ORIGINAL ARTICLE

Expression of circANKRD36 as A New Biomarker in Diabetic Nephropathy Patients with type 2 Diabetes Mellitus

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Diabetes Mellitus type 2, Diabetic nephropathy, tumor necrotic factor-alpha, interleukin-6 and Circular ankyrin repeat domain 36

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ABSTRACT

Background: *Type 2 diabetes mellitus (T2DM) is a long-term metabolic disease defined by reduced pancreatic β-cell function and impaired insulin action in peripheral tissues, which cause sustained irregularities in glucose homeostasis, mild inflammation.* ***Objectives:*** *Recent study aimed to detect levels of circANKRD36 in white blood cells of T2DM patients and examine their relationship with pro- inflammatory mediators (TNF-α and IL-6) in DN patients withT2DM and to evaluate the role of circANKRD36 in the initiation and development of DN.* ***Methodology:*** *Blood samples obtained from people with diabetes mellitus type 2 (69) cases and Control participants (23). Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was applied to evaluate levels of CircANKRD36. Levels of pro-inflammatory cytokines such as interleukin IL-6 via chemiluminescence and tumor necrosis factor (TNF)-α was determined via enzyme-linked immunosorbent assay (ELISA).* ***Results:*** *CircANKRD36 showed high expression levels in patients with T2DM and DN, contribute to the development of DN and it was superior to inflammatory markers in detection of early stages of DN.* ***Conclusion****: CircANKRD36 levels were markedly up-regulated in T2DM patients in comparison to control group (P=< 0.001).*

INTRODUCTION

Diabetes Mellitus Type 2 (T2DM) is among the most frequently occurring metabolic diseases and has emerged as a global medical concern, impacting more than 537 million cases globally as reported by the International Diabetes Federation (IDF) [1]. Diabetic nephropathy (DN) is a frequent secondary complication of diabetes and has become a major contributor to illness and death between diabetic patients2. DN is a complication related to small blood vessels that affects about 20–40% of individuals with T2DM[3]. DN is characterized as a glomerular disease that progresses through several stages, including glomerular hyper filtration, initial renal impairment, microalbuminuria, obvious protein in urine, and final stage of kidney disease [4]. The presence of microalbuminuria is widely accepted as the main approach for early diagnosis of DN. Mediators of inflammation are crucial in pathogenesis of DN, as renal cells can produce cytokines like tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6). These mediators act in a paracrine or autocrine fashion, playing a role in the initiation and progression of multiple kidney disorders[5]. However, numerous studies have indicated that they are insufficient and have certain limitations. Recently, new indicators were recognized for the Initial detection and monitoring of DN progression in T2DM patients[6]. Circular RNAs represent a class of intrinsic non-coding RNAs linked to various diseases, including T2DM. Circular ankyrin repeat domain 36 (circANKRD36) is implicated in T2DM and pathways related to inflammation through its connections with miRNAs such as hsa-miR-3614-3p, hsa-miR-498, and hsa-miR-501-5p.

METHODOLOGY

Our study involved sixty nine patients identified with diabetes mellitus Type 2 and twenty three healthy subjects in the period from April 2023 to April 2024. The control and patients group were age and sex matched. All patients were selected from Diabetes clinic and Internal medicine department, Assiut University Hospitals, Assiut University. Patients were identified with Type 2 diabetes mellitus dependent on the World Health Organization (WHO) criteria[7].

Ethical considerations:

Our study received authorization from the Institutional Review Board (IRB) of the Faculty of Medicine, Assiut University, before it was conducted with IRB.NO:17200640. Additionally, all participants gave their informed consent. Clinical trial ID: NCT05061459

Exclusion criteria:

- Other acute or chronic systemic inflammation disease/s.
- Immune system disorders.
- All endocrine diseases except T2DM.
- Malignancy.
- Persistent liver or kidney failure.

Classification of subjects:

T2DM patients were divided into three subgroups according to their urine albumin-to-creatinine ratio (UACR).

