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Original Article 1

Associations of circulating miR-93-5p and miR-197 levels and diabetic kidney disease

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Background
The progressive loss of kidney function associated with diabetes mellitus is known as diabetic kidney disease (DKD), and its morbidity and mortality are making it a major global issue. Monitoring is unavailable due to the lack of early and credible biomarkers. Several investigations have demonstrated the potential significance of microRNAs (miRNAs) in the field of DKD.

Objective
The current research aimsto evaluate the expression levels of circulating miRNA-93-5p and miR-197 in DKD patients with and without end-stage renal disease (ESRD) as well as their correlations with various clinical parameters. Additionally, to assess the potential utility of these examined miRNAs as biomarkers for detecting the risk of developing DKD.

Subjects and methods
A total of 120 subjects were enrolled in the study and classified into three groups: healthy non-diabetic controls (n = 40), the DKD without ESRD group (n = 40), and DKD patients with ESRD (n = 40). The expression levels of circulating miRNAs (miR-93-5p and miR-197) were detected through quantitative real-time PCR.

Results and conclusions
Our data revealed that the expression of miR-93-5p was significantly decreased in DKD groups with and without ESRD relative to the control group .Additionally, it was significantly reduced in DKD with ESRD compared to DKD without ESRD. However, no significant difference in the expression level of miR-197 was observed between the studied groups. In addition, the results of the correlation analysis indicated negative correlations of the miR-93-5p with urea, creatinine and potassium and positive correlations with glomerular filtration rate (GFR) and electrolyte levels (sodium and calcium). Moreover, miR-93-5p had high accuracy in the recessive operating characteristic (ROC) analysis, especially regarding the DKD group with ESRD (96.25%). According to our research, miR-93-5p may be a valuable biomarker for both diabetic kidney disease and the onset of end-stage renal disease in patients within DKD.

Keywords: Diabetic kidney disease (DKD);End-stage renal disease (ESRD); MicroRNAs (miRNAs); Biomarkers; Quantitative real-time PCR (qRT-PCR).

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Introduction

A chronic condition known as diabetic kidney disease (DKD) is defined as primary or secondary renal diseases that produce kidney damage lasting more than three months or a glomerular filtration rate (GFR) of less than 60 ml/min for longer than three months [1].DKD has a global incidence of approximately 13.4% and is predicted to be listed as the fifth most prevalent cause of death by 2045 [2]. Because diabetic kidney disease, especially in its early stages, lacks evident clinical manifestations, it is referred to as a silent disorder [3]. Consequently, the majority of those diseased are not aware of their disorder and are frequently only recognized as the disease has progressed [4]. As a whole, the pathophysiology of DKD remains unclear, but it is strongly linked to prevalent pathological characteristics such as glomerular sclerosis and interstitial fibrosis. The disease progresses irreversibly, eventually leading to chronic renal failure (CRF) [5]. The prevention of DKD progression and the significant reduction of associated adverse health consequences require early detection and efficient screening of high-risk people [6]. DKD is a leading cause of chronic kidney disease (CKD) worldwide and represents the primary driver of progression to end-stage renal disease (ESRD). Approximately 30–40% of patients with diabetes develop DKD, and a substantial proportion of these individuals

eventually progress to ESRD, requiring dialysis or kidney transplantation. Thus, identifying biomarkers that can predict DKD progression and the risk of ESRD is of major clinical importance [7,8].

To enhance the diagnosis and prognosis of DKD, more precise, non-invasive, highly sensitive, specific, and easily accessible biomarkers are required, as demonstrated by the diagnostic obstacles. Over the past ten years, there has been an increase in research on microRNAs (miRNAs) as prospective biomarkers of disease prognosis and diagnosis as well as potential therapeutic targets [9]. A class of small non-coding RNAs known as miRNAs is primarily responsible for controlling gene expression through the degradation of messenger RNA (mRNA) or the inhibition of translation of mRNA into essential proteins [10].They are essential for many cellular regulatory processes, including cell division, proliferation, development, and apoptosis [10], they are also involved in the growth as well as optimal function of the kidneys [11]. Exploring the importance of miRNAs in the onset and progression of DKD has gained attention [12-14].

MiRNA-93-5p, a member of the miR-106b-25 cluster, which is composed of three miRNAs, was determined to be up-regulated in the eyes of patients with diabetic retinopathy (DR). This suggests that miR-93-5p could possibly have an essential role in diabetes mellitus, including its progression and development of DR [15,16]. Furthermore, the downregulation of miR-93-5p enhanced the migration, proliferation and fibrosis of renal tubular epithelial cells, podocytes and mouse mesangial cells induced by high glucose. This indicates a role for miR-93-5p in the pathophysiology of DKD [17-19]. Moreover, it has been increasingly verified that miR-197 interacts with certain RNA species to play a critical role in drug resistance, metastasis, differentiation, apoptosis, and cell proliferation, as well as further biological processes [20]. Nevertheless, the prognostic significance of the expression level of circulating miR-197 in diabetic kidney disease has not been thoroughly established. Based on this information, the current study is intended to determine the expression levels of serum miR-93-5p and miR-197 in diabetic kidney disease patients with and without end-stage renal disease (ESRD)as compared to healthy controls,along with, their correlations with various clinical parameters of the studied groups. Furthermore, to evaluate the potential utility of circulating miR-93-5p and miR-197 as biomarkers for detecting the risk of developing DKD and progression of ESRD in patients with DKD.

