Journal of Research in Pharmacy

Research Article

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Serum soluble alpha klotho and periostin as a biomarker for detection of early diabetic nephropathy in Iraqi male with Type 2 Diabetes

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Received: 3 January 2024 / Revised: 21 January 2024 / Accepted: 23 January 2024

ABSTRACT: Diabetic nephropathy (DN) is one of the most prevalent diabetic microvascular complications, affecting up to 40% of type 2 diabetic patients (T2DM) and represents a general reason of chronic kidney disorder and end-stage kidney disease (ESKD). This study aimed to evaluate the soluble alpha klotho and periostin (POSTN) role as predictor of diabetic- nephropathy (DN) on 120 patients, 60 with T2DM and 60 with DN under hemodialysis (DN-HD) in a cross-sectional study. Serum-soluble alpha-klotho and periostin levels were evaluated using ELISA techniques. Receiver operating characteristic curves were used to evaluate the predictive value of soluble alpha-klotho and periostin, which revealed that soluble alpha-klotho significantly decreased in DN-HD patients compared to T2DM patients, while periostin significantly increased in DN-HD patients in comparison with T2DM patients. Specificity and sensitivity of the soluble alpha klotho in predicting DN were (62% and 59%), at cut-off value was 98.63 ng/mL, while periostin's sensitivity and specificity were (92% and 90%) at a cut-off value of 238.17 ng/mL which indicate that serum periostin can be considered as a valuable biomarker for early detection of DN in T2DM patients.

KEYWORDS: Diabetic mellitus; Diabetic-nephropathy; soluble alpha klotho; periostin.

1. INTRODUCTION

Diabetes mellitus (DM) is a set of heterogeneous metabolic diseases diagnosed by rise concentrations of glucose in blood which resulting from decreasing the secretion/ sensitivity to insulin, or it could be both. One of the primary destructive problems of DM is diabetic-nephropathy (DN) that become the main cause of end-stage kidney failure. DN happens in almost one third of patients diagnosed with T1DM and almost half of patients diagnosed with T2DM [1].

Klotho (KL) is single-pass transmembrane protein (135 k Da) which is encoded in human by the klotho gene. klotho gene divided in to three sub families: alpha klotho, beta klotho and gama klotho [2]. The α-klotho is expressed in multiple tissue including brain, reproductive organs, pituitary gland and parathyroid glands. However, it is more markedly produced in the renal, prominently in the distal convoluted tubules (DCT), proximal convoluted tubule (PCT), and even in some of the inner medullary collecting duct cells. α-klotho exists in two forms membrane-bound klotho alpha (mKL) and the soluble shape alpha klotho (sKL) [3,4]. Secreted α-klotho is produced by codomain shedding of mKL or alternative klotho mRNA splicing [5,6]. The main functionally active is the sKL and was detected in the blood, urine, and cerebrospinal fluid and its circulation half-life is approximated to be 7.5h. Unlike mKL ,that works as a co receptor for the fibroblast growth factor-23 (FGF23,which is a hormone derived from bone and belonging to the FGF family), sKL acts as a hormonal factor , controls the activity of calcium channels and sodium-phosphate transporter 2a , inhibits signaling pathways of insulin and insulin-like growth factor-1 (IGF-1),biomarker for diagnosis of chronic kidney disorders, improves the synthesis of endothelial nitric oxide, leading to Enhances endothelium-dependent vasodilation. Additionally, it acts as endogenous inhibitor of vascular calcification [7-9].

Periostin (POSTN), often referred to as osteoblast-specific factor-2 (OSF-2), is a kind of secrete extracellular matrix protein, It consists of 836 amino acids and has a 93.3 kDa of the molecular weight. POSTN is encoded by the human POSTN gene [10]. It contains an amino-terminal signal sequence, an EMI

How to cite this article: AL Temimi RB, Abdulla AH, Tahir NT. Serum soluble alpha klotho and periostin as a biomarker for detection of early diabetic nephropathy in Iraqi male with Type 2 Diabetes. J Res Pharm. 2024; 28(5): 1665-1673.

https://dx.doi.org/10.29228/jrp.2022.00
ISSN: 2630-6344

domain cysteine rich (first named after its presence in proteins of the EMILIN family), four tandem repeats of the Fascilin1 (FAS1) domains, and a carboxyl-terminal domain contains a heparin-binding [11]. It is expressed in connective tissues wealthy in collagens undergone that are subjected to mechanical stress conditions, like periosteum, periodontal ligaments, skin, lower digestive tract, thyroid gland, uterus, and breasts [12]. However, studies in humans have shown Peri POSTN ostin has higher expressed in biopsies obtained from patients with various renal disorders, such as diabetic nephropathy. Specifically, POSTN concentrations are elevated in both the kidney tissue and in the urine of individuals with T2DM. Additionally, POSTN concentrations are positively engaged with aging, high albumin in the urine (albuminuria), and the deterioration of kidney function. [13,14]. This study designed to assess the possibility of using s-αklotho and POSTN as markers for early detection of nephropathy in patients with T2DM.

