Journal of
Translational Regenerative Medicine

2026. Volume 1

Research Paper

Circulating miR20a5p and miR1495p as Inflammatory Biomarkers for Early Detection of Type 2 Diabetes Mellitus and PreDiabetes

Erfan Babahoseinpour[1], Reza Heidari[1, 2], Ali Shakerimoghaddam[3], Mahmoud Vahidi[4], Mehdi Shakouri Khomartash[1*]

1. Medical Biotechnology Research Center, AJA University of Medical Sciences, Tehran, Iran.
2. Cancer Epidemiology Research Center (AJA-CERTC), AJA University of Medical Sciences, Tehran, Iran.
3. Noncommunicable Diseases Research Center, Neyshabur University of Medical Sciences, Neyshabur, Iran.
4. Cancer Epidemiology Research Center (AJA-CERTC), AJA University of Medical Sciences, Tehran, Iran.

Use your device to scan and read the article online

Citation Babahoseinpour E, Heidari R, Shakerimoghaddam A, Vahidi M, Shakouri Khomartash M. Circulating miR20a5p and miR1495p as Inflammatory Biomarkers for Early Detection of Type 2 Diabetes Mellitus and PreDiabetes. Journal of Translational Regenerative Medicine. 2026; 1:E1011. http://dx.doi.org/10.32598/JTRM.1.1011

http://dx.doi.org/10.32598/JTRM.1.1011

ABSTRACT

Background: Chronic low-grade inflammation plays a central role in the development and progression of type 2 diabetes mellitus (T2DM). MicroRNAs (miRNAs) have emerged as promising regulators of inflammatory pathways and potential non-invasive biomarkers for early disease detection. This study aimed to investigate the expression levels of miR-20a-5p and miR-149-5p and their association with pro-inflammatory cytokines in individuals with T2DM, pre-diabetes, and non-diabetics.

Methods: A total of 90 participants were enrolled and divided into three groups: non-diabetic (n=30), pre-diabetic (n=30), and T2DM (n=30), according to the American Diabetes Association (ADA)'s criteria. Serum levels of miR-20a-5p and miR-149-5p were quantified using real-time polymerase chain reaction (PCR). The expression levels and protein concentrations of inflammatory cytokines (IL-6, IL-1β, TGF-β, and IFN-γ) were assessed by real-time PCR and enzyme-linked immunosorbent assay (ELISA), respectively. The receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic power of the selected miRNAs.

Results: The expression of miR-20a-5p was significantly upregulated in both pre-diabetic and T2DM groups, whereas the expression of miR-149-5p was significantly downregulated in the T2DM group. ROC analysis revealed excellent diagnostic power of miR-20a-5p for distinguishing T2DM (AUC=0.985) and pre-diabetics (AUC=0.895) from non-diabetic controls. Elevated expression and serum levels of IL-6, IL-1β, TGF-β, and IFN-γ were observed in pre-diabetic and T2DM groups. miR-20a-5p showed a significant positive correlation with pro-inflammatory cytokines (P<0.001). while miR-149-5p demonstrated a significant negative correlation (P<0.001).

Conclusion: The dysregulated expression of miR-20a-5p and miR-149-5p is closely associated with inflammatory responses in T2DM and pre-diabetes. miR-20a-5p has higher diagnostic accuracy and may serve as a promising circulating biomarker for early detection of diabetes-related metabolic and inflammatory alterations.

Keywords: Type 2 diabetes, Pre-diabetes, microRNA-20a-5p, microRNA-149-5p, Inflammation

Article info:
Received: 12 Jun 2026
Accepted: 13 Sep 2026
Publish: 10 Nov 2026

*** Corresponding Author:**
Mehdi Shakouri Khomartash, Assistant Professor.
Address: *Medical Biotechnology Research Center, AJA University of Medical Sciences, Tehran, Iran.*
Phone: *+98 (21) 43822923*
E-mail: *Shakourmehdi@gmail.com*

Highlights

- MicroRNAs are promising regulators of inflammatory pathways and potential non-invasive biomarkers for early disease detection

- Dysregulated expression of miR-20a-5p and miR-149-5p is closely associated with inflammatory responses in T2DM and pre-diabetes.

- This study revealed the excellent power of miR-20a-5p and miR-149-5p in distinguishing T2DM and prediabetes from non-diabetic controls.

Plain Language Summary

MicroRNAs (miRNAs) are becoming increasingly crucial in regulating gene expression networks. This study assessed the associations between the expression levels of miR-149-5p and miR-20a-5p and inflammatory factors to investigate their potential as biomarkers for the early identification of patients with T2DM and prediabetes. Dysregulated expression of miR-20a-5p and miR-149-5p, and elevated expression and serum levels of IL-6, IL-1β, TGF-β, and IFN-γ were observed in pre-diabetic and T2DM groups. miR-20a-5p had higher diagnostic accuracy than miR-149-5p and may serve as a promising circulating biomarker for early detection of diabetes-related metabolic and inflammatory alterations.

Introduction

By 2030, it is anticipated that there will be 438 million cases of diabetes worldwide, putting an economic burden (490 billion USD per year) on society. Some tests can be used to diagnose diabetes, including the oral glucose tolerance test (OGTT), fasting blood glucose (FBG), hemoglobin A1c (HbA1c), and the homeostatic model assessment of insulin resistance (HOMA-IR). However, these tests are only useful in predicting the development of type 2 diabetes mellitus (T2DM) in people who have previously undergone metabolic changes. As a result, understanding the molecular mechanisms involved in the development and progression of T2DM is important for early diagnosis and therapeutic strategies [1, 2].

According to studies, microRNAs (miRNAs), a form of short non-coding RNAs (ncRNAs), are becoming increasingly crucial in the regulation of gene expression networks [1-4]. They can degrade target messenger RNAs (mRNAs) or trigger post-transcriptional repression by attaching to their 3' untranslated regions (3'UTRs), which allows them to function as translational repressors [2, 5]. It has been found that, in addition to physiological functions, miRNAs have critical roles in the etiology of numerous diseases including cancer, diabetes, and cardiovascular disorders [6-9].

