Iraqi Journal of Biotechnology, December 2019, Vol. 18, No. 3, 97-103

Rate of microRNA-101 Level in Sample of Iraqi Patients with Chronic Hepatitis B Virus Infection

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Received: November 17, 2019 / **Accepted:** December 30, 2019 / **Published:** December 31, 2019

Abstract: Hepatitis B virus (HBV) Infection is highly widespread. Chronic hepatitis B infection (CHB) leads to expansion of complications, like cirrhosis, hepatocellular carcinoma (HCC) and death. microRNAs are emerging as biomarkers for liver disease development . this study aims to rated the level of serum miR-101 alteration in patients with CHB and inactive carrier comparing with healthy control. A total of 67 patient's blood samples collected during the period from February 2019 to September 2019 at Hepatology and Gastroenterology Teaching Hospital .The serum miRNA-101 expression were detected using real time PCR. The results showed that the serum miR-101 was downregulated in the chronic HBV and inactive carrier comparing with the healthy subjects but there was no significant difference (P = 0.630) was observed among them.

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

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Introduction

Hepatitis B virus (HBV) infection is the most common contagious diseases, with about 240 million people infected chronically all over the world (1) It is the major a etiological factor that cause acute or chronic hepatitis. Its infection results into well-known and lead to long-term consequence like cirrhosis and hepatocellular carcinoma (HCC) (2). The percentage of cirrhosis and liver tumor promote by HBV infection is 30% and 45% global (3). Therefore, it is very important to recognition virus-host interaction and developed new diagnostic marker that increases the HBV cure rate (4). The hepatitis B virus is a small pathogen transmitted by percutaneous exposure to contaminated blood ,serum or other body fluids. Through the blood , the virus can reaches the liver and infect the liver cells, which are the target cells for infection(5).

HBV is partly double stranded relaxed circular DNA (rcDNA) of 3.2 kb in length with envelope. HBV is member of the Hepadnaviridae family, with a strong preference for hepatocyte . After infecting liver cell, HBV rcDNA is released into the cell nucleus and creates a covalently closed circular DNA (cccDNA), that assist as the template for the transcription of viral RNAs(6). The genome of HBV has four over lapping open reading frames (ORFs). The preS and S region encodes the envelope proteins S (HBsAg) and M (pre-S2), and L (pre-S1), which provide to HBV binding to hepatocyte receptors and release from liver host

cells. The preC and C region encode the hepatitis B core antigen (HBcAg) and hepatitis B e antigen (HBeAg). HBcAg is a polypeptide of the viral capsid, but the function of HBeAg is obscure. The X part encodes the HBV x protein (HBVx), which regulates expression of viral and host gene to promote viral synthesis. The P region encodes viral reverse DNA polymerase, which get involved in DNA replication and RNA encapsidation. There are two enhancers region (enhancer I and II) and four promoters that directing transcription of the HBV(7).

MicroRNAs (miRNAs) are small, noncoding RNAs that regulate gene expression by affecting both the stability and translation of corresponding mRNAs(8). More than 700 human miRNAs have been discovered and all of them are involved in the regulation of various processes, including cell differentiation, apoptosis and development. miRNAs also affect virus replication and infection (9) serum miRNAs use as a new class of powerful markers for noninvasive diagnoses of patients with several diseases (10).

miR-101 is noncoding RNA located on chromosome 1p31.3 region . It is involved in regulation of post-transcriptional gene expression and is known as a main factor in host and virus interactions. (11) . Li et al. found in 2009 at least a two fold downregulation of miRNA-101 in 16 hepatocyte carcinoma HCC samples when compared with matching non-cancerous liver tissues, and the high expression of miRNA-101 suppressed the invasion and migration of cultured HCC cells(12). A genetic diversity in miR-101 and miR-338 can connect the clearance of HBV and progression of disease to the incidence of liver cirrhosis (LC) and hepatocyte carcinoma (HCC) in patients with chronic HBV infection(13).

In this study, the miR-101, was selected which is commonly deregulated and closely correlate with liver disease, to rate impact of this miRNA in inactive carrier (IC), chronic hepatitis (CH) and healthy controls.

Material and methods

This study was conducted from February 2019 to September 2019. A total number of 92 subjects, consisted of 51 chronic patients, 16 inactive carrier, and 25 healthy subjects which were referred to the Hepatology and Gastroenterology Teaching Hospital in the Medical City -Baghdad-Iraq. All the patients were positive for HBs Ag and did not have any other liver diseases, based on clinical reports.

Five mL of peripheral blood sample was withdrawn from each subject and taken in plain tube for serum separation that was used in the detecting of all serological markers(HBeAg, HBs Ag, HBc (Ab) ELISA assay) for HBV as well as RNA quantitation by real time PCR. Serum was stored at –80°C and maintained in 0.75 ml TRIzol reagent then used for micro-RNA extraction and detection of fold change of the microRNAs 101 using real time PCR. RNA was isolated from sample according to the protocol of TRIzol Reagent.

