

CENTRAL ASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES

https://cajmns.centralasianstudies.org/index.php/CAJMNS

Volume: 06 Issue: 04 | October 2025 ISSN: 2660-4159



Article

Role of miRNA122 in Patients with Chronic Hepatitis C Virus

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hepatitis C is examined. Patients in the virology and dialysis departments of Al-kindi Teaching Hospital in Baghdad and Al-imam Ali Teaching Hospital in Al-sadr City were randomly selected for both sexes and age groups from 2 January to 1 April 2023. Blood samples with 110 negative and 50 positive hepatitis C results. 50 blood samples were used in this study, 25 of which were from hepatitis C patients who had been diagnosed with a quick test and confirmed by an ELISA, and 25 came from healthy controls. miRNA-196 quantitative real-time PCR in hepatitis C patients and healthy individuals. There were 9 (36%), 4 (16%), and 9 (36%), with a patient age range of 33 to 65. There were 4 (16%) patients from rural regions and 21 (84%) individuals with chronic hepatitis C. The frequency distribution of patients based on the prevalence of some chronic diseases revealed that miRNA levels have an impact on them. Examples include diabetes, which affected 20% of patients as opposed to 16% of healthy individuals, chronic heart disease, which affected 16% of patients as opposed to 16% of healthy individuals, and adopted chronic blood pressure disease. Also accepted was hepatitis. 15% of patients abused alcohol, which is comparable to healthy people. Patients with low miRNA-122 levels and healthy controls Healthy controls had 1.60 0.86 mean levels compared to patients' 22.87 18.40 mean levels. Compared to healthy controls, patients had a level that was significantly greater (P 0.01). MiRNA-122 levels were 39.01 33.24 in HCV patients aged 50-59, compared to lower mean values (10.15 6.54) in those aged 40-49. HCV gender differences 122 miRNA levels The average values for men and women were 29,12 25,91 and 15,73 5,71, respectively. In HCV patients with diabetes, miRNA-122 levels were 58.48 41.40 and 13.97 5.81, respectively (P = 0.01). Levels of miRNA-122 and hypertension linked with HCV Its mean values were 16.03 18.06 in non-hypertensive people and 44.52 14.09 in persons with hypertension (P = 0.01). levels of miRNA-122 in ischemic heart disease caused by the HCV Individuals with and without ischemic heart disease had mean values of 49.89 12.34 and 17.72 27.24, respectively (P = 0.01). Significant (P = 0.01) miRNA-122 levels were found in HCV patients who misused alcohol to be 80.13 46.22 and 15.06 13.65, respectively.

Abstract: In this work, the gene expression of microRNAs 122 and 196 in people with chronic

Citation: Al-Helfi, M. J. M & Al-Ramahi, S. K. A. Recent Advances in Immunotherapy Role of miRNA122 in Patients with Chronic Hepatitis C Virus. Central Asian Journal of Medical and Natural Science 2025, 6(4), 2016-2025

Received: 10th May 2025 Revised: 16th Jun 2025 Accepted: 24th Jul 2025 Published: 20th Aug 2025



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Keywords: miRNA122, microRNA122, HCV, miRNA-122 level, ischemic heart disease, miRNA

1. Introduction

It is quite likely that the hepatotropic hepatitis C virus, which is commonly referred to as HCV, is the root cause of liver illness, a large amount of morbidity, and maybe even mortality in many parts of the globe. The virus, which is most often spread via the use of injectable drugs, blood transfusions, potentially unsafe injection techniques, and other medical procedures, has infected three percent of the world's population. Eighty percent

of those who are infected with HCV will initially develop asymptomatic acute hepatitis before progressing to chronic hepatitis [1], [2], [3]. Patients who are infected with HCV have an increased likelihood of developing cirrhosis, chronic liver disease, and primary hepatocellular carcinoma, which is more often referred to as HCC. According to a research that was conducted in 2021 by Benson and colleagues, HCV is to responsible for 27% of cases of cirrhosis and 25% of cases of HCC all over the world. A research that was conducted in 2022 by Harkus and colleagues found that the hepatotropic virus known as hepatitis C may be able to grow in mononuclear cells that are found in peripheral blood. It is possible that microRNAs, also known as miRNAs, which range in length from 19 to 25 nucleotides, have a role in post-transcriptional gene silencing [4]. When a microRNA is a part of a functional interaction network, it has the potential to target hundreds of different mRNAs and to have an effect on the expression of numerous genes [5], [6]. According to the findings of Weidner et al. 2021, the development of allergic rhinitis, eosinophilic esophagitis, asthma, and eczema are all influenced by microRNAs. There have been a number of papers that discuss the biological function of microRNAs. This paper addresses each and every one of the problems that are listed below: miRNA separation, target identification, expression level analysis, and both experimental and therapeutic target selection are some of the applications of this technology [7], [8], [9]. It was discovered that miR-122 was the first microRNA to have tissue specificity. The expression of between 70 and 80 percent of all miRNAs may be found in the liver. The whole sequence of the mature miR-122 microRNA can only be found in the genomes of vertebrates. The findings of a study conducted by Yu and Kim (2020) suggest that the microRNA known as MiR-122 is connected to hepatocellular carcinoma, HCV replication, and lipid metabolism [10].

