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The Association of Expression Patterns of Lncrna Taurine Upregulated Gene 1 (Tug1) with Progression and Severity of Diabetic Kidney Disease

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ABSTRACT

Background: Diabetic kidney disease (DKD) has become the leading cause of the end-stage renal disease (ESRD) and it is one of the most devastating complications of type 2 diabetes mellitus (T2DM). Its pathogenesis encompasses multiple dysregulated epigenetic mechanisms. Long non-coding RNAs (lncRNAs) have an important function in various diseases. However, their roles in DKD remain mainly unknown.

Objective: The present study was performed to explore the relative expression level of lncRNA taurine upregulated gene 1 (lncRNA Tug1) in Egyptian patients with T2DM and CKD and to investigate its associations with the progression of CKD.

Patients and methods: This cross-sectional-controlled study included a total of 50 patients with T2DM and 50 age and sex-matched controls, attending at Internal Medicine, Faculty of Medicine, Zagazig University Hospitals. Quantitative real-time reverse-transcription PCR (qRT-PCR) was used to detect the expression levels of lncRNA TUG1

Results: patients with T2DM had statistically significantly lower values of the relative expression level of lncRNA Tug1 compared to the control group (1.313±0.72, vs 2.354±0.97, P <0.001). Interestingly, patients with macroalbuminuria (0.48±0.311) had statistically significant lower values of the relative expression level of lncRNA Tug1compared to patients with microalbuminuria (1.42±0.49) and patients with normoalbuminuric (1.86±0.636), P <0.01. In patients with DKD among studied variables, serum creatinine and UACR were the main independent parameters associated with the relative expression of lncRNA Tug1.

Conclusion: It could be concluded that circulatory lncRNA Tug1 expression level is downregulated in patients with T2DM in a particular patient with macroalbuminuria. Thus, circulatory lncRNA Tug1 relative expression level may have a reno-protective role.

Keywords: DKD, lncRNA Tug1, qRT-PCR, T2DM, Macroalbuminuria

Introduction:

Diabetes mellitus is a major public health problem; and its prevalence will be more than 600 million by 2045 [(1)]. Diabetic kidney disease (DKD) is a microvascular complication and progresses gradually over many years in approximately 30–40% of individuals with T2D Mellitus. Approximately 45% of cases with ESRD result from DKD which is now the main cause of chronic kidney disease (CKD) worldwide and the leading cause of end-stage renal disease (ESRD) in addition, the presence of CKD is the single strongest predictor of mortality for persons with diabetes [(2)].

Interesting studies investigated the pathological mechanisms of DKD, and they detected glomerular hypertrophy, mesangial matrix growth, glomerulosclerosis, tubular atrophy, and tubulointerstitial fibrosis. Moreover, podocyte loss and epithelial dysfunction play important roles in DKD pathogenesis with further progression associated with inflammation but the exact molecular mechanisms responsible for DKD are not fully known. Accumulating evidence has indicated that Hyperglycemia, oxidative stress, and renal hemodynamic changes are important forms of metabolic stress capable of modifying epigenetic processes [(3)].

Long noncoding RNAs (lncRNAs) are a class of transcripts longer than 200 nucleotides without protein-coding function. Recent studies have identified the regulatory role of lncRNA in several gene expression processes, including nuclear importation, alternative splicing, DNA methylation, mRNA decay, as well as other transcriptional, post-transcriptional and epigenetic regulatory roles. The lncRNA taurine upregulated gene 1 (TUG1), located on chromosome 22q12, regulates podocyte health and glomerulosclerosis by altering the expression of peroxisome proliferator-activated receptor γ Coactivator 1α (PGC1A) [(4)].

Although recent studies were conducted to explore the pathogenic mechanism of dysregulated lncRNA, only limited examples of lncRNAs have been studied in DKD [(5)]. Thus, we aimed in the current study to investigate the relative expression level of lncRNA TUG1 in Egyptian patients with T2DM and CKD and to investigate its associations with the progression of CKD.

