

# Serum microRNA-122 as a potential biomarker for early detection and monitoring of type 2 diabetes mellitus: a cross-sectional study

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## Abstract

**Introduction:** MicroRNAs (miRNAs) are small noncoding RNAs with transcriptional repressive properties. Type 2 diabetes mellitus (T2DM) is closely associated with endothelial dysfunction and altered molecular signaling. Although microRNA-122 (miR-122) is highly abundant in the liver and contributes to lipid homeostasis, its significance in predicting long-term metabolic disease risk remains insufficiently understood.

**Materials and methods:** Circulating miR-122 levels were quantified in 85 patients with T2DM, stratified into: group 1: manifest T2DM (n=50), and group 2: T2DM diagnosed according to WHO criteria (n=35). Results were compared with 47 healthy controls. To assess the long-term predictive value of miR-122, findings were further compared with data from the prospective Bruneck study (n=810, baseline 1995). Multivariable Cox regression models were used to evaluate the association between log-transformed miR-122 levels and incident T2DM over a follow-up period of up to 15 years.

**Results:** Circulating miR-122 was significantly associated with T2DM status, with patient groups demonstrating altered expression patterns suggestive of its potential involvement in metabolic dysregulation. Notably, reduced miR-122 levels in patient groups emerged as a possible indicator of T2DM. In the Bruneck cohort, each 1-standard deviation (SD) increase in log(miR-122) was associated with a 37% higher risk of developing T2DM (HR=1.37, 95% CI: 1.03–1.82,  $p=0.021$ ) during the 15-year follow-up.

**Conclusion:** Decreased miR-122 levels may characterize individuals with existing T2DM, elevated long-term levels were predictive of future diabetes onset in a population-based cohort. These results underscore the utility of miR-122 as a promising biomarker for early identification of individuals at increased risk for T2DM.

## Keywords

diabetes mellitus, insulin resistance, microRNA-122

## Introduction

MicroRNAs (miRNAs) are a class of small noncoding RNAs that have transcriptional repressive properties.<sup>[1,2]</sup> They can control the transcriptional suppression or destruction of their target mRNAs by attaching via standard pair to a complement site in 3 untranslated regions of this

transcript. Angiogenesis, oncogenesis, stress responses and development all show important roles for miRNAs.<sup>[3]</sup>

Additionally, there is growing evidence that miRNAs play a major role in the cardiovascular system. For example, miRNAs regulate endothelial cell function, inflammatory response and angiogenic potential.<sup>[4,5]</sup> These miRNAs are encapsulated in microvesicle that protects them from

endogenous RNase action, despite the fact that they are not cell related. Interestingly, different expression profiles of plasma miRNAs can be observed: specific tumor miRNAs have been identified in cancer patients,<sup>[6,7]</sup> while tissue miRNAs function as a signal of harm. Heart failure, coronary artery disease and myocardial damage in cardiovascular diseases have all been explored in relation to circulating miRNAs.<sup>[8]</sup> One of the risk factors for heart diseases was type 2 diabetes mellitus, which results in micro- and macrovascular repercussions and endothelial dysfunctions.<sup>[9]</sup>

However, the investigations of serum miRNAs in DM are not detected yet. This work is the first to reveal a plasma miRNA signature for DM in a large population based sample. Our findings may provide new insights into the biology of diabetes and how its vascular effects manifest.

The liver's predominant miRNA, miR-122, is thought to play a key role in regulating the metabolism of fat and carbohydrate.<sup>[10]</sup> In non-human primates<sup>[11]</sup> and mice<sup>[12]</sup>, blocking miR-122 results in fatty acid oxidation, which reduces lipid synthesis and, ultimately, total cholesterol.

It is hypothesized that miR-122 may detrimentally have effects on metabolism and be connected to metabolic disease in humans. Data from recent epidemiological research is scarce and not very good. Although a lipid subtype split would help clarify and better understand how miR-122 regulates lipid homeostasis, published research has focused on relationships with primary lipids.<sup>[13,14]</sup> Importantly, previous studies used case-control or cross-sectional approaches.<sup>[15]</sup>

## Aim

We were unable to provide guidance on the long-term associations between circulating miR-122 and the evolution of new-onset illness outcomes over time. To bridge this gap in the current work, we conducted several experiments and investigations and published miR-122 data. Estimating the as-yet-unknown connections between circulating miR-122 and the long-term risk of type-2 diabetes (T2DM) was one of our goals.