Group A: normoalbuminuria (n=23) was defined as a UACR persistently <30 mg Alb/g creatinine. **Group B:** microalbuminuria (n=23) as a UACR between 30 and 300 mg Alb/g creatinine. **Group C:** macroalbuminuria (n=23) as a UACR >300 mg Alb/g creatinine.

Blood Specimens:

Ten ml of venous blood was collected and processed as follows:

- **6 ml** blood for Fasting blood glucose (FBG), Serum urea and creatinine, liver function, Total cholesterol (TC), Triglycerides (TG), HDL, IL6 and Pro-inflammatory cytokine TNF-α.
- **2 ml** blood into Ethylene-Diamine Tetra Acetic Acid (EDTA) covered tube, for complete blood count (CBC) and HbA1c (%)
- **2 ml** blood into tube coated with EDTA centrifuged at 1000 rpm for 10 min, and then plasma was cautiously transported into a tube free from RNase for RNA extraction. Plasma was stored at -80 °C until analysis.
- Random urine sample: was taken for Urine albumin creatinine ratio (UACR).

All cases were subjected to the following examinations:

a. Complete medical history involving family history of diabetes and therapeutic history.
b. Complete clinical examination.
c. Regular laboratory investigations:

Hematological investigations:

- Complete blood count (CBC) (ADVIA 2120, Siemens Healthineers).

Chemical investigations:

- Fasting plasma glucose (FPG), HbA1c (%), Kidney function tests, Lipid profile, Liver functions, Urine albumin creatinine ratio (UACR).
  (All these investigations were done by using ADVIA 1800 chemistry Auto-Analyzers,Siemens)
- eGFR using the Cockcroft-Gault equation was calculated as follows8.
- **eGFR** = [((140 − age) × (weight in Kg))/ Serum creatinie(mg/dl)X 72] × (0.85 if female)

- Special laboratory investigations including:

- Serum IL6, Serum Pro-inflammatory cytokine TNF-α, Plasma circANKRD36.

Determination of serum IL6:

Done by ADVIA Centaur-XPT Auto-Analyzer, Siemens Healthineers, USA that using chemiluminescent technology.

Detection of serum Pro-inflammatory cytokine TNF-α

TNF-α ELISA Kit catalog No: ELK1190 provided by Biotechnology was used for determination of serum TNF-α by sandwich enzyme immunoassay method.

Detection of plasma of circANKRD36 by reverse transcription quantitative polymerase chain reaction (RT-qPCR):

Relative quantification of plasma circANKRD36 levels were done in Clinical Pathology Department, Assiut University Hospital by quantitative RT-PCR.

(a) Purification of total RNA:

The GeneJet™ kit (Catalog Number: #K0731) by Thermo Fisher Scientific Inc,USA.

(b) Reverse Transcription:

The Thermo Scientific™ RevertAid™ First Strand cDNA Synthesis kit (Catalog Number: #K1622) by Thermo Fisher Scientific Inc,USA.

(c) Real time PCR

Carried out with the 7500 Fast Real- Time PCR system (Applied Biosystems, USA).

RTII CircRNA qPCR analysis and RTII SYPER Green Master mix kit was used in real-time PCR assay and cDNA generated through reverse transcription, employed as the template in the subsequent real-time PCR assay.

Forward primer for circANKRD36 GGAGGCCACAAGTGATGAGA, Reverse primer CCTGGTGGTTTCTCAGAAGAC and Forward primer for β-actin TTCCTTCCTGGGCATGGA, Reverse primer GAGGAGCAATGATCTTGA.

RT-PCR was conducted in a final volume of 25 µl, which included cDNA (≤500 ng), 12.5µl of Maxima SYBR Green qPCR Master Mix (2X), no ROX (Catalog Number: #K0251 by Thermo Fisher Scientific Inc, USA), 0.3 µM for primer of circANKRD36 and was completed by nuclease-free water following the manufacturer's guidelines.