Subjects and methods

Study population

This cross-sectional study included 120 participants who were classified into three groups. The first group is a renal group comprising 40 diabetic kidney disease patients without end-stage renal disease, and the second group is a renal failure group that includes 40 DKD patients with end-stage renal disease, in addition to 40 age- and sex-matched controls. The control group consisted of healthy adult individuals with no history of diabetes mellitus, active infections or malignancy, kidney, liver, cardiovascular, or other chronic illnesses. Patients with diabetic kidney disease were selected from the urology department of Kasr Elaini Hospital in Egypt. The medical research ethics committee (MREC) of the national research centre (NRC) authorized this study (13060121-2), which was conducted in accordance with the guidelines of the Declaration of Helsinki. All patients and controls provided written informed consent.

Definition of diabetic kidney disease (DKD)

By using the CKD-EPI creatinine equation, the glomerular filtration rate (eGFR) was calculated [21]. In epidemiological research, this equation was frequently applied. DKD was diagnosed among individuals with an a GFR <60 mL/min/1.73 m^2 (DKD stages; 3–5). The following inclusion criteria were used to identify DKD patients: (1) no transplants; (2) no cancer; (3) no hypertension or other chronic medical condition; and (4) non-acute or rapidly progressive renal diseases with diabetes mellitus. Pregnant women and patients with unstable clinical conditions or acute inflammation were not included in the study. Furthermore, non-related individuals without renal disease symptoms (GFR $\geq$ 90 ml/min/1.73 m^2) were chosen as controls.

Sample collection

Following an overnight fast, five milliliters of venous blood were extracted from each participant and placed in two tubes: one containing EDTA to evaluate HbA1c and the other containing the blood serum, which was separated by centrifugation at 3000 rpm for 15 minutes after 30 minutes of clotting at room temperature. For further biochemical analysis and miRNA investigations, the clear, non-hemolyzed supernatant sera were kept at -80°C.

Biochemical evaluations

All analyses were performed in serum or plasma in accordance with the guidelines provided by the various manufacturers. Using an enzymatic colorimetric approach, the levels of serum creatinine, urea, glycosylated hemoglobin A1c (HbA1c), and fasting plasma glucose (FPG), were evaluated (Stanbio Laboratory, Texas, USA). An automated system using the Cobas® 8000/6000 modular analyzer series (Roche Diagnostics,

Rotkreuz, Switzerland) was used to measure potassium, sodium, and calcium.

Detection of micro ribonucleic acids 93-5p and 197

Blood samples from each group was utilized to investigate the expression levels of circulating miRNAs (93-5p and 197). The TRIzol reagent (Invitrogen, USA) was applied to extract the total RNAs from serum. Using SYBR green master mix (Qiagen/SABiosciences Corporation, USA), 1 µg of RNA samples were reverse transcribed into complementary DNA (cDNA) via the RT kit (Applied Biosystems, Foster City, CA, USA). Quantitativereal-time PCR experiment (q RT-PCR) was performed using the Step one RT-PCR (Thermo Scientific, USA). For RT-PCR, the reaction conditions were 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. Every reaction was carried out in triplicate, with U6 acting as an endogenous control for miRNA. The $2^{-\Delta\Delta Ct}$ technique was employed to assess the relative gene expressions of various miRNAs[22].The purity and concentration of the RNA were evaluated using a Nanodrop spectrophotometer, and only samples with an A260/A280 ratio between 1.8 and 2.0 were included. To assess the quality of the RNA extraction and confirm its integrity, U6 small nuclear RNA was employed as the internal control in all q RT-PCR assays to normalize miRNA expression, since U6 expression did not differ significantly between groups. To reduce systematic bias, all extractions and q RT-PCR assays were performed in parallel using the same reagent batches and equipment. The primer sequences for PCR were as follow: miR-93-5p (F) 5′-AGGCCCAAAGTGCTGTTCGT -3 and (R) 5′-GTGCAGGGTCCGAGG -3′ [23]; miR-197 (F) 5′-ATTACTTTGCCCATATTCATTTTGA-3′ and (R) 5′-GCAACAGTGTGAATGTACTTAATGC-3′ [24]; U6 5′-CTCGCTTCGGCAGCACA-3′ and 5′-AACGCTTCACGAATTTGCGT-3′ [25].

Statistical analysis

SPSS version 22 (SPSS Inc., Chicago, IL, United States) was used to analyze the results. PASS 11 was applied to calculate the sample size and statistical power. The data were shown as mean ± standard error (SE). Clinical features and miRNA expression levels within and across groups were assessed via a one-way analysis of variance (ANOVA). Furthermore, the correlation coefficient (R) of the expression level between the examined miRNAs and parameters was ascertained using Pearson's correlation analysis. The relationship between several clinical variables and the expression levels of the investigated miRNAs (93-5p and 197) in patients with diabetic kidney disease was examined through multiple linear regression analysis. $P< 0.05$ is the threshold for a statistically significant P value. To determine the accuracy of the circulating miR-93 and miR-197 in the diagnosis of DKD, the area under the curve (AUC) of receiver operator characteristic (ROC) curves was obtained.

