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## ORIGINAL ARTICLE

# MICRORNA-122 AS A BIOMARKER ASSOCIATED WITH OXIDATIVE STRESS IN PATIENTS SUFFERING FROM METABOLIC DISEASES

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**Summary**

**Background:** Recent evidence has shown that circulating microribonucleic acid (miRNA) has been related to many diseases either as an inhibitor or a stimulant factor, among them miRNA-122 which has proven through studies its relationship with insulin resistance, an adversative lipid profile, obesity, type 2 diabetes, and metabolic syndrome in several studies; however, the mechanisms involved are unknown. This study investigates the role of miRNA-122 expression in overweight patients suffering from metabolic disorders such as diabetes and hypertension and its relationship to the development of oxidative stress in patient groups.

**Materials and Methods:** 30 patients with type 2 *diabetes mellitus* (T2DM), 30 people with hypertension (HTN), 30 patients with T2DM+HTN, and 30 healthy persons who served as controls were enrolled in this study. An ARCHITECT c4000 clinical chemistry analyzer was used to assess lipid profiles. The sandwich immunodetection approach was used to assess whole blood hemoglobinA1c. By colorimetric methodology, catalase activity (CAT), superoxide dismutase activity (SOD), malondialdehyde (MDA) levels, and advanced oxidation protein products (AOPPs) levels were measured. The expression of serum miRNA-122 was determined using the quantitative polymerase chain reaction.

**Results:** The activity of SOD and CAT in patient groups was found to be substantially lower than in the control group ($p < 0.05$), whereas MDA and AOPP concentrations were found to be significantly higher in patient groups compared to the control group ($p < 0.05$). When patient groups were compared to control groups, the miRNA-122 level was higher in the patients ($p< 0.05$).

**Conclusions:** miRNA-122 expression is involved in the pathogenesis of T2DM and HTN-induced oxidative stress, there is a reciprocal relationship between the increase in gene expression of the miRNA-122 and the increase in oxidative stress accompanied by a decrease in the effectiveness of antioxidant enzymes, which leads to the development of the disease.

*Key words: Metabolic Syndrome; Oxidative Stress; miRNA; Diabetes Mellitus; Hypertension*

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## Introduction

Central obesity, HTN, hyperglycemia, excessive triglycerides, and low levels of high-density lipoprotein in the blood (HDL) are all symptoms of the metabolic syndrome (MetS), which is defined by the presence of three or more of these conditions (1).

*Diabetes mellitus* (DM) is a metabolic disorder of carbohydrate metabolism disease characterized by hyperglycemia due to a deficiency of insulin hormone (Type 1 DM), either insufficient insulin secretion or an ineffectual response of cells to insulin (Type 2 DM) (2). Hypertension is correlated to diminished metabolic homeostasis, so it can be considered a metabolic disorder (3).

When the equilibrium of pro-oxidants and antioxidants is disrupted, oxidative stress occurs, and it plays a role in the development of diabetes and cardiovascular disease (4). Reactive oxygen species (ROS) are created via hyperglycemia and can harm cells in a variety of ways. Cell damage in DM eventually leads to secondary issues (5). In HTN, antioxidant activities, and lipid peroxidation by-products demonstrate an increase in ROS and a decrease in antioxidant defense activities in the blood and numerous cellular systems, including not just vascular wall cells (6), but also in circulating cells (7). Antioxidants can contribute hydrogen atoms to free radicals created by cellular metabolism or from outside sources, producing damage to lipids, amino acids, and deoxyribonucleic acid (DNA), eventually leading to cell death (8). Superoxide dismutase (SOD) family members operate as the first line of defense against ROS, eliminating highly reactive superoxide radicals and converting them to hydrogen peroxide ($H_2O_2$), which is then degraded by catalase (CAT), glutathione peroxidase (GPX), and peroxiredoxin (PRDX) (9). CAT is an antioxidant enzyme present in peroxisomes that contributes to the cell's antioxidant system.

Advanced oxidation protein products (AOPPs) have been hypothesized as one of the possible indicators of oxidative damage, which occurs due to oxidative and carbonyl stress and results in an increase in global inflammatory activity (10). In biological samples, malondialdehyde (MDA) is an excellent indicator of free radical-mediated damage and oxidative stress, with polyunsaturated fatty acid peroxidation being the main source of MDA (11).

