



Submitted: 06.05.2023  
Accepted: 26.06.2023  
Early publication date: 27.07.2023

Endokrynologia Polska  
DOI: 10.5603/EPa2023.0057  
ISSN 0423-104X, e-ISSN 2299-8306

# Clinical value of miR-23a-3p expression in early diagnosis of diabetic kidney disease

Xiaomei Meng, Xiao Yu, Peipei Lei

Department of Endocrinology, The Affiliated Yantai Yuhuangding Hospital of Qingdao University, Yantai, Shandong, China

## Abstract

**Introduction:** The objective was to observe the expression of miR-23a-3p in the serum of patients with type 2 diabetic nephropathy (T2DN) and to explore its clinical significance.

**Materials and methods:** 112 patients with type 2 diabetes were divided into a simple diabetes mellitus (NON) group, T2DN microalbuminuria (MIC) group, and T2DN macroalbuminuria (MAC) group, according to the urinary protein-creatinine ratio (uACR). Clinical data were collected, miR-23a-3p levels in serum were measured by quantitative reverse transcription polymerase chain reaction (qRT-PCR), and clinical parameters were measured by an automatic biochemical analyser; the influencing factors of diabetic kidney disease (DKD) and the correlation between miR-23a-3p expression and clinical parameters were analysed.

**Results:** The expression of miR-23a-3p in the serum of the DKD group was lower than that of the normal control (CON) and NON groups. Correlation analysis showed that miR-23a-3p was positively correlated with urinary albumin (Albu), glycosylated haemoglobin (HbA<sub>1c</sub>), total cholesterol (CHOL), glycated albumin (GA-L), serum creatinine (Scr), fasting blood glucose (GLU), and uric acid (UA), negatively correlated with uACR and high-density lipoprotein cholesterol (HDL-C), but not correlated with urinary creatinine (CREA). The area under the receiver operating characteristic (ROC) curve (AUC) of miR-23a-3p for the diagnosis of DKD was 0.686 [95% confidence interval (CI): 0.599–0.773], with a sensitivity of 64.5% and a specificity of 71.2%; the AUC for differentiating NON from DKD was 0.700 (95% CI: 0.598–0.802), with a sensitivity of 61.8% and a specificity of 77.8%. Multivariate logistic regression analysis showed that serum miR-23a-3p levels were not associated with the development of DKD after adjusting for other levels of influence and were not significant for the differentiation of NON and DKD.

**Conclusion:** Serum miR-23a-3p levels are decreased in T2DN patients, and this change becomes more significant with the severity of the disease, which may be a marker for the early diagnosis and progression of T2DN.

**Key words:** type 2 diabetic nephropathy; proteinuria; miR-23a-3p; diagnosis

## Introduction

Diabetic kidney disease (DKD) is the most serious and common microvascular complication of diabetes, and approximately 20–30% of patients with diabetes mellitus (DM) develop diabetic nephropathy, which leads to end-stage renal disease (ESRD) [1]. Modern medicine controls the progression of DKD mainly by controlling blood glucose, lowering blood lipid, and the application of angiotensin converting enzyme inhibitors and angiotensin receptor blockers, but some patients still fail to respond to the above treatment and progress to chronic renal failure or death. Because of the complexity of its aetiology, the molecular mechanisms involved in the pathogenesis of DKD remain poorly understood. At present, the early diagnosis of DKD and the judgment of disease progression still rely mainly on the excretion rate of urinary microalbumin and the detection of tissue biopsy. Proteinuria is a major marker of DKD and an independent risk factor for diabetic nephropathy and dia-

betic cardiovascular disease. Controlling the excretion of proteinuria has been found to be strongly associated with renal prognosis [2–4]. Proteinuria is not only a monitoring indicator of diabetic nephropathy, but also an important indicator of early intervention. However, the mechanism of proteinuria is not yet fully understood. Therefore, the prevention and treatment of proteinuria in the early stage of DKD is a key to the treatment of DKD. It is great of theoretical and practical significance to further reveal the pathogenesis of proteinuria in DKD and discover new targets for its effective intervention. Finding a new early diagnostic index of DKD and exploring its possible role in the occurrence and development of DKD are of great significance to deepen our understanding of the molecular mechanism of DKD treatment and better guide its early diagnosis and treatment. Therefore, it is imperative to find a specific and trustworthy marker of podocyte injury in DKD.

MicroRNAs (miRNAs) are novel small RNAs that regulate human genomics, and their abnormal

✉ Peipei Lei, Department of Endocrinology, The Affiliated Yantai Yuhuangding Hospital of Qingdao University, 20 Yuhuangding East Road, Yantai, Shandong, 264000, China; e-mail: Leipeipei19870824@163.com

expression is closely related to inflammatory response and glucose and lipid metabolism disorders, which may be involved in the development of DN [5, 6]. Some studies suggest that miR-23a-3p is abnormally expressed in patients with DKD, which is involved in the development of DKD by regulating a variety of signalling pathways and may provide a new field of view for the early diagnosis and targeted therapy of DKD. However, there are few reports at home and abroad on the expression of miR-23a-3p in the serum of patients with early type 2 diabetic nephropathy and its relationship with clinical parameters, and whether the expression of miR-23a-3p can be used as a biomarker for the early diagnosis of type 2 diabetic nephropathy and an indicator of disease progression.

In this study, we detected the changes of serum miR-23a-3p expression in patients with type 2 diabetes at different stages of the disease, and analysed its risk factors associated with the occurrence of DKD and its value in the diagnosis of DKD, so as to provide new ideas and new targets for the pathogenesis and early diagnosis and treatment of type 2 diabetic nephropathy.