The amplification protocol began with an initial denaturation and polymerase activation at 95°C for 10 minutes, subsequently 40 cycles of denaturation at 95°C for 15 seconds, and then 40 cycles of combined annealing and extension at 60 °C for 60 seconds (figure 1). Gene expression levels were standardized using β-actin, and relative expression of circANKRD36 was assessed and quantified utilizing RT-PCR. and Delta-Delta method for comparing Relative Quantitation results

i. $\Delta Ct\ Sample = Ct_{ANK36} - Ct_{Bactin}$
ii. $\Delta Ct\ Control = Ct_{ANK36} - Ct_{Bactin}$
iii. $\Delta\Delta Ct\ Sample = \Delta Ct\ Sample - \Delta Ct\ Control$
iv. Relative quantitation (Fold Change) of sample = $2^{-\Delta\Delta Ct}$

v. Relative quantitation (Fold Change) of control $_{Mean}$ = 1.

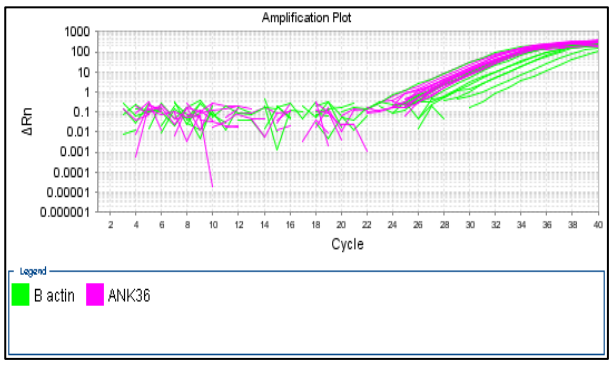

Fig. 1: CircANKRD36 by reverse transcription quantitative polymerase chain reaction (RT-qPCR).

Statistical analysis:

Data were analyzed through using SPSS version 26, with categorical variables expressed as occurrence rates and percentage values. The numerical dataset was reviewed using either the mean ± standard deviation or median and range based on their distribution and were analyzed using the independent Sample T test/Mann Whitney U test and the One-Way ANOVA/ Kruskal Wallis test. The Chi-square test was employed to assess differences in proportions among the groups. Pearson correlation analysis was conducted to identify correlation. Receiver operating characteristic (ROC) curve analysis was done.

RESULTS

Demographic data among studied groups:

- The age for T2DM patients ranged from 28-71 years with mean ±SD (50.42±10.47) in comparison to control group ranged from 29-60 years with mean ±SD (49.61±10.35 years), there was insignificant difference between two groups regarding mean age (P=0.748).
- In T2DM patients 17 male (24.6%) and 52 female (75.4%) and in control group 8 male (34.8%) and 15 female (65.2%) table (1).

Comparison of Laboratory investigations between T2DM patients and control group:

- Based on the findings of the present study, individuals with T2DM exhibited elevated metabolic risk factors and reduced kidney function compared to the control group. TNFα, IL6 and CircANKRD36 levels in T2DM patients were elevated than their levels in control group with (P value <0.001) (table 2).

Table 1: Demographic data among different examined groups:

Parameters	T2DM patients (*n* =69)	Control group (*n* =23)	*P* value
Age in years (mean ±SD)	(28-71) 50.42±10.47	(29-60) 49.61±10.35	0.748*
Gender			
Male	17 (24.6%)	8 (34.8%)	0.344**
FEMALE	52 (75.4%)	15 (65.2%)	

Table 2: Comparison of Laboratory investigations between T2DM patients and control group:

Parameters	T2DM patients (*n* =69)	Control group (*n* =23)	*P* value
HBA1c(%) Mean ± SD (range)	9.13±2.11 (6.2-14.0)	4.67±0.34 (3.7-5.5)	**<0.001***
RBG(mmol/l) Mean ± SD (range)	11.47±4.24 (5.1-21.9)	5.17±0.70 (4.0-7.0)	**<0.001***
TP(g/l) Median (range)	73.60 (48.7-84.5)	75.6 (67.9-83.0)	**0.023****
Albumin(g/l) Median (range)	44.00 (17-49)	45.00 (36-50)	**0.023****
Cholesterol (mg/dl) Median (range)	183.00 (70-304)	148.0 (8-183)	**<0.001****
TG (mg/dl) Median (range)	168.00 (42-367)	85.0 (46-324)	**<0.001****
HDL(mg/dl) Median (range)	42.100 (13.5-65.2)	40.6 (13.9-97.4)	0.626**
LDL(mg/dl) Median (range)	104.20 (32.0-198.0)	81.0 (35.3-111.1)	**<0.001****
urea (mmol/l) Median (range)	5.30 (1.6-40.0)	4.1 (2.9-5.8)	**0.004**
creatinine(µmol/l) Median (range)	73.00 (32-716)	64.0 (32-106)	**0.023**
Alb/creat ratio (mg Albumin/gm creatinine) Median (range)	52.80 (1.7-5038.1)	10.0 (4.5-28.0)	**<0.001**
eGFR(ml/min) Median (range)	91.00 (5-128)	102.0 (67-114)	**0.040**
TNFα pg/mL Mean ± SD (range)	20.17±2.42 (15.35-27.64)	18.05±0.87(16.64-19.56)	**<0.001**
IL6 pg/mL Median (range)	1.20 (0.0-1534.1)	0.0 (0.0-2.4)	**<0.001**
circANKRD36 Median (range)	174.03 (0.84-2304.12)	0.06 (0.00-4.14)	**<0.001**

Comparison of Laboratory investigations between T2DM subgroups:

- Based on the current study findings, patients with macroalbuminuria showed elevated metabolic risk factors and reduced kidney function relative to the other subgroups. TNFα in group with normoalbuminuria was lower significantly than group with microalbuminuria (P=0.007) and was lower significantly in group with nomroalbuminuria than macroalbuminuria group (P=0.005).
- IL6 was significantly lower in the normoalbuminuria group than macroalbuminuria group (P=<0.001) and was significantly lower in microalbuminuria group than macroalbuminuria group (P=<0.001). CircANKRD36 was statistically significant lower in normoalbuminuria group than microalbuminuria group (P=0.004) and was statistically significant lower in nomroalbuminuria group than macroalbuminuria group (P=<0.001) and was lower significantly in microalbuminuria group than macroalbuminuria group (P=0.004) (table 3).

Table 3: Comparison of Laboratory investigations between T2DM subgroups:

Variables	Normoalbuminuria (n =23)	Microalbuminuria (n =23)	Macroalbuminuria (n= 23)	P1	P2	P3
HBA1c% **Mean** ± SD (range)	8.27±1.34 (6.8-11.2)	9.03±2.28 (6.2-16.6)	10.09±2.24 (6.8-14.0)	0.141	**<0.001**	**0.043**
RBG(mmol/l) **Mean** ± SD (range)	10.73±3.86 (5.1-20.9)	10.90±4.37 (6.2-21.3)	12.78±4.35 (7.3-21.9)	0.879	0.061	0.084
TP(g/l) **Median (range)**	75.4 (69.1-82.7)	75.1 (48.7-80.9)	69.0 (49.9-84.5)	0.669	**<0.001**	**<0.001**
Albumin(g/l) **Median (range)**	45.00 (42-48)	44.00 (20-49)	35.00 (17-48)	0.999	**<0.001**	**0.002**
Cholesterol (mg/dl) **Median (range)**	183.0 (141-304)	180.0 (70-259)	187.0 (81-304)	0.372	0.740	0.575
TG(mg/dl) **Median (range)**	143.0 (63-367)	168.0 (42-310)	188.0 (81-342)	0.774	0.272	0.812
HDL(mg/dl) **Median (range)**	41.2 (29.0-60.4)	41.4 (13.5-65.2)	43.9 (13.9-56.4)	0.999	0.999	0.999
LDL(mg/dl) **Median (range)**	102.6 (66.3-198.0)	100.8 (32.0-168.4)	112.9 (35.3-198.0)	0.283	0.738	0.459
Urea (mmol/l) **Median(range)**	4.60 (1.7-11.3)	4.6 (2.4-33.5)	10.6 (1.6-40.0)	0.824	**<0.001**	**<0.001**
Creatinine (µmol/l) **Median(range)**	65.0 (36-111)	65.0 32-200)	167.0 (69-716)	0.493	**<0.001**	**<0.001**
Alb/creat ratio(mg Albumin/gm creatinine) **Median(range)**	11.7 (1.7-27.4)	52.8 (33.4-257.2)	1480.3 (309.1-5038.1)	**<0.001**	**<0.001**	**0.003**
eGFR(ml/min) **Median(range)**	108.0 (44-128)	103.0 (24-126)	35.00 (5-99)	0.164	**<0.001**	**<0.001**
TNFα pg/mL **(Mean ± SD)**	18.82±2.14 (15.35-25.0)	20.82±3.12 (16.46-27.64)	20.87±1.02 (19.64-22.96)	0.999	**0.005**	**0.007**
IL6 pg/mL **(Median(range))**	0.0 (0.0-5.2)	0.4 (0.0-388.3)	4.1 (0.0-1534.1)	0.358	**<0.001**	**<0.001**
circANKRD36 **Median(range)**	20.25 (0.84-106.15)	174.02 (64.45-495.88)	935.76 (360.76-2304.12)	**0.004**	**<0.001**	**0.004**

***P*1,** compared between **Normoalbuminuria** and **Microalbuminuria**
***P*2,** compared between **Normoalbuminuria** and **Macroalbuminuria**
***P*3,** compared between **Microalbuminuria** and **Macroalbuminuria**

Correlation between circANKRD36 and other investigations among patients with T2DM:

- CircANKRD36 in diabetic group had significant negative correlation with total protein, albumin and eGFR. Also had significant positive correlation HBA1C, urea, creatinine, album\create ratio, TNFα and IL6.
- TNFα in diabetic group had significant negative correlation with total protein, albumin and eGFR. Also had significant positive correlation HBA1C, creatinine, album\create ratio and IL6.
- IL6 in diabetic group had significant negative correlation with total protein, albumin and eGFR. Also had significant positive correlation with HBA1C, Urea, creatinine and album\create ratio (table 4).

Table 4: Correlation between circANKRD36, TNFα, IL6 and other investigation among patients with T2DM.

Variables	circANKRD36		TNFα		IL6	
	T2DM (n=69)		T2DM (n=69)		T2DM (n=69)	
	r	P	r	P	r	P
HBA1C %	**0.286**	**0.017**	**0.255***	**0.035**	**0.271**	**0.024**
RBG (mmol/l)	0.128	0.295	0.173	0.154	0.016	0.898
TP (g/l)	**-0.525**	**<0.001**	**-0.439**	**<0.001**	**-0.341**	**0.004**
Albumin (g/l)	**-0.475**	**<0.001**	**-0.271**	**0.025**	**-0.364**	**0.002**
Urea (mmol/l)	**0.464**	**<0.001**	0.216	0.074	**0.361**	**0.002**
Creatinine (µmol/l)	**0.648**	**<0.001**	**0.326**	**0.006**	**0.461**	**<0.001**
album\create ratio (mg Albumin/gm creatinine)	**0.869**	**<0.001**	**0.490**	**<0.001**	**0.558**	**<0.001**
eGFR (ml/min)	**-0.650**	**<0.001**	**-0.363**	**0.002**	**-0.491**	**<0.001**
Cholesterol (mg/dl)	0.003	0.981	0.028	0.822	0.008	0.950
Triglyceride (mg/dl)	0.160	0.188	0.043	0.728	-0.028	0.821
HDL (mg/dl)	-0.125	0.306	0.085	0.488	-0.101	0.409
LDL (mg/dl)	-0.007	0.955	0.013	0.913	0.092	0.451
circANKRD36			**0.424**	**<0.001**	**0.531**	**<0.001**
TNFα pg/mL	**0.424**	**<0.001**			**0.271**	**0.024**
IL6 pg/mL	**0.531**	**<0.001**	**0.271**	**0.024**		