Results

Anthropometric measurements and clinical data of diabetic kidney disease (DKD) groups and controls are shown in Table 1. There was a statistically significant increase (P<0.001) between different DKD patients without and with end-stage renal disease (ESRD) as compared to controls according to Random blood sugar (RBS) [180.7,193.5 and 94.63, respectively] glycated hemoglobin (HbA1c) [7.08, 7.26, and 4.8, respectively], urea [81.5, 143.05, and 23.63, respectively] along with creatinine [1.98,7.60, and 0.78, respectively]. On the other hand, there was a significant decrease (P<0.001) in hemoglobin (Hb) levels [11.95, 9.1, and 13, respectively], as well as in glomerular filtration rate (GFR) levels [33.96, 7.56, and 93.2, respectively]. For electrolyte levels; sodium [140.7,134.05, and 141.65, respectively]. Moreover, potassium level was significantly higher in DKD with ESRD (5.07) than DKD without ESRD (4.09) and control (4.24) with a P <0.001, as well as there was a slightly significant difference for calcium between DKD groups compared to controls (P=0.013).

The expression levels of circulating miRNA-93-5p and miRNA-197 are represented in Table 2 and Figure 1 (A & B). Our data revealed that the expression of miR-93-5p was significantly decreased in DKD groups with and without ESRD relative to the control group (1.052±0.03) (P<0.001) and it was significantly reduced in DKD with ESRD (0.179±0.025) compared to DKD without ESRD (0.6343±0.073). However, no significant difference in the expression level of miR-197 was observed between the different studied groups [1.0065±0.004, 1.2292±0.153, and1.1043±0.067, respectively) with P=0.265.

In addition, the findings of the correlation analysis are indicated in Table 3 and plotted in Figure 2. The negative correlations of the miR-93-5p with urea (r= -0.445, P<0.001) creatinine (r=-0.495, P<0.001), and potassium (r=0.407, P<0.001) were occurred. While, there were positive correlations of miR-93-5p with GFR (r=0.600, P<0.001), sodium (r= 0.372, P=0.001) and calcium (r=0.265, P=0.017). Conversely, miRNA-197 did not exhibit any correlation with the examined parameters.

Table 1 Descriptive and clinical data for studied groups.

Variables	Control (n=40)	DKD without ESRD (n=40)	DKD with ESRD (n=40)	*P* values
Age (years)	48.70±1.87	50.65±1.09	48.83±0.606	0.495
Sex M/F (%)	57.5%/42.5%	67.5%/32.5%	52.5%/47.5%	0.381
Random blood sugar (mg/dL)	94.63±1.394	180.7±2.894	193.55±9.721	**<0.001**
Glycated hemoglobin (HbA1c) %	4.868±0.068	7.082±0.073	7.26±0.165	**<0.001**
Hemoglobin (mg/dL)	13.005±0.125	11.953±0.157	9.133±0.2046	**<0.001**
Serum urea (mg/dL)	23.63±0.566	81.53±3.857	143.05±5.821	**< 0.001**
Serum creatinine (mg/dL)	0.7895±0.0146	1.9858±0.0486	7.608±0.3865	**<0.001**
GFR (mL/min/1.73 m^2)	93.21±0.7306	33.965±1.288	7.568±0.4812	**< 0.001**
Sodium (mEq/L)	141.65±0.652	140.7±0.412	134.05±0.586	**<0.001**
Potassium (mEq/L)	4.245±0.0653	4.092±0.043	5.07±0.1515	**<0.001**
Calcium (mmol/L)	1.1823±0.0076	1.2±0.095	1.155±0.0114	**0.013**

Data represented as mean ± standard error (SE) or as number %
GFR: glomerular filtration rate; DKD: diabetic kidney disease; ESRD: end stage renal disease
P values: comparison between groups; *P*<0.05 is significant; *P*<0.01 is highly significant

Table 2 The expression levels of miRNA-93-5p and miRNA-197 in the studied groups.

Variables	Normal Control (n=40)	DKD without ESRD (n=40)	DKD with ESRD (n=40)	*P* values
miRNA-93-5p	1.052±0.03	0.6343±0.073[a]	0.179±0.025[ab]	**<0.001**
miRNA-197	1.0065±0.004	1.2292±0.153	1.1043±0.067	0.265

Data represented as mean ± standard error (SE)
P values: comparison between groups; P<0.05 is significant; P<0.01 is highly significant
a: indicate a significant different from control group; b: indicate a significant different from DKD without ESRD group
DKD: diabetic kidney disease; ESRD: end stage renal disease

Table 3 Pearson correlation between miRNAs expression levels and clinical parameters in DKD.

