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MiR-194-5p might be a Potential Biomarker for Type 2 Diabetes Mellitus

Ali Dadbinpour[1,2], Mehran Dehghanian[3], Mohammad Yahya Vahidi Mehrjardi[4*]

[1]Genetic and Environmental Adventures Research Center, School of Abarkouh Paramedicine, Shahid Sadoughi University of Medical Science, Yazd, Iran.
[2]Department of Genetic, Medical School, Shahid Sadoughi University of Medical Science, Yazd, Iran.
[3]Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
[4]Diabetes Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Abstract

Objective: Type 2 diabetes mellitus (T2DM) incidence is increasing around the world as a progressive metabolic condition. The miRNA expression profile changes in the early stages of diabetes in body fluids. It can help in early diagnosis of diabetes, which reduces diabetes-related mortality. In this study, miR-194-5p gene expression levels in diabetic and pre-diabetic patients will be examined and compared to the healthy controls.

Materials and Methods: The expression levels of miR-194-5p were evaluated in 90 participants, referred to Yazd diabetes centers (Iran) in 2022, including 30 T2DM, 30 prediabetics, and 30 healthy subjects by real-time PCR. The potential pathways affected by microRNA were fitted to the Enrichr web server by applying target genes predicted to miR-194-5p in the Target Scan Human 7.2 database.

Results: The results of these studies indicate a gradual decrease in miR-194-5p expression levels in prediabetic and T2DM patients compared to healthy controls ($P< 0.001$). The role of the miR-194-5p target genes in T2DM-related signaling pathways such as the Wnt and TGF-beta pathways was also determined.

Conclusion: The results indicate that miR-194-5p is a potential biomarker for the early diagnosis of T2DM due to its down-regulation in the serum of prediabetics and diabetics compared to healthy subjects.

Keywords: T2DM, miR-194-5p, Gene expression, Biomarker

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Corresponding Author:
Mohammad Yahya Vahidi Mehrjardi, Yazd Diabetes Research Center, Talar Honar Alley, Shahid Sadoughi Blvd., Bahonar Sq., Yazd, Iran.
Tel: (98) 913 351 4629
Email: mmvahidi@gmail.com
Orcid ID: 0000-0003-0535-1590

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Introduction

The incidence of type 2 diabetes (T2DM), the most common chronic metabolic disease, is growing rapidly, which will be the seventh leading source of death in the world by 2030 based on the World Health Organization (WHO) studies (1,2). Insulin resistance, beta-cell apoptosis, and insulin secretion deficiency are the three main pathological defects causing T2DM (3). Although most patients will need insulin injections, hypoglycemic drugs like metformin are the first line of treatment (4). Diabetes is a multifactorial disease and a complex interaction between genetic, epigenetic, and environmental factors has a role in its pathogenesis (5-9).

The regulation of gene expression at the post-transcriptional level by microRNAs (miRNAs), as small non-coding ribonucleic acids, is a vital process in biological functions (10,11). Serious changes in the expression of different genes may be caused by small changes in the expression of a miRNA (12). MicroRNAs are potential targets for new therapies for diseases, including hepatitis C, cancer, and possibly diabetes (13-16). MiR-194, one of the most essential miRNAs in skeletal muscle, regulates the development of insulin resistance and takes action in glucose homeostasis (17,18). Due to the lack of studies on the change in the expression of this marker in blood samples of pre-diabetic and diabetic people compared to healthy people and investigating the pathways affected by this marker, playing a role in the occurrence of this disease, we aimed to evaluate and analogize miR-194-5p expression levels in the blood serum of type 2 diabetic and pre-diabetic patients contrasting with the healthy controls.

Material and methods

Sample collection

Data were collected and maintained from 90 participants who received no anti-diabetic treatments, including 30 T2DM, 30 pre-diabetic, and 30 healthy subjects (diagnosis based on the WHO criteria), referred to Yazd diabetes centers (Iran) in 2022. We assume the subjects with higher than 126 mg / dL fasting blood glucose level and HbA1c of over 6.5% are T2DM cases and those fasting blood glucose levels of 100 to 125 mg/dl and HbA1c of 5.7% to 6.4% as pre-diabetes, also cases with fasting glucose of lower than 100 mg / dL selected as normal subjects. The study's inclusion criteria include Adults ≥35 years of age, the lack of micro-vascular and macro-vascular complications, such as retinopathy, nephropathy, amputation, non-infectious disease, and coronary artery disease. Exclusion criteria include a dossier on infectious and inflammatory diseases (rheumatoid arthritis and lupus), a history of liver diseases, like hepatitis, a medical history of endocrine diseases, for instance; hyperthyroidism or hypothyroidism, and a family history of diabetes in first-degree relatives. 3 ml of whole blood in EDTA anticoagulants (for HbA1c) and 5 ml without EDTA (for biochemical parameters) were collected from each participant, after 12 hours of fasting.