## Materials and methods

### Study subjects

The Bruneck project, which began as a prospective population-based survey with the objective of examining the pathophysiology and epidemiology of atherosclerosis, has since expanded to encompass major human illnesses, such as diabetes.<sup>[16-18]</sup> At the baseline evaluations in 1990, the study population included a sex- and age-stratified random sample of individuals from Bruneck (Bolzano Province, Italy) aged 40–79 years. The first five-year period, from 1990 to the re-evaluation in 1995, saw the deaths or relocation

of 63 members of the subgroup. The results showed that the remaining population follow-up was 96.5% (n=822). RNA extractions were performed on serum samples collected from 822 participants as part of the 1995 follow-up. From 2000 to 2005, the follow-up was 100% complete for clinical goals and 91% complete for repeated laboratory testing. The Online Data Supplement contains a detailed description of how to evaluate the ankle brachial index. The Bruneck population, like that of other Western countries, reflects society as a whole in many ways. The average age of the participants was 62.9 years, with 49.9% being female and 9.7% having diabetes. Before the experiment began, each study participant provided written informed consent.

### Identifications of DM

Diabetes was defined by the World Health Organization as having glucose levels of 7 mmol/L (126 mg/dL) at fasting, 11.1 mmol/L (200 mg/dL) during the 2-hour oral glucose tolerance test, or being clinically diagnosed with the condition. The self-reported status of DM was unquestionably validated by reviewing general practitioners' medical records and Bruneck Hospital files.

### miRNA expressions of profile

RNA was extracted from serum specimens collected during the 1995 follow-up of 822 participants using the miRNA basic kit (Qiagen). TaqMan miRNA Arrays A and B were used to assess expression levels after miRNAs were reverse-transcribed with Megaplex primer pools (Human Pools A v. 2.1 and B v. 2.0). Individual miRNAs' expression was determined using TaqMan miRNA assays.

### Strategy of sampling and statistical analysis

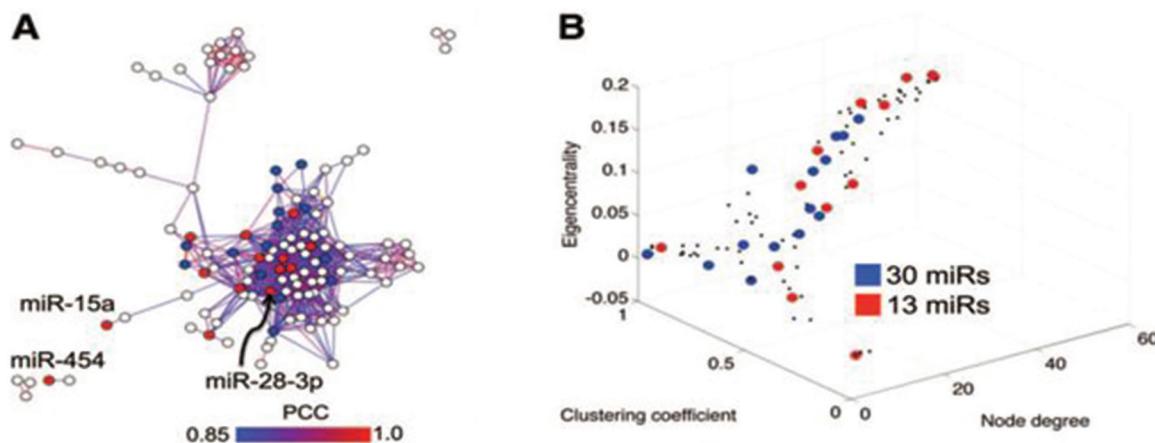
In the initial microarray screening, quantitative (q) PCR tests were performed on thirteen miRNAs with a link to DM. TaqMan assays were performed twice. Samples were collected from private clinics in Basrah, Iraq, between October 15 and November 15, 2024. Group 1 included 50 people with manifest diabetes from the Bruneck cohort, while group 2 included 35 people who developed diabetes between 2014 and 2024 (incident DM). Controls were 47 people of similar age and sex who had no history of diabetes and fasting glucose levels of 6.1 mmol/L (110 mg/dL) and 7.7 mmol/L (140 mg/dL), respectively. Finally, we analyzed the levels of miR-122 in 132 individuals. All qPCR findings were standardized to both miR-454 and RNU6b and analyzed as uncorrected Ct values because there were no widely accepted standards. The RNA of short nuclear samples had to meet the following criteria: first, it had to be detectable in all samples; second, it had to have a moderate range of expression levels; and third, it could not be linked to the presence of diabetes. Furthermore, miR-454's expression profile was found to be uncorrelated with the

rest of the microRNAs; the profile was located outside the coexpression module of the complex networks of microRNAs in serum. (Fig. 1).

