

Journal of Nephropathology



The predictive value of serum klotho in diabetes mellitus and hypertension

Kamal Khademvatani¹, Zahra Yekta², Mirhosein Seyed Mohammadzad³, Shahriar Khanahmadi⁴, Roghaiyeh Afsargharehbagh⁵, Leila Majdi⁴, Alireza Rostamzadeh⁶, Mojgan Hajahmadipoor Rafsanjani⁵, Ali Soleimany⁶, Elham Niknejad⁷, Mohammad Reza Zolfaghari⁸, Shima Khanahmadi⁴, Zeinab Pourmansouri⁹, Reza Karimi⁴

¹Seyyed-al-Shohada Heart Center, Urmia University of Medical Sciences, Urmia, Iran

²Department of Community Medicine, Urmia University of Medical Sciences, Urmia, Iran

³Patient Safety Research Center, Urmia University of Medical Sciences, Urmia, Iran

⁴Student Research Committee, Urmia University of Medical Sciences, Urmia, Iran

⁵Department of Cardiology, Shohada Hospital, Urmia University of Medical sciences, Urmia, Iran

⁶Urmia University of Medical Sciences, Urmia, Iran

⁷Department of Dentistry, Urmia University of Medical Sciences, Urmia, Iran

⁸Department of Exercise Physiology, Faculty of Physical Education and Sport Science, Urmia University of Medical Sciences, Urmia, Iran

⁹Department of Clinical Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

ARTICLE INFO

Article type:
Original Article

Article history:
Received: 15 April 2020
Accepted: 21 June 2020
Published online: 20 July 2020

Keywords:
Klotho
Diabetes
Hypertension
Predictive value

ABSTRACT

Introduction: Klotho allele status is associated with increased risk of cardiovascular diseases, diabetes and hypertension.

Objectives: To determine if serum klotho level was lower among diabetic and hypertensive patients compared to control group.

Patients and Methods: This was a cross-sectional study of 90 participants. Thirty pure diabetic patients and 30 participants with pure hypertension were compared with the healthy control group. Multiple logistic regressions were used to examine the association between serum klotho and diabetes and hypertension. We also tested the cut off point of serum klotho to predict hypertension and diabetes by using ROC (receiver operating characteristic) curve.

Results: The level of serum klotho was significantly lower in diabetic and hypertensive patients. Participants with higher klotho were less likely to have diabetes and hypertension [OR: 0.48, 95% CI (0.22-0.81)] even after adjustment for covariates. ROC curve for diabetes and hypertension indicated 0.8 area under the curve which was statistically significant.

Conclusion: This study found that serum klotho was associated with lower odds of diabetes and hypertension. Further longitudinal studies are necessary to confirm this finding.

Implication for health policy/practice/research/medical education:

Klotho is a recently known anti-aging protein. Diabetes and hypertension as two major public health issues associate with increased risk of cardiovascular complications. The level of serum klotho was significantly lower in diabetic and hypertensive patients. Serum klotho was associated with lower odds of diabetes and hypertension and participants with higher klotho were less likely to have diabetes and hypertension.

Please cite this paper as: Khademvatani K, Yekta Z, Seyed Mohammadzad M, Khanahmadi S, Afsargharehbagh R, Majdi L, et al. The predictive value of serum klotho in diabetes mellitus and hypertension. J Nephropathol. 2021;10(1):e07. DOI: 10.34172/jnp.2021.07.

Introduction

Klotho is a recently known anti-aging protein that encodes a single-pass transmembrane protein. It is mainly expressed

in the distal tubule cells of kidney, parathyroid glands and choroid plexus of the brain (1). Anti-aging effects of klotho by mediating inflammatory response have been studied

*Corresponding author: Shahriar Khanahmadi, Email: shahriar.khanahmadi@gmail.com, khademvatani.k@umsu.ac.ir

over several decades. A recent study in male rats revealed that the defect in *klotho* involving the process of randomly integrating a foreign gene into the mouse genome leads to change genomic map by introducing nonfunctional gene (2,3). *Klotho* deficient mice represent extensive and progressive arteriosclerosis attributed to endothelial dysfunction and medial calcification. Alternatively, in another study *klotho* gene delivering in a rat model with multiple atherogenic risk factors could repair vascular endothelial dysfunction and prevent perivascular fibrosis. Zhou et al proposed a mechanism in which *klotho* gene deficiency led to developing salt sensitive hypertension via inflammatory pathway in rats (4). Another study among diabetic rats suggests that *klotho* contributes to anti-inflammatory response which negatively regulates the production of nuclear factor activation (5).

