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Circulating microRNA-1, and IL-18 are associated with inflammation in patients with type 2 diabetes mellitus: A case control study

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Abstract

Background and objective: IL-18 and micRNA-1 can be differentially regulated in cardiomyopathy, nephropathy, and amputation that are among the many micro- and macrovascular issues known to be the complications of type 2 diabetes mellitus (T2DM). Thus, this study aims to detect the association of miRNA-1 and IL-18 with type 2 diabetes mellitus.

Methods: This study included 90 participants including 30 diabetic patients, 30 pre-diabetic patients and 30 healthy controls in Erbil City, Iraq between November 2021 and May 2022. Different parameters were assessed for each participant including levels interleukin-18, high sensitivity C-reactive protein, lipid profile, and miRNA-1 profiling. 200µL of fresh plasma was utilized for the extraction of microRNA, and the extracted RNA was subsequently transformed into cDNA. Utilizing quantitative Real-Time PCR, the miRNA profiling analysis was carried out.

Results: When comparing the mean age of the diabetic patients to the other studied groups, there was a statistically significant difference (P <0.05). Furthermore, there was a greater proportion of males, smokers, obese people, and people with hypertension among DM patients. When compared to the healthy controls, the concentration levels of IL-18 and hsC-reactive protein in both DM and pre-DM patients exhibited a substantial elevation (P <0.01). The expression of miRNA-1 was down-regulated in healthy controls and considerably up-regulated in both diabetes and pre-diabetic patients.

Conclusion: These findings demonstrated that T2DM patients had higher expression of miRNA-1 than did healthy persons. MiRNAs may be very important in the etiology of type 2 diabetes via inducing inflammation. More research is necessary to determine their implications as diagnostic and preventive biomarkers.

Keywords: MiRNA-1 expression; IL-18; Type 2 Diabetes Mellitus; Inflammation.

Introduction

Cardiomyopathy, nephropathy, and amputation are among the many micro- and macrovascular issues that are known to be complications of type 2 diabetes mellitus (T2DM), which increases the mortality rate among T2DM patients.(1) Therefore, identifying biological predictors for type 2 diabetes can help with early diagnosis and prognosis by illuminating novel molecular pathways linked to the illness.(2) miRNAs are small, single-stranded non-coding RNAs that are involved in practically every aspect of biological activity. Their most well-known function is regulating the post-transcriptional levels of gene expression.(3) Actually, around 60% of the protein-coding genes in mammalian cells have conserved targets for miRNAs. The 3'UTR of the mRNA targets and miRNAs partially base pair through their 5'-proximal seeding region.(4) This complex targeting process is strictly regulated in a variety of biological contexts, ultimately leading to decreased target gene expression.(5)

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Mechanistically, miRNAs restrict translation or activate mRNA deadenylation, which breaks down transcripts, to regulate gene expression in two distinct and separate ways.[6] As a result, miRNAs demonstrate a considerable effect in the diabetes mellitus development in humans.[7]

Interleukin-18 (IL-18) is a cytokine that belongs to the IL superfamily and is associated with the advancement of diabetes mellitus. It was initially discovered in the late 20th century as a molecule that induces interferon-gamma (IFNγ).[8] Smooth muscle cells, endothelial cells, monocytes/macrophages, and other cells produce it. IL-18 plays a major role in the etiology of atopic, autoimmune, and chronic inflammatory illnesses. It stimulates the expression of adhesion molecules, chemokine receptors, granulocyte macrophage colony-stimulating factor (GM-CSF), IFNγ, tumor necrosis factor (TNFα), and interleukin 1 (IL-1). It also activates type 1 or type 2 T helper immune response.[9] Apart from its role in immune defense against infectious agents. NK cells become more toxic when exposed to IL-18. A change in the ratio between IL-18 and IL-18 binding protein inhibits its function, leading to a state of illness. Like TNF-α and IL-6, high blood levels of IL-18 indicate a metabolic syndrome.[10] In view of that, the current study tries to investigate the possible association between IL-18 and miRNA-1 levels and the complications of T2DM in a sample of local population.

Methods

Study design

This prospective case control study was conducted on ninety participants from Nafie Akraey Health Center in Erbil City, Iraq between November 2021 and May 2022. The participants were all citizens of the Governorate of Erbil in Iraq. Individuals with a history of cancer, ongoing inflammatory or autoimmune disorders, bleeding and clotting problems, or recent surgery or trauma were not included.

