

Relationship of Serum Klotho Level With *ACE* Gene Polymorphism in Stable Kidney Allograft Recipients

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Keywords. Klotho, gene polymorphism, kidney transplantation

Introduction. The kidney is the main source of serum Klotho production. Immunosuppressive agents could affect the kidney in this regard. The effect of the *ACE* gene polymorphism on Klotho production is a less studied area. This study aimed to assess serum Klotho and *ACE* gene in a group of stable kidney transplant recipients.

Materials and Methods. In a cross-sectional study, 30 kidney transplant recipients with stable allograft function and 27 healthy young individuals were assessed for their serum Klotho levels. The *ACE* gene polymorphisms were studied in both groups.

Results. Klotho level was higher in kidney transplant recipients than the controls, but the difference was not significant (2.76 ± 2.41 ng/mL versus 2.01 ± 1.41 ng/mL, respectively). In both groups, serum Klotho level was higher in those with the I>I polymorphism, the men, those with higher glomerular filtration rate, and younger individuals, but the differences did not reach a significant level. Higher body mass index was significantly associated with lower serum Klotho level in both groups.

Conclusions. Klotho level after kidney transplantation meets the range in healthy individuals, and it is not affected by the *ACE* gene polymorphism.

IJKD 2017;11:151-6
www.ijkd.org

INTRODUCTION

In 1997, a Japanese group that worked on the mechanism of aging named *Klotho* on a gene that they discovered accidentally.^{1,2} This gene is located on chromosome 13q12 and it creates two transcription. One translation with normal length and its molecular weight is 130 kDa, and it works as membrane proteins (transmembrane). The other translation code is the N-terminal part and its molecular weight is 65 kDa to 70 kDa.^{2,3}

Klotho mRNA is found in the kidney, brain, reproductive organs, pituitary and parathyroid glands, bladder, skeletal muscle, placenta, thyroid, and colon. The kidney is the main source of serum

Klotho production, and Klotho mRNA and protein exist in the kidney in the distal tubule, which is associated with epithelial calcium channel transient receptor potential vanilloid member 5 and calbindin. Soluble Klotho is found in blood, urine, and cerebrospinal fluid.³⁻⁷

Lack of Klotho or fibroblast growth factor 23 (FGF23) causes shortness of life and a syndrome resembling the courses that occur in patients on dialysis and chronic diseases associated with aging.^{1,4,5,8-11} On the other hand, overexpression of Klotho causes slowing of aging process and increases life expectancy by 20% to 30% with the potential mechanism of oxidative stress resistance.²

Vitamin D, FGF23, and Klotho all form an endocrine axis for calcium and phosphate metabolism. Negative regulation of the renin gene is also performed by vitamin D. In chronic kidney disease, low levels of calcitriol, due to the loss of 1-alpha hydroxylase, increases renal renin production and subsequent renin-angiotensin-aldosterone system (RAAS) activation, which in turn reduces renal expression of Klotho and subsequent decrease in FGF23 signaling. This subsequently leads to high FGF23 levels that suppresses 1-alpha hydroxylase, and lowers calcitriol levels. This feedback loop results in vitamin D deficiency, RAAS activation, high FGF23 levels, and renal Klotho deficiency, all of which associated with progression of kidney damage.¹²

Multiple factors have effects on the regulation of Klotho, such as estrogen, calcium, phosphorus, 1,25-dihydroxyvitamin D₃, FGF23, oxidative stress, angiotensin II, age, serum creatinine level, reperfusion injury, altered level of blood pressure (especially hypotension), chronic kidney failure, inflammatory mediators, and peroxisome proliferator-activated receptors.^{2-6,8}

