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Urinary Activin A Level as a Predictor of Nephropathy in Type 2 Diabetic Patients

Asmaa E. I. Emam, Muhammad T. Abdel Ghafar, Wael F. M. Farrag and Khaled H. Shalaby

Department of Internal Medicine, Faculty of Medicine, Tanta University, Tanta, Egypt

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is a major health problem and its prevalence is increasing worldwide which results in high morbidity and mortality due to its wide spectrum of complications. Both diabetic nephropathy (DN) and diabetic retinopathy (DR) are serious microvascular complications and have been considered the major cause of blindness and end-stage renal failure. **Objective:** our study was conducted to assess the Urinary Activin A Level as a Predictor of Nephropathy in Type 2 Diabetic Patients **Patients and methods:** the study was conducted on 90 subjects 30 were healthy control and 60 had a diagnosis of T2DM according to American Diabetes Association Criteria (ADAC). Data were collected including, demographic, clinical, and laboratory data. Statistical analysis was carried out for all collected data using IBM SPSS. Statistical significance was determined at a p-value <0.05. **Results**: there was a significant increase between all studied groups with higher levels of urinary activin A in the macroalbuminuria group with a mean (252.14 ± 88.83). While, there was an insignificant difference between the control group and type 2 diabetic patients without albuminuria group as regard Activin A (*p*-value > 0.05). **Conclusion**: our current study suggests that urinary activin A is associated with the presence of DN so it can be used as a new marker for the prediction of DN as Activin A was positively correlated with the ACR in type 2 diabetic patients without albuminuria, type 2 diabetic patients with microalbuminuria, and type 2 diabetic patients with macroalbuminuria but there was no correlation between Activin A and ACR in normal control group.

Keywords: Type 2 diabetes mellitus, chronic kidney diseases and urinary activin A.

1. Introduction

Diabetes is a global health burden and its morbidity and mortality are increasing in recent decades (Pan *et al.,* 2017). Diabetes mellitus is the collective term for heterogeneous metabolic disorders whose main finding is chronic hyperglycemia due to disturbed insulin secretion or a disturbed insulin effect or usually both (Petersmann *et al.,* 2019).

Diabetic nephropathy, neuropathy, and retinopathy are the main microvascular complications induced by chronic hyperglycemia via several mechanisms such as the production of advanced glycation end products (AGEs), the creation of a proinflammatory microenvironment, and the induction of oxidative stress (Papatheodorou *et al.,* 2016).

Diabetic nephropathy is one of the major causes of end-stage renal failure worldwide (Qi *et al.,* 2017), it is defined according to the changes in renal structure and function. which include mesangial expansion, glomerular and tubular basement membrane thickening, and glomerular sclerosis and it usually manifests as a clinical syndrome including persistent albuminuria increased blood pressure and sustained reduction in glomerular filtration rate (GFR) (Lin *et al.,* 2018).

Urinary albumin excretion is the biomarker currently used to detect and stage DN because it is non-invasive; however, it lacks accuracy as it is normal in early DN (Tramonti *et al.,* 2013) while increased in other types of nephropathy (Haffner *et al.,* 1990) Therefore, the search for biomarkers with better accuracy is required for early detection of DN.

Corresponding Author: Asmaa E. I. Emam, Department of Internal Medicine, Faculty of Medicine, Tanta University, Tanta, Egypt. E-mail: asmaemam1994@gmail .com

Activin A, a member of the transforming growth factor-β (TGF-β) superfamily, composed of inhibin βA-βA subunits, initially discovered related to its capacity to induce the release of follicle-stimulating hormone (Sugatani, *et al.* 2018), not detected in normal kidney, was upregulated in tubular cells after ischemia/reperfusion injury (Yamashita, *et al.,* 2004).

Some studies have discovered that, in the early phase of DN, TGF-β exhibits upregulation in tubular epithelial and facilitates the differentiation of tubular epithelial cells into myofibroblasts (Ren *et al.,* 2009). Recently, studies have shown activin A to be induced in diabetic kidney disease (DKD) and to be a prominent mediator of hyperglycemia-induced profibrotic responses in mesangial cells. Moreover, Activin A inhibitor; follistatin, improved DKD in type 1 diabetic Akita mice (Soomro *et al.,* 2021).