Diagnostic criteria of circANKRD36, TNFα and IL6 for prediction of T2DM in comparison to control:

- circANKRD36: At a cut-off value >4.14, circANKRD36 were 99.5% accurate, 98.6% sensitive and 100 % specific for diagnosis of diabetic nephropathy, with area under the curve of 0.997, positive predictive value (PPV) of 100 % and negative predictive value (NPV) of 95.8% (P value<0.001).
- TNFα: At a cut-off value >19.07, TNFα were 81.0% accurate, 73.91% sensitive and 87.0 % specific for diagnosis of diabetic nephropathy, with area under the curve of 0.837, positive predictive value (PPV) of 94.4% and negative predictive value (NPV) of 52.6% (P value<0.001).
- IL6: At a cut-off value >0.3, circANKRD36 were 72.0% accurate, 60.9% sensitive and 82.6% specific for diagnosis of diabetic nephropathy, with area under the curve of 0.744, positive predictive value (PPV) of 91.3% and negative predictive value (NPV) of 41.3% (P value<0.001) (table 5) (figure 2).

Table 5: Diagnostic criteria of circANKRD36, TNFα and IL6 for prediction of T2DM in comparison to control:

Indices	Diagnostic criteria		
	circANKRD36	**TNFα**	**IL6**
AUC, 95% CI	0.997 (0.992-1.000)	0.837 (0.746-0.906)	0.744 (0.642-0.829)
Cut off	>4.14	>19.07	>0.3
Accuracy	99.5%	81.0%	72.0%
Sensitivity, %	98.6%	73.91%	60.9%
Specificity, %	100.0%	87.0%	82.6%
PPV %	100.0%	94.4%	91.3%
NPP %	95.8%	52.6%	41.3%
P Value	**<0.001**	**<0.001**	**<0.001**

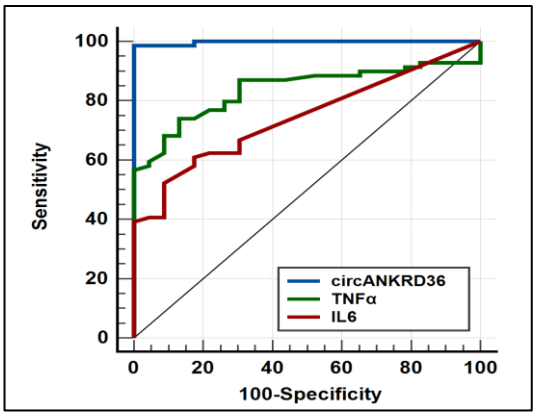

Fig. 2: Roc curve for the diagnostic ability of circANKRD36, TNFα and IL6 for prediction of T2DM in comparison to control.

DISCUSSION

Diabetes is a common long-term metabolic disease. Retinopathy, nephropathy, cardiomyopathy, neuropathy, and atherosclerosis serve as principal factors in morbidity and mortality in diabetic patients. So the macro- and microvascular complications are the bad prognostic features of DM. Rising studies suggests that low-grade inflammation, marked by elevated production of inflammatory factors, could have a major influence on the development of chronic diabetic complications[9].

In this research regarding demographic data age and sex didn't show a significant difference between T2DM patients and control group that is consistent with Fang et al.[10] that showed the same results.

