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MicroRNA-224 Up-Regulation: A Potential Risk Factor for Complications in Type 2 Diabetes Mellitus among Egyptian Patients

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Abstract

Type 2 diabetes mellitus (T2DM) is a significant global health challenge. Recent studies have shown that biomarkers such as microRNAs, especially miRNAs, are altered during the progression of T2DM and its associated complications, reflecting the mechanisms of the disease. This study focused on analyzing the expression of miRNA-224 in Egyptian T2DM patients and investigating its association with diabetic complications. A total of 205 participants were enrolled in this case-control study, of which 100 individuals were diagnosed with T2DM and 105 healthy controls. The study measured various biochemical, anthropometric, and psychometric parameters, alongside miRNA-224 expression via Real-time PCR. The results showed that T2DM patients exhibited significantly increased levels of glycated hemoglobin (HbA1C), fasting blood sugar (FBS), postprandial blood sugar (PPBS), international normalization ratio (INR), creatinine, urea, cholesterol, triglycerides (TG), and low-density lipoproteins (LDL), alongside significantly reduced high-density lipoproteins (HDL) compared to the control group. In addition, the expression of miRNA-224 was significantly upregulated in T2DM patients ($P < 0.001$) and showed promising diagnostic potential, predicting diabetes risk even in non-diabetic individuals. Diabetic complications such as retinopathy, neuropathy, eye issues, joint pain, and cardiovascular diseases were more common in T2DM patients, with statistically significant differences ($P < 0.05$). Increased expression of miRNA-224 (> 1.065) was associated with a higher incidence of complications. In conclusion, miRNA-224 emerges as a potential biomarker for diabetes, with elevated levels correlating with both the disease and its complications.

Keywords: Type 2 diabetes mellitus, Diabetic complications, miRNA-224 expression, Egypt

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Introduction

Type 2 diabetes mellitus (T2DM) is characterized by impaired insulin action, secretion, or both, leading to chronic hyperglycemia. The global prevalence of diabetes, especially T2DM, has surged in recent years,

with projections suggesting that by 2040, 629 million people worldwide will be affected [1]. T2DM is particularly widespread in developing countries [2], with the prevalence in Egypt reaching approximately 15.6% of adults aged 20-79.

Chronic hyperglycemia is believed to contribute significantly to damage across multiple organs, increasing both morbidity and mortality. Poor glycemic control is a primary cause of endothelial dysfunction, which subsequently affects various intracellular pathways associated with both microvascular and macrovascular complications [3]. The main microvascular complications of diabetes include nephropathy, neuropathy, retinopathy, cerebrovascular issues, and cardiovascular diseases. Around 25% of individuals with T2DM experience diabetic nephropathy and retinopathy [4]. The capillary basal lamina, which encompasses structures like the glomerular arterioles, heart, retina, muscle, and skin, thickens under conditions of poor glycemic control, leading to the development of microvascular complications [5].

Other significant risk factors for these complications include hyperlipidemia, genetic predisposition, advanced glycation end products, hypertension, growth factors, inflammatory mediators, and, of course, hyperglycemia [6].

In recent decades, there have been major advancements in our understanding of genetic material regulation [7]. The human genome contains approximately 2% protein-coding genes, with the remaining 90% comprising non-coding regions. While protein-coding genes are transcribed into RNA and translated into proteins, non-coding genes are transcribed into non-coding RNAs, which do not code for proteins but play important regulatory roles [8].

Among these non-coding RNAs, microRNAs (miRNAs) have been shown to play a crucial role in regulating various diseases [9]. MiRNAs are short RNA molecules, typically consisting of around 22 nucleotides [10]. They regulate gene expression post-transcriptionally through two main mechanisms: by suppressing mRNA translation and promoting mRNA degradation. It is estimated that over 60% of protein-coding genes have miRNA target sites in their 3′-UTR, indicating that these genes are under miRNA regulation [11].

In the context of glucose homeostasis, miRNAs represent a novel regulatory mechanism that complements the normal functions of insulin secretion and action to help maintain normal glucose levels in the body. Research has shown that specific miRNAs are upregulated in pancreatic endocrine cell lines, and these miRNAs are involved in regulating both insulin synthesis and secretion, as well as insulin signaling in target tissues [12, 13].

