의학박사 학위논문

만성콩팥병에서 혈청 Klotho와 심혈관계 인자와의 연관성

The Association Between Serum Klotho and Cardiovascular Parameters in Chronic Kidney Disease : Results From a Prospective Multicenter Cohort Study

2018 년 8 월

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Abstract

The Association Between Serum Klotho and Cardiovascular Parameters in Chronic Kidney Disease: Results From a Prospective Multicenter Cohort Study

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Background: Klotho, a protein linked to aging, has emerged as a pivotal player in mineral bone metabolism and might explain the relationship between chronic kidney disease (CKD) and cardiovascular disease (CVD). The present study aimed to investigate the association between serum klotho and cardiac

parameters in a large Korean CKD cohort.

Methods: The data of 2,101 individuals that participated in the KoreaN Cohort Study for Outcome in Patients With Chronic Kidney Disease (KNOW-CKD) cohort with available serum klotho levels. The serum klotho level was measured using an enzyme-linked immunosorbent assay kit (Immuno-Biological Laboratories Co., Gunma, Japan). Cardiovascular parameters were evaluated using left ventricular mass index (LVMI) and pulse wave velocity (PWV), which represent left ventricular hypertrophy (LVH) and arterial stiffness, respectively. The presence of LVH was defined using sex-specific LVMI cut-off values ($\geq$50 g/m$^{2.7}$ for men and $\geq$47 g/m$^{2.7}$ for women).

Results: Patients were 53.6 $\pm$ 12.2 years old and 61.1% were men. Mean estimated glomerular filtration rate (eGFR) was 53.0 $\pm$ 30.7 mL/min/1.73m^2. Median serum klotho level was 536 pg/mL (interquartile range [IQR]: 420-667 pg/mL). Advanced CKD stages were associated with lower serum klotho levels (P <0.001, P for trend <0.001). Ascending quartiles of klotho levels were significantly associated with LMVI decreases (P <0.001, P for trend <0.001). A multivariable linear regression model showed serum klotho had a significant inverse association with LVMI (β −0.04; 95% CI [confidence interval] −0.004, −0.00007; P = 0.041). The odds ratios of LVH in the 1st, 2nd, and 3rd klotho quartiles as compared with the 4th klotho quartile were not significantly different after adjustment. Klotho showed

a significant association with the presence of LVH only in men. After adjustment, no significant association was found between serum klotho and brachial–to–ankle PWV (β 0.003; 95% CI −0.04, 0.05; P = 0.876) or heart–to–femoral PWV (β −0.013; 95% CI −0.06, 0.034; P = 0.564).

Conclusions: Serum klotho was found to be an independent biomarker of LVMI, but not of arterial stiffness.

Keywords: Serum klotho, Soluble klotho, Chronic kidney disease, Left ventricular mass index, Left ventricular hypertrophy, Pulse wave velocity

Student Number: 2014–30609

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Introduction

Patients with chronic kidney disease (CKD) present with a higher burden of cardiovascular disease (CVD) and cardiovascular mortality than the general population [1, 2]. Left ventricular myocardial hypertrophy, the most commonly diagnosed cardiovascular abnormality in CKD patients, occurs secondary to volume and pressure overload [3]. Furthermore, cardiac hypertrophy is an important cause of cardiovascular morbidity and mortality in CKD patients because it can lead to congestive heart failure, arrhythmia, ischemic cardiomyopathy (even in the absence of coronary artery disease), and sudden death in CKD patients [4–6]. Arterial stiffness in CKD patients caused by arteriosclerosis with thickening and stiffening of the arterial wall [7] leads to cardiac hypertrophy and negatively influences prognosis in CVD [8, 9].

Mineral bone metabolism is important in CKD and progressive deterioration of calcium–phosphorus homeostasis is associated with cardiovascular complications. Impaired calcium–phosphorus homeostasis can cause cardiac hypertrophy and vascular calcification. Interestingly, klotho has emerged as a pivotal player in calcium–phosphorus homeostasis and mineral metabolism regulation in CKD, and might explain the relationship between CKD and CVD. The *Klotho* gene, which was originally identified

as an aging suppressor gene, has been shown to be closely associated with CKD. In a previous study, klotho knock-out mice exhibited hyperphosphatemia, ectopic soft tissue calcification, and arteriosclerosis [10], which are also observed in CKD patients, suggesting CKD might result from a state of klotho deficiency. In addition to serving as a biomarker for CKD, klotho deficiency is also a pathogenetic indicator of renal and extra-renal complications in CKD [11]. In previous experimental studies conducted in mouse models, restoration of serum klotho levels ameliorated cardiac hypertrophy and vascular calcification [12, 13], and haplodeficiency of the *Klotho* gene resulted in arterial stiffness [14]. Clinical data that support these experimental results are scarce and results are mixed [15]. In 86 CKD patients, cardiac hypertrophy evaluated using left ventricular mass index (LVMI) was found to be negatively associated with serum klotho levels but the analysis was not adjusted for potential confounders [16]. In another study conducted in dialysis patients, no significant association was observed between serum klotho and LVMI [17]. Previous studies have been conducted on small numbers of patients, and few studies have focused on CKD patients for the association between klotho and LVMI. Given the negative effects of klotho deficiency, its association with cardiovascular complications in preclinical studies, and the limited number of clinical studies, the current study aimed to investigate the association between serum klotho

and cardiovascular parameters in CKD patients, using baseline cross-sectional data obtained from a large Korean CKD cohort.