Diabetes and hypertension as two major public health issues associate with increased risk of cardiovascular complications. Chronic, systemic inflammation may be an important contributor to the development of diabetes and hypertension symptoms and their complications (6). Several studies indicate that *klotho* allele status is associated with increased risk of developing cardiovascular diseases, diabetes and hypertension (6). Donate et al demonstrated that expression of *klotho*-VS in a family is a reliable predictor for occult cardiovascular diseases (7). Another study among diabetic patients reported an association between *klotho* deficiency and risk of micro-albuminuria which led to increased risk of cardiovascular disease and end-stage renal failure (8). For this reason one of the potential pharmacodynamics pathways for antihypertensive medications like renin-angiotensin system blockade is a modulator of immune response by increasing soluble *klotho* (9).

Objectives

Given that diabetes and hypertension are two major risk factors for cardiovascular disease which is one of the most important leading cause of death in the world and based on the existing evidence of the role of *klotho* in developing diabetes and hypertension and their subsequent complications, we, therefore, aimed to determine the serum level of *klotho* in diabetic and hypertensive patients compared to healthy adults. We also hypothesized that whether serum *klotho* level can be used as a predictor factor to estimate the risk of diabetes and hypertension in the general population.

Patients and Methods

Patients and study design

This cross-sectional study was conducted in adults admitted at Seyod Al-Shohada teaching hospital in the province of West Azerbaijan, Iran in 2017. Participants

consist of three groups;

1. Patients with a history of at least six months hypertension which was confirmed by detecting the mean of blood pressure more than 140/90 mm Hg.
2. Patients with a history of diabetes for at least six months which was confirmed by serum glucose level more than 126 mg/dL or taking any anti-diabetic agents for at least 6 months.
3. Control group was volunteer participants with no history of high blood pressure or diabetes which were confirmed by measuring blood pressure and serum glucose before enrolling in the study. Since the level of *klotho* changes over life time all participants were matched by age.

We excluded participants with evidence of cancer, rheumatologic diseases, infectious diseases, chronic renal failure, acute or chronic hepatitis. Participants with having both diabetes and hypertension were also excluded. Patients were also examined by one cardiologist to rule out any cardiovascular diseases. Eligible participants were enrolled subsequently as they were referred to the hospital. The study sample consists of 30 participants per group.

A trained nurse was responsible to take blood samples and record participants' baseline characteristics. Demographic information including age, gender and body mass index were collected. The blood was transferred to the reference laboratory in order to measure the level of serum *klotho* (using Eastbiopharm kits) and lipid profile.

Ethical approval

This study was approved by the Urmia University of Medical Sciences institutional review Board and (Ethical code#IR.UMSU.REC.1395.20) was conducted in accordance with the Helsinki Declaration of 1975. The aim of study and possible harm were explained to each participant. All participants signed the consent form to confirm their awareness of procedure. This study was extracted from the medical thesis of Shahriar Khanahmadi at this university.

Statistical analysis

In this study, descriptive statistics were reported as mean (\pm standard deviation) or median for continuous variables and as frequency (percentage) for categorical variables. For comparisons of diabetic/hypertensive patients versus control participants, chi-square tests were applied to compare categorical variables. Student's t-test and the Wilcoxon rank sum test were used to compare continuous variables, as appropriate. Multivariable logistic regression models were fit to explore the association between serum *klotho* and the outcome of diabetes or hypertension separately. The model was then adjusted for demographic variables (age and gender) as potential confounder

variables. Next, we adjusted for clinical covariates (BMI [body mass index], triglyceride, low-density lipoprotein [LDL-C], high-density lipoprotein [HDL-C] and total cholesterol). Receiver operation characteristics (ROC) curve was also used to determine the cut-off point for klotho level to discriminate participants who had hypertension or diabetes with healthy participants. The overall performance was assessed by the area under the ROC curve, in which the area under the curve closer to one, represents the optimum capability to differentiate diabetic or hypertensive patients from control healthy group. STATA version 13 was used for all statistical analysis and all tests were considered statistically significant at $P < 0.05$.