PATIENTS AND METHODS

This cross-sectional-controlled study included a total of 50 patients with T2DM and 50 age and sex-matched controls, attending at Internal Medicine, Faculty of Medicine, Zagazig University Hospitals.

The design of the study is shown in figure (1).

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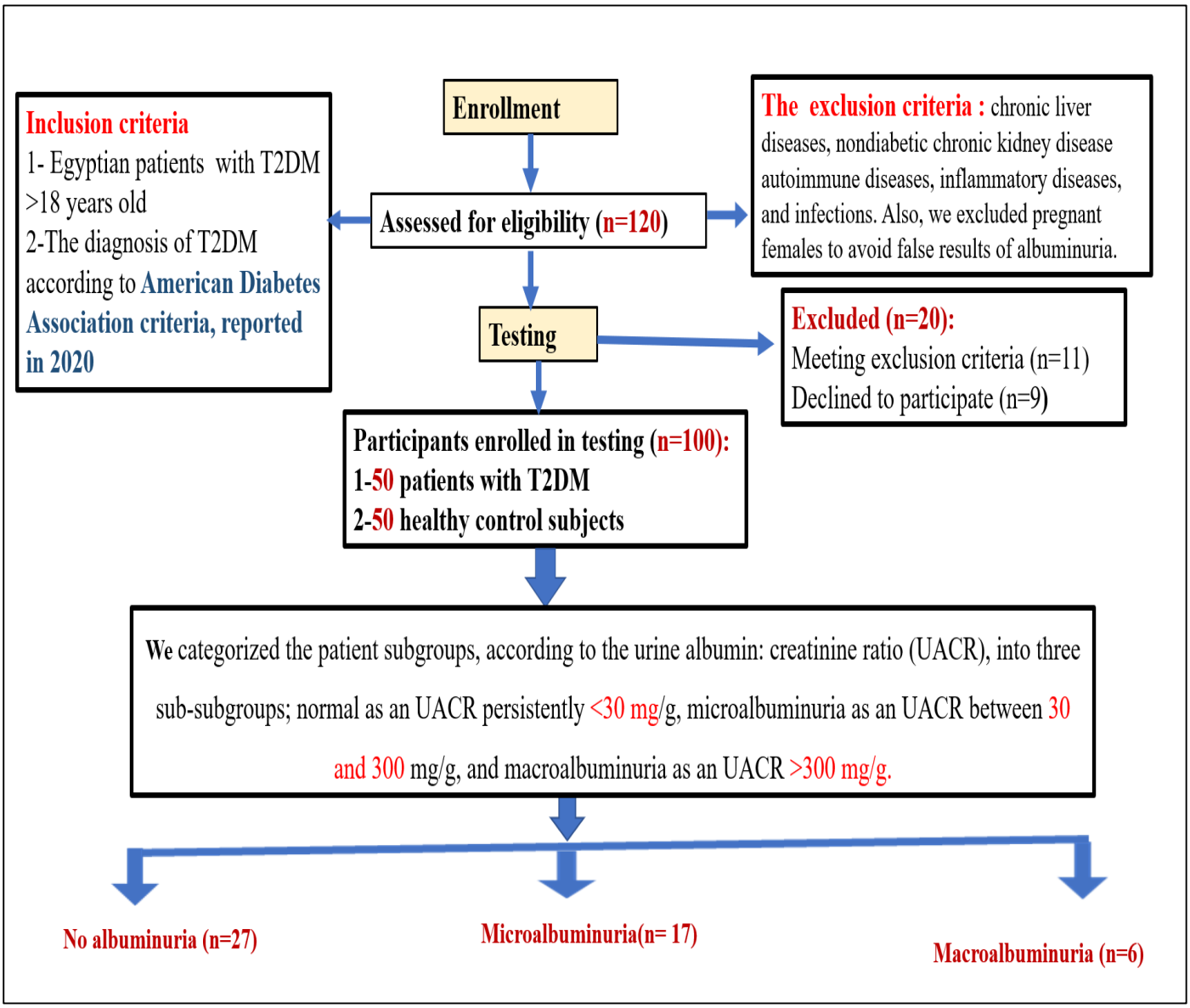

Figure (1): Flowchart of the study.

