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Research Article

Serum levels miR-29a-3p and miR-221-3p as potential biomarkers for diagnosis of diabetes

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ABSTRACT

Objectives: Type 2 diabetes (T2D) is a chronic metabolic disorder characterized by insulin resistance and progressive β-cell dysfunction. Early detection is crucial for effective management and the prevention of complications. Circulating microRNAs (miRNAs) have emerged as promising non-invasive biomarkers for various diseases, including metabolic disorders. This study aimed to assess the diagnostic potential of serum microRNA-29a-3p (miR-29a-3p) and microRNA-221-3p (miR-221-3p) in patients with T2D.

Methods: A case-control study was conducted, including 48 patients with T2D and 42 healthy controls. Serum levels of miR-29a-3p and miR-221-3p were quantified using real-time PCR.

Results: Both miR-29a-3p and miR-221-3p were significantly elevated in the sera of T2D patients compared to controls. Serum miR-29a-3p and miR-221-3p showed positive correlations with fasting blood glucose (r = 0.466, r = 0.403) and HbA1c (r = 0.375, r = 0.366), respectively. Additionally, miR-29a-3p exhibited a moderate correlation with triglycerides (TG) (r = 0.300) after adjusting for BMI, age, and gender. Both miRNAs also correlated with adiposity parameters, including body mass index, weight, and waist circumference.

Conclusion: Serum miR-29a-3p and miR-221-3p are significantly upregulated in T2D and correlate with key metabolic markers. Their diagnostic performance suggests that they may serve as valuable non-invasive biomarkers for the early detection and monitoring of T2D. Further research is required to validate these findings in larger and more diverse populations.

Keywords: miR-29a-3p, miR-221-3p, serum, diabetes, circulation

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Introduction

Type 2 diabetes (T2D) is a chronic metabolic disorder characterized by insulin resistance, impaired insulin secretion, and persistent hyperglycemia. It represents a significant global health burden, with its prevalence rising rapidly due to factors such as obesity, sedentary lifestyles, and aging populations (1). According to the International Diabetes Federation, approximately 537 million adults were living with diabetes in 2021, and this number is projected to reach 783 million by 2045 if current trends continue (2).

Early diagnosis and effective management of T2D are crucial in preventing complications such as cardiovascular disease, neuropathy, retinopathy, and nephropathy, which contribute to increased morbidity and mortality. However, current diagnostic tools—including fasting plasma glucose, oral glucose tolerance tests, and glycated hemoglobin (HbA1c)—have limitations regarding sensitivity, specificity, and the ability to detect early-stage disease (2, 3). Therefore, identifying novel biomarkers that enhance the accuracy and timeliness of T2D diagnosis is imperative.

MicroRNAs (miRNAs) are small, non-coding RNA molecules that play a central role in post-transcriptional gene regulation. They are involved in various biological processes, including glucose metabolism, insulin signaling, and pancreatic β-cell function, making them promising candidates for investigating the molecular mechanisms underlying T2D (4). Circulating miRNAs, which are stable and detectable in serum or plasma, have emerged as potential biomarkers for numerous diseases, including cancer, cardiovascular disorders, and metabolic conditions. Their dysregulation in T2D patients suggests they may serve as valuable indicators of disease onset, progression, and treatment response (5).

Among the miRNAs implicated in T2D, miR-29a-3p and miR-221-3p have garnered particular attention due to their roles in insulin resistance, inflammation, and β-cell dysfunction. miR-29a-3p, a member of the miR-29 family, has been shown to regulate glucose homeostasis and insulin sensitivity (6). Studies report its upregulation in T2D patients, where it contributes to insulin resistance by targeting key genes involved in insulin signaling pathways (7). Similarly, miR-221-3p has been associated with impaired insulin secretion and β-cell dysfunction, both hallmarks of T2D (8). Elevated levels of miR-221-3p have been observed in individuals with T2D, suggesting its potential role as a diagnostic marker (9).