2. RESULTS

2.1. Demographic and clinical characteristics of the patients

The mean age of the patients in DN under hemodialysis was (46.37±12.07 years) which did not differ significantly from that of T2DM patients (44.6±5.41years). Patients with T2DM had higher body mass index (BMI) (32.95±3.08) compared with both DN under HD patients (27.18±5.58). The median duration diabetes in DN under HD patients were significantly higher than T2DM patients (7.0 years and 3.5 years, respectively). The median duration of HD in patients with DN under HD was 14.5 years. As a marker for diabetic control, the mean value of HbA1c in patients with T2DM (8.31±1.34%) which was higher than DN under HD (6.6±1.73%). However, the mean of FBG did not differ significantly among the two groups (181.32±44.2 mg/dl and 181.68±39.41 mg/dl, respectively). The mean concentration of blood urea and creatinine was significantly higher in DN under HD (153.83±47.04 mg/dl and 8.08±2.54 mg/dl, respectively) than T2DM patients (35.80±7.55 mg/dl and 1.48±2.10 mg/dl, respectively). In contrast, patients DN under HD had significantly lower GFR (7.6±2.90 ml/min/1.73m^2) than those with T2DM (96.12±12.30 ml/min/1.73m^2) as shown in Table 1.

2.2. Serum levels of soluble alpha klotho and POSTN among groups

Table 2 shows that the median of sα-Kl in patients with T2DM was 364.83 pg/ml (range = 51.6–510.16 pg/ml), which was lower than that of patients with DN under HD (median = 335.25 pg/ml, range = 48.6-484.21 pg/ml).In contrast, POSTN was higher in patients with DN under HD (median= 108.07 ng/ml, range= 33.18-166.36 ng/ml) than patients with T2DM (median= 35.90 ng/ml, 26.81-54.23 ng/ml).

2.3. Predicting Value of Klotho and POSTN

ROC curve was utilized to assess the role of serum soluble alpha klotho and POSTN in predicting DN in patients with T2DM. For alpha Klotho, (AUC) was 0.623, 95% CI= 0.522-0.724, p= 0.042. The sensitivity and specificity 62% and 59%, respectively at cut-off value of = 98.63 ng/mL. For POSTN, the AUC was 0.975, 95% CI= 0.947-1.0, p<0.001. The cut-of levels were 238.17 ng/mL, with sensitivity and specificity 92% and 90%, respectively. (Figure 1)

2.4. Correlation analysis of soluble alpha klotho, POSTN with other biochemical parameters in study groups

Table 3 shows the results of correlation among patients with DN under HD. Alpha klotho displayed significant negative correlations with every of hemodialysis duration (r= -0.305, p= 0.018) and blood urea (r= -0.275, p= 0.034) (Figure 2 and 3, respectively), while POSTN had a negative correlation with GFR (r= -0.304, p = 0.018), as cleared in Figure 4.

Table 4 shows the results of correlation among patients with T2DM. Alpha klotho demonstrated a significant negative correlation with HbA1C (r= -0.275, p= 0.033), as shown in Figure 5. There was a high negative correlation among the concentration of POSTN with GFR (r=-0,272, P=0.035), as shown in Figure 6.