Blood samples and biochemical analysis were done for evaluation and classification of the studied participants. Urea and Creatinine levels were determined according to a Jaffe reaction method (Spinreact, Girona, Spain). Urinary albumin excretion was measured from an early-morning urine sample as the UACR. Total RNA was extracted by using Simply P Total RNA Extraction Kit (Cat. No. BSC52S1), according to the manufacturer's protocol.

The sequences of the primers used to amplify human lncRNA TUG1and GAPDH are shown in the following table. GAPDH was used as the internal control to normalize gene expression data. The relative mRNA expression was calculated using the $2^{-\Delta\Delta Cq}$ method.

Gene	Sequence, 5'-3'	Size, bp
TUG1	Forward: TAGCAGTTCCCCAATCCTTG Reverse: CACAAATTCCCATCATTCCC	150
GAPDH	Forward: CCCACTCCTCCACCTTTGAC Reverse: TGGTCCAGGGGTCTTACTCC	153

***Ethical Consideration*:**

This study was ethically approved by Zagazig University's Research Ethics Committee. Written informed consent of all the participants was obtained. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

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Statistical analysis

Analysis of data was performed using SPSS v.26. Data were expressed using descriptive statistics (mean ± standard deviation) and were analyzed using T-test (comparison between two groups) and F test (comparison between more than two groups) we tested the association between the expression profile of lncRNA Tug1 with UACR and other studied variables in patients with DKD and detected the independent variables by linear regression. Receiver operating characteristic (ROC) tested the diagnostic powers of lncRNA Tug1 relative expression level among studied subjects.

RESULTS

This cross-sectional controlled study was conducted on 50 Egyptian patients with T2DM, 65 % were female and 35% were male, their mean age was 44.53 ± 17.17 years, and 50 healthy subjects as control, 66% were female and 34% were male, their mean age was 42.55 ± 18.25 year. The diabetic and control group were matched for age, sex, and smoking. As expected, patients with T2DM had higher values of metabolic dysfunction and renal impairments compared to the control group, p <0.001* **(Table 1).**

Table (1): Anthropometric and biochemical characteristics of the studied groups.

Variables	Control group (n =50)	T2DM patients (n =50)	P value
Systolic blood pressure (mmHg)	117.5± 8.40	149.7± 20.54	<0.001*
Diastolic blood pressure (mmHg)	75.6±4.589	95.92± 14.33	<0.001*
FPG (mg/dl)	89.72± 6.304	220.97±30.04	<0.001*
HbA1c (%)	5.63±0.624	9.59±2.206	<0.001*
eGFR (mL/min)	98.37 ± 7.56	75.19±13.2	<0.001*
Serum creatinine (mg/dl)	0.916±0.27	1.96±0.48	<0.001*
UACR (mg/g)	20.82±2.6	188.97±13.4	<0.001*

T2DM, type 2 diabetes mellitus; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; Estimated glomerular filtration rate; UACR, urine albumin: creatinine ratio. * P < 0.05 .

General characteristics of diabetic patients:

As shown in figure (1) (study Flowchart), we classified our patients according to UACR, as 27 patients had normal UACR, 17 patients had microalbuminuria and 6 patients had macroalbuminuria. There was a statistically significant long duration of diabetes, diastolic blood pressure, FPG, HbA1c, and creatinine as well as higher values of UACR in patients with macroalbuminuria compared to other subgroups. On the other hand, patients with macroalbuminuria had a significantly lower value of estimated glomerular filtration rate (eGFR) compared to other groups. p <0.001* **(Table 2).**

Table (2): Clinical and laboratory variables of patients with T2DM.

