

Evaluation of miR-101 Level in Patients with Chronic Hepatitis B Virus Infection and Liver Cirrhosis

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ABSTRACT

Background and Objective: MiRNAs are small RNAs that are expressed in most eukaryotes, and can regulate gene expression by attaching to the 3' end of target mRNA. MicroRNA-101 (miR-101) post-transcriptional regulation is important for host-virus interactions. In addition, miR-101 has a tumor suppressive role in liver cancer and metastasis, and induces apoptosis in tumor cells. We examined miR-101 expression in patients with chronic hepatitis B, hepatitis B virus (HBV)-associated cirrhosis and healthy individuals.

Methods: The study was performed on 108 whole blood samples (36 samples from each group) collected in EDTA tubes. RNA was extraction by RNX-plus kit according to the manufacturer's protocol. Finally, miRNA expression was evaluated using relative real time PCR.

Results: A 2.4-fold increase was observed in miR-101 expression in patients with chronic hepatitis B, while there was a 3.5-fold increase in miR-101 expression in patients with HBV-associated cirrhosis compared with healthy controls ($P=0.003$). MiR-101 overexpression in patients with HBV-associated cirrhosis was more notable than in patients with chronic hepatitis B.

Conclusion: According to the results, evaluating miR-101 expression may predict disease progression from chronic hepatitis B to HBV-associated cirrhosis.

Keywords: MicroRNAs, Chronic Hepatitis B, Liver Cirrhosis, MiR-101.

INTRODUCTION

Hepatitis B infection is an inflammatory liver disease and a global health problem. The disease is caused by hepatitis B virus (HBV), a member of the *Hepadnavirus* family (1-4). Hepatitis B infection has various clinical presentations, ranging from a small infection to acute hepatitis, fulminant hepatitis and chronic hepatitis that leads to fibrosis, cirrhosis and hepatocellular carcinoma (4-6). Chronic hepatitis B is characterized by presence of HBsAg in serum for more than six months (7-10).

MicroRNAs (miRNAs) are small RNAs (about 22 nucleotide long) that are expressed in most eukaryotes, regulating gene expression via attachment to the 3' end of target mRNA (11-13). So far, more than 1000 mammalian miRNAs have been identified. MiRNA is also involved in complementary processes of metabolism, cell proliferation, apoptosis, gene expression, brain morphogenesis and stem cell division (14-16). There are some mechanism for suppressing gene expression after transcription by miRNA including suppression of translation initiation, post-translational suppression and removal of polyA tail, which result in CAP removal and destruction of the mRNA (16). Nucleotides 2-8 of the 5' end of the miRNA, called the "seed sequence" are especially important for attachment to the target mRNA. Expression of miRNAs is changed in viral infections. Moreover, both DNA and RNA viruses produce miRNAs in infected host cells, indicating the important role of miRNAs in virus-host interactions (17). MicroRNA-101 (miR-101) is an important post-transcriptional regulating factor for host-virus interactions. In addition, miR-101 has tumor-suppressive role in liver cancer and metastasis, and induces apoptosis in tumor cells (18-23). MiR-101 is transcribed from 31.3p1 (24). Circulating miRNAs have been suggested as diagnostic markers for various types of diseases. It has been thought that serum level of miR-101 can act as a noninvasive biomarker for discriminating HBV-related hepatocellular carcinoma (HCC) from HBV-related liver cirrhosis (LC). MiR-101 targets genes including *COX-2*, *Mcl-1*, *EZH2* and *FOS* (23-26). In addition, studies show that miR-101 controls expression of a set of targeted genes such as *Stathmin*, *c-jun*, and *C-X-C chemokine receptor type 7* (25). This

study aimed to determine the association of miR-101 expression with chronic hepatitis B and HBV-associated cirrhosis in Golestan Province, Iran.

MATERIAL AND METHODS

The study was performed on 108 complete blood samples (54 men and 54 women) collected from patients and healthy controls. The patients were divided into two groups of patients with chronic hepatitis and patients with HBV-associated cirrhosis. First, 5 ml whole blood samples was taken from each participant, and then collected in EDTA tubes. RNA was extracted from 2 ml whole blood using RNX-plus kit (Sinaclon, Iran) according to the manufacturer's protocol. Briefly, 1 ml of RNX-PLUS solution was added to 200 μ l of chloroform on ice. The mixture was centrifuged at 12000g at 4 °C for 15 min. While the middle phase was intact, equal volume of isopropanol was added to the mixture.

After centrifugation, the supernatant was removed, and 1 ml of 75% ethanol was added to the mixture. Then, the supernatant was removed and the sediment was dried at room temperature. Concentration of the RNAs extracted was measured by biophotometer. The optical density at 260/280 nm was 1.6-2.0, which was acceptable according to the RNX-plus kit.

MiRNA expression was evaluated by relative real time PCR. After removing DNA using DNase, assimilating primary extraction was done using miRNA diagnostic kit (Pars Genome Co.). MiRNA replication was done by relative real time PCR and using mir-Amp kit (Pars Genome Co.) according to the manufacturer's protocol. Thermal cycling conditions were as follows: Initial denaturation at 95°C for 30 seconds, 40 cycles at 60-63 °C for 20 seconds and 72 °C for 30 seconds.

The CT was obtained from Real-Time PCR based on the $\Delta\Delta$ CT method. Expression level of minas was analyzed using REST 2009 Software.