2. Materials and Methods

The Patient and Control Samples

Blood samples were collected at the Imam Ali Hospital in Sadr City, which is part of the Al-kindi Teaching Hospital in Baghdad, from the beginning of the year 2023 through the middle of the year. People between the ages of 20 and 60 were included in the samples, and an investigation into their medical history was also conducted individuals of all sexes and different ages were included in the study, and a total of 160 blood samples from those individuals were obtained at random. This experiment required a total of 50 blood samples: 25 from people who were ill and 25 from those who were well (serving as controls). In order to diagnose HCV and measure two different types of microRNA (miRNA122 and miRNA196), a venipuncture was used to withdraw 4 milliliters of blood. After placing the blood sample in a gel tube and letting it rest for around thirty minutes, the coagulation process was facilitated. After that, the sample was centrifuged at 3000 rpm for 15 minutes in order to separate the serum from the rest of the material. After all was said and done, the serum was poured into Eppendorf tubes and placed in the freezer.

3. Results and Discussion

Primers used in cureent study:

The miRNA primer design tool and the miRNA sequences that were selected from The Sanger Center miRNA database Registry were used in this study to construct the qPCR primers for the miRNAs l22 (MIMAT0000421) and miR-29a (MIMAT0004503). These primers were used to create the qPCR primers for the miRNAs. The MIMAT database has both of these primers in its collection [11], [12]. On the other hand, the qPCR housekeeping gene (GAPDH) (NM_001256799.3) for this study was generated with the use of Primer3 plus design online and the NCBI-Database. The South Korean company known as Macrogen was the provider of the primers and probe that are detailed in table (1).

Table 1. primers that used in this study.

PRIME	R	SEQUENCE			
miR-122 qPCR primer		F	AACCGGTGGAGTGTGACAAT	AACCGGTGGAGTGTGACAAT	
		R	R GTCGTATCCAGTGCAGGGT		
RT primer (specific) hsa- miR-122		GTCC	ATCCAGTGCAGGGTCCGAGGTATTCGCACGGATACGACCAAA CA		
GAPDH	F		AATTCCATGGCACCGTCAAG	1041	
qPCR primer	R		ATCGCCCCACTTGATTTTGG	104bp	

STEM-LOOP RT-qPCR

Real-time PCR was utilized to do an expression study and quantify the housekeeping gene (GAPDH) and miRNA 122 in patient serum and blood samples as well as in normal samples. This was done in order to compare the patient samples to the normal samples. This procedure was carried out in accordance with the guidelines that were supplied by Qattan et al, and it consisted of the stages that are outlined below:

Total RNA extraction

iNtRON took blood samples in Korea and extracted total RNA by following the directions given by the maker of the TRIzol® reagent kit and doing the process exactly as they said [13].

Estimation RNA yield and quality

To look at the recovered genome RNA, a Nanodrop spectrophotometer (THERMO, USA) was used. This device measures absorbance at (260/280 nm) to find out how much RNA there is and how good it is [14], [15].

DNase I Treatment

The retrieved RNA was treated with DNase I enzyme by using a DNase I enzyme kit and following the instructions given by the US-based Promega company. This was done to get rid of the small amounts of genomic DNA that were in the total RNA that had been extracted.

cDNA synthesis

Following the manufacturer's directions, RNA samples that had been treated with DNase-I were used to make miRNA with the M-MLV Reverse Transcriptase kit.

