

Expression of serum microRNA 30e-5p in type 2 diabetic patients with diabetic kidney disease

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Abstract

Type 2 diabetes (T2DM) is the most prevalent form of diabetes, and frequently associated with diabetic nephropathy, a leading contributor to chronic kidney disease (CKD). The presence of microalbuminuria serves as an early marker for diabetic kidney disease (DKD). Emerging evidence suggested that alterations in microRNA (miRNA) 30e-5p expression may be linked to T2DM progression, positioning it as a potential biomarker. This study investigated the clinical relevance of miRNA 30e-5p dysregulation by assessing its expression levels in T2DM patients with and without DKD. A total of 50 participants, including 25 T2DM patients with DKD and 25 T2DM patients without DKD, were examined. Serum miRNA 30e-5p levels were quantified using real-time polymerase chain reaction. Findings revealed a significant reduction in miRNA 30e-5p expression among patients with DKD compared to those without DKD (0.25 ± 0.26 vs. 0.89 ± 0.13 , $p < 0.001$). In conclusion, miRNA 30e-5p dysregulation appears to contribute to DKD development in Egyptian T2DM patients, suggesting its potential as a prognostic biomarker.

Keywords Type 2- diabetes mellitus (T2DM), Diabetic kidney disease (DKD), Micro RNA 30e-5p, qPCR.

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Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia, leading to severe complications, including cardiovascular and renal diseases.¹

Among these complications, diabetic kidney disease (DKD) is a significant microvascular disorder that affects approximately 40% of diabetic patients and is a major cause of end-stage renal disease (ESRD) worldwide.²

The Middle East, particularly Egypt, has emerged as a diabetes hotspot, and ranked

among the top ten countries in diabetes prevalence, affecting around 16.7% of the adult population.³ The early detection of DKD is crucial for preventing disease progression. Microalbuminuria has long been considered the primary biomarker for early DKD diagnosis; however, its sensitivity and specificity are limited, highlighting the need for novel biomarkers.⁴

Various risk factors, including genetic predisposition, epigenetic modifications, hypertension, prolonged dyslipidemia, and

chronic hyperglycemia, contribute to the development of DKD.⁵ In this context, epigenetic mechanisms, particularly microRNAs (miRNAs), have gained attention for their role in DKD pathogenesis and their potential as diagnostic and prognostic markers.⁶

Emerging evidence suggested that miRNA dysregulation plays a critical role in the initiation and progression of DKD. Notably, miRNA 30e-5p has been implicated in DKD due to its regulation of the uncoupling protein 2 (UCP2) gene which is involved in mitochondrial function and oxidative stress reduction.⁷

UCP2 mitigates oxidative stress by modulating mitochondrial reactive oxygen species (ROS) production, a key contributor to DKD pathophysiology.⁷ Given the strong association between UCP2 polymorphisms and diabetes complications, miRNA 30e-5p dysregulation may serve as an early indicator of DKD, facilitating timely intervention and disease management.⁸ This study aimed to address the role and clinical significance of miRNA 30e-5p dysregulation in DKD by comparing its expression in Type 2 diabetic patients with and without DKD.

Patients and Methods

The study included a total of 25 patients with T2DM and diabetic nephropathy (mean age: 60.16 ± 8.86 years, range: 32–74 years), recruited from the Department of Internal Medicine, Hospital of Beni-Suef University. Additionally, a control group of 25 diabetic patients without diabetic kidney disease (mean age: 52.76 ± 10.92 years, range: 32–74 years) was also included. Both groups were enrolled between November 2020 and December 2021.

All participants underwent the following assessments, including full history taking, thorough clinical examination, routine laboratory tests, which included random blood sugar levels (RBS), glycated hemoglobin (HbA1c), serum creatinine, and urinary microalbumin. Microalbuminuria was measured in randomly collected urine samples using the nephelometric method (Lot no. 32307362, Microalbumin MISPA I 2 AGAPPE, Diagnostics

Ltd, GmbH, Switzerland), according to the manufacturer's instructions.⁹

In addition, specific tests including polymerase chain reaction (PCR) and quantitative real-time reverse transcriptase (qPCR-RT) were analyzed.