Variables	Normal (n=27)	Microalbuminuria (n=17)	Macroalbuminuria (n=6)	P1	P2	P3
Duration of diabetes (years)	8.864±3.1	10.87±3.31	15.17±6.	<0.001*	<0.001*	<0.001*
Systolic blood pressure (mmHg)	140.2±11.43	143.7± 11.2	140.4±11.3	0.418	0.623	0.670
Diastolic blood pressure (mmHg)	75.6±8.58	95.9±8.3	115.9±9.3	<0.001*	<0.001*	0.839
FPG (mg/dl)	167.1±16.3	211.9±31.4	275.9±41.3	<0.001*	<0.001*	<0.001*
HbA1c (%)	8.63±1.4	9.59±2.3	10.59±1.5	<0.001*	<0.001*	<0.001*
eGFR (mL/min)	91.37 ± 7.5	79.19±13.2	66.19±13.2	<0.001*	<0.001*	<0.001*
Serum creatinine (mg/dl)	1.24±0.07	1.96±0.06	2.66±0.09	<0.001*	<0.001*	<0.001*
UACR (mg/g)	26.7±3.3	193.97±8.1	388.7±9.3	<0.001*	<0.001*	<0.001*

T2DM, type 2 diabetes mellitus; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; estimated glomerular filtration rate; UACR, urinary albumin-creatinine ratio, P < 0.05

Relative expression of lncRNA Tug1 level in the studied groups:

Our results show that diabetic patients had statistically significant lower values of the relative expression level of lncRNA Tug1compared to the control group (1.313±0.72, vs 2.354±0.97, P <0.001) (Figure 2a).

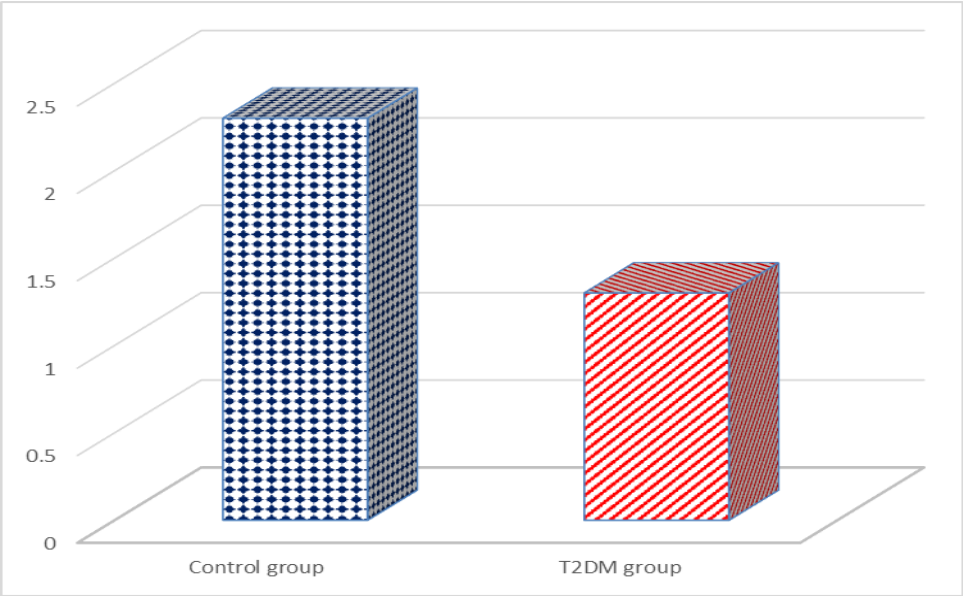

Figure (2a): Relative expression of lncRNA TUG1 level in the studied groups.

Relative expression of lncRNA Tug1 level in the patients with T2DM:

Our results show that patients with macroalbuminuria (0.48±0.311) had statistically significant lower values of the relative expression level of lncRNA Tug1compared to patients with microalbuminuria (1.42±0.49) and patients with normoalbuminuric (1.86±0.636), P <0.01 (Figure 2b).