However, despite these promising findings, the diagnostic utility of these miRNAs in T2D remains underexplored, and further validation in larger, diverse populations is needed.

This study aims to evaluate the potential of serum miR-29a-3p and miR-221-3p as biomarkers for the diagnosis of T2D. By reviewing existing literature and presenting new findings, we explore the expression patterns of these miRNAs in T2D diagnosis. Identifying reliable miRNA biomarkers could revolutionize early T2D detection, enabling timely interventions and improving patient outcomes. This study contributes to the growing body of evidence supporting the use of miRNAs as non-invasive biomarkers for metabolic disorders and highlights the potential of miR-29a-3p and miR-221-3p in T2D diagnostics.

Materials and methods

Study design and ethical approval

This case-control study was conducted to investigate the expression levels of circulating miR-29a-3p and miR-221-3p in patients with T2D and healthy individuals and evaluate their potential utility as diagnostic biomarkers. The study protocol was reviewed and approved by the Institutional Ethics Committee of Tehran University of Medical Sciences, and written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

Study Population

A total of 90 participants were recruited from the Diabetes Clinic of the Diabetes Research Center, Endocrinology and Metabolism Research Institute, Tehran University of Medical Sciences during 2014–2015. The T2D group included 48 individuals aged 40–65 years with a confirmed diagnosis of T2D based on the American Diabetes Association (ADA) criteria: fasting plasma glucose (FPG) ≥126 mg/dL, and/or HbA1c ≥6.5%. The control group comprised 42 healthy individuals without a history of diabetes with FPG <100 mg/dL and HbA1c <5.7% (10). Exclusion criteria included: pregnancy, type 1 diabetes, recent infections or inflammatory disease, malignancy, liver or renal dysfunction, and current use of immunosuppressive or anti-inflammatory medications.

Clinical and biochemical measurements

All participants underwent a standardized clinical evaluation on the day of sample collection. Anthropometric measurements were performed with participants wearing light clothing and no shoes. Body weight was measured to the nearest 0.1 kg using a calibrated digital scale, and height was measured to the nearest 0.5 cm using a wall-mounted stadiometer. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m²). Blood pressure was measured in the seated position after a 5-minute rest using an automated sphygmomanometer.

Three readings were taken at 1-minute intervals, and the average of the last two readings was recorded as the final systolic and diastolic blood pressure.

Venous blood samples were collected after an overnight fast (8–12 hours) for biochemical analysis. Fasting plasma glucose and fasting insulin levels were measured using standard enzymatic and immunoassay methods, respectively. Insulin resistance was estimated using the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) formula: HOMA-IR = [fasting insulin (μU/L) × fasting glucose (mmol/L)] / 22.5.

RNA extraction, cDNA synthesis and qRT-PCR

Total RNA, including small RNAs, was isolated from 200 μL of serum using the miRNeasy Serum/ Plasma Kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. For the quantification of miR-29a-3p and miR-221-3p, reverse transcription was performed using the miScript II RT Kit (Qiagen) in a final reaction volume of 20 μL, following the manufacturer's instructions. cDNA synthesis reactions were incubated at 37 °C for 60 minutes, followed by enzyme inactivation at 95 °C for 5 minutes.

Quantitative real-time PCR (qRT-PCR) was performed using the miScript SYBR Green PCR Kit (Qiagen) and specific miScript Primer Assays for miR-29a-3p, miR-221-3p, and U6 snRNA (endogenous control) on a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, USA). Each reaction was performed in triplicate, and no-template controls were included to check for contamination. The relative expression levels of miR-29a-3p and miR-221-3p were calculated using the 2^−ΔΔCt method, with U6 snRNA serving as the internal control. The ΔCt value was determined as the difference between the Ct values of miR-29a-3p or miR-221-3p and that of U6.

Statistical Analysis

Data were analyzed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). Normality was assessed using the Shapiro–Wilk test. Continuous variables were expressed as mean ± standard deviation (SD). Group comparisons were conducted using the unpaired Student's t-test or Mann–Whitney U test, as appropriate. A p-value < 0.05 was considered statistically significant.

