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Evaluation of serum MicroRNA 21, MicroRNA 192 and serum TGFβ1 in type 2 diabetes mellitus patients and their relation to diabetic nephropathy

Jumana Gamal Abou Eleila[1*], Amal Abdel Wahab Mohamed[2], Emam Abdalatif Waked[3], Laila Nessim Kamel[1], Hanan Shawky Amin[1] and Hadeel Mohammad Elhanafi[2]

Abstract

Background Diabetic nephropathy (DN) is a frequent and long-lasting microvascular consequence that has an established connection with diabetes. It serves as the primary etiological agent of end-stage renal disease, a critical renal disorder that develops on a worldwide level. The molecular pathophysiology of DN is multifactorial, such as transforming growth factor-beta [TGF-β] which affects the expression of miRNAs such as miRNA-21 and miRNA-192 during renal fibrosis. However, to date, the clinical application is inadequate due to discrepancies observed in the published data. This cross-sectional investigation aimed to assess the correlation between serum TGF-β1, miRNA-21 and 192, and glycemic control, metabolic abnormalities, and renal function in patients with type II diabetes.

Methods Based on the albumin/creatinine ratio (ACR), fifty subjects with type II diabetes were divided into three categories: Group I consisted of individuals with normoalbuminuria ($n=16$), Group II of microalbuminuria ($n=16$), and Group III of overt proteinuria ($n=18$). All participants were subjected to the estimation of mature miRNA-21 and miRNA-192 by TaqMan two-step stem loop qRT-PCR and serum TGFβ1 level by ELISA.

Results There was an upregulation in miRNA-21 expression in the 3 different groups of patients (p value $=0.043$). The serum fold change (FC) of miRNA-21 showed significantly greater median values in patients with overt proteinuria compared to those with normoalbuminuria (5.57 FC versus 1.11 FC, $p=0.017$). A positive correlation ($r=0.343$) ($p=0.013$) was observed between the ACR and the median levels of miRNA-21, which was statistically significant. No statistically significant distinctions were detected in the concentrations of serum TGF-β1 or miRNA-192 among the three patient groups (p values of 0.234 and 0.225, respectively).

Conclusion The findings of the present research implied that miRNA-21 might function as an early indicator of renal pathology associated with diabetes mellitus (DM).

Keywords Diabetic nephropathy, TGF-β, miR21, miR192

*Correspondence:
Jumana Gamal Abou Eleila
joeaboueleila@yahoo.com
[1] Department of Clinical Chemistry, Theodor Bilharz Research Institute, Giza, Egypt
[2] Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt
[3] Department of Nephrology, Theodor Bilharz Research Institute, Giza, Egypt

Background

Diabetes mellitus (DM) is a multifaceted and complicated metabolic disorder characterized by heightened glucose levels in the bloodstream due to impaired secretion of insulin, insulin activity, or both. There exists a correlation between diabetes-related chronic hyperglycemia and enduring dysfunction, injury, and failure of multiple systems, particularly neurological, optic, renal, and cardiovascular systems [1].

Diabetic nephropathy (DN) is a prominent sequel of DM distinguished by enlargement of glomeruli, deposits of extracellular matrix, and thickening of the membranes of the basement membrane, tubules and glomeruli. Chronic renal failure ultimately ensues as a consequence of these alterations, which manifest as tubulointerstitial and glomerular fibrosis and sclerosis [2, 3]. The International Diabetes Federation (IDF) estimates that 40% of diabetics may get end-stage renal failure [4].

TGF-β is an extremely versatile regulator that exerts control over an extensive array of cellular processes, including but not limited to adhesion, migration, extracellular matrix formation, and cell proliferation. The physiological presence of TGF-β is essential for support of normal development, tissue healing, and organ functionality [5].

Individuals with diabetes, irrespective of the form of the disease (type I or type II), demonstrate elevated levels of TGF-β expression in the tubules and glomeruli throughout the disease's early and late stages. A direct correlation has been identified between the level of glycemic control achieved by individuals with diabetes and the synthesis of TGF-β [6].

Intracellular signaling is initiated when TGF-β binds to receptor complexes composed of type I and type II transmembrane serine/threonine kinases that are closely related TβR-I and TβR-II, respectively. Smads function as intracellular mediators of TGF-β signaling by acting in the direction opposite to the TβR-I receptors [7, 8]. TGF-β1 acts as a transcriptional regulator of renal inflammation and fibrosis by stimulating signaling molecules, such as Smad3 and noncoding RNAs dependent on Smad3. This process is inhibited by Smad7 [9].

MiRNAs are endogenous single-stranded noncoding RNAs that are involved in post-transcriptional control of a variety of cellular biological functions. Base-pairing with the target messenger RNAs (mRNAs), commonly in the 3`UTR, might contribute to this control. When a miRNA attaches to the target mRNA, it usually causes translational repression as well as exonucleolytic mRNA degradation [10]. Prior studies have indicated that microRNAs (miRNAs) may influence the course of diabetic kidney disease, specifically by regulating fibrosis via the action of TGF-β1 [11].

