggesting HULC and UCA1 could be used as important targets for the treatment of liver.

Thus relative expressions of serum UCA1 and HULC might be valuable biomarkers for detection of cirrhosis and diagnosis of HCC from cirrhotic patients and also correlate with HCC stages.

This study is important because of high prevalence of HCC worldwide and the novelty of the work as, until now, very little is known about the effect of long non coding RNA on cancer in general as well as nothing is known about the association of relative expression of both serum IncRNA UCA1 and HULC and development of HCC . And they may be promising biomarkers for early diagnosis and prognosis of HCC. On the other hand, some limitations of the current study need to be addressed. First of all, the study subjects were from hospitals and may not be representative of the general population and restricted to Alexandria city, so it does not permit extrapolation of the results to other populations in other areas. Second, the sample size of this study is relatively small, which may not have enough statistical power to explore the true association. Therefore, large population- based prospective studies with ethnically diverse population are warranted to further elucidate the impact of relative expression of both serum IncRNA UCA1 and HULC on HCC.

Conclusion

The results of this study suggest that serum UCA1 and HULC could be novel biomarkers in diagnosis of early stages of HCC. Also their relative expression in the serum is significantly associated with tumor stages .

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