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ORIGINAL ARTICLE

ROLE OF MicroRNA-146A IN PATIENTS WITH CIRRHOSIS

Wisam S. Abood, Ali G. Radhi ✉

Department of Medical Microbiology, College of Medicine, University of Al-Qadisiyah, 58001, Al-Diwaniyah, Iraq

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Summary

Background and Objectives: Liver cirrhosis is a serious and chronic condition that affects the liver, causing irreversible damage and scarring. The present study is designed to find out a possible correlation of hepatitis B virus (HBV) with cirrhosis by microRNA.

Methods: The fold expression of the identified microRNAs by RT qPCR was determined to estimate the concentrations of circulating microRNAs in all samples. This study's main objective was to examine microRNA21-5p expression in liver cirrhosis patients. To extract RNA from samples of whole blood from all specimens in EDTA tubes, the study entailed collecting 60 specimens from the perspective of patients and samples pooled from 60 healthy participants (control group). Data on the patient are gathered for the study.

Result: Researchers compared the levels of miRNA-146a in individuals with hepatic cirrhosis and control subjects, and the findings were clear. individuals with hepatic cirrhoses and controls, correspondingly, had mean levels of miRNA-146a of 2.38 3.25 and 1.12 1.01; this alteration was statistically noteworthy (P = 0.002). Both the miRNA-146a cut-off value and the prediction of liver cirrhosis disease should be considered diagnostic or adjuvant diagnostic tests. With sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve (AUC) of 58.3%, 53.3%, 55.6%, 56.1%, and 0.610 (0.508 -0.711), the miRNA-146a cut-off value was > 0.91-fold. The current findings suggest that miRNA-146a is a subpar diagnostic marker.

Conclusion: Compared to controls, patients with cirrhosis had significantly higher levels of micro-RNA146A. When compared to those who are healthy, this finding demonstrates that micro-RNA 146A may influence the prognosis of cirrhosis.

Key words: Liver Cirrhosis; microRNA-146a; HBV

Introduction

Globally, chronic hepatitis B virus (HBV) infection is a significant contributor to viral hepatitis-induced liver cirrhosis (LC). More than 350 million people are thought to have prolonged HBV infection, and more than a million cases pass away every year (1). Despite excellent vaccination programmes that have reduced severe HBV rates in many nations, chronic HBV infection continues to be a major problem. Therefore, it is crucial to develop a reliable approach for safely and conveniently diagnosing and evaluating LC associated with persistent HBV infection (referred to as LC in the next section). Clinical indicators are frequently used to track the course of HBV infection

✉ University of Al-Qadisiyah, College of Medicine, Al-Diwaniyah, Iraq
kalibk82@gmail.com
☎ +9647807397283

and the disease. Although cell removal is an intrusive technique with strong investigative presentation, it is challenging to execute regularly due to the danger of numerous consequences. Blood testing and image analysis using aminotransferases (ALT, AST), fibrosis-associated variables, HBV DNA stages, and the existence of HBV antigens are noninvasive techniques (2-5). Numerous miRNAs have been linked to HCC, hepatic metabolism, hepatic fibrosis, and liver restoration, according to earlier research(6). Additionally, studies show that miRNAs in serum or plasma can distinguish between HCC and other hepatic disorders (7). Early detection and effective cirrhosis treatment might decrease the likelihood of HCC development (8-12).