Parameters		miRNA-93-5p	miRNA-197
Serum Urea (mg/dL)	Pearson Correlation	**-0.445****	-0.157
	Significant	**< 0.001**	0.164
Serum Creatinine (mg/dL)	Pearson Correlation	**-0.495****	-0.075
	Significant	**< 0.001**	0.510
GFR (mL/min/1.73 m^2)	Pearson Correlation	**0.600****	0.079
	Significant	**< 0.001**	0.485
Na^+ (mEq/L)	Pearson Correlation	**0.372****	0.106
	Significant	**0.001**	0.350
K^+ (mEq/L)	Pearson Correlation	**-0.407****	0.118
	Significant	**< 0.001**	0.297
Ca^{++} (mmol/L)	Pearson Correlation	**0.265***	0.169
	Significant	**0.017**	0.135

GFR: glomerular filtration rate; Na+: sodium; K+: potassium; Ca++: calcium
*P<0.05 is significant; **P<0.01 is highly significant

Multiple linear regression analysis was applied to study the association between miRNA-93-5p as well as miRNA-197 expression levels and the different clinical parameters in diabetic kidney disease groups (with and without ESRD) displayed in Tables (4 and 5). miR-93-5p revealed significant association with GFR with P=0.024 and 95% CI=0.002-0.035 (Table 4) as well as, miR-197 indicated significant association with K+ with P=0.050 and 95% CI=0.002-0.520 (Table 5).

Table 4 Multiple linear regression analysis for the association between miRNA-93-5p expression levels and the studied variables in DKD.

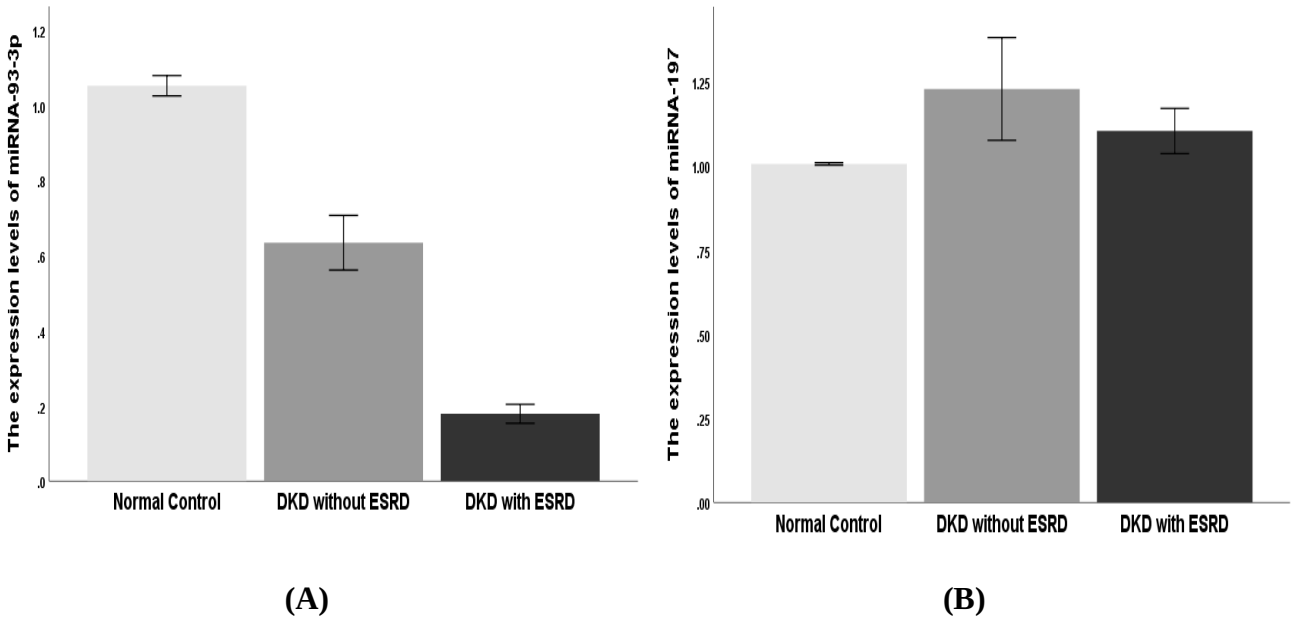
Variables	Unstandardized Coefficients		Standardized Coefficients	t	Significant	95% Confidence Interval for β	
	β	SE	Beta			Lower Bound	Upper Bound
Age	0.004	0.007	0.054	0.531	0.597	-0.011	0.019
Sex	0.020	0.110	0.024	0.181	0.857	-0.200	0.240
Random blood sugar	0.000	0.001	-0.026	-0.263	0.794	-0.002	0.002
Glycated hemoglobin	0.059	0.118	0.115	0.50	0.619	-0.176	0.293
Hemoglobin	-0.014	0.034	-0.062	-0.412	0.682	-0.082	0.054
Urea	-3.226	0.001	-0.003	-0.023	0.982	-0.003	0.003
Creatinine	0.018	0.028	0.146	0.653	0.516	-0.037	0.073
GFR	0.019	0.008	0.667	2.300	0.024	0.002	0.035
Sodium	-0.004	0.013	-0.042	-0.295	0.769	-0.029	0.022
Potassium	-0.079	0.060	-0.162	-1.312	0.194	-0.198	0.041
Calcium	0.890	0.624	0.149	1.427	0.158	-0.354	2.134

P < 0.05 is significant
Random blood sugar (RBS); Glycated hemoglobin (HbA1c); Hemoglobin (Hb); Glomerular filtration rate (GFR); Sodium (Na+); Potassium (K+); Calcium (Ca++)

Table 5. Multiple linear regression analysis for miRNA-197 expression levels and some studied variables in diabetic kidney disease patients.

