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α-Klotho as A Novel Biomarkers in Chronic Diabetic Nephropathy

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Authors' contributions

This work was carried out in collaboration among all authors. Author MEAE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors TBE and HMAEM managed the analyses of the study. Author HMAEM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Type 2 diabetes mellitus (DM) is the most common cause of end- stage renal disease. Albuminuria is the foremost commonly utilized marker to anticipate onset of diabetic nephropathy (DN) without sufficient affectability and specificity to identify early DN.
Aim: This study aimed to evaluate plasma α-Klotho as a new biomarker for chronic diabetic nephropathy.
Methods: This cross sectional study included 125 Egyptian subjects attending the out Patients Clinic of the Department of Internal Medicine, 10Th of Ramadan city Health Insurance Hospital and divided into:-control group, patient with diabetic mellitus, patients with Diabetic nephropathy and patient with diabetic nephropathy and other complications. Patients were subjected to measurement of plasma α- Klotho, FBS, HbAIC, serum creatinine, serum urea, serum uric acid, k, Na, serum phosphorus, Albumin: Creatinine Ratio, GFR, Chol, TG, LDL HDL, AST, ALT, T.BIL, D.BIL ALB, TP, GLB and A/G ratio.

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Results: Results showed that plasma a-klotho was significantly correlated with hemoglobin A1C, potassium, GFR, Albumin, TP and GLB. Meanwhile, plasma a- klotho was negatively correlated with duration of DM, CR, Urea, UR.A, Na, phosphorus, ACR, Chol, TG, LDL, AST, ALT, T.BIL, and D.BIL. However, there were no significant correlations between plasma α -klotho and FBS, HDL and A/G
ratio. At cut-off level ≥2.6, plasma α -klotho had 95% sensitivity and 81% specificity for diagnosing diabetic nephropathy.
Conclusion: α-klotho may be the chronic diabetic nephropathy markers for predicting renal injury in patients with type 2 diabetes.

Keywords: α-kloth; DN.

1. INTRODUCTION

Type 2 diabetes mellitus (DM) is the most common single cause of end-stage renal disease (ESRD) [1].

ESRD in nearly half of patients is due to diabetic nephropathy (DN), and these cases have the most exceedingly bad result compared to patients with other causes of ESRD. In spite of the fact that there are numerous novel drugs for DM, there are no particular healing medicines however for DN.

Reasons for destitute result incorporate insufficient markers and the complicated components of DN [2]. Now, severity of this disease is decided agreeing to the levels of albuminuria. Albuminuria is the foremost commonly utilized marker to foresee onset and movement of DN clinically. In any case, this conventional marker for DN needs both affectability and specificity to identify early organizes of DN [3].

However, some DN patients with ESRD do not present with significant albuminuria [4-6]. There is lack of association between glomerular filtration rate (GFR) and albuminuria suggests that an alternative to this albuminuria-based staging system is needed. Some studies have noted the existence of pathological change before microalbuminuria [4].

Therefore, even if microalbuminuria can be regarded as the earliest manifestation of DN, it is possible that a new biomarker for DN exists. Recently, different markers of DN were reviewed [7,8] including fibroblast growth factor 23, [9] tubular markers [10] (kidney injury molecule 1, neutrophil gelatinase-associated lipocalin, and liver-type fatty acid- binding protein [L-FABP]),[11] inflammatory markers (interleukin 6 [IL-6], IL-8, monocyte chemoattractant protein 1, and interferonγ–inducible protein), [12] urinary 8-hydroxy-20- deoxyguanosine, [13] serum cystatin C, [14] and so on. Among these, genetic susceptibility almost always leads to irreversible DN, and detection of the clinical markers mostly occurs too late to diagnose and monitor the progression of DN. As such, it is crucial to find an earlier and reliable marker for DN. Earlier diagnosis and intervention may provide an opportunity to stop the permanent damage caused by DN [14].

Although α -klotho was first described as an anti-aging factor, recent experimental and clinical studies suggest α -kloth als has important pleotroic effects on the kidneys. Soluble α -klotho is derived from the proteolytic cleavage o the extracellular portion of the membrane-bound α -klotho; alternatively, it can begenerated directly by the alterative splicing of the α -klothotranscript. It can be measured in blood, urine, and cerebrospinal [15].