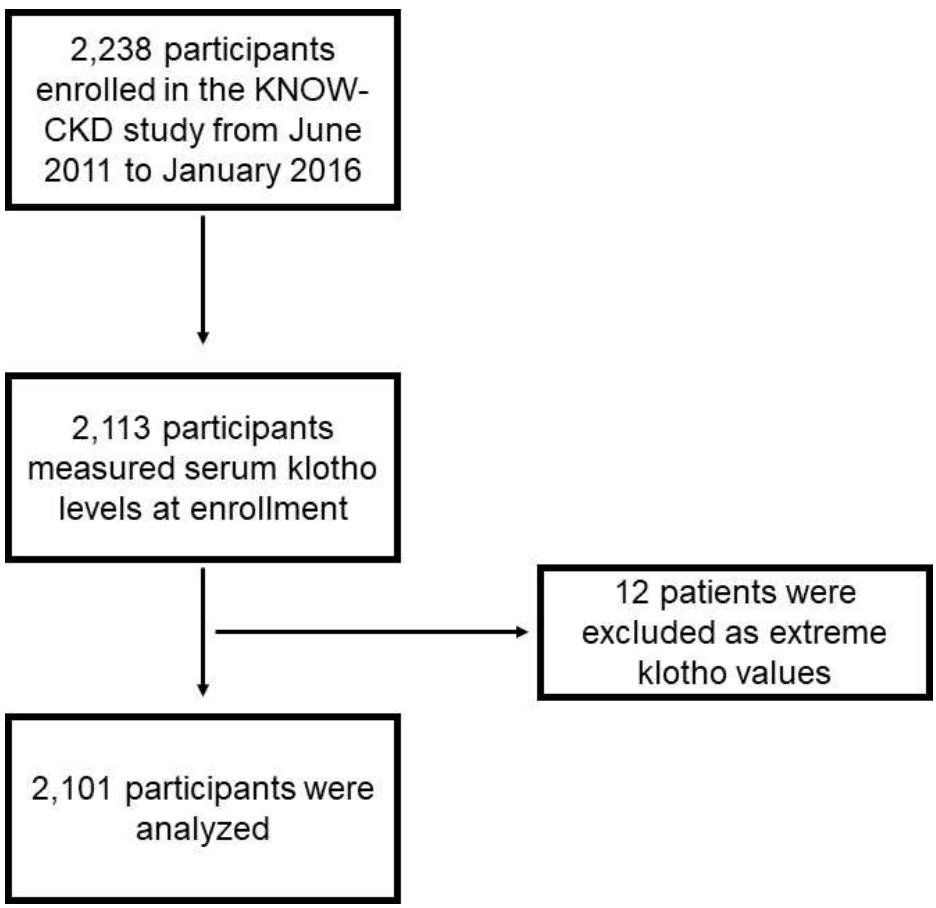
Materials and Methods

Study population

The KoreaN Cohort Study for Outcome in Patients With Chronic Kidney Disease (KNOW-CKD) was a Korean, multicenter, prospective, cohort study that enrolled participants with CKD from stage 1 to 5 (predialysis) from nine clinical centers of major university-affiliated hospitals in Korea. Details about the study design and methods are described elsewhere [18]. Subjects with severe heart failure (New York Heart Association Class III or IV) were excluded from enrollment according to study protocol. Among the 2,238 participants enrolled in the KNOW-CKD study from June 2011 to January 2016, 2,113 individuals had their serum klotho levels assayed (Figure 1). Twelve patients were excluded for extreme klotho values (serum klotho lower than the detectable range or >6000 pg/mL). These values were not influence value by Cook's distance analysis. Finally, 2,101 subjects were included in the cross-sectional analysis. The study protocol was approved by the ethical committee of each participating clinical center, including the Institutional Review Boards of Seoul National University Hospital, Severance Hospital, Kangbuk Samsung Medical Center, Seoul St. Mary's Hospital, Gil Hospital, Eulji General Hospital, Chonnam National University Hospital, and Busan Paik Hospital. All study

subjects provided written informed consent. The study protocol was in accordance with the principles of the Declaration of Helsinki.

Figure 1. Study participants algorithm



KNOW-CKD, The KoreaN Cohort Study for Outcome in Patients With Chronic Kidney Disease

Clinical data collection and laboratory analyses

Baseline demographic characteristics and laboratory values at enrollment were extracted from an electronic data management system (http://www.phactaX.org). Serum creatinine was measured using an isotope dilution mass spectrometry (IDMS)-traceable method [19] at a central laboratory. Estimated glomerular filtration rates (eGFR) were calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation [20]. Serum α-klotho levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Immuno-Biological Laboratories Co., Gunma, Japan) according to the manufacturer's protocol [21]. Intra-assay and inter-assay coefficients of variation were 2.7-3.5% (klotho levels 186.64-2968.78 pg/mL) and 2.9-11.4% (klotho levels 165.47-2903.01 pg/mL), respectively. The data used to determine intra-assay and inter-assay coefficients of variations were confirmed in our central laboratory by measuring serum control klotho levels in 20 repeats per ELISA plate. The intra-assay and inter-assay coefficients of variations were 0.57-1.78% and 3.01-6.12% (klotho levels 120.30-4468.50 pg/mL), respectively. The standard curve was found to be linear up to a klotho concentration 4000 pg/mL. C-terminal FGF23 levels were measured using a second generation human FGF23 ELISA kit (Immutopics, San Clemente, California, USA) according to the manufacturer's protocol. The intra-assay and

inter-assay coefficients of variation as reported by the manufacturer were 1.4-2.4% (FGF23 levels 33.7-302 RU/mL) and 2.4-4.7% (FGF23 levels 33.6-293 RU/mL), respectively.

Cardiovascular parameters

Cardiovascular parameters were evaluated using LVMI and pulse wave velocity (PWV), representing left ventricular hypertrophy (LVH) and arterial stiffness, respectively. Two-dimensional echocardiography was conducted by experts at each hospital, and the LVMI was calculated by dividing the left ventricular (LV) mass by the height$^{2.7}$ [22]. LVMI was calculated in the cohort center using the same formula. LV mass was calculated using the formula $0.8 \times \{1.04 \times [(\text{LVIDd} + \text{PWTd} + \text{SWTd})^3 - \text{LVIDd}^3]\} + 0.6$ (g), where LVIDd, PWTd, and SWTd are LV internal diameter at end diastole, posterior wall thickness at end diastole, and septal wall thickness at end diastole, respectively [23]. LVH was defined as a LVMI of $\geq$50 g/m$^{2.7}$ in men and of $\geq$47 g/m$^{2.7}$ in women [24]. Relative wall thickness (RWT) was calculated to as two times posterior wall thickness/LV internal linear dimension in diastole (RWT = [2 × PWTd]/LVIDd). RWT was considered to elevated if >0.42. LVMI and RWT were used to categorize LV geometry as follows; normal (normal LVMI and normal RWT), concentric remodeling (normal LVMI and RWT >0.42), concentric LVH (increased LVMI and RWT >0.42), eccentric LVH (increased LVMI and RWT $\leq$0.42). Systolic and

diastolic heart dysfunctions were defined as an LV ejection fraction of <50% and a ratio (E/E' ratio) of mitral peak velocity of early filling (E) to early diastolic mitral annular velocity (E') >15 on echocardiography, respectively [25, 26]. Arterial stiffness was measured using brachial-to-ankle PWV (baPWV) and heart-to-femoral PWV (hfPWV) [27]. Among the nine participating centers, hfPWV was measured only at five centers where the equipment was available. hfPWV represented central arterial stiffness, while baPWV represented peripheral arterial stiffness. Abdominal aorta calcification (AAC) scores were measured by simple lateral lumbar radiography and ranged from 0 to 24 (where 24 represents maximal calcification), as previously described [28]. Coronary artery calcium scores (CACS) were measured by computed tomography and were presented as Agatston scores, as previously described [29, 30].

Statistical analyses

Categorical variables were analyzed using the Chi-square test and results are presented as frequencies and percentages. Continuous variables were analyzed by one-way analysis of variance (ANOVA) or the Kruskal-Wallis test. The Kolmogorov-Smirnov test was used to determine the normality of variable distributions. Results are presented as the mean ± standard deviation (SD) for normally distributed variables and the median (interquartile range [IQR]) for variables with a

skewed distribution. Participants were classified into quartiles according to serum klotho level. The relation between serum klotho levels and CKD stages was analyzed using the Kruskall–Wallis test and *P* value is the difference between CKD stages. *P* – for trend of klotho level with advanced CKD stages was determined using the Jonckheere–Terpstra test (Jonckheere trend test). Association between LVMI or PWV and klotho quartiles were analyzed by ANOVA and *P* values indicate differences between klotho quartiles. *P* – for trend of LMVI and PWV value with higher klotho quartiles was measured using ANOVA trend analyses using polynomial contrasts. Multivariable linear regression model analysis with adjustment (enter method) was used to investigate associations between LVMI or PWV and serum klotho level. LVMI and PWV were viewed as continuous variables. LMVI and PWV (dependent variables) corresponding to the independent variables met the assumptions for linear regression model: normality, homoscedasticity, independence, and linearity. For all linear regression models, variables were selected based on the results of prior studies and physiologic reasoning [31–33]. Collinearity between variables was also tested. Logistic regression analysis was used to determine odds ratios (ORs) for the presence of LVH in association with different klotho quartiles as compared with the highest klotho quartile. *P* – values <0.05 were considered statistically significant. The SPSS statistical software (SPSS version 18.0,

Chicago, IL, USA) was used for all descriptive and outcome analyses.