## Material and methods

### Sample inclusion criteria

A total of 160 patients with type 2 diabetes and type 2 diabetic nephropathy (T2DN) hospitalized in the Nephrology Department and Endocrinology Department of Yantai Yuhuangding Hospital from December 2019 to August 2022 were selected, all of whom met the 2007 edition of the American Diabetes Association diagnostic criteria for diabetes. The research project was approved by the Medical Ethics Committee of Yantai Yuhuangding Hospital in accordance with the Declaration of Helsinki. The inclusion criteria were as follows: (1) age more than 18 years; (2) duration of type 2 diabetes mellitus (T2DM) more than 10 years; and (3) patients' consent and signed informed consent. Patients were excluded according to the following criteria: (1) patients with type 1 diabetes; (2) with malignant tumours; (3) with other chronic kidney diseases except diabetic nephropathy, such as glomerulonephritis and IgA nephropathy; (4) patients who had developed diabetic ketoacidosis (DKA) and other acute complications within the past 3 months; (5) patients with diabetic ketoacidosis; (6) abnormal liver function, primary hyperuricemia, thyroid dysfunction, fever, or urinary tract infection; (7) those who had recently used drugs affecting renal function; (8) those with immunodeficiency, suffering from systemic autoimmune diseases, such as organ transplantation history or acquired immunodeficiency syndrome (AIDS) diagnosis; (9) a history of major cardiovascular disease within 3 months: myocardial infarction, heart failure, left ventricular ejection fraction  $\leq 40\%$ , coronary angioplasty or bypass surgery, unstable angina, transient ischaemic attack, or cerebrovascular accident; and (10) pregnancy or lactation; poor compliance or blood glucose difficult to control. In addition, 30 healthy subjects from the physical examination centre of our hospital were selected as the normal control (CON) group. Inclusion criteria: Not taking any drugs recently, matched with diabetes group for age and sex. Exclusion criteria: Family history of diabetes and hypertension were excluded, except for diabetes, severe cardiopulmonary liver and kidney disease and benign (malignant) tumours, trauma,

and surgery. All included patients were informed about the benefits and risks of this pathway. Informed consent was obtained prior to enrolment.

Enrolled patients were divided into a simple diabetes group [NON, urinary protein-creatinine ratio (uACR)  $< 30$  mg/g], a T2DM microalbuminuria group (MIC,  $30$  mg/g  $< \text{UAER} < 300$  mg/g), and a T2DN macroalbuminuria group (MAC,  $\text{UAER} \geq 300$  mg/g or 24-hour urinary protein quantification  $\geq 0.5$  g) according to the staging criteria of Mogensen T2DN.

### Blood collection and processing

Six millilitres of venous blood were collected from all subjects under fasting state before 9 a.m., and the serum was collected immediately by centrifugation at 3000 rpm for 5 min at 4°C and cryopreserved at  $-80^\circ\text{C}$  for the detection of subsequent parameters.

### Biochemical indicators

Fasting blood glucose (GLU), serum creatinine (Scr), uric acid (UA), urea (UREA), triglyceride (TG), total cholesterol (CHOL), low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C), lipoprotein a (Lpa), and other biochemical indicators were measured by automatic biochemical analyser and supporting reagents. Glycosylated haemoglobin ( $\text{HbA}_{1c}$ ) was measured by high-pressure liquid chromatography. Urinary albumin (Albu) was measured using a Hitachi 7170A biochemical analyser and turbidimetry, urinary creatinine (CREA) was measured by rate method, and uACR was then calculated.

### Total RNA extraction and real-time fluorescence quantification

Serum was collected from the previous cryopreserved samples. AG RNAex Pro (Hunan Aikou Biological) was used to extract total RNA from samples, and total RNA was isolated and purified according to the manufacturer's protocol. Single-stranded DNA was obtained by reverse transcription using HiScript III 1<sup>st</sup> Strand cDNA Synthesis Kit (+ gDNawiper) (Vazyme Biotech Co., Ltd, Nanjing, China), referring to the following steps: 2  $\mu\text{L}$  of 5  $\times$  gDNawiper Mix, 1  $\mu\text{g}$  of total RNA, and RNase-free double-distilled water were added to 10  $\mu\text{L}$  in a 200  $\mu\text{L}$  centrifuge tube, mixed by pipette aspiration, and reacted at 42°C for 2 minutes after transient centrifugation; 2  $\mu\text{L}$  of 10  $\times$  RT Mix, 2  $\mu\text{L}$  of HiScript III Enzyme Mix, 1  $\mu\text{L}$  of Oligo (dT) 20VN, 1  $\mu\text{L}$  of Random hers, and 4  $\mu\text{L}$  of RNase-free double-distilled water were continued in a centrifuge tube, mixed by pipette aspiration, and reacted at 37°C for 15 minutes and at 85°C for 5 seconds. qRT-PCR experiments were performed according to ChamQ Universal SYBR qPCR Master Mix (Vazyme Biotech Co., Ltd, Nanjing, China) with the following systems: 2  $\times$  Mix 10  $\mu\text{L}$ , Primer-F (10  $\mu\text{M}$ ) 0.5  $\mu\text{L}$ , Primer-R (10  $\mu\text{M}$ ) 0.5  $\mu\text{L}$ , cDNA 1  $\mu\text{L}$ , and ddH<sub>2</sub>O 8  $\mu\text{L}$ . qPCR reaction conditions were as follows: pre-denaturation at 95°C for 30 sec; 40 cycles of reaction at 95°C for 10 sec, 60°C for 30 sec; melting curve at 95°C for 15 sec, 60°C for 60 sec, and 95°C for 15 sec. U6 was used as an internal reference. The primer sequences used in this study are detailed in Table 1.

