Evaluation of Sera Levels of miR-181b in Prediction of Prediabetic Patients in Iraq

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ABSTRACT

Background: Type 2 diabetes mellitus is the most common type of diabetes, accounting for 90-95% of all diabetes. It is epidemic in Asia. It is characterized by rapid rates of increase over short period and onset at a relatively young age and low body mass index. The epidemic is heterogeneous, varying according to different ethnic and cultural subgroups, degree of urbanization, and socioeconomic conditions in different Asian populations. MicroRNA-181b (miR-181b) is one of the best-known anti-inflammatory miRs involved in the inflammatory signaling pathway and recently it has been discovered as epigenetic changes contributor for the development of diabetes in the pre-diabetic stage.

Objectives: Evaluate the sera levels of miR-181b in type 2 diabetes mellitus prediabetic patients and investigate the relationship between prediabetic state and level of miR-181b gene expression.

Methods: This study was carried at Al-Hussan endocrinology center in Kerbala Provence in Iraq during the period from Oct, 2022 to April, 2023 on 70 patients with impaired fasting glucose level (34 males and 36 females) and 50 apparently healthy subjects (24 males and 26 females) who were dealt with as control group. Two ml of sera were collected from obese prediabetic patients (BMI ≥ 30, fasting serum glucose level 110-140 mg/dl) each patient / control subjects were investigated to detect miR-181b expression level with real time PCR.

Results: The results of the present study revealed a highly significant decline in mean of sera levels for miR-181b in prediabetic patients group in comparison with its levels in control group (p value ≤ 0.05).

Conclusion: miR-181b could be used as a novel diagnostic biomarker to predict diabetes in prediabetic state. Hence, to prevent diabetes by changing the life style or by using certain medications.

Keywords: miR-181b, Prediabetic, Type 2 diabetes mellitus.

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In parallel with economic development and nutrition transition, the rates of overweight and obesity have been increasing rapidly in Asian countries, abdominal or central adiposity, particularly detrimental to Type 2 diabetes mellitus (T2DM) and metabolic syndrome which are highly prevalent in Asians[1,2]. The high rates of gestational diabetes, childhood obesity, and over nutrition in later life, may contribute substantially to the increasing diabetes epidemic in Asia. MiRs are noncoding RNAs constituting 19 to 24 nucleotides, and they serve as hubs in gene regulatory network by controlling numerous targets via RNA silencing and posttranscriptional regulation of gene expression.

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MicroRNA-181b (miR-181b) is one of the best-known anti-inflammatory miRs involved in the inflammatory signaling pathway and recently it has been discovered as epigenetic changes contributor for the development of diabetes in the pre-diabetic stage[3]. Early detection of T2DM might be possible by identification and development of epigenetic biomarkers. MiRs, as evolutionary conserved molecules, regulate gene expression at the post transcription level by inhibiting the translation of the target mRNA molecule. miR-181b improves insulin signaling, inflammation, and endothelial cell dysfunction by targeting endothelial PH Domain And Leucine Rich Repeat Protein Phosphatase 2 (PHLPP2)[4-8].

The aim of the present study is to investigate the expression levels of miR-181b in sera of pre-diabetic patients and

healthy subjects to provide new strategies for early diagnosis of T2DM, Hence, prevent its complications.

Methods

This study was conducted at Al-Hussan endocrinology center in Kerbala Provence in Iraq. Samples were randomly selected from obese prediabetic patients with BMI ≥ 30 and fasting serum glucose level 110-140 mg/dl (Impaired fasting glucose), who were attending the diabetic consultation unit during the period from Oct, 2022 until April, 2023. The patients and control groups were with age ranged between 20-75 years. Study was carried out on 70 patients with impaired fasting glucose level (34 males and 36 females) and 50 apparently healthy subjects (24 males and 26 females) who were dealt with as control group.

Two ml of whole blood was collected from every patient and control subject and was put in EDTA tube with TRIZOL preservative material and stored in -70 °C until time of investigation. Work was done by Qiagen Rotor gene real time PCR.

This study was approved by scientific committee of Biochemistry Department, College of Medicine, University of Kerbala. A signed written consent was taken from each patient (or his / her relative) and each healthy individual participated in this study.