Biochemical parameters measurement

All biochemical parameters were measured by taking serum samples from the participants. To analyze glucose, LDL-C, HDL-C, triglycerides, and total cholesterol levels, we use established kits, namely the Pars test kits and the Tokyo Boeki Prestige analyzer (Japan). In addition, the NycoCard reader determined HbA1c.

Total RNA isolation

To extract total RNA from blood, we used RNA X-plus Solution (CinnaGen, Iran) and to remove external DNA contamination, the RNA samples were treated with DNase (Fermentas). The spectrophotometer (NanoDrop, Thermos Fisher) and OD 260/280 showed the purity and concentration of the RNA. Finally, the RNAs were stored in sterile 0.2 ml microtubes at 80 °C.

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cDNA Synthesis

Complementary DNA (cDNA) of miR-194-5p synthesis was based on the Bon-Mir RT kit (Bonyakhteh, Tehran, Iran) according to the manufacturer's instructions.

Real-Time PCR (RT-PCR)

During the experiment, real-time quantitative PCR (qPCR) reactions using the SYBR Green master mix (Bonyakhteh, Tehran, Iran) and performed in a thermal cycler (Applied Biosystem, ABI, Step One Plus, USA) played an important role. A few points for the experimental conditions are worth mentioning here: The thermal reaction conditions were 95°C for 15 minutes, followed by 40 cycles of 95°C for 15 seconds, 60°C for 15 seconds, and 72°C for 30 seconds. A final extension was also performed at 72°C for 5 minutes. Our final mix volume was 13 µL, including 1 µL of cDNA, 0.5 µL of miRNA-specific forward primer, 0.5 µL of universal reverse primer, and 6.5 µL of q-PCR master mix. DNase-RNase-free water was added to reach a final volume of 13 µL. All PCR reactions were duplicated and expression levels were assessed using $2^{-\Delta\Delta Ct}$. Small nucleolar RNAs (SNORD) 47 and miR-194-5p were used as reference and target genes, respectively (Table 1).

Statistical analysis

To compare the relative expression of the miR-194-5p in three groups including T2DM, prediabetic, and healthy subjects, Statistical analyses were conducted using SPSS 26, including the Kolmogorov-Simonov normality test and the one-way ANOVA post-hoc Tukey's HSD- Test. A $P< 0.05$ was considered as significant enrichment and the graphs were plotted with Graph Pad Prism 8.

Bioinformatics analysis

We selected and examined the contribution of 473 genes in different pathways using the Enrichr web server (https://maayanlab.cloud/Enrichr/) (19). It is also important to emphasize that these 473 genes were suggested by the Target Scan Human database as potential miR-194-5pp target genes.

Ethical considerations

This study was approved by the ethics committee of Shahid Sadoughi Universit y of Medical approved the study proposal (Code: IR.SSU.MEDICINE. REC. 1397. 028).

Results

Biochemical parameters

This study provides evidence that there is no notable difference in age, HDL, and age between the three groups. However, there is a notable difference in fasting blood glucose (FBG), HbA1c, triglyceride, and total cholesterol levels between the three groups. Measurement of the body mass index (BMI) in the three groups showed that the BMI in the diabetic group was significantly higher than in the healthy group, however, there was no significant difference between the prediabetic and the healthy group or the diabetic and prediabetic groups (Table 2).

Gene expression of miR-194-5p

We compared miR-194-5p expression levels between healthy (control), pre-diabetic, and T2DM patients. miR-194-5p expression declined in both pre-diabetes and T2DM groups compared to the healthy group, with $P< 0.001$.

In addition, miR-194-5p expression in the T2DM group was notably reduced compared

Table 1. qPCR primers employed in this study

Gene	Primers (5'→3')	Product size (bp)	TM (°C)
miR-194-5p	Forward: TTCATTATTACTTTTGGTACG	197	59
	Reverse*		60
SNORD 47	Forward: ATCACTGTAAAACCGTTCCA	194	58
	Reverse*		59

*Reverse primer was designed by Bon Yakhteh Company, Tehran, Iran
SNORD: Small Nucleolar RNAs.

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to the pre-diabetes group ($P< 0.01$).

Bioinformatics results

The Enricher web server examined the genes predicted as possible miR-194-5p targets. Based on its results, these genes are involved in numerous signaling pathways, like Wnt, TGF-beta, etc. The 10 paths identified with the highest *P* are presented in Table 3.

Discussion

Diabetes is extremely complicated and expensive to treat, with potentially fatal side effects (20). Insulin resistance can occur about 10 years before glycemic changes such as FPG and HbA1c (21). Therefore, a simple blood test, which can detect diabetes in the early stage could open new opportunities for early intervention and minimize the deleterious outcomes caused by T2DM before symptoms appear (22). Epigenetic mechanisms as a result of gene-environment interaction play a crucial role in the etiology of T2DM (23). The results of miR-194-5p expression in this study indicate a significant reduction in serum from T2DM patients compared to the healthy and prediabetic samples. Also, the expression level of miR-194-5p in the prediabetic group revealed a serious decline compared to healthy people. Bioinformatic studies show that miR-194-5p target genes can potentially alter the activity of Wnt, TGF-beta, and IL-1 signaling pathways. Several recent studies have also pointed to the involvement of these signaling pathways in T2DM development (24-26). Therefore, miR-194-5p may play a role in inhibiting the development and progression of type 2 diabetes.