Obesity and other aspects of MetS have yet to be fully understood molecularly, despite several studies indicating an expanding role for epigenetics, particularly concerning microRNAs 12). Through unique interactions with target genes, the non-coding single-stranded RNAs of 19–25 nucleotides (miRNAs) serve a function in transcriptional and post-transcriptional gene expression control (13). Adipocyte differentiation, metabolism, appetite control, and oxidative stress are only a few of the physiological and pathological processes in which miRNAs play a role (14). In teenagers with morbid obesity, ten circulating miRNAs have recently been identified as indications of (MetS), and miRNA-122 has been demonstrated to predict the chance of developing (T2DM) and (MetS) (15).

## Methods

### Sample Collection

The current study involved 120 individuals who were separated into 4 groups: individuals suffering from HTN, individuals suffering from T2DM, individuals suffering from both HTN and T2DM (HTN+T2DM) and healthy controls. All patients' information was recorded, such as sex, age, and duration of illness, and the control group was carefully selected to ensure that they did not have diabetes, hypertension, or other illnesses or disorders.

In the current study, the average age of all subjects varied from (39-66) years old. Patients visit the Murjan Teaching Hospital for routine checks out. The specialist doctor identified all the patients in this study, and the diagnosis was confirmed by clinical features and biochemical tests, like Cytosolic blood pressure (SBP), Diastolic blood pressure (DBP). Fasting blood sugar (FBS), Glycated hemoglobin (HbA1c), and lipid profile.

Information is also recorded from patients about age, gender, body mass index, smoking, family history, duration of disease, diabetes, and antihypertensive drugs. The current study was conducted in all laboratory test analyses performed at Nabu Scientific Foundation (Baghdad /Iraq), Murjan Teaching Hospital, Dr. Ammar Mohammed

Al-Amoud Lab in (Babylon / Iraq), and Biochemistry Laboratory in the College of Science (University of Al- Qadisiyah) at Al-Qadisiyah University.

Exclusion criteria: The study excluded patients suffering from COVID-19, liver disease, renal disease, thyroid disorders, and autoimmune disease.

**Storage**

Each subject's blood (5 mL) was obtained and separated into two tubes, (1 mL blood in K2EDTA tube for hemoglobin A1c (HbA1c), and 4 ml in gel tube). The serum was removed from the gel tube of blood by centrifuging it at 3600 rpm for 10-15 minutes. The isolated serum was divided into four portions using Eppendorf tubes. For the miRNA-122 study, one component was held at -40°C, while the others were maintained at -20°C for biochemical testing.

**Method**

Fasting blood sugar (FBS) was determined by spectrophotometry, while lipid profiles such as (Total cholesterol (TC), Triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), and Low-density lipoprotein-cholesterol (LDL-C) were determined by ARCHITECT c4000 clinical chemistry analyzer (Abbott, Japan) from Murjan Teaching Hospital. The glycated hemoglobin (HbA1c) level was measured by the sandwich immunodetection method (AFIAS HbA1c kit, Boditech, Korea). The activity of serum (SOD) was measured using the spectrophotometry technique (16). (CAT) was determined by ultraviolet (UV) spectrometry (17). (MDA) concentrations were determined by spectrophotometry (18). (AOPP) concentration was determined by UV spectrometry (10). The serum level expression of miRNA-122 was determined by quantitative polymerase chain reaction (qPCR). For RNA extraction, 0.3 mL of serum was employed using (TRIzol™ Reagent, Invitrogen, USA). MiRNA with miR-122-RT-primer was used to make the cDNA using (ProtoScript® First Strand cDNA Synthesis Kit, NEB, UK). PCR was conducted using Luna Universal qPCR MasterMix (NEB, UK). The resulting cDNA was mixed with miR-122-specific forward, reverse universal primers (Table 1), and cDNA Bright Green master mix. Gene U6 was employed as an internal control. A comparative threshold cycle (Ct) was used to calculate the relative levels of miR-122 and ($2^{-\Delta\Delta Ct}$), and the results indicated the fold change of expression.

**Table 1.** Primers used for qPCR experiments.

| Primers | Sequence | Size | Product Size (bp) |
|---|---|---|---|
| **miR-122_RT** | GTCGTATCCAGTGCAGGGTCCGAGGTGCACTGGATACGACCAAACAC | 47 | ------ |
| **miR-122For** | TGCGGTTGGAGTGTGACAATGG | 22 | |
| **miR-122Rev** | CAGTGCAGGGTCCGAGGT | 18 | 85 |
| **U6 For** | CTCGCTTCGGCAGCACA | 17 | |
| **U6 Rev** | AACGCTTCACGAATTTGCGT | 20 | 94 |

**Statistical Analysis**

The statistical analysis was carried out using version 28 of the Statistical Package for Social Sciences (SPSS). The information is given in the form of a mean and standard deviation. For normal and non-normal distribution data, (Kruskal Wallis) non-parametric ranking or one-way ANOVA with Tukey post hoc analysis was employed to analyze distinct groups. Throughout the study, a $P < 0.05$ was considered significant throughout. The correlation coefficient was utilized to identify the link between two continuous variables (r).