RNA Extraction and miRNA Quantification

Total RNA was extracted from serum samples collected from all the study participants. Blood samples (5 ml) were drawn into plain tubes, allowed to coagulate for 10 minutes, and centrifuged to separate the serum. The serum samples were stored at -80°C until miRNA expression was quantified using qPCR-RT. The extraction was performed using the miRNeasy Mini Kit (catalog number 217004, Qiagen, Germany).¹⁰

The qPCR-RT was carried out in two steps: (1) Reverse transcription of total RNA into complementary DNA (cDNA) using the TaqMan miRNA RT assay (Thermo Scientific, USA) specific for each miRNA of interest (assay reference number: 002223 for hsa-miR-30e-5p), with U6 small nuclear RNA (U6snRNA) serving as the reference gene (assay reference number: 001973); (2) Amplification of cDNA using qPCR in a Step One real-time PCR system. The cycling conditions included an initial denaturation at 95°C for 10 minutes, followed by 50 cycles, each of 95°C for 15 seconds and 60°C for 90 seconds. The relative expression of miRNA 30e-5p was calculated using the $2^{-\Delta\Delta\text{Cq}}$ method with U6snRNA as the reference gene.¹⁰

Statistical Analysis

Data were coded and entered into Microsoft Access database software, then analyzed using the Statistical Package for Social Science (SPSS) software version 22 (SPSS Inc., Chicago, IL, USA). Comparisons of quantitative data between two independent groups were conducted using the independent samples t-test, while comparisons of qualitative data were performed using the chi-square test. One-way ANOVA was applied to compare quantitative data of multiple groups. Descriptive statistics are presented as mean $\pm$ standard deviation (SD) for quantitative variables and frequency (percentage) for qualitative variables. The receiver operating

characteristic (ROC) curve was utilized to evaluate sensitivity, specificity, and cutoff values. A p -value <0.05 was considered statistically significant.¹¹

Results

The demographic and biochemical characteristics among participants were evaluated. There was no significant difference in

the estimated average glucose (eAG) or HbA1c between type 2 diabetic patients with diabetic kidney disease and those without diabetic kidney disease (p value > 0.05). However, there were statistical significant differences in serum creatinine and urinary microalbumin concentration between the same groups ($p < 0.05$) (Table 1).

Table 1. Comparison of the different biochemical studied parameters between Type 2 diabetic patients with diabetic kidney disease (Group I) and those without diabetic kidney disease (Group II).

	Group I (Mean $\pm$ SD)	Group II (Mean $\pm$ SD)	p value
HbA1C (%)	8.18 $\pm$ 0.40	8.06 $\pm$ 0.58	NS
eAG (mg/dl)	188.04 $\pm$ 11.36	184.72 $\pm$ 16.68	NS
Creatinine (mg/dl)	1.06 $\pm$ 0.12	0.83 $\pm$ 0.12	< 0.001
Microalbumin (mg/g creatinine)	189.52 $\pm$ 75.13	17.44 $\pm$ 5.79	< 0.001

$p > 0.05$ is not significant (NS).

There was a statistical significant difference in serum miRNA 30e-5p between Type 2 diabetic patients with diabetic kidney disease and those

without diabetic kidney disease ($p < 0.05$). (Table 2)

Table 2. Assessment of miRNA 30e-5p in Type 2 diabetic patients with diabetic kidney disease (Group I) and those without diabetic kidney disease (Group II).

	Group I (Mean $\pm$ SD)	Group II (Mean $\pm$ SD)	p value
miRNA 30e-5p	0.25 $\pm$ 0.26	0.89 $\pm$ 0.13	< 0.001

$p \leq 0.05$ is significant.

By studying the correlation between miRNA 30e-5p and different variables of the studied groups, we found a significant negative correlation between urinary microalbumin and serum miRNA 30e-5p ($r = -0.726$, $p < 0.001$). Also there was a significant negative correlation between serum creatinine level (mg/dl) and miRNA 30e-5p ($r = -0.569$, $p < 0.001$) (Figures 1 and 2).

We performed the ROC curve analysis, to determine the sensitivity and specificity of miRNA 30e-5p as a diagnostic tool of DKD. It showed a sensitivity of 96% and a specificity of 68%, at an area under the curve (AUC) of 0.964, 95% CI = 0.915–1.000, $p < 0.001$ (Table 3).