Results

Demographic and baseline clinical characteristics of the study subjects

The clinical characteristics of the 2,101 study subjects at enrollment are shown in Table 1. Mean age was 53.6 ± 12.2 years, and 61.1% were men. Mean eGFR was 53.0 ± 30.7 mL/min/1.73m^2. Patients with diabetes mellitus (DM) and hypertension (HTN) comprised 33.9% and 96.1% of the subjects, respectively. Median serum klotho level was 536 pg/mL (IQR: 420–667). Serum klotho levels according to CKD stages are shown in Figure 2. Advanced CKD stages were found to be significantly associated with lower serum klotho levels (P <0.001, P for trend <0.001).

Table 1. Clinical characteristics of the study subjects at enrollment stratified by serum klotho quartile

Characteristics	Total (N = 2,101)	Klotho groups				*P* - value	*P* for trend††
		1st quartile (n = 524) (99-419 pg/mL)	2nd quartile (n = 528) (420-536 pg/mL)	3rd quartile (n = 523) (537-666 pg/mL)	4th quartile (n = 526) (667-3641 pg/mL)		
Age (mean ± SD)	53.6 ± 12.2	54.0 ± 11.8	54.6 ± 11.9	53.5 ± 12.2	52.2 ± 12.5	0.015	0.007
Sex, men, n (%)	1284 (61.1)	330 (63.0)	319 (60.4)	317 (60.6)	318 (60.5)	0.795	0.440
BMI (kg/m^2)	24.5 ± 3.4	24.8 ± 3.5	24.6 ± 3.2	24.5 ± 3.4	24.2 ± 3.3	0.036	0.005
DM, n (%)	712 (33.9)	171 (32.6)	192 (36.4)	172 (32.9)	177 (33.7)	0.560	0.962
HTN, n (%)	2020 (96.1)	507 (96.8)	517 (97.9)	501 (95.8)	495 (94.1)	0.011	0.007
SBP (mmHg)	128.5 ± 16.4	129.3 ± 16.1	128.5 ± 16.6	127.9 ± 16.9	128.5 ± 16.1	0.599	0.370
DBP (mmHg)	76.9 ± 11.2	76.7 ± 11.8	77.0 ± 11.1	76.6 ± 11.2	77.2 ± 10.5	0.786	0.667
ACEi or ARB, yes, n (%)	1795 (85.6)	458 (87.4)	461 (87.5)	442 (84.7)	434 (82.7)	0.075	0.013
eGFR ($ml/min/1.73m^2$)	53.0 ± 30.7	48.2 ± 28.6	47.6 ± 27.8	54.6 ± 31.6	61.7 ± 32.5	<0.001	<0.001
Hemoglobin (g/dL)	12.8 ± 2.0	12.6 ± 1.9	12.6 ± 2.0	12.9 ± 2.0	13.3 ± 2.1	0.001	<0.001
Uric acid (mg/dL)	7.0 ± 1.9	7.4 ± 2.0	7.2 ± 1.9	6.9 ± 1.9	6.6 ± 1.8	<0.001	<0.001
Albumin (g/dL)	4.2 ± 0.4	4.1 ± 0.4	4.2 ± 0.4	4.2 ± 0.4	4.2 ± 0.5	0.316	0.068

Table 1. Clinical characteristics of the study subjects at enrollment stratified by serum klotho quartile *(Continued)*

Characteristics	Total (N = 2,101)	Klotho groups				*P* - value	*P* for trend††
		1st quartile (n = 524) (99-419 pg/mL)	2nd quartile (n = 528) (420-536 pg/mL)	3rd quartile (n = 523) (537-666 pg/mL)	4th quartile (n = 526) (667-3641 pg/mL)		
Total cholesterol (mg/dL)	174.3 ± 39.4	174.0 ± 39.3	172.0 ± 38.0	172.5 ± 40.3	178.8 ± 39.6	0.021	0.053
CRP, median, (Q1, Q3) (mg/dL)	0.06 (0.02, 0.17)	0.08 (0.03, 0.20)	0.06 (0.03, 0.16)	0.06 (0.02, 0.16)	0.05 (0.02, 0.13)	<0.001	<0.001
Phosphorus (mg/dL)	3.7 ± 0.7	3.8 ± 0.7	3.7 ± 0.7	3.7 ± 0.7	3.6 ± 0.6	0.004	0.001
Corrected Ca (mg/dL)	9.0 ± 0.4	9.0 ± 0.4	9.0 ± 0.5	9.0 ± 0.4	8.9 ± 0.4	0.505	0.530
Klotho, median (Q1, Q3) (pg/mL)	536 (420, 667)	335 (269, 383)	479 (449, 505)	593 (562, 626)	788 (714, 913)	<0.001	<0.001
25(OH)VitD, median (Q1, Q3) (ng/mL)	16.5 (12.7, 21.3)	16.7 (13.5, 21.7)	16.6 (13.1, 21.2)	16.4 (12.6, 21.3)	16.1 (12.1, 21.0)	0.040	0.005
$1,25(OH)_2$VitD, median (Q1, Q3) (pg/mL)	25.4 (20.1, 33.7)	26.4 (20.1, 36.2)	24.6 (19.1, 31.6)	24.3 (19.4, 32.5)	26.6 (21.4, 34.4)	<0.001	0.599
iPTH, median (Q1, Q3) (pg/mL)	51.5 (33.2, 84.0)	55.0 (35.0, 86.1)	53.0 (34.5, 88.8)	52.6 (33.9, 86.8)	46.3 (30.2, 74.8)	0.005	0.003
C-terminal FGF23, median (Q1, Q3) (RU/mL)	17.9 (0.4, 31.3)	18.5 (0.3, 30.2)	19.0 (1.0, 32.3)	18.6 (0.8, 31.5)	10.4 (0.2, 31.4)	0.094	0.419
UACR, >300 mg/g, n (%)	884 (49.9)	217 (52.3)	255 (56.0)	205 (45.5)	207 (45.9)	<0.001	<0.001
LVMI ($g/m^{2.7}$)	42.0 ± 11.7	43.3 ± 12.0	42.7 ± 12.2	42.1 ± 11.9	40.1 ± 10.7	<0.001	<0.001
LVH, n (%)	509 (24.8)	145 (28.3)	139 (27.0)	128 (24.8)	97 (19.2)	0.004	0.001

Table 1. Clinical characteristics of the study subjects at enrollment stratified by serum klotho quartile *(Continued)*

