Diagnostic Potential of miRNA-122 in Liver Disease Iraqi Patients Sample

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Abstract
Background: Given the liver's large size, it performs many essential functions that the body needs and cannot do without. Conversely, any dysfunction in these functions can lead to a specific liver disease. To determine the type of disease, it must be diagnosed, and this diagnosis is made through several well-known tests. However, in this study, we will attempt to explore the diagnostic role of miRNA-122 in these diseases, as it can be used as a comprehensive, definitive diagnostic tool.
Subjects and Methods: One hundred samples were collected from patients with liver disease and 100 samples from healthy individuals for comparison. The mean ages of the patients and controls ranged from 52.12 ± 12.470 to 49.23 ± 13.465, respectively. Five milliliters of blood were drawn from all participants, and 1 ml of each sample was used for real time polymerase chain reaction (RT qPCR) analysis to detect miRNA 122. Remaining amount of each sample was used to measure interleukin-18 using enzyme-linked immunosorbent assay (ELISA), in addition to other biochemical tests performed in this study.
Results: Regarding the amount of miRNA-122, the results showed statistical significance between the two groups ($P < 0.05$). Patients' serum levels of interleukin-18 were higher than those of the healthy group ($P = 0.001$). Statistical differences were clear and significant between the two groups in all other biochemical tests ($P < 0.001$).
Conclusions: In conclusion, the results showed that miR-122 is closely associated with various liver diseases, demonstrating its potential as a definitive diagnostic tool in this regard.

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Keywords: Liver Diseases, miRNA-122, Interleukin-18, Hepatic Disease

1. Introduction

With the capacity to self-renew its cells, the liver is the biggest and most enormous organ in the body. This size enables it to contribute to most of the body's vital functions, which involve infinitely regular biochemical processes. Most studies indicate that miRNAs are critical for regulating liver growth, regeneration, and metabolic functions [1]. Followed by changes in miRNA networks in hepatocytes in various liver diseases and ailments, including hepatitis, hepatic steatosis, cirrhosis, and hepatocellular carcinoma [2]. By cloning various mouse tissue samples, the first version of miR-122 was identified, with elevated levels of the miRNA in these tissues. A number of new miRNAs were discovered in specific tissues, including miR-122, which is concentrated exclusively in liver cells and tissues [3, 4]. miR-122 is closely associated with liver function in adults [5]. MicroRNA-122 has been found to be a biomarker for a number of recognized liver conditions, including non-alcoholic fatty liver disease (NAFLD) and chronic hepatitis B or C, and liver diseases caused by drug abuse and addiction, after being discovered in the bloodstream [6]. By encouraging infection, the majority of cytokines at development of acute hepatic diseases [7]. For example, interleukin-18 (IL-18) is a pro-inflammatory cytokine that activates interferon gamma and stimulates T cell proliferation [8]. It is produced primarily by hepatic macrophages and acts to combat bacteria, parasites, drug residues, and various viruses in the liver. It also stimulates natural killer cells, leading to the destruction of dying liver cells [9-12].

Given the increasing burden of various liver diseases, which require easy and early diagnosis for treatment, our study evaluated the diagnostic potential of miRNA-122 levels in the serum of patients with these diseases.

2. Methodology

Study Design and Ethical Approval

Investigation took place between March and July 2025 at Al-Qadisiyah University Teaching Hospital, Iraq. The study followed the ethical guidelines established by the 1975 Helsinki declaration and was reviewed and cleared by the ethics committee of the College of Dentistry, Al-Qadisiyah University (Approval No. 881, dated 12-03-2025). All participants provided signed informed consent before taking part in the study, Figure.1 show design of this study.

Sample Size Calculation:

The number of participants was determined based on Herbert Arkin's equation for estimating sample size in comparative studies:

$$n = \frac{N.Z^2.\sigma^2}{(N-1).E^2 + Z^2.\sigma^2}$$

Where: n: sample size, N: population size, Z: 1.96 (95%), σ: standard devotion, E:0.05(5%).

Study Population:

Group 1 (Patients): 100 patients clinically diagnosed with liver diseases, involving hepatitis A, B, or C; non-alcoholic fatty liver disease (NAFLD); autoimmune hepatitis; hemochromatosis; alpha-1 antitrypsin deficiency; liver failure; and hepatocellular carcinoma (HCC). Diagnosis was confirmed through a combination of laboratory findings and radiological imaging.

