Original Paper

Blood Purification

Blood Purif
DOI: 10.1159/000381937

Received: April 17, 2014
Accepted: March 27, 2015
Published online: ■■■

Serum Soluble Klotho Level Is Associated with Abdominal Aortic Calcification in Patients on Maintenance Hemodialysis

Hong Cai Renhua Lu Mingfang Zhang Huihua Pang Mingli Zhu
Weiming Zhang Zhaohui Ni Jiaqi Qian Yucheng Yan

Department of Nephrology, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

Key Words
Abdominal aortic calcification · Soluble Klotho · Maintenance hemodialysis · Cardiovascular disease · Vascular calcification

Abstract
Background: Klotho is a single transmembrane protein originally identified as an 'aging suppressor'. Emerging evidence reveals that soluble Klotho (sKl) in the circulation plays important roles in anti-aging, anti-oxidation, anti-apoptosis and Wnt signaling. However, the role of serum sKl in the vascular calcification in hemodialysis patients is not clear. The aim of this study was to determine the associations of sKl with abdominal aortic calcification in patients on maintenance hemodialysis (MHD). ***Methods:*** 129 MHD patients were enrolled prospectively. Serum sKl level was detected by ELISA. Abdominal aortic calcification was measured by abdomen lateral plain radiograph, and the abdominal aorta calcification (AAC) score was calculated. The sKl levels were observed in patients with different degrees of calcification. Logistic regression analysis was used to determine the risk factor of abdominal aortic calcification in MHD patients. The diagnostic value of sKl for abdominal aortic calcification was assessed using receiver operator characteristic (ROC). ***Results:*** Abdominal aortic calcification was seen in 87 of 129 patients. The median AAC score was 4.0 (0.00, 11.00) and the median sKl level was 616.29 (378.19, 821.61) pg/ml. Serum sKl levels were inversely associated with AAC. When evaluated as AAC categories (<5, 5–15, >15) with ordinal logistic regression, each SD higher sKl was associated with 37.1% lower odds of AAC severity (proportional odds ratio: 0.629; 95% confidence interval: 0.413–0.959, p = 0.031) in models adjusted for demographic data, lifestyle factors, traditional CVD risk factors and uremic risk factors. Multivariate logistic regression analysis showed that serum sKl levels and smoking were independent risk factors for severe AAC. The area under the receiver-operating characteristic curve (AUC) of serum sKl for severe abdominal aortic calcification was 0.746 (0.612–0.880, p = 0.001), sensitivity was 0.885, and specificity was 0.562 for a cutoff value of 265.39 pg/ml. ***Conclusions:*** Lower serum sKl levels are independently associated with severe AAC. Serum sKl might have a diagnostic value for the severe AAC in MHD patients.

Introduction

Cardiovascular disease (CVD) is one of the main causes of mortality in end-stage renal disease (ESRD) patients. Several studies suggested that vascular calcifications play a main role in CVD. Studies have shown that apart from traditional Framingham risk factors, some nontraditional

0253–5068/15/0000–0000$39.50/0

E-Mail karger@karger.com
www.karger.com/bpu

Yucheng Yan, MD, PhD
Renal Division, Renji Hospital, Shanghai Jiao Tong University
School of Medicine
Shanghai (China)
E-Mail yucheng.yan@163.com

risk factors, such as the imbalance of calcium and phosphorus metabolism, the micro-inflammatory state, protein-energy malnutrition, anemia, oxidative stress, and endothelial dysfunction were contributing factors of vascular calcification [1]. Epidemiological studies have linked vascular calcifications to bone loss and fractures in chronic kidney disease (CKD) patients and also in the general population, stressing the fact that both can share pathogenic pathways. The mechanisms involved in vascular calcification are complex and not yet fully understood. Current knowledge suggests that a large number (and an imbalance between them) of circulating promoters and inhibitors of the calcification process, that is, fetuin-A, matrix-Gla protein, osteoprotegerin, osteopontin, bone morphogenetic proteins, and inorganic pyrophosphate, are involved in the deterioration of vascular tissue.

The *klotho* gene, originally identified as an 'aging suppressor' gene in mice, encodes for a 130 kDa of Klotho protein and is widely expressed in the kidney, parathyroid, and brain. It is considered part of a new bone-kidney endocrine axis to maintain phosphorus and vitamin D balance [2, 3]. Klotho exists in two forms as membrane-bound klotho and secreted klotho; it is more concentrated in the membrane-bound forms than secreted forms in the study of vascular calcification. A secreted form of Klotho of 70 kD is a product of alternative splicing, which is the extracellular domain of membrane Klotho and can be released into blood, thus functioning as a circulating substance to exert multiple systemic biological actions on distant organs. This cleaved extracellular domain of membrane Klotho is referred to as soluble Klotho (sKl) [4, 16]. Recently, sKl has been found to suppress Na^+-dependent uptake of phosphate and mineralization induced by high phosphate and preserves differentiation in vascular smooth muscle cells (VSMC) in vitro. That suggests a role as a calcification inhibitor. However, the relationship between sKl concentration and vascular calcification in humans is not clear. It is also unknown whether low sKl levels are associated with abdominal aortic calcification in maintenance hemodialysis (MHD) patients. In this study, we aimed at exploring the role of sKl in the pathogenesis of vascular calcification by observing the relationship between sKl level and abdominal aortic calcification in MHD patients.