An analysis of microRNA expression patterns has revealed a majority of a specific cluster of miRNAs in the kidneys of adult humans: miRNA 215, miRNA 146a, and miRNA 886. Furthermore, the kidney contains greater quantities of additional miRNAs, including miRNA 192, miRNA 194, miRNA 21, miRNA 200a, and miRNA 204, in comparison with the other organs [12]. Through the establishment of an intricate network comprising targeted genes and signaling cascades, miRNA-21 contributes to the advancement of DN. That network facilitates various biological processes, including fibrosis, inflammation, extracellular matrix deposition and epithelial-to-mesenchymal transition [13]. The upregulation of smad3 expression and downregulation of smad7 expression contribute to the enhancement of epithelial-to-mesenchymal transition (EMT) provoked via TGF-β1. The application of inhibitors that specifically target miRNA-21 not only impedes the advancement of renal fibrosis and EMT in patients with DN but also enhances the structural integrity and functional capacity of the kidney. Suppression of miRNA-21 has been described as an optional therapy target for DN renal fibrosis [14].

MiRNA-192 demonstrates a notable degree of expression within the renal cortex of the kidney. Numerous studies have provided evidence that miRNA-192 contributes to the pathogenesis of hepatic as well as renal fibrosis. Nevertheless, the precise influence of miRNA-192 on diabetic nephropathy (DN) remains an issue of controversy [15].

Regarding the contribution of serum TGFβ1, miRNA 21 and 192 in DN pathogenesis, we aimed in this cross-sectional investigation to assess the correlation between them and glycemic control, metabolic abnormalities, and renal function in patients with type II diabetes.

Methods

Patients

The present research study was executed on fifty patients diagnosed with type II diabetes (mean age 58.1 ± 6.1 years, 42% males) recruited from the Nephrology Department in Theodor Bilharz Research Institute (Giza, Egypt) in the period from November 2018 to February 2019.

Study population

Diabetic patients diagnosed with fasting plasma glucose ≥ 126 mg/dl or 2 h postprandial ≥ 200 mg/dl during oral glucose tolerance test (OGTT) or hemoglobin A1c ≥ 6.5% were included.

Patients with nephropathy not caused by diabetes, acute inflammation, tuberculosis, autoimmune diseases, cancer, cardiovascular diseases, or patients with other

endocrinal disorders apart from diabetes were excluded from this study.

The studied individuals were classified according to ACR into 3 groups:

Group (I): individuals with type II diabetes and normoalbuminuria ($n=16$, mean age 57.69 ± 5.79 years, 7 males and 9 females, ACR < 30 mg/g).

Group (II): individuals with type II diabetes and microalbuminuria ($n=16$, mean age 56.25 ± 4.17 years, 6 males and 10 females, ACR > 30 and < 300 mg/g).

Group (III): individuals with type II diabetes and overt proteinuria ($n=18$, mean age 60.22 ± 7.35 years, 8 males and 10 females, ACR > 300 mg/g).

Patients were identified as having diabetes if they exhibited fasting plasma glucose levels of at least 126 mg/dL or 2 h postprandial plasma glucose levels of at least 200 mg/dL during an OGTT. Additionally, the condition was established if hemoglobin A1c levels exceeded 6.5%.

Patients were categorized as having diabetic nephropathy if they exhibited two out of three consecutive urinary ACRs of more than 30 mg/g.

Individuals with diabetes who presented with nephropathy due to causes such as heart failure, liver disease, neurological or other endocrinological disorders, malignancies, acute or chronic infections, or autoimmune diseases were considered ineligible for participation in the study.

Age, gender, BMI, and diabetes duration were among the demographic and clinical data collected.

The patient's medical history was examined to identify any prior myocardial infarction or stroke. The identification of concurrent hypertension was achieved by monitoring blood pressure measurements that exceeded 140/90 mmHg or by detecting the administration of medications for hypertension. Standard fundoscopy and neurological examinations were employed to ascertain the absence of retinopathy and neuropathy, respectively.

Sampling

Serum samples necessary for routine laboratory testing include plasma fasting glucose, postprandial glucose, kidney function tests and lipid profile. To analyze HbA1c, EDTA samples were obtained, whereas urine samples were acquired to determine the urinary albumin/creatinine ratio (ACR). A Beckman Coulter AU480 analyzer was utilized for the analysis (Beckman Coulter, Brea, California, USA). The calculation of estimated GFR (eGFR) applied the Modification of Diet in Renal Disease (MDRD) formula:

"eGFR = 175 × (Serum creatinine) − 1.154 × (Age) − 0.203 × (0.742 if female) × (1.212 if African American) (mL/min/1.73 m^2)" [16].