Table 1. Clinical diagnostics of the patients

Variable	DN under HD (n=60)	T2DM (n=60)	p-value
Age, years			
Mean ±SD	46.37±12.07	44.6±5.41	**0.256**
Range	30-72	30-70	
BMI, Kg/m²			
Mean ±SD	27.18±5.58	32.95±3.08	**0.021**
Range	16.16-46.81	24.72-36.73	
Duration of DM, M			
Mean ±SD	114±3.85	4.11±3.12	**<0.001**
Median	7.0	3.5	
Range	1.0-15.0	0.1-12.0	
Duration, M			
Mean ±SD	21.63±23.31	-------	**-----**
Median	14.5		
Range	2.0-144		
FBS, mg/dl			
Mean ±SD	181.32±44.2	181.68±39.41	0.815
Range	133-320	130-248	
HbA1c, %			
Mean ±SD	6.6±1.73	8.31±1.34	**<0.001**
Range	4.3-11.5	6.3-11.3	
Urea, mg/dl			
Mean ±SD	153.83±47.04	35.80±7.55	**<0.001**
Range	28.7-270.41	20.0-64.0	
Creatinine, mg/dl			
Mean ±SD	8.08±2.54	1.48±2.10	**<0.001**
Range	4.04-13.48	0.50-9.0	
GFR, ml/min/1.73m²			
Mean ± SD	7.6±2.90	96.12±12.30	**<0.001**
Range	4.0-14.0	64.0-121.0	

DN: Diabetic nephropathy; T2DM: type 2 diabetic mellitus; BMI: Body mass index; FBS: Fasting blood sugar; Hb1Ac: Hemoglobin A1C; GFR: Glomerular filtration rate.

Table 2. Median levels of sα-Klotho and POSTN among study population.

Variable	DN under HD (n=60)	T2DM (n=60)	p-value
sα-Klotho, pg/ml			
Mean ±SD	319.53±98.11	349.38±100.44	**0.047**
Median	335.25	364.83	
Range	48.6-484.21	51.6-510.16	
POSTN, **ng/ml**			
Mean ±SD	106.2±33.52	37.26±7.0	**<0.001**
Median	108.07	35.90	
Range	33.18-166.36	26.81-54.23	

DN: Diabetic nephropathy; T2DM: type 2 diabetic mellitus

Table 3. Correlation of soluble alpha klotho and POSTN with different study parameters.

Variables	**sα-Klotho, pg/ml**		POSTN, **ng/ml**	
	Coefficient	**p-value**	**Coefficient**	**p-value**
Age, years	0.095	0.470	0.169	0.311
BMI, kg/m²	0.055	0.676	0.243	0.061
DM duration, M	-0.003	0.980	0.148	0.259
HD duration, M	**-0.305**	**0.018**	0.014	0.914
FBS, mg/dl	0.057	0.666	-0.130	0.324
HbA1c, %	0.062	0.635	0.154	0.239
Urea, mg/ dl	**-0.275**	**0.034**	0.173	0.228
Creatinine, mg/ dl	-0.060	0.647	0.137	0.321
GFR, ml/min/1.73m²	0.055	0.679	**-0.304**	**0.018**
POSTN	-0.108	0.413		

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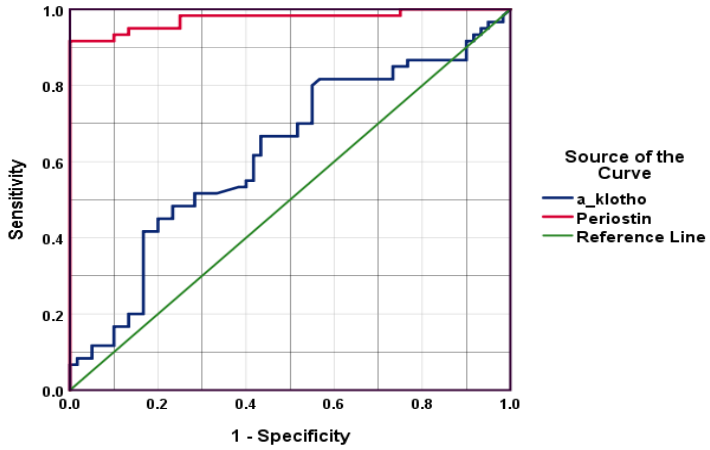

Figure 1. ROC diagram of sα-klotho and POSTN in patients with DN under hemodialysis as compared with T2DM.

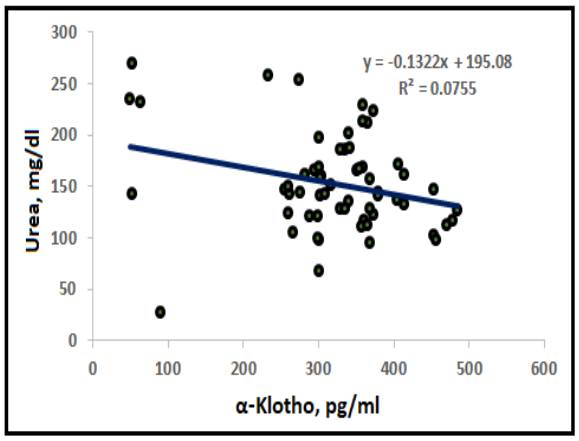

Figure 2. Scatter plot and regression line between sα-klotho and urea in patients with DN under HD