### Bioinformatics analysis

To better understand the functional involvement of miR-23a-3p in DKD, it was submitted to bioinformatics analysis to investigate its putative target genes and find possible biological pathways under its regulation. Using miRDB (Fig. <http://mirdb.org/>), TargetsCan (Fig. [http://www.targetscan.org/vert\\_72/](http://www.targetscan.org/vert_72/)), and microRNA.org (<http://www.microrna.org/microrna/home.do>), the database predicts target genes for differentially expressed miRNAs. Target genes appearing simultaneously in all 3 databases are given by <http://bioinformatics.psb.ugent.be/webtools/Venn/>, and at the DAVID website (Fig. <https://david.ncifcrf.gov/home.jsp>), and intersections were analysed for enrichment pathway analysis.

Table 1. Primers used in this study

Primer name	Sequence
RT-U6	GTCGTATGCAGAGCAGGGTCCGAGGTATTCGCACTGCATACGACAAAATATGG
U6-F	AAGGATGACACGCAAATC
U6-R	GAGCAGGGTCCGAGGT
RT-miR-23a-3p	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGGAAT
miR-23a-3p-F	GCGATCACATTGCCAGGG
miR-23a-3p-R	AGTGCAGGGTCCGAGGTATT

### Data analysis

GraphPad 9.5 statistical software was used for analysis, measurement data were expressed as mean  $\pm$  standard deviation ( $\bar{x} \pm s$ ), one-way analysis of variance was used for comparison between multiple groups, and Tukey test was used for further pairwise comparison. Enumeration data were compared using the  $\chi^2$  test. Multivariate logistic regression was applied to analyse the risk factors affecting the occurrence of DKD. Pearson correlation analysis was used to determine the relationship between various indicators. Receiver operating characteristic (ROC) curves were plotted to analyse the value of miR-23a-3p levels in the diagnosis of DKD.  $P < 0.05$  was considered statistically significant.

## Results

### General data of enrolled patients

Clinical information was collected from each enrolled patient for one-way statistical analysis of variance, and the results are shown in Table 2. The levels of GLU, GA-L, Scr, UA, HbA<sub>1c</sub>, and CHOL in the NON, MIC,

and MAC groups were significantly higher than those in the CON group ( $P < 0.05$ ).

### Comparison of serum miR-23a-3p levels

Serum miR-23a-3p levels were gradually decreased in the CON, NON, MIC, and MAC groups ( $p = 0.0002$ ) (Fig. 1). This suggests that the expression levels of serum miR-23a-3p in T2DN patients at different stages are associated with T2DN disease progression and provide new targets for T2DN treatment.

### Correlation analysis between serum miR-23a-3p levels and clinical parameters of patients

Spearman correlation analysis showed that serum miR-23a-3p was positively correlated with Albu ( $r = 0.951, p \leq 0.001$ ), HbA<sub>1c</sub> ( $r = 0.331, p < 0.001$ ), CHOL ( $r = 0.243, p = 0.004$ ), GA-L ( $r = 0.398, p < 0.001$ ), Scr ( $r = 0.215, p = 0.010$ ), GLU ( $r = 0.399, p < 0.001$ ), and UA