Chronic inflammation plays an important role in the pathogenesis of T2DM and its relevant complications [10-12]. It has been demonstrated that inflammatory cytokines, including interleukin-6 (IL-6), interleukin-1β (IL-1β), transforming growth factor- β (TGF- β), and interferon-γ (IFN-γ), are enhanced in individuals with T2DM and contribute to insulin resistance and beta-cell dysfunction [13, 14]. Therefore, the identification of molecular markers to show the state of inflammation and the pathogenesis of T2DM is of great importance. It has been reported in several studies that miR-20a-5p and miR-149-5p may contribute to the regulation of inflammatory processes and may be regarded as potential biomarkers in various diseases [15-17]. Thus, we hypothesize that dysregulated expression of miR-149-5p and miR-20a-5p contributes to the inflammation observed in individuals with pre-diabetes and T2DM. To address this hypothesis, in the present study, we assessed the expression levels of miR-149-5p and miR-20a-5p as potential biomarkers for the early identification of patients with T2DM and pre-diabetes. We also aimed to investigate the association between the aforementioned miRNAs and inflammatory factors such as IL-6, IL-1β, TGF-β, and IFN-γ in participants.

Materials and Methods

Participants

The participants were Iranian people who visited specialized diabetes clinics in Tehran for medical attention and clinical evaluation. They were aged 20-60 years old, had no microvascular or macrovascular issues, no infection or coronary artery disease, nonsmokers, with a body mass index ranging from 25 to 35 kg/m^2. The inclusion criteria were based on the American Diabetes Association (ADA) recommendations. The participants were divided into three groups of 30: (a) Normoglycemic group, who had fasting plasma glucose (FPG) levels <100 mg/dL (5.6 mmol/L) and HbA1c values <5.7% (39 mmol/mol) and were not taking medication; (b) pre-diabetic group, who had FPG values between 100 and 125 mg/dL (5.6 to 6.9 mmol/L) and/or HbA1c levels between 5.7 and 6.4% (39 to 47 mmol/mol); and (c) T2DM group, who had FPG levels >126 mg/dL (7.0 mmol/L) and/or HbA1c values >6.5% (48 mmol/mol).

Blood sampling and serum preparation

Fasting venous blood samples of 8 mL per participant were rigorously collected in accordance with strict standards. We used 4 mL of ethylenediaminetetraacetic acid (EDTA) tubes for RNA extraction. Among these, 2 mL were placed in a full blood count (CBC) tube containing EDTA, which is required for the determination of HbA1c. Simultaneously, 4 mL of blood was taken in a dedicated test tube containing a clot activator to aid in the separation of serum components [18]. To achieve optimal serum separation, the collected samples were incubated at room temperature (RT) for 1 hour, followed by centrifugation at 2,000 RCF (relative centrifugal force) for 5 minutes. To ensure that any remnant debris was completely removed, the serum samples were centrifuged at 12,000 RCF for 15 minutes. The serum was carefully transferred into a 1.5 mL microtube and stored at -80 ˚C for further tests.

Biochemical tests

Serum concentrations of FBG and HbA1c were measured using an ELISA kit (MyBioSource, USA), and lipid profiles were measured using a direct lipid profile Kit (Pishtaz Teb Zaman, Iran). Fasting insulin levels were determined using the Human INS (Insulin) Elisa kit (Elabscience, USA) according to the manufacturer′s instructions [19].

Real-time polymerase chain reaction

Primers for the genes *IL-1β*, *IFN-γ*, *TGF-β*, *IL-6, miR-149-5P*, and *miR-20a-5P* were designed using Primer3 plus Software. Total RNA was extracted using TRIzol reagent (CA:15596018; Thermo Fisher, USA) in accordance with the manufacturer′s instructions. Briefly, 1 mL of triazole was added to 250 µL of blood, followed by adding 200 µL of chloroform to separate the macromolecules. After centrifugation, the aqueous phase containing RNA was carefully removed and the RNA was precipitated with isopropanol and washed with 70% ethanol. Finally, the dried RNA precipitate was dissolved in diethylpyrocarbonate-treated water [20]. Purity and concentration of the extracted RNA were determined by analyzing the A260/A280 ratio using NanoDrop spectrophotometry (Bio-Rad 550, USA). The RNA integrity was assessed using electrophoresis on a 1% agarose gel.

cDNA synthesis was carried out using the SMOBIO kit (Hsinchu, Taiwan) according to the manufacturer's instructions [21]. Briefly, the amount of 500 nanograms of RNA from each sample was subjected to cDNA synthesis using oligo(dt) and random hexamer primers. Subsequently, the target genes were amplified by real-time polymerase chain reaction (PCR) using the specific primers (Table 1) and SYBR Green Master mix (Amplicon, Denmark). The ΔΔCT (cycle threshold) method was used to quantify miRNA expression differences between groups. The Equation 1 was used to calculate the fold changes of miRNA expression as well as inflammatory genes within each sample, including both T2DM and pre-diabetic groups, in comparison with the healthy control group:

1. Fold change=$2^{(-\Delta\Delta Ct)}$

ΔΔCT: ($CT_{\text{gene or miRNA}}$ - $CT_{\text{reference or SNORD}}$) T2DM or pre-diabetes - ($CT_{\text{gene or miRNA}}$ - $CT_{\text{reference or SNORD}}$) controls.

To investigate the expression of cytokine genes, the *GAPDH* gene was used as a reference gene. To assess each miRNA's sensitivity, specificity, and prediction power for both T2DM and pre-diabetes, we used the receiver operating characteristic (ROC) curve for each group and then calculated the area under the ROC curve (AUC). The AUC is a number between 0 and 1. The closer this number is to 1, the higher the diagnostic power. In addition, we investigated the correlation of the levels of inflammatory genes and microRNAs in the T2DM group.

Table 1. List of primer sequences designed for amplifying their respective gene targets

Gene Name	Accession Number	Primer Sequence 5′ 3′
IL-1β	NM_000576.3	ATTCTCTTCAGCCAATCTTC AACAAGTCATCCTCATTGC
IFN-γ	NM_000619.3	GGCATTTTGAAGAATTGGAAAG CTTGATGGTCTCCACACTC
TGF-β	NM_000660.7	TACCTGAACCCGTGTTGCTC GTGACATCAAAAGATAACCACTCT
IL-6	NM_000600.5	CAACCTGAACCTTCCAAA GTATACCTCAAACTCCAAAAG
GAPDH	NM_002046.7	CACCACACTGAATCTCCCCT TGGTTGAGCACAGGGTACTT
miR-149-5P	miRBase: MIMAT0000450 NR_029702.1	AGCTCTGGCTCCGTGTCTTCACTCCC GCGAGCACAGAATTAATACGACTC
miR-20-5P	miRBase :MIMAT0000075 NR_029492.1	CGCTAAAGTGCTTATAGTGCAGGTAG GCGAGCACAGAATTAATACGACTC
U6	NR_004394.1 MIM:180692	CGCAAGGATGACACGCAAATTC GCGAGCACAGAATTAATACGACTC

Bioinformatics analysis

Bioinformatic databases such as miRDB and TargetScan were used to identify putative mRNA targets of miRNA-20a and miRNA-149. Only the genes involved in insulin control and inflammation were selected from the target gene results, and their role and applications were evaluated.