Results similar to our results were obtained from a study that investigated the expression of microRNAs in the skeletal muscle tissue of mice and humans with prediabetes and diabetes by microarray.

Modulation of miR-194 underscores its pivotal role in various facets of skeletal muscle glucose metabolism, including augmented glucose uptake, glycolysis, glycogenesis, and glucose oxidation, mediated through pathways

Table 2. Demographic information of the analyzed subjects

Parameters	Healthy (n = 30)	Pre-diabetics(n=30)	T2DM (n = 30)	*P*-value
Age (years)	53.5 (± 7.2)	56.7 (± 7)	55.4 (± 5.3)	$P = 0.32$
BMI (Kg/m2)	25.03(±0.46)	25.97 (± 0.26)	26.47 (± 0.34)	$P = 0.01$
FBG (mg/dl)	90.1 (± 6.4)	108.8 (± 8.5)	191.1 (± 59.24)	$P < 0.001$
HbA1c	5.1 (± 0.24)	6.04 (± 0.28)	8.62 (± 1.74)	$P < 0.001$
Triglycerides (mg/dl)	118.11 (± 30.84)	136.82 (± 50.34)	206.1 (± 87.12)	$P < 0.001$
Cholesterol (mg/dl)	147.61 (± 21.21)	174.75 (± 44.72)	189.5 (± 53.85)	$P = 0.04$
HDL	43.11 (± 10.6)	39.9 (± 9.5)	35.31 (± 10.9)	$P = 0.12$

Values are expressed as mean ± SEM. P-values were determined through the One-Way ANOVA test. HDL: high-density lipoprotein, HbA1c: glycosylated hemoglobin, FBG: fasting blood glucose, BMI: Body mass index.

Table 3. Predicted signaling pathways

Index	Name	*P*-value	Adjusted *P*-value	Odds Ratio	Combined score
1	Wnt signaling pathway	6.669e-7	0.0005982	4.04	57.48
2	TGF-beta signaling pathway	0.000002032	0.0009111	4.30	56.31
3	Ubiquitin-mediated proteolysis	0.000004325	0.001140	4.85	59.92
4	TGF-beta regulation of skeletal system development	0.000005085	0.001140	6.26	76.29
5	Adipogenesis	0.00007694	0.01316	4.17	39.55
6	Circadian rhythm	0.0001018	0.01316	6.20	57.03
7	Pathways in cancer	0.0001027	0.01316	2.78	25.55
8	Thyroid-stimulating hormone signaling pathway	0.0001592	0.01701	5.78	50.50
9	Developmental biology	0.0001829	0.01701	2.46	21.20
10	Interleukin-1 signaling pathway	0.0001896	0.01701	4.05	34.75

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implicating AKT, GSK3, and mitochondrial oxidative phosphorylation mechanisms (27). In contrast to the results obtained in this study, the study conducted by Andrea Jaeger et al reported that serum levels of miR-192 and miR-194 are associated with the occurrence of T2DM, independent of fasting glucose and HbA1c. Furthermore, they showed that miR-194 and miR-192 serum levels were also elevated in diabetic Akt2 knockout mice compared to wild-type mice (28). Demirsoy et al highlight a potential link between miR-194-5p expression and the Wnt signaling pathway, known to be associated with the development of T2DM. Specifically, the significant alteration of miR-194-5p expression following metformin treatment in patients with T2DM suggests a potential role for this microRNA in mediating the therapeutic effects of metformin in T2DM management (29).

Considering the limitations of this study, such as the relatively small sample size and contradictory results compared to previous studies, it is suggested that future studies aim to improve the reliability and interpretability by utilizing larger sample sizes, and other methods of gene expression evaluation in blood and other tissue samples. Additionally, given the complexity and diversity of microRNA activities in biological pathways, the use of novel methods such as gene knockout and RNA interference (RNAi) for investigating the effects of microRNAs in future studies is recommended. Furthermore, the assessment of the expression levels of downstream genes in the affected pathways by these microRNAs should be incorporated, which could provide further insights into their regulatory roles. Such approaches may aid in a more precise and trustworthy examination of pivotal factors in the pathophysiology of T2DM.

Conclusion

The implications of the results of this study confirm that miR-194-5p expression was transformed at different stages of diabetes. Furthermore, expression of miR-194-5p was significantly reduced in diabetics and prediabetics compared to healthy controls. Therefore, it can be considered as a potential biomarker in the early diagnosis of T2DM.

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Conflict of Interest

All authors interpret that they have no conflict of interest.

Authors' contributions

A.D. and MY.VM. researched literature and conceived the study. MY.VM and M.D. were involved in protocol development, gaining ethical approval, patient recruitment and data analysis. M.D. wrote the first draft of the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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