Moreover, various diabetic complications are significantly influenced by epigenetic changes, including histone modifications, DNA methylation, and non-coding RNAs, especially miRNAs [14]. MiRNAs are essential for processes like cell division, differentiation, proliferation, and apoptosis. Previous studies on miRNAs have also highlighted their role in various diseases, including the SARS-CoV-2 infection [15] and as potential non-invasive biomarkers for hepatocellular carcinoma in chronic hepatitis C virus-infected Egyptian patients [16].

MiRNAs are generated from specific DNA sequences as primary miRNAs (pri-miRNAs), which are processed into precursor miRNAs (pre-miRNAs) and finally mature miRNAs. These mature miRNAs typically interact with the 3′-UTR of their target mRNAs, leading to mRNA degradation and suppression of translation. However, studies have also shown that miRNAs can interact with other regions, such as the 5′-UTR and promoter regions of coding sequences [4].

An imbalance in miRNA expression is associated with various biological processes, including cell proliferation, apoptosis, and development. MiRNAs are considered excellent biomarkers for diabetes and its complications. Specific miRNAs influence cellular viability, insulin secretion, insulin resistance, and both microvascular and macrovascular complications. Research has demonstrated that miRNA dysregulation occurs in vascular, renal, and retinal cells, and in vivo models of diabetes have confirmed these associations. The role of miRNAs in the onset and/or prevention of diabetic complications is critical, as they specifically target key genes involved in these processes [6].

This study further investigates the significance of miRNAs, particularly their potential as biomarkers for diabetic complications, as prognostic indicators, and as therapeutic targets. Retinopathy and other ocular issues are among the most common complications of T2DM and are specifically addressed in this context.

Materials and Methods

Study Participants

This research included 205 participants, consisting of 100 individuals diagnosed with type 2 diabetes mellitus (T2DM) and 105 healthy controls, all recruited from the outpatient clinic of the National Institute of Diabetes and Endocrinology. Ethical approval was obtained from the GOTHI Ethical Committee (General Organization of Teaching Hospital and Institution) under the reference number IDE00288.

The T2DM diagnosis followed the American Diabetes Association's 2020 criteria, which require a fasting plasma glucose level of at least 7.0 mmol/L (126 mg/dL) [17]. Patients included in the study had existing diabetes complications, such as retinopathy, neuropathy, and cardiovascular diseases. Additionally, demographic and risk factor data were collected from all participants.

Key physiological and anthropometric measurements, such as waist circumference (WC), pulse pressure (PP), systolic blood pressure (SBP), diastolic blood pressure (DBP), height, and weight, were recorded. Informed consent was obtained from each participant. Anthropometric measurements, including height and weight, were taken while participants wore light clothing and stood barefoot. Heights and weights were measured to the nearest 0.5 cm and 0.1 kg, respectively. Body mass index (BMI) was calculated using the standard weight (kg) divided by height squared (m^2). WC was measured to the nearest 0.1 cm. Blood pressure readings were taken after the participants rested for 5-10 minutes, with an average of three readings used for analysis.

Biochemical Assessments

Fasting blood samples (15 mL) were collected, and serum samples were analyzed to determine postprandial blood glucose, high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), total cholesterol (TC), and random blood glucose levels using an Olympus AU 400 automated biochemistry analyzer. Low-density lipoprotein cholesterol (LDL-C) levels were assessed using the Firewall method [18].

Blood Sample Handling and Storage

Blood samples were drawn into yellow gel vacutainers, and after a 30-minute clotting period, the samples were centrifuged at 4000 rpm for 10 minutes to separate serum. Two serum aliquots were prepared, with one aliquot designated for RNA extraction. Serum samples were stored at -80°C until further processing.

miRNA-224 Assay

Total RNA was isolated from serum using the Qiagen miRNeasy mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. RNA concentrations were quantified using a NanoDrop 1000 spectrophotometer (NanoDrop Tech, Wilmington, DE). The expression of miRNA-224 was quantified using quantitative reverse transcription PCR (qRT-PCR), employing the TaqMan® microRNA reverse transcriptase kit (Applied Biosystems, Cat. No. 4366596) and miR-224-specific primers (Applied Biosystems, Cat. No. 4427975). RNU6B served as the internal control. RNA concentrations in serum were adjusted by dilution in RNase-free water for consistent reverse transcription (RT) reactions. The RT reaction mixture (15 µL) consisted of 7 µL of master mix, 3 µL of primer, and 5 µL of RNA (1-10 ng). The reverse transcription process was carried out using a Mastercycler Gradient with the following conditions: 16 °C for 30 minutes, 42 °C for 30 minutes, 85 °C for 5 minutes, and held at 4 °C. Real-time PCR was then performed using TaqMan® Universal Master Mix 40R (Applied Biosystems, Cat. No. 4440043) on the MX 3000 Applied Biosystems system.