Enzyme-linked immunosorbent assay (ELISA) assay

All serum samples were subjected to cytokine assay using the ELISA Flex human IFN-γ horseradish peroxidase (HRP), human IL-1β HRP, human IL-6 HRP, and human TGF-β HRP (Mabtech, Sweden) according to the manufacturer's protocol [22]. Briefly, to quantify the concentration of cytokines IL-1β, IFN-γ, TGF-β, and IL-6, 100 µL of each diluted serum sample and standards were added to each well of a 96-well plate in triplicate and incubated for 2 hours at RT. Then, the wells were washed, and 100 µL of biotin-conjugated mAb antibody was added to each well and incubated for 2 hours at room temperature (RT), followed by washing and adding 100 µL of HRP-conjugated streptavidin to each well. After extensive washing, TMB (3,3′,5,5′-Tetramethylbenzidine) substrate was added to each well, and the reaction was stopped after 15 minutes. The optical density was measured by an ELISA reader (BioTek 800 TS, USA) at a wavelength of 450 nm. Finally, the concentration of the mentioned cytokines was obtained from the standard curves.

Statistical analyses

GraphPad Prism software, version 9 (San Diego, CA) was used for statistical analysis. The data were reported as Mean±SD and were analyzed using one-way analysis of variance (ANOVA). Significant differences between groups were determined using Tukey's test. $P<0.05$ was considered statistically significant.

Results

Biochemical analyses

Considering the importance of lipid parameters in enhancing the risk of pre-diabetes and T2DM, the relationship between the lipid profile and diabetes was examined. According to the findings, the mean HDL levels were determined for each group as follows: Control=39.4±15.58 mg/dL, pre-diabetes=35.65±9.09 mg/dL, and T2DM=34.42±9.39 mg/dL. Also, the average triglyceride levels for each group were as follows: Control=167±28.14 mg/dL, pre-diabetes=175.4±45.88 mg/dL, and T2DM=186±60.10 mg/dL. Moreover, the mean cholesterol levels for each were as follows: Control=167.7±43.13 mg/dL, pre-diabetes=180.3±52.6 mg/dL, and T2DM=191.2±58.38 mg/dL. As shown in Figure 1A, there were no statistically significant differences in the concentrations of Triglycerides (P=0.287), cholesterol (P=0.144), and HDL (P=0.342) among the three groups.

Hematological analyses

Measuring FBG and fasting insulin levels is the main indicator in the diagnosis of T2DM. Insulin resistance (HOMA-IR results) plays an important role in the development of T2DM. Also, HbA1c indicates the average level of blood sugar during 2-3 months, which helps in the diagnosis of T2DM [23, 24]. Our findings showed that the average fasting insulin in the control, pre-diabetic, and T2DM groups were 10.3±0.63, 11.41±0.94, and 12.87±0.6, respectively. The mean FBG levels in the three groups were 87.42±4.746, 118.4±3.628, and 139.8±3.204 mg/dL, respectively. The mean HOMA-IR value in the three groups was 2.222±0.17, 3.336±0.3, and 4.1±0.25, respectively. Finally, the average HbA1c

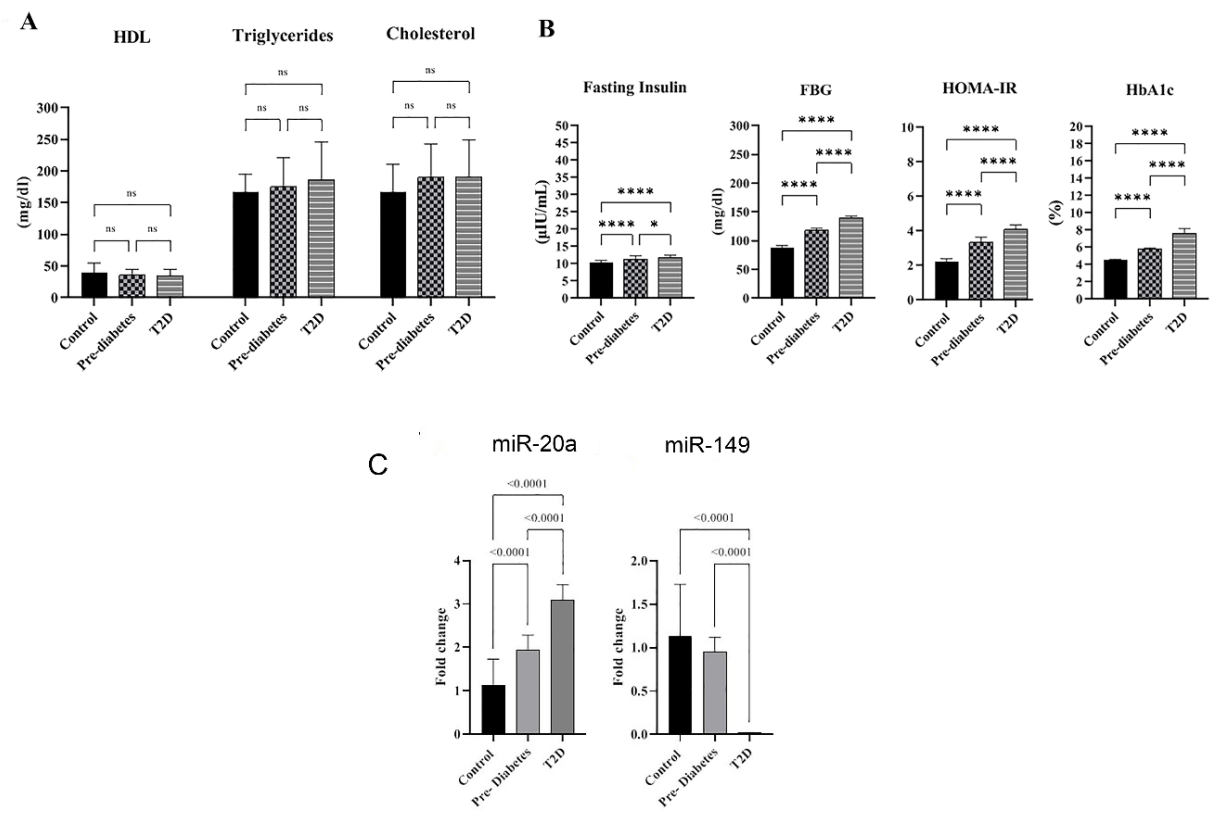

Figure 1. Determination of the lipid profiles, glucose-related indices, and expression of the miRNAs in the study groups