Table 2. General data of enrolled patients

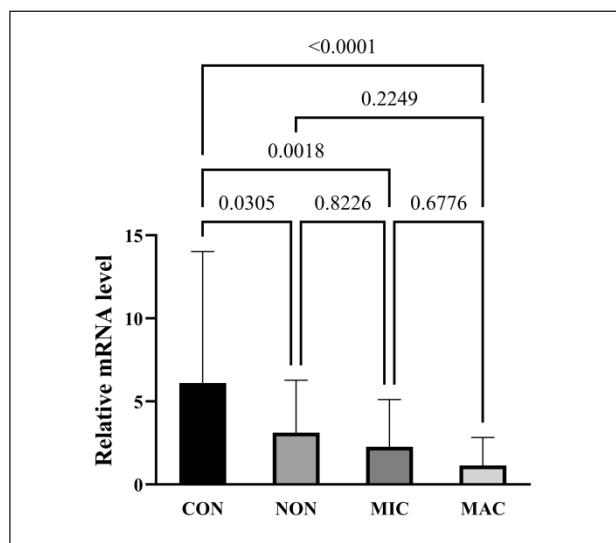
	CON (n = 30)	NON (n = 36)	DKD		F	p
			MIC (n = 41)	MAC (n = 35)		
Age	63.83 $\pm$ 12.82	60.17 $\pm$ 12.43	59.44 $\pm$ 11.37	55.43 $\pm$ 15.89	2.23	0.0875
Gender, M/F	14/16	19/17	24/17	18/17		
BMI [kg/m <sup>2</sup> ]	22.92 $\pm$ 3.01	24.81 $\pm$ 4.41	25.38 $\pm$ 3.51	24.25 $\pm$ 4.68	2.383	0.0721
SBP [mmHg]	131.73 $\pm$ 7.90	134.72 $\pm$ 12.81	136.44 $\pm$ 13.09	138.66 $\pm$ 10.42	2.114	0.1012
DBP [mmHg]	79.37 $\pm$ 6.87	80.64 $\pm$ 8.12	82.44 $\pm$ 9.15	83.20 $\pm$ 8.98	1415	0.2411
GLU [mmol/L]	5.24 $\pm$ 0.70 <sup>bcd</sup>	10.47 $\pm$ 3.56 <sup>ad</sup>	11.10 $\pm$ 4.69 <sup>a</sup>	13.71 $\pm$ 6.99 <sup>ab</sup>	18.47	< 0.0001
GA-L [%]	13.93 $\pm$ 1.98 <sup>bcd</sup>	25.04 $\pm$ 5.77 <sup>ad</sup>	26.22 $\pm$ 8.36 <sup>ad</sup>	30.79 $\pm$ 9.24 <sup>abc</sup>	32.06	< 0.0001
UREA [mmol/L]	5.95 $\pm$ 1.43	5.61 $\pm$ 1.71	6.49 $\pm$ 2.29	6.30 $\pm$ 2.44	1.36	0.2576
Scr [mol/L]	58.00 $\pm$ 11.15	58.28 $\pm$ 16.88 <sup>c</sup>	70.34 $\pm$ 24.51 <sup>b</sup>	66.49 $\pm$ 21.99	3.50	0.0173
UREA:Scr	0.11 $\pm$ 0.03	0.10 $\pm$ 0.03	0.10 $\pm$ 0.03	0.10 $\pm$ 0.03	0.4546	0.7145
UA [ $\mu$ mol/L]	281.77 $\pm$ 53.97 <sup>cd</sup>	348.33 $\pm$ 114.52	394.49 $\pm$ 132.54 <sup>a</sup>	388.43 $\pm$ 108.72 <sup>a</sup>	7.387	0.0001
LDH [U/L]	206.61 $\pm$ 35.38	193.61 $\pm$ 38.44	208.05 $\pm$ 57.05	224.60 $\pm$ 95.42	1.495	0.2187
HbA <sub>1c</sub> (%)	5.07 $\pm$ 0.58 <sup>bcd</sup>	9.24 $\pm$ 1.82	9.21 $\pm$ 1.86	9.84 $\pm$ 1.72	57.36	< 0.0001
CHOL [mmol/L]	4.75 $\pm$ 0.91 <sup>d</sup>	5.08 $\pm$ 1.66	5.57 $\pm$ 1.75	6.14 $\pm$ 2.47 <sup>a</sup>	3.753	0.0125
TG [mmol/L]	5.92 $\pm$ 24.76	3.05 $\pm$ 4.94	3.12 $\pm$ 4.01	7.82 $\pm$ 24.15	0.6854	0.5624
HDL-C [mmol/L]	1.49 $\pm$ 0.34 <sup>abc</sup>	1.18 $\pm$ 0.30 <sup>a</sup>	1.23 $\pm$ 0.34 <sup>a</sup>	1.14 $\pm$ 0.35 <sup>a</sup>	6.971	0.0002



Table 2. General data of enrolled patients

	CON (n = 30)	NON (n = 36)	DKD		F	p
			MIC (n = 41)	MAC (n = 35)		
LDL-C [mmol/L]	2.71 ± 0.60	2.86 ± 1.06	3.09 ± 1.3.4	3.44 ± 1.73	2.087	0.1047
Lpa [mg/L]	151.08 ± 91.22	197.06 ± 217.28	157.73 ± 131.06	191.67 ± 170.86	0.7289	0.5364
Albu [mg/L]	11.77 ± 7.54 <sup>d</sup>	5.49 ± 6.24 <sup>d</sup>	82.46 ± 75.55 <sup>d</sup>	496.68 ± 317.55 <sup>abc</sup>	71.87	< 0.0001
CREA [mmol/L]	9.82 ± 3.85 <sup>b</sup>	5.76 ± 4.73 <sup>a</sup>	7.15 ± 4.13	6.62 ± 3.34	3.741	0.0127
uACR [mg/g]	11.91 ± 7.69 <sup>d</sup>	8.17 ± 6.51 <sup>d</sup>	109.70 ± 85.66 <sup>d</sup>	836.13 ± 773.18 <sup>abc</sup>	36.92	< 0.0001

Note: Data are presented as mean (SD) unless otherwise stated. CON — control group; NON — diabetes mellitus group; MIC — type 2 diabetic nephropathy microalbuminuria group; MAC — type 2 diabetic nephropathy macroalbuminuria group; DKD — diabetic kidney disease; BMI — body mass index; SBP — systolic blood pressure; DBP — diastolic blood pressure; GLU — fasting blood glucose; GA-L — glycated albumin; UREA — serum urea; Scr — serum creatinine; UREA:Scr — serum urea-creatinine ratio; UA — serum uric acid; HbA<sub>1c</sub> — glycated haemoglobin; CHOL — total cholesterol; TG — triglycerides; LDH — lactate dehydrogenase; HDL-C — high-density lipoprotein; LDL-C — low-density lipoprotein; Lpa — lipoprotein; Albu — urinary microalbumin; CREA — urinary creatinine; uACR — urine protein-to-creatinine ratio. Compared with CON group, <sup>a</sup>p < 0.05; compared with NON group, <sup>b</sup>p < 0.05; compared with MIC group, <sup>c</sup>p < 0.05; compared with MAC group, <sup>d</sup>p < 0.05