The difference in levels between, miR-181b in prediabetic patients and control group has been compared to find the effect of prediabetic state on that parameter. The correlation coefficient t-test was used to describe the association between the variable studied parameter in this study; $p \leq 0.05$ was considered statistically significant. Data were statistically analyzed by utilizing SPSS for Windows, version 22 (SPSS Inc. Chicago, Illinois, United States). Data were expressed as mean ± standard deviation (SD). Shapiro–Wilk normality test was used to determine whether the studied parameter followed a Gaussian distribution. Independent samples t-test was used to compare between means of the studied groups. Additionally, Categorical variables were analyzed by $\chi 2$ tests. The Scheffé, Tukey, Hochberg's GT2 Post Hoc tests for multiple comparisons was applied after ANOVA tests. Variable in which the distribution of data did not conform to normality were first log transformed for analysis and then converted back to standard units for presentation. The association degrees between variables were analyzed by Pearson correlation analysis. A two-tailed P-value less than 0.05 ($P \leq 0.05$) was considered significant.

Results

Seventy patients with prediabetes (36 females and 34 males), and 50 apparently healthy subjects (26 females and 24 males) with comparable percentage of both sex. The mean±SD value of age of prediabetic patients (35±12 years), and healthy controls (40±16 years), without significant differences among them, (Table 1).

The nuclear control transcript (housekeeping gene) that have been used to search for miR expression in this study was miR-U6, which give information about the environment of storage of miR from day of blood collection 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 between each of prediabetic patients group and healthy controls, (Tables 2 and 3 and Figure 1).

The results of the present study revealed a highly significant decline in mean ± SD of sera levels for miR-181b in prediabetic patients group in comparison with its levels in control group (p value ≤ 0.05), (Table 4 and Figure 2).

The ΔCt of miR-181b in prediabetic patients = (means Ct of miR-181b) – (means Ct of miR-U6). Subsequently, the fold of miR-181b gene expression in prediabetic patients was decrease (0.007 fold) in comparison with its levels in control group, with receiving operation characteristic ROC value of 92 % (sensitivity =88%, specificity = 95%), cutoff value = 0.0558 (P value ≤ 0.05, 95%, AUC=0.92), (Table 5 and Figures 2 and 4).

Table 1: Mean ±SD value of age and number of studied subjects according gender.

Group	Age (year) Mean (±SD)	Females No. (%)	Males No. (%)
Prediabetic patients (n=70)	35±12	36 (51.5)	34 (48.5)
Healthy subjects (n=50)	40±16	26 (52)	24 (48)

Table 2: Mean ±SD values of Ct of miR-U6 of studied groups.

Group	Mean Ct of miR-U6 ± SD	Range*
Prediabetic patients (n=70)	21.56±0.45	20-22
Apparently healthy (n=50)	21.55±0.43	20-22

* Accepted rang by supplied kit

Table 3: Comparison of miR-U6 fold expression between studied groups.

Group	Means ±SD Ct of miR-U6	2^{-Ct}	Experimental group/Control group	Fold of gene expression
Prediabetic patients (n=70)	21.57±0.44	3.21E-07	3.21E-07/3.24E-07	0.98
Healthy subjects (n=50)	21.55±0.43	3.24E-07	3.24E-07/3.24E-07	1

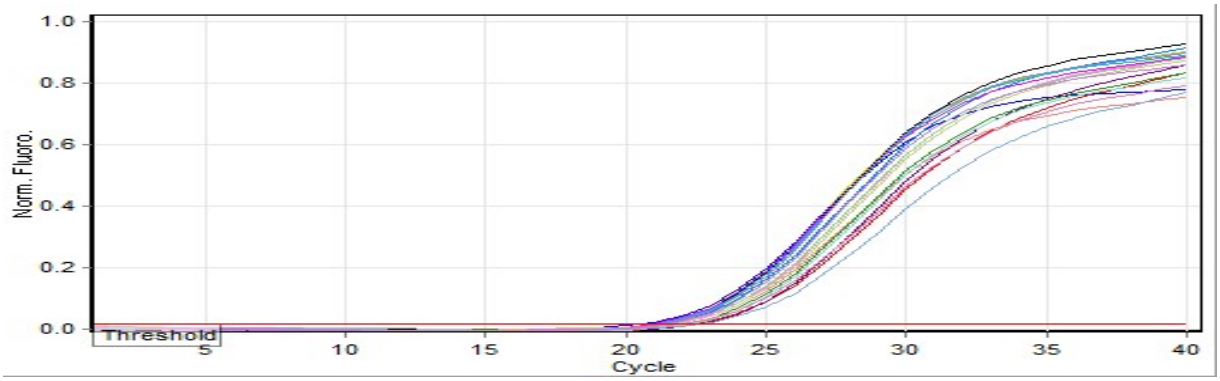

Figure 1: miR-U6 amplification plots by qPCR samples included all studied groups. The photograph was taken directly from Qiagen Rotor gene qrtPCR machine.