Results

A total of ninety participants were enrolled in the study, including 48 patients diagnosed with T2D and 42 healthy controls. The levels of several parameters—such as age, FBS, HbA1c, HOMA-IR, insulin, systolic blood pressure (SBP), diastolic blood pressure (DBP), triglycerides (TG), BMI, and waist circumference (WC)—were significantly greater in patients with T2D compared to healthy subjects. No significant differences were found in the levels of cholesterol, LDL-C, and HDL-C between the two groups (Table 1).

Serum levels of miR-29a-3p and miR-221-3p were quantified using real-time PCR. Mean serum miR-29a-3p expression was significantly higher in the T2D group compared to controls (2.8 ± 0.5 vs. 1.1 ± 0.3, p < 0.001). Similarly, miR-221-3p levels were elevated in diabetic patients (2.4 ± 0.6 vs. 0.9 ± 0.2, p < 0.001) (Fig. 1).

Correlation analysis was performed to investigate the relationship between serum miR-29a-3p and miR-221-3p levels and key clinical and metabolic parameters

Table 1. Clinical and biochemical characteristics of the study population

Parameter	Non-diabetic subjects (n=42)	T2D subjects (n=48)	P value
Gender (M/F)	13/29	26/22	0.038
Age (years)	47.2 ± 5.4	56.9 ± 7.6	0.000
SBP (mmHg)	121.2± 13.9	123.4 ±15.0	0.083
DBP (mmHg)	71.7 ± 9.7	75.9 ± 8.7	0.060
WC (cm)	99.0 ± 9.9	96.4 ± 11.7	0.165
HC (cm)	105.4 ± 7.1	101.4 ± 11.8	0.061
BMI (Kg/m2)	27.4 ± 3.4	28.5 ± 4.6	0.032
WHR	0.93 ± 0.05	0.95 ± 0.05	0.610
FBS (mg/dL)	85.0 ± 8.4	131.1 ± 31.0	0.000
Cholesterol (mg/dL)	150.9 ± 18.6	154.8 ± 49.9	0.670
TG (mg/dL)	120.4 ± 60.4	134.8 ± 55.3	0.203
HDL-C (mg/dL)	43.5 ± 7.1	42.1 ± 9.0	0.394
LDL-C (mg/dL)	118 ± 19.8	125.7 ± 20.7	0.710
Insulin (μIU/mL)	5.70 ± 2.2	9.37 ± 5.6	<0.0001
HbA1c (%)	5.3 ± 0.4	7.23 ± 0.6	<0.0001
HOMA-IR	1.24 ± 0.3	2.76 ± 0.9	<0.0001
Metformin (n)	-	46	-
Statins (n)	-	27	-
Antihypertensive (n)	-	15	-

SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, WC: Waist Circumference, HC: Hip Circumference, FBS: Fasting Blood Sugar, TG: Triglyceride, BMI; Body Mass Index, WHR: Weight to Hip Ratio, HDL: High-density Lipoprotein, LDL: Low-density Lipoprotein. Data are shown as "mean ± SD".