Participants (n=90), ages ranging from 37 to 63, were divided into three groups: pre-diabetic (HbA1c: 5.7% – 6.4%), T2DM (HbA1c > 6.4) based on ADA guidelines[11], and healthy individuals (n = 30/group).

Samples handling and storage

Approximately 5 ml of venous blood sample was taken right away using the venipuncture technique. After being collected, blood samples were centrifuged at 1000–2000 rpm for 15 minutes at room temperature (RT) (using coagulation sodium citrate 3.2% tubes) to extract plasma. After that, the collected plasma was divided into several aliquot in 2 mL tubes and kept for later examination at -80°C.

Study investigations

The study groups underwent tests for the quantitative assessment of IL-18 using the enzyme linked immune sorbent assay (ELISA) in BioLab laboratory and the qPCR analysis of MiRNA-1 expression inZheen Genetic Center for both healthy controls and diabetes patients.

Enzyme-linked immunosorbent assay

The ELISA method was utilized to evaluate the plasma levels of IL-18, an inflammatory cytokine, in patients with diabetes. In specifics, commercial ELISA kits from the (Invitrogen, USA) were used for the test. The methods were carried out strictly in compliance with the manufacturer's experiment protocol. As soon as the stop solution was added, the absorbance at 450 nm was measured. With every assay, a standard curve was created, and the concentration of the tested materials was determined by referencing the standard curve.

Isolation of microRNA

Following the manufacturer's instructions, 200µL of plasma was used to isolate miRNA using the FavorPrep^{TM}small RNA isolation kit (Biofluids Corp - Korea).

MiRNA Expression analysis by qRT-PCR

The Add-Bio kit (Korea) was used to reverse transcribe the extracted miRNA. Using SNOR miRNA as a reference, quantitative real-time PCR was used to

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measure the expression levels of miRNA-1. For every sample, three replicates were used in order to attain a greater level of reliability with a coefficient of variation of less than 10%. The differential expression was only computed using the Ct values that fell between 15 and 40. By normalizing the target miRNA expression levels against SNOR miRNA, the ΔCt technique was utilized to quantify the relative expression level of each particular miRNA as: $\Delta ct = (miRNA\text{-}1^{Ct} - miRNA\text{-}SNOR^{Ct})$.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 23.0 was used to examine the data that had been obtained. Fisher's exact test and One-way ANOVA was used to compare the means of studied groups. The linear relationship between each of the two variables was examined using the Pearson correlation coefficient test. *P* values less than 0.05 indicated statistical significance in the results. For miRNA-1 differential expression, Graph Pad Prism version (8) was utilized.

Ethical consideration

According to the study protocol that was filed to the ethical committee at Hawler Medical University's College of Medicine (No. 2), each subject was granted the choice of taking part in the study or not. Furthermore, participants were free to leave the research at any moment.

Results

The results of this study are portrayed at two levels of comparison including the participants' personal attributes such as age and gender and also their disease related status. Table 1 and table 2 show the socio-demographic clinical features and baseline information of all study groups (DM, pre-DM, and HC). Diabetic patients revealed a higher mean age (59.14, ranging between 38 and 63 years) when compared to the other two groups with a statistically significant difference (*P* = 0.02). Additionally, DM patients represented a higher percentage of males, smokers, obese and HTN (60%, 66.6%, 70% and 63.3%, respectively) compared to other study groups. Concentration levels of IL-18 and hsCRP showed significant elevation (*P* <0.01) in both DM and pre-DM patients compared to the HC group. The mean values of the following terms: age, BMI, HbA1c, fasting insulin, and lipid profiles revealed statistically significant elevations (*P* <0.01) for both DM and pre-DM when compared to HC.

There was no statistically significant difference in miRNA-1 expression between obese DM and obese pre-DM (*P* = 067). In contrast, there was a significant difference in the expression of miRNA-1 between non-obese diabetic and non-obese pre-diabetic patients (*P* <0.001). (Figure 1)

Table 3 shows the correlation of miRNA-1 with other inflammatory markers and biochemical parameters in diabetic patients**.** Results of Pearson correlation in diabetic patients revealed a positive correlation of miRNA-1 with IL-18 (r = 0.295, *P* = 0.019), hsCRP (r = 0.284, *P* = 0.031), HbA1c (r=0.252, r=0.04) and Insulin (r = 0.351, *P* = 0.012).