A) Lipid profiles, including the levels of HDL, triglycerides, and cholesterol; B) Glucose-related indices, including fasting insulin, FBG, and HbA1c, measured in serum samples of all subjects; C) Comparison of fold changes in the expression of miR-20a-5p and miR-149-5p in serum samples of three groups, measured by real-time PCR using the ΔΔCt method

Ns: No significant difference between groups.

*P<0.05; ****P<0.001.

Note: Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated via the following formula: Fasting insulin (µlU/mL) × fasting glucose (mg/dL)/405) [35]. Data are presented as Mean±SD.

in the three groups was 4.521±0.1122, 5.834±0.091, and 7.61±0.55 %, respectively (Figure 1B). Notably, a statistically significant increase in fasting insulin, FBG, HOMA-IR, and HbA1c levels was detected in both T2DM and pre-diabetic groups compared to the control group (P<0.001).

Real-time PCR

The expression level of miR-20a in the T2DM group was 3.09-fold higher than that of the control group (P<0.001) as shown in Figure 1C. The expression level of miR-20a-5p in the pre-diabetic group increased 1.93-fold compared to the control group (P<0.001). Furthermore, there was a significant difference in the expression of miR-20a-5p between T2DM and pre-diabetic groups (P=0.016). On the other hand, the expression of miR-149 in the T2DM group was 1.6-fold lower than that of the control group (P<0.001). In contrast, there was no significant decrease in the expression of miR-149 compared to the control group (P=0.069). Furthermore, a significant difference in the expression of miR-149 was observed between T2DM and pre-diabetic groups (Figure 1C).

The ROC curve analysis was used to determine the efficacy of miRNAs as diagnostic biomarkers for T2DM and pre-diabetes. As can be seen in Figure 2A, an AUC of 0.9856 for miR-20a-5p (95% CI, 0.9573%, 1%) was observed in the T2DM group compared to the control group. The ideal sensitivity and specificity were determined to be 100% and 97%, respectively, with a cut-off value of 2.48 (Figure 2A). Furthermore, the AUC for miR-149-5P in the T2DM group was found to be 0.9356 (95% CI, 0.8739%, 0.9973%), with a cutoff value of 0.03486, yielding 78% and 100% sensitivity and specificity, respectively (Figure 2C). The AUC for miR-20a-5p in the pre-diabetic group compared to the control group was determined to be 0.8956 (95% CI, 0.8153%, 0.976%). The ideal sensitivity and specificity were 92.5% and 82.5%, respectively, with a cutoff value of 1.571 (Figure 2B). For miR-149-5P, the AUC was 0.54 (95% CI, 0.3988%, 0.6812%), with a cutoff value of 0.9883, yielding ideal sensitivity and specificity of 80% and 52.5%, respectively (Figure 2D).

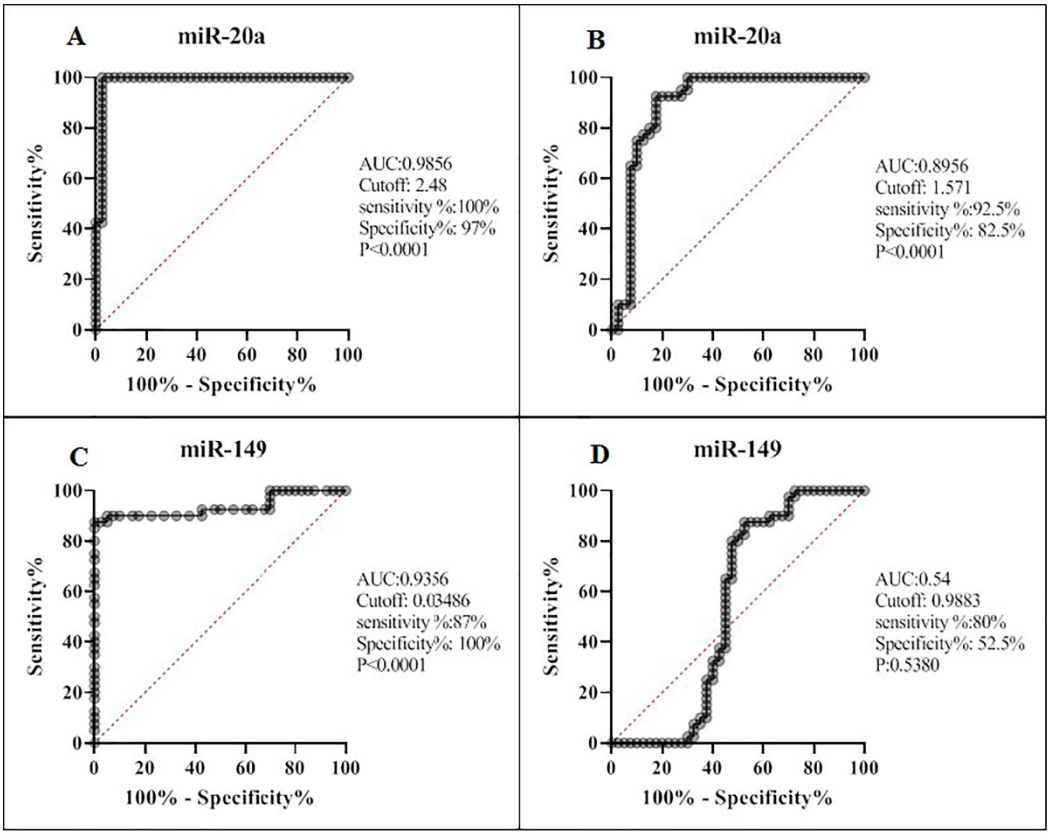

Figure 2. The analysis of ROC curves for each miRNA (miR-20a-5p or miR149) in the T2DM group (A and C) and the pre-diabetic group (B, D) in GraphPad Prism 9, to assess the sensitivity and specificity of each miRNA

P<0.05 indicates that microRNAs can be introduced as biomarkers.

