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Original Article

Evaluation of lncRNA-HULC Level in the Plasma of Chronic HCV-Infected Patients

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Abstract

Background and Aims: The main role of ncRNAs (non-coding RNAs) is to regulate various cellular activities. LncRNAs (long non-coding RNAs) are a group of ncRNAs that are over 200 base pairs in length. It has been shown that lncRNAs regulate and control various cellular functions. Disruption of the expression of lncRNAs can cause various disease and deficiency in the cell function. LncRNA-HULK is one of lncRNA, which is greatly increased in liver disorders, including hepatitis C. Recently, the use of HULK as a biomarker has been suggested as a prognostic factor for liver disease such as hepatocellular carcinoma. Therefore, this study aimed to investigate the level of lncRNA-Hulk in chronic HCV-infected patients.

Materials and Methods: The present study included 50 patients with chronic hepatitis C. After transferring the samples, total RNA was extracted and the quantity of HCV-RNA and lncRNA-HULK were determined using the real-time PCR assay. Finally, the relationship between HCV-RNA and lncRNA-HULK levels was evaluated.

Results: Of the total patients, 13 were female and 37 were male. All patients were HIV Ag/Ab and HBs Ag negative. Results showed that HCV-RNA level was 4,500-2,300,000 copies per mL of plasma. In addition, threshold cycles of lncRNA-HULK were calculated 28-38. Statistical analyses showed that there was a significant relationship between HCV-RNA level and lncRNA-HULK in the plasma of chronic patients.

Conclusion: In the recent study, the relationship between HCV-RNA quantity and lncRNA-HULC level in chronic hepatitis C patients was investigated. It is suggested that lncRNA-HULC levels could be considered as a biomarker in such patients. Accordingly, lncRNA-HULC quantification could be utilized to predict the progression of liver disease and the outcome in chronic HCV-infected patients.

Keywords: non-coding RNA, lncRNA, HULC, HCV, chronic

Introduction

HCV (hepatitis C virus) is a small, enveloped, single-stranded RNA virus that belongs to the Flaviviridae family (1).

According to the WHO (World Health Organization), about 71 million people are annually infected with HCV and it causes 0.4 million deaths every year.

Currently, only 15-20% of infected people are aware of their disease, and few informed people have been treated.

Cirrhosis and HCC (hepatocellular carcinoma) can be caused by CHC (chronic HCV) infection (2, 3). Two biochemical markers in the blood that frequently are used to diagnose liver tissue injury are AST (aspartate amino-transferase) and ALT (alanine aminotransferase). AST is not only specific to liver damage because is expressed in the cytosol of red blood cells and muscles besides the mitochondria of the liver. While increased plasma ALT activity is associated with liver disorders because its levels are lower outside the liver (4).

Therefore, the use of lncRNAs as a biomarker can play an important role in the accurate and easier diagnosis of liver injury (5).

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Table 1. Sequences of primers and probe selected from the study of Panzitt et al.	
HULC-F primer	5'-ATCTGCAAGCCAGGAAGAGTC-3'
HULC-R primer	5'-CTTGCTTGATGCTTTGGTCTGT-3'
TaqMan Probe	5'-HEX-CCAGACCATGCAGGAACTCTGATCGTGGAC-BHQ-3'

LncRNAs (long non-coding RNAs) are a class of non-coding RNAs longer than 200 bp that are involved in many cellular processes by interfering with transcriptional and post-transcriptional regulation (6). So, this indicates their effect on the pathogenesis of cancer and other diseases. For a variety of reasons, lncRNAs can be used as biomarkers in the diagnosis, control of disease, and therapy with molecular targeting; including a) they are detectable in the serum and plasma. b) They are stable after extraction from the blood for molecular analysis. This means that they are not significantly damaged when subjected to physical reactions (prolonged incubation at room temperature, freezing and thawing cycles), chemical reactions (high or low pH), and biological reactions (RNase). c) Their presence and expression are changed in pathological conditions (7).

One of the lncRNAs is HULC (highly up-regulated in liver cancer) which was identified for the first time in patients with HCC as the highest lncRNA by Panzitt et al in 2007.

HULC with 500 nucleotides in long is located on chromosome 6p24.3 and have two non translation exons (8, 9). Many studies suggest that numerous features of HULC are involved in the initiation of growth and metastasis of HCC cells (10).

Our main goal in this research was an evaluation of the relation between lncRNA-HULC level and HCV-RNA quantity in the plasma of chronic hepatitis C patients.