Renin-angiotensin system is another important system that affects the kidney (normal and transplanted).¹³ Several studies have shown that the high activity of this system is associated with poor survival of transplanted kidneys. The RAAS plays a pivotal role in renal progression and its blockade is an important renoprotective intervention, which seems that it hinges with FGF23, Klotho, and vitamin D in an endocrine axis. Angiotensin-converting enzyme (ACE) is an important member of this system and this enzyme has 3 important polymorphisms that have effects on its activity: I>I, I/>D, and D>D, of which D>D has the highest, I>I has the lowest, and I>D has intermediate activity.¹⁴⁻¹⁷ High levels of angiotensin II reduce expression of protein and mRNA of the Klotho.^{4,15} Klotho level in I>I polymorphism cell can be increased by administration of captopril and this will not happen in D>D polymorphism.¹⁵

Administration of the V₂-receptor agonist 1-desamino-8-D-arginine vasopressin leads to a marked decrease in pro-inflammatory mediators and a dramatic increase in the bacterial burden in the kidney. Klotho transcript and protein N levels are reduced by dehydration in mice and in serum deprived vasopressin-exposed human embryonic

kidney cells.¹⁸

This study was aimed to examine the interaction between the RAAS and the vitamin D-FGF23-klotho axis as well as its possible implications for progression of chronic kidney disease.

MATERIALS AND METHODS

This cross-sectional study included 30 transplanted kidney patients with stable kidney function and 27 healthy and demographically matched young individuals. The inclusion criteria were age between 18 to 50 years, kidney transplant for at least 2 years, and normal kidney function defined as a serum creatinine level less than 1.6 mg/dL. Patients with concomitant diagnoses of malignancy, infection, urinary tract obstruction, symptomatic heart failure, multi-organ transplant, use of erythropoietin, and smoking were excluded. Informed consent was obtained from all participants and the study protocol was approved by the local ethics committee (10728/4/5).

Measurements were done in both groups, including serum Klotho levels and ACE gene polymorphisms. For all participants, anthropometric data and medical and drug history were recorded, and 4 mL of blood was obtained and kept in different tubes for genetic study and serologic measurements. After separating serum, samples stored in -80°C. At the biochemistry laboratory, serum Klotho was measured using a Klotho enzyme-linked immunosorbent assay kit (Hangzhou Eastbiopharm, Hangzhou, China). Genetic study of ACE polymorphism (I/D) was performed on the genomic DNA that was isolated from peripheral blood leukocytes.

The SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Chicago, IL, USA) was used for statistical analysis. Data were expressed by mean ± standard deviation and frequencies and percentages. Comparison of quantitative variables was performed by the Kruskal-Wallis test, Mann-Whitney U test, 1-way analysis of variance, and the Tukey post hoc test. Comparison of qualitative variables was performed by the chi-square test or the Fisher exact test. *P* values less than .05 were considered significant.

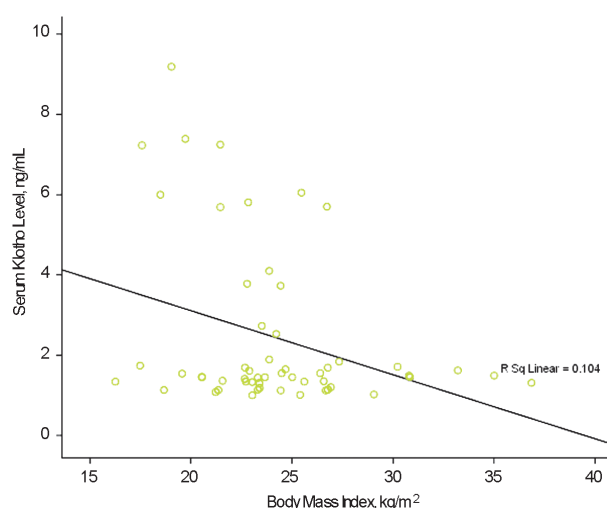
RESULT

The mean serum concentration of Klotho was 2.76 ± 2.42 ng/mL in the kidney transplant