**Results**

There were 90 patients and 30 healthy controls in this research. Table 2 exhibits the patients' features as well as the results of the biochemical testing. When compared to the other groups, the body mass index (BMI) values in T2DM were greatly increased. SBP and DBP levels in the (T2DM+HTN) group were considerably higher than

in the other groups. HbA1c and FBS levels were considerably greater in T2DM and (T2DM+HTN) patients than in HTN patients and the control group. The (T2DM+HTN) group exhibited considerably greater TC and TG levels than the other groups. The T2DM group had much greater LDL-C than the other groups, while the control group had significantly higher HDL-C. ($P < 0.05$).

**Table 2.** The study groups' clinical and laboratory feature.

| Parameters | Groups (Mean ± SD) | | | | p-value |
|---|---|---|---|---|---|
| | Control | T2DM | HTN | T2DM +HTN | |
| **Number** | 30 | 30 | 30 | 30 | |
| **Age (years)** | 48.27 ± 6.47 | 51.00 ± 6.27 | 52.86 ± 5.82 * | 55.04 ± 6.059 * | 0.001 |
| **Sex(M/F)** | 15/15 | 16/14 | 15/15 | 17/13 | --- |
| **BMI (Kg/m²)** | 24.80 ± 2.07 | 28.49 ± 1.62 * | 28.04 ± 1.29 * | 27.27 ± 2.05 * | 0.001 |
| **SBP (mmHg)** | 113.59 ± 8.12 | 122.40 ± 5.37 * | 139.36 ± 3.836 * | 143.13 ± 3.68 * | 0.001 |
| **DBP (mmHg)** | 75.40 ± 3.09 | 80.36 ± 3.45 * | 88.22 ± 4.18 * | 91.81 ± 4.59 * | 0.001 |
| **FBS (mg/dl)** | 92.18 ± 8.85 | 236.44 ± 56.0 * | 97.86 ± 9.47 | 226 ± 81.85 * | 0.001 |
| **HbA1c (%)** | 4.80 ± 0.49 | 9.30 ± 2.54 * | 5.17 ± 0.78 | 7.85 ± 1.90 * | 0.001 |
| **TC (mg/dl)** | 137.45 ± 22.68 | 166.09 ± 25.7 * | 176.63 ± 28.38 * | 187.45 ± 28.49 * | 0.001 |
| **TG (mg/dl)** | 103.86 ± 17.75 | 139.04 ± 19.8 * | 125.36 ± 16.95 * | 165.22 ± 24.86 * | 0.001 |
| **HDL-C (mg/dl)** | 51.86 ± 7.79 | 42.36 ± 7.30 * | 47 ± 7.20 * | 35.31 ± 4.99 * | 0.001 |
| **LDL-C (mg/dl)** | 50.27 ± 4.70 | 113.5 ± 17.65 * | 49.90 ± 5.23 | 80.86 ± 9.87 * | 0.001 |

*Mean statistically significant differences between patient groups as compared to the control group ($P < 0.05$). SBP=systolic blood pressure, DBP=diastolic blood pressure, BMI=body mass index, FBS=fasting blood sugar, HbA1c=glycated haemoglobin, TC=total cholesterol, TG=triglyceride, HDL-C=high density lipoprotein, LDL=low density lipoprotein

When compared to the control group, SOD activity was decreased in the T2DM+HTN, T2DM, and HTN groups (Figure1). When compared to the control group, CAT activity was decreased in T2DM+HTN, T2DM, and HTN (Figure 2). The AOPP levels in the (T2DM+HTN), HTN, and T2DM groups were all significantly higher than in the control group (Figure 3). When compared to the control group, MDA levels were higher in (T2DM+HTN), T2DM, and HTN (Figure 4). qPCR miRNA analysis showed serum levels of miRNA-122 expression increased significantly in the T2DM group in comparison to a control group, but there was a discrepancy in the HTN group, with 50% of patients showing an increase and 50% showing a decrease in miRNA-122 gene expression in comparison to a control group. The level of miRNA-122 was slightly higher in T2DM+HTN in comparison to the control group (Figure 5).