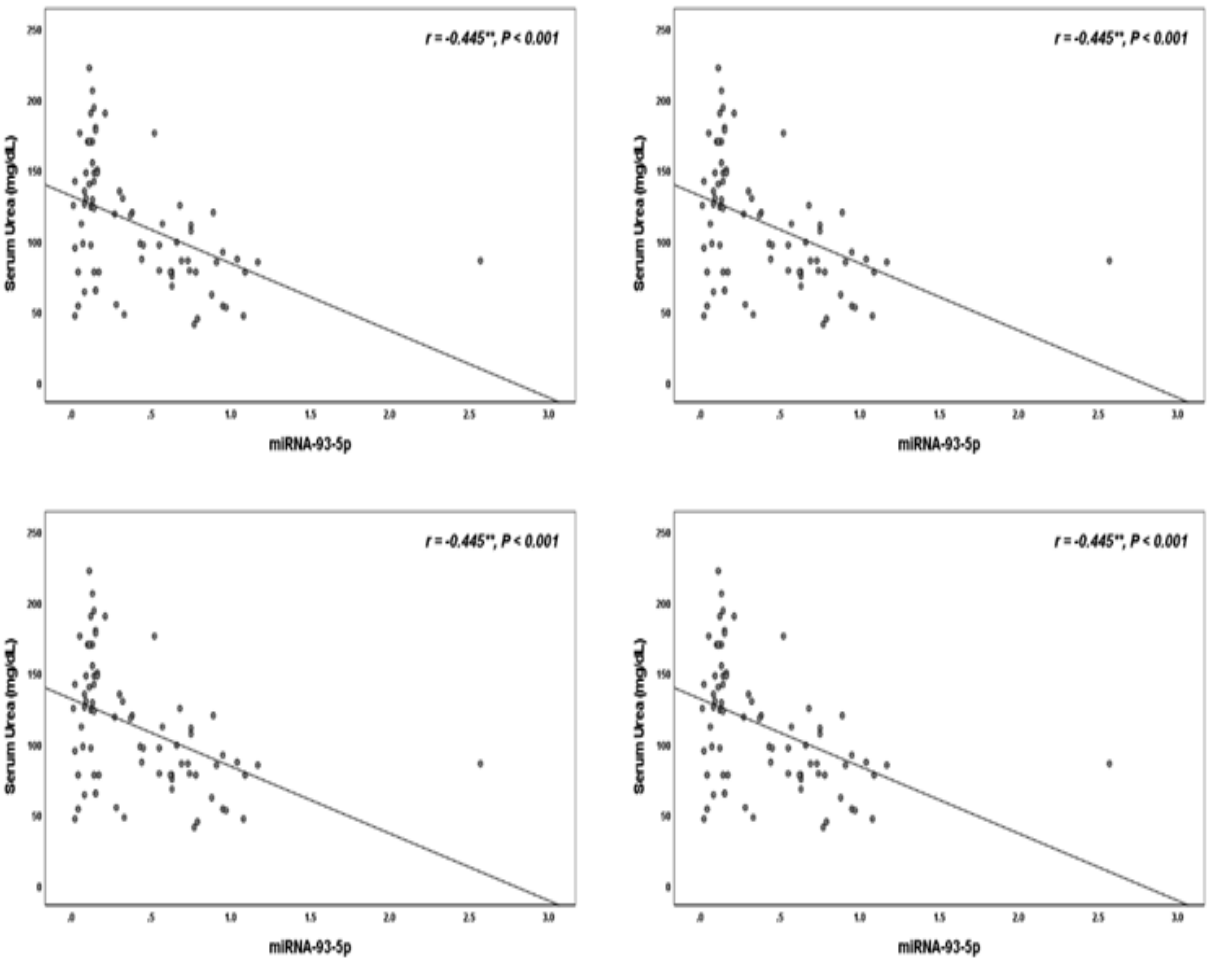
Variables	Unstandardized Coefficients		Standardized Coefficients	t	Significant	95% Confidence Interval for β	
	β	SE	Beta			Lower Bound	Upper Bound
Age	-0.017	0.016	-0.125	-1.025	0.309	-0.049	0.016
Sex	-0.043	0.239	-0.028	-0.179	0.858	-0.520	0.434
RBS	-0.001	0.002	-0.036	-0.306	0.761	-0.004	0.003
HbA1c	-0.327	0.253	-0.355	-1.296	0.199	-0.831	0.177
Hb	-0.031	0.074	-0.076	-0.419	0.676	-0.178	0.116
Urea	-0.005	0.003	-0.306	-1.698	0.094	-0.011	0.001
Creatinine	-0.021	0.060	-0.093	-0.348	0.729	-0.141	0.099
GFR	-0.006	0.018	-0.115	-0.331	0.742	-0.041	0.029
Na^+	0.022	0.028	0.138	0.810	0.420	-0.033	0.077
K^+	0.261	0.130	0.297	2.007	0.050	0.002	0.520
Ca^{++}	1.269	1.354	0.118	0.938	0.352	-1.431	3.969