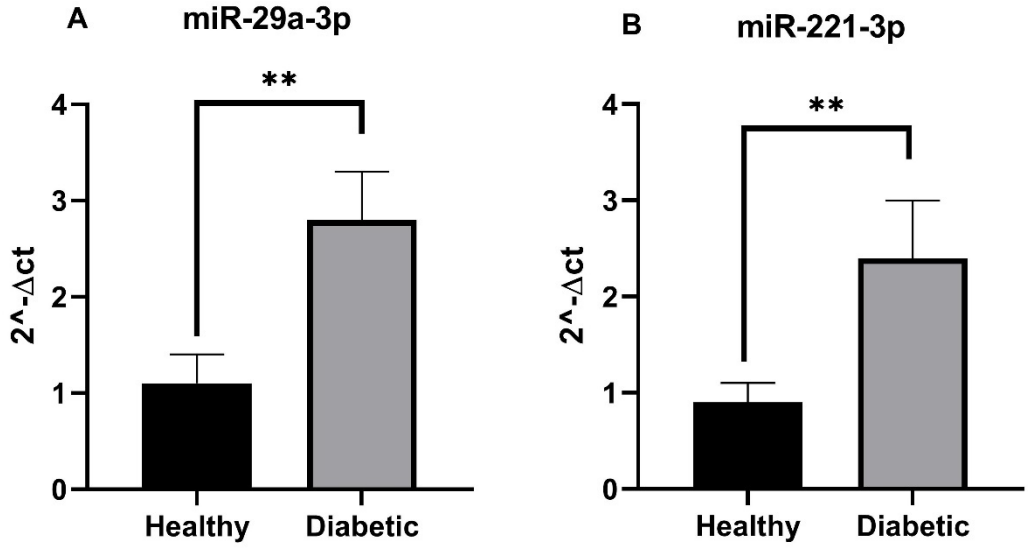

Figure 1. Comparison of circulating miR-29a-3 (A) and miR-221-3p (B) level in type 2 diabetic and non-diabetic subjects. The relative expression of miRNAs was measured by real-time PCR. Statistical differences are based on analyses of log-transformed data, but the means of untransformed data are presented in tables. Data are shown as mean ± SD.

Table 2. Correlation between miR-155 and clinical and biochemical characteristics

Parameter	miR-29a-39		miR-221-3p	
	R1	R2	R1	R2
Age (years)	0.212	0.285	0.241	0.253
SBP (mmHg)	0.160	0.133	0.158	0.138
DBP (mmHg)	0.092	0.080	0.126	0.102
Weight (Kg)	**0.343***	**0.297***	**0.380***	**0.291***
WC (cm)	**0.364***	**0.333***	**0.335***	**0.324***
HC (cm)	0.190	0.127	0.198	0.206
BMI (Kg/m2)	**0.454***	**0.452***	**0.467***	**0.479***
WHR	0.263	0.178	0.131	0.129
FBS (mg/dL)	**0.499****	**4.660****	**4.116***	**4.036***
Cholesterol (mg/dL)	0.246	0.211	0.162	0.147
TG (mg/dL)	**0.335***	**0.300***	0.223	0.278
HDL-C (mg/dL)	0.114	0.144	0.176	0.164
LDL-C (mg/dL)	0.188	1.269	0.106	0.129
Insulin (µIU/mL)	**0.365****	**0.312***	**0.381****	**0.406****
HbA1c (%)	**0.372***	**0.375***	**0.389***	**0.366***
HOMA-IR	**0.475****	**0.512****	**0.479***	**0.491***

SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, WC: Waist Circumference, HC: Hip Circumference, FBS: Fasting Blood Sugar, TG: Triglyceride, BMI; Body Mass Index, WHR: Weight to Hip Ratio, HDL: High-density Lipoprotein, LDL: Low-density Lipoprotein., R1: unadjusted, R2: adjusted for age, gender and BMI. Data were analyzed using log of 2-delta CT of miR-29a-3p and miR-221-3p and variables in log transformed forms (FBS, TG, Cholestrol, HDL-C, LDL-C, Insulin, HOMA-IR, and HbA1c).*= P value less than 0.05, **= p value less than 0.01

associated with T2D. A positive and statistically significant correlation was found between serum miR-29a-3p levels and several key metabolic markers related to T2D. Specifically, miR-29a-3p showed correlations with FBG ($r = 0.466$, $p < 0.001$) and HbA1c ($r = 0.475$, $p < 0.001$), indicating that higher miR-29a-3p levels were associated with elevated glucose levels. Additionally, miR-29a-3p was strongly correlated with insulin ($r = 0.312$, $p < 0.001$) and HOMA-IR ($r = 0.512$, $p < 0.001$), suggesting that miR-29a-3p may reflect insulin resistance. Moreover, miR-29a-3p was also associated with adiposity parameters such as BMI ($r = 0.452$, $p < 0.001$), weight ($r = 0.343$, $p < 0.001$), and WC ($r = 0.364$, $p < 0.001$). All these positive correlations remained statistically significant after adjustment for age and gender. A positive correlation with TG ($r = 0.300$, $p < 0.001$) was also observed, further supporting the potential role of miR-29a-3p in lipid metabolism.