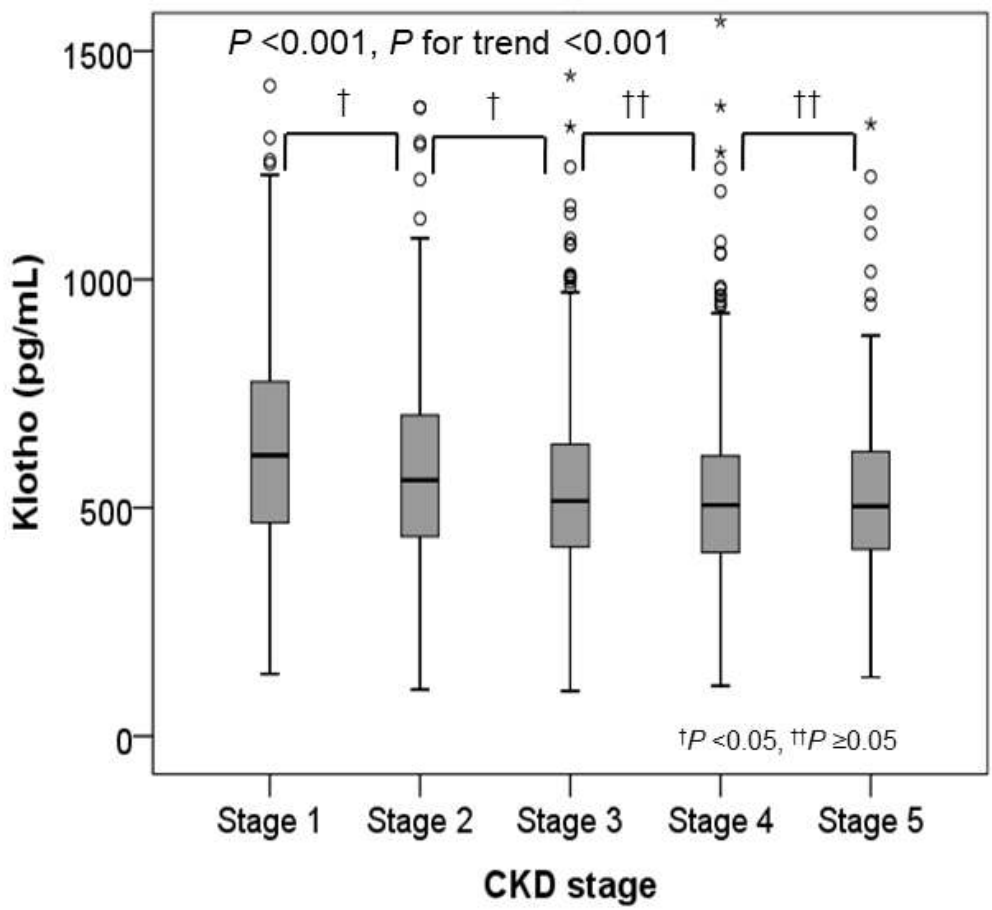
Characteristics	Total (N = 2,101)	Klotho groups				*P* - value	*P* for trend††
		1st quartile (n = 524) (99-419 pg/mL)	2nd quartile (n = 528) (420-536 pg/mL)	3rd quartile (n = 523) (537-666 pg/mL)	4th quartile (n = 526) (667-3641 pg/mL)		
LV geometry, n (%)						0.084	0.006
Normal	1248 (60.9)	303 (59.1)	304 (59.0)	316 (61.2)	325 (64.2)		
Concentric remodeling	293 (14.3)	65 (12.7)	72 (14.0)	72 (14.0)	84 (16.6)		
Eccentric LVH	254 (12.4)	76 (14.8)	70 (13.6)	60 (11.6)	48 (9.5)		
Concentric LVH	255 (12.4)	69 (13.5)	69 (13.4)	68 (13.2)	49 (9.7)		
baPWV (cm/s)	1534 ± 344	1552 ± 348	1552 ± 322	1527 ± 357	1507 ± 347	0.127	0.024
†hfPWV (cm/s)	1018 ± 274	1053 ± 300	1028 ± 257	1005 ± 277	989 ± 258	0.022	0.002
AAC ≥1, n (%)	703 (35.0)	172 (35.0)	194 (38.0)	183 (36.6)	154 (30.6)	0.079	0.127
CACS >100, n (%)	498 (24.6)	133 (26.2)	131 (25.7)	121 (24.0)	113 (22.6)	0.528	0.143
LVEF <50%	30 (1.5)	8 (1.5)	5 (1.0)	7 (1.3)	10 (2.0)	0.607	0.497
E/E' >15	176 (8.6)	46 (9.0)	47 (9.1)	42 (8.2)	41 (8.1)	0.923	0.536

†1,243 patients had a measured hfPWV (*vs.* 1,907 patients with a measured baPWV) at study enrollment

††P for trends were calculated for klotho quartiles

SD, standard deviation; BMI, body mass index; DM, diabetes mellitus; HTN, hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; eGFR, estimated glomerular filtration rate using the CKD-EPI creatinine equation; CRP, C-reactive protein; Ca, calcium; 25(OH)VitD, 25-hydroxy vitamin D; $1,25(OH)_2$VitD, 1,25-hydroxyvitaminD; iPTH, intact parathyroid hormone; FGF23, fibroblast growth factor23; UACR, random urine albumin-creatinine ratio; LVMI, left ventricular mass index; LVH, left ventricular hypertrophy; baPWV, brachial-to-ankle pulse wave velocity; hfPWV, heart-to-femoral pulse wave velocity; AAC, Abdominal aorta calcification; CACS, coronary artery calcium score; LVEF, left ventricular ejection fraction; E/E', ratio of mitral peak velocity at early filling (E) to early diastolic mitral annular velocity (E').

Figure 2. Serum klotho levels across CKD stages



Advanced CKD stages were found to be significantly associated with lower serum klotho levels (P <0.001, P for trend <0.001).

CKD, chronic kidney disease

Comparison of baseline characteristics between serum klotho quartiles

Comparison of baseline characteristics of subjects between serum klotho quartiles was shown in table 1. Patients in the highest (4^{th}) quartile group were younger and had a lower mean body mass index (BMI). eGFR and hemoglobin levels were higher in the 4^{th} klotho quartile. Uric acid, C-reactive protein (CRP), phosphorus, and intact parathyroid hormone (iPTH) levels were lower in the 4^{th} klotho quartile.

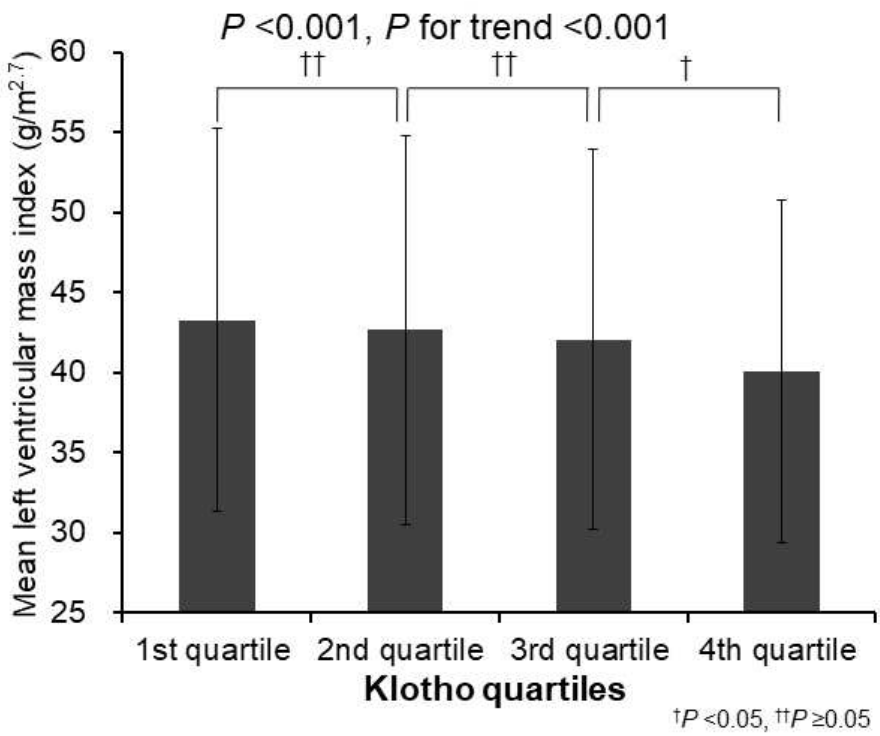
Serum klotho and cardiovascular parameters