Despite these recent studies, information about activin A in DKD is incomplete, with little known about the relationship between Activin A and kidney function in individuals with DKD (Bian, *et al.,* 2019) therefore, this study aims to clarify the potential role of Activin A as a predictor of diabetic nephropathy in type 2 diabetic patients and to evaluate its relation to various clinical and laboratory parameters.

This study aims to assess the role of urinary Activin A level as a possible predictive factor for the detection of nephropathy in type 2 diabetic patients**.**

2. Patients and Methods

Study Design: A cross-sectional study.
Study population: This was carried out on 90 subjects divided into four groups:

- **Group I**: 30 apparently healthy subjects will be included to serve as healthy control,
- **Group II**: Type 2 diabetic patients without albuminuria (n=20),
- **Group III**: Type 2 diabetic patients with microalbuminuria (n=20),
- **Group IV**: Type 2 diabetic patients with macroalbuminuria (n=20).

The patients were recruited from the internal medicine Department, at Tanta University hospitals in the period between September 2021 to August 2022.

Ethical consideration

Permission was obtained from Research Ethics Committee as a part of the Quality Assurance Unit in the Faculty of Medicine at Tanta University to conduct this study and to use the facilities in the hospital. Informed written consent was obtained from all patients.

Provision of privacy

Privacy of all data was guaranteed as the following: There was a code number for each patient, so the data of patients were strictly confidential.

Inclusion criteria

Patients diagnosed with type 2 diabetes mellitus according to the American Diabetes Association criteria were selected from the Internal Medicine department inpatient wards outpatient clinics of Tanta University Hospitals.

Exclusion criteria

All patients with the following will be excluded from this study:

- Type 1 Diabetes mellitus.
- Glomerulonephritis or nephrotic syndrome.
- Renal dialysis.
- Autoimmune diseases and inflammatory bowel diseases.
- Chronic infection.
- Active immunosuppressive therapy.
- Pregnancy.
- Malignancy.

Possible Hazards during the research

The slight risks of bleeding or infection during blood sampling for investigations, it was avoided by good compressing or by using sterile techniques. No other hazards are expected during the period of research. There was safe disposal of waste products e.g., needles…etc. Any unexpected risks that appeared during the research were cleared to participants and the ethical committee on time**.**

2.1. Methods

All the participants will be subjected to

- **Full history taking** Including age, sex, cigarette smoking, duration of DM, history of receiving medication for chronic diseases, and family history.

- **Complete clinical examination**

A. Measurement of systolic and diastolic blood pressure.
B. Chest, cardiac and abdominal examination to exclude subjects with any abnormal findings.
C. Measurement of body weight and height with calculation of body mass index (BMI) which is a person's weight in kilograms divided by the square of their height in meters.
D. Fundus Examination.

- **Laboratory investigations**

1. Serum urea and creatinine levels.
2. Urinary albumin/creatinine ratio.
3. Estimated Glomerular Filtration Rate(eGFR).
4. Lipid profile (total cholesterol, high-density lipoprotein-cholesterol, low density, lipoprotein-cholesterol, and triglycerides).
5. Fasting and 2h post prandial blood glucose.
6. HbA1c.
7. Complete urine analysis.
8. Liver functions test.
9. Complete blood count test.

Specific laboratory investigations:

Measurement of Activin A level in the urine using human activin A ELISA (Enzyme-Linked Immunosorbent Assay) kit with catalogue No 201-12-0115 from Shanghai Sunred SRB) Technology Company . Collecting urine samples in a sterile container, centrifugation for 20 minutes at a speed of 2000-3000 r.p.m then the supernatant was removed.

Test principle

The kit uses a double – antibody sandwish enzyme-linked immunosorbent assay (ELISA) to assay the level of human activin A in samples. By adding activin A to monoclonal antibody enzyme which is pre coated with human activin A monoclonal antibody, incubation then, adding activin A antibodies labled with biotin, and combined with streptavidin –HRP to form immune complex then carrying out and washing again to remove the uncombined enzyme. Then adding chromogen solution A, B, the color of the liquid will change inyo the blue, and at the effect of acid, the color finally becomes yellow .the chroma of color and the concentration of the human substance Activin A of sample were positively correlated.