RESULTS

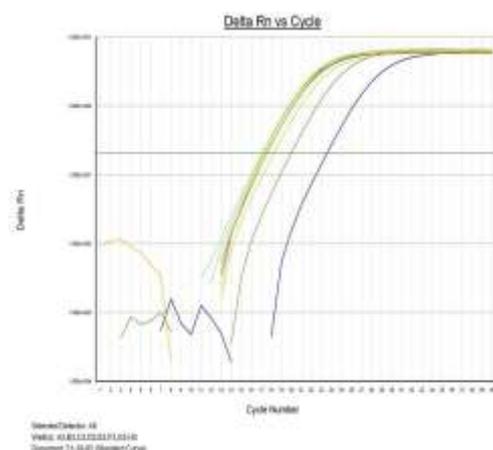
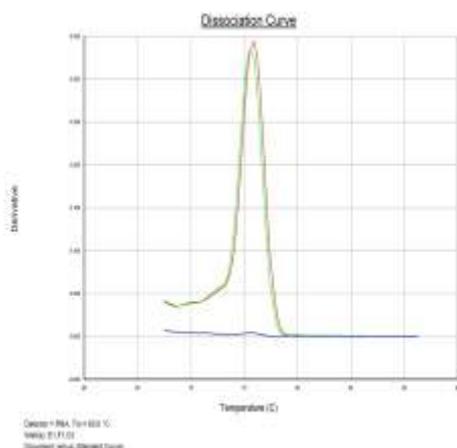
According to the results, miRNA-101 expression is not affected by age and gender in patients with chronic hepatitis B and HBV-associated cirrhosis.

Table 1- MiRNA-101 expression in patients with chronic hepatitis B and HBV-associated cirrhosis and healthy controls based on age and gender

Group	Average	Age	P-value	Average	Gender	P-Value
Chronic hepatitis B	20.3760	<30	.852	25.0919	Male	.240
	30.1850	30-35				
	37.7438	35-40				
	49.1580	40-45				
	17.1400	45-50				
	17.0017	50>				
HBV-associated cirrhosis	12.86600	< 30	.783	35.3931	Male	.145
	14.89000	30-35				
	8.06250	35-40				
	22.10650	40-45				
	40.96500	45-50				
	17.49000	50>				
Control	9.5433	<30	.874	11.543	Male	.855
	16.6333	30-35				
	10.7643	40-45				
	12.2583	45-50				
	12.1200	50>				
	13.9383	40-45				

The rate of expression in patient and control groups was compared. In this study, miRNA-101 expression was examined in 108 samples from patients with chronic HBV infection and HBV-related cirrhosis and healthy controls. Δ CT values (miRNA-101/ U6 gene ratio) was

calculated and the results were evaluated by t-test. MiRNA-101 was significantly overexpressed in patients compared to the control ($P=0.003$). Figures 1 demonstrates the melting curve and amplification plot obtained in the real-time PCR experiment.



DISCUSSION

MiRNAs act as key regulators of gene expression in cells. As mentioned previously, HBV is a global health problem. In 2014, Yun Xie et al. found that miR-101 is overexpressed in patients with chronic hepatitis B and HBV-associated cirrhosis, which is consistent with our results. Predicting the risk of disease progression in patients with chronic hepatitis B is of great importance. Studies have reported that serum level of miR-101 relates to HBsAg directly, which is an indicator for viral translation. In addition, miR-101 serum level is important for progression of the disease. Therefore, miR-101 could be considered as a new noninvasive biomarker for identification of the risks of HBV infection (6).

In 2014, Yun Xie et al. investigated the profile of miR-101 expression in serum of patients with chronic HIV-related hepatitis, LC and HCC in Pakistan. They found that miR-101 is significantly overexpressed in patients with HBV-related LC compared to patients with chronic hepatitis B and healthy controls. However, miR-101 expression was suppressed in patients with HBV-related HCC compared to patients with HBV-related LC, chronic hepatitis B and healthy controls. They also investigated 237 samples with qPCR and found that miR-101 expression decreased in patients with HBV-related HCC and increased in patients with HBV-related HCC and LC. They concluded that miR-101 could be used as a noninvasive biomarker for discriminating HBV-HCC from HBV-LC (6).

Study of Jinmai Jiang et al. reported that several miRNAs are overexpressed by two- to three-fold in patients with hepatitis B and HBV-associated cirrhosis. They also reported that Real-Time PCR analysis showed increased level of miR-101 in the patients.

In this study, we found that miR-101 expression increased 2.4-fold and 3.5-fold in patients with chronic hepatitis B and patients with HBV-associated cirrhosis compared to healthy controls. Overexpression of miR-101 in patients with HBV-associated cirrhosis was more notable than that of in patients with chronic hepatitis B. Thus, miR-101 expression

level could be used as a noninvasive biomarker for discriminating HBV-LC from HBV-HCC (19). It can also play an important role in molecular etiology and treatment of cancer (22).

In 2013, Yu Fu et al. found that miR-101 level in patients with HCC was significantly lower than in control group. Moreover, serum level of miR-101 had a negative correlation with the level of miR-101 expression. The mentioned study used Real-Time quantitative reverse transcription PCR for evaluation of miR-101 expression, and suggested that miR-101 expression level is correlated with tumor size, HBV DNA and HBsAg level.

The difference between the results of our study with other studies is due to the fact that most studies have investigated miR-101 expression in patients with HCC, while we evaluated patients with chronic hepatitis B and HBV-associated cirrhosis.

The result of the present study could be used for further investigation of hepatitis B progression. Therefore, the serum level of miR-101 is different depending on the stage of the disease. It is suggested that miR-101 expression be examined in a larger sample size to validate the results of the present study.

CONCLUSION

Various studies have revealed that miRNAs play a key role in viral physiological and pathological processes. The pattern of change in expression of miR-101 in blood and tissue samples of patients with HBV infection differs from that of in healthy individuals. Therefore, miR-RNA could be considered as a therapeutic option for HBV therapy. HBV infection could be a biological indicator and powerful tool for cancer (24).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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