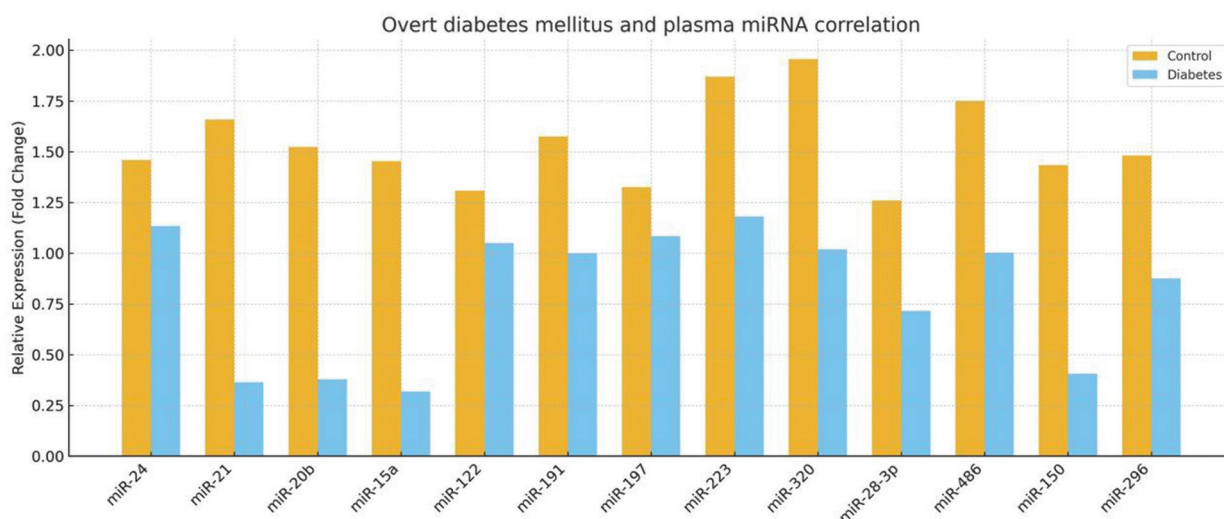
Data were analyzed using STATA version 10 and SPSS version 26.0. Continuous variables are displayed as dichotomous data, with the median represented as a percentage and numbers. The median fold change is shown in Fig. 2.

In order to determine precise probability values, this study used the nonparametric Wilcoxon test for related samples to compare the miRNA levels of people with incident

or prevalent diabetes to similar groups of matched controls. Logistic regression analyses were also performed for data, including loge-transformed expression levels of miRNAs (1 per model), C-reactive protein, body mass indexes, social status, waist-to-hip ratio, physical activity, smoking status, and high sensitivity to account for the potentially confusing lifestyle effect feature and other parameters associated with DM. Hosmer and Lemeshow show the detailed process of creating models.<sup>[19]</sup> The inclusion of suitable interaction terms allowed for the calculation of first-order interaction



**Figure 1.** Coexpression networks and miRNA topological values. Network of undirected and weighted miRNA coexpression. PCC indicates that nodes and edges (links) have similarity in miRNA expression. Strong similarity is shown by a gradient in the red-blue edges hue. At PCC values of 0.87, there were 1020 coexpression links and 120 miRNAs in the coexpression network. The clustering coefficients and the relationship with the node degrees are presented for 120 miRNAs. Thirteen of the thirty miRNAs that were expressed differently (blue) occupied locations that were essential for the overall upkeep of the network.



**Figure 2.** Correlation between overt diabetes mellitus and plasma miRNA. Thirteen serum mi-RNAs quantified using qPCR in matched controls and in patients with diabetes mellitus. Each graph's central bars show the relative difference in fold between the plasma levels of mi-RNAs in diabetes patients and control subjects. The fold alterations between the plasma of hyperglycemic patients and the control group are contrasted in the bars on the left. Using multivariable logistic regression analysis of matched data, odds ratios (95% CIs) are displayed in the lines and squares on the right. The nonparametric Mann-Whitney test for unrelated sample and Wilcoxon test for related sample were used to determine probability values.

between miRNAs and the previously listed factors, as well as sex and age. None of these theories were statistically significant. Using a linear model, the variations in miR-122 between glucose tolerance groups were compared. All of the above probability values were two-sided.

### Inference and analysis of MiRNA coexpression network

We have used network inference techniques to evaluate the general expression characteristics of miRNAs in DM. Either the context likelihood of relatedness or the Pearson correlation coefficient was used to examine the similarity in miRNA expression profiles among all possible miRNA pairs.<sup>[20]</sup> Nodes in undirected weighted networks represent miRNAs, whereas connections (edges) show how comparable pairs remaining dependent above a specified threshold are. Since the context likelihood of relatedness depends on a reciprocal information metric and doesn't assume linearity, it offers some flexibility in identifying biological correlations that could otherwise go unnoticed, whereas Pearson correlation coefficients evaluate linear links between features (miRNAs).<sup>[21,22]</sup> Pearson correlation coefficients were used to find clusters of similarly expressed miRNAs, whereas context likelihood of relatedness found all nonrandomly associated qPCR-validated miRNA profiles. Context likelihood of relatedness was selected for miRNAs that were validated by qPCR since it is more adaptable to nonlinear dynamics of miRNA expressions than Pearson correlation coefficients and performs noticeably better than other network inference techniques in detecting physiologically significant associations, despite the fact that the two methods can occasionally produce results that are similar.<sup>[23,24]</sup> A scale-free design, which is a feature of most real-world networks, including biological ones, was first shown by the miRNA coexpression network after the Pearson correlation coefficient threshold was chosen.<sup>[25]</sup> To guarantee repeatability, thirty differentially expressed miRNAs were assessed according to their network characteristics rather than the degree of over- or underexpression.<sup>[26]</sup> The context likelihood of relatedness thresholds were set to allow for the representation of all thirteen miRNAs in the network while minimizing the number of connections between them.