Serum samples used were stored at − 80 °C until RNA extraction for determination of the expression level of mature miR-21 and miR-192.

Written informed consent was acquired from every patient involved. The research methodology followed the Helsinki Declaration of 1975, as amended in 2012, and obtained permission from the A institutional review board before initiating subject enlistment [17].

TGF-β1 level

The amount of TGF-β1 was measured by utilizing the Human TGF beta1 platinum ELISA kit from Affymetrix eBioscience, catalog number BMS249-4. The purpose of developing an ELISA for human TGF-β1 solid-phase sandwich was to determine the amount of target that is attached between two matched antibodies.

Analysis of the expression of miRNA genes

Serum RNA was extracted utilizing the miRNeasy Mini Kit (Catalog no. 217004) to profile miRNA expression. miRNA complementary DNA (cDNA) was synthesized via reverse transcription of RNA using the stem-loop RT primer and the TaqMan® MicroRNA Reverse Transcription reagent. This enabled the reverse transcription of synthetic controls and target miRNAs simultaneously. Real-time PCR was conducted utilizing TaqMan microRNA assays that are specific to the mature sequence under evaluation. miR-21 (hsa-miR-21-3p) MIMAT0004494 with mature sequence CAACAC CAGUCGAUGGGCUGU, miR-192 (hsa-miR-192-3p) MIMAT0007017 with mature sequence CUGCCAAUU CCAUAGGUCACAG and Cel-miR-39 (Cel-mir-39-3p) MIMAT0000010 with mature sequence UCACCGGGU GUAAAUCAGCUUG.

The qPCRs were performed employing an Applied Biosystems StepOne real-time PCR instrument. The cycle threshold was implemented to figure out the level of miRNA expression. To determine the degree of expression demonstrated by a particular miRNA, the CT value of that miRNA is subtracted from the average CT value of reference genes per sample in a provided set of samples. As a standard, the synthetic control gene was utilized. Using equation $2^{-\Delta\Delta CT}$, the relative expression (fold change) of each putative miRNA in every group was calculated, with Cel-miR-39 serving as the reference gene. The ΔCT value was calculated for each miRNA in each sample utilizing the subsequent formula: to determine the ΔCT sample, the CT value of the miRNA is subtracted from the CT value of Cel miR 39. Next, the ΔΔCT value was determined using the formula: ΔΔCT = (CT miRNA − CT Cel miR 39) for the patient group − (CT miRNA − CT Cel-miR 39) for the control group.

Chemicals used

TGF-β1 was measured using the Human TGF beta1 platinum ELISA kit from Affymetrix eBioscience, catalog number BMS249-4. Serum RNA extraction was done utilizing the miRNeasy Mini Kit (Catalog no. 217004). cDNA was synthesized via reverse transcription of RNA using the stem-loop RT primer and the TaqMan® MicroRNA Reverse Transcription reagent. Real-time PCR was conducted utilizing TaqMan microRNA assays and performed on an Applied Biosystems StepOne real-time PCR instrument.

Statistical analysis

For classification and data entry, version 26 of the Statistical Package for the Social Sciences (SPSS) (IBM Corp., Armonk, NY, USA) was used. The quantitative data were specified by the minimum and maximum values, the mean, standard deviation, and median. An alternative approach was used to describe the categorical data by employing frequency (count) and relative frequency (percent). The Kruskal–Wallis and Mann–Whitney tests, which are nonparametric, have been used to compare quantitative variables. Categorical data were compared utilizing the chi-squared test (χ^2). The precise test was used in situations where the expected frequency was less than five. To determine the correlations between quantitative variables, the Spearman correlation coefficient was applied. We considered P-values less than 0.05 to be statistically significant. An analysis of the area under the receiver operating characteristic (ROC) curve was performed to ascertain the most effective cutoff value.

Results

Table 1 contains demographic, clinical, and biochemical information regarding the study participants.

Table 1 Demographic, clinical and biochemical characteristics of studied participants

