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BLOOD LEVELS OF MICORNA-21 IN NEWLY DIAGNOSED PATIENTS WITH TYPE 2 DIABETES MELLITUS IN IRAQI POPULATION

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ABSTRACT

Background: *Diabetes mellitus (DM) is now regard as a worldwide health issue affecting hundreds million of people. It is a metabolic disease targeting glucose metabolism and insulin homeostasis, Type 2 diabetes mellitus (T2DM) is the most prevent type of this disease accounting about 90-95% of the disease as whole. microRNAs (miRNAs) which are a small non-coding RNAs, they revolutionized as potential new biomarkers for the diagnosis and prognosis of a wide range of disorders.*

Recent studies prove the relationship between miR-21 and inflammatory process and insulin resistance, our emphasis suggest there is a role of miR-21 in insulin resistance in T2DM. Studies have explored the potential of miR-21 as diagnostic biomarkers for T2DM.

Aim of study: *The purpose of this study is to compare the sera levels of miRNA-21 among newly diagnosed patients e with T2DM patients with their levels in apparently healthy subjects using blood samples.*

Methods*: The time frame for this case-control research is August 2022 through March 2023, and it took place at Al-Sadr Teaching Hospital/ Masin / Iraq. There were 100 patients home recent diagnosed with T2DM (50 females and 50 males) with age ranged between (20 -70 years) and 100 apparently healthy subject who dealt with as control group. 2 ml of blood drown from each patient and healthy person, putted it in EDTA tube with 1 ml of Trizole as a preservative, and put the tubes in the freezer at -70 degrees Celsius until analyze the samples.*

1- INTRODUCTION

Diabetes Mellitus

Diabetes mellitus emerge as global problem around the world, an age-related metabolic condition. As its incidence grows outside of industrialized nations, DM becomes a major public health issue worldwide. The International Diabetes Federation (IDF) has released an estimate of the global prevalence of diabetes (Whiting et al., 2011). Diabetes develops mainly in one of two ways. Ineffective insulin response causes T2DM, while inadequate insulin production causes type 1 diabetes mellitus T1DM (Chien et al., 2015).

MiR-21

Mature miR-21 originates from a full-length pri-miR-21 molecule with a sequence of 3433 nucleotides. Chromosome 17q contains the miR-21 gene (DNA of premiR-21) (Carthew et al.,2009).

In the nucleus, Drosha acts as an endonuclease to generate pre-miR-21 from pri-miR-21. The cytosolic enzyme Dicer subsequently snips this pre-miR-21 into a little RNA duplex. The transcriptional abundance of both strands is equivalent, but the thermodynamic stability of either end of the duplex selects for processing as mature miRNA only one strand (miR-21). (Carthew et al.,2009).

2- METHODOLOGY

Patients and Control Groups

The participants in this case-control research were patients at Al-Sadr Teaching Hospital/Madina in Masin between August 2022 and November 2022. One hundred newly diagnosed T2DM patients (50 females and 50

men) with age ranged from 20-75 years old. In contrast, one hundred seemingly healthy participants (50 females and 50 males) dealt with as control group, matching of age of both groups have been done.

Blood Collection and Storage

Two milliliters (ml) of peripheral venous blood sample was collected from every patient. The collected blood was mixed with 1 ml of Trizole preserving material, kept in EDTA tube, and stored at - 70 °C until the time of laboratory investigations of blood miR-21 was performed.

Quantitative Real-Time PCR (qRT–PCR)

The expression levels of miRNAs were measured by the reverse transcription-quantitative polymerase chain reaction, quantitative real-time qRT-PCR SYBR Green assay was used. The microRNA levels of endogenous control gene U6 were amplified and used to normalize the miRNA levels of the miRNA.

The measured parameters are:

1- Mir-U6 by real-time PCR.

2- MiR-21 by real-time PCR.

Analysis of Data

We used SPSS for Windows, version 22 to do statistical analysis. Data were presented as mean ± standard deviation (SD). Researchers checked for Gaussianity in the parameters they examined using the Shapiro-Wilk normality test. For this study, we compared the means of the groups using an independent samples t-test. In addition, 2 tests were used to examine categorical variables. After an analysis of variance (ANOVA), we used Scheffé, Tukey, and Hochberg's GT2 Post Hoc tests for multiple comparisons.

3- Result

MicroRNA Results

MiR-U6 Results

The nuclear control transcript (housekeeping gene) that was used in this study was miR-U6 (gives information about the storage conditon of miR from the day of blood taking to the day of laboratory work and about the normalization of the studying tools and materials). The results showed non-significant differences in mean ± SD value of Ct as well as in fold of gene expression of miR-U6 shown in tables (1), and figure (1).