### Investigating topology

During prescreening, the architecture of the global miRNA coexpression network was considered, as well as the presence or absence of overexpression or underexpression of each miRNA. Topological parameters like clustering coefficient, node degree, and eigenvector centrality were carefully computed for every miRNA. An individual miRNA's node degrees are determined by the total number of edges that are related to it. The cluster coefficients show the extent to which miRNAs are likely to form groups. Strong relationships with other miRNAs that are also important in the

network increase a miRNA's eigenvector centrality, which is a measure of miRNA significance.

### Cell culture: endothelial cell cultures

After being purchased from Cambrex, the human umbilical vein endothelial cell (HUVEC) was cultured on gelatin-coated flasks in M199 media supplemented with 1 ng/mL endothelial cell growth factor (Sigma), 3 g/mL endothelial growth supplement from bovine neural tissue (Sigma), 10 U/mL heparin, 1.25 g/mL thymidine, 5% FBS, and 100 g/mL penicillin and streptomycin, as previously documented.<sup>[27,28]</sup> For six days, HUVECs with a high glucose concentration (25 mmol/L) were cultivated in complete medium. To counteract the effects of osmotic stress, mannitol was added to the full medium (5 mmol/L glucose) in which HUVECs were grown. After counting the cells on day five, a matching number were seeded onto T75 flasks, which were subsequently incubated for a further day.

### Separating vesicle

Vesicles were cut apart as before.<sup>[29]</sup> Before the conditioned media was gathered, HUVECs were briefly lysed in QIAzol reagent following a 24-hour period during which they were denied serum and growth factors. To examine the expression of miRNA, cellular lysate was stored at  $-20^{\circ}\text{C}$ . First, the conditioned media was precleared for 10 minutes at 800 g to get rid of floating cells. After 20 minutes of centrifugation at 10,600 rpm, endothelium particles—also referred to as optative bodies—were able to be separated. Small microparticles (less than 1  $\mu\text{m}$  in size) that were shed from endothelial cells were then isolated using a second centrifugation stage that was conducted for two hours at 20,000 and 500 rpm. The same centrifugation methods were used to create vesicles from serum. After being revived in PBS, the separated vesicle was kept at  $-80^{\circ}\text{C}$ . The miRNeasy kit was used to extract total RNA, as previously mentioned. A NanoDrop spectrophotometer was used to measure the amount of RNA.

## Results

Thorough miRNA profiling applied to Applied Biosystems' Card A v. 2.1 and Card B v. 2.0 human TaqMan miRNA arrays, two individuals with diabetes mellitus, and six appropriate controls were used for the initial screening. All subsequent research focused on this data set and discovered 13 differently expressed plasma miRNAs in diabetics out of the 132 miRNAs with Ct values that were detected by using the fluidic Card A.

### Analysis of miRNA networks

Correlation value (PCC>0.91) in this level, the networks were dominated by a few hubs connected to a large num-

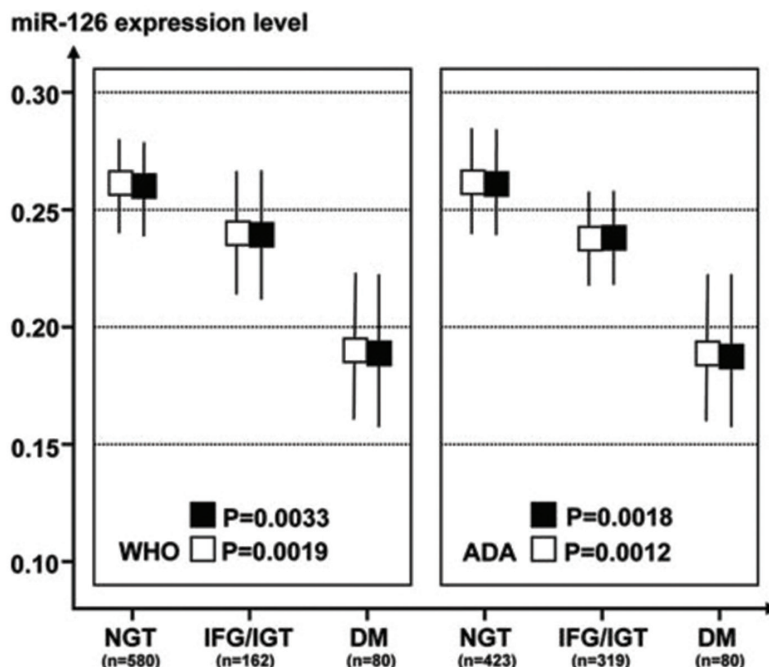
ber of loosely connected nodes, as is typical in biological networks. The miRNA network consisted of 1020 coexpression connections edges and 120 miRNAs nodes.