Statistical Evaluation

Data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 24. Descriptive statistics, such as means, medians, ranges, and standard deviations, were used for continuous data, while frequencies and percentages were used for categorical variables. Chi-square (Fisher's exact) tests were applied to examine the relationships between categorical variables. A logistic regression model was used to calculate odds ratios and 95% confidence intervals for factors that were significant in univariate analysis. A receiver operating characteristic (ROC) analysis was employed to evaluate the diagnostic accuracy, including sensitivity, specificity, positive predictive value, and negative predictive value, along with 95% confidence intervals. A P-value ≤ 0.05 was considered statistically significant, and all tests were two-tailed.

Results and Discussion

A total of 205 participants were included in the study, comprising 100 individuals with type 2 diabetes mellitus (T2DM) and 105 healthy controls. Of the diabetic participants, 61 (61%) were male and 39 (39%) were female, while the control group consisted of 61 (58.1%) males and 44 (41.9%) females. Among the T2DM

participants, 52 (52%) had values less than or equal to the median, and 48 (48%) had values greater than the median. In the control group, 53 (50.5%) had values less than or equal to the median, and 52 (49.5%) had values greater than the median (**Table 1**). No significant differences were found between the diabetic and control groups regarding gender, age, smoking status, or BMI, with p-values of 0.672, 0.372, 0.581, and 0.827, respectively.

Table 1. Demographic comparison between control and diabetic groups

Variable	Control group (n = 105)	Diabetic group (n = 100)	P-value
Gender			0.672
Male	61 (58.1%)	61 (61.0%)	
Female	44 (41.9%)	39 (39.0%)	
Age (years)			0.827
≤ Median Value	53 (50.5%)	52 (52.0%)	
> Median Value	52 (49.5%)	48 (48.0%)	
Smoking			0.581
No	84 (80.0%)	83 (83.0%)	
Yes	21 (20.0%)	17 (17.0%)	
BMI			0.372
≤ 25	31 (29.5%)	24 (24.0%)	
> 25	74 (70.5%)	76 (76.0%)	

Significant positive correlations were observed between various laboratory parameters such as white blood cell count (WBC), hematocrit percentage (HCT%), mean corpuscular hemoglobin concentration (MCHC), neutrophil count, HbA1C percentage, fasting blood sugar (FBS), pulse pressure (PP), international normalized ratio (INR), creatinine, urea, triglycerides (TG), and low-density lipoprotein (LDL) between the control and diabetic groups (**Table 2**).

Table 2. Laboratory findings comparison between control and diabetic groups

Parameter	**Control group (n = 105)**	**Diabetic group (n = 100)**	**P-value**
Hemoglobin (Hb)			0.575
Normal	45 (42.9%)	39 (39.0%)	
Abnormal	60 (57.1%)	61 (61.0%)	
RBC count (Million/mm^3)			0.878
Normal	41 (39.0%)	38 (38.0%)	
Abnormal	64 (61.0%)	62 (62.0%)	
WBC count			< 0.001
Normal	72 (68.6%)	0 (0.0%)	
Abnormal	33 (31.4%)	100 (100.0%)	
HCT%			< 0.001
Normal	73 (69.5%)	30 (30.0%)	
Abnormal	32 (30.5%)	70 (70.0%)	
MCH (pg)			0.201
Normal	76 (72.4%)	80 (80.0%)	
Abnormal	29 (27.6%)	20 (20.0%)	
MCHC (g/dl)			< 0.001
Normal	31 (29.5%)	56 (56.0%)	
Abnormal	74 (70.5%)	44 (44.0%)	
Neutrophil (g/L)			< 0.001
Normal	84 (80.0%)	2 (2.0%)	
Abnormal	21 (20.0%)	98 (98.0%)	
Lymphocyte (g/L)			0.141
Normal	82 (78.1%)	86 (86.0%)	