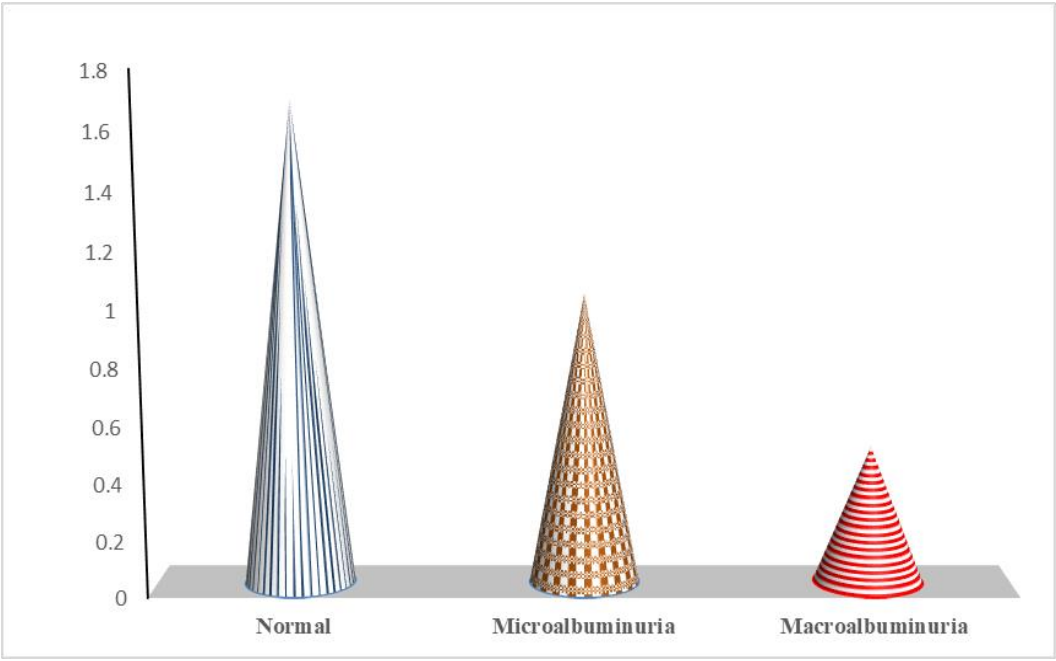

Figure (2b): Relative expression of lncRNA TUG1 level in groups with T2DM.

Correlations between relative expression of lncRNA Tug1 with clinical and laboratory characteristics in patients with DKD:

The current study revealed that the relative expression of lncRNA Tug1significantly negatively correlated with duration of diabetes, diastolic blood pressure, HbA1c, creatinine, and UACR and significantly positively correlated with eGFR, P<0.001 (Table 3).

Table (3): Correlations between relative expression of lncRNA TUG1 with clinical and laboratory characteristics in patients with DKD

Variables	lncRNA TUG1	
	r	p
Duration of diabetes (years)	0.460	<0.001*
Systolic blood pressure	0.107	0.112
Diastolic blood pressure	0.582	<0.001*
FPG (mg/dl)	0.459	<0.001*
HbA1c (%)	0.645	<0.001*
eGFR (mL/min)	-0.484	<0.001*
Serum creatinine (mg/dl)	0.567	<0.001*
UACR (mg/g)	0.589	<0.001*

T2DM, type 2 diabetes mellitus; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; estimated glomerular filtration rate; UACR, urinary albumin-creatinine ratio. * P < 0.05.

Linear regression analyses in patients with DKD:

A linear regression analysis test was done to assess the main independent parameters associated with the relative expression of lncRNA Tug1levels. Our results showed that serum creatinine, as well as UACR, were independently correlated with lncRNA Tug1, P<0.001 (Table 4).