These findings highlight the potential of serum miR-20a-5p as a useful biomarker for identifying T2DM and pre-diabetes. However, it is prudent to explore using this biomarker along with other diagnostic approaches for higher accuracy in screening and diagnosis of pre-diabetes.

Gene expression levels of pro-inflammatory cytokines

As shown in Figure 3A, the expression levels of *IL-6* in the T2DM group were significantly higher than those of the control group (fold change=5.33±0.36 vs 1.36±1.26; P<0.001). Similarly, the T2DM group had significantly greater levels of *IL-1B* expression compared to the control group (fold change=2.96±0.8 vs 1.06±0.35; P<0.001). The levels of expression of *TGF-β* in the T2DM patients were also significantly higher than those of the control group (fold change=4.07±1.17 vs 1.26±0.69; P<0.001). Furthermore, *IFN-γ* expression levels in the T2DM group were considerably greater than in the control group (fold change=3.63±1.14 vs 1.05±0.34; P<0.001).

Cytokine ELISA

According to the data in Figure 3B, the levels of IL-6 protein in the T2DM (230.56±13.45 pg/mL) and pre-diabetic (69.70±7.86 pg/mL) groups were significantly higher than those in the control group (45.03±1.4 pg/mL); P<0.001. The expression levels of IL-1B protein in the T2DM (14.95±1.73 pg/mL) and pre-diabetic (6.04±0.7 pg/mL) groups also significantly increased compared to the control group (5.23±0.33 pg/mL); P<0.0001 and P=0.004, respectively. The expression levels of TGF-B

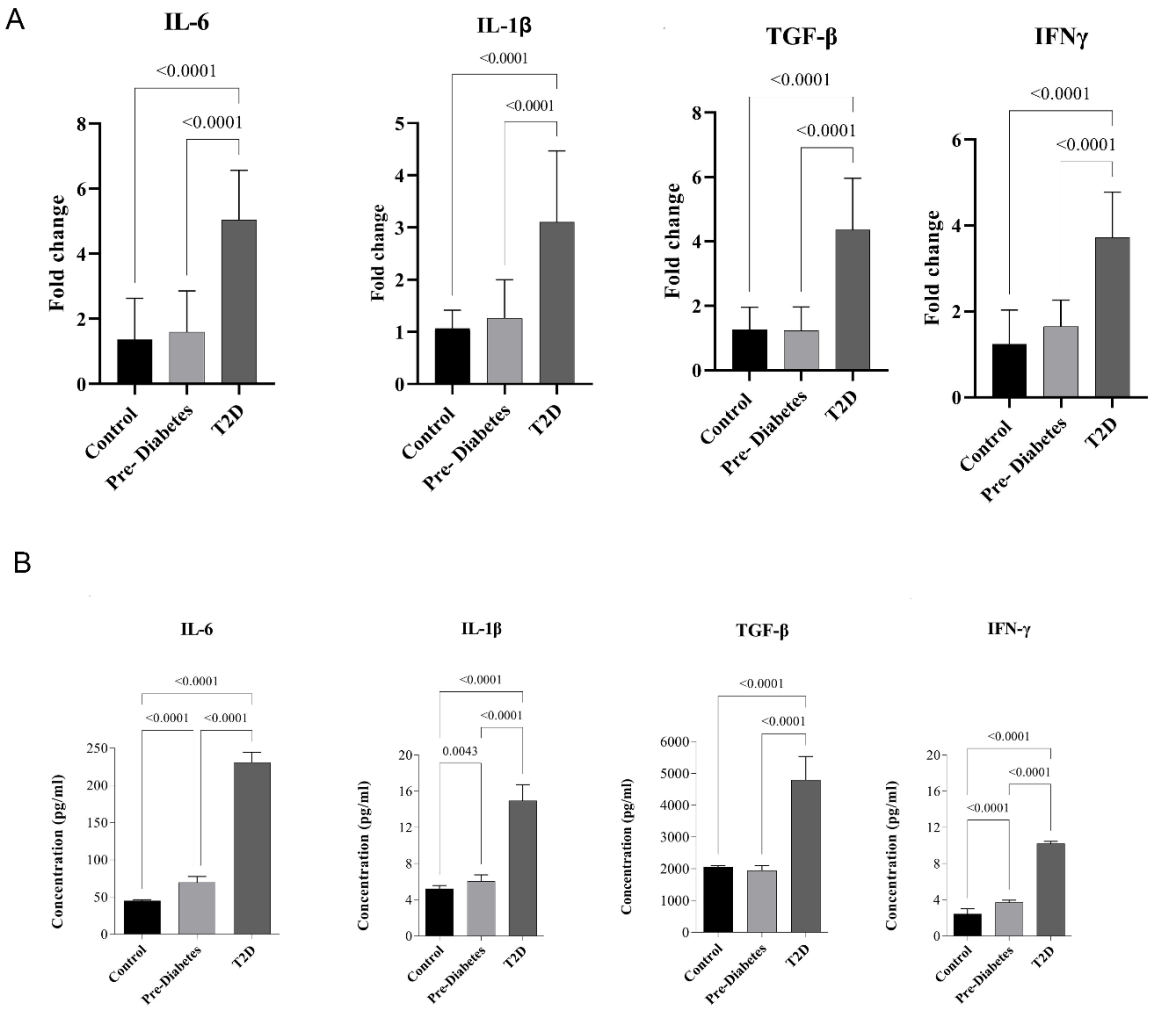

Figure 3. The fold changes in the expression (A) and the concentrations (B) of the circulating pro-inflammatory cytokines in serum samples of three groups.

Note: Data are shown as Mean±SD. Since P<0.05, the differences between groups were significant.

in serum of the T2DM group (4796.94±724.78 pg/mL) were significantly higher than those in the control group (2060.8±41.71 pg/mL); P<0.001. The expression levels of IFN-γ in the T2DM (10.21±0.24 pg/mL) and pre-diabetic (3.71±0.24 pg/mL) groups were significantly higher than in the control group (2.43±0.57 pg/mL); P<0.001. Overall, the expression levels of all selected pro-inflammatory cytokines in individuals with T2DM and prediabetes were significantly higher than in healthy individuals.