Molecular detection of gene expression of *miRNA-101*

cDNAs for *miRNA-101*and *RNU* (reference gene) were produced using

SYBRR Green, TRIzol® LS Reagent, RT-qPCR system (Promega-USA), primers (Table-1and according to the manufacturer's protocol. Quantus Florometer was used to detect the concentration of extracted cDNA in order to detect the goodness of samples for downstream applications. For 1 µl of RNA or cDNA, 199 µl of diluted QuantyFlour Dye was mixed. After 5min incubation at room temperature in dark place, RNA concentration values were detected. Amplification of *miRNA-101* and *RNU* reference genes was done by Two Step RT-PCR as follow: first step: 16C for 30 min, 42C for 30min, 85C for 5 min and 4C for 10 min for 1 cycle. second step 95C for 5 min for 1 cycle. , 95C for 15 sec, 55C for 30 sec and 72C for 30 sec for 40 cycle.

Table (1): Sequences of primers used for amplification miR-101 and RNU reference gene

Primer	Sequence
RNU -RT	5`-GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACAATCAG-3`
RNU-F	5`-GTGAACTTATTGACGGGCG-3`
RNU- R	5`-GTGCAGGGTCCGAGGT-3`
miR-101-3p-RT	5`-GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACTTCAGT-3`
miR-101-F	5`-GTTTGGGTACAGTACTGTGATA-3`
miR-101-R	5`-GTGCAGGGTCCGAGGT-3`

Results and disscution

The gender and age groups of the patients are summarized in Table 3 and 2. It was found that most of the patients were located within (20-30) year with (33.8%) table 1. These results harmonized with studies done in Iraq as (14) and(15) adduced that the common age group for hepatitis B was in Third decade. In Iraq most infections occur young adults (15-29 years) (16). The infected patients likely will taken antiviral drug and the probability for mutations occurrence will be great in these patients in any age if the treatment continue. also, the ages of many patients who participated in the study extend from 20-30 years, due to the fact that most of the Iraqi population who's infection with HBV are youth and this age is active and more insecure to HBV infections.

Table (2): The patient's age groups.

Age Group	Frequency	Percent%
< 20	10	14.7
20-29	23	33.8
30-39	10	14.7
40-49	7	10.3
50-59	12	17.6
>=60	6	8.8
Total	68	100.0

This study showed (Table 2) that 63.2% of patients males and 36.8% females. The sex distribution of patients in the study was similar to other study in Iraq (13) and (14). This may be related to the fact that males in Iraq generally are more active and more exposed to risk factors than female.

Table (3): Gender distribution of the patients.

Gender	Frequency	Percent%
Male	43	63.2
Female	25	36.8
Total	68	100.0

Expression profile of miRNA-101

To investigate the levels of circulating miR-101 are altered in patients with CHB, inactive carrier and healthy control , the concentrations of miR-101 were measured in the sera from patients and healthy controls using quantitative real-time PCR .Real-time RT-PCR data were quantified depend on Ct values that are inversely associated with amount of starting template so high Ct values parallel with low levels of gene expression, and vice versa Figure (1and 2).

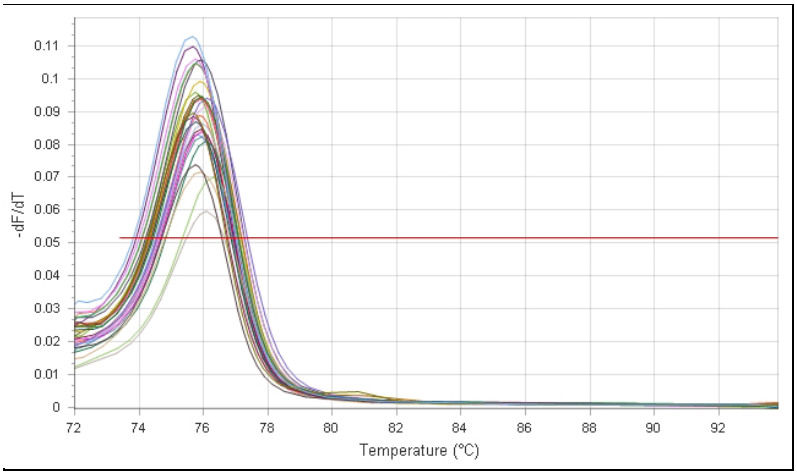

Figure (1): The miR-101 expression Melt on Green Melt from 72°C to 95°C.