Serum klotho and left ventricular hypertrophy

LMVI significantly decreased as klotho quartile increased (P <0.001, P for trend <0.001; Figure 3). 509 (24.8%) of the 2,101 study subjects had LVH (281 [22.4%] men, 228 [28.6%] women). LV geometry was not significantly different between klotho quartiles (P = 0.084). Table 2 summarizes the results of multivariable linear regression analysis of the association between klotho and LVMI. After adjustment for age, sex, DM, HTN, BMI, systolic blood pressure, eGFR, hemoglobin, phosphorus, corrected calcium, and FGF23, serum klotho had a significant inverse association with LVMI (β − 0.04; 95% CI

−0.004, −0.00007; P = 0.041; Table 2).

Figure 3. Mean left ventricular mass index across klotho quartiles



Left ventricular mass index significantly decreased as klotho quartile increased (P <0.001, P for trend <0.001).

Table 2. Multivariable linear regression analysis presenting associations between klotho and left ventricular mass index

Variable	Model A		Model B		Model C
	β (95% CI)	*P*	*β* (95% CI)	*P*	*β* (95% CI)
Klotho (pg/mL)	-0.09 (-0.006, -0.002)	<0.001	-0.079 (-0.005, -0.002)	<0.001	-0.04 (-0.004, -0.00007)

Model A: Adjusted for age and sex

Model B: Adjusted for age, sex, DM, HTN, BMI, and SBP

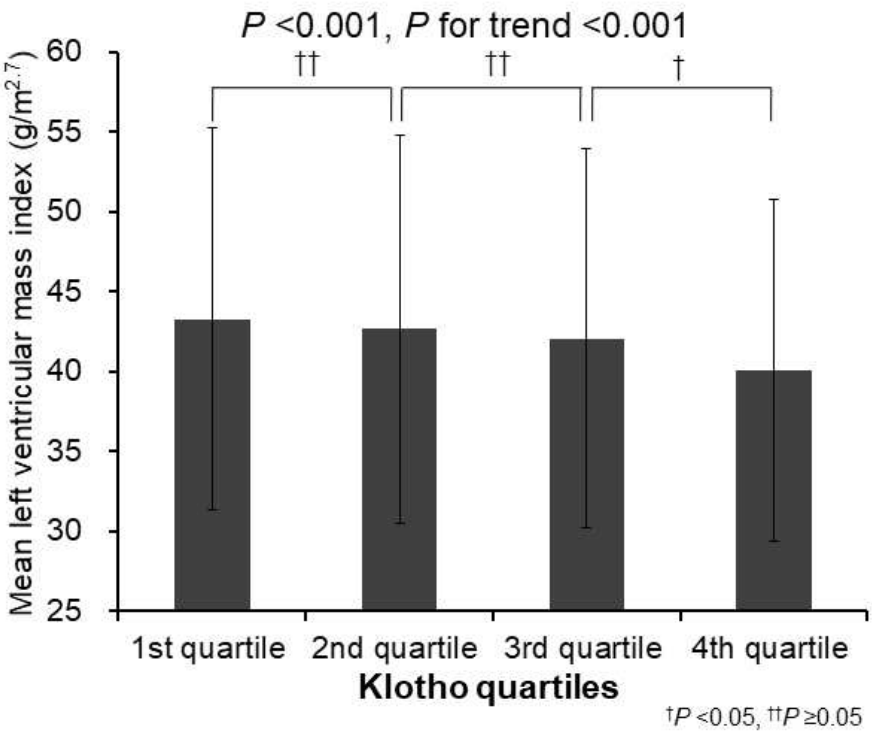
Model C: Adjusted for age, sex, DM, HTN, BMI, SBP, eGFR, hemoglobin, phosphorus, corrected Ca, and FGF23

β, Standardized coefficient; CI, confidence interval; DM, diabetes mellitus; HTN, hypertension; BMI, body mass index; SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate using the CKD-EPI creatinine equation; Ca, calcium; FGF23, fibroblast growth factor 23

When analyzed according to the presence of LVH, increased in klotho quartiles were significantly associated with decreases in the presence of LVH (P = 0.004, P for trend = 0.001; Figure 4). The results of the multivariable logistic analysis of the association between klotho and the presence of LVH are shown in Table 3. The ORs of LVH in the 1st, 2nd, and 3rd klotho quartiles as compared with the 4th klotho quartile were not significantly different using fully adjusted model C (Table 3). When univariate analysis of LVH was performed stratified by sex, men exhibited a significant inverse relation between klotho quartile and the presence of LVH (P = 0.002; Figure 5), and for men, the 4th klotho quartile had the lowest LVH percentage. The clinical characteristics of the study subjects stratified by sex are summarized in Table 4. Men were older (P = 0.030) and had a higher BMI (P <0.001) than women. In addition, men had a higher prevalence of DM (P <0.001) and HTN (P <0.001) and a lower eGFR (P = 0.032) than women. Total cholesterol (P <0.001) and phosphorus (P <0.001) levels were higher in women. Subgroup analysis stratified by sex and adjusted for age, DM, HTN, BMI, systolic blood pressure, eGFR, hemoglobin, phosphorus, corrected calcium, and FGF23, showed that the 1st (OR 1.79; 95% CI 1.15, 2.79; P = 0.010) and 3rd (OR 1.73; 95% CI 1.11, 2.69; P = 0.016) klotho quartiles were significantly associated with the presence of LVH than the 4th klotho quartile in men (Figure 6). No significant effect was

observed in women.

Figure 4. Presence of left ventricular hypertrophy in klotho quartiles



Increased in klotho quartiles were significantly associated with decreases in the presence of LVH (P = 0.004, P for trend = 0.001).
LVH, left ventricular hypertrophy

Table 3. Association between klotho quartiles and the presence of left ventricular hypertrophy

Variable	Model A		Model B		Model C	
Klotho quartile	Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*
1st	1.61 (1.19, 2.17)	0.002	1.53 (1.11, 2.10)	0.009	1.24 (0.89, 1.72)	0.211
2nd	1.43 (1.06, 1.94)	0.020	1.40 (1.02, 1.92)	0.040	1.16 (0.83, 1.61)	0.386
3rd	1.34 (0.99, 1.82)	0.061	1.34 (0.97, 1.85)	0.079	1.20 (0.86, 1.67)	0.292
4th	Reference		Reference		Reference	

Left ventricular hypertrophy was defined as a LVMI of ≥50 g/m$^{2.7}$ in men and of ≥47 g/m$^{2.7}$ in women.