Animals with chronic kidney disease have very low renal, plasma, and urinary α-klotho levels [16]. Furthermore, humans with chronic kidney disease exhibit markedly reduced α -klotho in serum and urine in the early stages of kidney disease, progressively decreasing in more advanced stages. However, with regard to diabetic nephropathy, the role of α -klotho in the pathogenesis of kidney injury has not been fully studied. Renal α -klotho expression is markedly decreased in diabetic nephropathy in humans and mice [17].

A similar decline is observed in kidney cells treated with methylglyoxal- modified albumin. These findings collectively suggest renal α –klotho deficiency is part of an underlying mechanism involved in diabetic kidney injury. However, the actual role of soluble α -klotho in

diabetic kidney disease has not been evaluated [18].

So this study aimed to evaluate Plasma α-klotho as biomarkers in chronic diabetic nephropathy.

1.1 Aim of the Work

The study aimed to evaluate Plasma α -klotho as biomarkers in chronic diabetic nephropathy.

2. PATIENTS AND METHODS

2.1 Study Design

Cross sectional study, aiming to evaluate plasma α -klotho as biomarkers in chronic diabetic nephropathy.

2.2 Study Setting

The study was carried out at Clinic of the Department of Internal Medicine, 10^{Th} of Ramadan city Health Insurance Hospital.

2.3 Target Population

Diabetic patients attending the Out Patients Clinic of the Department of Internal Medicine, 10^{Th} of ramadan city Health Insurance Hospital. This study included 125 Participants who were divided into:-

Group A:

Control group:- 20 healthy subjects whose age ranged between 30-50 years old were taken as control group.

Study group:- including 105 patients divided into.

Group B: Group 1:- 20 patient with diabetic mellitus whose age ranged between 30-50 years old.
Group C: 65 patients Diabetic nephropathy whose age ranged between 30-50 years old.
Group D: Diabetic nephropathy and other complications whose age ranged between 30-50 years old.

2.4 Inclusion Criteria

1. Patients were free from infectious disease,
2. Patients were free from inflammatory disease,
3. Patients were free from liver disease,
4. Patients were free from malignancy, and
5. All were nonsmokers.

2.5 Exclusion Criteria

1. Patients with active urinary tract infection;
2. Patients with renal disease other than diabetic nephropathy; neoplastic disorders; severe liver dysfunction; active or chronic infection or inflammatory disorders;
3. Pregnancy;
4. Patients with a recent (i.e., within 6 months) history of acute myocardial infarction, stroke, or occlusive peripheral vascular disease. All patients were subjected to the following;

a) Collection of demographic data as required in the attached sheet including age, occupation, anthropometric measurements of height, weight, waist circumference, and history of disease.
b) Collection of morning urine samples in vacutaniner cup and also collection of 10 venous blood samples from over night fasted 5 ml blood were
c) Collected on plane tubes and other 5 ml blood were collected on EDTA tubes by vacutaniner system under complete aseptic conditions and HbAIC firest done and the samples centrifuged for 10 min at 2,500 g within 30 min separated serum and plasma were stored at 20 ○C for the measurement of measurement of plasma α – klotho concentration, serum fasting glucose concentration, serum creatinine, serum urea n, serum uric acid, serum potassium k , serum sodium Na serum phosphorus, Albumin:CreatinineRatio,GFR concentration, serum cholesterol and serum triglycride . AST, ALT, T.BIL, D.BIL ALB, TP, GLB and A/G ratio.

The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 23). Data was presented and suitable analysis was done according to the type of data obtained for each parameter. The following tests were used:

2.6 Descriptive Statistics

Mean, Standard deviation (± SD) and range for parametric numerical data, while Median and Interquartile range (IQR) for non-parametric

numerical data. Frequency and percentage of non- numerical data.

2.7 Analytical Statistics

ANOVA test of significance was used when comparing between means of more than two groups.

Post-hoc test after ANOVA for significance between each two groups.

Chi-Square test was used to examine the relationship between two qualitative variables.