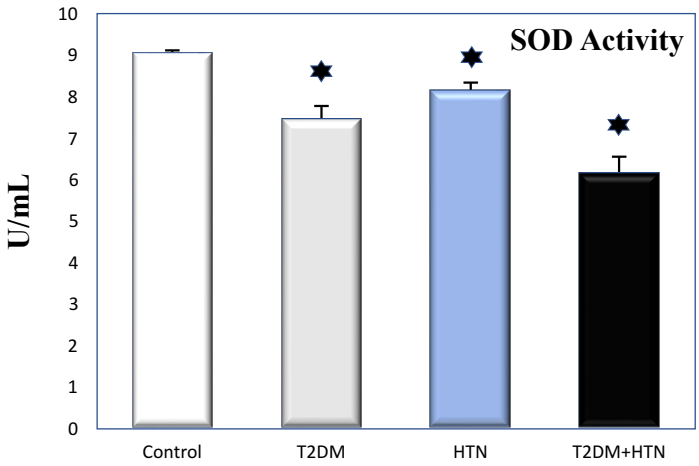


**Figure 1.** Comparison of SOD activity U/ml in different studied groups, control, T2DM, HTN, and T2DM+HTN P-value < 0.001. * Mean statistically significant differences between patient groups as compared to the control group ($P < 0.05$).

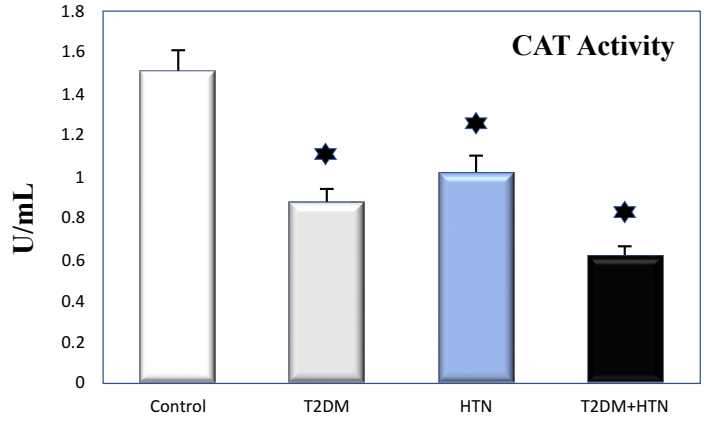


**Figure 2.** Comparison of CAT activity U/ml in different studied groups, control, T2DM, HTN, and T2DM+HTN (P-value < 0.001).
* Mean statistically significant differences between patient groups as compared to the control group (P < 0.05).

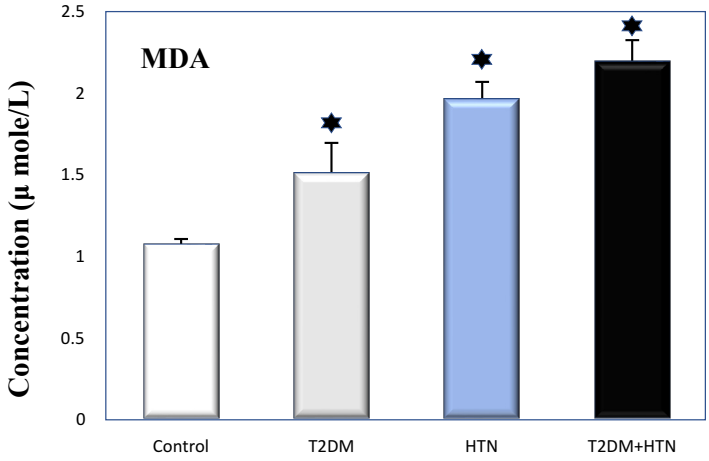


**Figure 3.** Comparison of MDA level µmol/L in different studied groups, control, T2DM, HTN, and T2DM+HTN (P-value < 0.001).
* Mean statistically significant differences between patient groups as compared to the control group (P < 0.05).