	DM with normoalbuminuria (*n* = 16)	DM with microalbuminuria (*n* = 16)	DM with overt proteinuria (*n* = 18)	*p* value
Age (years)	57.69 ± 5.79	56.25 ± 4.17	60.22 ± 7.35	0.404
Gender (male %)	7 (43.8%)	6 (37.5%)	8 (44.4%)	0.906
Duration of diabetes (years)	5.0 (0.0–9.0)	5.5 (0.0–20.0)	6.5 (0.0–20.0)	0.386
SBP (mmHg)	138.75 ± 12.45	135.00 ± 15.06	142.78 ± 10.60	0.193
DBP (mmHg)	89.06 ± 7.58	87.19 ± 6.57	91.94 ± 5.46	0.114
BMI	35.14 ± 2.89	34.35 ± 3.03	35.66 ± 1.89	0.603
Urea (mg/dl)	26.0 (19.0–44.0) (a)	32.10 (16.0–106.7) (a)	104.5 (49.0–222.5) (b)	**< 0.001**
Creat (mg/dl)	0.87 (0.75–1.3) (a)	0.86 (0.60–4.17) (a)	3.0 (1.34–10.16) (b)	**< 0.001**
eGFR (ml/min/1.73m^2)	77.0 (58.0–99.0) (a)	78.5 (16.0–102.0) (a)	19.00(4.0–45.0) (b)	**< 0.001**
FBS (mg/dl)	158.0 (99.0–292.0)	210.5(112.0–484.0)	173.5 (130.0–289.0)	0.066
PP (mg/dl)	237 (182.0–395.0)	312.5 (170.0–543.0)	233.0 (184.0–360.0)	0.125
HbA1c (%)	7.5 (6.5–12.6) (a)	9.3 (6.5–14.9) (b)	7.15 (5.1–11.3) (a)	**0.007**
Chol (mg/dl)	206.56 ± 47.50	207.00 ± 51.53	205.28 ± 49.59	0.806
TG (mg/dl)	150.5 (66.0–245.0)	137.5(83.0–266.0)	110.0 (46.0–202.0)	0.135
HDL (mg/dl)	44.00 ± 9.19	42.12 ± 11.89	41.83 ± 4.90	0.698
LDL (mg/dl)	132.5 (74.0–198.0)	139.5 (29.0–191.0)	133.5 (78.0–251.0)	0.888
miR21 (FC)	1.11 (0.04–4.41) (a)	3.01 (0.31–101.83)	5.57 (0.28–39.73) (b)	**0.043**
miR192 (FC)	1.20 (0.24–6.28)	0.72 (0.16–8.37)	0.51 (0.04–8.75)	0.234
TGF-β1 (pg/ml)	10,838 (1535–35,402)	15,691 (1356–27,037)	18,692 (1356–37,698)	0.225

Data are stated as mean ± SD or median (25th–75th percentile range) or frequency (percent). *p* Value is significant < 0.05. a, b Groups sharing the same letters don't differ from one another at *p* = 0.05 degree of significance. SBP: systolic blood pressure. DBP: diastolic blood pressure. BMI: body mass index. eGFR: estimated glomerular filtration rate. FBS: fasting blood sugar. PP: postprandial glucose Chol: cholesterol. TG: triglyceride. HDL: high-density lipoprotein. LDL: low-density lipoprotein. FC: fold change. The results were presented for each candidate miRNA as a fold change within each group, which was estimated using equation $2^{-\Delta\Delta CT}$

Table 2 Serum expression of miR21, miR192 and serum TGF-β1 in diabetic normoalbuminuric group and diabetic microalbuminuric + proteinuric group

	DM with normoalbuminuria (*n* = 16)	DM with microalbuminuria, overt proteinuria (*n* = 34)	*p* value
miR21 (FC)	1.11 (0.04–4.41)	3.88 (0.28–101.83)	0.013
miR192 (FC)	1.20 (0.24–6.28)	0.56 (0.04–8.75)	0.167
TGF-β1 (pg/mL)	10,837 (1534–35,402)	16,772 (1356–37,698)	0.140

Results are represented as median (25th–75th percentile range). FC = fold change. *p* value is significant < 0.05

Table 3 Serum expression of miR21, miR192 and serum TGF-β1 based on the absence or presence of complications (in the form of retinopathy, peripheral neuropathy, chronic kidney disease or ischemic heart disease)

	No complications (*n* = 18)	Complications (*n* = 32)	*p* value
miR21(FC)	1.97 (0.04–23.49)	2.11 (0.28–101.83)	0.505
miR192(FC)	0.54 (0.18–6.28)	0.85 (0.04–8.75)	0.385
TGF-β1 (pg/mL)	12,016 (1356–35,402)	16,772 (1356–37,698)	0.067

Data represented as median (25th–75th percentile range; results were expressed as fold change (FC); *p* value is significant < 0.05

A statistically significant difference in miR21 expression was identified across the three groups under investigation (p value = 0.043). The median values of miRNA21 FC in the serum of the overt proteinuric group were significantly higher than those of the normoalbuminuric group (5.57 FC versus 1.11 FC, $p = 0.017$). There were no statistically significant variations observed in the expression of miR192 or TGF-β1 serum levels across the three groups under investigation (Tables 1, 2, 3, 4, 5, and Figs. 1, 2, 3).

As a potential diagnostic marker for the progression of kidney insult, serum expression of miR21 and miR192, as well as blood level of TGF1, were assessed using a receiver operating characteristic curve (ROC) analysis (Fig. 4). Serum miR21 exhibited a sensitivity of 55.9% and specificity of 88% in detecting the progression of kidney insult at a cut-off value of 3.258 FC; the AUC was 0.719 (p = 0.013, 95% CI 0.579–0.858).