Fisher's exact test was used to examine the relationship between two qualitative variables when the expected count is less than 5 in more than 20% of cells.

Correlation analysis (using Pearson's method) to assess the strength of association between two quantitative variables. The correlation coefficient denoted symbolically "r" defines the strength (magnitude) and direction (positive or negative) of the linear relationship between two variables.

- r= 0-0.19 is regarded as very weak correlation
- r=0.2-0.39 as weak correlation
- r=0.40-0.59 as moderate correlation
- r=0.6-0.79 as strong correlation
- r=0.8-1 as very strong correlation

Regression model to predict an outcome from independent factors. ROC curve for prediction of independent value effect on the outcome.

2.8 P- Value: Level of Significance

-P>0.05: Non significant (NS).
-P< 0.05: Significant (S).

3. RESULTS

Table 1 shows that A total of 125 subjects were enrolled in this study; their mean age was 55.8610.4 years (range, 24–82 years), and there were 71 men and 54 women. Age, BMI, Duration of D.M, F.B.G, C.P.A, HBAIC, S. creatinine, S. urea, UR.A, Na, ACR, GFR, Cholesterol, Triglycerides, HDL, LDL, AST, ALT, ALB, T.BIL, and D.BIL were significantly higher in diabetic patients than non-diabetic control. Meanwhile, K, Ph, T.P, AG ratio and KL were significantly lower in diabetic patients than in non-diabetic controls. Other parameters did not differ significantly between the diabetes group and non-diabetic controls. Table 2 shows that Age, BMI, Duration of D.M, F.B.G, C.P.A, HBAIC, S. creatinine, S. urea, UR.A, Na, ACR, GFR, Cholesterol, Triglycerides, HDL, LDL, AST, ALT, ALB, T.BIL, D.BIL, K, Ph, T.P, AG ratio and KL were significantly different between four groups. Table 3 shows that plasma α -klotho was significantly correlated with hemoglobin A1C, potassium, GFR, Albumin, TP and GLB. Meanwhile, plasma α -klotho was negatively correlated with duration of DM, CR, Urea, UR.A, Na, phosphorus, ACR, Chol, TG, LDL, AST, ALT, T.BIL, and D.BIL. However, there were no significant correlations between plasma a-klotho and FBS, HDL and A/G ratio. Table 4 and Fig. 1 show that at cut- off level ≥2.6, plasma a-klotho had 95% sensitivity and 81% specificity for diagnosing diabetic nephropathy.

4. DISCUSSION

α-Klotho is a single-pass transmembrane protein that is highly expressed in the kidneys and is known to act as a co-receptor for fibroblast growth factor-23 [19]. Circulating soluble α-klotho can be generated directly by the alterative splicing of the α-klotho transcript or the extracellular domain of membrane α-klotho can be released from membrane-anchored α-klotho on the cell surface . Unlike membrane α- klotho, which functions as a co- receptor for fibroblast growth factor- 23, soluble α-klotho acts as a hormonal factor and plays important roles in anti-aging, anti-oxidation, ion transport modulation, and Wnt signaling [18].

Previous studies aiming to clarify the role of α-klotho as a potential biomarker of kidney injury show the blood and urinary concentrations of α-klotho decrease early in the course of chronic kidney disease in mice with experimentally induced chronic kidney disease as well as humans [20]. As blood α-klotho concentration was found to be linearly associated with eGFR in previous studies , plasma α- klotho was associated with the eGFR in the present study ($r=0.888$, $p<0.001$) [19,17].

Urinary α-klotho is known to be correlated with eGFR and is reported to be a surrogate marker of functioning nephrons in the patients with chronic kidney disease [21]. The present finding that plasma α-klotho was higher in the diabetic patients whose eGFR is also higher than that of normal people, further corroborates this.