In our study regarding comparison between T2DM patients and control group showed that there's significant difference in total protein and albumin level, this is explained by the characteristic histomorphological features of DN involve increase thickness of the glomerular basement membrane (GBM), extension of the mesangial matrix, nodular sclerosis, widespread effacement of podocyte foot processes, and ultimately progression to kidney failure[11]. This is in agreement with the results reported in the study by Hasan et al.[12].

Also kidney function tests (urea, creatinine, Alb/creatinine ratio and eGFR) showed significant difference because hyperglycemia influences both primary glomerular and vascular lesions involved in the progression of kidney disease. Alongside diabetes, additional factors like hypertension, smoking, obesity, and genetic predisposition also play a role in the pathological alterations[13]; these findings are in line with those of the study by Rashad et al.[14].

Lipid profile showed significant difference this results from elevated plasma levels of VLDL and LDL, which may be due to increase hepatic production of VLDL or reduced clearance of VLDL and LDL from the bloodstream, that is consistent with Sabahekhier et al.[15].

In our study IL6 and TNF-α levels were significant difference between both groups due to in diabetic or overweight individuals, there was a persistent low-grade inflammation, reflected by increased levels of cytokines such as TNF-α and IL6[16], that is consistent with Malenica et al.[17] and Al-Sarray et al. [18] respectively.

Our findings demonstrated that expression of circANKRD36 was elevated significantly in T2DM patients in comparison to the healthy group that is in agreement with the study done by Rashad et al.[14] and Pan et al.[19].

In the recent research regarding the comparison between normoalbuminuria and microalbuminuria with macroalbuminuria showed a significant difference in level of HbA1c, urea and creatinine these results correspond to those obtained by Gomaa et al.[20], a significant difference in TP, albumin and eGFR these findings are in line with the study carried out by Elsheik et al.[21] and a significant difference in levels of TNF-α and IL6 these results correspond to those obtained by Moriwaki et al.[22].

In comparison among 3 subgroups we found statistically a significant difference in both Alb/creat ratio and circANKRD36 these findings are in line with the study carried out by Rashad et al.[14].

Our results showed significant positive correlations between the levels of circANKRD36 and HBA1c, urea, creatinine, alb/ creatinine ratio, TNF-α and IL6 also showed significant negative correlations with TP, albumin and this suggests that circANKRD36 could serve as a noninvasive hematological indicator in diabetic nephropathy.

In our study the pro inflammatory markers (TNF-α and IL6) showed significant positive correlations with HBA1c, urea, creatinine, alb/ creatinine ratio due to chronic inflammation in nearly all patients with diabetes type 2 and is strongly linked to the pathophysiological changes that occur through the development of the disease[23], also showed significant negative correlations with TP, albumin and eGFR, .

ROC analysis was employed to assess the diagnostic ability of circANKRD36, TNF-α and IL-6 in distinguishing DN patients with T2DM from the control group. Our findings showed that the sensitivity was 98.6 % and the specificity was 100% for circANKRD36 that is consistent with the research done by Rashad et al.[14] which showed the sensitivity and the specificity were 90% and 93% for circANKRD36.

Our findings indicated that the sensitivity was 73.91% and the specificity was 87% for TNF-α in diagnosis of DN this is in agreement with the results

reported in the study by Zhang et al. [24] that showed sensitivity and specificity for TNF-α 65.96%, 76.22% in the same order in the diagnosis of early-stage diabetic nephropathy in type 2 diabetes patients.

Our findings indicated that sensitivity was 60.9% and specificity was 82.6% for IL6 this is in agreement with the results reported in the study by Singh et al.[25] that showed sensitivity and specificity for IL6 70.6%, 60.2% in predicting of diabetic nephropathy in diabetic patients.

CONCLUSION

In conclusion, CircANKRD36 showed high expression levels in patients with T2DM and DN, and contribute to the development of DN. CircANKRD36 can detect early stages of DN and it was superior to inflammatory markers (TNF-α and IL6) in detection of early stages of DN.

Declarations:

Consent for publication: Not applicable

Availability of data and material: Data are available upon request.

Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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