Table (4): Linear regression analyses to test the influence of the main independent variables against relative expression of lncRNA TUG1 levels (dependent variable) in patients with DKD

Model		Unstandardized Coefficients		Standardized Coefficients	t	p	95% C.I.	
		B	SE	Beta			Lower Bound	Upper Bound
lncRNA TUG1	(Constant)	-0.760	0.533		-1.42	0.157	-1.818	-0.760
	Duration of diabetes	0.001	0.004	0.021	0.237	0.813	-0.007	0.001
	S. creatinine	0.020	0.007	0.235	2.833	<0.001*	0.006	0.020
	UACR	0.011	0.002	0.828	5.708	<0.001*	0.007	0.011
	eGFR	-0.101	0.069	-0.231	-1.46	0.147	-0.239	-0.101

The accuracy of relative expression of lncRNA Tug1for discriminating patients with T2DM from the control group by ROC analysis:

We investigated the potential diagnostic value of the relative expression of lncRNA Tug1 (Figure 3a), the cutoff value was 1.354 and the AUC was 0.958 (95% CI =0.915-1.000). Furthermore, the sensitivities and the specificities were 96% and 72% respectively.

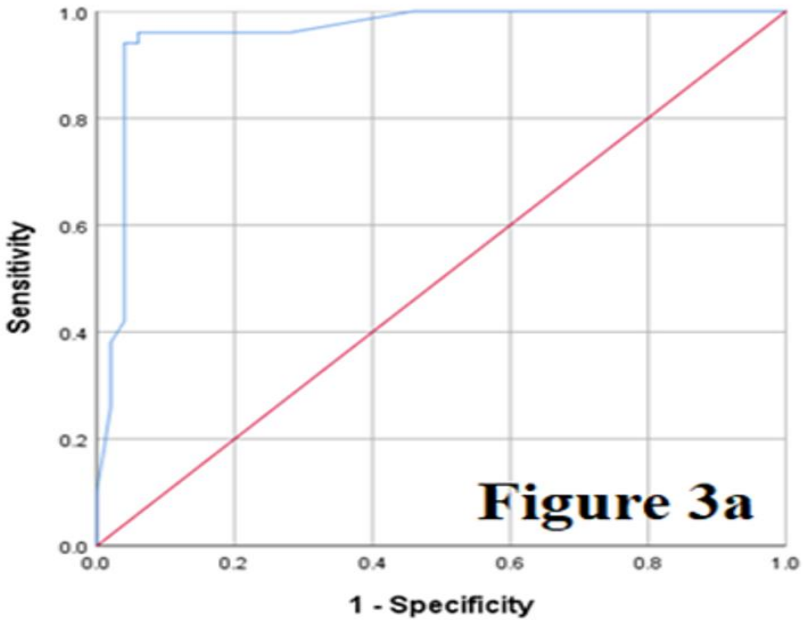

Figure (3a): The accuracy of Relative expression of lncRNA TUG1 level for discriminating patients with T2DM from control group by ROC analysis.

The accuracy of relative expression of lncRNA Tug1 for discriminating patients with microalbuminuria from patients with normoalbuminuric by ROC analysis:
We aimed to evaluate the potential diagnostic value of lncRNA Tug1, the cutoff value was 1.02 and the AUC was 0.726 (95% CI =0.564-0.982). Furthermore, the sensitivities and the specificities were 82.7% and 68.6% respectively (Figure 3b).