Table 1. Baseline characteristics among control, diabetics and diabetics with nephropathy groups

	Group A	Group B	Group C&D	P-value
Age(years)	36.2±4.5	41.7±5.2	47.4±2.7	<0.001 [1]
Sex				
Male	12(60%)	12(60%)	47(53.8%)	0.810[2]
Female	8(40%)	8(40%)	38(46.2%)	
BMI(Kg/m)	27.3±2.3	28.7±2.6	29.7±2.7	0.001*[1]
Duration of D.M	--	4.82±2.1	4.95±2.4	<0.001 [1]
F.B.G	89.05±8.6	151.67±17	157.9±23.3	<0.001 [1]
HBAIC	4.95±0.29	9.06±0.96	9.09±0.9	<0.001 [1]
S. creatinine	0.82±0.13	1.01±0.15	4.52±2.5	<0.001 [1]
S. urea	34.75±3.77	40.3±4.76	130.73±2.56	<0.001 [1]
UR.A	4.34±0.63	5.9±0.95	7.5±0.78	<0.001 [1]
Na	4.36±0.39	4.2±0.38	5.39±0.59	<0.001 [1]
K	141.95±2.96	140.4±2.3	139.8±2.24	<0.001 [1]
Ph	3.7±0.48	3.69±0.48	3.61±0.42	<0.001 [1]
ACR	10.9±1.9	21.6±3.41	22.1±3.72	<0.001 [1]
GFR	128.97±27	110.5±8.8	130.8±6.6	<0.001 [1]
Cholesterol	169.15±13.4	170.9±12.1	194.6±33	<0.001 [1]
Triglycerides	127±8.9	156.2±15.9	157.1±29.9	<0.001 [1]
HDL	47.8±2.6	40.8±3.35	42.8±6.5	<0.001 [1]
LDL	96.9±13.1	99.2±11.78	122.3±33.6	<0.001 [1]
AST	26.85±3.37	37.15±7.7	38.82±7.9	<0.001 [1]
ALT	26.85±2.62	36.9±6.9	43.7±14.6	<0.001 [1]
ALB	4.05±0.2	4±0.20	4.73±0.22	<0.001 [1]
T.BIL	0.61±0.07	0.68±0.09	0.76±0.093	<0.001 [1]
D.BIL	0.16±0.19	0.18±0.02	0.19±0.03	<0.001 [1]
T.P	7.03±0.21	7.01±0.22	6.66±0.38	<0.001 [1]
GLB	3.02±0.17	3.02±0.17	2.93±0.27	0.383[1]
AG ratio	1.76±0.21	1.35±0.1	1.29±0.2	<0.001 [1]
KL	3.86±0.84	3.2±0.58	1.13±0.41	<0.001 [1]

*1. ANOVA test; 2. Chi-square test. *Statistical significant when p-value <0.05*

Meanwhile, little is known about circulating α-klotho levels in diabetes- related nephropathy. Recent studies in patients with diabetes report conflicting data. One study found serum α-klotho level was not significantly different between patients with diabetes without nephropathy and non-diabetic controls [21].

In contrast, another study reports a significant reduction in serum α- klotho levels in patients with glycated hemoglobin (HbA1c) levels ≥6.5% compared to control samples (HbA1c <6.5%) [22]. Kacso et al. [20] report α-klotho decreases in early chronic kidney disease and increases thereafter in the diabetic patient. However, they did not evaluate the association between soluble α-klotho levels and the extent of albuminuria in the early stage of diabetic nephropathy, specifically in patients with normal renal function. Asai et al. [23] previously showed that renal α-klotho levels were significantly decreased in early diabetic nephropathy patients; however, they've never compared renal α-klotho levels between diabetic patients and normal control. They've just showed reduction in renal α- klotho levels in diabetic nephropathy patients than patients with minimal change disease or IgA nephropathy. Furthermore, the mean age of diabetic nephropathy patients was significantly older than patients with minimal change disease and IgA nephropathy. They also showed that renal α-klotho levels were significantly decreased in diabetic mice at 8 weeks after development of diabetes mellitus. They showed that albuminuria was increased at 2, 4, 6, and 8 weeks after onset of diabetes, however, renal α- klotho levels were not decreased until 4 weeks after development of diabetes. And they've not reported the renal α-klotho levels in early stage of albuminuric diabetic mice before 4 weeks of diabetes. Zhao et al. [24] showed decreased renal klotho expression in db/db mouse. Although they did not indicate the levels of albuminuria or renal function data, they used db/db mouse at 20 weeks of age, which is regarded as relatively late stage of diabetic nephropathy. Deveraj et al. [22]