The Korean company Bioneer made cDNA for the GAPDH gene.

cDNA synthesis for miRNA

The business in Korea Bioneer used an RNA sample treated with DNase-I and an M-MLV reverse transcriptase kit, as directed by the manufacturer, to make cDNA for the GAPDH gene.

cDNA synthesis for GAPDH gene by Bioneer company in Korea

master mix preparation for qPCR The qPCR master mix was made with the iNtRON RealMODTM Green SF 2X qPCR mix kit, which was made in Korea. Gene amplification can be found in Real-Time PCR devices with the help of SYBER Green dye and this kit. master mix preparation for qPCR

Quantitative Real-Time PCR (qPCR) is a method that bio-rad-CFX96 in India uses to measure the expression of the GAPDH gene in line with industry standards.

qPCR data analysis

The CT Method Using a reference gene, which was created by Livak and Schmittgen in 2001, was used to look at the results of q RT-PCR for target and housekeeping genes. The statistical program for social sciences (SPSS) version 26 was used to collect, organize, describe, analyze, and show the data. The mean and standard deviation were given after the Kolmogorov-Smirnov test was run to see if the numbers were spread out properly or not. If the variable is spread out in a regular way, an independent sample t-test was used to figure out the difference in mean between any two groups. An ANOVA test can be used to compare the mean difference between more than two groups when a measure has a normal distribution. The chi-square test was used to look for links between any two categories of factors. The chances ratio and 95% confidence interval were used to figure out how dangerous something was. Receiver operator characteristic (ROC) curve analysis, along with its accuracy level, sensitivity, specificity, and degree of significance (P), was used to find the cutoff value that correctly predicts a good result. Daniel (2009) said that the level of significance was a P-value of 0.05 or less, and the level of high significance was a P-value of 0.01 or less.

miRNA-122 level

According to the present findings, levels of miRNA-122 were 22.87 18.40 and 1.60 0.86 in HCV-infected patients and healthy controls, respectively. This level difference between the two groups was very significant (P 0.01), as shown in table (2).

Table 2. miRNA-122 level in patients with HCV and healthy control.

	Cases –control comparison			
	Patients n = 25	Healthy control n = 25	P	
miRNA-122				
Mean± SD	22.87 ± 18.40	1.60 ± 0.86	< 0.01 †	
Range	0.19 – 131.23	0.20- 3.20	HS	

n: number of cases; **SD**: standard deviation; †: independent samples t-test; HS: Highly significant at P \leq 0.001.

In a finding that is comparable to that of Bandiera et al, the current study found elevated levels of mirna122 in HCV patients in comparison to healthy controls. The researchers explained this finding by noting that mirna122 is essential for the capacity of the hepatitis C virus to reproduce. discovered that people who were infected with HCV had a larger percentage of microRNA 122 in their bodies compared to those who did not have the illness. There was a considerable difference, which is a solid indication and might perhaps serve as a guide for detecting whether or not someone has this infection. This difference can be attributed to the major role it plays in HCV replication. Gebert and MacRae found that this conclusion is consistent with their findings. They provided a model for it. A significant reduction in HCV RNA was seen in human liver cells that were positive for HCV replicons when endogenous miR-122 was inhibited. HCV RNA was lower when endogenous miR-122 was inhibited from functioning. This is due to direct contact between miR-122 and two neighboring binding sites that have complementing seed matching. This is brought about as a result of this interaction, since the critical mass has almost reached (Figure 1).

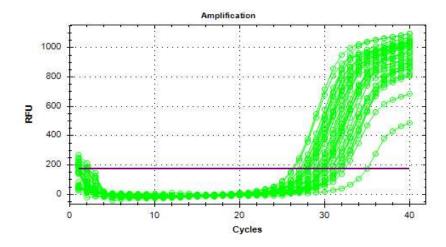


Figure 1. Real Time PCR amplification plots for miRNA122 in patients and healthy control samples.

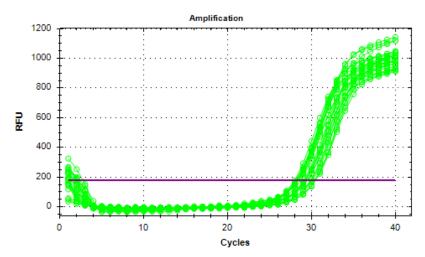


Figure 2. Real Time PCR amplification plots for housekeeping GAPDH gene in patients and healthy control samples.

Evaluation of miRNA-122.