Results

A total of 30 participants per each group were included in the analysis. The median age of participants with diabetes was 52.3 ± 8.3 versus 51.13 ± 8.7 years among hypertensive patients. There were no statistical significant differences based on age and gender among diabetic and hypertensive patients compared to healthy participants. Table 1

summarizes the demographic and clinical characteristics of study participants by each group. Diabetic patients were more likely to have higher BMI than the control group, however patients with hypertension did not show any significant differences based on the mean of BMI with the control group. Triglyceride and total cholesterol were significantly higher among patients with hypertension compared to the control group. Diabetic participants were more likely to have higher HDL-C compared to the control group. The mean of serum klotho was 1.6 ± 0.9 ng/mL in diabetic patients versus 1.2 ± 0.5 ng/mL in hypertensive patients compared to 2.7 ± 1 ng/mL among the control group ($P < 0.001$).

We examined the association of serum klotho and having hypertension or diabetes using logistic regression analysis. Table 2 shows the results of regression among diabetic patients. The first unadjusted model showed lower odds of having diabetes associated with increasing serum klotho [OR: 0.48 CI 95% (0.22-0.81)]. A similar result was found after adjusting for demographic variables (age, gender and smoking status). Further adjustment for

Table 1. Baseline characteristics by each group (diabetic, hypertension and healthy participants)

Variable	Total sample (N=90)	Diabetes (n=30)	Hypertension (n=30)	Control (n=30)	P value	
					Dia /con	BP/con
Age (year)	50.51±9	52.33±8.3	51.13±8.7	48.06±9.6	0.07	0.2
Gender (male %)	50	46.7	36.7	66.7	0.09	0.02
Ex-smoker/current smoker	24.4	33.3	40	0	0.00	0.00
BMI (kg/m ²)	25±2.7	25.7±1.9	24.8±2	24.68±1.8	0.06	0.7
Total cholesterol (mg/dL)	162±39.1	156±44.8	151.1±26.1	179±38.9	0.03	0.00
LDL-C (mg/dL)	87.57±23.9	83.1±27.92	86.6±22.1	93±20.18	0.12	0.25
HDL (mg/dL)	40.6±9.4	39.3±8.7	39.4±9.4	43±9.8	0.13	0.16
TG (mg/dL)	145.8±45.9	202.9±47	113.5±45.4	121.1±70	0.00	0.61
Klotho (ng/mL)	1.9±1	1.6±0.9	1.2±0.5	2.7±1	<0.001	<0.001

Table 2. Results of multivariable adjusted logistic regression examining the association of diabetes/hypertension with serum klotho

Group	Variable	Unadjusted OR, P value	Socio-demographic adjusted OR (CI95%), P value	Clinical covariates adjusted OR (CI95%), P value
Diabetic participants	Serum klotho (ng/mL)	0.48 (0.22-0.81), 0.001	0.48 (0.21-0.7) 0.001	0.45 (0.29-0.78), 0.01
	Female	-	0.47(0.12-1.7), 0.25	0.35(0.07-1.6), 0.18
	Age ≥55 (year)	-	0.94(0.88-1.0), 0.16	0.91(0.83-1.0), 0.09
	BMI (kg/m ²)	-	0.93(0.65-1.33), 0.7	0.94(0.61-1.4), 0.24
	HDL-C (mg/dL)	-	-	0.98(0.89-1.07), 0.73
	LDL-V (mg/dL)	-	-	1.01(0.97-1.04), 0.47
Hypertensive participants	Serum klotho (ng/mL)	0.37(0.12-0.87) 0.00,	0.35(0.17-0.91), 0.000	0.37(0.2-0.88), <0.001
	Female	-	0.11(0.1-1), 0.06	0.7 (0.4-1.1), 0.06
	Age ≥55 (year)	-	0.95 (0.86-1.05), 0.38	0.96(0.83-1.12), 0.66
	BMI (kg/m ²)	-	1.33 (0.75-2.2), 0.2	1.22(0.66-2.3), 0.44
	TG (mg/dL)	-	-	0.99(0.97-1.02), 0.92
	HDL-C (mg/dL)	-	-	1.05(0.89-1.2) 0.51
	LDL-C (mg/dL)	-	-	0.95 (0.85-1.0) 0.32

clinical characteristics such as triglyceride, BMI, HDL-C, LDL-C, total cholesterol did not meaningfully change the association. Subsequently, logistic regression analysis was used to determine the association of serum klotho and hypertension (OR: 0.37, CI: 0.12-0.87). The odds of hypertension were less than one which identified the protective effect of klotho in healthy population (Table 2).