Serum miR-221-3p also showed significant correlations with various metabolic parameters. miR-221-3p levels

were correlated with fasting blood glucose ($r = 0.403$, $p < 0.01$), indicating a relationship between miR-221-3p and glycemic status in T2D patients. Similarly, miR-221-3p demonstrated a positive correlation with HbA1c ($r = 0.440$, $p < 0.01$), further supporting the role of miR-221-3p in reflecting long-term glucose control. Additionally, miR-221-3p was positively correlated with BMI ($r = 0.479$, $p < 0.05$), weight ($r = 0.291$, $p < 0.001$), and WC ($r = 0.324$, $p < 0.05$), suggesting that higher levels of miR-221-3p are associated with obesity. These results remained statistically significant after adjustment for age and gender.

Discussion

The results of this study highlight the promising potential of serum miR-29a-3p and miR-221-3p as biomarkers for the diagnosis of T2D. Our data demonstrate that both miRNAs are significantly elevated in T2D patients compared to healthy controls, with levels correlating with key metabolic parameters such as FBG, HbA1c, insulin resistance, and obesity. These findings suggest that these miRNAs could serve as valuable diagnostic tools for T2D, offering a new approach to the management and early detection of this increasingly prevalent condition.

Our study presents compelling evidence that miR-29a-3p is elevated in the serum of T2D patients, supporting its potential as a biomarker for the diagnosis of T2D. These findings are consistent with studies that have demonstrated the utility of miR-29a-3p as a diagnostic tool in various metabolic diseases, including diabetes (6, 7, 11, 12). In a meta-analysis profiling circulating miRNAs in T2D subjects (n = 14 studies), obese individuals (n = 6 studies), and pre-diabetic individuals (n = 7 studies), miR-29a-3p was found to be upregulated in T2D (7). Similarly, miR-29a-3p was reported to be elevated in pre-diabetic subjects. In the context of obesity, miR-29a-3p was reported to be significantly increased in the circulation of obese compared to non-obese subjects (7).

In vitro and in vivo studies also support these findings. In several animal models of impaired fasting glucose or T2D, miR-29 family miRNAs were observed to be upregulated (13–15). The transcription factor FOXA2 has been reported to have binding sites in the promoter region of the miR-29 gene. Foxa2 mRNA increases along with miR-29 family miRNAs in the liver tissues of mouse models of prediabetes, and silencing Foxa2 inhibited the expression of miR-29a, miR-29b, and miR-29c. Manipulating miR-29a levels in hepatocyte cell lines altered key lipid metabolism genes (e.g., ABHD5, HMGCS2, and PPARGC1A) that are also known to be regulated by FOXA2 (16).

Abnormal activation of gluconeogenesis in the liver also leads to hyperglycemia in T2D. Important regulators of gluconeogenesis were found to be targeted by miR-29a. Adenoviral overexpression of miR-29a in the livers of diabetic and high-fat diet-induced obese mice reduced the expression of PEPCK, a key gluconeogenic gene, and lowered hepatic glucose production (14). Liver-specific miR-29a knockout (KO) animals confirmed PI3K as a target for miR-29 and demonstrated prolonged insulin signaling in liver tissue (17).