Group 2 (Controls): 100 apparently healthy individuals recruited from hospital staff with no known history of liver disease, chronic illness, or on-going medication.

Inclusion Criteria:

Adults aged 30–70 years.

For patients: confirmed liver disease by a physician.

For controls: no history of hepatic, metabolic, or systemic disorders.

Exclusion Criteria:

Diabetes mellitus, cardiovascular disease, malignancies (other than HCC), autoimmune disorders not related to liver disease.

Recent infections, alcohol use, or on-going immunosuppressive therapy.

Sample Collection and Processing:

From each participant, 5 mL of peripheral blood was collected under aseptic conditions:

1 mL was used for quantification of circulating miRNA-122 using quantitative reverse transcription real- time PCR (RT-qPCR) (Bio-Rad, USA). Total RNA, including small RNA fractions, was extracted using a standardized commercial kit. Reverse transcription was performed using a miRNA-specific cDNA synthesis kit. The expression of miRNA-122 was standardized using U6 small nuclear RNA as an internal reference. The resulting expression levels were analysed applying the comparative Ct approach (2^- ΔΔCt).

Quantitative Reverse Transcription Real-Time PCR:

The miRNA-122 primers used in this study were designed by Primer 3plus, version 4, and were carefully reviewed by the UCSC Student Code of Conduct Software, with their reference sequences in the National Centre for Biotechnology Information (NCBI) database, The qPCR Primers with their nucleotide sequence and product size, Table.1.

Primer Preparation:

The primers were taken from the manufacturing site and freeze-dried and dissolved in nuclease-depleted water according to the manufacturer's recommendations. We then prepared a 100 µM stock solution, which was stocked at -20°C. Then 10 µL of every primer supplies solution was diluted in 90 µL of denatured water to produce 10 µM sol, and this was kept at -20°C till utilize.

Total RNA Extraction:

Eppendorf tubes were used to mix 250 ml of serum and 500 ml of TRIzol, and stocked at -20°C till screening. Upon conversion of gore to serum, gross RNA was separated and extracted using TRIzol™ reagent (ER501-01), based on producing company recommendations.

Rating of RNA Quantum and Pureness:

Nanodrop (Thermo Fisher Scientific USA) was used to determine the quantity and purity of the extracted DNA, based on the manufacturer's recommendations. This process is selective and reliable for measuring least-abundant RNA specimens. miRNA content in every specimen ranged between 73 and 147 ng/µL, indicating that miRNA has high selectivity compared to other forms of RNA. Absorbance was measured at 260 and 280 nm to determine purity. An A260/A280 ratio between 1.95 and 2.0 indicates the purity of the RNA sample.

Complementary DNA (cDNA) Synthesis, with Specific Primer:

The Easy Script® One-Step gDNA elimination and cDNA makings Super Mix Kit supply a plenary method for synthesizing miRNA from first-strand total RNA templates. Based producing company recommendations, gross RNA is reverse transcribed to produce complementary DNA (cDNA).

Quantification of microRNAs:

In accordance with producing company recommendations, the kit is used to measure the amount of small RNA (approximately 20 nucleotides or base pairs). Kit facilitates the quick disclosure of every kinds of little RNA, including small RNA, as well as single, double-stranded RNA. It is eclectic adequate for little RNAs when compared to large mRNAs, and can bear contaminants like salts, solvents, and cleaners.

Calculate the Gene Expression:

Gene expression of miR-122 was gauged in sick specimen through qRT-PCR based on the proportional cycle threshold (2^-ΔΔCt) methodology and healthy control samples, with GAPDH as an interior control (housekeeping gene).

The remaining 4 mL was centrifuged at 3000 rpm for 10 minutes to obtain serum. The serum was liquated into four

parts and stored at –80°C in sterile Eppendorf tubes until further analysis.

Biochemical and Cytokine Analysis:
One aliquot was used to measure interleukin-18 (IL-18) using a commercial ELISA kit (Mabtech, USA), following the manufacturer's instructions. The kit's sensitivity, detection range, and intra-/inter-assay variability were recorded.