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Table 1 Socio-demographic, clinical features and baseline information of study groups

Variables	DM (n=30) No (%)	Pre-DM (n=30) No (%)	HC (n=30) No (%)	*P*-value
Age range (years)	39-63	37-62	35-60	-
Male / Female	18 (60) / 12 (40)	16 (53.3) / 14 (46.6)	15 (50) / 15 (50)	0.600
Smoker / Non-smoker	20 (66.6) / 10 (33.3)	18 (60) / 12 (40)	8 (26.6) / 22 (73.3)	0.004
Obese / non-obese	21 (70) / 9 (30)	17 (56.6) / 13 (43.4)	7 (23.3) / 23 (76.6)	< 0.001
HTN / non-HTN	19 (63.3) / 11 (36.6)	16 (53.3) / 14 (46.6)	5 (16.6) / 25 (83.3)	< 0.001

Table 2 Comparison of IL-18, hsCRP, and biochemical parameters among study groups

Variables	DM (n=30) Mean±SE	Pre-DM (n=30) Mean±SE	HC (n=30) Mean±SE	*P*-value
Age (years)	58.14±1.03^{a}	56.32±1.19^{a}	51.55±1.235^{b}	0.020
BMI (kg/m²)	28.56±0.647^{a}	26.23±0.612^{a}	24.67±0.276^{b}	0.006
HbA1c	7.062±0.191^{a}	6.028±0.161^{b}	5.221±0.067^{c}	0.002
Fasting Insulin (µIU/mL)	13.06±1.39^{a}	11.16±0.89^{b}	9.47±0.86^{c}	0.001
Inflammatory Biomarkers				
IL-18 (pg/mL)	90.1±0.647^{a}	81.819±0.789^{b}	60.786±0.487^{c}	0.001
hsCRP (mg/L)	12.349±0.219^{a}	8.469±0.153^{b}	1.7.34±0.136^{c}	0.001
Lipid profile and lipid ratios				
TC (mg/dL)	208.6±7.014^{a}	198.1±5.348^{b}	157.8±4.586^{c}	0.004
TG (mg/dL)	237.1±11.58^{a}	228.9±10.55^{b}	133.025±5.643^{b}	0.0023
HDL-C(mg/dL)	31.29±0.634^{a}	36.32±0.876^{b}	41.44±1.567^{c}	0.004
LDL-C (mg/dL)	147.9±4.457^{a}	146.89±6.787^{a}	121.8±3.465^{b}	0.0368

Similar letters in a row indicate for statistical significant association while different letters indicate for statistical significant difference between cells in a row.

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Table 3 Pearson correlation analysis of miRNA-1 with other inflammatory markers and biochemical parameters in diabetic patients

Parameters	MiRNA-1 DM	
	R	*P*
IL-18	0.295	0.019
HsCRP	0.284	0.031
Cholesterol	0.185	0.234
Triglyceride	0.239	0.012
HDL-C	-0.119	0.402
LDL-C	0.059	0.831
HbA1c	0.252	0.04
Insulin	0.351	0.012