recipients, 2.98 ± 2.37 ng/mL among 10 participants with a D>D ACE gene polymorphism 2.50 ± 2.42 ng/mL among 17 with D>I polymorphism, and 3.52 ± 3.21 ng/mL among 3 with I>I polymorphism (Table 1). The healthy controls had a mean serum Klotho concentration of 2.01 ± 1.41 ng/mL, and their subgroups with the D>D, D>I, and I>I polymorphism has serum Klotho levels of 1.80 ± 1.01 ng/mL, 1.79 ± 1.29 ng/mL, and 3.23 ± 2.27 ng/mL, respectively (Table 1). Serum Klotho level was higher in the kidney transplant recipients compared to the controls, but not to a significant level. Klotho concentration was higher among those with I>I polymorphism in both the kidney transplant and the control groups, although the difference was not significant. Serum Klotho level was inversely correlated with BMI in both groups (Table 2; Figure). Male sex and higher GFR levels were directly but not significantly correlated with Klotho concentration.

DISCUSSION

The mean plasma Klotho level in our kidney transplant recipient was higher (2.76 ± 2.41 ng/mL) than the levels reported by 2 previous studies by Fukino and colleagues and Leone and colleagues (0.613 ng/mL and 0.68 ng/mL,



Correlation between body mass index and serum Klotho level

respectively).^{7,9} The reason for this finding can be the time of measurement in our study that was performed at least 2 years after transplantation, which was long after the effect of initial high-dose immunosuppressants and probable episodes of acute kidney injury (AKI). Furthermore, majority of our transplant recipients were young individuals with normal allograft function. The mean plasma Klotho level in our study of healthy individual was higher (2.01 ± 1.41 ng/mL) than that reported

Table 1. Serum Klotho in Kidney Transplant and Control Groups by ACE Gene Polymorphism

ACE Gene Polymorphism	Kidney Transplant Recipients		Healthy Controls	
	Number of Participants (%)	Mean Klotho (Range), ng/mL	Number of Participants (%)	Mean Klotho (Range), ng/mL
I>I	3 (10.0)	3.52 ± 3.21 (1.62 to 7.23)	4 (14.8)	3.23 ± 2.27 (1.33 to 6.05)
I>D	17 (56.7)	2.50 ± 2.42 (1.00 to 9.19)	13 (48.1)	1.79 ± 1.29 (1.01 to 5.81)
D>D	10 (33.3)	2.98 ± 2.37 (1.13 to 7.39)	10 (37.0)	1.83 ± 1.03 (1.08 to 3.78)
All	30 (100)	2.76 ± 2.41 (1.00 to 9.19)	27 (100)	2.01 ± 1.41 (1.01 to 6.05)

Table 2. Mean Values of Kidney Transplant Recipients' Characteristics and Clinical parameters and Their Correlations With Serum Klotho Level

Parameter	Mean Value (Range)	P for Correlation With Klotho
Age, y	30.9 ± 5.3 (19 to 39)	> .05
Years after transplantation	6.42 ± 2.44 (3 to 11)	> .05
Serum phosphorus, mg/dL	3.45 ± 0.44 (2.8 to 4.1)	> .05
Serum calcium, mg/dL	9.16 ± 0.70 (7.8 to 10.3)	> .05
Blood urea, mg/dL	36.64 ± 10.27 (19 to 61)	> .05
Serum creatinine, mg/dL	1.28 ± 0.26 (0.7 to 1.8)	> .05
Glomerular filtration rate, mL/min	64.53 ± 17.83 (38.5 to 103.1)	> .05
Alkaline phosphatase, u/L	253.20 ± 115.44 (116 to 411)	> .05
Body mass index, kg/m ²	24.12 ± 5.63 (16.26 to 36.85)	.049
Cyclosporine, mg/d	164.71 ± 38.58 (0 to 225)	> .05
Mycophenolate mofetil, mg/d	1763.15 ± 305.88 (0 to 2000)	> .05
Prednisolone, mg/d	5.12 ± 1.89 (0 to 10)	> .05

by others,^{19,20} but the mean age was significantly lower in our study compared with the previous reports, and this could be the explanation for those differences.

