



Effect of Statin on *MiR122-5p* Gene Expression and Some Biochemical Markers in Iraqi Cardiovascular Disease Patients

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Abstract

Background. Cardiovascular disease (CVD) is the category of diseases that impact the heart and arteries. Statins are commonly administered to people who are at higher risk of cardiovascular disease. *MiR122* has been identified as a key mediator of cholesterol balance. **Aim.** To evaluate the effects of *miR122-5p* expression on statin response in Iraqi patients with cardiovascular disease. **Methods.** The study included 100 CVD patients, male and female, taking statins and ranging in age from 45 to 72 years. The patients divided into two groups, non-response group (50 patients) and the response group (50 patients), that are used as control group. Blood samples were taken and the total RNA was extracted from plasma samples by TRIzolTM Reagent extraction method for real-time PCR analysis to determine the gene expression of the *miR122-5p*. Biochemical markers Low-density lipoprotein, High density lipoprotein, Triglyceride and Glucose (LDL, HDL, TG, Glucose) were also assessed. **Results.** Age and family history both had substantial results ($P=0.028$ and $P=0.011$, separately). Low-density lipoprotein levels were elevated in statin-non-responsive patients and were substantial ($P=0.0001$). The glucose, TG and HDL were no an important variance. The gene expression of *miR122-5p* was significant variance between response group and non-response group ($P=0.0001$). Association between *miR122-5p* and LDL in station non-response CVD patients was observed (0.0001), with positive correlation (r-value 0.84), HDL was significant correlation (0.0002) and positively correlated (r-value 0.50). Triglyceride and glucose were no significant correlation (0.029, 0.112 individually). **Conclusion.** The results of this study suggest that the non-responsive group with CVD may have a different statin response due to upregulated of *miR122-5p*, which may impact cholesterol levels.

Keywords: Cardiovascular Disease, Statin, Gene expression, *miR122-5p*.

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Introduction

Cardiovascular disease (CVD) is a group of conditions that impact the heart and arteries such as coronary artery disease, heart failure, valve disease, and hypertension (1). Low-density lipoprotein and residual lipoprotein particles that accumulate inside arteries cause atherosclerosis, which is characterized by the formation of plaques (2). The global prevalence

rate of CVDs increased from 0.061% to 0.070% between 2010 and 2019 (3). CVD is responsible for 32% of deaths worldwide, with heart attacks and strokes accounting for 85% of these deaths (4). In Iraq, cardiovascular disease was estimated to be the cause of 33% of deaths (5). Traditional risk factors for CVD include hypertension, smoking, obesity, diabetes mellitus, and elevated cholesterol (6). Statins reduce

low-density lipoprotein cholesterol (LDL-C). Statins function by competitively blocking the action of 3-hydroxy-3-methylglutaryl-CoA reductase (7). Non-coding, single-stranded RNAs with a length of 21–25 nucleotides are called microRNAs, or miRNAs (8). miRNAs are involved in the control of several processes, such as development, apoptosis, and cell differentiation (9). Many gene expressions are regulated by miRNAs at the posttranscriptional level of mRNA. Using the 3' untranslated regions (UTR) as an attachment. MiRNAs function by inhibiting translation and digesting mRNA (10). Many miRNAs are essential for the growth and development of the human heart and vascular system, including angiogenesis, lipid metabolism regulation, plaque development, cardiac cell contractility and cell proliferation (11). *Mir122* is a liver specific miRNA. It is present at approximately 66,000 copies per cell in adult liver, representing over 70% of the total liver miRNA pool, *mir122* derives from a single genomic locus on chromosome 18 in humans. Hepatocyte nuclear factor 4 (HNF-4), a liver-enriched transcription factor, stimulates the synthesis of *mir122*. Within the liver, *mir122* regulates cholesterol metabolism, iron homeostasis and is a tumor suppressor (12). *Mir122* has been found to be important in regulating cholesterol homeostasis, based on a study of gene expression, mice administered *mir122* inhibitors displayed decreased expression of genes related to cholesterol production, including 3-hydroxy-3-methylglutaryl-CoA synthase 1 (*HMGCS1*) (13). However, the upregulated *mir122* increases the cholesterol synthesis by increasing sterol regulatory element-binding proteins (SREBPs) expression

indirectly. Sterol regulatory element-binding proteins (SREBPs) are transcription factors, which cling to the endoplasmic reticulum's membrane. In the presence of low cholesterol, SREBPs are carried to the Golgi, where they undergo proteolytic processing and trigger the transcription of genes linked to cholesterol (13). By inhibiting the activity of *mir122* in liver cells, *SOCS3* expression was reduced, which subsequently caused *STAT3* activation. after which *STAT3* inhibits SREBP expression. Because SREBP is crucial for regulating the generation of fatty acids and cholesterol, decreasing expression of *mir122* could be the cause of these processes' decline (14).