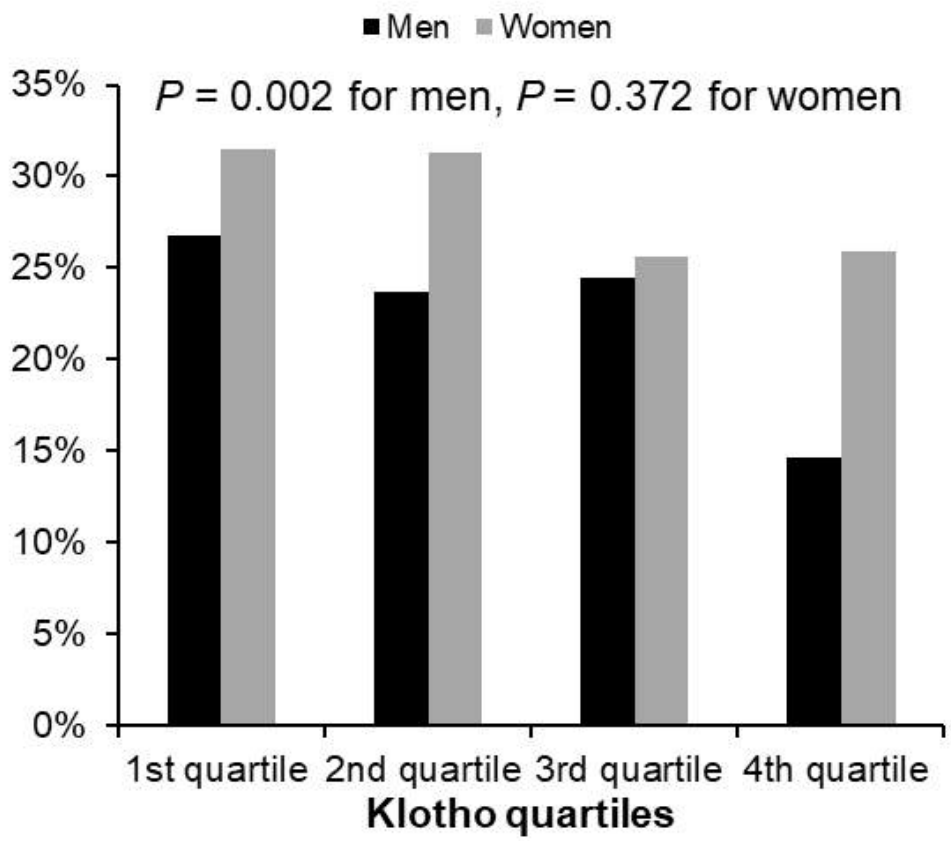
Model A: Adjusted for age and sex

Model B: Adjusted for age, sex, DM, HTN, BMI, and SBP

Model C: Adjusted for age, sex, DM, HTN, BMI, SBP, eGFR, hemoglobin, phosphorus, corrected Ca, and FGF23

LVMI, left ventricular mass index; CI, confidence interval; DM, diabetes mellitus; HTN, hypertension; BMI, body mass index; SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate using the CKD-EPI creatinine equation; Ca, calcium; FGF23, fibroblast growth factor 23

Figure 5. Presence of left ventricular hypertrophy in klotho quartiles stratified by sex



When univariate analysis of LVH was performed stratified by sex, men exhibited a significant inverse relation between klotho quartile and the presence of LVH (P = 0.002).

LVH, left ventricular hypertrophy

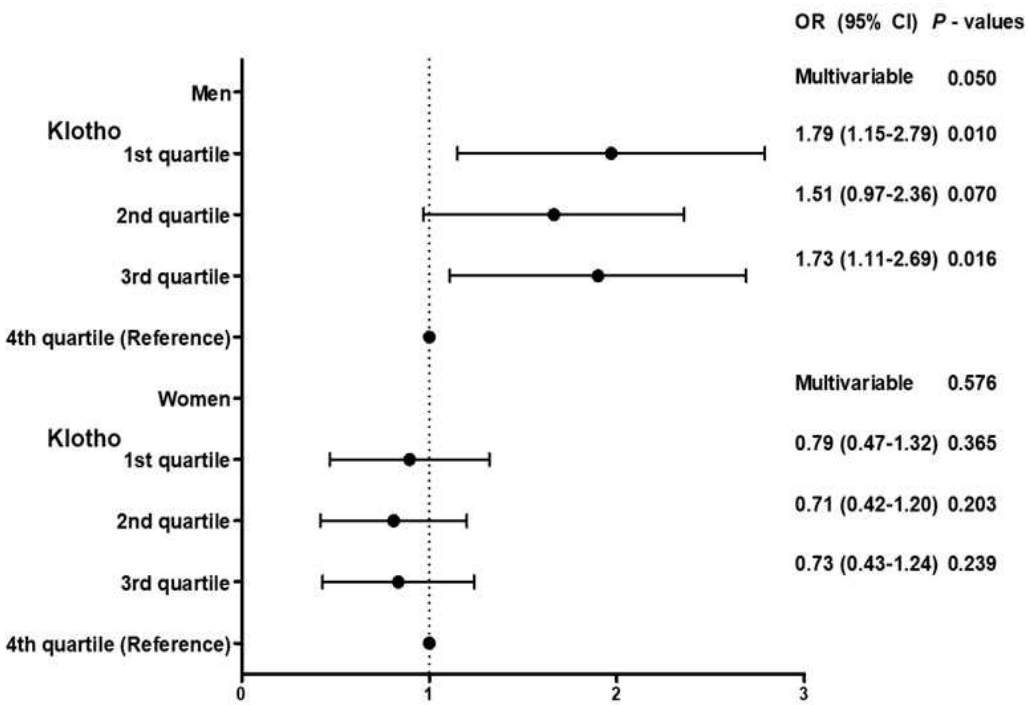
Table 4. Clinical characteristics of study subjects stratified by sex

Characteristics	Men (n = 1284)	Women (n = 817)	*P* - value
Age (mean ± SD)	54.0 ± 12.7	52.9 ± 11.4	0.030
BMI (kg/m^2)	24.8 ± 3.1	24.1 ± 3.7	<0.001
DM, n (%)	475 (37.0)	237 (29.0)	<0.001
HTN, n (%)	1262 (93.8)	758 (92.8)	<0.001
SBP (mmHg)	129.7 ± 16.5	126.8 ± 16.1	<0.001
DBP (mmHg)	77.4 ± 11.3	76.0 ± 10.9	0.006
ACEi or ARB, yes, n (%)	1124 (87.5)	671 (82.1)	0.002
eGFR ($ml/min/1.73m^2$)	51.8 ± 28.7	54.9 ± 33.6	0.032
Hemoglobin (g/dL)	13.4 ± 2.1	11.9 ± 1.5	<0.001
Uric acid (mg/dL)	7.4 ± 1.9	6.5 ± 1.9	<0.001
Albumin (g/dL)	4.2 ± 0.4	4.1 ± 0.4	0.003
Total cholesterol (mg/dL)	169.7 ± 39.7	181.6 ± 37.7	<0.001
CRP, median, (Q1, Q3) (mg/dL)	0.07 (0.03, 0.17)	0.05 (0.02, 0.16)	0.001
Phosphorus (mg/dL)	3.6 ± 0.7	3.9 ± 0.6	<0.001
Corrected Ca (mg/dL)	9.0 ± 0.4	9.0 ± 0.4	0.005
Klotho, median (Q1, Q3) (pg/mL)	534 (416, 665)	539 (425, 671)	0.362
25(OH)VitD, median (Q1, Q3) (ng/mL)	17.6 (13.7, 22.3)	15.1 (12.0, 19.8)	<0.001
$1,25(OH)_2$VitD, median (Q1, Q3) (pg/mL)	25.5 (20.3, 33.9)	25.3 (19.7, 33.2)	0.239