	Area under the curve	*p* value	95% CI		Cut off	Sensitivity %	Specificity %
			Lower bound	Upper bound			
miR21	0.719	0.013	0.579	0.858	3.258	55.9	87.5
miR192	0.378	0.167	0.215	0.540	–	–	–
TGF-β1	0.631	0.140	0.451	0.810	–	–	–

Table 4 Correlation analysis between the studied markers and both demographic and laboratory data in studied patients

	TGF-β1		miR-21		miR-192	
	R	*p*	*r*	*p*	*r*	*p*
Age (year)	−0.023	0.872	−0.001	0.972	0.033	0.822
Duration of diabetes (years)	−0.017	0.909	−0.122	0.399	−0.303	0.033
Urea (mg/dl)	0.227	0.113	0.163	0.258	−0.204	0.154
Creat (mg/dl)	0.157	0.276	0.102	0.480	−0.201	0.162
eGFR (ml/min/1.73 m^2)	−0.210	0.143	−0.142	0.326	0.165	0.252
A/C ratio (mg/g)	0.255	0.074	0.343	0.015	−0.155	0.283
FBS (mg/dl)	0.043	0.767	0.261	0.067	0.180	0.212
PP (mg/dl)	−0.064	0.657	0.121	0.403	0.120	0.407
HbA1C (%)	−0.205	0.154	−0.041	0.775	0.042	0.771
Chol (mg/dl)	−0.045	0.758	0.127	0.381	0.095	0.513
TG (mg/dl)	−0.276	0.053	−0.030	0.838	−0.005	0.973
LDL (mg/dl)	−0.009	0.949	0.121	0.402	0.117	0.419
HDL (mg/dl)	0.149	0.303	0.140	0.331	0.113	0.433

r = correlation coefficient. *P* = *p* value. *r* < 0.3: no correlation. *r* = 0.3– < 0.5: weak correlation. *r* = 0.5: fair correlation. *r* = > 0.5–0.75: good correlation. *r* > 0.75: very good correlation. eGFR: estimated glomerular filtration rate. *A/C* ratio: albumin/creatinine ratio. FBS: fasting blood sugar. PP: postprandial glucose Chol: cholesterol. TG: triglyceride. HDL: high-density lipoprotein. LDL: low-density lipoprotein

Table 5 Correlation between serum expression of miR21, miR192 and serum TGF-β1

	TGF-β1		miR-21	
	r	*p*	*R*	*p*
miR-21	0.428	0.002	–	–
miR-192	0.139	0.335	0.276	0.052

r = Correlation Coefficient, *P* = *p* value. *r* < 0.3: no correlation. *r* = 0.3– < 0.5: weak correlation. *r* = 0.5: fair correlation. *r* = > 0.5–0.75: good correlation. *r* > 0.75: very good correlation

Discussion

Serum levels of miRNA 21, miRNA 192 expression and TGF-β1 were assessed in type II diabetic patients in the current study to determine their relationship with glycemic control, metabolic abnormalities, and renal function.

The molecular pathogenesis of diabetic nephropathy is multifactorial, involving pro-fibrotic and pro-inflammatory cytokines (e.g., TGF-β), pro-inflammatory factors (as interleukin IL-1, 6 and 18), endothelin systems, in addition to protein kinase C and other biochemical aberrations [18]. Multiple pathological processes, including adhesion, migration of multiple cell types, cellular proliferation, differentiation, programmed cell death and extracellular matrix protein synthesis, are activated and regulated by TGF-β [19]. During renal fibrosis, the regulation of the expression of TGF-β dependent miRNAs (miR21, miR192, and the miR29 family) is intricately controlled by TGF-β1 through the Smad3 pathway [20]. It was found that TGF/Smad proteins are involved in the biosynthesis and regulation of microRNAs. Prior research has established the regulatory function of microRNAs in the pathophysiology and development of the kidneys. It has been demonstrated that dysregulation of several of these miRNAs contributes to the pathophysiology and progression of DN. The discovery that miRNAs are consistently detected in the bloodstream outside of cells indicates that they function as extracellular signaling molecules and potentially function as noninvasive biomarkers for an extended array of diseases [21].

There has been discussion regarding the potential of microRNAs (miRNAs) to act as therapeutic targets and diagnostic or prognostic indicators in incidences of chronic renal disease [22].

Nevertheless, the utilization of miRNA signatures in clinical settings is challenging due to the conflicting findings in the research that examines the expression profile of these miRNAs in chronic kidney disease (CKD) [11].