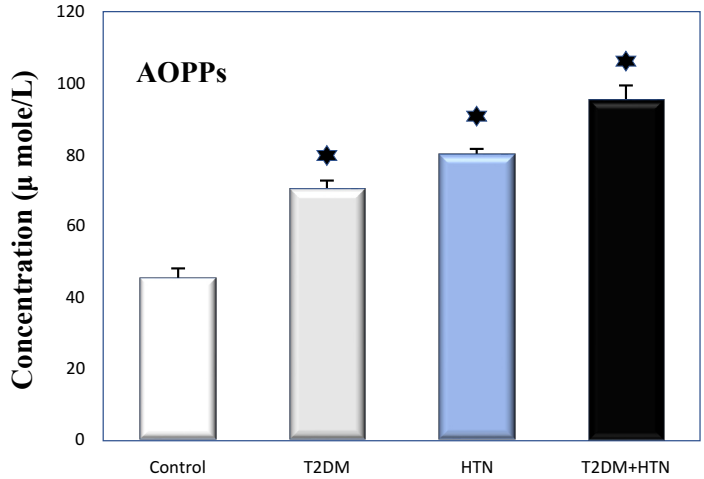


**Figure 4.** Comparison of AOPPs level µmol/L in different studied groups, control, T2DM, HTN, and T2DM+HTN (P-value < 0.001).
* Mean statistically significant differences between patient groups as compared to the control group (P < 0.05).

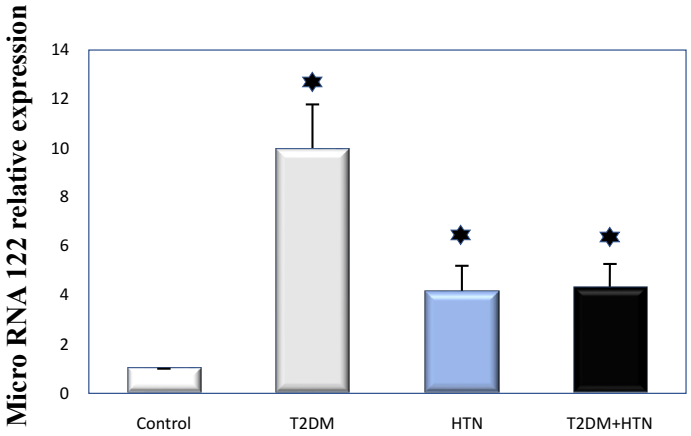


**Figure 5.** The level of expression of microRNA-122 in the various study groups was compared, control, T2DM, HTN, and T2DM+HTN (P-value = 0.049). * Mean statistically significant differences between patient groups as compared to the control group ($P < 0.05$).

In patient groups, there were significant positive associations between miRNA-122 expression levels and TG, LDL-C, MDA, and HbA1c, while negative correlations between miRNA-122 expression levels and HDL-C, and CAT (Table 3).

**TABLE 3.** Correlation of miR-122 with other parameters in T2DM, HTN, and T2DM+HTN patients.

| Compared parameters | T2DM | | HTN | | T2DM+HTN | |
|---|---|---|---|---|---|---|
| | Value of correlation coefficient | P-value | Value of correlation coefficient | P-value | Value of correlation coefficient | P-value |
| **miR-122 vs HbA1c** | + 0.801 ** | < 0.001 | + 0.826 ** | < 0.001 | + 0.818 ** | < 0.001 |
| **miR-122 vs LDL-C** | + 0.529 * | 0.0196 | + 0.677 ** | 0.001 | + 0.504 * | 0.027 |
| **miR-122 vs TG** | + 0.313 | 0.192 | + 0.431 | 0.065 | + 0.401 | 0.088 |
| **miR-122 vs HDL-C** | - 0.413 | 0.079 | - 0.686 ** | 0.001 | - 0.585 ** | 0.008 |
| **miR-122 vs MDA** | + 0.636 ** | 0.003 | + 0.432 | 0.064 | + 0.359 | 0.13 |
| **miR-122 vs CAT** | - 0.34 | 0.154 | - 0.26 | 0.278 | - 0.22 | 0.365 |

* (P-values < 0.05): significant different, ** (P-values < 0.01): highly significant different

## Discussion

SOD and CAT activities were found to be considerably reduced in the group with both HTN and diabetes. SOD and CAT, which scavenge free radicals, are the initial line of defense against oxidative damage in normal conditions, digesting superoxide and $H_2O_2$ beforehand and combining to create the more dangerous hydroxyl radical. The reduction in SOD and CAT activity might be explained by cross-linking or depletion of the enzymes due to increased lipid peroxidation (19). Because hypertensive and diabetic individuals are unable to eliminate circulating superoxide anion, ROS-induced vascular damage increases, and a decrease in SOD activity suggests a deficiency in antioxidant defense mechanisms. As a result, low serum SOD activity has been linked to a higher risk of vascular damage. The results agreed with Gómez-Marcos *et al*., (2016), and Okoduwa *et al*., (2015) (20, 21).