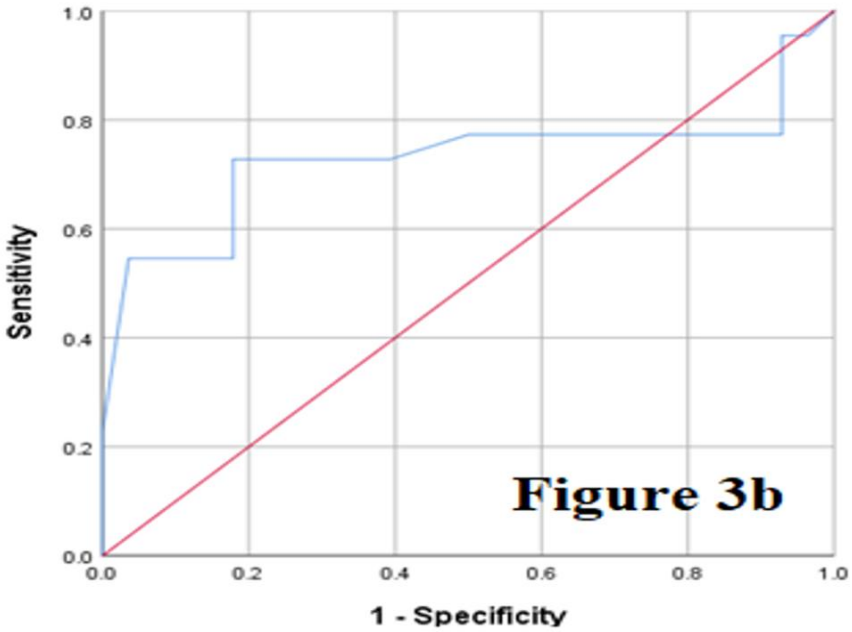

Figure (3b): The accuracy of relative expression of lncRNA TUG1 for discriminating patients with microalbuminuria from patients with normoalbuminuric by ROC analysis.

The accuracy of relative expression of lncRNA Tug1for discriminating patients with macroalbuminuria from microalbuminuria by ROC analysis:
For further evaluation of the diagnostic power of lncRNA Tug1 (the cutoff value was 0.52 and the AUC was 0.799 (95% CI =0.577-1.000). Furthermore, the sensitivities and the specificities were 87.7% and 77.3% respectively (Figure 3c).

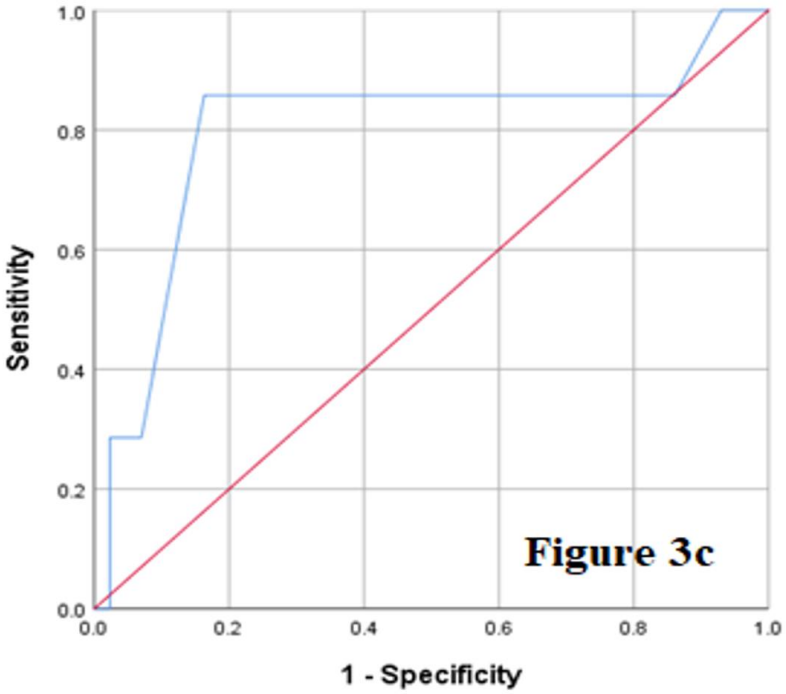

Figure (3c): The accuracy of relative expression of lncRNA TUG1 for discriminating patients with macroalbuminuria from microalbuminuria by ROC analysis.

DISCUSSION

A growing number of reports confirmed that uncontrolled hyperglycemia can result in progression of chronic microvascular complications, retinopathy, nephropathy, and neuropathy as well as macrovascular, coronary artery, peripheral, and cerebral vascular disease) [(6)]. Also, more evidence demonstrated that DKD is the main cause of end-stage CKD globally and an important cardiovascular risk factor [(7)].

DKD starts with microalbuminuria, defined as albumin excretion of 30–299 mg/day, and without treatment, it evolves into macroalbuminuria (>500 mg albumin/day) and progressive decline in the glomerular filtration rate. The molecular pathogenesis of DKD could be genetic, metabolic, inflammatory, and other mechanisms. However, the underlying mechanism leading to these changes has not been fully identified [(8)]. Thus, we aimed in the current study to investigate the relative expression level of lncRNA Tug1in Egyptian patients with T2DM and CKD and to investigate its associations with the progression of CKD.