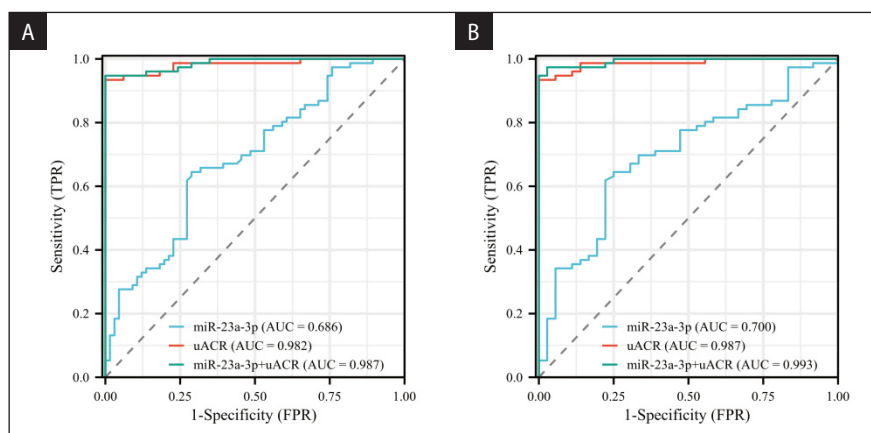


**Figure 1.** Comparison of serum miR-23a-3p levels ( $x \pm SD$ ). CON — control group; NON — diabetes mellitus group; MIC — type 2 diabetic nephropathy microalbuminuria group; MAC — type 2 diabetic nephropathy macroalbuminuria group

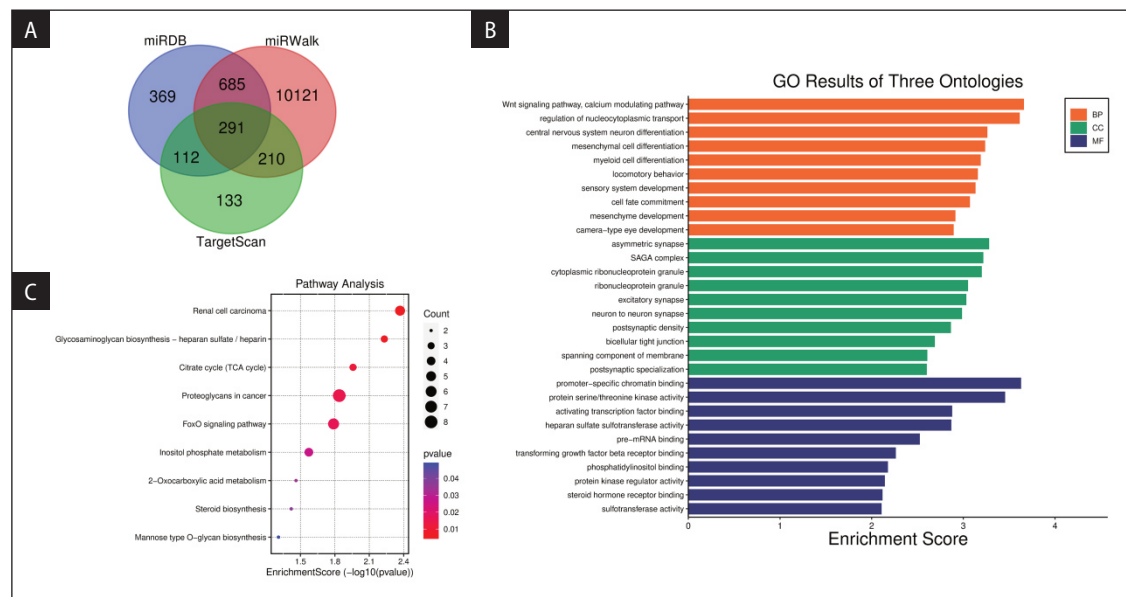
( $r = 0.277$ ,  $p < 0.001$ ), and negatively correlated with uACR ( $r = -0.624$ ,  $p < 0.001$ ) and HDL-C ( $r = -0.188$ ,  $p = 0.025$ ), but not with CREA ( $r = -0.105$ ,  $p = 0.215$ ).

#### Diagnostic value of miR-23a-3p in patients with DKD

ROC analysis showed that the area under the curve (AUC) of miR-23a-3p was 0.686 [95% confidence interval (CI): 0.599–0.773] among all enrolled members, indicating that miR-23a-3p was able to better distinguish healthy, diabetic DKD patients. Thus, we demonstrated that quantification of miR-23a-3p in serum can be used as a primary or at least auxiliary criterion for the diagnosis of DKD. Similarly, using serum miR-23a-3p as a diagnostic reference index, the sensitivity of diagnosing DKD in diabetes was 61.842%, the specificity was 77.778%, and the area under the ROC curve was 0.700 (95% CI: 0.598–0.802), and the results indicated that miR-23a-3p had some accuracy in predicting whether patients had diabetic nephropathy,



**Figure 2.** Diagnostic value of miR-23a-3p in patients with diabetic kidney disease (DKD). **A.** Diagnostic value of miR-23a-3p for DKD in the human population; **B.** Diagnostic value of miR-23a-3p for DKD in diabetes. TPR — true positive rate; FPR — false positive rate; AUC — area under the curve; uACR — urine protein-to-creatinine ratio



**Figure 3.** Target gene prediction and enrichment pathway analysis of miR-23a-3p. **A.** Venn diagram of target genes predicted by 3 databases; **B.** Gene ontology (GO) deals with classification of target genes. **C.** Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways enriched of target genes. BP — biological process; CC — cellular component; MF — molecular function

**Table 3.** Receiver operating characteristic (ROC) results for diabetic kidney disease (DKD) versus miR-23a-3p and urinary protein-creatinine ratio (uACR)