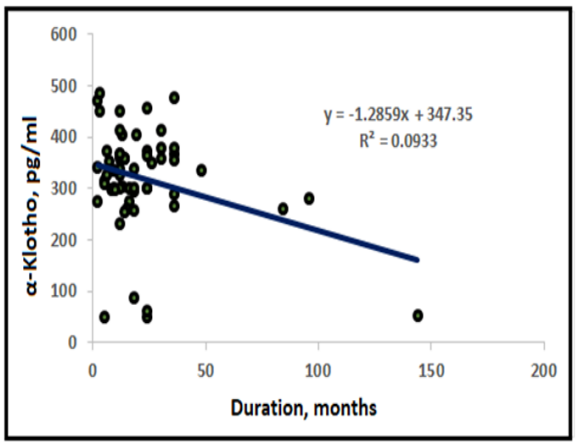

Figure 3. Scatter plot and regression line between disease duration and sα-klotho in patients with DN under HD.

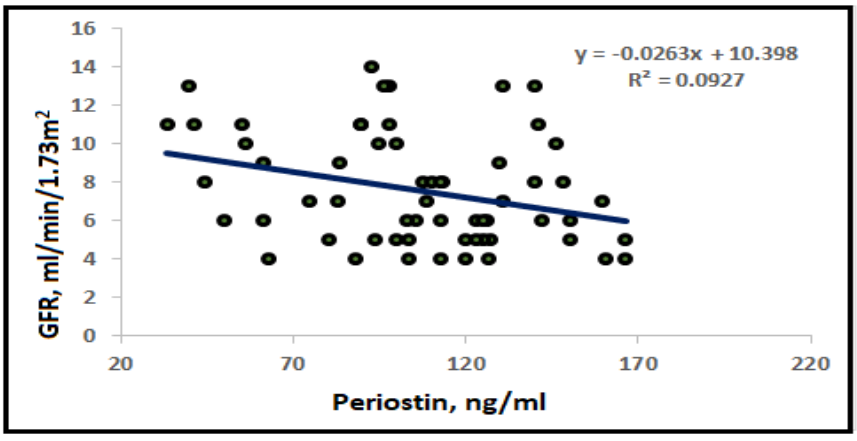

Figure 4. Scatter plot and regression line between POSTN and GFR in patients with DN under HD.

https://dx.doi.org/10.29228/jrp.2022.00
J Res Pharm 2024; 28(5): 1665-1673

Table 4. Correlation of soluble alpha klotho and POSTN with different study parameters.

	sα-Klotho, pg/ml		POSTN, ng/ml	
Variables	**Coefficient**	**p-value**	**Coefficient**	**p-value**
Age, years	0.161	0.219	0.100	0.447
BMI, kg/m²	-0.055	0.678	-0.169	0.197
DM duration, M	-0.142	0.278	0.098	0.456
FBS, mg/dl	0.141	0.282	0.046	0.729
HbA1c, %	**-0.275**	**0.033**	0.178	0.173
Urea, mg/ dl	0.063	0.635	0.146	0.266
Creatinine, mg/ dl	0.014	0.913	0.135	0.305
GFR, ml/min/1.73m²	0.130	0.323	**-0.272**	**0.035**
POSTN	-0.200	0.126		

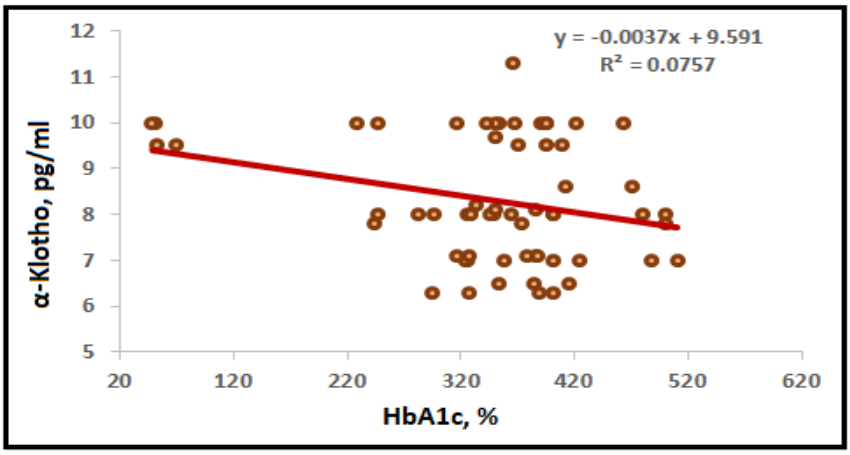

Figure 5. Scatter plot and regression line between HbA1c and sα-klotho in patients with T2DM