A receiver operator characteristic (ROC) curve analysis was performed in order to identify the miRNA-122 cutoff value and predict the existence of the HCV infection as diagnostic tests or adjuvant diagnostic testing. Both of these goals were accomplished via the use of adjuvant diagnostic testing. Figures 3-4 and 3-5, respectively, present the outcomes of this experiment. The threshold value for miRNA-122 is set at 1.2. was more than 2.45-fold, with values for sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve of 92%, 84.0%, 85.2%, 91.3%, and 0.922 (0.817-1.000), respectively .(Figure 2)

Table 3. Sensitivity and specificity of miRNA-122level (> 2.45-fold) in HCV infection

miRNA-122 level	HCV patients $n=25$	Healthy control n = 25	
> 2.45	23 (%)	4 (%)	
< 2.45	2 (%)	21 (%)	
Sensitivity %	92.0 %		
Specificity %	84.0%		
PPV %	85.2%		
NPV %	91.3%		
AUC (95% CI)	0.922 (0.817- 1.000)		

CI: Confidence interval, AUC: Area under curve.

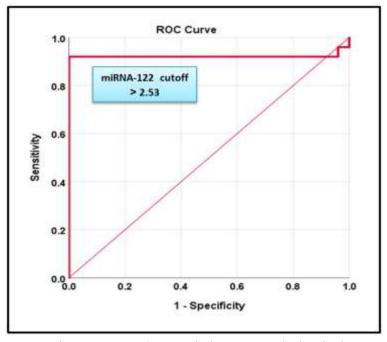


Figure 3. Receiver operator characteristic curve analysis of miRNA-122 for the calculation of possible diagnostic cutoff value.

The interaction between miR-122 and HCV was demonstrated by the curve, which demonstrated that both the high levels of mRNA 122 in patients with HCV disease and the low levels in healthy individuals play a significant role in considering it as a diagnostic or diagnostic aid for detecting viral hepatitis C virus. This was demonstrated by the fact that the curve demonstrated that both the high levels of mRNA 122 in patients with HCV disease and the low levels in healthy individuals. In a similar vein, Bajan et al. shown that people with HCV infection had greater levels of mirna122 than non-infected persons. This is due to the fact that HCV needs mirna122 to double in order to replicate.(table 3) miRNA-122 levels according to age of patients with HCV.

According to the present findings, levels of miRNA-122 were greater in patients with age groups (50-59) than in other age groups, with the category (40-49) having the lowest percentage at $10.81 \, 6.54$. However, the difference was not statistically significant (P = 0.05). as shown in table (4).

Table 4. miRNA-122 levels according to age groups of patients with HCV

	Age group comparison				
	< 40 years n=5	40-49 years n=9	50-59 years n=7	≥ 60 years n=4	P value
miRNA-122					
Mean± SD	18.15± 16.34	10.81 ± 6.54	39.01 ± 33.24	27.65±20.43	0.255
Range	0.19 –67.93	3.60 –24.02	7.87-131.21	7.98-47.70	NS

n: number of cases; **SD**: standard deviation; †: one way ANOVA; NS: not significant at P \leq 0.05.

In a recent research, Lambert et al. (2016) found that miRNA 122 was positively correlated with age. One possible explanation for this finding is that ages tend to converge as people become older. This conclusion was comparable to that observed by Luo, J. et al. (2016), who discovered that patients aged 50 to 59 years had the greatest incidence of HVC infection, with the influence of mirna122 determining this.

miRNA-122 levels according to gender of patients with HCV.

According to the data that were just presented, the levels of miRNA-122 were higher in male patients with HCV (29.46 25.91 and 15.73 5.71) than they were in female patients (P = 0.05), as the table (5) shows.

Table 5. miRNA-122 levels according to gender of patients with HCV.

	Male/Female comparison			
	Male n = 13	Female n = 12	P	
miRNA-122				
Mean± SD	29.46 ± 25.91	15.73 ± 5.71	0.235 +	
Range	3.60 – 131.23	0.19- 47.70	S	

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

this result was similar to Bartel, (2004), he explained in his study that there is no relationship between the level of miRNA 122 and the human race .

miRNA-122 levels according to diabetes disease of patients with HCV.

According to the most recent research, levels of miRNA-122 were shown to be significantly higher in HCV patients who also had diabetic illness, with values ranging from 58.48 to 41.40 and 13.97 to 5.81. According to table (6), there was a statistically significant difference in the levels of miRNA-122 between patient groups that had diabetes disease and those who did not have diabetes. This difference had a P value of 0.01.

Table 6. miRNA-122 levels according to diabetes disease of patients with HCV.

Table of min 11 122 levels according to diabetes disease of patients with the vi-			
	Diabetes disease		
	Yes n = 5	No n = 20	P
miRNA-122			
Mean± SD	58.48 ± 41.40	13.97 ± 5.81	0.01

Range 27.21 – 131.23	0.19- 67.93	† S
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n: number of cases; **SD**: standard deviation; †: independent samples t-test; S: significant at P \leq 0.05.