DN is characterized by a dramatic increase in miRNA21 expression in plasma, urine, and renal tissue; this increase has continued with the progression of DN. By interacting with multiple signaling cascades and binding to target proteins, miRNA21 establishes a complex network that promotes DN [13]. Hung et al., found that miR21 levels are upregulated in type I diabetic patients with overt proteinuria and microalbuminuria ($p = 0.001$, $p = 0.0024$, respectively) as compared to patients with normoalbuminuria [23]. In agreement, miR21 was only significantly higher in the overt proteinuric group when compared to the normoalbuminuric group in the current

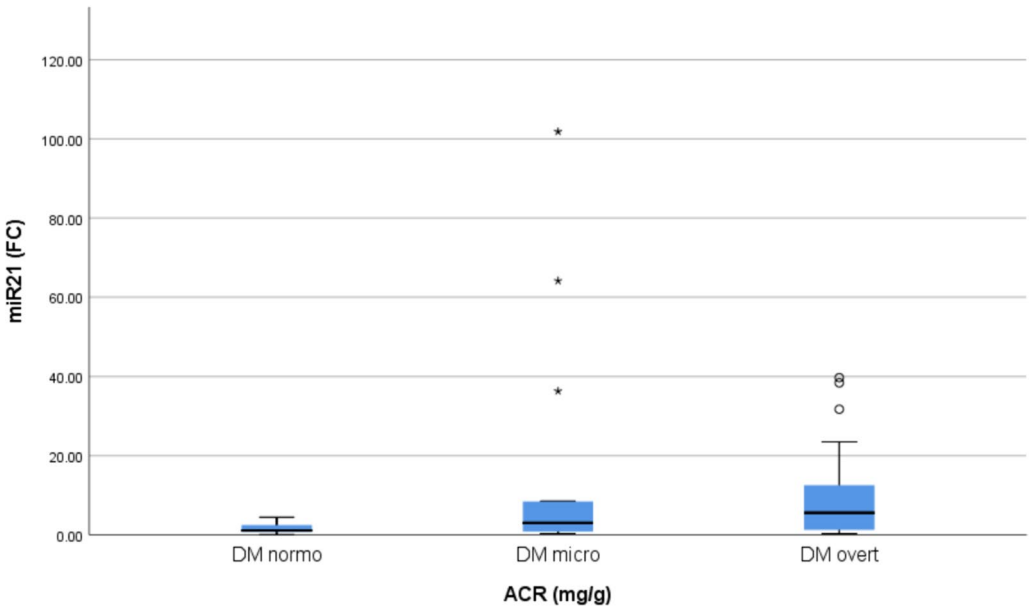

Fig. 1 miR21 expression (FC) levels within the studied groups

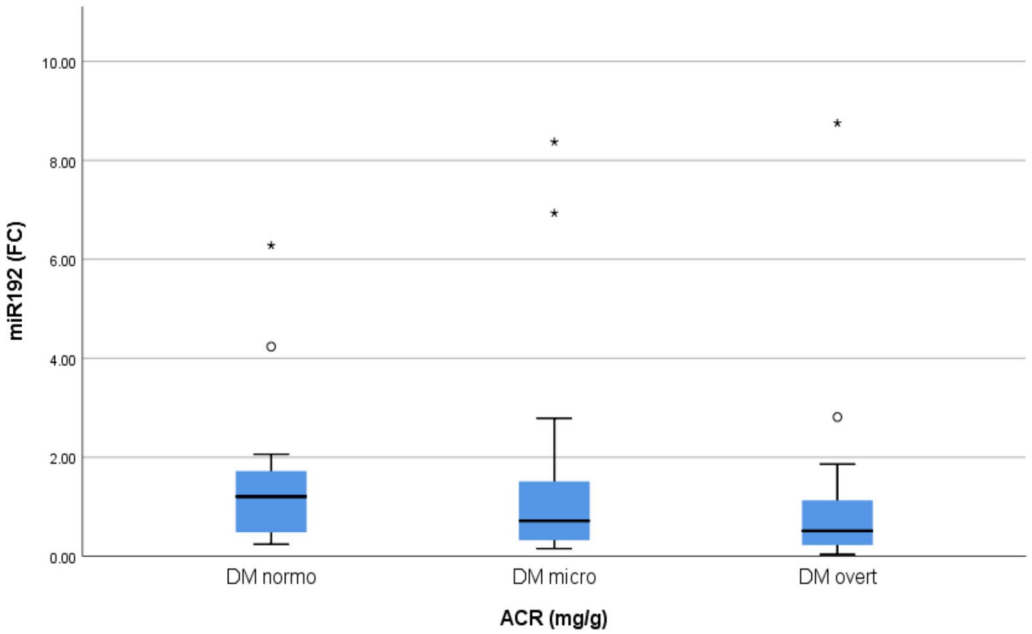

Fig. 2 miR192 expression (FC) levels within the studied groups

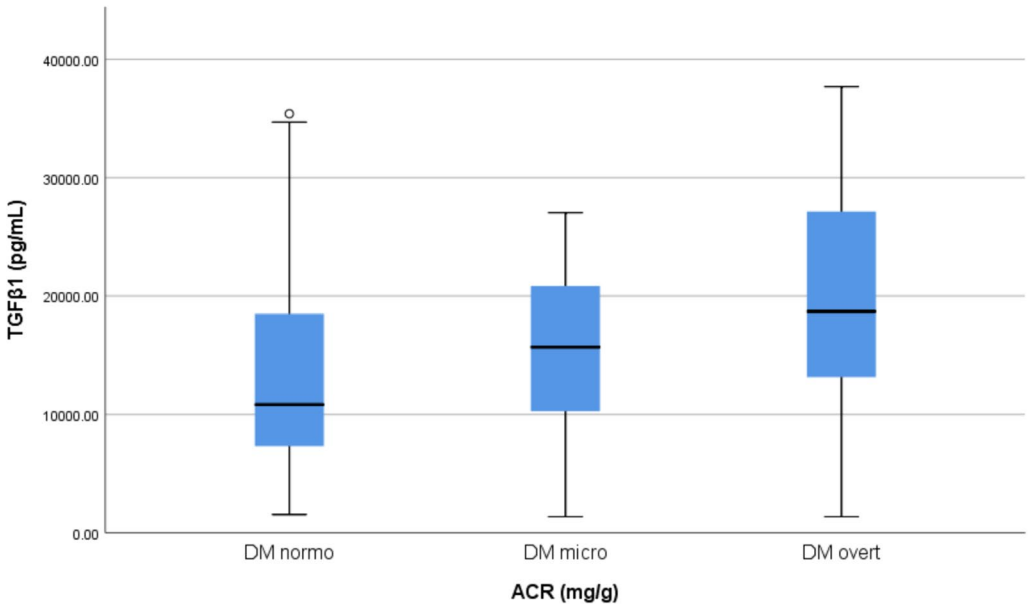

Fig. 3 Serum TGF-β1 (pg/ml) levels within the studied groups

study. According to Fouad et al. study, when compared to ACR, plasma miRNA21 demonstrated higher sensitivity (94.1%) and specificity (100%) in diagnosing DN at a cut-off of 0.01. ACR cutoff levels of 45 mg/gm and 89% specificity and 88.2% sensitivity were observed [24].