Table (1): (Mean ± SD) values of Ct of miR-U6 of studied group

Group	No.	Mean Ct of miR-U6 ± SD	Rang*
T2DM Patient	100	13.706±0.45	12-14
Control group	100	13.78±0.43	12-14

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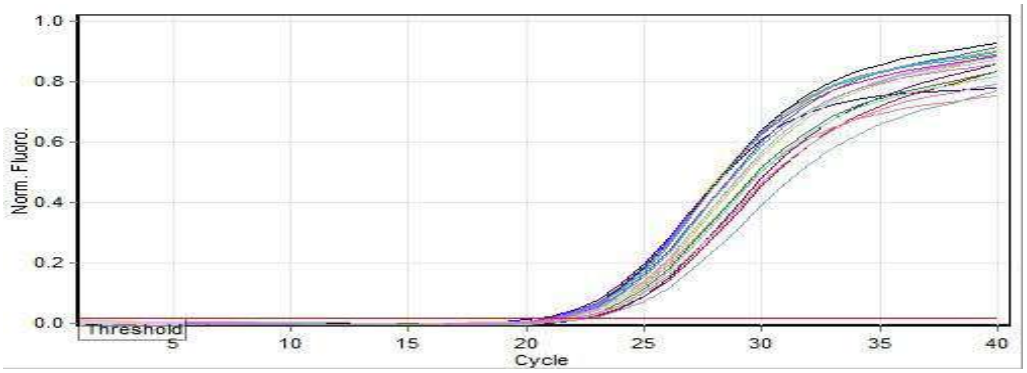

Figure (1): miR-U6 amplification plots by qPCR Samples included studied groups.

MiR-21 results

The results showed a significant elevation in sera levels of miR-21 in the T2DM patients group in comparison with its levels in the control group ($p < 0.05$), as shown in table (2) and figures (2,3).

ΔCt of miR-21 and subsequent fold of miR-21 gene expression of patients revealed a significant fold of gene expression (2.1) in comparison with their levels in the control group. The ROC value of miR-21 was found to be 93 % (sensitivity =85%, specificity = 100%) cutoff value = 1.7265 (P value 0.001, 95% AUC=0.96) as shown in figure (2,3).

Table (2): Fold of miR-21 expression Depending on $2^{-\Delta Ct}$ Method
* ΔCt (Means Ct of miRNA21- Means Ct of miRNAU6)

Groups	Means Ct of miR-21 ±SD	Means Ct of miR-U6 ±SD	ΔCt*	$2^{-\Delta Ct}$	experimental group/ Control group	Fold of gene expression	P value
Glioma patients (n=28)	21.666± 0.62	13.706± 0.45	7.960	0.004	0.004/0.0019	2.1	<0.05
Healthy subjects (n=30)	22.81±0 .81	13.78±0. 43	9.03	0.00191	0.00191/0.00191	1	

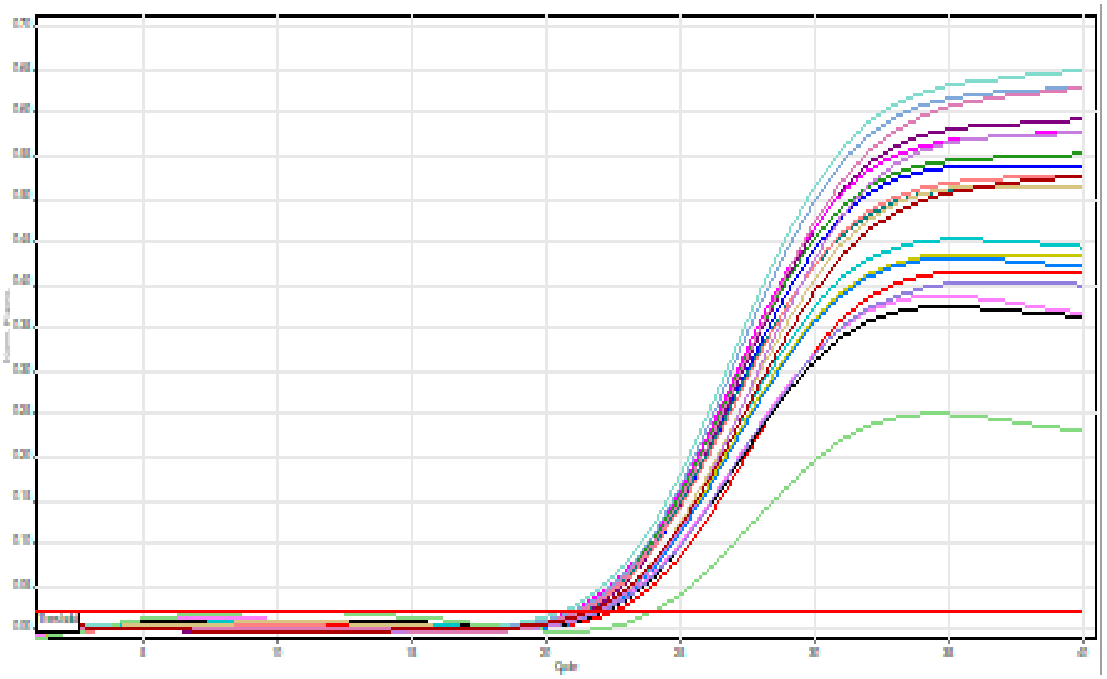

Figure (2): MiR-21 amplification plots by qPCR Samples included studied groups.