Methods

Samples and patients: The present study included 50 patients with chronic hepatitis C. These patients were confirmed and referred by the gastroenterologist. Patients samples were collected in tubes containing EDTA, The samples were sent to the laboratory of hepatitis and AIDS department of the Institute Pasteur of Iran. Immediately after receiving the blood samples, the plasma was separated and kept at -80°C until the next step.

HCV real-time PCR: Total RNA was extracted using the High Pure RNA Isolation Kit (Roche, Germany) according to the kit instructions and stored at -80°C. In the next step, the plasma HCV-RNA level was quantitation. This step was carried out using the GeneProof HCV PCR kit (GeneProof, Czechia) according to the kit manual.

This kit did not require cDNA synthesis separately and the cDNA synthesis step and real-time PCR were performed in one-step.

Each test run included negative control and the tests were performed in duplicate.

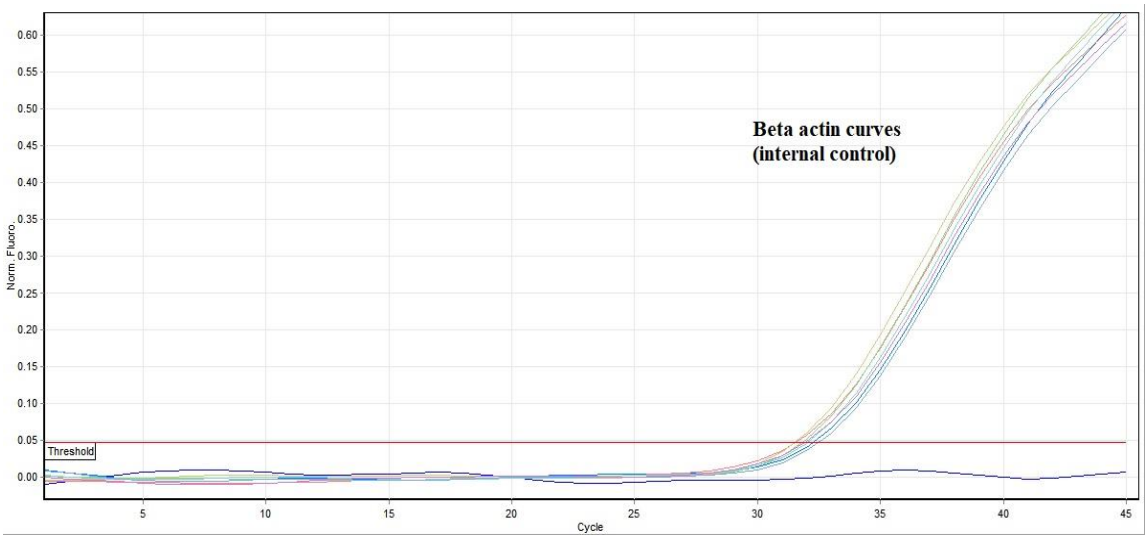
LncRNA-HULC real-time PCR: Because a commercial kit was not available to determine the level of LncRNA-HULC, it was decided to prepare the primer and probe sequence from the previous studies as a home-brew test and use the obtained C_T (threshold cycle) to evaluate the HULC level. The concentration of nucleic acid is inversely related to the number C_T. TaqMan real-time PCR was used to evaluate HULC level. Sequences of primers and probe were selected from the study of Panzitt *et al*. (Table 1). The primers and probe were made by Bioneer (Korea). The β-actin gene was used as an internal control to confirm the RNA extraction steps and the accuracy of the real-time PCR assay. qPCRBIO Probe 1-Step Master Mix (PCR Biosystems, UK) was used for real-time PCR assay. cDNA synthesis and real-time PCR were performed in one-step. Each test run included negative control and the tests were performed in duplicate.

Results

From the total patients, 13 were female and 37 were male. Patients ranged in age from 19 to 67 years. Patient characteristics were summarized in the Table 2. All patients in this study were negative for HIV and HBsAg.

Table 2. Summarized characteristics of the studied patients					
Clinical status	Sex (Female/Male)	Age range (year)	ALT (IU/L)	LncRNA-HULC level (Threshold cycle)	HCV-RNA level (copies/ml of plasma)
Chronic hepatitis C	13/37	19-67	10-300	28-38	4,500– 2,300,000

Fig. 1. Real-time PCR amplification plot of some internal controls. The CT values of the internal controls were 25-35.