RBS: random blood sugar; HbA1c: glycated hemoglobin; Hb: hemoglobin; GFR: glomerular filtration rate; Na^+: sodium; K^+: potassium; Ca^{++}: calcium
$P \leq 0.05$ is significant



(A) **(B)**

Fig. 1 Mean expression levels of the studied miRNAs among groups.
DKD: diabetic kidney disease; ESRD: end stage renal disease



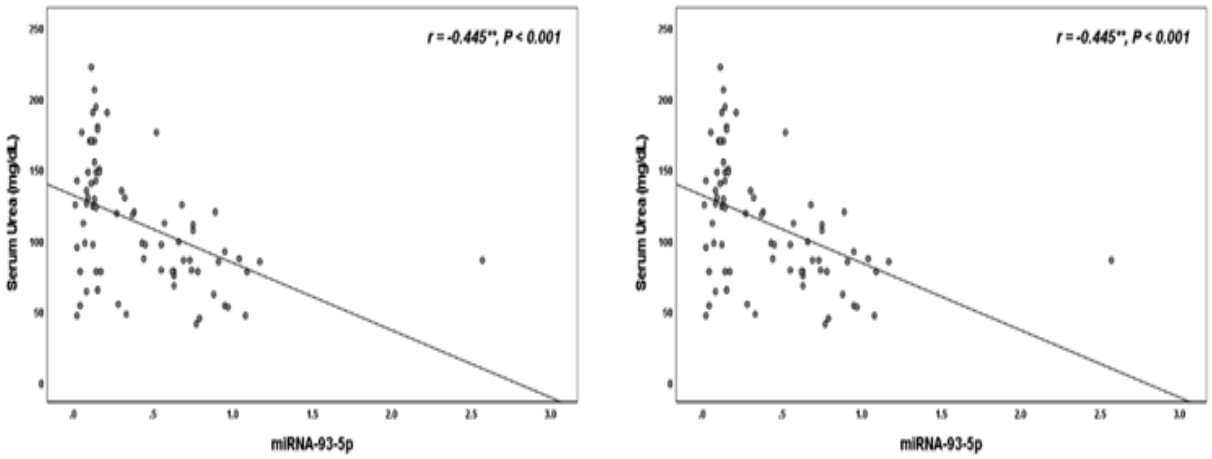
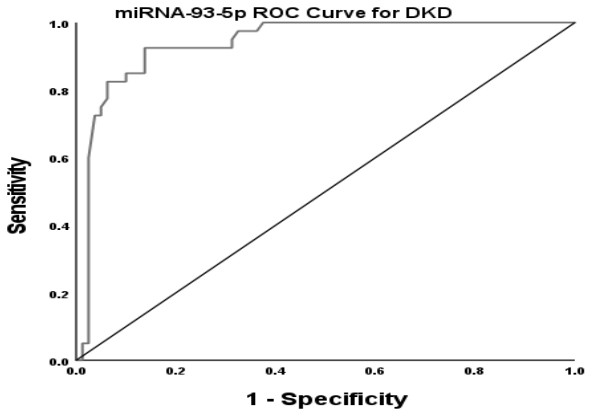


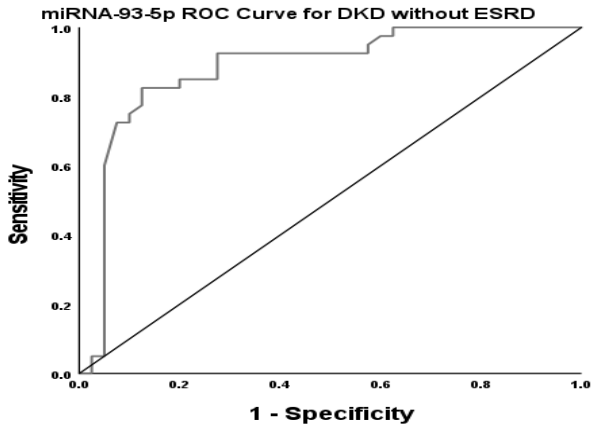
Fig. 2 Correlations of miRNA-93-5p with significant parameters in DKD patients.
DKD: diabetic kidney disease; GFR: glomerular filtration rate; Na^{+}: sodium; K^{+}: potassium; Ca^{++}: calcium

Recessive operating characteristic (ROC) analyses were carried out and plotted in Figure 3 (A, B, C, and D) to discriminate between the DKD groups from healthy controls and within the two groups (with & without ESRD). The results were as follows: for miRNA-93-5p in DKD patients with ESRD from controls (3A); [P<0.001, accuracy=96.25% with 95% CI=0.986-1]. for miR-93-5p in DKD patients with ESRD from without ESRD (3B); [P<0.001, accuracy=82.5% with 95% CI=0.8-0.96]. for miR-93-5p in DKD patients with ESRD from controls (3C); [P<0.001, accuracy=96.25% with 95% CI=0.986-1]. Finally, for miR-93-5p in DKD patients with ESRD from without ESRD (3D); [P<0.001, accuracy=82.5% with 95% CI=0.73-0.93].