Statistical analysis of the data

Data were analyzed using the IBM® SPSS statistical software, version 21. We used the one-sample Kolmogorov-Smirnov test to check the normality of the data some data were parametric and others were non-parametric. Numerical data were presented as mean and standard deviation (SD) and categorical data were presented as numbers and percentages. Chi-squared test was used for comparing the qualitative data. One Way of Anova test (ANOVA) test was used to compare the means in different groups if the data were parametric. While Kruskal-Wallis test was used also to compare the means in different groups if the data were non-parametric. Significance between groups was done

using Post Hoc Test (Tukey HSD test) linear correlation analysis was done by spearman coefficient correlation and used to test the positive or negative associations between different variables. For the risk estimated, Linear regression was used to detect the predictor variables Receiver operating characteristic (ROC) curve analysis was done to detect the sensitivity and specificity of the studied marker. The level of significance was adopted at $p<0.05$. The ROC (receiver operating characteristic) curve: The ROC curve is a graphical representation of the interaction between sensitivity and specificity for a diagnostic test at various cutoff points. The left upper corner of the curve is a good diagnostic test. Sensitivity and specificity are both 100% at this point. The point of the curve closest to the upper left corner provides the highest test potential and minimizes the sum of incorrect diagnoses. The area below a curve is the overall accuracy of the test; the bigger the zone, the better the results. The curve nearest to the upper left corner is more sensitive and specific, which means that both curves are more accurate.

3. Results

In terms of age and gender, there were no significant difference between the studied groups ($p>0.05$) with a significant difference regarding BMI ($p<0.001$). The control group had the lowest BMI.

Regarding the duration of diabetes and smoking, there were no significant differences between the studied groups ($p>0.05$). However, regarding hypertension and use of ACE or ARBs there were significant differences among all groups (all $p < 0.001$).

HbA1c, FBG, 2hPP, urea, creatinine, ACR, TGs, LDL, cholesterol, and CRP levels showed significantly increasing trends across all the studied groups (all $p < 0.001$), with the highest levels in macroalbuminuria group. However, Hb, eGFR, and HDL levels showed significantly decreasing trends across the studied groups (all $p <0.001$), with the lowest levels in macroalbuminuria group. There was no significant difference among the studied groups regarding TLC and platelet count (all $p > 0.05$) (Table 1).

Activin A levels showed significantly increasing trends among the studied groups, with the highest level in macroalbuminuria group (all $p < 0.001$), However, there was no significant difference between the control group and T2DM patients without albuminuria ($p > 0.05$) (Table 2).

urinary activin A level had a positive correlation with ACR in T2DM patients with and without albuminuria. However, there was no significant correlation between urinary activin A levels and ACR in the normal control group. Additionally, there were no significant correlation between activin A levels, DN grades, creatinine, eGFR and CRP levels in each studied group (Table 3).

Table 1: Comparison between the studied groups regarding laboratory investigations

	Normal (n = 30)	**Normoalbuminuria (n = 20)**	**Microalbuminuria (n = 20)**	**Macroalbuminuria (n = 20)**	**Test of significance**	***P***
FBG (mg/dl)						
Mean ± SD.	84.60 ± 7.44	125.4 ± 15.81	141.4 ± 39.97	226.6 ± 62.04	F =64.152*	<0.001*
p_0		0.001	<0.001*	<0.001*		
Sig. bet. grps.		p_1=0.493,p_2<0.001*,p_3<0.001*				
2hPP(mg/dl)						
Mean ± SD.	161.1 ± 184.6	174.3 ± 31.87	209.4 ± 53.83	298.6 ± 81.94	F =6.074*	0.001*
p_0		0.980	0.489	0.001		
Sig. bet. grps.		p_1=0.781,p_2=0.007*,p_3=0.085				
HbA1C (%)						
Mean ± SD.	5.21 ± 0.30	7.39 ± 0.59	8.02 ± 1.83	11.63 ± 2.26	F =84.272*	<0.001*
p_0		<0.001*	<0.001*	<0.001*		
Sig. bet. groups.		p_1=0.487,p_2<0.001*,p_3<0.001*				
HB(gm/dl)						
Mean ± SD.	11.92 ± 1.22	11.82 ± 0.99	11.31 ± 1.48	10.19 ± 1.06	F =9.430*	<0.001*
p_0		0.991	0.292	<0.001*		
Sig. bet. grps.		p_1=0.534,p_2<0.001*,p_3=0.023*				
PLT(Thousands/cmm)						
Mean ± SD.	260.5 ± 72.03	245.3 ± 48.03	242.8 ± 49.43	248.9 ± 55.69	F =0.458	0.713
TLC(Thousands/cmm)						