Correlation between miRNAs and cytokine levels

To assess the correlation between the miRNAs and cytokines in the T2DM group, fold changes in the expression of miRNA-20a and miRNA-149 were compared to those of the cytokines. As shown in Figure 4A, the expression of miR-20a-5p had a positive relationship with levels of cytokines IL-6 (r=0.646), IL-1B (r=0.802), TGFB (r=0.853), and IFN-γ (r=0.774). However, as indicated in Figure 4B, there was a negative association between the miR-149-5p expression and the expression of cytokines IL-6 (r=-0.614), IL-1β (r=-0.811), TGF-β (r=-0.769), and IFN-γ (r=-0.695). The heat map was used further to demonstrate the relationship between the miRNAs and cytokines. As shown in Figure 4C, there was a complete agreement between the expression of miR-20a-5p and all selected cytokines, while an adverse colour was observed for miR-149-5p, which indicates a notable decrease in its expression compared to miR-20a-5p.

Identification of key target genes

Our findings suggest that miRNA-149 may target the genes *IFN-γ*, *IL-6*, *IL-1α*, and *TGFB2*. In contrast, no miRNA-20a binding sites were found within the inflammation-related genes. Additional significant targets were descried in supplementary Table 1.

Discussion

In the present study, we assessed the differential expression of miRNAs, including miR-20a-5p and miR-149-5p, and their association with inflammatory cytokines in individuals with T2DM, pre-diabetics, and healthy people as controls. The findings showed that the expression of miR-20a-5p was significantly higher in patients with T2DM and pre-diabetics, while the expression of miR-149-5p was significantly lower. Consistent with these findings, some studies have reported that the expression of miR-20a-5p was significantly higher in patients with diabetic kidney disease, T2DM, and type 1 diabetes compared to healthy people [25-28]. The results of this study showed that miR-20a-5p and miR-149-5p

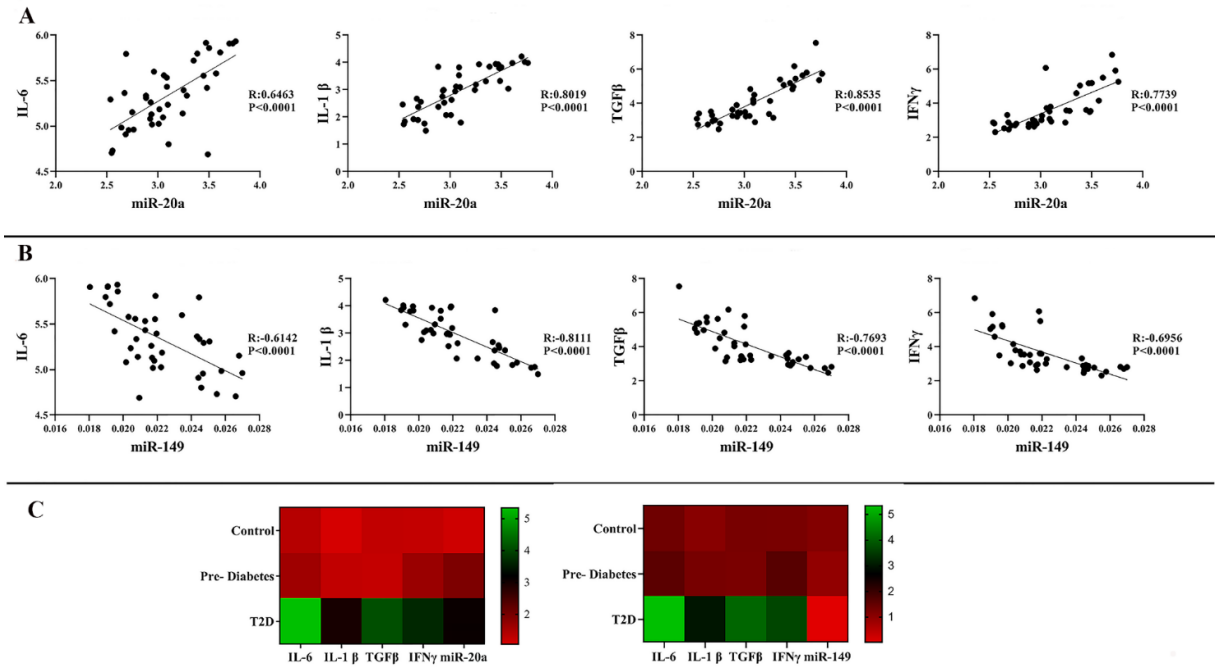

Figure 4. The scatter plot of fold changes in the expression of miR-20a, miR-149, and inflammatory cytokines in the T2DM group (A, B): P<0.05 indicates a significant correlation between miRNA expression and cytokine levels; C) The heat map diagram of fold changes in the expression of miR-20a, miR-149, and inflammatory cytokines in the T2DM group: A red hue indicates decreased gene expression, while a green hue indicates increased gene expression.

could be used as potential molecular biomarkers for the diagnosis of T2DM and pre-diabetes. The ROC analysis was used to examine this capacity. Our findings showed that miR-20a-5p had an AUC of 0.9856, sensitivity of 100%, and specificity of 97% in discriminating T2DM individuals from non-diabetic individuals, whereas miR-149-5p had an AUC of 0.9356, sensitivity of 78%, and specificity of 100%. These results show that miR-20a-5p may be a better candidate for the diagnosis of T2DM than miR-149-5p. Consistent with these data, a prior study reported a remarkable increase in the expression level of miR-20a-5p and miR-20b in patients with T2DM and in individuals with T2DM plus non-alcoholic fatty liver disease [15]. More studies are required to validate this outcome.