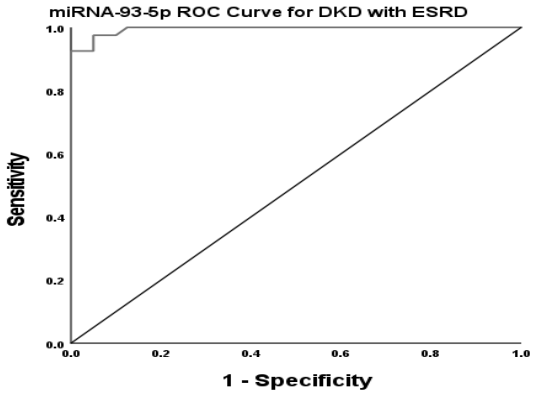
Cut off	AUC	*P*-value	Sensitivity	Specificity	Accuracy	95% CI
0.82	0.94	**<0.001**	92.5 %	86.3 %	89.4 %	0.89–0.98

Fig. 3 (A) ROC curve for the discriminatory ability of miR-93-5p to identify DKD patients from those without DKD.



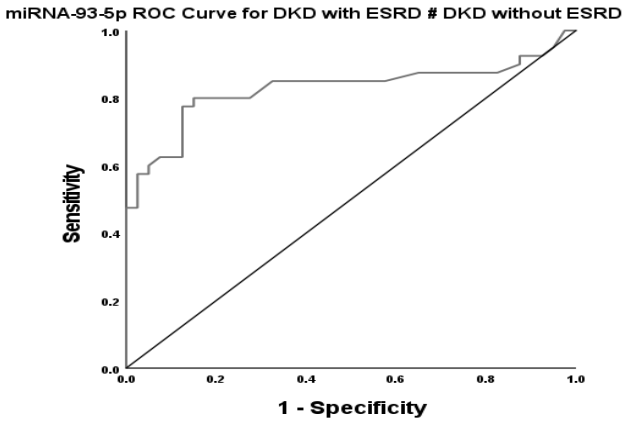
Cut off	AUC	*P value*	Sensitivity	Specificity	Accuracy	95% CI
0.82	0.88	**<0.001**	92.5 %	72.5 %	82.5 %	0.8-0.96

Fig. 3 (B) ROC curve for the discriminatory ability of miRNA-93-5p to identify DKD patients without ESRD from normal controls.



Cut off	AUC	*P* value	Sensitivity	Specificity	Accuracy	95% CI
0.56	0.995	**<0.001**	97.5 %	95%	96.25%	0.986-1

Fig. 3 (C) ROC curve for the discriminatory ability of miRNA-93-5p to identify DKD patients with ESRD from controls.



Cutoff	AUC	*P* value	Sensitivity	Specificity	Accuracy	95% CI
0.2275	0.827	**<0.001**	80%	85%	82.5 %	0.73-0.93

Fig. 3 (D) ROC curve for the discriminatory ability of miRNA-93-5p to identify DKD patients with ESRD from without ESRD.

miRNA: micro-RNA; DKD: diabetic kidney disease; ESRD: end stage renal disease; AUC: area under the curve

Discussion

Diabetes mellitus (DM) affects an estimated 463 million individuals globally and continues to pose a significant public health challenge [26]. Among its complications, diabetic kidney disease (DKD) is the leading contributor to end-stage renal disease (ESRD) and imposes a considerable economic burden on healthcare systems worldwide [27]. Despite advancements in diabetes management, the ability to predict and understand the early stages of DKD remains limited [28]. MicroRNAs (miRNAs), as epigenetic regulators, play essential roles in modulating gene expression and are implicated in various physiological and pathological processes [29, 30]. Some miRNAs are predominantly expressed in the adult kidney, while others are

detected in multiple tissues, with their expression varying by organ type and developmental stage [31, 32]. These molecules are involved in critical renal functions such as glomerular filtration, electrolyte homeostasis, and cellular signaling [33]. Notably, alterations in the expression of circulating miRNAs have been linked to the progression of DKD [34]. Thus, the objective of the present study is to investigate the expression profiles of circulating miR-93-5p and miR-197 in Egyptian patients with diabetic kidney disease, with and without ESRD, and to evaluate their potential role as biomarkers of disease severity.

In this study, a marked reduction in miR-93-5p expression was observed in both DKD groups-those with and without ESRD-when compared to healthy controls. Furthermore, its levels were significantly lower in patients with ESRD compared to those without. These results align with findings from Ulbing et al. [35], who reported decreased miR-93-5p levels in individuals with advanced stages of chronic kidney disease (CKD), suggesting that downregulation of this miRNA may play a role in disease progression. Similarly, Wang et al. [23] highlighted a notable decline in miR-93-5p expression in DKD, implicating it in the pathophysiological mechanisms of the disease. Additional studies have also shown reduced levels of circulating miR-93 among diabetic individuals compared to non-diabetic controls [34, 36]. This downregulation might be related to hyperglycemia-induced alterations in chromatin architecture, particularly affecting kidney podocytes [37]. MiR-93 is known to influence angiogenic pathways, including the modulation of vascular endothelial growth factor (VEGF), which is critical for vascular function and integrity [36]. Therefore, its dysregulation may contribute to vascular complications in diabetes [34]. Interestingly, contrary to our findings, elevated miR-93 levels have been noted in diabetic retinopathy [38], possibly reflecting distinct tissue-specific regulatory mechanisms. This discrepancy underscores the need for further investigation into the role of miR-93 across different diabetic complications and blood tissue samples.