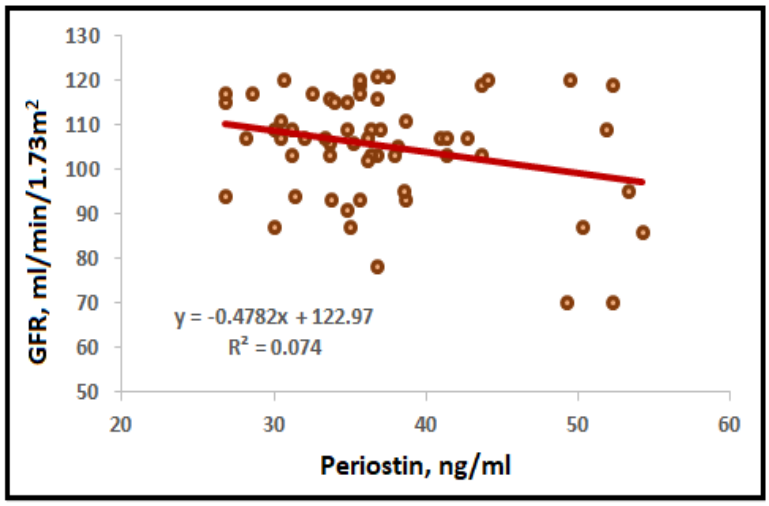

Figure 6. Scatter plot and regression line between POSTN and GFR in patients with T2DM.

3. DISCUSSION

Regarding the raised incidence and low prognosis of DN, a sensitive, conveniently applicable and inexpensive screening way is needed for the earlier diagnosis and detection of DN. In the present study, we targeted two kidney biomarkers soluble alpha klotho and POSTN that might help in earlier detection of the disease in patients with T2DM.

According to the results of the study, α-KL levels significantly decreased in patients with DN under HD. Since α-KL is expressed mainly in the kidney, it is logical to assume that a decrease in the number of nephrons can result in a decline in the synthesis and secretion of α-KL. Studies conducted on rodents demonstrate a strong correlation between soluble α-KL levels in urine and blood and the expression of renal

klotho [15]. Furthermore, human studies have exhibited that the soluble α-KL level in serum and urine is fall down in CKD patients, and progressively decreasing in more advanced stages [16]. Notably, the decrease in α-KL in our results was less than 50%, indicating that α-kl may also be generated by organs other than the kidneys. This is consistent with recent studies which have indicated that the thyroid gland and pituitary gland are also sites of α-KL reproduction, which could clarify the less marked reductions in soluble α-KL levels in patients with chronic kidney disease[17,18].

The primary interesting finding in current study that POSTN levels were significantly higher in patients with DN under HD. These results were agreed with the study conducted by Alesutan et al.,2022, who revealed that serum POSTN concentration didn't differ significantly in the CKD participants (p = 0.4375), while there were significantly elevated in the HD patients [22]. Also, the present work in agreement with another study performed by Abbad et al. who employed a mouse model of T2DM, combining transcriptomic analysis, immunostaining, and protein/mRNA expression quantification to investigate POSTN's contribution to DN development. The study found a relationship between increased POSTN expression, reduced kidney function, advanced-stage kidney harm and fibrosis, and Nuclear factor kappa B (NF-κB) activation [14]. On the other hand, several previous studies emphasized that POSTN levels alter earlier compared with creatinine or urine albumin levels, making an increasing concentration of POSTN in the urine might serve as an earlier sign of diabetes-induced renal stress and, conversely, reduced concentration may indicate an improvement in renal function [23,24].

Various hypotheses have explained the elevated serum POSTN in DN patients. One hypothesis suggests that DN, is linked to chronic decreased-grade inflammation and angiogenesis, with studies showing that inflammatory mediators like interleukin-13 and Transforming growth factor -β can induce POSTN production [25,26]. POSTN also plays a role in regulating inflammatory responses [27], indicating its involvement in various disease-related inflammatory microenvironments [28]. Moreover, Hakuno et al. demonstrated POSTN's ability to Encourage angiogenesis through Akt and focal adhesion kinase (FAK), suggesting that raised serum POSTN in DN patients may be linked to the processes of inflammation and angiogenesis in diabetes [29].