Marker selection was employed to choose the 13 differently expressed miRNAs since it is more repeatable to identify their location in the miRNA coexpression network than to identify individual over or under expressions.<sup>[26]</sup> Thirteen networks of differential miRNAs were topologically central. Out of the many miRNAs with different expression levels, 13 were chosen because they showed a broad range of node degrees, clustering coefficients and eigenvector centrality values. miR-454, which was located outside of each network module, was the only miRNA that was demonstrated to be unconnected to the expression of other miRNAs. This is how it was selected as an extra normalizing control.

### qPCR validations

qPCR helped to improve the quantification of the 13 topographically distinct miRNAs. Every patient with evident diabetes had their age and sex matched. In diabetics, plasma level of miR-21, miR-24, miR-20b, miR-122, miR-191, miR-15a, miR-197, miR-320, miR-223, miR-486, miR-29b, and miR-150 were all lower; however, miR-28-3p was frequently greater (Fig. 2). Results for expressions level standardized to either RNU6b or miR-454 and non-standardized miRNA levels were in agreement (Fig. 2).

Four miRNAs, including endothelium miR-122, remained significant after controlling for the multiple comparison that were performed (probability value 0.000140). There were significant differences in nine miRNAs standardized to RNU6b between DM patients and controls. However, because individual miRNAs in this situation are highly related rather than independent of one another, the Bonferroni correction is overly conservative. Multivariate analysis revealed that all miRNAs, with the exception of miR-29b, were significantly associated with manifest DM. There was a positive inverse relationship between eleven and miR-28-3p. In both diabetes patients with and without treatment, the results for miRNAs standardized to miR-454 are shown in Fig. 3. In a subsequent run, miR-122 was quantified and compared to miR-454. Once again, miR-122 was a strong predictor of manifest DM, according to logistic regression studies (odd ratios [93% CI] for a 1 SD unit reduction of log-transformed expressions levels of miR-122: 1.98 [1.41–2.78]). Furthermore, plasma levels of miR-122 showed a progressive decline in impaired fasting glucose/impaired glucose tolerance (n=35), normal glucose tolerance (n=47) and manifest diabetes (n=50) across categories. The levels of miRNAs and fasting glucose levels were inversely correlated in both diabetic patients and control subjects ( $r=-0.175$  to  $-0.369$ ). Plasma samples from hyperglycemic patients aged 8–12 weeks (Fig. 3) were able to independently reproduce the majority of the miRNA changes seen in DM.



**Figure 3.** miR-122 plasma levels in groups with diabetes. Normal glucose tolerance (NGT) and impaired fasting glucose/impaired glucose tolerance (IFG/IGT) are both possible. White squares are values that have been corrected for age and sex; black squares are values that have been adjusted for high-sensitivity C-reactive protein, body mass index, waist-to-hip ratio, smoking status, age, sex, social status, and family history of diabetes mellitus. All 132 members of the research population were used in this analysis. IFG/IGT, DM, and NGT category differences in miR-122 were assessed using trend probability values and general linear models.

## Incident DM

Importantly, prior to the onset of DM, certain miRNAs were already altered. Over the course of the ten year follow-up period, 50 people in total developed diabetes (mean interval to diagnosis of DM from 2014 to 2024). Values of miR-29b, miR-15a, miR-223 and miR-122 were significantly lower in patients group individuals, but miR-28-3p was higher in matched control group (Fig. 4).