Materials and methods

The investigation was carried out between February and June (2024) at the Genetic Engineering and Biotechnology Institute, University of Baghdad. One hundred cardiovascular disease patients, the age from 45 to 72, were chosen from the Baghdad Teaching Hospital and the Iraqi Center for Cardiovascular Diseases. Divided into two groups: the response group, which acted as the control group, and the non-response group. Five milliliters of peripheral venous blood were drawn, then two milliliters of blood were moved into an EDTA tube, and the plasma put straight into TRIzol (TransZolUp, TRANS, China) preservation for RNA extraction and frozen, kept at -20°C. Three milliliters of blood were moved into a five milliliter sterile gel tube, and the clotted blood was separated by centrifuging it for ten minutes at 3000 rpm, every sample's serum was kept at -20°C to biochemical tests (LDL, HDL, TG, Glucose). Every set of tubes was assigned a unique code that

corresponded to every single serum and whole blood sample.

Protocol of microRNA Extraction

The TRIzol™ Reagent extraction method was used to extract total RNA from the plasma samples; Liquid samples were directly treated with 1 milliliter of TRIzol reagent. The RNA-containing aqueous phase was mixed with 200 µl of chloroform, precipitated as a white gel-like pellet with 500 µl of isopropanol, and then washed with 1 ml of 75% ethanol. Then in 50 µl of nuclease-free water and cooked in a water bath at 60 °C for 10 minutes.

Reverse transcription for complementary DNA (cDNA) synthesis

EasyScript® First-Strand cDNA Synthesis SuperMix kit (AE301-02, TRANS, China) contains all the necessary components to effectively synthesize first strand miRNA from whole RNA templates. After thawing from ice, the template RNA and all of the reagents were quickly centrifuged to gather the contents and additional components to cDNA preparation.

Quantitative real-time polymerase chain reaction (RT-qPCR)

A real-time PCR reaction was utilized to convert RNA to cDNA; cDNA served as the template, and RNA was detected using RNA-specific primers, for primer design, the *miR122-5p* sequence was obtained from MiRBASE database. sRNAPrimerDB software considered for primer design. RT-qPCR primers are provided from Macrogen company (Korea), as indicated in (Table 1). To normalize the results, use the *U6* as housekeeping gene (15). The $2^{-\Delta\Delta C_t}$ was used to determine the comparing RQ level (fold change) for *miR122-5p* expression among the statin response group other than those that statin non-response group. The lyophilized primers were dissolved in distilled water, to create a stock RNA solution, resulting in a final concentration of 100 pmol. Subsequently, a working solution of the primers at 10 pmol/µl was prepared by combining 10 µl of the primer stock solution with 90 µl of deionized distilled water. The volume of components of the qPCR mixture displayed in (Table 2).

Table (1): sequence of primers uses to *miR122-5p* and *U6* gene expression.

Primers		Sequences
<i>MiR122-5p</i>	Forward	ACCGAGGTTGGAGTGTGACAA
	Reverse	GTCGTATCCAGTGCAGGGTCC
<i>U6</i>	Forward	GTTTGTAGTTTTGGAGTTAGTGTGTGT
	Reverse	CTCAACCTACAATCAAAAACAACACAAACA

Table (2): The volume of the qPCR mixture's components

Components	Volume/Reaction
Master Mix	10 µl
cDNA	5 µl
Forward Primer (10 µM)	0.6 µl
Reverse Primer (10 µM)	0.6 µl
Nuclease-free water	3.8 µl
Complete Volume	20 µl

Statistical analysis

International Business Machine Statistical package for the Social Sciences (IBM SPSS– version 28) was

employed to determine how the study parameters were affected by the various groups (responders and non-responders). The results were analyzed

statistically, and the values were expressed as Mean. correlation test between the parameters and folding was employed. To compare two samples significantly, the t-test was employed. Significant comparisons between percentages were made using the chi-square test. P values that were equal to or less than 0.05 and 0.01 probability were deemed statistically significant.

Results

The age range of CVD patients in this study was from (45 to 72) years, the results of age distribution was

significant (0.028). The high frequency distribution of response group (32) occurs in age groups (45_50) years old, while more than 50 years in non-response group (29). as shown in the table (Table 3)

During the study, family history was evaluated for all study groups. The family history distribution was significant (0.011). The family history percentage of non-response CVD patients was higher. as shown in the table (Table 4)

Table (3): Age distribution in the studied groups.