reported that soluble fraction of klotho was decreased in diabetic patients than non-diabetic controls, however they never mentioned the albuminuria status of their diabetic patients. According to our data, there was a clear inverse association between plasma α-klotho level and albuminuria level in diabetic patients (r=-0.380, p<0.001). Van Ark J et al. (2013) measured circulating α-klotho levels in diabetes patients. Although they reported that circulating α-klotho levels were not changed in diabetic patients compared to control, their sample size was very small (n = 35) and they never mentioned the albuminuria status of their diabetic patients.

In the present study, plasma α-klotho levels in patients with diabetes were highest in the normoalbuminuria (3.2±0.58) and decreased with increasing urinary albumin excretion in group 3 and 4 (1.31±0.26 and 0.53±0.15). It is surprising that the plasma α-klotho levels in the macroalbuminuria group were still comparable with those in the non- diabetic controls. In agreement with anotherstudy in which Plasma α-klotho (572.4 pg/mL [95% CI, 541.9–604.6 pg/mL] vs. 476.9 pg/mL [95% CI, 416.9–545.5 pg/mL]) was significantly higher in diabetic patients than non-diabetic controls. Among diabetic patients, plasma α-klotho concentration was inversely associated with albuminuria stages (normoalbuminuria, 612.6 pg/mL [95% CI, 568.9–659.6 pg/mL], microalbuminuria, 551.8 pg/mL [95% CI, 500.5–608.3 pg/mL], and macroalbuminuria, 505.7 pg/mL [95% CI, 439.7–581.7 pg/mL] (p for trend= 0.0081) [20]. Both acute kidney injury and chronic kidney disease exhibit renal and systemic α-klotho deficiency. Levels of α-klotho plummet very early and severely in acute kidney injury, representing a pathogenic factor that exacerbates acute kidney damage [19]. In chronic kidney

Table 2. Baseline characteristics among study groups

	Group A	Group B	Group C	Group D	P-value
Age(years)	36.2±4.5	41.7±5.2	47.5±2.3	47±2.8	<0.001 [1]
Sex					
Male	12(60%)	12(60%)	34(52.3%)	13(65%)	0.935[2]
Female	8(40%)	8(40%)	31(47.7%)	7(35%)	
BMI(Kg/m)	27.3±2.3	28.7±2.6	30.2±3.2	30±2.9	0.002*[1]
Duration of D.M	--	4.95±2.4	8.82±2.9	9.15±2.7	<0.001 [1]
F.B.G	89.05±8.6	151.67±17	167.55±31.8	178.45±29	<0.001 [1]
C.P.A	37.35±6	84.14±7	59.89±4.76	105.5±5.26	<0.001 [1]
HBAIC	4.95±0.29	9.06±0.96	9.64±2.03	9.16±0.69	<0.001 [1]
S. creatinine	0.82±0.13	1.01±0.15	4±2.45	6.22±1.95	<0.001 [1]
S. urea	34.75±3.77	40.3±4.76	122.46±39.9	157.6±28.3	<0.001 [1]
UR.A	4.34±0.63	5.9±0.95	7.3±0.75	8.1±0.58	<0.001 [1]
Na	4.36±0.39	4.2±0.38	5.3±0.58	5.7±0.49	<0.001 [1]
K	141.95±2.96	140.4±2.3	133±4.9	123.4±5.88	<0.001 [1]
Ph	3.7±0.48	3.69±0.48	3.77±0.37	5.58±0.37	<0.001 [1]
ACR	10.9±1.9	21.6±3.41	324.12±328.3	3071.9±3241.4	<0.001 [1]
GFR	128.97±27	110.5±8.8	20.88±10.3	10.63±4.5	<0.001 [1]
Cholesterol	169.15±13.4	170.9±12.1	181±20.5	238.7±27.2	<0.001 [1]
Triglycerides	127±8.9	156.2±15.9	146.5±16.1	191.2±38.6	<0.001 [1]
HDL	47.8±2.6	40.8±3.35	44.8±5.94	36.4±3.49	<0.001 [1]
LDL	96.9±13.1	99.2±11.78	107.6±19.75	170.3±22.45	<0.001 [1]
AST	26.85±3.37	37.15±7.7	37.17±6.8	64.7±13.15	<0.001 [1]
ALT	26.85±2.62	36.9±6.9	36.7±5.9	37.5±14.9	<0.001 [1]
ALB	4.05±0.2	4±0.20	3.8±0.23	3.5±0.19	<0.001 [1]
T.BIL	0.61±0.07	0.68±0.09	0.72±0.07	0.87±0.04	<0.001 [1]
D.BIL	0.16±0.19	0.18±0.02	0.18±0.02	0.22±0.02	<0.001 [1]
T.P	7.03±0.21	7.01±0.22	6.8±0.28	6.2±0.31	<0.001 [1]
GLB	3.02±0.17	3.02±0.17	2.95±0.16	3±0.23	<0.001 [1]
AG ratio	1.76±0.21	1.35±0.1	1.28±0.15	1.32±0.17	<0.001 [1]
KL	3.86±0.84	3.2±0.58	1.31±0.26	0.53±0.15	<0.001 [1]