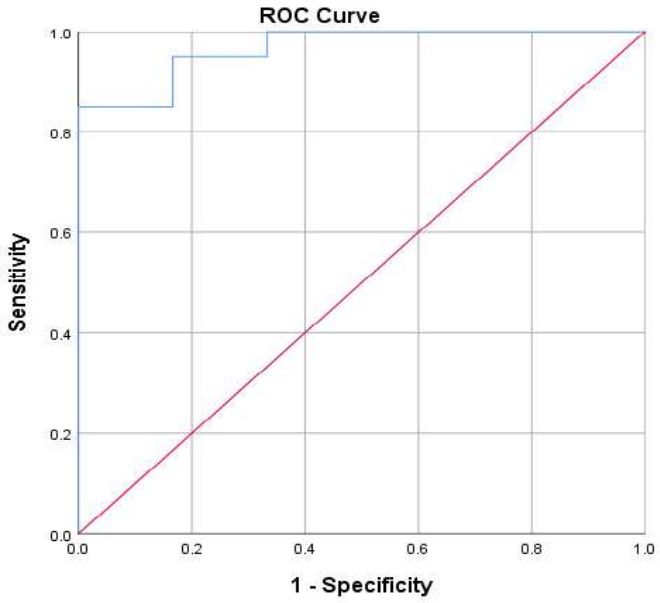

Figure (3): ROC value of miR-21 in T2DM patients group

4- DISCUSSION

As hyperglycemia is the pathognomonic clinical manifestation of DM, it can induce pancreatic-cell damage and apoptosis and decrease -cell proliferation (Wang Set et al., 2015). Low insulin production and the associated rapid progression of diabetes are the results of -cell dysfunction (Zhong X, Chung AC, Chen HY et al., 2013) (Chen Q, Qiu F, Zhou K et al., 2017). In addition, as obesity is a pathognomonic sign of DM, LDL-Cho elivation in the blood and HDL-Cho decline are related to insulin secretion (Fryirs MA, Barter PJ, Appavoo M, et al., 2010). An elevated level of HDL-Cho could reduce the blood glucose level, and down-regulation of HDL-Cho could increase the blood glucose level (Song X, Teng J, Wang A, et al., 2016). miR-21 antagomir (antagomirs,

also known as anti-miRs, are a class of chemically engineered oligo nucleotides designed to silence endogenous miRNAs). Down-regulated the contents of TG, TC, LDL-Cho, and up-regulated HDL (Song X, Teng J, Wang A, et al .,2016).

Recent investigations demonstrated a significant involvement of miR-21 in inflammation and hepatic metabolism. In NAFLD (non-alcoholic fatty liver disease), miR-21 seems to regulate triglycerides, free cholesterol, and total cholesterol levels through the inhibition of 3-hydroxy-3-methyl glutaryl-coenzyme A reductase (HMGCR)(Sun, C.; et al., 2015) and fatty acid-binding protein7(FABP7), which induces FAs uptake and accumulation (Ahn, J.; Lee, H.; Jung, C.H.; Ha, T et al ., 2012) (Meng, F. et al., 2007) (Wu, H.; Ng, R.; Chen, X.; Steer, C.J.; Song, G. et al., 2016) are two of MiR-21's targets that play a role in preventing the progression of liver steatosis. Recent evidence indicates that miR-21 plays a function in lipid metabolism by directly suppressing steatosis development in mice via overexpression of numerous miR-21 targets involved in lipid metabolism (Gjorgjieva, M. et al ., 2019)(Calo, N.; et al ., 2016).

Furthermore, new research using a miR-21-based technique to detect glycaemic deficits in individuals at high risk for developing diabetes revealed likely pathogenic processes, including associations between circulating miR-21 and plasmatic Ox-S-induced damage. In contrast to our hypothesis (La Sala L et al., 2016). Hyperglycemia disrupts the defense system. Hence, diabetic patients have lower levels of endogenous antioxidants and greater levels of lipid peroxidation than healthy controls (Ceriello A. et al.,2000; Ceriello A. et al.,2008).

CONCLUSION

We demonstrate significant elivation in blood level of miR-21 which is a rapied non-invasive method for giagnosis, However, future efforts to examine miRs as a distinct group of blood-based biomarkers in T2DM patients.

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