## miRNAs as DM biomarker

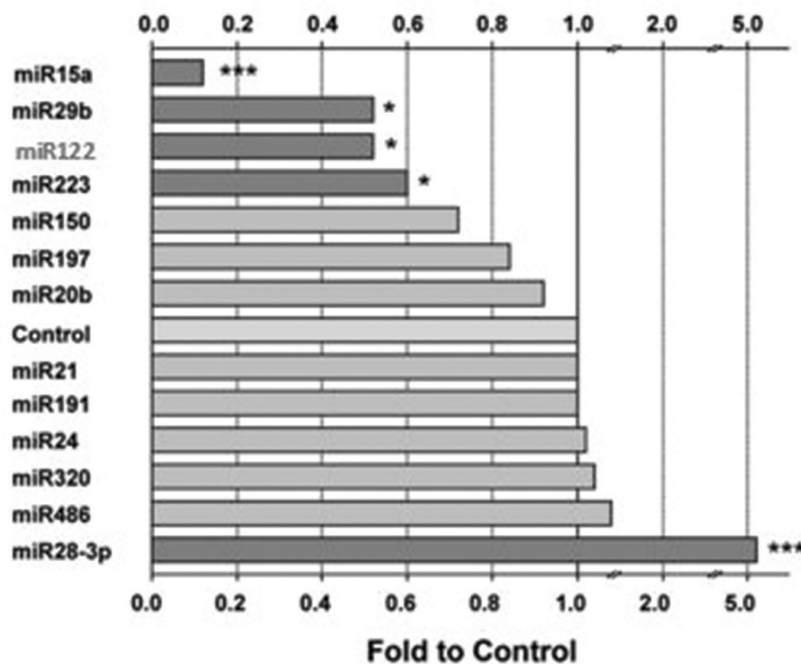
To find out if miRNAs can accurately differentiate between people with incident or prevalent DM and healthy controls, we broke down miRNAs into principle components (PC). The expression patterns of the five most significant miRNAs (miR-122, miR-15a, miR-28-3p, miR-223, and miR-320) allowed for the accurate diagnosis of 60/85 (73%) DM patients and 40/47 (94%) controls (Fig. 5A,B,C). In comparison to a sample of patients with well-controlled diabetes, the 35 DM cases classified as normal subjects had significantly lower fasting glucose (mean  $\pm$  SD, 130.0 $\pm$ 25.9 mg/dL against 150.7 $\pm$  50.0 mg/dL,  $p=0.0047$ ) and HbA1c (mean  $\pm$  SD, 5.94 $\pm$ 0.81% versus 6.54 $\pm$ 1.86%,  $p=0.014$ ) values. The performance of the classifier was not enhanced by the inclusion of more miRNAs (Fig. 5B). Therefore, it may be concluded that there is minimal need for miRNA signature-based classification for these five miRNAs. The miRNA relevance network's inference further supported the potential use of miRNAs as diabetes diagnostic tools.

## miRNA-122 in DM

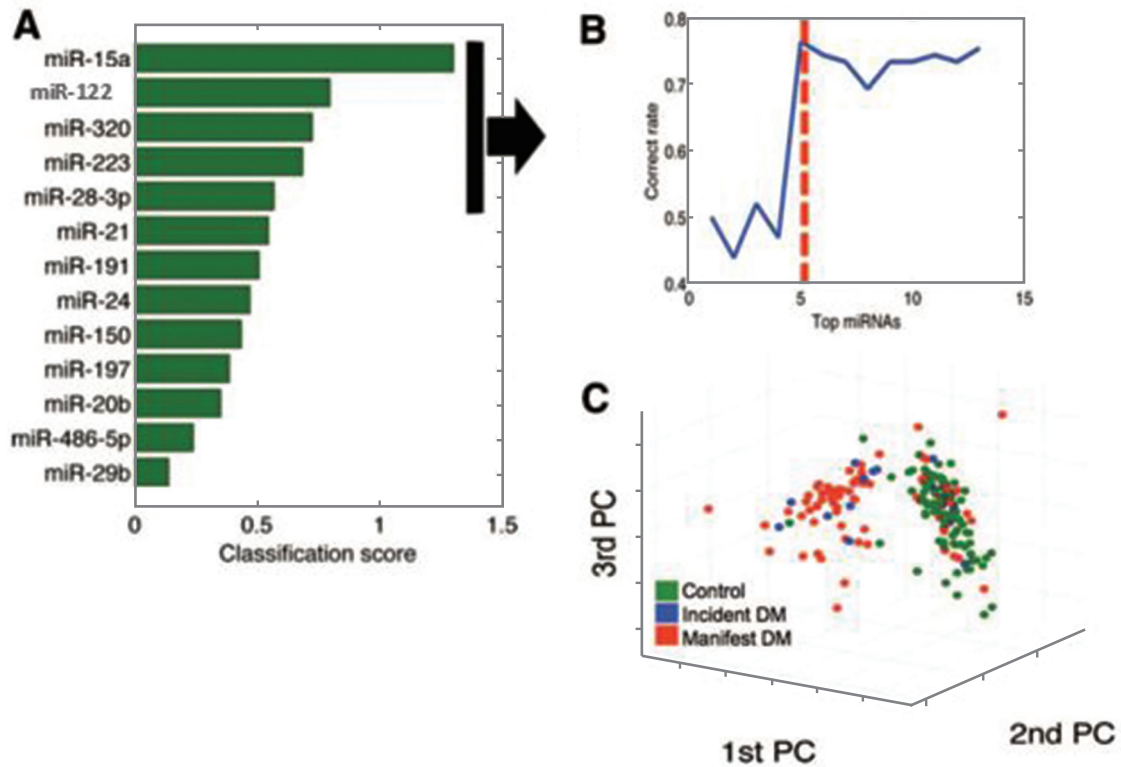
The miRNA most commonly linked to diabetes mellitus is miR-122. Angiogenesis, wound healing, and the maintenance of vascular integrity are all regulated by this miRNA. It has previously been demonstrated that endothelial cells and endothelial apoptotic bodies contain a significant concentration of miR-122.<sup>[30]</sup> In order to ascertain if hyperglycemia influences this process, the miRNA level of microparticle produced under standard and shed endothelium particle (5 mmol/L) along with elevated (25 mmol/L) glucose concentration was examined using miR-122 release from endothelial cells. Severe hyperglycemia had no effect on cellular miRNA concentrations, but it dramatically decreased the level of miR-122 in endothelial dead cells (Fig. 6A). Other than miR-24, the shedding of other miRNAs remained unchanged. The lower levels of miR-122 in diabetics limited to the plasma particulate fraction (Fig. 6B) are in line with these in vitro studies. Lastly, data from our study sample indicates that a decrease in miR-122 in plasma is linked to both preclinical and overt DM illness.