Abnormal	23 (21.9%)	14 (14.0%)	
Monocyte (g/L)			0.081
Normal	78 (74.3%)	63 (63.0%)	
Abnormal	27 (25.7%)	37 (37.0%)	
HbA1C %			< 0.001
< 5.7	105 (100.0%)	20 (20.0%)	
5.7 - 6.4	0 (0.0%)	12 (12.0%)	
> 6.4	0 (0.0%)	68 (68.0%)	
FBS (mg/dL)			< 0.001
Normal	63 (60.0%)	0 (0.0%)	
Abnormal	42 (40.0%)	100 (100.0%)	
PPBS (mg/dL)			< 0.001
Normal	97 (92.4%)	1 (1.0%)	
Abnormal	8 (7.6%)	99 (99.0%)	
Creatinine			0.012
Normal	105 (100.0%)	94 (94.0%)	
Abnormal	0 (0.0%)	6 (6.0%)	
Urea			0.024
Normal	103 (98.1%)	91 (91.0%)	
Abnormal	2 (1.9%)	9 (9.0%)	
Cholesterol			< 0.001
Normal	103 (98.1%)	79 (79.0%)	
Abnormal	2 (1.9%)	21 (21.0%)	
TG			0.026
Normal	97 (92.4%)	82 (82.0%)	
Abnormal	8 (7.6%)	18 (18.0%)	
HDL			< 0.001
Normal	72 (68.6%)	17 (17.0%)	
Abnormal	33 (31.4%)	83 (83.0%)	
LDL			< 0.001
Normal	0 (0.0%)	33 (33.0%)	
Abnormal	105 (100.0%)	67 (67.0%)	

Additionally, the study revealed significant differences between the groups in terms of retinopathy, with P-values less than 0.001 (**Table 3**). A strong association was also observed for neurological complications.

Table 3. Comparison of diabetic complications between control and diabetic groups

Complication	Control group (n = 105)	Diabetic group (n = 100)	P-value
Retinopathy			< 0.001
No	104 (99.0%)	83 (83.0%)	
Yes	1 (1.0%)	17 (17.0%)	
Eye complications			< 0.001
No	105 (100.0%)	74 (74.0%)	
Yes	0 (0.0%)	26 (26.0%)	
Neurological complications			0.001
No	105 (100.0%)	91 (91.0%)	
Yes	0 (0.0%)	9 (9.0%)	
Cardiac diseases			*
No	105 (100.0%)	97 (97.0%)	
Yes	0 (0.0%)	3 (3.0%)	

A receiver operating characteristic (ROC) curve was constructed to assess the sensitivity and specificity values for each micro-RNA 224 level detected in the type 2 diabetes mellitus study sample (**Figure 1; Table 4**).

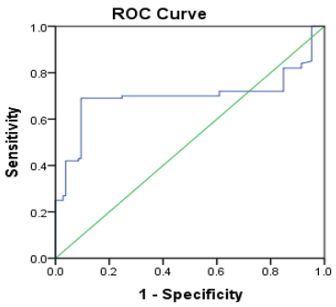

Figure 1. ROC curve displayed from the sensitivity and specificity values determined for each micro-RNA 224 level measured in the study sample, with type 2 diabetes mellitus.

Table 4. miRNA 224 cut-off values

Metric	Value
Sensitivity	69.0%
Specificity	90.5%
Positive predictive value (PPV)	87.3%
Negative predictive value (NPV)	75.4%
Area under the curve (AUC)	0.698
P-value	< .001
Total accuracy	80%
Standard error (SE)	0.041
Confidence interval (CI)	Lower = 0.617, Upper = 0.779

The expression of microRNA-224 in the population studied showed significant correlations with clinical characteristics and laboratory results. These include HCT% (P = 0.001), MCHC (P = 0.002), urea (P = 0.017), cholesterol (P = 0.001), and retinopathy (P = 0.010), which were all notably associated with microRNA-224 expression.

The relationship between microRNA-224 expression and both clinical features and laboratory data is summarized in **Table 5**.

Table 5. Association between serum microRNA-224 expression and laboratory findings