After RNA extraction, the purity of the isolated RNA was measured by NanoDrop Spectrophotometer (Thermo Scientific, USA).

The purity of extracted RNA and nucleic acid quality was acceptable and the ratio of OD_{260}/OD_{280} was determined to be 1.8-2.0. A one-step real-time PCR assay was used to determine the quantity of HCV-RNA.

In this study, the amount of HCV-RNA was determined between 4,500-2,300,000 copies per mL of serum. Internal control C_T was 25-35, thus the accuracy of RNA extraction and the absence of PCR inhibitory were confirmed. The amplification plot of real-time PCR of some samples can be seen in Figures 1, 2, and 3. DNase-free water was used for the negative control reaction and the absence of PCR contamination was confirmed. As mentioned, all reactions were performed in duplicate and the C_T mean was considered. According to the kit manual, the absolute quantification method was used to determine the HCV-RNA level of the samples and the efficiency of each run was above 90% and R2 was about one.

One-step real-time PCR test and TaqMan method were used to measure lncRNA-HULC level. At this step, Huh-7 (human hepatoma) cells were utilized as a positive control. The C_T number of the positive control was 15.

Huh-7 is a human cell line derived from liver cancer that has a high level of lncRNA-HULC expression. The C_T values of the lncRNA-HULC were 28-38. In this case, by increasing the concentration of lncRNA-Hulk, the number C_T decreases, or in other words, by increasing the concentration, detection of fluorescence due to the amplification of RNA (cDNA) is done earlier.

The quality of RNA extraction and the absence of the PCR inhibitors were also confirmed by the β-actin gene as an internal control.

Statistical analysis showed that there was a significant relationship between HCV-RNA quantity and lncRNA-HULC level (p-value <0.05) in the chronic HCV-infected patients.

Fig. 2. Real-time PCR amplification plot of HCV-RNA quantification of some samples.

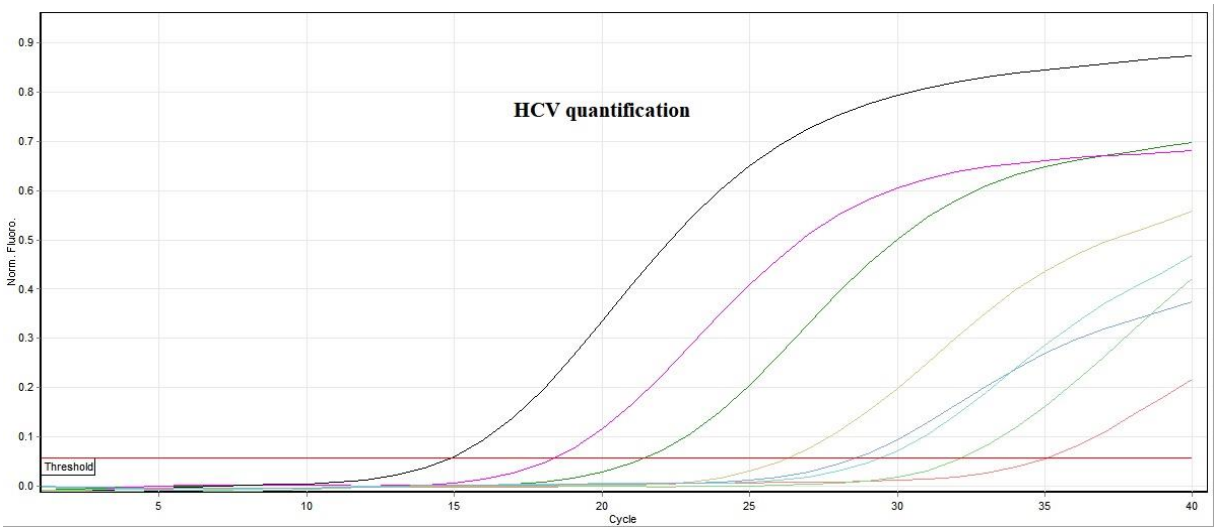
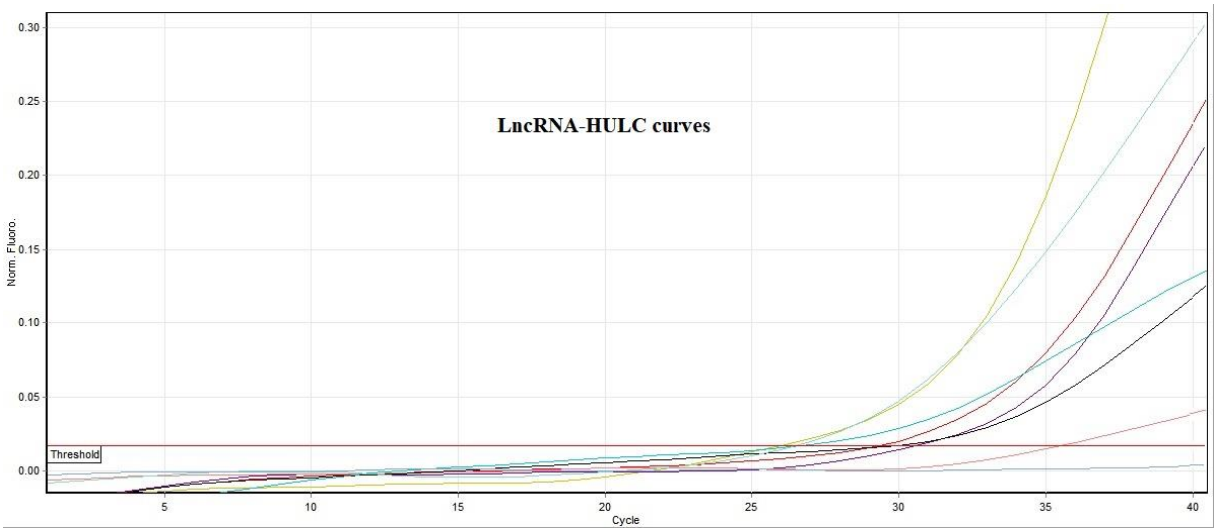


