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The Crosstalk between Interferon and Transforming Growth Factor Beta Signaling Pathways in Hepatitis C Patients

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ABSTRACT

Background: there is an increasing interest in using microRNAs (miRNAs) as biomarkers in liver disease. Diagnostic biomarkers in hepatitis C serve as a great benefit for the early treatment of HCV. **Aim of the Work:** the aim of this study was to evaluate the expression of miRNA-16 in Egyptian patients infected with HCV and their relation to different biochemical and clinicopathological parameters. **Patients and Methods:** twenty-five subjects were chosen, 16 HCV infected and 9 healthy controls. Collection, processing and storage of serum for evaluation of miRNA16 using RT-qPCR was done. We evaluated the power of miRNA as a diagnostic tool using ROC curve analysis. The prognostic significance of the investigated parameters in HCV patients was explored. **Results:** there is a highly significant difference of miRNA 16 expression between HCV patients and healthy controls. miRNA 16 has great sensitivity and specificity for differentiating between patients with HCV and healthy controls. **Conclusion:** miRNA 16 can be used as a potential diagnostic biomarker for HCV. In addition, it could be used for staging of the disease.

Keywords: HCV, miRNA, miRNA 16.

INTRODUCTION

Hepatitis C virus (HCV) infection is by far considered one of the main reasons of chronic liver disease throughout the world. The extended effect of HCV infection ranges from minimal change to extensive fibrosis, cirrhosis, liver cell failure with the risk of progression to hepatocellular carcinoma (HCC) [(1)]. HCV genotype 4 infection is common in the Middle East and Africa, especially northern and sub-Saharan area. According to recent estimates 10.4 million patients are positive for HCV genotype 4 accounting for 13% of all HCV infections worldwide. In Egypt alone, 15% of the Egyptians are sero-positive, 93% of which are infected with genotype 4 [(2)]. MicroRNAs (miRNAs) are small non-coding RNAs that affect the function a wide variety of other genes by inhibiting the translation of their complementary mRNAs. Some miRNAs play an important role in immunity against viral infection. Changes in the levels of hepatic miRNAs have been reported in many liver diseases as HCC and liver fibrosis [(3)]. The miRNA16 family consists of miRNA15a, 15b, and 16. Downregulation of miRNA15a and miRNA 16 is a valuable predictor of HCC spread and patient survival [(4)]. Moreover, the expression levels of miRNA 16 are significantly high in HCV infected patients and in hepatic fibrosis [(5, 6)]. In this study, we tested the hypothesis that miRNA 16 is upregulated in HCV patients in comparison to healthy controls.

AIM OF THE WORK

The aim of this study was to evaluate the expression of miRNA-16 in Egyptian patients infected with HCV and their relation to different biochemical and clinicopathological parameters.

PATIENTS AND METHODS

Enrollment of patients: we recruited patients infected with hepatitis C virus (HCV patients, n = 16) and individuals (controls, n = 9) who were matched with patients for age and gender as possible in the study. he Ethics Committee of Ain Shams Faculty of Medicine, Egypt, approved all procedures involving human material, and all patients signed an informed consent. From November 2016 to June 2017, a total of 25 participants were enrolled into the study (Internal Medicine Department, Faculty of Medicine, Ain Shams University) including 16 patients with HCV infection and 9 healthy controls recruited from interested hospital employees who were excluded from the diagnosis of hepatitis B virus (HBV), HCV, and HCC. We excluded any patient with acute infections, clinically detected jaundice, symptoms of liver cell failure, HBV and HCC. Male and female participants were included with an age range of 20–40 years (median, 35 years). Clinical characteristics of patients were retrieved from the clinical records available and were assessed retrospectively (Table 2). In addition, the clinicopathologic parameters were collected such as gender, smoking, HBV surface antigen, HCV antibodies, and serum alpha fetoprotein (AFP) and performed ultrasound. **Samples collection and processing:** five milliliters of venous blood were drawn and collected in a serum separator tube centrifuged at 3,000 rpm for 10 min, and the serum was separated, aliquoted, and stored immediately at -80°C. **Extraction of total RNA, including miRNA:**