Laboratory finding	microRNA-224 expression	P-value
	≤ 1.065	> 1.065
WBC	Normal (64, 50.8%)	Normal (8, 10.1%)
	Abnormal (62, 49.2%)	Abnormal (71, 89.9%)
HCT%	Normal (75, 59.5%)	Normal (28, 35.4%)
	Abnormal (51, 40.5%)	Abnormal (51, 64.6%)
MCH (pg)	Normal (95, 75.4%)	Normal (61, 77.2%)
	Abnormal (31, 24.6%)	Abnormal (18, 22.8%)
MCHC (g/dl)	Normal (43, 34.1%)	Normal (44, 55.7%)
HbA1C %	< 5.7 (100, 79.4%)	< 5.7 (25, 31.6%)
	5.7-6.4 (3, 2.4%)	5.7-6.4 (9, 11.4%)
	> 6.4 (23, 18.3%)	> 6.4 (45, 57.0%)
FBS (mg/dl)	Normal (54, 42.9%)	Normal (9, 11.4%)
	Abnormal (72, 57.1%)	Abnormal (70, 88.6%)
PP (mg/dl)	Normal (87, 69.0%)	Normal (11, 13.9%)
	Abnormal (39, 31.0%)	Abnormal (68, 86.1%)
INR	Normal (70, 55.6%)	Normal (15, 19.0%)
	Abnormal (56, 44.4%)	Abnormal (64, 81.0%)
Creatinine	Normal (124, 89.4%)	Normal (75, 94.9%)
	Abnormal (2, 1.6%)	Abnormal (4, 5.1%)
Urea	Normal (123, 97.6%)	Normal (71, 89.9%)
	Abnormal (3, 2.4%)	Abnormal (8, 10.1%)
Cholesterol	Normal (119, 94.4%)	Normal (63, 79.7%)
	Abnormal (7, 5.6%)	Abnormal (16, 20.3%)
TG	Normal (114, 90.5%)	Normal (65, 82.3%)
	Abnormal (12, 9.5%)	Abnormal (14, 17.7%)
HDL	Normal (72, 57.1%)	Normal (17, 21.5%)
	Abnormal (54, 42.9%)	Abnormal (62, 78.5%)
LDL (U/L)	Normal (10, 7.9%)	Normal (23, 29.1%)
	Abnormal (116, 92.1%)	Abnormal (56, 70.9%)

Retinopathy	No (120, 95.2%)	No (67, 84.8%)
	Yes (6, 4.8%)	Yes (12, 15.2%)
Eye complication	No (120, 95.2%)	No (59, 74.7%)
	Yes (6, 4.8%)	Yes (20, 25.3%)
Neurological complication	No (123, 97.6%)	No (73, 92.4%)
	Yes (3, 2.4%)	Yes (6, 7.6%)
Joint pain	No (119, 94.4%)	No (74, 93.7%)
	Yes (7, 5.6%)	Yes (5, 6.3%)
Waist measurement	≤ median (68, 54.0%)	≤ median (41, 51.9%)
	> median (58, 46.0%)	> median (38, 48.1%)
Cardiac diseases	No (125, 99.2%)	No (77, 97.5%)
	Yes (1, 0.8%)	Yes (2, 2.5%)

MicroRNA-224 expression was also observed to have a highly significant correlation (P < 0.001) to PPBS (**Figure 2**).

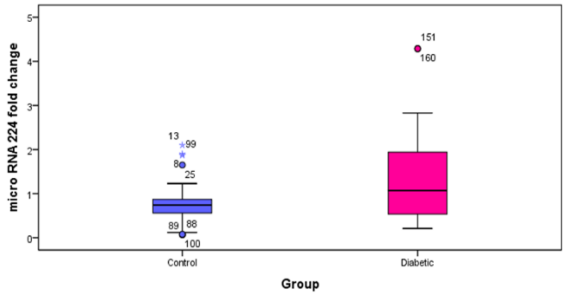

Figure 2. Comparison between the studied groups regarding microRNA-224 expression.

The study found a lack of significant correlation between microRNA-224 expression and the presence of complicated or uncomplicated diabetes mellitus (DM) in the studied patients (P = 0.480). However, significant relationships were observed between complicated and uncomplicated diabetic patients and the levels of hemoglobin (Hb) (P = 0.024) and red blood cell count (RBC) (P = 0.015). The expression of microRNA-224 was found to correlate directly with both control and diabetic patients (Figure 2).

The study included 205 participants: 100 individuals with type 2 diabetes mellitus (T2DM), of whom 61% were male and 39% female, and 105 control individuals, with 58.1% males and 41.9% females. A male predominance was observed in the participants, which contrasts with a similar study that found a majority of female participants [19]. There were no significant differences between the diabetic and control groups in terms of gender, age, smoking, and BMI. This aligns with another study that reported no notable relationship between smoking, BMI, and diabetes risk [20], although some research suggests a higher BMI is linked to an increased diabetes risk [21].

Several positive significant correlations were observed between clinical parameters such as HbA1c (%), FBS, PPBS, TG, cholesterol, HDL, and LDL. This is consistent with other studies that report positive associations between HbA1c, FBS, cholesterol, LDL-C, TG, and LD [22]. Furthermore, positive significant correlations were found between WBC, HCT (%), MCHC, and neutrophils, as observed in another study comparing diabetic and healthy children [23].