	Normal (n = 30)	**Normoalbuminuria (n = 20)**	**Microalbuminuria (n = 20)**	**Macroalbuminuria (n = 20)**	**Test of significance**	***P***
Mean ± SD.	7.52 ± 1.93	8.17 ± 2.03	8.42 ± 1.55	7.34 ± 2.22	F =1.484	0.225
Urea(mg/dl)						
Mean ± SD.	28.77 ± 10.47	40.60 ± 13.32	54.92 ± 21.69	77.65 ± 32.17	F =25.062*	<0.001*
p₀		0.186	<0.001*	<0.001*		
Sig. bet. grps.		p_1=0.121,p_2<0.001*,p_3=0.003*				
Creatinine (mg/dl)						
Mean ± SD.	0.74 ± 0.13	0.89 ± 0.16	1.35 ± 0.30	2.33 ± 1.33	F =26.80*	<0.001*
p₀		0.842	0.009*	<0.001*		
Sig. bet. grps.		p_1=0.129,p_2<0.001*,p_3<0.001*				
ACR(mg/g cre)						
Min. – Max.	4.50 – 27.0	4.50 – 27.70	54.0 – 230.0	318.0 – 1684.0	H= 71.924	<0.001*
Median (IQR)	15.50 (12.0 – 21.0)	16.0 (12.50 – 20.50)	93.75 (62.23 – 153.20)	727.5 (435.0 – 816.50)		
p₀		0.765	<0.001*	<0.001*		
Sig. bet. grps.		p_1<0.001*,p_2<0.001*,p_3=0.015*				
e GFR						
Mean ± SD.	98.07 ± 12.63	83.58 ± 14.02	53.03 ± 16.22	34.26 ± 18.89	F= 83.415*	<0.001*
p₀		0.008*	<0.001*	<0.001*		
Sig. bet. grps.		p_1<0.001*,p_2<0.001*,p_3=0.001*				
TGs(mg/dl)						
Min. – Max.	70.0 – 129.0	54.0 – 180.0	80.0 – 325.0	61.0 – 420.0	H= 29.334	<0.001*
Median (IQR)	97.50 (86.80 – 107.0)	94.0 (87.80 – 125.0)	147.5 (137.50 – 168.0)	175.0 (98.50 – 193.50)		
p₀		0.587	<0.001*	<0.001*		
Sig. bet. groups.		p_1=0.002*,p_2<0.001*,p_3=0.670				
HDL(mg/dl)						
Mean ± SD.	68.03 ± 5.92	66.75 ± 16.70	59.22 ± 17.53	42.59 ± 10.79	F =17.801*	<0.001*
p₀		0.986	0.092	<0.001*		
Sig. bet. grps.		p_1=0.261,p_2<0.001*,p_3=0.001*				
LDL (mg/dl)						
Mean ± SD.	97.33 ± 13.24	97.0 ± 37.62	126.7 ± 43.21	155.7 ± 73.21	F=8.716*	<0.001*
p₀		1.000	0.107	<0.001*		
Sig. bet. grps.		p_1=0.155,p_2<0.001*,p_3=0.172				
Cholest(mg/dl)						
Mean ± SD.	185.6 ± 15.90	184.8 ± 43.30	216.2 ± 45.86	235.5 ± 93.51	F=4.636*	0.005*
p₀		1.000	0.208	0.010*		
Sig. bet. grps.		p_1=0.260,p_2=0.019*,p_3=0.670				
CRP(mg/l)						
Min. – Max.	1.0 – 5.50	1.0 – 11.0	0.50 – 132.0	1.50 – 45.0	H =27.783	<0.001*
Median (IQR)	3.25 (2.50 – 4.50)	5.0 (2.50 – 6.50)	8.0 (5.0 – 19.0)	13.0(4.75 – 26.50)		
p₀		0.095	<0.001*	<0.001*		
Sig. bet. groups.		p_1=0.025*,p_2=0.009*,p_3=0.700				

CRP ,c reactive protein; FBG,fasting blood gloucose; 2hPP, 2 hour post prandial; TLC, total leucocytic count; PLT,platelets; HB,heamoglobin; ACR,albumin creattinie ratio;e GFR: estimated glomerular filtration rate; IQR,inter quartile range; SD, standard deviation.