Predicted outcome	Predictor variables	AUC	CI	Cut-off value	Sensitivity	Specificity	Accuracy
CON	miR-23a-3p	0.686	0.599-0.773	0.96737	0.64474	0.71212	0.67606
	uACR	0.982	0.963-1.000	29.97	0.93421	1	0.96479
NON vs. DKD	miR-23a-3p + uACR	0.987	0.972-1.000	0.56489	0.94737	1	0.97183
NON vs. DKD	miR-23a-3p	0.700	0.598-0.802	0.94716	0.61842	0.77778	0.66964
	uACR	0.987	0.970-1.000	29.947	0.93421	1	0.95536
	miR-23a-3p + uACR	0.993	0.983-1.000	0.80397	0.94737	1	0.96429

AUC — area under the curve; CI — confidence interval; CON — control group; NON — diabetes mellitus group

while miR-23a-3p combined with uACR had high accuracy in detecting diabetic nephropathy.

Logistic regression analysis showed that low serum miR-23a-3p levels were associated with the occurrence of DKD [odds ratio (OR) = 0.837,  $p < 0.001$ ] using CON as the reference category, and decreased serum miR-23a-3p levels could also distinguish simple diabetes from diabetic nephropathy (OR = 0.841,  $p = 0.019$ ) using the NON group as the reference category. In the multivariate model, in addition to serum miR-23a-3p, annual GLU, GA-L, Scr, UA, HbA<sub>1c</sub>, CHOL, HDL-C, Albu, CREA, and uACR were also included, and the results showed that serum miR-23a-3p levels were not associated with the occurrence of DKD after adjusting for GLU, GA-L, Scr, UA, HbA<sub>1c</sub>, CHOL, HDL-C, Albu, CREA, and uACR effects (OR = 0.000,  $p = 0.998$ ), and the differentiation between

CON and DKD remained statistically insignificant (OR = 0.000,  $p = 0.963$ ).

### Target gene prediction and enrichment pathway analysis of miR-23a-3p

Predicting the genes and pathways that may be regulated by miR-23a-3p by bioinformatics provides a direction for the further search for the pathogenesis of DKD. We predicted the target genes of miR-23a-3p in 3 databases: miRWalk, TargetScan, and miRDB, respectively, and analysed the intersections for enrichment pathway analysis on the DAVID website. The results showed that the intersection involved 291 mRNAs (Fig. 3). Assuming these 291 screened genes are target genes of miR-23a-3p, we performed GO and KEGG analysis to search for potential biological processes or pathways. The results showed that the enrichment pathway included

**Table 4. Multivariate logistic regression analysis of the diagnostic value of serum miR-23a-3p levels for diabetic kidney disease (DKD)**

	Univariate analysis			Multivariate analysis		
	$\chi^2$	OR	p	$\chi^2$	OR (95%CI)	p
Model 1	11.089	0.837	0.001	0.000	0.000	0.998
Model 2	5.502	0.841	0.019	0.002	0.000	0.963

OR — odds ratio; CI — confidence interval; CON — control group; NON — diabetes mellitus group

Note: In Model 1, non-independent variables were dichotomous variables of the "DKD and CON groups", with the "CON group" as the reference category; in Model 2, non-independent variables were dichotomous variables of the "NON and DKD groups", with the "NON group" as the reference category; univariate analysis, independent variables were serum miR-23a-3p; multivariate analysis, independent variables were serum miR-23a-3p and GLU (fasting blood glucose), GA-L (glycated albumin), Scr (serum creatinine), UA (serum uric acid), HbA<sub>1c</sub> (glycated haemoglobin), CHOL (total cholesterol), HDL-C (high-density lipoprotein), Albu (urinary microalbumin), CREA (urinary creatinine), and uACR (urine protein-to-creatinine ratio)

9 pathways: renal cell carcinoma, glycosaminoglycan biosynthesis-heparin sulphate/heparin, citrate cycle (TCA cycle), proteoglycans in cancer, FOXO signalling pathway, inositol phosphate metabolism, 2-oxocarboxylic acid metabolism, steroid biosynthesis, and mannose type O-glycan biosynthesis. According to our above findings, it is believed that miR-23a-3p may aggravate renal injury by activating FOXO-mediated oxidative stress pathway.

## Discussion

DKD is the most serious and common microvascular complication of diabetes, and DKD has become the second leading cause of end-stage renal disease. However, due to the complexity of the aetiology of diabetic nephropathy, multiple factors are involved, and the molecular mechanisms involved in its pathogenesis remain not well understood. The development of DKD is related to immune inflammatory response, oxidative stress, metabolic abnormalities and epigenetics, and the pathogenesis of epigenetics in DKD is currently the focus of research. With the rapid development of miRNA detection and quantitative technology, including gene chip, quantitative PCR, and high-throughput sequencing, the development of miRNAs as potential biomarkers and therapeutic targets for human diseases has become a research hotspot. miRNAs are stably present in human plasma and urine, and miRNAs present in blood are sensitive biomarkers of cancer, tissue damage, and heart failure [7, 8]. As a class of non-coding small RNAs, miRNAs affect various life activities of organisms by participating in the regulation of cellular gene expression, and they play an important role in the development of DKD and are expected to be potential markers for the diagnosis of DKD as well as new targets for drug therapy [9].