Additionally, positive correlations were found with creatinine and urea, consistent with a study showing a positive relationship between FBS, uric acid levels, and blood parameters [24]. Significant variations were noted in retinopathy between the groups, with P-values < 0.001, as well as between the duration of diabetes and the severity of retinopathy (P < 0.01), HbA1c (P < 0.01), and fasting blood glucose (P < 0.01) [25]. Neurological complications also showed a significant correlation (P = 0.001), with increased HbA1c levels identified as a predictor of polyneuropathy in diabetic patients [26].

These findings are consistent with a recent study suggesting that microRNA-224 is detectable in the urine of diabetic patients and may serve as an indicator of β-cell destruction, with clinical and biochemical variables linked to its expression levels [27]. Combining parameters like HbA1c and microRNA expression could be useful in distinguishing diabetic patients from healthy individuals and could serve as predictors for diabetes. Multivariate logistic regression analysis revealed significant connections between elevated FBS, low HDL, and high LDL in both patients and controls, with notable associations between cholesterol and HDL.

MicroRNA-224 expression is regulated by the NF-kB inflammatory signaling pathway and TGF-signaling pathways [28]. Research on miRNA profiles in T2DM

identified 40 consistently dysregulated miRNAs, with eight potentially serving as blood biomarkers and two showing significant tissue-specific regulation [29].

In this study, the ROC curve for microRNA-224 in the T2DM cohort showed a sensitivity of 69.0%, specificity of 90.5%, PPV of 87.3%, NPV of 75.4%, and total accuracy of 80%, with an area under the curve (AUC) of 0.698. This is in line with previous research suggesting that circulating miRNAs could be useful in predicting T2DM onset [30], with other studies identifying miRNAs such as miR-15a, miR-29b, and miR-126 as disrupted before T2DM development [31]. Recent research further suggests that combining miRNAs with HbA1c enhances diagnostic accuracy, with an AUC of 0.8342 compared to 0.6950 for HbA1c alone [30].

Moreover, ROC analysis of pooled diabetic groups indicated that miR-224 could differentiate between diabetic and control groups [27]. Therefore, circulating miRNAs, including miR-224, show promise as tools for predicting T2DM development in clinical settings.

MicroRNA-224 expression also correlated significantly with WBC, neutrophils, HbA1c (%), FBS, PPBS, INR, HDL, LDL, and eye complications. Previous studies have suggested that varying concentrations of circulating miRNAs could offer valuable insights into T2DM therapy monitoring, prognosis, and diagnosis [32]. Research also indicates that several miRNAs are disrupted in T2DM and may contribute to the development of diabetic complications [33]. A study comparing miRNA expression in newly diagnosed T2DM, pre-T2DM, and T2DM-susceptible individuals found higher miRNA expression in T2DM patients than in those at risk but with normal glucose tolerance [34].

Conclusion

Circulating miRNAs, such as microRNA-224, could serve as valuable markers in medical practice for predicting the onset of T2DM and detecting diabetic complications.

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Ethics Statement: None

References

1. Ortiz-Martínez M, González-González M, Martagón AJ, Hlavinka V, Willson RC, Rito-Palomares M. Recent Developments in Biomarkers for Diagnosis and Screening of Type 2 Diabetes Mellitus. Curr Diab Rep. 2022;22(3):95-115. doi:10.1007/s11892-022-01453-4
2. Akhtar S, Nasir JA, Ali A, Asghar M, Majeed R, Sarwar A. Prevalence of type-2 diabetes and prediabetes in Malaysia: A systematic review and meta-analysis. PLoS One. 2022;17(1):e0263139. doi:10.1371/journal.pone.0263139
3. Adamis AP, Shima DT, Yeo KT, Yeo TK, Brown LF, Berse B, et al. Synthesis and secretion of vascular permeability factor/vascular endothelial growth factor by human retinal pigment epithelial cells. Biochem Biophys Res Commun. 1993;193(2):631-8. doi:10.1006/bbrc.1993.1671
4. Faselis C, Katsimardou A, Imprialos K, Deligkaris P, Kallistratos M, Dimitriadis K. Microvascular Complications of Type 2 Diabetes Mellitus. Curr Vasc Pharmacol. 2020;18(2):117-24. doi:10.2174/1570161117666190502103733
5. Al-Kharashi AS. Role of oxidative stress, inflammation, hypoxia, and angiogenesis in the development of diabetic retinopathy. Saudi J Ophthalmol. 2018;32(4):318-23. doi:10.1016/j.sjopt.2018.05.002
6. Ji Z, Luo J, Su T, Chen C, Su Y. miR-7a Targets Insulin Receptor Substrate-2 Gene and Suppresses Viability and Invasion of Cells in Diabetic Retinopathy Mice via PI3K-Akt-VEGF Pathway. Diabetes Metab Syndr Obes. 2021;14:719-28. doi:10.2147/DMSO.S288482
7. Nešić K, Habschied K, Mastanjević K. Possibilities for the Biological Control of Mycotoxins in Food and Feed. Toxins (Basel). 2021;13(3):198. doi:10.3390/toxins13030198
8. Statello L, Guo CJ, Chen LL, Huarte M. Gene regulation by long non-coding RNAs and its biological functions. Nat Rev Mol Cell Biol. 2021;22(2):96-118. doi:10.1038/s41580-020-00315-9
9. García-Fonseca Á, Martin-Jimenez C, Barreto GE, Pachón AFA, González J. The Emerging Role of Long Non-Coding RNAs and MicroRNAs in Neurodegenerative Diseases: A Perspective of