Table (5). Age distribution in the studied groups.				
Age Groups	Cases		Pearson Chi square (χ^2)	P-value
	Response	Non-response		
45-50	32	21	4.857	0.028*
More than 50	18	29		
*(P≤0.05) Significant				

Table (4): Family History distribution in the studied groups.

Family History	Cases		Chi square (χ^2)	P-value
	Response	Non-response		
Yes	12	16	6.48	0.011*
No	38	34		
*(P≤0.05) Significant				

In this study, serum levels of low-density lipoprotein (LDL) were elevated in the statin non-response group (168.44±2.25 mg/dl). Although serum LDL levels in the response group were (78.80±2.45 mg/dl), there was a statistically significant difference between the two groups (0.0001). Additionally, the study showed that the differences in serum levels of HDL, triglycerides, and glucose between the

two groups were not statistically significant. The mean of HDL, Triglyceride and Glucose was (34.40±1.28 mg/dl), (89.08±2.48 mg/dl) and (90.98±1.46 mg/dl) in response group while the mean of HDL, Triglyceride and Glucose was (31.56±1.29 mg/dl), (93.06±3.50 mg/dl) and (91.12±1.54 mg/dl) in non-response group. As displayed in (Table 5).

Table (5): Evaluation of biochemical markers between responder and non-responder CVD patients.

Group	Mean ± SE (mg/dl)			
	LDL	HDL	TG	Glucose
Response patients	78.80±2.45	34.40±1.28	89.08±2.48	90.98±1.46
Non-response patients	168.44±2.25	31.56±1.29	93.06±3.50	91.12±1.54
P-value	0.0001*	0.12	0.35	0.94
T-test	-26.913	1.557	-0.926	-0.066
*(P≤0.01) Significant				

As per the results of the present investigation, patients with CVD who did not respond to statins had noticeably greater levels of *miR122-5p* expression than those who did. The fold change in

the expression of *miR122-5p* in response patients (fold change 1.06), and non-responding CVD patients (fold change 9.16), as illustrated in (Table 6).

Table (6): levels of *miR122-5p* in responder and non responder CVD patients.

Group	Ct of <i>miR122-5p</i>	Ct of <i>U6</i>	ΔCT	ΔΔCt	Folding	T-test	P-value
Response patients	25.73±0.24	20.68±0.26	5.05±0.07	0.0	1.06±0.058	-15.92	0.0001*
Non-response patients	23.66±0.23	21.70±0.21	1.96±0.07	-3.09±0.07	9.16±0.50		
*(P<0.01) Highly Significant							

In this study, the changes in *miR122-5p* expression levels and biochemical markers were assessed in statin non-response group. The *miR122-5p* expression levels increased with the LDL levels in non-response CVD patients, the r-value was positive correlation (r-value 0.84) and the correlation was significant (0.0001).

And the HDL levels were positively correlated with *miR122-5p* levels (r-value 0.50) and the correlation was significant (0.0002). Triglyceride and glucose levels were not significantly correlated with *miR122-5p* levels (0.029, 0.112 individually). As shown in (Table7)

Table (7): Correlation between changes in *miR122-5p* levels with biochemical markers in non-response CVD group

Folding of <i>miR122-5p</i>	LDL	HDL	TG	Glucose
Pearson Correlation	0.84	0.50	0.33	-0.25
p-value	0.0001*	0.0002*	0.029	0.112
N	50	50	50	50

*. Correlation is significant at the 0.01 level (2-tailed).

Discussion

As people age, several physiological processes gradually weaken, raising their risk of disease and other health problems. Pathological alterations include amplified arterial stiffness, reduced LV systolic reverse capacity, hypertrophy (16). structural changes, produces fewer nitric oxide (NO) that damaged arteries'

endothelium due to lowers NO-dependent inflation (17).

This result was shown that the non-response group was connected with age and had a high frequency in the age group of more than 50 years, and this may be associated with the changes in aging. Older patients often have altered pharmacokinetics and pharmacodynamics (18).

Speciously, the age distribution between response group and non-response group was significant difference ($P=0.028$), this outcome is consistent with Irene's results, which showed a statistical substantial variation between the age groups of patients with CVD (4).

In this study, in accordance with family history, the statin response varied considerably, the results indicate ($P=0.011$), and the percentage was higher in non-responder CVD patients. The risk of CHD rises linearly as the number of afflicted family member's rises. Claimed family history of cardiovascular disease (CVD) is an effective warning sign for later coronary heart disease (CHD) events. Cardiovascular illness is associated with premature coronary artery disease in the family (19).

These results are consistent with studies of Seung (20) and Christy (21), which further corroborate that a parent's history of cardiovascular disease increased the probability of developing CVD.