C-terminal FGF23, median (Q1, Q3) (RU/mL)	19.6 (1.7, 32.6)	19.5 (1.7, 38.3)	0.364
UACR, >300 mg/g, n (%)	514 (47.8)	370 (53.2)	0.071

SD, standard deviation; BMI, body mass index; DM, diabetes mellitus; HTN, hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; eGFR, estimated glomerular filtration rate using the CKD-EPI creatinine equation; CRP, C-reactive protein; Ca, calcium; 25(OH)VitD, 25-hydroxy vitamin D; $1,25(OH)_2$VitD, 1,25-hydroxy vitamin D; iPTH, intact parathyroid hormone; FGF23, fibroblast growth factor 23; UACR, random urine albumin-creatinine ratio

Figure 6. Multivariable logistic analysis of the association between klotho levels and the presence of LVH stratified by sex



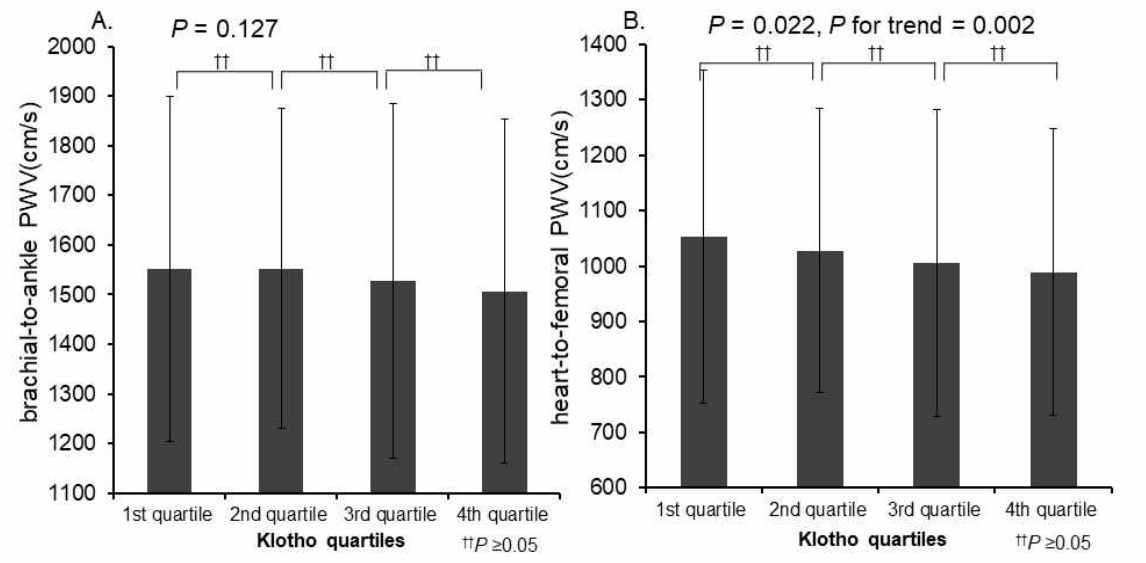
Subgroup analysis stratified by sex and adjusted for age, DM, HTN, BMI, systolic blood pressure, eGFR, hemoglobin, phosphorus, corrected calcium, and FGF23, showed that the 1st and 3rd klotho quartiles were significantly associated with the presence of LVH than the 4th klotho quartile in men. No significant effect was observed in women.

OR, odds ratio; DM, diabetes mellitus; HTN, hypertension; BMI, body mass index; SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate using the CKD-EPI creatinine equation; Ca, calcium; FGF23, fibroblast growth factor 23

Serum klotho and arterial stiffness

baPWV showed a tendency to decrease with klotho quartile (P = 0.127; Figure 7A), but hfPWV decreased significantly (P = 0.022, P for trend = 0.002; Figure 7B). However, after adjustment, no significant association was observed between serum klotho level and baPWV (β 0.003; 95% CI −0.04, 0.05; P = 0.876) or hfPWV (β −0.013; 95% CI −0.06, 0.034; P = 0.564) (Table 5).

Figure 7. Mean pulse wave velocity in klotho quartiles



A. Brachial-to-ankle pulse wave velocity tended to decrease with klotho quartile (P = 0.127). B. Heart-to-femoral PWV significantly decreased with klotho quartile (P = 0.022, P for trend = 0.002).
PWV, pulse wave velocity

Table 5. Multivariable linear regression analysis results for associations between klotho level and pulse wave velocity

Variable		Model A		Model B		Model C
		β (95% CI)	*P*	*β* (95% CI)	*P*	*β* (95% CI)
baPWV	**Klotho (pg/mL)**	-0.011 (-0.07, 0.04)	0.582	-0.017 (-0.07, 0.02)	0.329	0.003 (-0.04, 0.05)
hfPWV	**Klotho (pg/mL)**	-0.029 (-0.09, 0.02)	0.228	-0.037 (-0.09, 0.01)	0.087	-0.013 (-0.06, 0.034)

Model A: Adjusted for age and sex

Model B: Adjusted for age, sex, DM, HTN, BMI, and SBP

Model C: Adjusted for age, sex, DM, HTN, BMI, SBP, eGFR, hemoglobin, phosphorus, corrected Ca, and FGF23

β, Standardized coefficient; CI, confidence interval; baPWV, brachial-to-ankle pulse wave velocity; hfPWV, heart-to-femoral pulse wave velocity; DM, diabetes mellitus; HTN, hypertension; BMI, body mass index; SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate using the CKD-EPI creatinine equation; Ca, calcium; FGF23, fibroblast growth factor 23

Serum klotho levels and arterial calcification and heart dysfunction

AAC score and CACS values were not significantly different between klotho quartiles (Table 1). Systolic or diastolic heart function values as LVEF and E/E' were not significantly different between klotho quartiles (Table 1).

Discussion

The kidney is the principal organ responsible for the production of klotho, and CKD is known to be associated with a klotho-deficiency state. Furthermore, CKD patients suffer from a high burden of CVD. In the present study, serum klotho levels were lower in advanced CKD stages. Klotho exhibited an independent negative association with LVMI. However, serum klotho was significantly associated with the presence of LVH only in men. No significant association was observed between klotho levels and PWV after adjustment. Abdominal aorta calcification and coronary artery calcification were not significantly different between klotho quartile. Systolic or diastolic heart dysfunction were similar in klotho quartiles.

Previous studies have shown that CKD patients are more likely to exhibit cardiac structural changes in the absence of a LV ejection fraction reduction [34, 35]. However, lower prevalences of systolic and diastolic heart dysfunctions found in the present study were not surprising, given that subjects with severe heart failure (New York Heart Association Class III or IV) were excluded.