Materials and Methods

Patients

This investigation was approved by the institutional review board of the Renji Hospital. Patient's inclusion criteria include signing informed consent, older than 18 years, on MHD between August 2010 and December 2011 with the vintage of longer than 3 months. Patients who had connective tissue disease, acute infection, trauma, malignant tumor, severe malnutrition disease, mental illness and those who needed antibiotics, corticosteroids or immunosuppressive agents and surgery within a month were excluded. Of 147 patients who met inclusion criteria, 4 were excluded due to usage of corticosteroids or immunosuppressive agents, 3 due to infection or antibiotic use within a month, 2 due to surgery or trauma within a month, 5 due to comorbidity of malignant tumors, 2 due to severe malnutrition disease and 2 due to mental illness or mobility problems. At last, 129 individuals were enrolled in the final analytic sample.

Abdominal Aorta Plain Roentgenography

A lateral plain radiograph of the abdomen was obtained that included the last two thoracic vertebrae and the first two sacral vertebrae. The aorta was identified as the tubular structure coursing in front of the anterior surface of the spine. A semi-quantitative scoring system was utilized as suggested in the original manuscript by Kauppila et al. [6]. Only the segments of abdominal aorta in front of the first to the fourth lumbar vertebra were considered. Calcific deposits in the abdominal aorta adjacent to each lumbar vertebra were assessed separately for the posterior and anterior wall of the aorta using the midpoint of the intervertebral space above and below the vertebrae as the boundaries. Lesions were graded as follows: 0, no aortic calcific deposits; 1, small scattered calcific deposits filling less than one-third of the corresponding length of the vertebral level; 2, medium quantity of calcific deposits about one-third or more, but less than two-thirds of the corresponding vertebral length; 3, severe quantity of calcifications of more than two-thirds or more of the corresponding vertebral lengths. With this numerical grading, the score could vary from a minimum of 0 to a maximum of 24 points. All X-rays were read and graded by two investigators. The average of the two scores was considered to be the final score. On the basis of the CORD study [7], abdominal aorta calcification (AAC) was divided into none or mild calcification group (AAC score <5), moderate calcification group (5≤ AAC score ≤15) and severe calcification (AAC score ≥16).

Laboratory Tests

Blood samples were collected after capturing an abdominal lateral plain film and at the time of pre-dialysis. Serum and plasma were separated and frozen at –80°C. sKl was measured in plasma using a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) (Immuno-Biological Laboratories, Takasaki, Japan, range from 93.75 to 6,000 pg/ml). Biochemical data were obtained using local routine laboratory methods. Serum markers measured were those addressing mineral metabolism, including total calcium, phosphate, intact parathyroid hormone (PTH), as well as hemoglobin, albumin, fasting glucose, C-reactive protein and lipid profiles. Total serum calcium was adjusted for albumin levels using the conversion factor: corrected calcium = calcium + 0.02 mmol/l × (40 – albumin).

According to the quartile distribution in the patients with sKl level, patients were divided into four groups: I group, sKl level in the lowest quartile; II group, sKl level in the second quartile;

III group, sKl level in the third quartile; IV group, sKl level in the highest quartile. The AAC scores were compared between the four groups.

Clinical Evaluation

The patients' history of ESRD cause, concomitant diseases (diabetes mellitus, hypertension, CVD), smoking, medications, predialysis blood pressure, height, weight and body mass index (BMI) were collected.

All patients had been dialyzed with 500 ml/min of bicarbonate dialysate flow and the F80 (Fresenius, Germany) or REXEED (Asahi Kasei Corporation) polysulfone membrane dialyzers for more than three months. Blood flows were 200–350 ml/min and dialysis time of 10–12 h per weeks. Target ultrafiltration was to achieve clinically estimated dry weight.

Statistical Analysis

Kolmogorov-Smirnov test was used for estimating Gaussian distribution of the data and $p > 0.05$ was considered normal distribution. For normal distribution variables, continuous variables were expressed as means and standard deviations, while for skewed variables, expressed as medians and interquartile range. Categorical variables were expressed as number (or as percentage, %). We categorized patients into quartiles of sKl defined by the distribution within our study population. Differences in demographic data and clinical variables across the sKl quartiles were analyzed with the independent-samples t-test or Mann-Whitney U test for continuous variables and with the Chi-square test or Fisher's exact test for categorical variables. sKl concentration and clinical biochemical indicators, AAC were entered as covariates into the stepwise multiple linear regression model. The ordinal logistic regression model was used to analyze the risk factors in patients with AAC. ROC curves under the curve (AUC) of sensitivity and specificity was used to evaluate sKl diagnosis of abdominal aortic calcification. $p < 0.05$ was considered statistically significant. SPSS 15.0 (SPSS Inc., Chicago, Ill., USA) was used to compute all statistical analyses and figures.