## Discussions

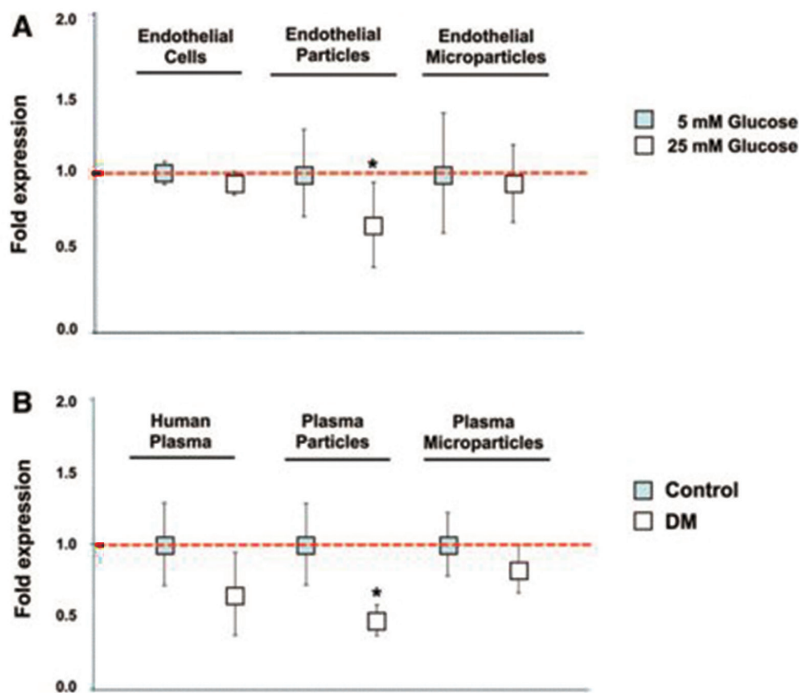
In this study, we present preliminary data supporting a plasma miRNA profile in diabetic patients, which may have predictive value. Our findings warrant additional research into the role of miRNAs in diabetes-related issues.



**Figure 4.** An association between incident DM and plasma miRNAs. Thirteen qPCR measurements of plasma miRNAs were made in patients who developed diabetes throughout a ten-year monitoring period and matched controls. The probability values are  $p^* < 0.05$ ,  $p^{***} < 0.001$ .



**Figure 5.** PCA, categorization, and network characteristics. Thirteen miRNAs were effectively categorized among individuals with incident diabetes (n=50), manifest diabetes (n=35), and control subjects (n=47). (A) A stronger ability to classify is shown by higher scores, which also reflect a higher degree of differential expression; (B) classification accuracy. The top 5 variably expressed miRNAs were used to get the highest classification accuracy; (C) determines if control patients can differentiate from cohort with manifest and incident DM. Using PCA decomposition of the top 5 mi-RNAs, 60/85 (73%) patients with manifest DM and 40/47 (94%) controls could be classified together.



**Figure 6.** Increasing glucose level impact on miR-122 content of vesicle. miR-122 contents of endothelial derived particle (A) circulating vesicles in plasma (B) decreasing by high glucose levels. QPCR aided in the assessment of miRNA expression. miR-454 was the standard control. The data, which come from four distinct investigations, mean  $\pm$  SD. \*  $p < 0.05$ .