Assay Procedure:
Using the ELISA Flex kit for research purposes, level of IL-18 in human serum was estimated. For serological analysis, the ELISA diluent is very important. This solution has the ability to prevent the binding of non-identical antibodies to the antibodies for analysis, which in turn prevents inaccurate positive readings. As these asymmetric bodies are available in samples of living organisms. This diluent has been tested and approved using specimen from healthy human donors and is therefore ideal for use.
Other aliquots were used to perform routine biochemical tests, including liver enzymes (ALT, AST, ALP), total and direct bilirubin, fasting blood glucose (FBG), HbA1c, and lipid profile (total cholesterol, triglycerides, LDL, HDL). Tests were conducted using automated analysers at the hospital's central laboratory.

Statistical Analysis
The Statistical Package for the Social Sciences (SPSS), version 26, was used to organize, statistically process, and present the results of the data. Regarding the numerical variables, these were expressed through the calculation of the arithmetic mean and standard deviation. The classificatory variables were presented using frequencies and percentages. A comparison was made between the means of two independent groups. The strength and direction of the relationship between two quantitative variables were assessed using Pearson's correlation coefficient. A significance level of ($P \leq 0.05$) was used as the cutoff for significance.

Table 1: The qPCR Primers with their nucleotide sequence and product size

qPCR primer		Sequence (5'-3')	qPCR product size
miRNA-122	F	TGGAGTGTGACAATGGTGTTTG	70bp
	R	GCGAGCACAGAATTAATACGAC	

3. Results
Age distribution data for each study group are displayed in [Table 2]. Group one and group two did not differ statistically significantly in the age variable, [P=0.801]. The study included a number of males and females for each group shown in [Table 3]. The frequency distribution between two groups did not differ significantly by gender, as shown by the probability value (P = 0.258), [Table 4] compares the metabolic parameters (Fasting Blood Glucose FBG, Hemoglobin A1c HbA1c, and C-peptide) of the patients with the control group, [Table 5] compares the lipid values (triglycerides, cholesterol, Very Low Density Lipoprotein VLDL, Low Density Lipoprotein LDL and High-Density Lipoprotein HDL) of patients with the control group, [Table 6] shows the results of comparing the liver function tests (AST, ALT, ALP and TSB) of the patients with the control group, [Figure 2] displays the findings from a comparison of the serum levels of [IL-18] in liver disease patients and the control group. Serum IL-18 concentrations within patients were significantly rise than those within healthy control set, according to current data [44.65±8.2 vs 21.03±4.73, P < 0.001).

miRNA-122 Expression
Relative expression levels of miRNA-122 were assessed using the 2^-ΔCt method, with U6 as the internal control. One control sample with high miR-122 expression was used as a calibrator.
The mean ΔCt values for the control and patient groups were –6.517 and –8.27, respectively. The corresponding mean 2^-ΔCt values were 91.58 in controls and 310.26 in patients, indicating markedly elevated expression in the liver disease group [Table 7, Figure 2].
The fold-change analysis revealed that miRNA-122 expression was approximately 3.4-fold higher in liver disease patients than in healthy controls (P < 0.05), suggesting a strong association between elevated miR-122 expression and hepatic pathology.

Table 2: Comparison between two study groups in Age

Group	Mean ±	SD	p-Value
Patients	52.12	12.470	0.801
Control	49.23	13.465	NS

SD: standard deviation; NS: not significant at P > 0.05

Table 3: Comparison of the two study groups by sex

Groups	Sex		Total	p-Value
	Male	Female		
Patients	53 (53.0%)	47 (47.0%)	100	0.258 NS
Control	45 (45.0%)	55 (55.0%)	100	
Total	98 (98.0%)	102 (102.0%)	200	

NS: not significant at P > 0.05

Table 4: Metabolic parameters (FBG, HBA1c and C-peptide) in patients and healthy controls

Groups		FBG (mg/dl)	HBA1c (%)	C-peptide (ng/mL)
Patients	Mean	223.47	9.66	0.41
	SD	30.23	2.12	0.10
Control	Mean	92.39	4.77	1.2
	SD	12.30	0.77	0.12
p-Value		$1x10^{-3}$*	$1x10^{-3}$*	$1x10^{-3}$*

SD: standard deviation; *: significant at $P \leq 0.05$

Table 5: Results of (Triglycerides, Cholesterol, VLDL, LDL and HDL) between two study group