In the skeletal muscle of T2D patients and ob/ob mice, miR-29a levels were increased (18). Moreover, miR-29a expression was elevated by saturated fatty acids in cultured myocytes (19). Overexpression of miR-29a in myotubes resulted in decreased glucose uptake and palmitate oxidation, while inhibition of miR-29a reversed these effects. miR-29a induced insulin resistance in primary human skeletal muscle cells. Furthermore, miR-29a upregulation was observed in the skeletal muscles of diet-induced obese mice as well as in rat myotubes following palmitate exposure (19). Overexpression of miR-29a in myocytes significantly reduced IRS-1 protein levels, leading to decreased glucose uptake and impaired insulin signaling (19).

In adipose tissue, miR-29 family members are upregulated by diabetes and obesity (20), and forced expression of miR-29a impairs adipocyte differentiation (24). Moreover, overexpression of miR-29a in 3T3-L1 adipocytes reduced glucose uptake in the presence of insulin (21).

Our findings regarding miR-221-3p are also in line with previous studies. MicroRNA-221-3p has been reported to be involved in adipocyte differentiation, metabolism, and insulin signaling (22, 23). miR-221 expression is elevated in obesity and is induced by adipose tissue inflammation (22, 24). Elevated expression of miR-221-3p impairs adipocyte lipid storage and differentiation, and modifies the content of key signaling lipids such as ceramide and diacylglycerol—alterations relevant to metabolic diseases such as T2D (25). Serum miR-221-3p levels were significantly upregulated in diabetic patients with retinopathy (9). In addition, adipose and plasma miR-221 levels have been associated with obesity, insulin resistance, and new-onset diabetes after peritoneal dialysis (26). Abdel-Tawab et al. also reported that enhanced circulating miR-221 may serve as a potential diagnostic and prognostic biomarker for diabetic nephropathy (27). Another study reported that diabetes is accompanied by increased arterial miR-221 expression, which promotes intimal thickening, and that inhibition of miR-221 may be efficacious in preventing the cardiovascular complications of diabetes (28).

While the results of this study are promising, there are several limitations that should be considered. First, the sample size was relatively small, and a larger cohort—including individuals at different stages of T2D—would provide more robust data on the utility of miR-29a-3p and miR-221-3p as diagnostic biomarkers. Additionally, the majority of participants in this study were from a specific geographic region, and further validation in diverse

populations is necessary to assess the generalizability of these findings. Moreover, it would be important to explore the potential of miR-29a-3p and miR-221-3p as biomarkers for monitoring T2D complications, such as diabetic nephropathy and retinopathy.

Conclusion

In conclusion, this study provides evidence that serum miR-29a-3p and miR-221-3p are elevated in T2D patients and correlate with key clinical markers of the disease, including glucose metabolism, insulin resistance, and obesity. These findings suggest that miR-29a-3p and miR-221-3p could serve as valuable biomarkers for the diagnosis of T2D. Future studies are needed to further investigate the mechanistic roles of these miRNAs in T2D pathogenesis and to explore their potential as tools for assessing disease progression and treatment response.

Conflict of Interest

The authors have nothing to declare.