F: F for One way ANOVA test, Pairwise comparison between each of 2 groups was done using Post Hoc Test (Tukey)

H: H for Kruskal Wallis test, Pairwise comparison between each of2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test)

p: p-value for comparing the four studied groups

p₀: p-value for comparing between Normal and each other group

p₁: p-value for comparing between Normoalbuminuria and Microalbuminuria groups

p₂: p-value for comparing between Normoalbuminuria and Macroalbuminuria groups

p₃: p-value for comparing between Microalbuminuria and Macroalbuminuria groups

*: Statistically significant at $p \leq 0.05$

Table 2: Comparison among the four studied groups according to Activin A

Activin A (ng/l)	Normal (n = 30)	Normoalbuminuria (n = 20)	Microalbuminuria (n = 20)	Macroalbuminuria (n = 20)		*P*
Mean ± SD.	140.42 ± 32.47	140.57 ± 54.63	197.93 ± 83.0	252.14 ± 88.83	F=14.652*	<0.001*
p_0		0.990	<0.001*	<0.001*		
Sig. bet. grps.		p_1=0.007*, p_2<0.001*,p_3=0.01*				

SD: Standard deviation
F: F for One way ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)
p: p value for comparing between the four studied groups
p_0: p value for comparing between Normal and each other group
p_1: p value for comparing between Normoalbuminuria and Microalbuminuria
p_2: p value for comparing between Normoalbuminuria and Macrolbuminuria
p_3: p value for comparing between Microalbuminuria and Macroalbuminuria
*: Statistically significant at $p \leq 0.05$

Table 3: Correlation between Activin A levels and different parameters in each group

	Activin A							
	Normal		Normo		Micro		Macro	
	R	P	r	p	R	P	R	P
Creatinine(mg/dl)	0.304	0.103	0.261	0.267	0.010	0.967	0.103	0.666
ACR(mg/g cre)	0.270	0.150	0.655	0.002*	0.761	<0.001*	0.711	<0.001*
eGFR	-0.267	0.154	-0.171	0.470	-0.064	0.790	-0.240	0.307
CRP(mg/l)	-0.032	0.868	0.011	0.962	-0.025	0.915	0.124	0.603

r: Pearson coefficient
*: Statistically significant at $p \leq 0.05$

4. Discussion

Diabetic nephropathy is one of the most common complications of diabetes. The prevalence of DN is increasing steeply along with the diabetes epidemic. Approximately one-third to half of the patients with DM develop renal manifestations (Muddu, *et al.,* 2018).

Much attention has been paid recently to the role of the transforming growth factor-beta (TGF-β) superfamily members in renal disease and in particular of activin A (ActA). ActA is an important regulator of the normal development of fetal kidneys. While it is not expressed in healthy adult kidneys, it seems to be involved in the progression of several renal diseases and related complications (Cianciolo *et al.,* 2021)

This study aims to assess the role of urinary Activin A level as a possible predictive factor for the detection of nephropathy in type 2 diabetic patients.

As regard FBG (p-value < 0.001). Type 2 diabetic patients with the macroalbuminuria group showed higher FBG (226.6 ± 62.04). Also, there was a significant decrease when comparing the normal control group and type 2 diabetic patient groups as regard FBG (p-value < 0.001).

Regarding 2hPP there was a significant increase between all studied groups (p-value < 0.001). Type 2 diabetic patients with the macroalbuminuria group showed higher 2hPP (298.6 ± 81.94). Also, there was a significant difference when comparing the normal control group and type 2 diabetic patients with the macroalbuminuria group as regard 2hPP (p-value < 0.001). While there was an insignificant difference between the normal control group and type 2 diabetic patients without albuminuria and type 2 diabetic patients with microalbuminuria as regard 2hPP (p-value > 0.05).