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total RNA was extracted from sera using (mirVana™ PARISTM Kit, ambion, USA) according to the manufacturer's instructions. Total RNA was eluted by 50 µL of ribonuclease-free water. The quality of total RNA was detected by A260 to A280 ratio. **Quantitative real time-PCR(qPCR) for miRNA:** miRNA expressions in serum from HCV patients were assessed using quantitative reverse transcriptase polymerase chain reaction assay. Complementary DNA was synthesized from total RNA using TaqMan® MicroRNA Reverse Transcription Kit using thermal cycler (Thermo Electron Waltham, Mass) according to the manufacturer's instructions. qPCR amplification was performed using an TaqMan® MicroRNA Assay together with the TaqMan® Universal PCR Master Mix StepOnePlus System (Applied Biosystems Inc, Foster, Calif) according to the manufacturer's instructions. Relative quantification of miRNA expression was calculated using the 2-ΔΔCT method. miRNA 16 expression was normalized to endogenous control, in this study it was normalized to snRNA U6 and related to healthy controls. **Statistics:** all statistical analyses were performed using SPSS 20. Comparisons were performed using Mann-Whitney test and chi-square test, as appropriate. To evaluate the predictive Value of miRNA 16 for HCV, we performed the receiver operating characteristic (ROC) curve. The strengths of the associations between miRNA expression and clinicopathologic parameters were tested with the Pearson rank correlation. 2-tailed P value of 0.05 or less was considered statistically significant.

RESULTS

The demographic characteristics of the patients are detailed in Table 1.

Table (1): Study population demographic data (N = 25)

Demographic factors	HCV (n = 16)	Normal (n = 9)	P	χ^2
Age				
<35	8	4	0.789	χ^2=0.071
≥35	8	5		
Gender				
Male	7	3	0.263	χ^2=0.61
Female	9	6		

Clinical characteristics of study population: no significant difference was observed in age and gender ratio among the 2 groups (P > 0.05). Other clinicopathological factors were also conducted (details of the clinicopathological data are provided in Table 2) in the blood test for liver function; elevated level of aspartate transaminase, alanine transaminase in HCV patients (P< 0.05), with no significant difference in Total bilirubin, direct bilirubin, serum albumin and alpha fetoprotein between the 2 groups.

Table (2): Study population Clinicopathological characteristics (N = 25)

Clinicopathological factors	HCV (n = 16)	Normal (n = 9)	P	U
ALT				
Mean ± SD	34.4±15.4	17±5.97	0.001**	U=15
Median	30	17		
IQR	29.75	3.5		
Mean Rank	16.65	6.67		
AST				
Mean ± SD	32.1±12.98	20±7.7	0.012*	U=28
Median	25	17		
IQR	23.75	10.5		
Mean Rank	15.75	8.11		
Total bilirubin				
Mean ± SD	0.83±0.3	0.76±0.24	0.346	U=55.5
Median	0.9	0.7		
IQR	0.38	0.4		
Mean Rank	14.03	11.17		
Direct bilirubin				
Mean ± SD	0.20±0.1	0.156±0.07	0.256	U=53.5
Median	0.2	0.1		
IQR	0.1	0.1		
Mean Rank	14.16	10.94		
Serum albumin				
Mean ± SD	3.5±0.41	3.97±0.56	0.077	U=41
Median	3.5	3.9		
IQR	0.4	0.95		
Mean Rank	11.06	16.44		
α-fetoprotein				
Mean ± SD	4.97±2.57	3.67±1.9	0.222	U=50.5
Median	5	3.5		
IQR	5.5	3.25		
Mean Rank	14.34	10.61		
miRNA 16				
Mean ± SD	7.34±6	0.995±0.064	0.008**	U=25
Median	6.68	0.999		
IQR	9.48	0.06		
Mean Rank	15.94	7.78		

ALT, alanine transaminase; AST, aspartate transaminase; HCV, hepatitis C virus infection. χ^2 Chi-square test. U Mann–Whitney U test.

**Highly significant difference was detected between investigated groups at P<0.01.

*Significant difference was detected between investigated groups at P<0.05.