Material and Methods

During the time from the first of December 2022 to the end of March 2023, case-control research was done on the following study groups. The patient group for this study consists of (60), of which (53) are men and (7) women, ranging in age from 43 to 78 years old. The diagnosis of LC were conducted by gastroenterologist at Baghdad Medicine City. A questionnaire covering age, sex, hypertension, treatment, duration of cirrhosis, and family history was used during direct interviews with the patients. As a control group, 60 people who appeared clinically healthy (49 men and 11 women) and have no history of systemic disease were also included in the study. All subjects verbally consented to the study, which complied with Medicine City Gastroenterology Hospital's ethical standards. In this study, quantitative reverse transcription PCR (RTqPCR) and miRNA146a RNA isolation were evaluated. Total RNA was extracted from whole blood samples from patients with 60 samples of cirrhosis and 60 samples of healthy controls using the TRIzol reagent and stored at 4 or -80 °C. The RNA kept at 4 C was utilised to make cDNA, whereas the RNA stored at -80 C was for long-term storage. Total RNA concentration was determined using a biological spectrophotometer (NanoDrop 2000). 1 g of total RNA was inversely copied into cDNA using a First-strand cDNA Creation kit, as directed by the manufacturer, at 37 °C for 60 min and 4 °C for 5 min. RT-qPCR was carried out using a Bio-Rad real-time PCR machine and the SYBR PremixEx Taq II kit. The samples were conducted using the following thermocycling settings: 15 min of first breakdown at 95 °C, 15 sec at 94 °C, 40 cycles at 60 °C, and lastly 30 sec of extension at 72 °C. The primer sequences and U6 (for miR126a), which were used as internal references, are listed in Table 1. The relative levels of gene expression were calculated using.

Primers: The Sanger Center miRNA database Registry and the miRNA primer design tool were used in this study to select the miRNA sequences for the qPCR primers for microRNA146 (13). In contrast, the qPCR housekeeping gene (GAPDH) was designed in this work using online tools Primer3 plus design and the NCBI-Database. The (Macrogen firm, Korea).

Table 1. Primers used for reverse transcription quantitative PCR.

Gene	Primers (5'→3')
MicroRNA 146a	
Forward	GCCGCTGAGAACTGAATTCCA
Reverse	GTGCAGGGTCCGAGGT
U6	
Forward	CTCGCTTCGGCAGCACA
Reverse	AACGCTTCACGAATTTGCGT

Statistical analysis: The data was analysed using the Statistical Package for Social Sciences (SPSS) version 26 and Microsoft Office Excel 2010. To present the numeric data, we employed a range of techniques including calculating the mean, and standard deviation, and performing the Kolmogorov-Smirnov normality test. This helped us determine whether the variables followed a normal distribution or not. To examine differences in means between various groups, we utilized the independent sample t-test, assuming that the variable in question exhibited a normal distribution. For exploring associations between categorical variables, we employed the Chi-square test. This allowed us to investigate the relationship between different factors. To measure risk, we estimated the odds ratio and 95 % confidence interval. This provided valuable insights into the potential outcomes. Furthermore, we utilized receiver

operator characteristic (ROC) curve analysis to identify the cut-off value that predicted positive findings. This analysis was accompanied by metrics such as area under the curve (AUC), accuracy level, sensitivity, specificity, and level of significance (P). To determine statistical significance, we considered a P-value of less than 0.05, with an even higher level of significance set at 0.01. These thresholds helped us draw meaningful conclusions from the data.

Results

In the current study, 60 patients with LC (30 with HBV cirrhosis and 30 with alcoholism cirrhosis) versus 60 control subjects were enrolled in the present study. The mean age of the patients was 56.95 ± 7.80 and the mean age of the control subjects was 54.81 ± 6.98; there was non-significant difference in the mean ages of the two subjects (P=0.117) Table 1.

Table 2 displayed the frequency of distribution of cirrhosis and control by age groups. There was non-significant differences among hepatic patients and control group in terms of frequency of distribution by age of subject (P=0.568). There was non-significant distribution of the patient and control subjects by gender (P=0.306), which included 49 (81.7%) men and 11 (18.3%) women in the hepatic cirrhosis group and 53 (88.3%) males and 7 (11.7%) females in the control group. To minimise bias related to age and gender in such case-control research, the current finding—that there is no statistically significant in the distribution of people in the two subjects according to age and gender—is a prerequisite.

Table 2. Demographic characteristics of patients with liver cirrhosis and healthy control subjects.