*1. ANOVA test; 2. Chi-square test. *Statistical significant when p-value <0.05*

disease, α-klotho deficiency significantly impacts the progression of renal disease as well as extrarenal complications. Meanwhile, soluble α-klotho levels in plasma and/or urine may serve as early biomarkers of kidney parenchymal injury [19]. Another studies indicate the potential contribution of absolute α-klotho deficiency to acute and chronic kidney injury. Emerging evidence suggests α-klotho deficiency is an early biomarker of kidney disease as well as a pathogenic factor. α-Klotho deficiency is associated with progression and chronic complications in chronic kidney disease, including vascular calcification, cardiac hypertrophy, and secondary hyperparathyroidism; in particular, α-klotho deficiency induces resistance to fibroblast growth factor-23 and predisposition to hyperphosphatemia, which represents a critical feature of chronic kidney disease [20]. In the present study, plasma α-klotho concentrations tended to decrease with increasing degrees of albuminuria. The results of the present study may help further elucidate the role of α-klotho in the development and progression of albuminuria in type 2 diabetes. Also, at cut-off level ≥2.6, plasma a-klotho had 95% sensitivity and 81% specificity for diagnosing diabetic nephropathy. Nevertheless, the underlying mechanisms explaining the present results require further investigation. The present results may be explained by increased α-klotho synthesis or its cleavage process, although requires further study. The extracellular domain of α-klotho protein is subject to ectodomain shedding and is released into the blood and urine; therefore, it may function as a hormone [17]. Hyperglycemia does not affect renal α-klotho production per se, because high glucose does not alter α-klotho expression in kidney cells and diabetes does not affect renal α-klotho mRNA expression in mice . The lack of an association between HbA_{1c} or glycated albumin with soluble α- klotho concentrations in the present study also corroborates previous observations. Insulin can increase soluble α-klotho concentration through the cleavage and release of the extracellular domain of α-klotho [21]. In type 2 diabetes at early stage, soluble α-klotho level is increased in plasma that may result in increased amount of α-klotho protein in urine. α-Klotho protein is expressed in both apical and basolateral membrane of kidney tubule. Soluble α-klotho level may be determined by two possible mechanisms; 1) cleavage of α-klotho protein by proteases such as ADAM10 or 17 and 2) secretion of splice variant form of α-klotho into blood or urine [17].

Table 3. Correlations between plasma α-Klotho levels and other parameters in patients with diabetes

	KL	
	R	**P-value**
Duration of DM	-0.444	<0.001*
F.B.S	0.164	0.095
HBAIC	0.197	0.044*
CR	-0.562	<0.001*
Urea	-0.713	<0.001*
UR.A	-0.663	<0.001*
Na	-0.631	<0.001*
K	0.693	<0.001*
Ph	-0.676	<0.001*
ACR	-0.380	<0.001*
GFR	0.888	<0.001*
Chol	-0.477	<0.001*
TG	-0.197	0.044*
HDL	-0.033	0.739
LDL	-0.483	<0.001*
AST	-0.456	<0.001*
ALT	-0.453	<0.001*
ALB	0.501	0.001*
T.BIL	-0.442	<0.001*
D.BIL	-0.271	0.005*
T.P	0.513	<0.001*
GLB	0.210	0.032*
A/G ratio	0.095	0.333