r: Pearson Correlation Coefficient

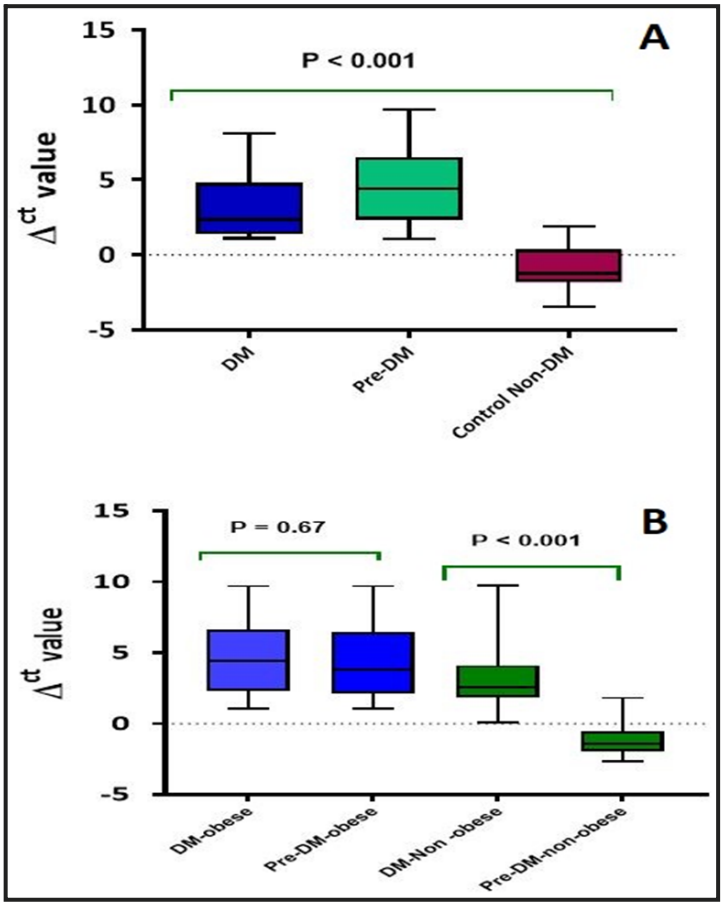

Figure 1 The differential expression of miRNA-1 in (**A**) diabetic, pre-diabetic, and Non-diabetic controls and (**B**) obese diabetic, obese pre-diabetic, non-obese diabetic and non-obese pre-diabetic. Δ^{ct} value is the normalized expression of miRNA-1. *P* values denote for LSD test (**A**) and unpaired t-tests (**B**). **Error bars** represent range and mean values

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Discussion

The plasma samples were subjected for multiplex RT-qPCR tests for quantification of circulating miRNA and further verification of the plasma miRNAs profile. We identified miRNA-1 by carefully assessing the plasma miRNA expression levels in patients with and without diabetes. Compared to HC, patients with diabetes and pre-diabetes had higher levels of miRNA-1. The current result is in line with similar work done by Banjeree and coworkers[12] who reported that Plasma levels of miR-1 was lower in patients with diabetic and pre-diabetic by doing a thorough network analysis, a unique plasma miRNA profile for DM was found, which included a lower level of miR-1. It's noteworthy to observe that there was a drop in certain miRNA-1 years prior to the development of diabetes. The most often identified miRNA-1 produced from endothelial cells was associated with diabetes mellitus (DM), and the current study discovered a strong correlation between miRNA-1 and HbA1c.

We discovered a strong direct correlation between miRNA-1 and inflammatory cytokines, such as IL-18 and hsCRP, in this population-based, case-control study. This is the first observational study looking at the relationship between serum levels of inflammatory cytokines, specifically IL-18, in T2DM patients, and inflammation-related miRNAs. Assessing these relationships is increasingly important for creating diagnostic and treatment strategies for chronic illnesses, such as type 2 diabetes.[13] An increasing body of research has linked certain miRNAs to chronic inflammation in type 2 diabetes. The inflammatory state is a major factor in these patients' hyperglycemia and insulin resistance.[14] The core of inflammatory cascades connected to insulin resistance is an increase in inflammatory cytokines, particularly IL-18,[15] as we observed in present study in which levels of IL-18 and hsCRP were significantly higher in diabetic and pre-diabetic patients compared to healthy controls.

Our results were consistent with a similar research that demonstrated T2DM patients' plasma had higher than normal levels of IL-18.[16] Similarly, in terms of IL-18, increased plasma concentration of IL-18 was seen in diabetic patients by other previous studies.[17] According to experimental research, JNK and MAPK signaling pathways are activated when IL-18 is overexpressed, which leads to the disruption of the insulin signaling pathway and the development of insulin resistance.[18] In view of that, a study done by Lana and coworkers has shown the vulnerability to obesity and metabolic dysfunction in a group of IL-18 knockout mice[19] that greatly supports the findings of this study.

Conclusion

In conclusion, these findings demonstrated that T2DM patients had higher expression of miRNA-1 than did healthy persons. Furthermore, this study found a strong correlation between insulin resistance, inflammatory variables, and miRNA-1. MiRNAs may be very important in the etiology of type 2 diabetes via inducing inflammation. More research is necessary to determine their implications as diagnostic and preventive biomarkers.

Competing interests

The authors declare that they have no competing interests.

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