Machine Learning. Biomolecules. 2021;11(8):1132. doi:10.3390/biom11081132
10. Calore F, Lovat F, Garofalo M. Non-Coding RNAs and Cancer. IJMS. 2013;14(8):17085-110. doi:10.3390/ijms140817085
11. Valinezhad Orang A, Safaralizadeh R, Kazemzadeh-Bavili M. Mechanisms of miRNA-Mediated Gene Regulation from Common Downregulation to mRNA-Specific Upregulation. Int J Genomics. 2014;2014:970607. doi:10.1155/2014/970607
12. Poy MN, Hausser J, Trajkovski M, Braun M, Collins S, Rorsman P, et al. miR-375 maintains normal pancreatic alpha- and beta-cell mass. Proc Natl Acad Sci U S A. 2009;106(14):5813-8. doi:10.1073/pnas.0810550106
13. Fang H, Wang X, Liu X, Michaud JP, Wu Y, Zhang H, et al. Molecular characterization of insulin receptor (IR) in oriental fruit moth, *Grapholita molesta* (Lepidoptera: Tortricidae), and elucidation of its regulatory roles in glucolipid homeostasis and metamorphosis through interaction with miR-982490. Insect Mol Biol. 2022;31(5):659-70. doi:10.1111/imb.12794
14. Amr KS, Abdelmawgoud H, Ali ZY, Shehata S, Raslan HM. The potential value of circulating microRNA-126 and microRNA-210 as biomarkers for type 2 diabetes with coronary artery disease. Br J Biomed Sci. 2018;75(2):82-7. doi:10.1080/09674845.2017.1402404
15. de Sanctis JB, García A, Garmendia J, Moreno D, Hajduch M, Radzioch D. Importance of miRNA in SARS-CoV2 infection. Gac Med Caracas. 2020;128(1S):S17-S22.
16. Mohamed AA, Omar AA, El-Awady RR, Hassan SM, Eitah WM, Ahmed R, et al. MiR-155 and MiR-665 Role as Potential Non-invasive Biomarkers for Hepatocellular Carcinoma in Egyptian Patients with Chronic Hepatitis C Virus Infection. J Transl Int Med. 2020;8(1):32-40. doi:10.2478/jtim-2020-0006
17. American Diabetes Association. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2021. Diabetes Care. 2021;44(Suppl 1):S15-S33. doi:10.2337/dc21-S002
18. Davis PJ, Liu M, Sherman S, Natarajan S, Alemi F, Jensen A, et al. HbA1c, lipid profiles and risk of incident type 2 Diabetes in United States Veterans. PLoS One. 2018;13(9):e0203484. doi:10.1371/journal.pone.0203484
19. Bains V, Kaur H, Badaruddoza B. Association analysis of polymorphisms in LEP (rs7799039 and rs2167270) and LEPR (rs1137101) gene towards the development of type 2 diabetes in North Indian Punjabi population. Gene. 2020;754:144846. doi:10.1016/j.gene.2020.144846
20. Cullen MW, Ebbert JO, Vierkant RA, Wang AH, Cerhan JR. No interaction of body mass index and smoking on diabetes mellitus risk in elderly women. Prev Med. 2009;48(1):74-8. doi:10.1016/j.ypmed.2008.10.008
21. Chan JCY, Chee ML, Tan NYQ, Cheng CY, Wong TY, Sabanayagam C. Differential effect of body mass index on the incidence of diabetes and diabetic retinopathy in two Asian populations. Nutr Diabetes. 2018;8(1):16. doi:10.1038/s41387-018-0018-0
22. Hussain A, Ali I, Ijaz M, Rahim A. Correlation between hemoglobin A1c and serum lipid profile in Afghani patients with type 2 diabetes: hemoglobin A1c prognosticates dyslipidemia. Ther Adv Endocrinol Metab. 2017;8(4):51-7. doi:10.1177/2042018817692296
23. Tihić-Kapidžić S, Čaušević A, Fočo-Solak J, Malenica M, Dujić T, Hasanbegović S, et al. Assessment of hematologic indices and their correlation to hemoglobin A1c among Bosnian children with type 1 diabetes mellitus and their healthy peers. J Med Biochem. 2021;40(2):181-92. doi:10.5937/jomb0-25315
24. Amartey NA, Nsiah K, Mensah FO. Plasma Levels of Uric Acid, Urea, and Creatinine in Diabetics Who Visit the Clinical Analysis Laboratory (CAn-Lab) at Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. J Clin Diagn Res. 2015;9(2):BC05-09. doi:10.7860/JCDR/2015/10905.5530
25. Elgemai E, Zeriban N, Soliman S. Prevalence of diabetic retinopathy among children with type 1 diabetes mellitus treated by insulin. Delta J Ophthalmol. 2018;19(3):196-200. doi:10.4103/DJO.DJO_15_18
26. Lee WJ, Jang S, Lee SH, Lee HS. Correlation Between the Severity of Diabetic Peripheral Polyneuropathy and Glycosylated Hemoglobin Levels: A Quantitative Study. Ann Rehabil Med. 2016;40(2):263-70. doi:10.5535/arm.2016.40.2.263
27. Bacon S, Engelbrecht B, Schmid J, Pfeiffer S, Gallagher R, McCarthy A, et al. MicroRNA-224 is Readily Detectable in the Urine of Individuals with