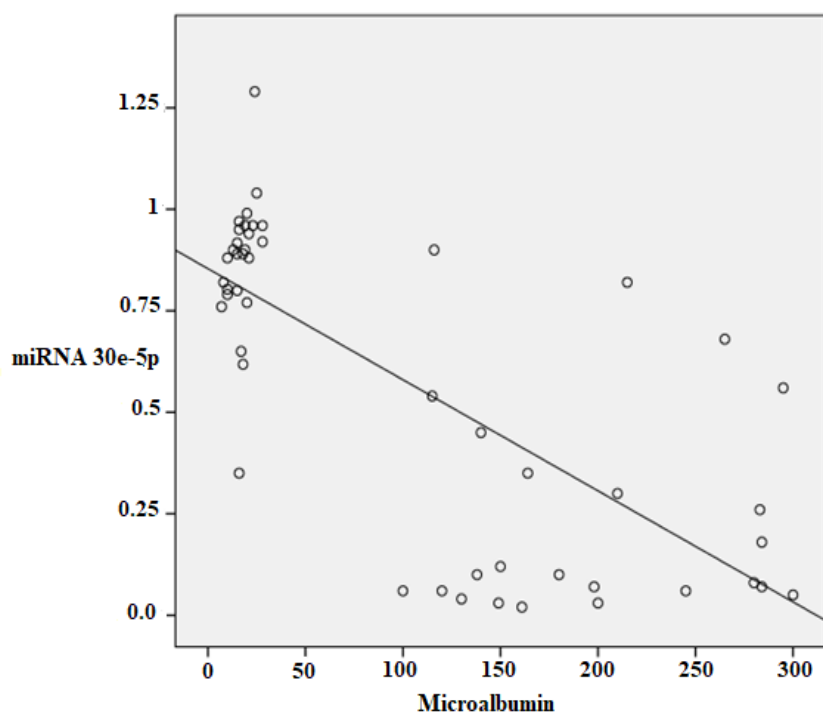


Figure 1. Scatter plot showing correlation between urinary microalbumin and serum miRNA 30e-5p ($r = -0.726, p < 0.001$).

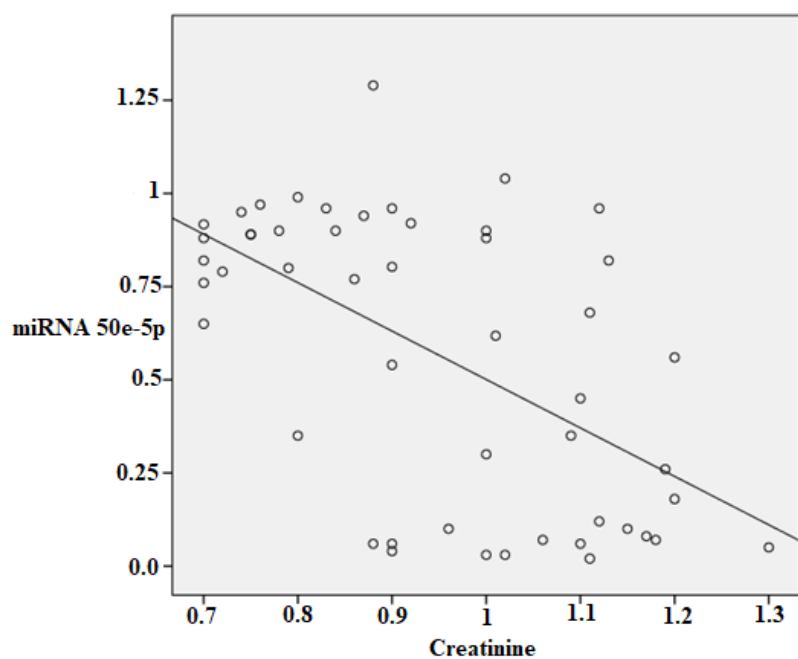
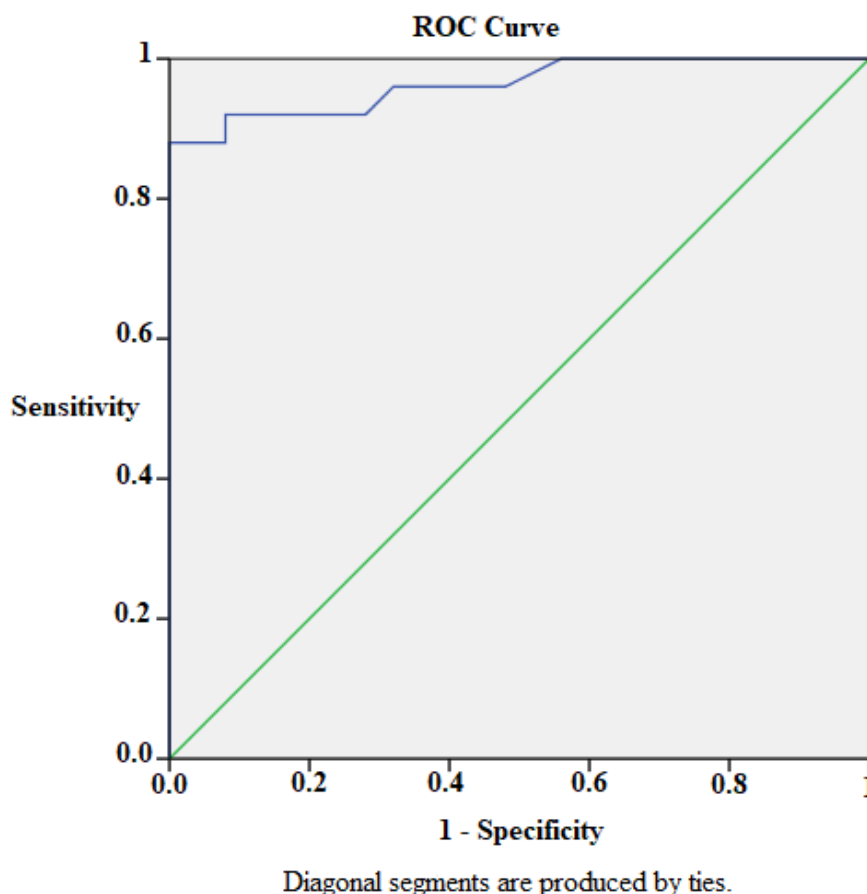