Several studies have explored the role of chronic inflammation in the pathogenesis of diabetes and its complications [29, 30]. In this regard, it has been reported that miRNAs play an important role in the development of chronic inflammation, pancreatic dysfunction, insulin resistance, and diabetic complications [31, 32]. According to our results, there was a significant increase in the expression levels of mRNAs encoding cytokines IL-6, IL-1β, TGF-β, and IFN-γ, as well as their protein levels in serum samples of patients with T2DM compared to controls. Furthermore, there was a positive correlation between the expression levels of miR-20a-5p and inflammatory cytokines IL-6, IL-1β, TGF-β, and IFN-γ. On the contrary, there was a negative relationship between the expression of miR-149-5p and the mentioned cytokines. These findings propose that miR-20a-5p and miR-149-5p may play a role in the development of T2DM and pre-diabetes through induction of pro-inflammatory responses. In agreement with these results, some studies have reported that miR-20a-5p modulates the pro-inflammatory cytokines IL-6, IL-1β, and TNF-α through regulating ASK1, a key component of the toll-like receptor 4 (TLR-4) pathway [15, 16]. In contrast to our findings, a study showed that an overexpression of miR-20a-5p could inhibit the T-cell receptor-mediated signaling in CD+ T-cells and production of the inflammatory cytokines [17]. Thus, miR-20a-5p can be a potential target to control the inflammatory responses in autoimmune diseases and diabetes [17]. However, more studies are required to identify the molecular targets of miR-20a-5p and explore its mechanisms of action using in vitro experiments as well as in vivo models.

According to our knowledge, there are no reports on the association between the expression level of miR-149-5p and the induction of chronic inflammation in diabetes. However, in the present study, there was a notable decrease in both mRNA and protein levels of miR-149-5p in people with T2DM compared to controls. Consistent with these results, Palmieri et al reported a reduction in miR-149-5P levels in HUVEC cells as a result of TNF-α down-modulation, and transfection with miR-149-5P mimic successfully blocked the TNF-α-induced expression of IL-6, iNOS, and MMP-9 [33]. It has also been demonstrated that miR-149-5P may regulate inflammation elicited by IL-1β, and that TAK1 and the NF-Kb signaling pathway play crucial roles in this regulation [34].

One limitation of this study was that it only determined the relationship between the expression of miRNAs and inflammatory cytokines, but it could not prove the causality. Furthermore, it could be more informative to follow up on the results. Therefore, more evidence is needed to accurately predict the influence of miR-20a-5p and miR-149-5p on the development and progression of T2DM and prediabetes.

Conclusion

The expression level of miR-20a-5p is higher, and that of miR-149-5p is lower, in patients with T2DM compared with non-diabetic individuals. There is a positive relationship between the expression level of miR-20a-5p and inflammatory cytokines, while they have a negative association with miR-149-5p. More studies are recommended to investigate the relationship between miR-20a-5p and miR-149-5p expression and the development and progression of T2DM and pre-diabetes. Furthermore, more work is needed to clarify the mechanisms by which the inflammatory biomarkers are regulated by miRNAs such as miR-20a-5p and miR-149-5p. These data can be used to develop more effective treatments for T2DM and pre-diabetes.

Ethical Considerations

Compliance with ethical guidelines

Written informed consent was obtained from all participants, and the study was conducted in accordance with the institutional ethics committee on human research, which complied with the Declaration of Helsinki.

Funding

This study was funded by Aja University of Medical Sciences, Tehran, Iran.

Authors' contributions

Conceptualization and study design: Erfan Babahoseinpour and Mehdi Shakouri Khomartash; Experiments: Mahmoud Vahidi and Reza Heidari; Data interpretation: Mehdi Shakouri Khomartash, Mahmoud Vahidi and Reza Heidari; Writing: Erfan Babahoseinpour.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors sincerely thank AJA University of Medical Sciences for its scientific support. We also extend our appreciation to Geniran Lab for providing laboratory facilities and an excellent research environment that contributed to the successful completion of this study.

References

[1] Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993; 75(5):843-54. [DOI:10.1016/0092-8674(93)90529-Y] [PMID] [PMCID]

[2] Seo J, Jin D, Choi CH, Lee H. Integration of microRNA, mRNA, and protein expression data for the identification of cancer-related microRNAs. PLoS One. 2017; 12(1):e0168412. [DOI:10.1371/journal.pone.0168412] [PMID] [PMCID]

[3] Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. Nature Reviews. Molecular Cell Biology. 2009; 10(2):126-39. [DOI:10.1038/nrm2632] [PMID]

[4] Heidari R, Akbariqomi M, Asgari Y, Ebrahimi D, Alinejad-Rokny H. A systematic review of long non-coding RNAs with a potential role in Breast Cancer. Mutation Research. Reviews in Mutation Research. 2021; 787:108375. [DOI:10.1016/j.mrrev.2021.108375] [PMID]

[5] Pillai RS, Bhattacharyya SN, Filipowicz W. Repression of protein synthesis by miRNAs: How many mechanisms? Trends in Cell Biology. 2007; 17(3):118-26. [DOI:10.1016/j.tcb.2006.12.007] [PMID]

[6] Ebert MS, Sharp PA. Roles for microRNAs in conferring robustness to biological processes. Cell. 2012; 149(3):515-24. [DOI:10.1016/j.cell.2012.04.005] [PMID] [PMCID]

[7] Mendell JT, Olson EN. MicroRNAs in stress signaling and human disease. Cell. 2012; 148(6):1172-87. [DOI:10.1016/j.cell.2012.02.005] [PMID] [PMCID]

[8] Fernandez-Valverde SL, Taft RJ, Mattick JS. MicroRNAs in β-cell biology, insulin resistance, diabetes and its complications. Diabetes. 2011; 60(7):1825-31. [DOI:10.2337/db11-0171] [PMID] [PMCID]

[9] Karami M, Mousavi SH, Rafiee M, Heidari R, Shahrokhi SZ. Biochemical and molecular biomarkers: Unraveling their role in gestational diabetes mellitus. Diabetology & Metabolic Syndrome. 2023; 15(1):5. [DOI:10.1186/s13098-023-00980-8] [PMID] [PMCID]

[10] Akash MS, Rehman K, Chen S. Role of inflammatory mechanisms in pathogenesis of type 2 diabetes mellitus. Journal of Cellular Biochemistry. 2013; 114(3):525-31. [DOI:10.1002/jcb.24402] [PMID] [PMCID]

[11] Lontchi-Yimagou E, Sobngwi E, Matsha TE, Kengne AP. Diabetes mellitus and inflammation. Current Diabetes Reports. 2013; 13(3):435-44. [DOI:10.1007/s11892-013-0375-y] [PMID]

[12] Soares NS, Pereira F, Reis F. The role of inflammation in diabetic cardiomyopathy. International Journal of Interferon, Cytokine and Mediator Research. 2012; 59-73. [DOI:10.2147/IJICMR.S21679]