Diabetes Mellitus and is a Potential Indicator of Beta-Cell Demise. Genes (Basel). 2015;6(2):399-416. doi:10.3390/genes6020399

28. Scisciani C, Vossio S, Guerrieri F, Schinzari V, De Iaco R, de Meo PD, et al. Transcriptional regulation of miR-224 upregulated in human HCCs by NFκB inflammatory pathways. J Hepatol. 2012;56(4):855-61. doi:10.1016/j.jhep.2011.11.017
29. Zhu H, Leung SW. Identification of microRNA biomarkers in type 2 diabetes: a meta-analysis of controlled profiling studies. Diabetologia. 2015;58(5):900-11. doi:10.1007/s00125-015-3510-2
30. Jiménez-Lucena R, Rangel-Zúñiga OA, Alcalá-Díaz JF, López-Moreno J, Roncero-Ramos I, Molina-Abril H, et al. Circulating miRNAs as Predictive Biomarkers of Type 2 Diabetes Mellitus Development in Coronary Heart Disease Patients from the CORDIOPREV Study. Mol Ther Nucleic Acids. 2018;12:146-57. doi:10.1016/j.omtn.2018.05.002
31. Zampetaki A, Kiechl S, Drozdov I, Willeit P, Mayr U, Prokopi M, et al. Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. Circ Res. 2010;107(6):810-7. doi:10.1161/CIRCRESAHA.110.226357
32. Pordzik J, Jakubik D, Jarosz-Popek J, Wicik Z, Eyileten C, De Rosa S, et al. Significance of circulating microRNAs in diabetes mellitus type 2 and platelet reactivity: bioinformatic analysis and review. Cardiovasc Diabetol. 2019;18(1):113. doi:10.1186/s12933-019-0918-x
33. Shi R, Chen Y, Liao Y, Li R, Lin C, Xiu L, et al. Research Status of Differentially Expressed Noncoding RNAs in Type 2 Diabetes Patients. Biomed Res Int. 2020;2020:3816056. doi:10.1155/2020/3816056
34. Kong L, Zhu J, Han W, Jiang X, Xu M, Zhao Y, et al. Significance of serum microRNAs in pre-diabetes and newly diagnosed type 2 diabetes: a clinical study. Acta Diabetol. 2011;48(1):61-9. doi:10.1007/s00592-010-0226-0