The results of this investigation indicated that serum miR21 exhibited a sensitivity of 55.9% and specificity of 88% at a cut-off value of 3.258 FC, as measured by an AUC of 0.719 ($p = 0.013$, 95% CI 0.579–0.858). The inconsistency in the findings may be accounted for by the limited sample size utilized in our research and the distinct methodologies employed for miRNA retrieval, isolation, storage and analysis.

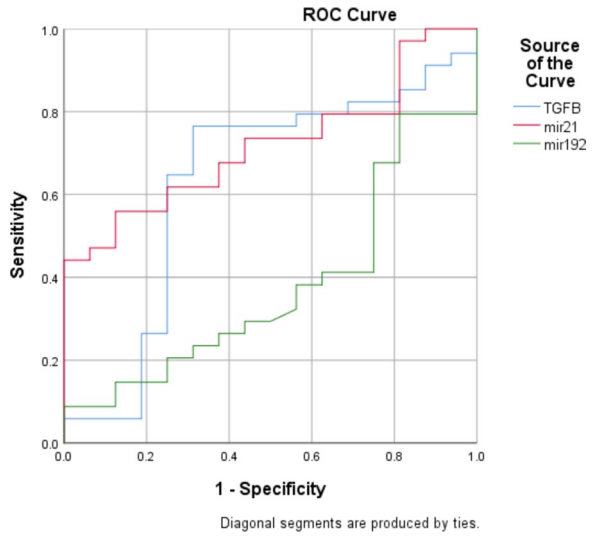

Fig. 4 ROC curve for miR21, miR192 and TGFβ1

MiRNA 192 expression did not differ statistically between normoalbuminuric, microalbuminuric and overt proteinuric type II diabetic patients (p value = 0.234). In agreement with this study, Hung et al. reported that there was no significant variation in miRNA192 levels between T2DM subjects with normoalbuminuria versus subjects with microalbuminuria and overt proteinuria [23]. Furthermore, Al-Kafaji and Al-Muhtaresh found no statistically significant difference between microalbuminuric and normoalbuminuric diabetic groups in terms of miRNA192 expression. In contrast, the study authors documented a statistically significant decrease in miR192 expression between the macroalbuminuric and normoalbuminuric groups ($p < 0.005$) [21].

Contrary to this study, Lotfy and coworkers found a statistically significant decrease in miRNA192 expression level in microalbuminuric and macroalbuminuric groups when compared to the normoalbuminuric group [25].