Fig. 3. Real-time PCR amplification plot of lncRNA-HULC quantification of some samples. The CT values of the lncRNA-HULC were 28-38.



Discussion

Recent progress in sequencing technology, genome, and transcription analysis has demonstrated that more than 90% of the human genome is transcribed. While only, nearly 2% of RNAs are translated into proteins and more than 98% are non-coding RNAs (ncRNA).

One class of the ncRNAs is lncRNAs which play a vital role in regulating gene expression (11). Hitherto, more than 3,000 lncRNAs have been identified and a small number of them have important role in many diverse biological processes, such as cell proliferation, differentiation, cell cycle, apoptosis and invasion, marker of cell fate and parental imprinting (9). Reports have shown that level of lncRNAs increase in many cancers (such as breast, colorectal, pancreatic, and HCC) and diseases (diabetes, cardiovascular, neurological disorders, and autoimmune diseases) (12).

HULC is one of the lncRNAs that have over-expression in HCC and colorectal cancer that metastasized to livers compared to healthy individuals (9). Up regulation of HULC was associated with survival, lymph node metastasis, metastasis and tumor stage (13).

In this research, we studied the level of HULC in the blood of chronically HCV infected patients and the relationship between HCV-RNA level and lncRNA-HULC with ALT enzyme.

It has been reported earlier that downregulation of HULC reduced ALT and AST in the rat serum which was associated with inhibition of liver lesions and fibrosis (14). Our results also showed a direct relationship between ALT activity and HULC level that was similar to the results reported by Shaker et al (15).

We used one-step real-time PCR test and TaqMan method for detected lncRNA-HULC level that was similar to the method research of Zhang et al, while is different from the method in other studies (6).

The results of our research are showed that HCV infection increases the level of HULC expression like Huh-7 cell line that has a high level of HULC expression. Even though, previous studies on HCV-related HCC and lncRNA-HULC are scarce (16) but this data was similar to the results of Sharma et al which reported overexpression of HULC by HCV in a cell culture model (17). Also, our results are supported by Kitabayashi et al and Sharma et al (17, 18). While in some studies it has been reported no significant relationship (6).

Although the number of samples was limited, important results were obtained from the research. In this study, the relationship between HCV-RNA quantity and lncRNA-HULC level in chronic hepatitis C patients was investigated.

Conclusion

Since the relation of elevated lncRNA-HULC level with liver disease has been investigated, it is suggested that lncRNA-HULC levels could be considered as a biomarker in such patients.

Accordingly, lncRNA-HULC quantification can be utilized to predict the progression of liver disease and worsening in chronic HCV-infected patients.

Acknowledgment

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Conflict of interest

No conflict of interest is declared.

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