In a previous experimental study [12] klotho-deficient CKD mice exhibited more cardiac hypertrophy and cardiac fibrosis than wild-type CKD mice. Furthermore, intravenous delivery of a transgene encoding soluble klotho attenuated cardiac hypertrophy in these klotho-deficient CKD mice. The authors

suggested downregulation of the stress-induced transient receptor potential canonical 6 (TRPC6)-mediated gene amplification loop by soluble klotho might play a role in its cardioprotective effects on uremic hearts [36]. Another experimental study reported klotho protected against indoxyl sulphate-induced cardiac hypertrophy in CKD mice [16]. They also showed that serum klotho levels were associated with LVMI in CKD patients but this analysis was not adjusted for potential confounders. The majority of animal studies on the topic have suggested that klotho deficiency is associated with cardiac hypertrophy, but clinical studies have produced mixed results for the relation between serum klotho levels and cardiac hypertrophy. In one study, the lowest klotho tertile was associated with LVH or systolic dysfunction only among patients with stage G3a or G3b CKD, respectively [37]. In another study, serum klotho was not found to be independently associated with CVD, including LVMI, in 127 dialysis patients [17], which is considerably less than the number of patients enrolled in the present study. These authors suggested associations between soluble klotho and cardiovascular parameters may have been diminished because their patients had already developed end stage renal disease [17]. In a study conducted on 444 patients with CKD stage 2-4, soluble klotho was not significantly associated with cardiovascular outcomes [38]. However, cardiovascular parameters were not included in the analysis and

mean eGFR was lower than that observed in the present study (45 ± 16 *vs.* 53.0 ± 30.7 ml/min/1.73m^2). The present study included all stages of CKD patients and showed serum klotho might be a marker of LVMI in predialysis CKD patients. The reasons for discrepancies between previous studies and the present study remain uncertain. However, there are possible explanations. First, subjects enrolled differed in terms of race and kidney function, and different numbers of subjects were recruited. Second, in the present study, cardiovascular parameters were examined rather than cardiovascular outcomes. Third, patients with severe heart failure (New York Heart Association Class III or IV) were excluded from the present study. However, the present study was conducted on a much larger number of CKD subjects, and thus, has greater statistical power. Soluble klotho is known to have anti-aging, anti-oxidant, and anti-vascular calcification effects [39], and CKD (a klotho-deficient state) may be associated with chronic cardiovascular complications. In the present study, a significant association was identified between klotho levels and LVMI in a large number of CKD patients, after adjusting for markers of mineral bone metabolism.

Interestingly, the association between klotho and the presence of LVH was found to be significant in men but not women. Unfortunately, studies have defined LVH in different ways [6, 23, 40] and LVH definition might be race-dependent, which

suggests racial-and sex-specific echocardiographic criteria for LVH may be needed for Asians.

Previous studies have shown comorbidities, such as obesity and metabolic syndrome, affect LVH in women more than in men [41, 42], and it has been suggested insulin resistance might counterbalance the favorable cardiovascular effects of estrogen in women [43], and that biological factors specifically associated with visceral fat might be responsible for observed sex-related differences [41]. In my opinion these comorbidities are likely to have a greater impact on LVH than klotho in women. In the present study, sex did not influence klotho or FGF23. Previous studies have reported sex related difference in FGF23 levels due to hormone changes in women [44-46]. However, data is lacking on klotho levels in men and women and on whether the effects of klotho on cardiovascular events are sex dependent.

In an experimental study, *Klotho* gene delivery into skeletal muscle inhibited medial hypertrophy of the aorta in an animal model of atherosclerotic disease [47]. In another experimental study, klotho deficiency-induced arterial stiffening was mediated by the upregulation of aldosterone [14]. Furthermore, soluble klotho has been shown to protect endothelial integrity by regulating calcium entry into vascular endothelial cells [47, 48]. In a study involving 114 CKD patients, serum klotho level was identified to be a significant determinant of arterial stiffness (defined as a baPWV of ≥1400 cm/sec) [49]. In the present

study, hfPWV was also analyzed as a central arterial stiffness marker. In a clinical study, arterial stiffness as determined by baPWV measurements was observed to be elevated in 109 CKD patients, but not to be related to serum klotho levels [50]. However, this study was performed on small number of diabetic CKD patients. Further studies are needed to elucidate the association between serum klotho levels and arterial stiffness in CKD patients.

The present study has a number of limitations that warrant consideration. First, owing to the cross-sectional nature of the study, it was not possible to determined the cause-effect inferences of the relationship between serum klotho levels and cardiac hypertrophy or arterial stiffness. Second, serum klotho exhibits circadian variation, and thus measurements should have been taken at a fixed time [51]. Third, c-terminal FGF23 levels were measured in the present study. Lack of agreement between c-terminal and intact FGF23 measurements and also differences in their association with other biochemical parameters have been reported [52]. Nevertheless, higher c-terminal and intact FGF23 values have both been associated with increased mortality and poor outcomes in CKD patients.

Conclusion

Serum klotho was found to be an independent biomarker of LVMI but not of arterial stiffness or vascular calcification. Further studies are warranted to elucidate the clinico-pathogenic relevances of relations between serum klotho levels and cardiovascular parameters, and to determine whether interventions intented to maintain or increase serum klotho levels can prevent cardiovascular events and reduce mortality in CKD patients.

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만성콩팥병에서 혈청 Klotho 와 심혈관계 인자와의 연관성

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배경: Klotho는 노화와 연관된 단백질로 미네랄 뼈 대사 장애에 중추적인 역할을 담당하며 만성콩팥병과 심혈관 질환의 연관성에 대해서 설명할 수 있다. 본 연구는 대규모 한국인 만성콩팥병 코호트에서 혈청 klotho와 심혈관 인자와의 연관성에 대해서 살펴보고자 한다.

방법: KNOW-CKD (KoreaN Cohort Study for Outcome in Patients With Chronic Kidney Disease) 코호트 환자 중에서 혈청 klotho 값이 측정된 2,101 명의 환자를 분석하였다. 혈청 klotho는 효소 면역법으로 측정하였다. 심혈관 인자로 좌심실 질량지수(left ventricular mass index)와 동맥 맥파 속도(pulse wave velocity)를 평가하였으며 이는 각각 좌심실 비대와 혈관경직도를 나타낸다. 좌심실 비대 유무는 성별에 따른 좌심실 질량지수 기준으로 정의하였다(남자≥ 50 g/$m^{2.7}$, 여자≥47 g/$m^{2.7}$).

결과: 환자들의 평균 나이는 53.6 ± 12.2세 였고 61.1%가 남자였다. 평균 추정 사구체 여과율은 53.0 ± 30.7 mL/min/1.73m^2 였다. 혈청 klotho의 중앙값은 536 pg/mL(사분위 범위: 420-667 pg/mL) 였다.

진행된 만성콩팥병 단계에서 klotho 값이 유의하게 낮았다(P <0.001, P for trend <0.001). 단변량 분석에서 klotho 값이 높은 사분위 그룹에서 좌심실 질량지수가 유의하게 감소하는 소견을 보였다(P <0.001, P for trend <0.001). 다변량 선형 회귀 분석에서 혈청 klotho는 좌심실 질량지수와 유의한 음의 상관관계를 보였다(β −0.04; 95% CI [confidence interval] −0.004, −0.00007; P = 0.041). 좌심실 비대에 대한 교차비는 klotho 4분위 그룹을 기준으로 비교하였을 때 klotho 1,2,3 분위 그룹과 보정 후 유의한 차이가 없었다. Klotho는 남자에서만 좌심실 비대와 유의한 연관성을 보였다. 혈청 kloth와 상완−발목 동맥 맥파 속도(β 0.003; 95% CI −0.04, 0.05; P = 0.876) 및 심장−대동맥 맥파 속도는(β −0.013; 95% CI −0.06, 0.034; P = 0.564) 보정 후 유의한 연관성이 없었다.

결론: 혈청 klotho는 좌심실 질량지수의 독립적인 바이오마커이나 혈관 경직도와는 유의한 연관성을 보이지 못하였다.

주요어: 혈청 클로토, 가용성 클로토, 만성콩팥병, 좌심실 질량지수, 좌심실비대, 동맥 맥파 속도

학 번: 2014-30609