Figure 2. Scatter plot showing correlation between serum creatinine level (mg/dl) and miRNA 30e-5p ($r = -0.569, p < 0.001$).

Table 3. Sensitivity and Specificity of miRNA 30e-5p as a diagnostic tool for diabetic kidney disease (DKD).

Gene	Sensitivity	Specificity	AUC	Cut off point	<i>p</i> value	95% CI
miRNA 30e-5p	96%	68%	0.964	0.88	< 0.001	0.915–1.000

$p \leq 0.05$ is significant.

**Figure 8.** The Receiver Operating Characteristic (ROC) curve of miRNA 30e-5p as a diagnostic tool of DKD.

Discussion

Diabetes mellitus (DM) is a metabolic disorder that leads to chronic hyperglycemia, contributing to complications such as cardiovascular and renal diseases. There are multiple forms of DM, including type 1, type 2, maturity-onset diabetes of the young (MODY), gestational diabetes, and secondary diabetes due to endocrinopathies or medication use.¹²

This study aimed to evaluate the expression of miRNA 30e-5p in T2DM patients with and

without DKD, assessing its potential clinical significance. Our results demonstrated a significant difference in serum miRNA 30e-5p levels between the two groups ($p < 0.05$), with a marked downregulation in DKD patients. This aligns with findings by Motshwari et al. 2023, who reported consistent miRNA 30e-5p downregulation in CKD patients, highlighting its possible protective role in kidney function.¹³

Similarly, the study by Park et al. 2022, observed significantly lower plasma miRNA 30e-5p levels in DKD patients ($p < 0.001$), reinforcing

its diagnostic value.¹⁴ Additionally, the study by Jihménez et al. 2021, found decreased urinary miRNA 30e-5p levels in diabetic patients with microalbuminuria. A strong negative correlation was noted between urinary microalbumin and miRNA 30e-5p ($r = -0.726$, $p < 0.001$).¹⁵ On the other hand, the study by Dieter et al. 2019, demonstrated no significant correlation between them in T1DM patients.¹⁶

The ROC curve analysis indicated that miRNA 30e-5p is a promising diagnostic tool for DKD, with high sensitivity (96%) and specificity (68%). These findings align with those reported by Wang et al. 2019, who demonstrated a diagnostic potential for miRNA 30e-5p with an AUC (> 0.7).¹⁷

The meta-analysis by Fujii 2023, reported comparable findings, indicating that miRNA 30e-5p could effectively differentiate normal individuals from those with DKD, with an AUC exceeding 0.8.¹⁸ Additionally, the study by Lv et al. 2023, suggested that miRNA 30e-5p might have therapeutic potential for DKD due to its predicted high expression in bone marrow mesenchymal stem cell-derived exosomes.¹⁹ Furthermore, the study by Pana et al. 2023, demonstrated a significant downregulation of miRNA 30e-5p in the urine of patients with polycystic kidney disease, reinforcing its potential role in kidney-related pathologies.²⁰

This study has few limitations such as the relatively small sample size, and the cross-sectional nature of the study. Further large-scale, multicenter, and longitudinal studies are recommended to validate these findings and explore the potential therapeutic applications of miRNA 30e-5p.

In conclusion, our findings indicate that miRNA 30e-5p expression is significantly reduced in the serum of DKD patients. This suggested that miRNA 30e-5p could serve as a reliable biomarker for diagnosing DKD with high sensitivity and specificity.