ROC curve was also used to determine the level of serum klotho to predict hypertension and diabetes. ROC curve for diabetic participants indicated the 0.8 area under the curve which was statistically significant (Figure 1). The similar analysis among patients with hypertension revealed the 0.6 area under the curve significantly (Figure 2). Serum klotho of 1.77 was set as the cut off which is able to perfectly discriminate the patients with diabetes and healthy people with 93% sensitivity and 81% specificity. The optimum level of klotho to predict hypertension was 1.73 with 93% sensitivity and 78% specificity.

Discussion

Diabetes and hypertension are two major public health issues in the world. According to epidemiological studies, the prevalence of hypertension and diabetes is increasing among elderly (4). Although diet and health behaviors have been suggested as an essential well-known modifiable factor in developing diabetes and hypertension, over the last decade, a bunch of *in vivo* and *in vitro* studies have examined the correlation of klotho with diabetes and hypertension and their adverse outcomes. (3,5,10-12). The recent hypothesis is that klotho protein as an anti-aging hormone can modulate the expression level of antioxidant enzymes (5,6,13,14). While inflammation and endothelial dysfunction are in the biological pathway to develop diabetes and hypertension, a protective function of klotho against endothelial dysfunction has been reported by several studies (5,9,15). Animal studies showed that the treatment effect of antihypertensive medications among diabetic rats is associated with accelerating klotho level in serum (9). Our study showed the serum klotho level is significantly lower among diabetic and hypertensive patients than healthy people. The level of klotho meaningfully discriminates diabetic and hypertensive patients from the control group. These results suggest that the serum klotho could be an important tool in the prediction of detecting diabetes and hypertension, although additional prospective studies are needed to confirm these results.

In our study, the level of serum klotho was significantly higher among control healthy people. The odds of diabetes and hypertension were significantly lower among those with higher level of serum klotho even after adjusting for some covariates such as lipid profile, age, gender and BMI. The level of klotho is a new interest in the risk

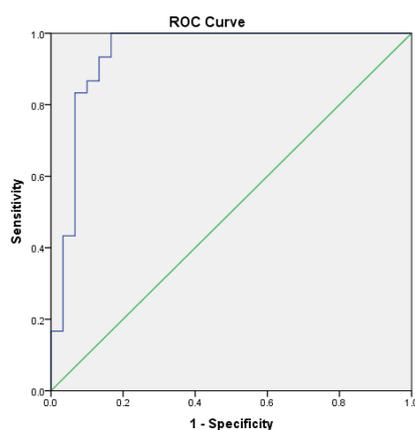


Figure 1. ROC curve to discriminate the klotho level for developing diabetes.

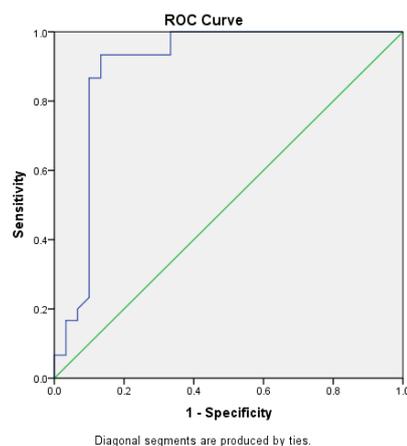


Figure 2. ROC curve to discriminate the klotho level for developing hypertension.

management of cardiovascular disease. Atherosclerosis, oxidative stress and endothelial dysfunction have been revealed to be related to the level of klotho expression in mice (1,16,17). Several studies suggested the correlation between serum klotho and prevalence of diabetes and hypertension and their complications (8,18-21). In rat model with multiple atherogenic risk factors such as obesity and hyperlipidemia, *in vivo* Klotho gene delivery can repair endothelial dysfunction and perivascular fibrosis (8).

Semba et al showed that among adult participants, higher plasma klotho is associated with a lower odds of having cardiovascular disease (1). Ming et al reported hypertension among old people contributed to the reduction of the serum klotho which led to endothelial dysfunction as the predisposing factor for cardiovascular diseases (20). There is also contradictory evidence regarding the association between klotho levels and diabetic patients compared to non-diabetic controls. Lee et al found that klotho level was significantly higher in diabetic patients (22). One

possible explanation is some important variables such as blood pressure and age values were significantly different in diabetic patients and control group in the study by Lee et al (10,22). However, no significant differences based on these parameters were detected in our study. Another reason is that Lee et al measured plasma klotho levels, while serum klotho was examined in our study. It appears the main difference between the klotho concentration in plasma and serum is due to clotting factors in the plasma (10, 23).