Moreover, there was a significant increase between all studied groups, as regard HbA1C (p-value < 0.001). Type 2 diabetic patients with the macroalbuminuria group showed higher HbA1C (11.63 ± 2.26). Also, there was a significant decrease when comparing the normal control group and type 2 diabetic patient groups as regard HbA1C (p-value < 0.001).

This finding is in line with a systematic review conducted in Oman Obtained by Alrawahi *et al.,* 2012 reported that the incidence of diabetic nephropathy was 42.5% and the significant risk factors

associated with it include long duration of diabetes, family history of diabetic nephropathy, and poor glycemic control (high HbA1c).

Also agreed with a 3-year retrospective cohort study of 604 Korean patients with type 2 diabetes mellitus by Ki-Ho Song *et al.* (2019) that showed HbA1c variability and dyslipidemia were risk factors for the progression of DN independent of eGFR and urine ACR.

Regarding Heamoglobin there was a significant decrease between all studied groups, as regard HB (p-value < 0.001). Type 2 diabetic patients with the macroalbuminuria group showed lower HB (10.19 ± 1.06). Also, there was a significant increase when comparing the normal control group and type 2 diabetic patients with the macroalbuminuria group as regard HB (p-value < 0.001). While there was an insignificant difference between the normal control group and type 2 diabetic patients without albuminuria and type 2 diabetic patients with microalbuminuria as regard HB (p-value > 0.05).

This finding is in line with A meta-analysis conducted to determine the pooled prevalence and associated factors of anemia in diabetic patients in Ethiopia. (2021) which reported that The pooled prevalence of anemia in diabetic patients in Ethiopia using the random-effects model was estimated to be 24.81%

Observational studies indicated that lower Hb levels in diabetic nephropathy/DKD were associated with adverse outcomes, including increased risks for the progression of kidney disease, cardiovascular morbidity, and mortality. Nevertheless, most of these studies did not consider the renal pathology. Recently, pathological studies focusing on diabetic nephropathy, post-transplant nephropathy, and ANCA-associated renal vasculitis demonstrated that the progression of renal anemia was associated with advanced renal tubulointerstitial lesions.

Regarding platelets and TLC, there was an insignificant difference between all studied groups (p-value > 0.05).

Regarding renal function tests, there was a significant difference between all studied groups, as regard urea, and creatine (p-value < 0.001). Type 2 diabetic patients with the macroalbuminuria group showed higher urea, and creatine (77.65 ± 32.17), and (2.33 ± 1.33) respectively. While there was an insignificant difference between the normal control group and type 2 diabetic patients without albuminuria as regard urea, and creatine (p-value > 0.05).

Regarding ACR and eGFR there was a significant difference between all studied groups. Type 2 diabetic patients with the macroalbuminuria group showed higher ACR with a median (727.5). While there was an insignificant difference between the normal control group and type 2 diabetic patients without albuminuria as regard ACR (p-value > 0.05). Type 2 diabetic patients with the macroalbuminuria group showed lower eGFR (34.26 ± 18.89) that agreed with a prospective and controlled study conducted in the Biochemistry Laboratory of Izmir Ataturk Training and Research Hospital by ozgur Aslan *et al.,* 2009 which reported that Patients in the albuminuric group had longer diabetes duration and lower eGFR compared with other groups.

Regarding Triglycerides, there was a significant difference between the studied groups. Type 2 diabetic patients with the macroalbuminuria group showed higher TGs with a median (175) While there was an insignificant difference between the normal control group and type 2 diabetic patients without albuminuria. (p-value > 0.05).

Similarly regarding Cholesterol Type 2 diabetic patients with the macroalbuminuria group showed higher TGs with a median (235.5 ± 93.51)
Also, LDL Type 2 diabetic patients with the macroalbuminuria group showed higher TGs with a median (of 155.7 ± 73.21).

On the other hand, regarding HDL there was a significant difference between all studied groups (p-value < 0.001). Type 2 diabetic patients with the macroalbuminuria group showed lower HDL (42.59 ± 10.79). Also, there was a significant increase when comparing the normal control group and type 2 diabetic patients with the macroalbuminuria group as regard HDL (p-value < 0.001). While there was an insignificant difference between the normal control group and type 2 diabetic patients without albuminuria and type 2 diabetic patients with microalbuminuria as regard HDL (p-value > 0.05).