Increased serum level of miRNA16 in HCV patients than normal: the miRNA 16 level, based on relative quantity (RQ) values in serum, are summarized in Table II. The median RQ value was 0.99 and 6.68 in healthy donors and HCV groups, respectively. Compared with the control groups, the HCV group had a higher expression of miRNA 16 (P< 0.01). **Correlation between the serum level of miRNA 16 and clinicopathologic factors:** there was a significant correlation between miRNA 16 expression with viral load, ALT, AST, total bilirubin and direct bilirubin in the study population (Table 3).

Table (3): Spearman's correlation between miRNA 16 and different clinicopathologic factors among the study population (N=25)

Parameters	miRNA 16	
	r	p-value
PCR(IU/ml)	0.544	0.005**
ALT	0.572	0.003**
AST	0.541	0.005**
Total bilirubin	0.489	0.013*
Direct bilirubin	0.434	0.03*

r Spearman's correlation coefficient
*Significant correlation was detected between investigated groups at P<0.05.
**Highly significant correlation was detected between investigated groups at P< 0.01.

Accuracy of miRNA 16 in predicting HCV by ROC analysis: we further evaluated the diagnostic value of miRNA 16 by ROC curve and area under the curve. ROC curve was illustrated in figure 1. When compared HCV patients with healthy people, the threshold of miRNA 16 was 1.58. The sensitivity was 81.25% indicating that this threshold could be used to discriminate between HCV and healthy volunteers (Table 4).

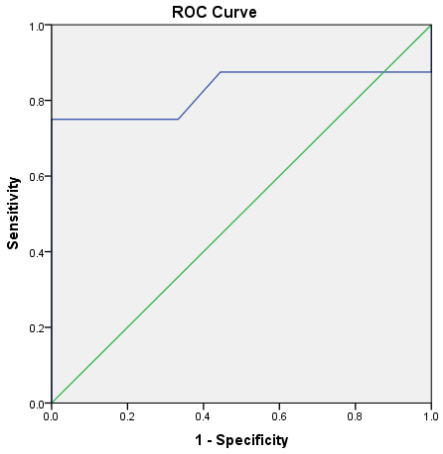

Fig 1. (A) ROC curve analysis for miRNA16 to calculate the best cutoff point that discriminates between HCV and healthy groups. Best cutoff point of miRNA 16 is 1.58 (sensitivity = 81.25 % and specificity = 100 %; AUC (SE) = 0.826 [0.088], 95% confidence limits range = 0.655-0.998, P =0.008).

Table (4): Performance characteristics of the serum miRNA 16 among different groups of the study (n = 25)

Biomarker	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
miRNA 16	81.25%	100%	100%	75%	80%

miRNA 16, microRNA16; NPV, negative predictive value; PPV, positive predictive value

DISCUSSION

miRNAs have a physiological importance in metabolic pathways, immunity, viral hepatitis, cancers, liver fibrosis [8] and may have clinical relevance as pathological markers for early diagnosis of HCV infection. In this study, we have characterized the role of miRNA 16 as diagnostic and prognostic markers for HCV patients compared to healthy controls. miRNA 16 is one of the most prominent miRNAs implicated in cell-cycle regulation and induction of apoptosis. In this study, we demonstrated that miRNA 16 was significantly higher in HCV than in healthy controls (P = 0.008). At the cut-off value of 1.58, serum miRNA 16 was able to discriminate between HCV and healthy control groups with a sensitivity of 81.25 %, specificity of 100 %, and diagnostic accuracy of 80%. Our finding of increased circulating levels of miRNA 16 in HCV patients is in agreement with **Cermillia *et al.*** [3] have also reported the potential role of miRNA 16 to detect the stage of HCV infection. There is significantly increase in expression levels of miRNA 16 in HCV patients with liver fibrosis [9] and HCC [10]. In contrast to our study **El-Abd *et al.*** [7] showed no significant difference in miRNA expression among both HCV and healthy control groups. The discrepancy between the results of our study and other studies may be attributed to the large ethnic and geographic variability in the incidence of HCV among the different populations. Moreover, there are different HCV genotypes other than genotype 4, which represents over 90 % of the cases in Egypt.

CONCLUSION

miRNA 16 was highly expressed in the serum of HCV patients when compared to healthy controls. This lead to an indication that miRNA 16 might be useful potential biomarkers and therapeutic target for HCV.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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