Another hypothesis proposes that NF-κB activation and nuclear translocation of the p65 sub-unit in DN patients play a critical role in renal dysfunction progression [30]. However, the specific mechanisms driving to NF-κB activation in DN remain unclear. Under inflammatory or pathological circumstances, NF-κB can be activated by various stimuli, from Toll-like receptors to cytokine and growth factor receptors, leading to the transcriptional regulation of genes coupled with chronic inflammation, fibrosis, and tissue remodeling [31].

To the best of our knowledge this is the first study which investigated the predictive value of sα-KL, and POSTN for DN in Iraqi patients with T2DM.

4. CONCLUSION

The results of the present study suggest that serum POSTN may be as earlier marker for predicting kidney injury in patients with T2DM. Longitudinal prospective studies are required in order to expound the role of POSTN in the patho-physiological mechanisms of the evolution and advancement of albuminuria in T2DM.

5. MATERIALS AND METHODS

5.1. The Study Population

This study included 120 patients with T2DM, ages range 30-72 years. They were attending AL-Karama Teaching Hospital, AL- Yarmouk Teaching Hospital, and AL-Kindy Teaching Hospital in Baghdad / Iraq for the period from January 2022 to June 2022. The participants were divided into two primary categories: 60 patients with T2DM and 60 patients with DN undergoing hemodialysis treatment 3 times a week for 3 hours/ session.

Exclusion criteria included the individuals which Type I diabetes mellitus, hypo or hyperthyroidism, clinically confirmed depression, and has undergone renal transplantation. A comprehensive questionnaire comprising specific demographic information such as gender, age, and duration of diabetes, hemodialysis (HD) duration, each participant's treatment type (whether dietary, oral, insulin, or combined therapy) and parents' history of diabetes were collected. Anthropometric measurements, including height and body weight, and BMI were evaluated in all patients that participants in this study regarding to standard protocols.

5.2. Sample collection

Ten milliliter of venous blood samples were collected from patients with T2DM and DN under HD (before taking the dose of heparin) after an overnight fast of at least 8 hours, and then divided into two equal parts: A volume of 5 milliliters was placed in a tube that had a concentration of 1.5 milligrams per milliliter of (K3EDTA) for elevation of HbA1c%. The remaining blood was put in a gel tube which contain activator, without anti-coagulants, and was left at the temperature of the room (20-25) °C for 30 minutes, and centrifuged at 3000 rpm for 5 minutes to collect serum. Then a portion of the serum was utilized to assessthe routine biochemical tests promptly .The remaining portion of the serum was frozen in securely sealed Eppendorf tubes and stored at a temperature of -20°C for clinical tests later.

5.3. Biochemical Tests

The estimation of soluble alpha klotho and POSTN was performed by sandwich ELISA kit, according to the information provided by the manufacturer (MELSIN/ China EKHU-3077). Absorbance was evaluated at 450 nm and a standard curve was taken from a calculated dilutions of alpha klotho and POSTN.

5.4. Statistical analysis

The data collected were analyzed by using SPSS software version 25.0 (SPSS, Chicago). Continuous results were expressed as mean and SD. Normality was confirmed using Shapiro Wilk test. Accordingly, Student t-test or Mann Whitney U test were utilized for analysis of normally and non-normally distrusted data, respectively. Receiver operating characteristic (ROC) curve analysis was utilized to estimate the predictive level of soluble alpha klotho and POSTN in predicting DN among patients with T2DM. Pearson's correlation test was utilized to explore the potential association between soluble alpha klotho and POSTN with other factors. A p-value < 0.05 was deemed to show a statistically significant difference.

Acknowledgements: The authors deeply thank the department of chemistry/college of sciences/ mustansiriah university / Baghdad-Iraq for their help in providing the scientific laboratories to make this work done. A special thanks to all the participants in this study.

Author contributions: Concept – A.A., N.T.; Design – R.T., A.A., N.T.; Supervision – A.A., N.T.; Resources – R.T., A.A.; Materials – R.T.; Data Collection and/or Processing – R.T.; Analysis and/or Interpretation – R.T., A.A.; Literature Search – R.T., N.T.; Writing – R.T.; Critical Reviews A.A., N.T.

Conflict of interest statement: "The authors declared no conflict of interest" in the manuscript

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