Characteristic	Patients *n*=60`	Healthy control *n*=60	*P*
Age (years)			
Mean ± SD	56.95 ± 7.80	54.81 ± 6.98	0.117 † NS
Range	43-78	42-72	
< 50, *n* (%)	14 (23.3%)	14 (23.3%)	0.568 ¥ NS
50-59, *n* (%)	25 (41.7%)	30 (50.0%)	
≥ 60, *n* (%)	21 (35.0%)	16 (26.7%)	
Gender			
Male, *n* (%)	53 (88.3 %)	49 (81.7 %)	0.306 ¥ NS
Female, *n* (%)	7 (11.7%)	11 (18.3%)	

n: number of cases; SD: standard deviation; †: independent samples t-test; ¥: Chi-square test; NS: not significant at P > 0.05

miRNA-146a level in patients and healthy control: The findings of the comparison of the levels of miRNA-146a in individuals with hepatic cirrhoses and healthy participants are shown in Table 3. Individuals with hepatic cirrhosis and controls, respectively, had mean levels of miRNA-146a of 2.38 ± 3.25 and 1.12 ± 1.01, correspondingly; this difference was statistically significant (P=0.002).

Table 3. Serum miRNA-146a level in patients with liver cirrhosis and healthy control.

	Patients *n* = 60	Healthy control *n* = 60	*P value*
miRNA-146a			
Mean ± SD	2.38 ± 3.25	1.01 ± 1.12	0.002 † S
Range	0.05 - 15.75	0.03 - 5.45	

n: number of cases; SD: standard deviation; †: independent samples t-test; S: significant at P ≤ 0.05.

Evaluation of miRNA-146A: Receiver operator characteristic (ROC) curve analysis was done to assess the miRNA-146a cut-off value and predict the presence of liver cirrhosis as investigative tests or adjuvant investigative tests. The results are presented in Table 4 and Figure 1. With sensitivity, specificity, positive prognostic value (PPV), negative prognostic value (NPV), and area under the curve (AUC) of 58.3%, 53.3%, 55.6%, 56.1%, and 0.610 (0.508-0.711), the miRNA-146a cut-off value was > 0.91-fold. The current findings suggest that miRNA-146a is a subpar diagnostic marker.

Table 4. Sensitivity and specificity of miRNA-146a level (> 0.91-fold) in liver cirrhosis disease.

miRNA-146a level	patients *n* = 60	Healthy control *n* = 60
> 0.91	35 %	28 %
> 0.91	25 %	32 %
Sensitivity %	58.3 %	
Specificity %	53.3 %	
PPV %	55.6 %	
NPV %	56.1 %	
AUC (95% CI)	0.610 (0.508 - 0.711)	

CI: Confidence interval, AUC: Area under curve.

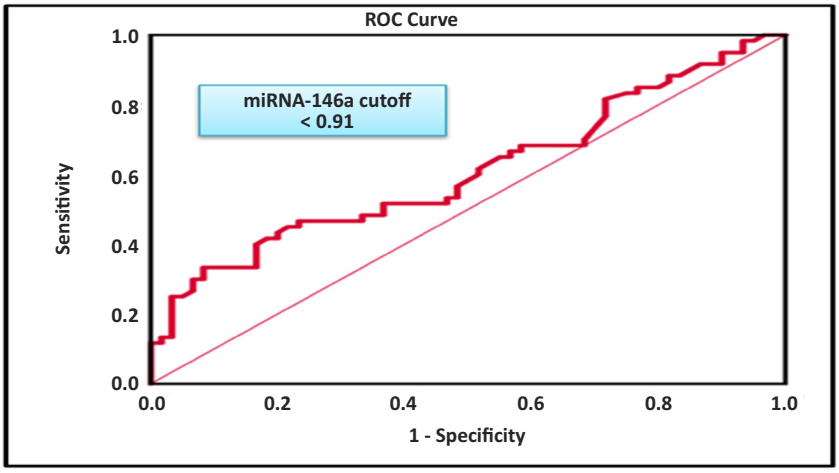

Figure 1. Receiver operator characteristic curve analysis of miRNA-146a for the calculation of possible diagnostic cut-off value.