ROC curve indicated the 0.8 area under the curve which was statistically significant. Serum klotho 1.77 was set as the cut off which perfectly discriminates the diabetic patients with 93% sensitivity and 81% specificity. Almost similar cut off of 1.73 was found for hypertensive patients with 93% sensitivity and 78% specificity.

There were some limitations in this study which need to be acknowledged. The cross-sectional design subjected our finding to some biases. Since cause and effect variables would be evaluated at a single point of time, the cross-sectional design prevents any inferences of causality. However, a cross-sectional study is appropriate for hypothesis generation. Selection bias might be another limitation in this study which affects our study generalizability. Although our study could find a significant difference in serum klotho among patients and the control group, small sample size is another important limitation in this study. Unmeasured confounder variables are another challenging issue in this study. In addition, all participants did not have a follow-up plan which limits our ability to assess the prognosis of the disease.

Conclusion

In this cross-sectional study, the level of serum klotho was significantly lower among patients with diabetes and hypertension than the healthy population. Higher serum klotho was associated with lower possibility of diabetes and hypertension. In ROC curve analysis serum Klotho was a significant predictor of having diabetes and hypertension. We recommend additional studies to confirm these findings. If confirmed, mechanistic studies will be useful to determine whether the effect of serum klotho is due to reduced inflammation or other pathways related to unmeasured variables.

Limitations of the study

The relatively small sample was the main limitation of this research. The finding of this study should be further confirmed by larger and longer clinical research.

Authors' contribution

SKH, MS and KKH conceived and designed the study.

ZY, MH and SKH developed the study protocol, designed and tested the study data. RK, MRZ, RA, AR, ShKH and AS collected the study data. EN and ZY analyzed the data. SHKH and KKH confirmed the analysis and approved the manuscript.

Conflicts of interest

All authors declare no conflicts of interest.

Ethical considerations

Ethical issues (including plagiarism, double publication) have been completely considered by the authors.

Funding/Support

This study was supported by Urmia University of Medical Sciences (Grant# 94-01-67-2101).

References

1. Semba RD, Cappola AR, Sun K, Bandinelli S, Dalal M, Crasto C, et al. Plasma klotho and cardiovascular disease in adults. *J Am Geriatr Soc.* 2011;59(9):1596-601. doi: 10.1111/j.1532-5415.2011.03558.x
2. Wang Y, Sun Z. Current understanding of klotho. *Ageing Res Rev.* 2009;8(1):43-51. doi: 10.1016/j.arr.2008.10.002.
3. Wang Y, Sun Z. Klotho gene delivery prevents the progression of spontaneous hypertension and renal damage. *Hypertension.* 2009;54(4):810-7. doi: 10.1161/HYPERTENSIONAHA.109.134320
4. Zhou X, Chen K, Lei H, Sun Z. Klotho gene deficiency causes salt-sensitive hypertension via monocyte chemotactic protein-1/CC chemokine receptor 2-mediated inflammation. *J Am Soc Nephrol.* 2015;26(1):121-32. doi: 10.1681/ASN.2013101033.
5. Zhao Y, Banerjee S, Dey N, LeJeune WS, Sarkar PS, Brobey R, et al. Klotho depletion contributes to increased inflammation in kidney of the db/db mouse model of diabetes via RelA (serine)536 phosphorylation. *Diabetes.* 2011;60(7):1907-16. doi: 10.2337/db10-1262.
6. Martin-Nunez E, Donate-Correa J, Lopez-Castillo A, Delgado-Molinos A, Ferri C, Rodriguez-Ramos S, et al. Soluble levels and endogenous vascular gene expression of KLOTHO are related to inflammation in human atherosclerotic disease. *Clin Sci (Lond).* 2017;131(21):2601-9. doi: 10.1042/CS20171242
7. Donate-Correa J, Martin-Nunez E, Mora-Fernandez C, Muros-de-Fuentes M, Perez-Delgado N, Navarro-Gonzalez JF. Klotho in cardiovascular disease: Current and future perspectives. *World J Biol Chem.* 2015;6(4):351-7. doi: 10.4331/wjbc.v6.i4.351
8. Arking DE, Becker DM, Yanek LR, Fallin D, Judge DP, Moy TF, et al. KLOTHO allele status and the risk of early-onset occult coronary artery disease. *Am J Hum Genet.* 2003;72(5):1154-61. doi: 10.1086/375035
9. Karalliedde J, Maltese G, Hill B, Viberti G, Gnudi L. Effect of renin-angiotensin system blockade on soluble Klotho in