Groups		Cholesterol (mg/dl)	TG (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Patients	Mean	184.0	205.6	39.9	112.2	33.7
	SD	29.6	26.2	6.99	20.7	3.7
Control	Mean	127.7	144.5	26.6	34.8	40.0
	SD	29.6	14.5	3.17	8.1	5.9
p-Value		$1x10^{-3}$*	$1x10^{-3}$*	$1x10^{-3}$*	$1x10^{-3}$*	$211x10^{-3}$

SD: standard deviation; *: significant at $P \leq 0.05$

Table 6: Comparison of (AST, ALT, ALP) between two study group

Groups		ALT	AST	ALP
Patients	Mean	31.22	44.4	88.92
	SD	1.42	6.8	12.23
Control	Mean	14.3	10.81	31.21
	SD	1.46	2.35	6.7
p-Value		$1x10^{-3}$*	$1x10^{-3}$*	$1x10^{-3}$*

SD: standard deviation; *: significant at $P \leq 0.05$

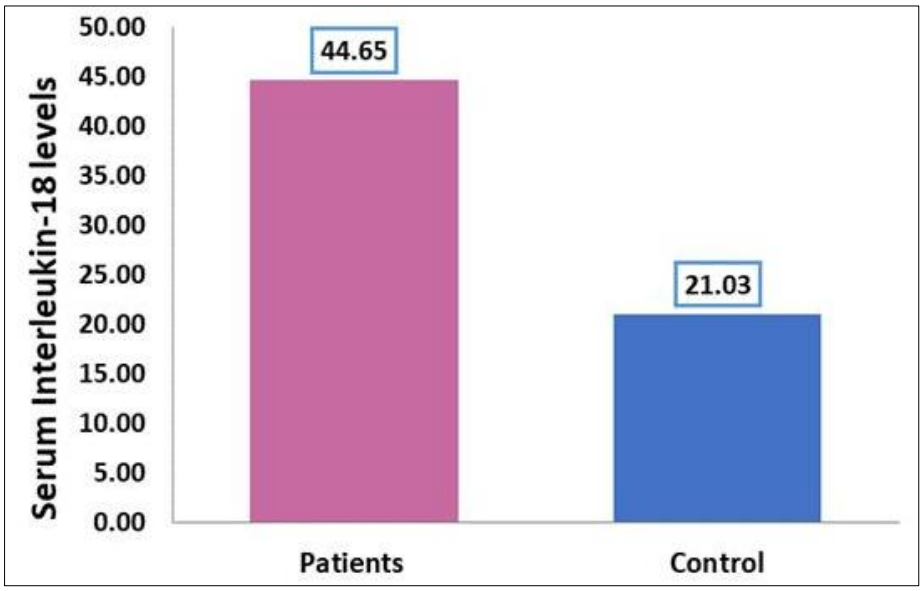

Fig 1: serum Interleukin-18 level in patients and healthy control subjects

Table 7: Comparison of (Ct, $2^{-}\Delta ct$ and Folding) between two study groups

Groups	Means Ct of miRNA-122)	Means Ct of U6	ΔCt (Means Ct of miR122)	2-ΔCt	experimental group/ Control group	Fold of gene expression
Patients	13.20	21.47	-8.27	310.26	310.26/91.58	3.4
Control	15.05	21.56	-6.52	91.58	91.58/91.58	1.00