Previous studies have shown that miRNAs are involved in the pathogenesis of DKD by affecting the expression of multiple signalling molecules in mul-

ti-ple signalling pathways and are associated with renal impairment, interstitial fibrosis, and podocyte apoptosis in DKD patients, which may provide new ideas for understanding the pathogenesis of DKD [10, 11]. TAYEL et al. found that miR-126 and miR-192 expression was down-regulated in DKD patients compared with healthy controls, and further analysis revealed that miR-126 and miR-192 expression was negatively correlated with creatinine levels and urinary albumin creatinine ratio (ACR) [12]. In DKD mouse kidney, miR-21 is one of the more expressed miRNAs, and inhibition of this miRNA in vitro and in vivo reduces mesangial matrix expansion, interstitial fibrosis, macrophage infiltration, podocyte loss, proteinuria, and expression of inflammatory and fibrotic molecules. miR-21 antagonist can improve the structure and function of kidney in DKD mice and is an effective drug for the treatment of diabetic complications [13]. CIVANTOS et al. found that sitagliptin, as a dipeptidyl peptidase-4 (DPP-4) inhibitor, alleviated miRNA-mediated oxidative stress in DKD rats by downregulating the miR-200a/Keap-1/Nrf2 signalling pathway [14]. The above findings suggest that miRNAs can be used as molecular markers and therapeutic targets for DKD.

In this study, we showed that serum miR-23a-3p levels significantly decreased in the CON, NON, MIC, and MAC groups, suggesting that low miR-23a-3p expression may be involved in the development of DKD. MA et al. showed that miRNAs regulate a variety of biological cell functions, and altered expression of miRNAs is closely related to DKD development and may be an independent factor predicting the development of DKD [15]. YU et al. suggested that miRNAs may be involved in the development and progression of DKD by promoting renal fibrosis and neovascularization and can be used as noninvasive biological indicators for the diagnosis of DKD [16]. In this study, we showed that miR-23a-3p is an important risk factor affecting the development of DKD in patients with type 2 diabetes. These results suggest that serum miR-23a-3p levels

are decreased in patients with type 2 diabetes, and their risk of developing DKD is high. Urinary microalbumin to creatinine ratio uACR is an effective indicator for the diagnosis of glomerular microangiopathy, and elevated uACR levels can promote the development of DKD and are risk factors for predicting the development of DKD.

In this study, ROC curve analysis showed that miR-23a-3p combined with uACR had the largest area under the curve for the diagnosis of DKD, indicating that the combined detection of miR-23a-3p and uACR had a better efficacy for the diagnosis of DKD, and miR-23a-3p is expected to be a novel biomarker for the diagnosis of DKD. In addition to serum miR-23a-3p, annual GLU, GA-L, Scr, UA, HbA<sub>1c</sub>, CHOL, HDL-C, Albu, CREA, and uACR were also included in the logistic regression multivariate model, and the results showed that serum miR-23a-3p levels were not associated with the occurrence of DKD after adjusting for the effects of other parameters, and the differentiation between CON and DKD remained statistically insignificant. In summary, serum miR-23a-3p levels were significantly lower in DN patients, which is a risk factor for DKD in type 2 diabetic patients, but not an independent risk factor, and its combination with uACR has good value in the diagnosis of DKD.

In summary, serum miR-23a-3p levels in T2DN patients gradually decrease with the progression of the disease, and serum miR-23a-3p is closely related to blood glucose, lipid metabolism, and proteinuria in T2DN patients and plays an important role in the progression of T2DN, which may become a new marker for the diagnosis of T2DN and indicate the progression of the disease, as well as providing new ideas for the treatment of T2DN proteinuria. However, the comparison of serum miR-23a-3p with microalbuminuria parameters and its specific pathophysiological mechanism during the development of DKD still need to be studied in depth. Given the limited number of cases in this study, further sample size expansion, prospective studies, and clinical follow-up are still needed to revalidate and determine the clinical value of serum miR-23a-3p in patients with DKD.

### Acknowledgments

XM participated in the literature search, data collection, experimental operation, data analysis and manuscript preparation throughout the study. XY participated in the sample collection and analysis; Both XM and PL were involved in the entire study design and manuscript preparation and review. All authors have read and approved the final version for publication.

### Funding

None.

### Conflict of interest

None.