That agreed with A cross-sectional study by Moyad Jamal Shahwan *et al.* (2019). that was carried out the 291 diabetes patients recruited 22.3% had hypercholesterolemia (TC ≥ 200) and 61.9% had hypertriglyceridemia. Abnormal LDL-C levels (≥130) were found in 8.9% of patients and HDL-C

was less than 40 mg/dl in 54.3%. Patients with HbA1c values ≥ 7.0% had significantly higher values of total cholesterol (TC) and abnormal LDL-C compared with the patients who had HbA1c < 7.0%
The results of the lipids profile in our study are similar to the results of a study done in Anambra state South-East Nigeria by Jisieike-Onuigbo *et al.* (2011). except regarding HDL that showed dyslipidemia in the study and control subjects. Dyslipidemia was more prevalent in diabetics with overt nephropathy. Elevated TC and hypertriglyceridemia were significantly more prevalent in DN subjects compared to subjects without DN Also LDL-C was more elevated in those with DN, compared with controls. Lower levels of serum HDL-C were more prevalent in the controls than in the DN group.

But on the other hand, they disagreed with Huang *et al.* (2015) in China a study that include 253 patients with T2DM, 115 of whom have early-stage diabetic nephropathy compared to 210 healthy age- and sex-matched subjects and found no significant differences in total cholesterol, HDL, and LDL levels were detected between the patient and control groups ($P > 0{\cdot}05$ for all).
In this study, we measured urinary activin A by ELISA to assess its role as a predictor for the detection of nephropathy in type 2 diabetic patients and showed there was a significant increase between all studied groups (p-value < 0.001). Type 2 diabetic patients with the macroalbuminuria group showed higher Activin A (252.14± 88.83). Also, there was a significant decrease when comparing the normal group with (type 2 diabetic patients with microalbuminuria and type 2 diabetic patients with macroalbuminuria) (p-value < 0.001). While, there was an insignificant difference between control group and type 2 diabetic patients without albuminuria group (p-value > 0.05).

That agreed with a German study by Lorenzo Catanese *et al.* (2023). that was performed on 51 patients with ANCA-associated vasculitis (AAV), 41 of whom had kidney involvement. Urinary Activin A was significantly increased in patients with kidney involvement compared to non-kidney AAV and correlated with other biomarkers of CKD such as proteinuria, liver-type fatty-acid-binding protein, and N-acetyl-beta-D-glucosaminidase. Furthermore, urinary Activin A was significantly higher in patients with a glomerular crescent formation (in a kidney biopsy), indicating ongoing glomerular inflammation and severe damage. After immunosuppressive treatment, urinary Activin A decreased rapidly.

Also agreed with a study of two adult diabetes cohorts and controls by Bian *et al.* (2019) on the relationships between plasma (or urine) activin A, estimated glomerular filtration rate (eGFR), and DKD injury biomarkers were tested with logistic regression and correlation coefficients And found that Plasma activin A levels were elevated in diabetes compared with controls and correlated inversely with eGFR.After eGFR adjustment, only albuminuria and tumor necrosis factor receptor-1 were associated with the highest activin tertile. Albuminuria is also related to urinary activin.

Also agreed with an experimental study on human and mice tissues by Li *et al.* (2003) which demonstrate the role of the TGF-β Smad signaling pathway in Extracellular matrix production induced by high glucose in a variety of cell types. We further show that high glucose–activated Smad signaling is TGF-β dependent. Importantly, the observation that inhibition of Smad signaling by overexpression of Smad 7 can block high glucose–mediated collagen matrix production indicates that targeting the TGF-β Smad signaling pathway may provide a novel therapeutic strategy for the prevention and treatment of diabetic complications.

5. Conclusion

Urinary activin A was significantly higher in diabetic patients, especially with macroalbuminuria, Urinary activin A could be used as an easy method for the prediction of diabetic nephropathy, DN has a positive correlation with urinary activin A, Activin A was positively correlated with the ACR in type 2 diabetic patients.

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