Table 4: Fold of miR-181b expression depending on $2^{-\Delta Ct}$ method.

Groups	Means Ct of miR-181b	Means Ct of U6	ΔCt (Means Ct of miR-181b	$2^{-\Delta Ct}$	experimental group/ Control group	Fold of gene expression	p-value
Patients	**27.09**	**21.57 ±0.44**	**5.52**	**0.021657**	**0.021657/2.770218**	**0.007**	**≤ 0.05**
Control	**20.92**	**21.55 ±0.43**	**-1.47**	**2.770218**	**2.770218/2.770218**	**1.00**	

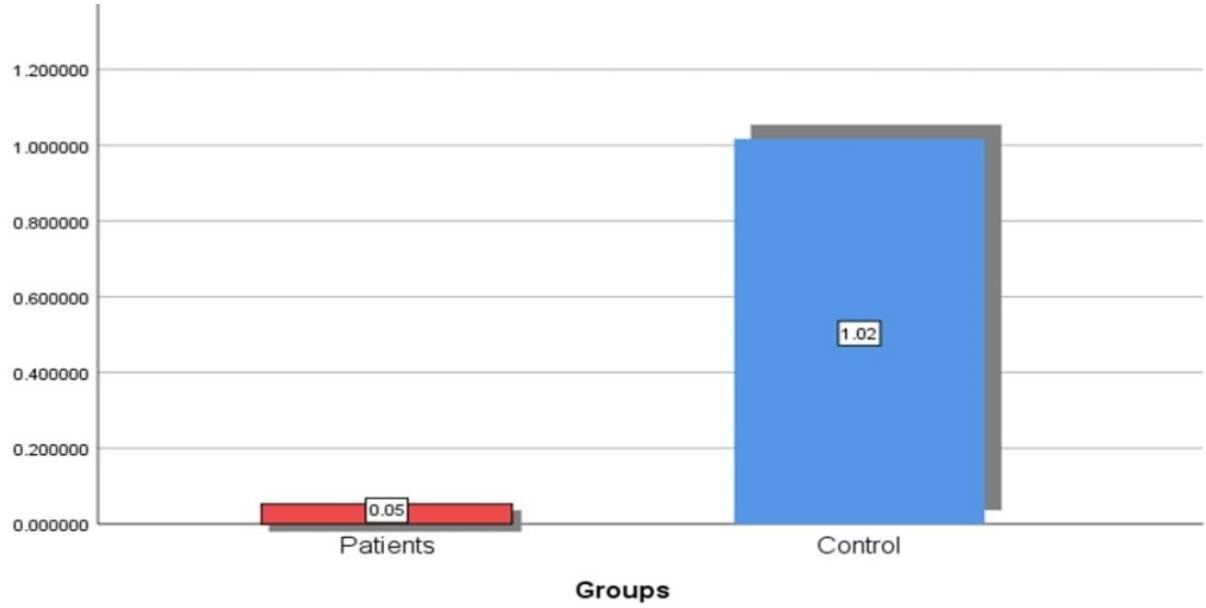

Figure 2: miR-181b in prediabetic patients group and control group.

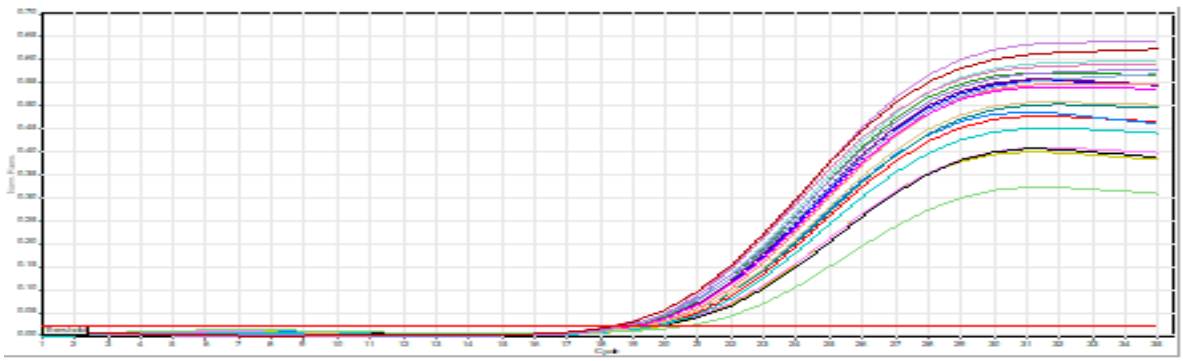

Figure 3: MiR-181b amplification plots by qPCR Samples included studded groups. The photograph was taken directly from Qiagen Rotor gene qrtPCR machine.