[13] Mavridis G, Souliou E, Diza E, Symeonidis G, Pastore F, Vassiliou AM, et al. Inflammatory cytokines in insulin-treated patients with type 2 diabetes. Nutrition, Metabolism, and Cardiovascular Diseases. 2008; 18(7):471-6. [DOI:10.1016/j.numecd.2007.02.013] [PMID]

[14] Reinehr T, Roth CL. Inflammation markers in type 2 diabetes and the metabolic syndrome in the pediatric population. Current Diabetes Report. 2018; 18(131):1-12. [DOI:10.1007/s11892-018-1110-5]

[15] Ye D, Zhang T, Lou G, Xu W, Dong F, Chen G, et al. Plasma miR-17, miR-20a, miR-20b and miR-122 as potential biomarkers for diagnosis of NAFLD in type 2 diabetes mellitus patients. Life Sciences. 2018; 208:201-7. [DOI:10.1016/j.lfs.2018.07.029] [PMID]

[16] Philippe L, Alsaleh G, Pichot A, Ostermann E, Zuber G, Frisch B, et al. MiR-20a regulates ASK1 expression and TLR4-dependent cytokine release in rheumatoid fibroblast-like synoviocytes. Annals of The Rheumatic Diseases. 2013; 72(6):1071-9. [DOI:10.1136/annrheumdis-2012-201654] [PMID]

[17] Reddycherla AV, Meinert I, Reinhold A, Reinhold D, Schraven B, Simeoni L. miR-20a inhibits TCR-mediated signaling and cytokine production in human naïve CD4+ T cells. PLoS One. 2015; 10(4):e0125311. [DOI:10.1371/journal.pone.0125311] [PMID] [PMCID]

[18] Zeinali F, Aghaei Zarch SM, Jahan-Mihan A, Kalantar SM, Vahidi Mehrjardi MY, Fallahzadeh H, et al. Circulating microRNA-122, microRNA-126-3p and microRNA-146a are associated with inflammation in patients with pre-diabetes and type 2 diabetes mellitus: A case control study. Plos One. 2021; 16(6):e0251697. [DOI:10.1371/journal.pone.0251697] [PMID] [PMCID]

[19] Elabscience. Human INS (Insulin) ELISA Kit (E-EL-H2665). Houston: Elabscience; 2026. [Link]

[20] Thermo Fisher. TRIzol™ Reagenz. Winchester: Thermo Fisher; 2026. [Link]

[21] SMOBIO. ExcelRT™ Reverse Transcription Kit (RP1300). Hsinchu: SMOBIO; 2026.

[22] Mabtech. ELISA Flex: Human IFN-γ (HRP) 3420-1H-6 /3420-1H-20. Lund: Mabtech; 2021 [Link]

[23] Pajunen P, Peltonen M, Eriksson JG, Ilanne-Parikka P, Aunola S, Keinänen-Kiukaanniemi S, et al. HbA(1c) in diagnosing and predicting type 2 diabetes in impaired glucose tolerance: The Finnish diabetes prevention study. Diabetic Medicine. 2011; 28(1):36-42. [DOI:10.1111/j.1464-5491.2010.03183.x] [PMID]

[24] Kerr D, Partridge H, Knott J, Thomas PW. HbA1c 3 months after diagnosis predicts premature mortality in patients with new onset type 2 diabetes. Diabetic Medicine. 2011; 28(12):1520-4. [DOI:10.1111/j.1464-5491.2011.03443.x] [PMID]

[25] Filipów S, Łaczmański Ł. Blood circulating miRNAs as cancer biomarkers for diagnosis and surgical treatment response. Frontiers in Genetics. 2019; 10:169. [DOI:10.3389/fgene.2019.00169] [PMCID]

[26] Azhir Z, Dehghanian F, Hojati Z. Increased expression of microRNAs, miR-20a and miR-326 in PBMCs of patients with type 1 diabetes. Molecular Biology Reports. 2018; 45(6):1973-80. [DOI:10.1007/s11033-018-4352-z] [PMID]

[27] Yang ZM, Chen LH, Hong M, Chen YY, Yang XR, Tang SM, et al. Serum microRNA profiling and bioinformatics analysis of patients with type 2 diabetes mellitus in a Chinese population. Molecular Medicine Reports. 2017; 15(4):2143-53. [DOI:10.3892/mmr.2017.6239] [PMID] [PMCID]

[28] Khan SH. Blood miRNA signature in Type-2 Diabetes Mellitus? Possible futuristic molecular biomarkers. The Professional Medical Journal. 2023; 30(01):1-9. [DOI:10.29309/TPMJ/2023.30.01.6984]

[29] Virella G, Lopes-Virella MF. The role of the immune system in the pathogenesis of diabetic complications. Frontiers in Endocrinology. 2014; 5:126. [DOI:10.3389/978-2-88919-352-3]

[30] Nguyen DV, Shaw LC, Grant MB. Inflammation in the pathogenesis of microvascular complications in diabetes. Frontiers in Endocrinology. 2012; 3:170. [DOI:10.3389/fendo.2012.00170]

[31] McClelland AD, Kantharidis PJCs. microRNA in the development of diabetic complications. Clinical science (London, England : 1979). 2014; 126(2):95-110. [DOI:10.1042/CS20130079] [PMID]

[32] Karolina DS, Armugam A, Sepramaniam S, Jeyaseelan K. miRNAs and diabetes mellitus. Expert review of Endocrinology & Metabolism. 2012; 7(3):281-300. [DOI:10.1586/eem.12.21] [PMID]

[33] Palmieri D, Capponi S, Geroldi A, Mura M, Mandich P, Palombo D. TNFα induces the expression of genes associated with endothelial dysfunction through p38MAPK-mediated down-regulation of miR-149. Biochemical and Biophysical Research Communications. 2014; 443(1):246-51. [DOI:10.1016/j.bbrc.2013.11.092] [PMID]

[34] Chen Q, Wu S, Wu Y, Chen L, Pang Q. MiR-149 suppresses the inflammatory response of chondrocytes in osteoarthritis by down-regulating the activation of TAK1/NF-κB. Biomedicine & Pharmacotherapy. 2018; 101:763-8. [DOI:10.1016/j.biopha.2018.02.133]

[35] Choudhry A, Guddaiah R, Prabhu KJJoP, Sciences B. HOMA IR/C peptide ratio-A better tool for predicting prognosis of type II diabetes mellitus. Journal of Pharmaceutical and Biomedical Sciences 2015; 5(8):650-4. [Link]