Through differential expression analysis and network topology principles, we identified 13 plasma miRNAs, including miR-122 deletion, in diabetes mellitus. The results were corroborated by hyperglycemic patients and multi-variable analyses of individuals with diabetes mellitus and age- and sex-matched controls. Before diabetes mellitus developed, some plasma miRNAs were dysregulated.

Among the 13 miRNAs studied using principal component analysis (PCA), five—miR-122, miR-15a, miR-28-3p, miR-223, and miR-320—have the highest scores and are both necessary and sufficient for nonredundant classification. The top-scoring miRNA, miR-15a, has previously been linked to apoptosis and neoplastic cell cycle regulation, but its exact role in diabetes mellitus is unknown.<sup>[30]</sup> It was demonstrated that the expression of B-cell lymphoma 2, a crucial antiapoptotic proteins, was negatively regulated and inversely correlated with that of cyclin D1. Perhaps due to low plasma concentrations, miR-15a did not substantially vary from the control in patients. Whether miRNA levels can forecast the onset of diabetes mellitus (DM) in high-risk populations, such as those with impaired fasting glucose, borderline, metabolic syndrome or HbA1c, as well as whether miRNAs can aid in forecasting the microvascular and macrovascular complications of DM, are pertinent clinical questions.<sup>[31]</sup> According to our research, plasma miRNAs may be a good indicator of DM, but before they can be validated in larger cohorts of people with DM and prediabetes, more comprehensive comparisons with other established risk variables are required. MiR-122 is particularly significant.

Each member of the Bruneck cohort had their plasma level of miR-122 measured. We are aware of no other large population-based investigation that has quantified miRNA. In contrast to the majority of miRNAs, which are widely produced, miR-122 is essential for maintaining endothelium homeostasis and vascular integrity since it is highly concentrated in endothelial cells.<sup>[32]</sup> It enhances the signaling of vascular endothelial growth factor by inhibiting the sprout-related protein SPRED1 and phosphoinositol-3 kinase regulatory subunit 2 (PIK3R2/p85), two adverse regulators of the VEGF pathway.<sup>[33]</sup> Plasma miRNAs reflect these changes and are packaged in membranous vesicles that fluctuate in number, cellular origins, and compositions according to illness condition.<sup>[34]</sup>

These vesicles are not just a result of cell activity or death, according to mounting evidence. Rather, they create a new kind of cell-to-cell communication. For instance, miR-122 is the most prevalent miRNA in endothelium-dead bodies.<sup>[35]</sup> Endothelial cells release miR-122, which has been demonstrated to provide paracrine vasoprotection and control the VEGF response. Our findings showed that the concentration of miR-122 in endothelium apoptotic bodies decreased in a glucose-dependent manner and that diabetes mellitus was consistently linked to loss of miR-122. Low plasma levels may lead to decreased delivery of miR-122 to monocytes and contribute to insulin resistance and endothelial dysfunction since apoptotic

bodies and microparticles can be distributed to several cell types.<sup>[29,35]</sup>

We found that loss of miR-122 increases the risks of subclinical and symptoms of type 2 DM, which is consistent with previous research that showed monocytes and miR-122 of diabetic patients exhibit decreased responsiveness to insulin resistance.<sup>[36]</sup>

Numerous strengths of our study include its size, representativeness for the general population, high methodological standards, control for multiple testing, network analysis, stringent replication using numerous methodologies, a variety of standards (miR-454, RNU6b), and a variety of systems (plasma, cell culture). Limitations include the fact that particulate fractions in plasma contain particles other than endothelium's dead bodies and that the microarray utilized for the first screening did not capture all miRNAs currently identified. Therefore, in order to evaluate the potential of the provided miRNA signature and identify miRNA-drug interactions, research involving sizable cohorts of patients with diabetes and prediabetes is necessary. As a result, we cannot assert that the miRNA profile among people with DM is comprehensive.

## Conclusions

First evidence of this study that the plasma miRNAs, specifically endothelium's miR-122, are dysregulated in diabetes mellitus patient is presented in this study. This could help develop new biomarker for risks assessment and classifications. And could use for miRNA-based treatment approaches that target the vascular complications associated with the disease.

## Ethical approval

The local Ethics Committee of the University of Basra gave ethical approval for the study (Protocol No. EU/142 of October 10, 2024).

## Conflict of interest

The authors declare no conflict of interest regarding the publication of this study.

## Ethical statements

- The authors declared that no clinical trials were used in the present study.
- The authors declared that certain experiments on human tissues were performed for the present study.
- The authors declare that informed consent was obtained from the participants of the study.
- The authors declared that no experiments on animals were performed for the present study.

- The authors declared that no commercially available immortalized human and animal cell lines were used in the present study.

## Use of AI

No use of AI was reported.

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All authors declare that they received no financial support from any institution or university.

## Author contributions

All authors have contributed equally.

## Data availability

All data used are referenced or included in the article.

## Acknowledgements

Not applicable

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