Table 5: Receiver operating characteristic curve data of the studied genes.

Parameters	AUC	Explanation	P-value	The best Cut off	Sensitivity %	Specificity%
miR-181b	0.92	Excellent	≤ 0.05	0.0558	88	95

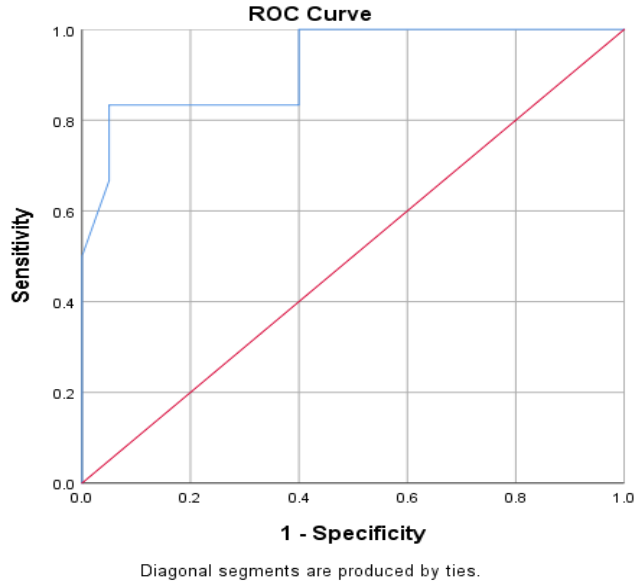

Figure 4: Receiver operating characteristic curve of the miR-181b.

Discussion

The known vital cause of type 2 diabetes mellitus development is insulin resistance, the process that start with adipose tissue dysfunction and low-grade inflammation in adipocyte[9-12]. Imbalance dietary habit and excessive diet and its metabolic substrate end product expose adipose tissue and other tissues to primary low grade inflammation[13], enhancing the release of cytokines[14,15]. Recent studies support the idea that chronic inflammatory process in white adipose tissue is involved in the pathogenesis of obesity-associated insulin resistance[16-19].

Furthermore, endothelial cells dysfunction is a big contributor of development of insulin resistance, metabolic syndrome and type 2 diabetes mellitus[20-22], which was experimentally established.

Over production of these cytokines and leptin hormone might play an additional impact on inflammatory process that have been noticed in obese patient as we mentioned above because the binding site for miR-181b in the 3′ UTR region of the interlukin-6 molecule; thus, miR-181b reduces the expression of interleukin-6 this interleukin act as anti-inflammatory agent during inflammation[23].

Additionally, Tomé-Carneiro, studied T2DM patients and found that miR-181b expression levels significantly increased in the patients who consumed one daily dose of resveratrol, an anti-inflammatory drug[24]. It was recently identified that miR-181b plays an important role in inhibiting the NF-κB signaling pathway through direct targeting of importin α3, a protein that is essential for nuclear transmission of the NF-κB[25]. Overexpressing miR-181b markedly improved glucose uptake and glucose homeostaesis in adipocytes via paracrine mechanisms with adipocytes.

In other hand, miR-181b targets PHLPP2, a phosphatase that dephosphorylates Rho-associated serine/threonine kinase ROCK1[26]. ROCK1

that is a key downstream target of the small GTPases. ROCK is involved in diverse cellular activities including actomyosin and cytoskeleton dynamics contractility and organization that is involve in inflammatory process in insulin resistance[27]. The ROCK signaling pathway plays a critical role in a range of diseases[28]. Knockdown of PHLPP2 phenocopies miR-181b's effect on glucose homeostasis, insulin sensitivity. The elevated level of ROCK1mRNA may be due to its major cytoskeletal role in proliferation and adhesion of malignant cells[29]. Thus, it attracted great interest to value the clinical role of ROCK1mRNA in glioma. ROCK1 mediates various cellular responses, including cell proliferation, growth, and apoptosis via microtubule network organization and effects on the cytoskeleton[30]. Hallgren et al. demonstrated that the ROCK signaling pathway was regarded as a sensor of tissue compliance[31]. Elevated level of ROCK1mRNA may also be due to down regulation of miR181b resulted in the present study as the demonstrated unambiguously that miR-181b targeted specifically the 3′UTR region of ROCK1.

In conclusion, miR-181b could be used as a novel diagnostic biomarker to predict diabetes in prediabetic state.

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