This research was analogous to the one that was conducted by Gill et al. (2016) since it established a causal relationship between elevated levels of mirna122 and hyperglycemia. This link was proven by an alteration in the body's metabolism, especially in individuals who were infected with viral HCV..

miRNA-122 levels according to hypertension disease of patients with HCV.

According to the most recent research, the levels of miRNA-122 in HCV patients with hypertension illness measured 44.52 14.09, whereas the levels in HCV patients without hypertension disease was 16.03 18.06. According to table (7), this difference was statistically significant at a level of 0.01, which stands for probability.

Table 7. miRNA-122 levels according to hypertension disease of patients with HCV.

	Hypertens		
	Yes	NO 10	P
miRNA-122	n = 6	n = 19	
Mean± SD	44.52 ± 14.09	16.03 ± 18.06	0.01 +
Range	24.02 – 67.93	0.19- 131.23	S

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

This result was comparable to that of Adinolfi et al, who demonstrated that higher levels of miRNA 122 were present in hypertensive individuals. Hypertension blood pressure is one of the markers that impact HCV infection.

miRNA-122 levels according to Ischemic heart disease of patients with HCV.

The most recent study found that the levels of miRNA-122 in HCV patients with ischemic heart disease were significantly greater *than* the levels in HCV patients without the condition, which were 49.89 12.34 and 17.72 27.24, respectively. This difference was statistically significant with a value of (P = 0.01), as shown in table 8.

Table 8. miRNA-122 levels according to Ischemic heart disease of patients with HCV.

	Ischemic h		
	Yes n = 4	No n = 21	P
miRNA-122			
Mean± SD	49.89 ± 12.34	17.72 ± 27.24	0.01 +
Range	41.24 - 67.93	0.19- 131.23	S

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

It became abundantly evident that there is a link between the amount of microRNA 122 and individuals who have heart and blood vessel problems, and that the latter plays a significant role in myocardial infarction. This was linked to the connection between miRNA 122 and the production of collagen, as well as the connection between miRNA

122 and the creation of blood vessels. This conclusion was comparable to that of Adinolfi et al, who demonstrated that the level of miRNA 122 was elevated in individuals suffering from cardiac disease, cardiomyopathy, and other related conditions.

miRNA-122 levels according to Alcohol abuse of patients with HCV.

As indicated in table (9), the present findings indicate that levels of miRNA-122 are greater in HCV patients who misuse alcohol; levels of miRNA-122 were 80.13 46.22 and 15.06 13.65 in patients who did not abuse alcohol.

Table 9. Frequency distribution of miRNA-122 levels according to alcohol abuse of patients with HCV.

randon nemero n			
	Alcohol abuse		
	Yes n = 3	No n = 22	P
miRNA-122			
Mean± SD	80.13 ± 46.22	15.06 ± 13.65	0.01 +
Range	41.24 – 131.23	0.19- 47.70	S

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

This result was equivalent to that which was found by Haque et al, who established that excessive drinking has an effect on the levels of miRNA 122. They did this by showing that alcohol abuse has an influence on the levels of miRNA 122 due to the fact that alcohol abuse has a major impact on the liver and that miRNA 122 is created in the liver. This result was comparable to their findings. In addition to this, he found a significant relationship between high levels of microRNA 122 and heavy drinkers. Consumption of alcohol is one of the high-risk variables that has been linked to an accelerated course of chronic hepatitis C (CHC).

4. Conclusion

Chronic Hepatitis C Virus (HCV) infection remains a significant public health challenge globally, often progressing to liver cirrhosis and hepatocellular carcinoma. In this study, we focused on the role of miRNA-122, a liver-specific microRNA, as a biomarker for HCV infection. Our findings suggest that miRNA-122 levels are significantly elevated in HCV patients compared to healthy controls, with correlations observed between miRNA-122 levels and various clinical factors such as age, gender, diabetes, hypertension, ischemic heart disease, and alcohol abuse. These results support miRNA-122's potential as a diagnostic biomarker and a therapeutic target in HCV management. The study also highlights the need for further research into the mechanisms through which miRNA-122 influences HCV replication and its broader implications for liver-related diseases. The clinical application of miRNA-122 in diagnostic and therapeutic settings could lead to more effective management strategies for chronic hepatitis C, ultimately improving patient outcomes.

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