Our study revealed clear evidence that patients with T2DM had metabolic and renal dysfunction. Among patients with T2DM, patients with macroalbuminuria, there were longer duration of diabetes as well as higher values of diastolic blood pressure, FPG, HbA1c, and UACR compared to patients without T2DM. Moreover, they had significantly the lowest values of eGFR compared to other groups.

Similar to our results **Molitch *et al.*** [(9)]**,** observed that poor glycemic control was associated with albuminuria. Additionally, **Cooper *et al.*** [(10)]**,** observed that the degree of microalbuminuria was significantly correlated with hypertension. And these findings are consistent with the results of the UKPDS study (United Kingdom Prospective Study), in which a reduction of HbA1c levels by 0.9% was correlated with a reduction in the relative risk of albuminuria by 30% [(11)].

It is increasingly recognized that lncRNAs play vital roles in many diseases in particular kidney diseases through complicated mechanisms [(12)]. In this context, emerging evidence suggests that lncRNAs were found to have potential use in the prediction of renal diseases and could be used as diagnostic and prognostic maskers [(13)]. From the above-mentioned, it is evident that lncRNA is an important regulatory molecule that can be targeted to modulate cellular physiology and functions in the pathophysiological progress of different diseases. Regarding the regulatory roles of lncRNAs in kidney disease, recent studies observed their role in renal ischemia, injury, inflammation, fibrosis, and other renal diseases [(14)].

The results presented herein are innovative as this study performs a robust estimation of lncRNA Tug1relative expression levels as an epigenetic marker of DKD. Our results show that diabetic patients had statistically significantly lower values of lncRNA Tug1 compared to controls. Interestingly, in patients with DKD, there were statistically significant lower values of lncRNA Tug1in patients with macroalbuminuria compared to patients with microalbuminuria and normoalbuminuria

The current study revealed that the relative expression of lncRNA Tug1 significantly negatively correlated with duration of diabetes, diastolic blood pressure, HbA1c, creatinine, and UACR and significantly positively correlated with eGFR. Interestingly, a linear regression analysis test was applied to assess the main independent parameters associated with the relative expression of lncRNA Tug1 levels. The current results showed that serum creatinine, as well as UACR, were independently correlated with lncRNA Tug1.

According to **Long *et al.* [15],** the tissue expression level of lncRNA Tug1was downregulated in renal tissues of both diabetic mice and diabetic patients and the authors detected that lncRNA Tug1 had a protective role from diabetes-induced CKD, suggesting that this lncRNA may be a possible therapeutic target to treat DKD.

Similar findings were observed by **Li *et al.* [16],** as they detected the functional role of the lncRNA Tug1/PGC1α axis on mitochondrial homeostasis and urea cycle metabolites in experimental models of diabetes. lncRNA Tug1 regulates podocyte health and glomerulosclerosis by altering PGC1A which could lead to metabolic dysfunction [17].

Long *et al.* [15], discovered the regulatory role of the Tug1/PGC-1α axis in the progression of DN. They confirmed that Tug1, a lncRNA, positively controls Ppargc1a gene transcription and its target genes in podocytes.

Accordingly, we analyzed our data by ROC to estimate the cutoff, AUC, sensitivity, and specificity of lncRNA TUG1 relative expression as an epigenetic biomarker, our results detected that the diagnostic power of lncRNA TUG1level in differentiating diabetic patients from the control group was higher than the power of lncRNA TUG1level in differentiating discriminating patients with albuminuria among diabetic patients.

CONCLUSION

It could be concluded that patients with T2DM had statistically significantly lower values of the relative expression level of lncRNA Tug1 compared to the control group, interestingly, patients with macroalbuminuria had lower values of the relative expression level of lncRNA Tug1 compared to other groups. Thus, circulatory lncRNA Tug1 relative expression level may have a reno-protective role. More further studies are needed to document the value of lncRNA Tug1 as a diagnostic biomarker as well as a therapeutic target in DKD.

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