In regard to circulating miRNA-197, our findings did not reveal a significant difference in expression levels between diabetic kidney disease patients and healthy controls, nor between those with and without end-stage renal disease. Contrarily, an experimental investigation involving earthquake survivors indicated a significant association between circulating miR-197 levels and CKD, potentially linked to post-disaster changes in vascular function [39]. However, such results

should be interpreted with caution. Prior studies have also reported altered expression of miR-197 in individuals with type 2 diabetes mellitus, suggesting a possible involvement in cardiovascular disease development [40]. Although the underlying mechanisms remain unclear, it has been hypothesized that miR-197-abundant in platelets-may contribute to cardiovascular complications through platelet activation [41]. Nonetheless, the biological role of this miRNA within platelet function is still poorly characterized, necessitating additional research to clarify its potential implications in CKD pathophysiology.

Our data revealed that miR-93-5p levels were positively associated with urea and creatinine, estimated glomerular filtration rate (eGFR), and key electrolytes such as calcium, potassium, and sodium. Conversely, no significant correlations were observed between miR-197 expression and the measured clinical parameters. Supporting our findings, Zou et al. reported a relationship between elevated plasma miR-93 levels and increased risk of diabetic retinopathy in individuals with type 2 DM [38]. Moreover, vascular calcification in chronic kidney failure is frequently attributed to imbalances in mineral metabolism, particularly involving calcium and phosphate [42]. Research by Ma et al. has suggested that miR-93 may influence biliary tract stone formation by modulating the Wnt/β-catenin signaling pathway, implicating its role in calcium regulation and bone remodeling [43]. Additionally, miRNAs have been shown to contribute to osmoregulatory processes and may play a role in ion transport regulation under hypertonic stress conditions [44]. Consistent with this, reduced levels of miR-93-5p have been linked to CKD progression and were found to correlate with both inflammation and renal impairment in advanced disease stages [35].

In addition, our data demonstrated that circulating miR-93-5p exhibited strong diagnostic performance based on receiver operating characteristic (ROC) curve analysis, showing a high level of accuracy (96.25%) in distinguishing diabetic kidney disease patients with ESRD from healthy individuals and a notable accuracy (82.5%) in differentiating DKD patients with ESRD from those without. These results highlight the potential of miR-93-5p as a useful biomarker for identifying individuals at elevated risk of advanced DKD. Although miR-93-5p demonstrated strong diagnostic performance with high sensitivity and specificity, these results must be interpreted with caution given the limited sample size of this study. Larger, independent cohorts are required to validate the clinical utility of miR-93-5p as a biomarker for DKD and related ESRD. Similarly, another study reported that miR-93 yielded an area under the curve (AUC) of 72% when distinguishing diabetic patients from healthy controls, although it was not as effective in identifying diabetic nephropathy among diabetic individuals [34]. Furthermore, our findings suggest that, unlike miR-93-5p, miR-197 may have limited utility as a biomarker in DKD.This negative result provides useful information for future biomarker studies by highlighting miR-93-5p as a more promising candidate.

Limitations of study

This study has certain limitations, most notably the relatively small sample size within each patient group. Future research involving larger and more diverse populations is necessary to confirm these results and to explore additional circulating miRNAs that may contribute to the understanding of diabetic kidney disease. Furthermore, since the study population was relatively uniform in terms of ethnicity, age range, and geographic background, it is essential that these findings be validated in broader, more heterogeneous cohorts that reflect variations in genetic, environmental, and lifestyle factors.

Conclusion

Diabetic kidney disease often progresses unnoticed in its initial stages due to the absence of clear clinical symptoms. As a result, early detection and targeted screening of individuals at increased risk are essential to delay progression and minimize associated health burdens. Based on our results, micro ribonucleic acid 93-5p may serve as a promising candidate for prognostic evaluation in patients diagnosed with diabetic kidney disease, both with and without end-stage renal disease. However, further functional studies are warranted to clarify the molecular mechanisms by which miR-93-5p may contribute to the pathogenesis of DKD and to explore whether it could be targeted therapeutically. Additionally, our findings suggest that, in contrast to miR-93-5p, the utility of miR-197 as a biomarker in DKD may be limited. These preliminary findings underscore the need for broader investigations in larger, diverse populations to validate its potential clinical utility.

Conflicts of interest

The authors declare there are no conflicts of interest.

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Authors' contributions

Concepts and design: M.A., M.D.E.A., W.G., and L.M.; Definition of intellectual content: S.A.;

literature search, clinical studies, and data acquisition: A.E., W.G., L.M., and N.S.E.; data analysis and statistical analysis: W.G. and L.M.; manuscript preparation: A.E., N.S.E., L.M. and W.G.; manuscript editing and review: M.A., M.D.E.A., L.M., and W.G.; Guarantor: M.A. and M.D.E.A.

Availability of data

The datasets used and /or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical considerations

The medical research ethics committee (MREC) of the National Research Centre (NRC) authorized this study (13060121-2), which was conducted in accordance with the guidelines of the declaration of Helsinki.

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