### References

- Gross JL, de Azevedo MJ, Silveiro SP, et al. Diabetic nephropathy: diagnosis, prevention, and treatment. *Diabetes Care*. 2005; 28(1): 164–176, doi: [10.2337/diacare.28.1.164](https://doi.org/10.2337/diacare.28.1.164), indexed in Pubmed: [15616252](https://pubmed.ncbi.nlm.nih.gov/15616252/).
- Adler AI, Stevens RJ, Manley SE, et al. UKPDS GROUP. Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64). *Kidney Int*. 2003; 63(1): 225–232, doi: [10.1046/j.1523-1755.2003.00712.x](https://doi.org/10.1046/j.1523-1755.2003.00712.x), indexed in Pubmed: [12472787](https://pubmed.ncbi.nlm.nih.gov/12472787/).
- American Diabetes Association. Standards of medical care in diabetes—2008. *Diabetes Care*. 2008; 31 Suppl 1: S12–S54, doi: [10.2337/dc08-S012](https://doi.org/10.2337/dc08-S012), indexed in Pubmed: [18165335](https://pubmed.ncbi.nlm.nih.gov/18165335/).
- Ninomiya T, Perkovic V, de Galan BE, et al. ADVANCE Collaborative Group. Albuminuria and kidney function independently predict cardiovascular and renal outcomes in diabetes. *J Am Soc Nephrol*. 2009; 20(8): 1813–1821, doi: [10.1681/ASN.2008121270](https://doi.org/10.1681/ASN.2008121270), indexed in Pubmed: [19443635](https://pubmed.ncbi.nlm.nih.gov/19443635/).
- Sankrityayan H, Kulkarni YA, Gaikwad AB. Diabetic nephropathy: The regulatory interplay between epigenetics and microRNAs. *Pharmacol Res*. 2019; 141: 574–585, doi: [10.1016/j.phrs.2019.01.043](https://doi.org/10.1016/j.phrs.2019.01.043), indexed in Pubmed: [30695734](https://pubmed.ncbi.nlm.nih.gov/30695734/).
- Li Mo, Guo Q, Cai H, et al. miR-218 regulates diabetic nephropathy via targeting IKK- and modulating NK- B-mediated inflammation. *J Cell Physiol*. 2020; 235(4): 3362–3371, doi: [10.1002/jcp.29224](https://doi.org/10.1002/jcp.29224), indexed in Pubmed: [31549412](https://pubmed.ncbi.nlm.nih.gov/31549412/).
- Cho HJ, Eun JW, Baek GOK, et al. Serum Exosomal MicroRNA, miR-10b-5p, as a Potential Diagnostic Biomarker for Early-Stage Hepatocellular Carcinoma. *J Clin Med*. 2020; 9(1), doi: [10.3390/jcm9010281](https://doi.org/10.3390/jcm9010281), indexed in Pubmed: [31968558](https://pubmed.ncbi.nlm.nih.gov/31968558/).
- Han R, Li Ke, Li Li, et al. Expression of microRNA-214 and galectin-3 in peripheral blood of patients with chronic heart failure and its clinical significance. *Exp Ther Med*. 2020; 19(2): 1322–1328, doi: [10.3892/etm.2019.8318](https://doi.org/10.3892/etm.2019.8318), indexed in Pubmed: [32010305](https://pubmed.ncbi.nlm.nih.gov/32010305/).
- Kim H, Bae YU, Jeon JS, et al. The circulating exosomal microRNAs related to albuminuria in patients with diabetic nephropathy. *J Transl Med*. 2019; 17(1): 236, doi: [10.1186/s12967-019-1983-3](https://doi.org/10.1186/s12967-019-1983-3), indexed in Pubmed: [31331349](https://pubmed.ncbi.nlm.nih.gov/31331349/).
- Yang F, Cui Z, Deng H, et al. Identification of miRNAs-genes regulatory network in diabetic nephropathy based on bioinformatics analysis. *Medicine (Baltimore)*. 2019; 98(27): e16225, doi: [10.1097/MD.00000000000016225](https://doi.org/10.1097/MD.00000000000016225), indexed in Pubmed: [31277135](https://pubmed.ncbi.nlm.nih.gov/31277135/).
- Peters LJE, Floege J, Biessen EAL, et al. MicroRNAs in Chronic Kidney Disease: Four Candidates for Clinical Application. *Int J Mol Sci*. 2020; 21(18), doi: [10.3390/ijms21186547](https://doi.org/10.3390/ijms21186547), indexed in Pubmed: [32906849](https://pubmed.ncbi.nlm.nih.gov/32906849/).
- Tayel SI, Saleh AA, El-Hefnawy SM, et al. Simultaneous Assessment of MicroRNAs 126 and 192 in Diabetic Nephropathy Patients and the Relation of these MicroRNAs with Urinary Albumin. *Curr Mol Med*. 2020; 20(5): 361–371, doi: [10.2174/1566524019666191019103918](https://doi.org/10.2174/1566524019666191019103918), indexed in Pubmed: [31629394](https://pubmed.ncbi.nlm.nih.gov/31629394/).
- Kölling M, Kaucsar T, Schauer C, et al. Therapeutic miR-21 Silencing Ameliorates Diabetic Kidney Disease in Mice. *Mol Ther*. 2017; 25(1): 165–180, doi: [10.1016/j.ymthe.2016.08.001](https://doi.org/10.1016/j.ymthe.2016.08.001), indexed in Pubmed: [28129112](https://pubmed.ncbi.nlm.nih.gov/28129112/).
- Civantos E, Bosch E, Ramirez E, et al. Sitagliptin ameliorates oxidative stress in experimental diabetic nephropathy by diminishing the miR-200a/Keap-1/Nrf2 antioxidant pathway. *Diabetes Metab Syndr Obes*. 2017; 10: 207–222, doi: [10.2147/DMSO.S132537](https://doi.org/10.2147/DMSO.S132537), indexed in Pubmed: [28652790](https://pubmed.ncbi.nlm.nih.gov/28652790/).
- Ma J, Wang Y, Xu HT, et al. MicroRNA: a novel biomarker and therapeutic target to combat autophagy in diabetic nephropathy. *Eur Rev Med Pharmacol Sci*. 2019; 23(14): 6257–6263, doi: [10.26355/eurrev\\_201907\\_18446](https://doi.org/10.26355/eurrev_201907_18446), indexed in Pubmed: [31364128](https://pubmed.ncbi.nlm.nih.gov/31364128/).
- Yu J, Yu C, Feng B, et al. Intrarenal microRNA signature related to the fibrosis process in chronic kidney disease: identification and functional validation of key miRNAs. *BMC Nephrol*. 2019; 20(1): 336, doi: [10.1186/s12882-019-1512-x](https://doi.org/10.1186/s12882-019-1512-x), indexed in Pubmed: [31455266](https://pubmed.ncbi.nlm.nih.gov/31455266/).