MiRNA-146a levels have been compared based on the kind of liver cirrhosis, and the findings are shown in Table 5. Patients with alcoholism liver cirrhosis have mean serum miRNA-146a levels that were higher than those with HBS liver cirrhosis, but the variance was non-significant (P=0.059). The mean serum miRNA-146a levels were 1.59 ± 1.27 and 13.18 ± 3.08 in individuals with HBS hepatic cirrhosis and individuals with alcoholism hepatic cirrhosis, respectively.

Table 5. Frequency distribution of miRNA-146a levels according to type of liver cirrhosis.

	HBS cirrhosis *n* = 30	Alcoholism cirrhosis *n* = 30	*P*
miRNA-146a			
Mean ± SD	1.59 ± 1.27	3.18 ± 3.08	0.836 †
Range	0.05 - 10.10	0.10 - 15.75	NS

n: number of cases; SD: standard deviation; †: independent samples t-test; NS: not significant at P>0.05

Discussion

MicroRNAs are indeed remarkable gene regulators that play a crucial role in orchestrating a wide range of cellular processes. They are small non-coding RNA molecules that hold the key to understanding complex conditions like liver cirrhosis (14). By manipulating the expression of their target genes, microRNAs act as conductors in a genetic context, ensuring a harmony between cellular activities. Among the countless microRNAs, miRNA-146 stands out as an influential player, attracting scientists with its profound impact on the immune response and its association with various diseases. miRNA-146 dampens the inflammatory signalling pathways by selectively targeting molecules such as IRAK-1 and TRAF-6, which are instrumental in innate immune activation. This ability to modulate the immune response highlights the significant role microRNAs play in maintaining immune homeostasis and preventing excessive inflammation (14). MicroRNAs, in the grand scheme of cellular fate, emerge as both guardians and influencers. Their regulatory functions shape cellular processes and unravel the mysteries of disease. With each discovery, our understanding of these important molecules deepens, revealing a new world of molecular pathways (15). The role of microRNAs in liver cirrhosis, for instance, is of paramount importance. This complex condition involves various cellular and molecular changes, leading to liver fibrosis and impaired liver function. Understanding the role of microRNAs, such as miRNA-146, in liver cirrhosis can provide valuable insights into the disease mechanism and potentially pave the way for new therapeutic approaches. Moreover, microRNAs have been implicated in a wide range of diseases beyond liver cirrhosis. Their dysregulation has been associated with cancer, cardiovascular diseases, neurodegenerative disorders, and many others. By studying the expression patterns and functional roles of microRNAs in different diseases, scientists can uncover potential diagnostic biomarkers and therapeutic targets (16). The significance of microRNAs lies not only in their ability to regulate gene expression but also in their potential as therapeutic tools. Researchers are exploring the possibility of using synthetic microRNAs or microRNA mimics to modulate gene expression in a controlled manner, opening up new avenues for targeted therapies.

The present results show a significant increase in the expression of miRNA-146a in patients with liver cirrhosis (2.38 ± 3.25) compared to healthy controls (1.12 ± 1.01). Elevated miRNA-146a levels in liver cirrhosis may be indicative of an activated inflammatory response, as miRNA-146a is known to regulate inflammatory signalling pathways (17). Exciting discoveries have unveiled a multitude of binding sites of NF-κB (nuclear factor κB) within the promoter region of the remarkable miRNA-146a gene. We explore how IL-1 and TNF-α affect the expression of miRNA-146a, relying on the powerful influence of NF-κB (18). miRNA-146a may affect the activation and function of hepatic stellate cells (HSCs), which are major contributors to fibrosis development. It is possible that elevated miRNA-146a levels in cirrhosis patients could influence the balance between HSC activation and deactivation (16). Dysregulation of miRNA-146a in liver cirrhosis could disrupt immune system homeostasis and contribute to aberrant immune responses (19). The dysregulated immune responses associated with high miRNA-146a levels in cirrhosis may contribute to the perpetuation of inflammation and liver injury. Interaction with other miRNAs and signaling pathways; miRNA-146a may interact with other miRNAs and signaling pathways involved in liver cirrhosis (20).