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Author Contributions

REI; Conceptualization, study design, data collection, manuscript drafting. SOAK; Laboratory analysis, data interpretation, manuscript review. KA; Statistical analysis, results interpretation, critical manuscript revision. AMR; Literature review, methodology refinement, manuscript editing. MMA; Supervision, final approval of the manuscript, correspondence. All authors have reviewed and approved the final version of the manuscript and agree to its submission.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Scientific Research Ethical Committee of the Faculty of Medicine, Beni-Suef University (Approval No. FMBSURCE/07062020/Khalil, 2020).

Informed consent

An informed consent was obtained from each participant before being included in the study.

References

1. American Diabetes Association (2019). Microvascular complications and foot care: standards of medical care in diabetes." *Diabetes Care*; 42.Supplement 1: S124-S138.
2. Khalil, S. H. A., Megallaa, M. H., Rohoma, K. H., et al., (2018). Prevalence of type 2 diabetes mellitus in a sample of the adult population of Alexandria, Egypt. *Diabetes research and clinical practice*, 144, 63-73.
3. American Diabetes Association (2019). "Standards of medical care in diabetes". *Diabetes Care* 42. Supplement_1: S1-S2.
4. Assmann, T. S., Recamonde-Mendoza, M., et al., (2018). MicroRNAs and diabetic kidney disease: Systematic review and bioinformatic analysis. *Molecular and cellular endocrinology*, 477, 90-102.

5. Wanner, N., and Bechtel-Walz, W. (2017). Epigenetics of kidney disease. *Cell and tissue research*, 369(1), 75-92.
6. Jiang, L., Qiu, W., Zhou, Y., et al., (2013). A microRNA-30e/mitochondrial uncoupling protein 2 axis mediates TGF- β 1-induced tubular epithelial cell extracellular matrix production and kidney fibrosis. *Kidney international*, 84(2), 285-296.
7. Souza, B. M. D., Assmann, T. S., Kliemann, L. M., et al., (2011). The role of uncoupling protein 2 (UCP2) on the development of type 2 diabetes mellitus and its chronic complications. *Arquivos Brasileiros de Endocrinologia & Metabologia*, 55, 239-248.
8. Souza, B. M. D., Michels, M., Sortica, D. A., et al., (2015). Polymorphisms of the UCP2 gene are associated with glomerular filtration rate in type 2 diabetic patients and with decreased UCP2 gene expression in human kidney. *PLoS One*, 10(7), e0132938.
9. Schmitz, A. (1988). Microalbuminuria and mortality in non-insulin-dependent diabetes. In *The kidney and hypertension in diabetes mellitus* (pp. 65-70). Boston, MA: Springer US.
10. Bustin, S. A., Benes, V., Garson, J. A., et al., (2009). The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments.
11. Dawson B and Trapp RG. (2001): Basic and clinical biostatistics. 3rd ed. New York: McGraw-Hill.
12. Sapra, A., & Bhandari, P. (2024). Diabetes.[Updated 2023 Jun 21]. *StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing*.
13. Motshwari, D. D., Matshazi, D. M., Erasmus, R. T., et al., (2023). MicroRNAs associated with chronic kidney disease in the general population and high-risk subgroups—a systematic review. *International journal of molecular sciences*, 24(2), 1792.
14. Park, S., Kim, O. H., Lee, K., et al., (2022). Plasma and urinary extracellular vesicle microRNAs and their related pathways in diabetic kidney disease. *Genomics*, 114(4), 110407.
15. Jiménez-Avalos, J. A., Fernández-Macías, J. C., & González-Palomo, A. K. (2021). Circulating exosomal MicroRNAs: New non-invasive biomarkers of non-communicable disease. *Molecular Biology Reports*, 48(1), 961-967.
16. Dieter, C., Assmann, T. S., Costa, A. R., et al., (2019). MiR-30e-5p and MiR-15a-5p expressions in plasma and urine of type 1 diabetic patients with diabetic kidney disease. *Frontiers in genetics*, 10, 563.
17. Wang, J., Wang, G., Liang, Y., et al., (2019). Expression profiling and clinical significance of plasma microRNAs in diabetic nephropathy. *Journal of diabetes research*, 2019(1), 5204394.
18. Fujii, Y. R. (2023). *The microRNA quantum code book*. Springer.
19. Lv, J., Hao, Y. N., Wang, X. P., et al., (2023). Bone marrow mesenchymal stem cell-derived exosomal miR-30e-5p ameliorates high-glucose induced renal proximal tubular cell pyroptosis by inhibiting ELAVL1. *Renal Failure*, 45(1), 2177082.
20. Pana, C., Stanigut, A. M., Cimpineanu, B., et al., (2023). Urinary Biomarkers in Monitoring the Progression and Treatment of Autosomal Dominant Polycystic Kidney Disease—The Promised Land? *Medicina*, 59 (5), 915.