In this study, the median level of miRNA192 exhibited a substantial negative relationship with the duration of diabetes ($r = -0.303$, $p = 0.033$). That was similar to El-Monem et al. who determined that there is a correlation between a reduction in miRNA192 and an increase in renal fibrosis in living organisms. Additionally, it was seen that the expression of miRNA192 decreases as the duration of the disease becomes longer which ranges from 5 to 15 years [26]. Ezzat et al. found significantly higher levels of miR192 in individuals with long-standing disease (43.5 ± 24 months), contrary to the current study [27].

In this work, there was no statistical difference in serum TGF-β1 levels between normoalbuminuric, microalbuminuric and overt proteinuria type II diabetic patients (p value = 0.225). In disagreement, Xiaoyu et al. showed that the levels of TGF-β1 in the overt proteinuric group were significantly higher than in the microalbuminuric and normoalbuminuric groups (p value < 0.01) [28]. Moreover, a study done by Mou et al., documented that individuals with type 2 diabetes mellitus who presented with microalbuminuria had elevated serum TGF-β1 levels in comparison with those with normoalbuminuria. Similarly, individuals with macroalbuminuria had elevated serum TGF-β1 levels in comparison with those with microalbuminuria [29]. Tianbiao et al. reached at the similar conclusions [30]. The results of the previous research contradicted the findings of the present investigation.

TGF-β1 and miRNA21 exhibited a significant positive correlation in this study (r = 0.428, p = 0.002). There are functional interactions between miRNA21 and TGFβ. Smad 7 is a direct target for miR21, leading to its inhibition and enhancement of TGFβ signaling [7–9]. This finding may provide insight into the etiology of DN, as there is evidence that in vitro cultured endothelial cells are susceptible to miRNA modifications induced by hypoxia and elevated glucose. The correlation between miRNA21 upregulation and mitochondrial dysfunction, as well as oxidative stress, is not unexpected. Similarly, hyperglycemia contributes to diabetic nephropathy through direct or indirect hemodynamic mechanisms that cause renal injury. Contributing to tubulointerstitial injury and glomerular sclerosis, it stimulates intrinsic glomerular cells to secrete TGF-β1, activates protein kinase C, and increases AGE production (31, 32).

There were limitations in this study that restricted the precise interpretation of the parameters tested during diabetic nephropathy progression. The previously mentioned restrictions involve a comparatively small sample size, the absence of any apparent correlation between levels of miR21 and renal histopathological condition as assessed through renal biopsies, and the absence of subsequent repeated measurements of candidate miRNAs.

Conclusions

In summary, the findings of the present investigation revealed a statistically significant increase in the expression level of miRNA21 among diabetic individuals presenting with both overt proteinuria and microalbuminuria, as opposed to those with normoalbuminuria. There was an absence of statistically significant variation observed in the serum levels of TGF-β1 and miRNA192 among the three groups that were investigated. A significant positive correlation was observed between median levels of miRNA21 and ACR, while a

significant negative correlation was observed between serum expression of miRNA192 and the duration of diabetes. The examined parameters exhibited no correlation with BMI, BP, FBS, PP, HbA1c, cholesterol, triglyceride, HDL, or LDL.

In diabetic patients, miRNA21 may serve as a useful noninvasive biomarker for the early detection of ongoing endothelial dysfunction, according to our findings. That may provide insights into the diagnostic, predictive, and therapeutic role of miRNA 21 in treating DN. The current evidence highlights an important area for future research focusing on the effective biomarkers for DN, which may facilitate early diagnosis and the judgment of prognosis for DN. Here, the findings on the pivotal role of miR-21 in the pathogenesis of DN may indicate that miR-21 could serve as a potential therapeutic target.

Abbreviations

ACR	Albumin/creatinine ratio
DM	Diabetes mellitus
DN	Diabetic nephropathy
ECM	Extracellular matrix proteins
EDTA	Ethylenediaminetetraacetic acid
eGFR	Estimated GFR
EMT	Epithelial to mesenchymal transition
FC	Fold change
IDF	The International Diabetes Federation
miRNA	MicroRNA
mRNA	Messenger RNA
TBRI	Theodor Bilharz Research Institute
TGF-β	Transforming growth factor-beta
UTR	3′ Untranslated region

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Author contributions
JGAE and HME participated in study design. JGAE carried out the molecular studies and the immunoassays. EAW assisted in samples collection and patients' history taking and clinical examination. HME performed the statistical analysis. HSA revised the manuscript. AAWM and LNK conceived of the study and participated in coordination of it. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Ethical approval and consent to participate
A written informed consent was obtained from all patients. The study protocol was in accordance with the declaration of Helsinki 1975 and as modified in 2012 and approved by the institutional review board of TBRI before the start of enrolling participants.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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