The increase in MDA in both sexes of patients with (HTN) and (T2DM) is attributed to oxidative stress caused by the damage caused by high blood pressure and diabetes in body tissues, as well as the drugs used to treat both

diseases, which cause the generation of free radicals and increase lipid oxidation reactions in cell membranes, according to the research results (MDA) (22). The results of the present study also agree with Okoduwa *et al*., (2015) and Mahreen *et al*., (2010), who found that serum MDA is elevated in a patient with T2DM, HTN, and (T2DM+HTN) compared to control (21, 23).

The results revealed a strong increase in the level of AOPPs in patients with (HTN) and (T2DM) when compared to the control group. It was also discovered that the level of AOPPs was advanced in patients with (HTN) when compared to patients with (T2DM) (T2DM). Molecular processes connected to ROS formation and oxidative stress in response to high glucose concentrations include increased flow via the polyol and glucosamine pathways, activation of protein kinase C, and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (23-25). Chronic AOPP production in T2DM patients may promote renal inflammation by activating renal NADPH oxidase, according to the findings. HTN is an oxidative stressor in and of itself, and it has been related to end-organ damage and atherosclerosis due to HTN (26). AOPPs are a novel oxidative stress marker in hypertension. By stimulating neutrophils, monocytes, and T-lymphocytes, AOPPs might have a role in oxidative stress and inflammation. When the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is active, AOPPs decrease nitric oxide (NO) production in macrophages and enhance ROS production in endothelial cells *in vitro* (26). The activation of NADPH oxidase, which stimulates angiotensin II type 1 (AT1) receptors, may result in increased production of ROS and a decrease in NO in arterial HTN (27). The results are in agreement with Conti *et al*., (2019) (28).

In the liver, miR-122 is a prevalent microRNA that accounts for more than 75% of total microRNA expression (29). Furthermore, Willeit *et al*., (2017) found that persons with T2DM had significantly greater levels of serum miR-122 (30), which matches the results. Indeed, miR-122 has been associated with T2DM, HTN, atherosclerosis, and potentially heart failure. The renin-angiotensin-aldosterone system, vascular endothelial dysfunction, and vascular smooth muscle are all affected by miRNAs, which have a role in the genesis and progression of HTN (31). According to one study, miR-122 expression in the plasma of HTN African green monkeys was significantly higher than in the plasma of normal stress African green monkeys (32). According to the average miR-122 gene expression, it increased in HTN compared to the control group, which is consistent with what Zhou *et al*., said. However, there is a noticeable discrepancy in miR-122 expression in the same group (HTN). It was observed that the reason for the difference in HTN patients might be related to the level of lipids, where a strong relationship was observed between miRNA 122 and lipid markers, as it was found that patients with higher levels of TG and lower levels of HDL showed an increase in miR-122 expression. There is a significant increase in the expression level of miR-122 in (T2DM) patients compared to the control group, microRNA-122 gene expression showed instability in the group with (HTN), resulting in lower expression levels in (T2DM+HTN) and (HTN) patients compared with (T2DM).

There were strong positive relationships between miR-122 expression and TG, LDL, and HbA1c levels, but there arenegative correlations with HDL-C, according to our findings. The results are in agreement with Rashad *et al*., (33), who found a favorable correlation between miR-122 levels with TC, TG, HDL-C, and LDL-C levels in T2DM patients with and without coronary artery disease (CAD). Negative associations were found between miR-122 with CAT activity, while positive associations with MDA concentrations, while no studies have been found on these associations.

**Study limitations**

Issues with research samples and selection, the lack of sufficient previous research studies on the subject of miRNA-122, insufficient sample size, cost, and time constraints.

**Conclusion**

This study found a tight association between increasing the gene expression of miRNA-122 and its stimulus to oxidative stress through the inhibition of antioxidant enzymes. The antioxidant system may be affected directly by miRNA. Several investigations should be carried out to understand the mechanism of miRNA-122's detrimental influence on the antioxidant enzymatic system, hence raising the amount of oxidative stress, which has an impact on the development and worsening of illnesses caused by metabolic imbalances.

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**Conflict of Interest Statement**

The authors have no conflicts of interest regarding the publication of this article.

**Adherence to Ethical Standards**

The study was approved by the ethical committee at the University of Al-Qadisiyah (registration code CMUQ 3150 on 19.10.2021).

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