Based on their LDL serum levels, individuals in this study were divided into statin therapy responders and non-responders. The findings indicated that the statin response varied greatly depending on LDL levels ($P=0.0001$). However, there are a number of possible reasons. The four categories of potential reasons for statin hyporesponsiveness are as follows: primary statin resistance, common lipid disorders, compliance issues, and analytical problems. Most labs continue to compute LDL-C using the equation, which is based on the count of lipids in a typical lipid screen (22) Primary statin resistance may be caused by a variety of gene variations that are linked to the efficacy of statins (23). Multiple studies

have shown that a percentage of those receiving treatment will give up using statins after a few months (24).

Apparently, the mean of responders and non-responders was (78.80 ± 2.45) and (168.44 ± 2.25) respectively, and the mean percentage was higher in the non-responder CVD patients. The result aligns with Bjorn's research (25) and Suma's research (26) that the LDL-C reduction varies significantly among CVD patients who use statins.

The findings indicated that there was no discernible variation between HDL levels and the statin response ($P=0.12$), and the mean percentage of HDL was higher in the responder CVD patients.

Different statins have varying degrees of HDL-C increasing effects. Given that statins often boost the liver's production of apolipoprotein (Apo) A-I and HDL neogenesis, their effect on HDL-C may be due to reverse cholesterol transport (RCT) activation (27).

The mean of these cases in this study was (34.40 ± 1.28 vs 31.56 ± 1.29), which distresses with Philip's research that found the ability of statins to raise HDL-C significantly (28).

Triglyceride levels did not suggestively variation with statin therapy ($P=0.35$), according to this research, and the mean percentage was higher in patients with CVD who did not respond to treatment. The mean of these cases was (89.08 ± 2.48 vs 93.06 ± 3.50).

In fact, VLDL triglyceride levels can drop by as much as 35–45% in patients with severe hypertriglyceridemia when statins are used. Statins cause the hepatocyte cell surface to express low-density lipoprotein (LDL) receptors. Because liver LDL receptors are overexpressed, there is a rise in the

breakdown of VLDL and chylomicrons, which lowers TG levels. Consequently, statins' ability to lower TG has been viewed as a negative consequence (29).

These findings are distressing with Andrew's research (30). The CVD patients in this study with normal triglyceride levels and statins lower the high levels of triglycerides.

There was no discernible change in the glucose serum levels between CVD patients who responded and those who did not ($P=0.94$), the mean of these cases (90.98 ± 1.46 vs 91.12 ± 1.54). Statin-associated diabetes risk has been linked to a number of pathways, most of which are connected to either reduced insulin production or increased insulin resistance. HMG CoA suppression has several downstream consequences that may raise the risk of diabetes. The CVD patients in this study with normal blood sugar levels and the increased risk of diabetes is limited to people who are already at risk of getting the disease (31). These results are distressing, with Tanushree's research which shown that statins caused a statistically important increase in fasting glucose (32).

According to the study, the expression levels of *miR122-5p* varied significantly across the groups of patients with CVD ($p=0.0001$), with fold changes of 1.06 in the response group and 9.16 in the non-response group. The outcomes of this study are consistent with those of Etxebarria's study, which discovered that hepatocytes treated with statins express more *miR-122* (33). In non-response group, the expression levels of *miR122-5p* increased in parallel with the LDL levels, the r-value was positive (r-value 0.84) and the correlation was significant (0.0001), and correlation between HDL levels and *miR122-5p* levels was positive (r-value 0.50) and significant

(0.0002). The increase of *miR122-5p* expression level in statin non-response group raises the possibility of *miR122-5p* effect on the statin response in lowering of LDL serum levels. Statin inhibition the action of 3-hydroxy-3-methylglutaryl-CoA reductase (7). And transcription of 3-hydroxy-3-methylglutaryl coenzyme A reductase regulated by sterol regulatory element-binding proteins (SREBPs). In the presence of low cholesterol, SREBPs are undergo proteolytic processing and trigger the transcription of HMG-CoA reductase (14). The *miR-122* control cholesterol homeostasis and increases the cholesterol synthesis by increasing SREBP expression indirectly (12). The use of statins inhibits HMG-CoA reductase, which raises the expression of *miR122*. This, in turn, causes the upregulated *miR122* will have a feedback effect that raises cholesterol levels as result of statin use.

Conclusions:

The level of *miR122-5p* expression was significantly increased in non-response CVD patients who have elevated LDL serum levels, the results of the statistical analysis shown significant positive correlation between *miR122-5p* expression levels and cholesterol serum levels in non-response group. According to the results of this study, overexpression of *miR122-5p* may affect the cholesterol level and thus, the statin response in the non-responsive group with CVD.

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