This study found no significant difference in serum Klotho levels between kidney transplant recipients and healthy individuals. In congruent with our study, Akimoto and colleagues and Bleskestad and coworkers reported the same findings.^{19,21} However, Leone and colleagues reported that Klotho concentration was higher in kidney transplant recipients than in healthy individuals, and they contributed it to the higher erythropoietin level in kidney transplant patients.⁷

The relationship between the *ACE* gene I>I polymorphism and higher Klotho level was reported by Hamdi and Castellon in a sample of tissue culture, but our study was the first report about this effect in kidney transplant recipients.¹⁵ Mitani and colleagues applied long-term infusion of angiotensin II to mice and detected reduced klotho expression,⁴ which is in line with our study that participants with D>D polymorphism had higher levels of angiotensin II and lower Klotho levels.⁴ Data suggests that angiotensin II negatively regulates the renal expression of klotho in an animal model. Mitani and colleagues demonstrated downregulation of renal klotho expression in response to angiotensin II infusion.⁴ In a compressor dose is angiotensin II type 1 receptor-dependent and some parts of non-pressure-driven angiotensin II induced proteinuria, is explained by its negative effect on Klotho level.⁴ In cultured tubular epithelial cells, angiotensin II-induced angiotensin II type 1 receptor-mediated downregulation of Klotho was confirmed.²² Yoon and colleagues demonstrated that salt restriction, a RAAS-activating intervention, reduces Klotho expression, which was reversed by losartan. Cyclosporine-induced damage is also associated with downregulation of renal Klotho in association with upregulation of renal RAAS activation; addition of losartan completely prevented the loss of Klotho expression.¹² The mechanisms of angiotensin II-induced Klotho downregulation may be explained by direct downregulation by angiotensin II type 1 receptor or enhanced oxidative stress-induced negative effect.^{23,24}

As RAAS reduces reactive oxygen species production,²⁵ angiotensin II and tumor necrosis

factor- α converting enzyme are upregulated in the vitamin D deficiency state and may be involved in Klotho downregulation, and this could be an explanation for inflammation-induced renal Klotho downregulates and vascular calcification in chronic kidney disease.^{26,27} Calcitriol has been shown to enhance renal Klotho expression; the possible mechanism is reduced RAAS activation. Conversely, tubular injury leads to Klotho downregulation. This condition happens in kidney allograft, but we are unaware whether this is mediated by RAAS activation or not.^{23,24} Angiotensin II could theoretically be responsible for a vicious circle formation; its induction of Klotho deficiency leads to FGF23 resistance and suppression of vitamin D 1-alpha hydroxylase, mediated by extracellular signal-regulated kinase.²⁸⁻³⁰ Inhibition of RAAS and supplementation of vitamin D are well-established interventions to interrupt the vicious circle of Klotho deficiency-induced FGF23 resistance.³¹

In our study male sex had positive effects on plasma Klotho level. The same result was reported in a study on elderly population,³² but our study is the first to our knowledge that shows positive effects of male sex on Klotho levels in kidney transplant recipients. In some studies, there are no relationship between sex and serum Klotho level or even its level was higher in women.³³⁻³⁷ In our study, also a higher GFR was directly correlated with serum Klotho concentration, but not significantly³⁸⁻⁴⁰ Klotho level was inversely correlated with BMI in both kidney transplant recipients and healthy groups. The same result was reported by Amirtani and coworkers and Sze and coworkers,^{35,41} but some other studies did not demonstrate this relationship.^{36,37,42-44}

CONCLUSIONS

Klotho level after kidney transplantation meets the range in healthy individuals, and it is not affected by the *ACE* gene polymorphism.

CONFLICT OF INTEREST

None declared.

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Received April 2016
Revised August 2016
Accepted September 2016