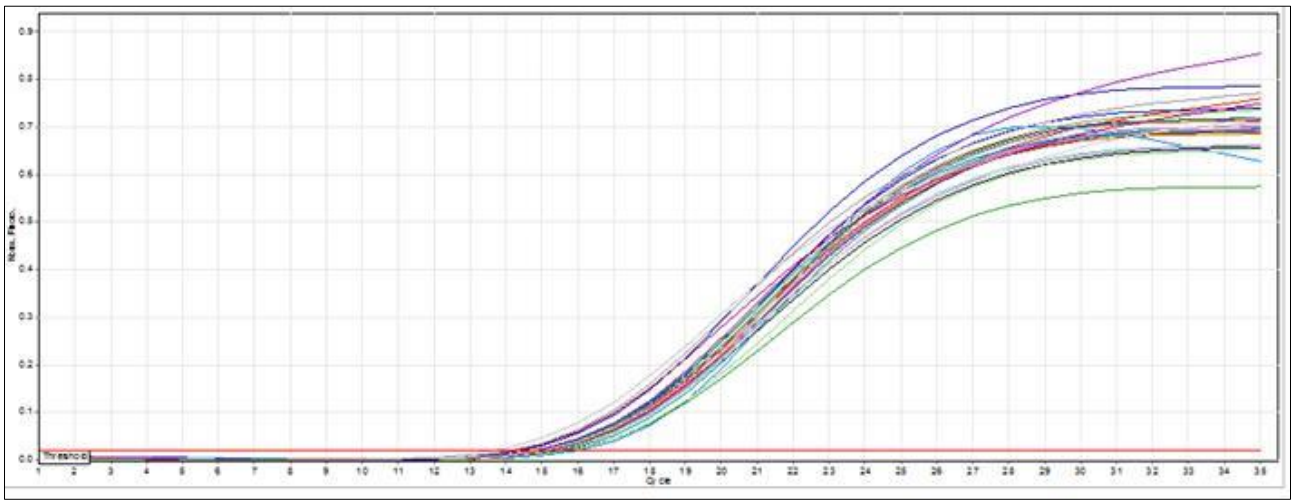

Fig 3: miR122 gene amplification plots by qPCR. Samples included all study groups. The photograph was taken directly from Agilent qPCR machine

4. Discussion

The main results of the study showed higher levels of microRNA in blood samples of liver patients compared to samples of healthy people. This aligns with the findings of a research conducted by Sharapova and associates [13]. Serum miRNA-122 levels may be considered a biomarker indicating liver toxicity. This has been confirmed by numerous studies examining acute and sub-acute toxicity in rat populations, and it is likely to compete with and outperform other diagnostic markers, including enzyme biomarkers, most notably liver transaminase. Additionally, the study found that patients with liver illness had higher serum levels of IL-18 than did healthy people. It is also in line with the findings of Falasca et al, who demonstrated that elevated IL-18 levels in the plasma of sick suffering of hepatic illnesses, like, non-alcoholic fatty liver disease and hepatitis C, have a unique role as an inflammatory marker and worsen damage to the liver and biliary system [14]. There was also a positive relationship between IL-18 concentrations in plasma and signs of cholestasis. According to the study's findings, liver patients' serum levels of FBG were higher than those of healthy people. According to studies by Deng et al, high FBG increases the hazard of non-alcoholic fatty hepatic disorder, which requires immediate treatment. Tests for liver function (ALT, AST, and ALP) showed modest elevation [15]. These tests aid in determining which part of the liver is most impacted and can aid in a differential diagnosis depending on the pattern of elevations. Liver cell disease is indicated by a disproportionate increase in (ALT) and (AST) relative to increases in bilirubin and (ALP). The production of albumin and vitamin K-dependent clotting factors are two indicators of actual liver function. Soon diagnosis and treatment can minimize morbidity and mortality from chronic liver disease. However, these laboratory values are not closely related to liver disease [16]. The results of lipid tests were also high in patients compared to healthy people. This is expected because lipids are essential components that control cell functions and internal balance, as the liver has the main role in metabolizing, producing, and sending them. Therefore, it is natural for an imbalance in lipid levels to appear in these tests in patients with various liver diseases [17]. Increased levels of miR-122 in liver cells may be linked to its potential role in important biological functions. Targets of miR-122 have been identified, particularly its association with cholesterol metabolism [4]. Increased liver enzymes like ALT, AST, and GLDH were closely linked to higher serum miRNA-122 levels. According to certain research, miRNA-122 performed similarly to AST and GLDH as a biomarker of liver damage, but better than ALT. The prediction accuracy rate when miRNA-122 was combined with ALT, AST, and GLDH was 4% [13].

5. Conclusion

The results of the study were promising regarding miRNA-122 and its increase in serum of patients with various hepatic problems and diseases, which stimulates the possibility of using it as a diagnostic tool and possibly a therapeutic tool in the future.

Conflict of Interest

There was no significant conflict of interest.

ACKNOWLEDGMENTS
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List of Abbreviations

RTPCR	quantitative Real-Time Polymerase Chain Reaction
ELISA	Enzyme-linked Immunosorbent Assay
IL-18	Interleukin-18
FBG	Fasting Blood Glucose
HBA1c	Hemoglobin A1c
ALT	Alanine Transferase
AST	Aspartate Transferase
ALP	Alkaline phosphatase
TSB	Total serum bilirubin
GLDH	Glutamate dehydrogenase
TG	Triglycerides
HDL	High-Density Lipoprotein
LDL	Low Density Lipoprotein
VLDL	Very Low-Density Lipoprotein

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