The present results disagree with the results of Tarao *et al*. (2019), who demonstrated that miR-146a is downregulated in the serum and liver tissue of patients with liver cirrhosis (21). Furthermore, recent research has proposed a link between the levels of miR-146a in the serum and the severity of inflammatory responses and tissue damage as the liver progresses from fibrosis to cirrhosis. This discovery adds a new layer of understanding to processes involved in these conditions. Moreover, miR-146a, known for its involvement in inflammation, has significant attention in the scientific community and has been shown to play a crucial role in the world of hematopoiesis. These findings highlight the multifaceted nature of miR-146a and its potential as a key player in various biological pathways (22). Previous studies also reported the down-regulation of miR-146a in hepatocellular carcinoma and ischemia/reperfusion damage (23). The latest discoveries challenge the findings of a previous study that explored the impact of liver injury on miR-146a levels in inflammatory cells. Surprisingly, the current research reveals a discrepancy, as it suggests that these levels do not significantly fluctuate in response to liver injury. However, the initial study indicated that miR-146a plays a crucial role in regulating inflammatory cell recruitment and liver injury (24). These contrasting results indicate that the dysregulation of miRNAs in circulating inflammatory cells might be the underlying cause for the observed changes in circulatory miRNAs in chronic liver disease.

The current study validated the miRNA-146a in predicting liver cirrhosis which is less important for detection of the progression and diagnosis of patients with liver cirrhosis. The present study shows that 35 of 60 patients (58.3%) had miRNA-146a lower than the cut-off value (> 0.91) compared to 28 of 50 healthy subjects (46.7%) have miRNA-146a lower than the cut-off value (> 0.91), and the difference was non-significant (P<0.05). ROC curve analysis shows that the miRNA-146a cut-off value was > 0.91 with sensitivity, specificity, PPV and NPV levels of 58.3%, 53.3%, 55.6% and 56.1% respectively. The area under the curve (AUC) of the receiver operating characteristic (ROC) was 0.610 (0.508 - 0.711) for these miRNAs, indicating that these miRNA can't predict the disease severity of liver cirrhosis.

The latest findings have unveiled an intriguing connection between miRNA-146a levels and liver cirrhosis in patients with alcoholism. Surprisingly, the mean levels of miRNA-146a were found to be significantly higher in individuals suffering from alcoholism-associated liver cirrhosis compared to those with HBV liver cirrhosis. Although the difference observed did not reach statistical significance (P = 0.059). It was discovered that the presence of IFN-α, a potent antiviral agent, resulted in a cascade of events within the cells. Notably, it triggered the degradation of viral RNAs through a proteasome-dependent pathway. Additionally, IFN-α successfully thwarted the formation of core particles, effectively impeding the synthesis of viral proteins. Ultimately, this remarkable phenomenon culminated in the suppression of virion production. These remarkable findings shed light on the molecular mechanisms underlying alcoholism-related liver cirrhosis and HBV infection (25).

Conclusion

The study revealed important insights into the role of micro-RNA146A in patients with cirrhosis. The findings indicated that individuals with cirrhosis exhibited significantly higher levels of micro-RNA146A compared to the control group. This discovery holds immense significance as it suggests that micro-RNA146A may play a crucial role in influencing the prognosis of cirrhosis. The elevated levels of micro-RNA146A in patients with cirrhosis highlight its potential as a biomarker for disease progression and severity. Furthermore, this finding opens up new avenues for research and the development of targeted therapies aimed at modulating micro-RNA146A levels to improve the prognosis and treatment outcomes for individuals living with cirrhosis. By unravelling the molecular mechanisms through which micro-RNA146A operates, scientists and healthcare professionals can gain a deeper understanding of the underlying pathophysiology of cirrhosis and potentially identify novel therapeutic targets. Overall, this study underscores the importance of micro-RNA146A in cirrhosis and its potential as a prognostic marker, paving the way for future advancements in the field of liver disease research.

Conflict of interest

The authors declare no conflict of interest concerned in the present study.

Adherence to Ethical Standards

The study was approved by the College of Medicine/ University of Al-Qadissiya with approval number (UoQ/CoM 30/4712 on 15.12.2022).

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