References

1. Alipoor B, Ghaedi H, Meshkani R, Omrani MD, Sharifi Z, Golmohammadi T. The rs2910164 variant is associated with reduced miR-146a expression but not cytokine levels in patients with type 2 diabetes. J Endocrinol Invest. 2018;41(5):557-66. https://doi.org/10.1007/s40618-017-0766-z
2. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9(th) edition. Diabetes Res Clin Pract. 2019;157:107843. https://doi.org/10.1016/j.diabres.2019.107843
3. Ghorbani S, Mahdavi R, Alipoor B, Panahi G, Nasli Esfahani E, Razi F, et al. Decreased serum microRNA-21 level is associated with obesity in healthy and type 2 diabetic subjects. Arch Physiol Biochem. 2018;124(4):300-5. https://doi.org/10.1080/13813455.2017.1396349
4. Mahdavi R, Ghorbani S, Alipoor B, Panahi G, Khodabandehloo H, Esfahani EN, et al. Decreased serum level of miR-155 is associated with obesity and its related metabolic traits. Clin Lab. 2018;64. https://doi.org/10.7754/Clin.Lab.2017.170618
5. Kraczkowska W, Stachowiak L, Pławski A, Jagodziński PP. Circulating miRNA as potential biomarkers for diabetes mellitus type 2: should we focus on searching for sex differences? J Appl Genet. 2022;63(2):293-303. https://doi.org/10.1007/s13353-021-00678-5
6. Dalgaard LT, Sørensen AE, Hardikar AA, Joglekar MV. The microRNA-29 family: role in metabolism and metabolic disease. Am J Physiol Cell Physiol. 2022;323(2):C367-77. https://doi.org/10.1152/ajpcell.00051.2022
7. Villard A, Marchand L, Thivolet C, Rome S. Diagnostic value of cell-free circulating microRNAs for obesity and type 2 diabetes: a meta-analysis. J Mol Biomark Diagn. 2015;6(6):251. https://doi.org/10.4172/2155-9929.1000251
8. Gentile AM, Lhamyani S, Mengual-Mesa M, García-Fuentes E, Bermúdez-Silva FJ, Rojo-Martínez G, et al. MiR-221-3p/222-3p cluster expression in human adipose tissue is related to obesity and type 2 diabetes. Int J Mol Sci. 2023;24(24). https://doi.org/10.3390/ijms242417449
9. Zhao L, Pan Q. Highly-Expressed MiR-221-3p Distinctly Increases the Incidence of Diabetic Retinopathy in Patients With Type 2 Diabetes Mellitus. Transl Vis Sci Technol. 2023;12(10):17. https://doi.org/10.1167/tvst.12.10.17
10. American Diabetes Association. 2. Diagnosis and classification of diabetes: Standards of care in diabetes-2024. Diabetes Care. 2024;47(Suppl 1):S20-42. https://doi.org/10.2337/dc24-SDIS
11. Udesen PB, Glintborg D, Sørensen AE, Svendsen R, Nielsen NLS, Wissing MLM, et al. Metformin decreases miR-122, miR-223 and miR-29a in women with polycystic ovary syndrome. Endocr Connect. 2020;9(11):1075-84. https://doi.org/10.1530/EC-20-0195
12. Jimenez-Lucena R, Alcala-Diaz JF, Roncero-Ramos I, Lopez-Moreno J, Camargo A, Gomez-Delgado F, et al. MiRNAs profile as biomarkers of nutritional therapy for the prevention of type 2 diabetes mellitus: From the CORDIOPREV study. Clin Nutr. 2021;40(3):1028-38. https://doi.org/10.1016/j.clnu.2020.06.035
13. Pandey AK, Verma G, Vig S, Srivastava S, Srivastava AK, Datta M. miR-29a levels are elevated in the db/db mice liver and its overexpression leads to attenuation of insulin action on PEPCK gene expression in HepG2 cells. Mol Cell Endocrinol. 2011;332(1-2):125-33. https://doi.org/10.1016/j.mce.2010.10.004
14. Liang J, Liu C, Qiao A, Cui Y, Zhang H, Cui A, et al. MicroRNA-29a-c decrease fasting blood glucose levels by negatively regulating hepatic gluconeogenesis. J Hepatol. 2013;58(3):535-42. https://doi.org/10.1016/j.jhep.2012.10.024
15. Hung YH, Kanke M, Kurtz CL, Cubitt R, Bunaciu RP, Miao J, et al. Acute suppression of insulin resistance-associated hepatic miR-29 in vivo improves glycemic control in adult mice. Physiol Genomics. 2019;51(8):379-89. https://doi.org/10.1152/physiolgenomics.00037.2019
16. Kurtz CL, Peck BC, Fannin EE, Beysen C, Miao J, Landstreet SR, et al. MicroRNA-29 fine-tunes the expression of key FOXA2-activated lipid metabolism genes and is dysregulated in animal models of insulin resistance and diabetes. Diabetes. 2014;63(9):3141-8. https://doi.org/10.2337/db13-1015
17. Dooley J, Garcia-Perez JE, Sreenivasan J, Schlenner SM, Vangoitsenhoven R, Papadopoulou AS, et al. The microRNA-29 family dictates the balance between homeostatic and pathological glucose handling in diabetes and obesity. Diabetes. 2016;65(1):53-61. https://doi.org/10.2337/db15-0770
18. Massart J, Sjögren RJ, Lundell LS, Mudry JM, Franck N, O'Gorman DJ, et al. Altered miR-29 expression in type 2 diabetes influences glucose and lipid metabolism in skeletal muscle. Diabetes. 2017;66(7):1807-18. https://doi.org/10.2337/db17-0141
19. Yang WM, Jeong HJ, Park SY, Lee W. Induction of miR-29a by saturated fatty acids impairs insulin signaling and glucose uptake through translational repression of IRS-1 in myocytes. FEBS Lett. 2014;588(13):2170-6. https://doi.org/10.1016/j.febslet.2014.05.011
20. He A, Zhu L, Gupta N, Chang Y, Fang F. Overexpression of micro ribonucleic acid 29, highly up-regulated in diabetic rats, leads to insulin resistance in 3T3-L1 adipocytes. Mol Endocrinol. 2007;21(11):2785-94. https://doi.org/10.1210/me.2007-0167
21. Glantschnig C, Koenen M, Gil-Lozano M, Karbiener M, Pickrahn I, Williams-Dautovich J, et al. A miR-29a-driven negative feedback loop regulates peripheral glucocorticoid receptor signaling. FASEB J. 2019;33(5):5924-41. https://doi.org/10.1096/fj.201801385RR