*Pearson correlation test;*Statistical significant when p-value <0.05*

Table 4. Validity of plasma α -Klotho for diabetic nephropathy

	AUC	Sensitivity	Specificity	Cut-off value
KL	0.855	95%	81%	2.6

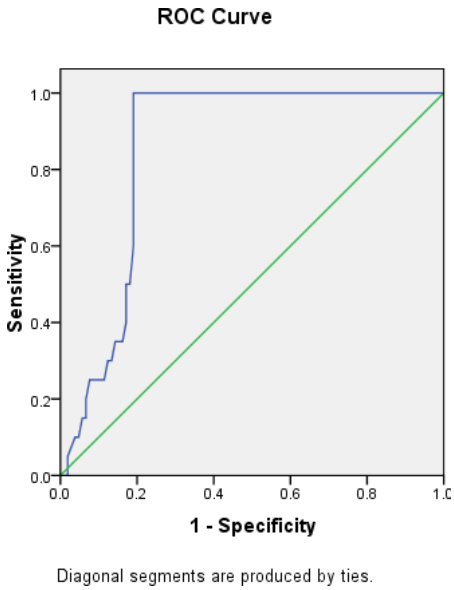

Fig. 1. ROC curve of plasma α-Klotho for diabetic nephropathy

Extracellular domain of α-klotho canbe released into urine and blood from apical and basolateral membranes, respectively. Insulin receptor is also expressed in both apical and basolateral membrane of kidney tubular cells. At early stagy of type 2 diabetes, blood insulin level is increased that can stimulate the cleavage and release of the extracellular domain of α-klotho into blood and/or urine. In this study, blood insulin level was also increased in diabetic patients than non-diabetic controls as expected. Increased level of soluble α-klotho may be filtered in glomeruli and present in urine [24]. Cha et al. [25] reported that intraperitoneal administration of soluble klotho increased urinary K^+ excretion in rat by ROMK channel activation which is expressed in apical membrane. Interestingly, epitope- tagged klotho was appeared in urine at 2 hr after intravenous administration indicating that klotho protein may be filtered in glomeruli and regulates ROMK channel from luminal side.

Exogenous supplementation or stimulation of endogenous α-klotho may prevent and/or ameliorate kidney injury and mitigate chronic kidney disease development. The correction of α-klotho deficiency may delay the progression and forestall the development of extrarenal complications in chronic kidney disease. Angiotensin II receptor blocker treatment was recently shown to increase blood α-klotho levels while reducing albuminuria in type 2 diabetes with nephropathy [17].

The findings tha both exogenous soluble α-klotho administration and overexpression of membranous α- klotho in kidney cell culture suppress NF-κB activation and subsequent inflammatory cytokine production in the response to TNF-α stimulation suggest α-klotho serves as an anti- inflammatory modulator. Therefore, preventing deceases in α-klotho and α-klotho supplementation are potential novel therapeutic strategies for chronic diabetic nephropathy. In multiple experimental models of chronic kidney disease, the replacement or endogenous upregulation of α-klotho protects the kidneys from renal insults, preserves kidney function, and suppresses renal fibrosis. Thus, α-klotho is a highly promising candidate early biomarker as well as a novel therapeutic agent for chronic kidney disease [19].

Blood α-klotho concentrations can easily be checked and used to assess the development of diabetic nephropathy prior to the onset of microalbuminuria, which is the earliest sign of

diabetic nephropathy in clinical settings. To best of our knowledge, this study is the first study to assess validity of α-klotho for diagnosing diabetic nephropathy.

5. CONCLUSION

In conclusion, the results of the present study suggest plasma and urinary α -klotho may be the early markers for predicting renal injury in patients with type 2 diabetes and we need to do long-term prospective study in order to elucidate the role of α -klotho in the pathophysiological mechanisms of the development and progression of albuminuria in type 2 diabetes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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