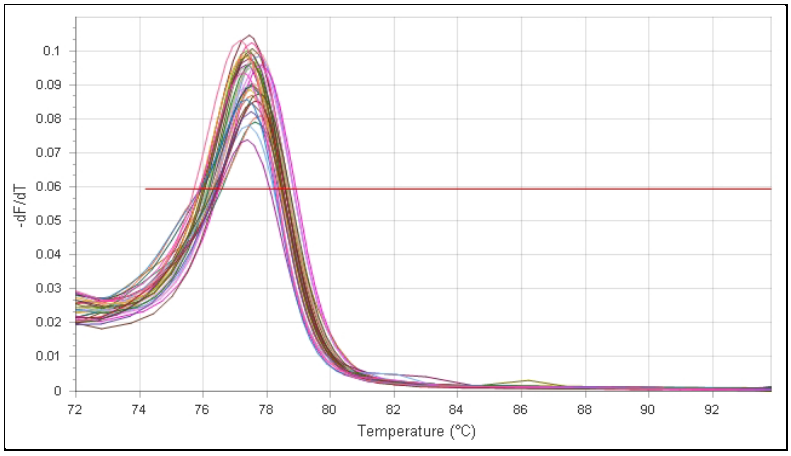

Figure (2): The RNU expression Melt on Green Melt from 72°C to 95°C.

The results showed that The serum miR-101 was downregulated in the chronic HBV and inactive carrier compared with the healthy subjects Figure (3).

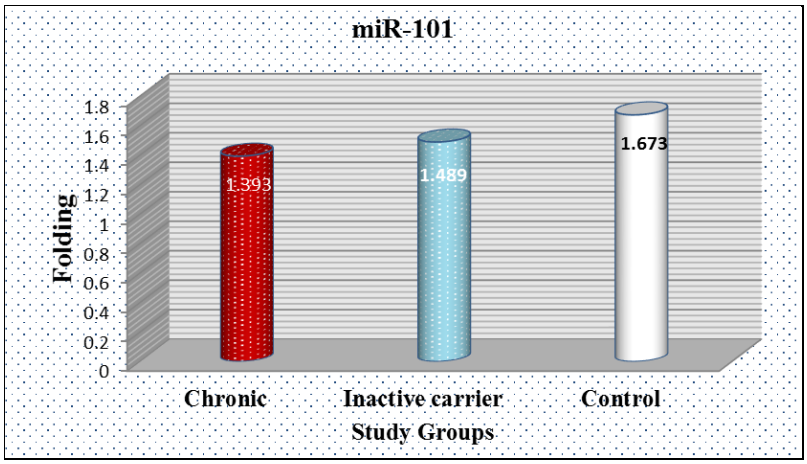

Figure (3): The miR-101 expression in the chronic HBV and inactive carrier compared with the healthy subjects.

No significant difference (*P* = 0.630) was observed in the miR-101 serum levels between the chronic HBV, inactive carrier and control (Table 4).

Table (4): miR-101 serum levels in chronic HBV , inactive carrier and control.

Folding miR-101	Chronic	Inactive carrier	Control	p-value ©
Mean	1.393	1.489	1.673	0.630 N.S
Standard Deviation	1.120	0.894	1.465	
Median	1.631	1.489	1.449	
Standard Error	0.395	0.732	0.293	
Range	4.862	3.106	4.451	
N	51	16	25	

miRNAs have been identified in many fluid of humon body, as in serum and plasma(17), studies have been indicated that serum circulating miRNAs can be use as diagnostic biomarkers for many disease states (18). miRNAs can therefore considered to be representative of certain pathological stats Dueto their accessibility and stability in the circulatory system (19) so that make them ideal biomarkers, particularly for diagnostic diseases in at risk patients (20). but, little is known about the source of miRNAs and the control mechanisms of their biogenesis. It is theorized that miRNAs may enter the blood circulation through secretion from blood cells , tissues and cells that are affected by human disease (19).

The differences in miR-101 expression associated with progression of liver diseases was attentived to detecting could miR-101 serve as a non-invasive diagnostic biomarker. For this purpose, we analysed expression of miR-101 in the serum samples from CHB, inactive carrier and control groups by real-time PCR . In this study, we demonstrated that miR-101 was downregulated in CHB, inactive carrier compared with healthy control. This finding correlates with many studies(21, 22).in 2013, wei et al .demonstrate that HBx can up regulate DNMT3Aactivity by suppress miR-101 expression. However, the mechanism underlying how HBx inhibits the miR-101 expression remains obscure(23).

In adation, the result showed that there was no significant difference in the expression of miR-101 in serum samples from CHB patients compared

with healthy controls , In our view the lack of a difference significance between the CHB and control samples in the present study can be refer to the following causes. First, low staging and grading scores, of CHB patients. second possible explanation is that the transcription factors concerned with inflammation of liver may not be targets of miR-101; the previously reported targets of miR- 101 that are liver-associated transcription factors have been those included in hepatocarcinogenesis and advanced stage of liver diseases.(24, 25) .In 2014, Xie et al. also show no significant difference in the expression of miR-101 in tissue and serum samples from CHB patients compared with healthy controls which is consistent with our results(22).

Conclusion

The findings of current study indicate that althoug the fluctuation in circulating miR-101level during chronic heptitis infection but it cannot be serve as a potential non-invasive biomarker and it can be useful in cas of hepatocarcinogenesis and advanced of stage liver diseases.

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