- patients with type 2 diabetes, systolic hypertension, and albuminuria. *Clin J Am Soc Nephrol*. 2013;8(11):1899-905. doi: 10.2215/CJN.02700313.
10. Nie F, Wu D, Du H, Yang X, Yang M, Pang X, et al. Serum klotho protein levels and their correlations with the progression of type 2 diabetes mellitus. *J Diabetes Complications*. 2017;31(3):594-8. doi: 10.1016/j.jdiacomp.2016.11.008.
 11. Devaraj S, Syed B, Chien A, Jialal I. Validation of an immunoassay for soluble Klotho protein: decreased levels in diabetes and increased levels in chronic kidney disease. *Am J Clin Pathol*. 2012;137(3):479-85. doi: 10.1309/AJCPGPMAF7SFRBO4.
 12. Kuro-o M. Klotho, phosphate and FGF-23 in ageing and disturbed mineral metabolism. *Nat Rev Nephrol*. 2013;9(11):650-60. doi: 10.1038/nrneph.2013.111.
 13. Kuro-o M. Klotho as a regulator of oxidative stress and senescence. *Biol Chem*. 2008;389(3):233-41. doi: 10.1515/BC.2008.028.
 14. Yamamoto M, Clark JD, Pastor JV, Gurnani P, Nandi A, Kurosu H, et al. Regulation of oxidative stress by the anti-ageing hormone klotho. *J Biol Chem*. 2005;280(45):38029-34. doi: 10.1074/jbc.M509039200
 15. Donate-Correa J, Mora-Fernandez C, Martinez-Sanz R, Muros-de-Fuentes M, Perez H, Meneses-Perez B, et al. Expression of FGF23/KLOTHO system in human vascular tissue. *Int J Cardiol*. 2013;165(1):179-83. doi: 10.1016/j.ijcard.2011.08.850.
 16. Saito Y, Nakamura T, Ohyama Y, Suzuki T, Iida A, Shiraki-Iida T, et al. In vivo klotho gene delivery protects against endothelial dysfunction in multiple risk factor syndrome. *Biochem Biophys Res Commun*. 2000;276(2):767-72. doi: 10.1006/bbrc.2000.3470
 17. Kurosu H, Yamamoto M, Clark JD, Pastor JV, Nandi A, Gurnani P, et al. Suppression of aging in mice by the hormone Klotho. *Science*. 2005;309(5742):1829-33. doi: 10.1126/science.1112766
 18. Maltese G, Fountoulakis N, Siow RC, Gnudi L, Karalliedde J. Perturbations of the anti-ageing hormone klotho in patients with type 1 diabetes and microalbuminuria. *Diabetologia*. 2017;60(5):911-4. doi: 10.1007/s00125-017-4219-1
 19. Maltese G, Karalliedde J. The putative role of the antiageing protein klotho in cardiovascular and renal disease. *Int J Hypertens*. 2012;2012:757469. doi: 10.1155/2012/757469
 20. Su XM, Yang W. Klotho protein lowered in elderly hypertension. *Int J Clin Exp Med*. 2014;7(8):2347-50.
 21. Cheng MF, Chen LJ, Cheng JT. Decrease of Klotho in the kidney of streptozotocin-induced diabetic rats. *J Biomed Biotechnol*. 2010;2010:513853. doi: 10.1155/2010/513853.
 22. Lee EY, Kim SS, Lee JS, Kim IJ, Song SH, Cha SK, et al. Soluble alpha-klotho as a novel biomarker in the early stage of nephropathy in patients with type 2 diabetes. *PLoS One*. 2014;9(8):e102984. doi: 10.1371/journal.pone.0102984.
 23. Liu JJ, Liu S, Morgenthaler NG, Wong MD, Tavintharan S, Sum CE, et al. Association of plasma soluble alpha-klotho with pro-endothelin-1 in patients with type 2 diabetes. *Atherosclerosis*. 2014;233(2):415-8. doi: 10.1186/1471-2369-15-147

Copyright © 2021 The Author(s); Published by Society of Diabetic Nephropathy Prevention. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.