22. Mysore R, Ortega FJ, Latorre J, Ahonen M, Savolainen-Peltonen H, Fischer-Posovszky P, et al. MicroRNA-221-3p regulates angiopoietin-like 8 (ANGPTL8) expression in adipocytes. J Clin Endocrinol Metab. 2017;102(11):4001-12. https://doi.org/10.1210/jc.2017-00453
23. Peng J, Zhou Y, Deng Z, Zhang H, Wu Y, Song T, et al. miR-221 negatively regulates inflammation and insulin sensitivity in white adipose tissue by repression of sirtuin-1 (SIRT1). J Cell Biochem. 2018;119(8):6418-28. https://doi.org/10.1002/jcb.26589
24. Meerson A, Traurig M, Ossowski V, Fleming J, Mullins M, Baier L. Human adipose microRNA-221 is upregulated in obesity and affects fat metabolism downstream of leptin and TNF-α. Diabetologia. 2013;56:1971-9. https://doi.org/10.1007/s00125-013-2950-9
25. Ahonen MA, Asghar MY, Parviainen SJ, Liebisch G, Höring M, Leidenius M, et al. Human adipocyte differentiation and composition of disease-relevant lipids are regulated by miR-221-3p. Biochim Biophys Acta Mol Cell Biol Lipids. 2021;1866(1):158841. https://doi.org/10.1016/j.bbalip.2020.158841
26. Chan GCK, Than WH, Kwan BCH, Lai KB, Chan RCK, Teoh JYC, et al. Adipose and plasma microRNAs miR-221 and 222 associate with obesity, insulin resistance, and new onset diabetes after peritoneal dialysis. Nutrients. 2022;14(22). https://doi.org/10.3390/nu14224889
27. Abdel-Tawab MS, Mohamed MG, Doudar NA, Rateb EE, Reyad HR, Elazeem NAA. Circulating hsa-miR-221 as a possible diagnostic and prognostic biomarker of diabetic nephropathy. Mol Biol Rep. 2023;50(12):9793-803. https://doi.org/10.1007/s11033-023-08846-y
28. Lightell DJ Jr, Moss SC, Woods TC. Upregulation of miR-221 and -222 in response to increased extracellular signal-regulated kinases 1/2 activity exacerbates neointimal hyperplasia in diabetes mellitus. Atherosclerosis. 2018;269:71-8. https://doi.org/10.1016/j.atherosclerosis.2017.12.016