Results

Patients' Characteristics

Among the 129 MHD patients study sample, the mean age was 58.18 ± 13.72 55.8% (n = 72) patients were men and 23.3% had diabetes.

Table 1 shows the basic demographic characteristics, CVD risk factors and biochemical indicators by serum sKl quartiles. The results showed no significant differences in basic demographic data, comorbidities, and laboratory data among groups.

Abdominal Aortic Calcification

On lateral plain radiograph of the abdomen, 87 patients had abdominal aortic calcification, the median AAC score was 4.00 (0.00, 11.00). Sixty-nine (53.5%) patients had a no or mild calcification (0≤ AAC score ≤4); 44 (34.1%) patients had a moderate calcification (5≤ AAC score ≤15); 16 (12.4%) patients had severe calcification (AAC score ≥15). Compared with the highest quartile, individuals with lower sKl levels had high AAC scores (table 1).

The Relationship between Serum Soluble Klotho and Clinical Parameters

Pearson's correlation analysis showed that the serum sKl level was correlated with dialysis vintage ($r = -0.204$, $p < 0.05$), fasting glucose ($r = 0.282$, $p < 0.05$) and $\log_{10}$ PTH ($r = -0.205$, $p < 0.05$). Age was possibly correlated with sKl ($r = 0.173$, $p = 0.05$), while no relationship was found with BMI, Kt/V, corrected calcium, phosphate, albumin, triglyceride, total cholesterol and hsCRP.

The Relationship between Serum Soluble Klotho and AAC

Among the 129 patients, the median sKl concentration was 612.56 (379.17, 816.63) pg/ml. Compared with the mild or moderate calcification patients, the severe calcification one had lower sKl concentration (677.27 (445.87, 858.94) vs. 259.16 (190.33, 539.76) and 570.25 (303.73, 889.43) vs. 259.16 (190.33, 539.76), both $p < 0.05$).

Pearson's correlation analysis showed that patients' serum sKl levels were inversely correlated with AAC score ($r = -0.214$, $p = 0.015$). Quartile analysis demonstrated a dose response relationship between sKl and AAC categories (Chi-square, $p = 0.011$) (fig. 1). Among patients with no or mild calcification, 14.5% were in the lowest sKl quartile, whereas 27.5% were in the highest quartile. Conversely, among those severe classification patients identified by AAC score >15, 56.3% were in the lowest sKl quartile, whereas only 6.3% were in the highest.

Table 2 shows the association of sKl, both by quartiles and as a continuous predictor variable, with each marker of AAC. Klotho was inversely associated with AAC score. This finding remained consistent in models that adjusted for age and sex, lifestyle factors, biochemical indicators. Each SD higher sKl level was associated with 36.2% lower odds of high AAC in model adjusted for lifestyle factors, and this association was still rendered statistically significant when adjusted for lifestyle factors and biochemical indicators (OR = 0.629, $p = 0.031$).

Analysis of Risk Factors Affected with Severe Calcification of Abdominal Aorta in MHD Patients

Multivariate logistic regression analysis showed that the serum sKl concentration decreased (OR = 3.559, 95% CI 1.453–8.717) is an independent risk factor for severe calcification of the abdominal aorta, although adjusted by age, gender, smoking (table 3).

Table 1. Baseline characteristics of community-living individuals and laboratory data by quartiles of serum Klotho

	All (n = 129)	Soluble Klotho quartiles, soluble Klotho range, pg/ml			
		I ≤379 (n = 32)	II 379–613 (n = 33)	III 613–817 (n = 32)	IV >817 (n = 32)
Age, years	58.18±13.72	57.31±13.66	56.15±15.21	58.75±12.99	60.56±13.10
Male, n (%)	72 (55.8)	19 (59.4)	18 (54.5)	18 (56.3)	17 (53.1)
Smoking, n (%)	86 (66.7)	21 (65.6)	23 (69.7)	22 (68.8)	20 (62.5)
Diabetes, n (%)	30 (23.3)	9 (28.1)	7 (21.2)	4 (12.5)	10 (31.3)
Hypertension, n (%)	105 (81.4)	24 (75)	28 (84.8)	27 (84.4)	26 (81.3)
CVD, n (%)	62 (48.1)	15 (46.9)	17 (51.5)	15 (46.9)	15 (46.9)
Dialysis duration, months	70.50 (28.00, 120.75)	118.00 (23.00, 141.00)	56.50 (19.25, 96.25)*	44.50 (29.25, 121.50)	57.00 (28.00, 96.00)*
BMI, kg/m^2	20.79 (18.70, 23.18)	20.79 (19.69, 23.26)	20.62 (18.33, 23.51)	20.75 (18.16, 22.67)	20.87 (18.68, 23.28)
SBP, mm Hg	139.47±20.73	139.28±19.94	138.61±21.10	141.06±18.64	138.94±23.78
DBP, mm Hg	74.88±13.09	75.00±15.38	75.85±12.01	75.53±14.28	73.13±10.63
Kt/V	1.73±0.35	1.69±0.33	1.78±0.36	1.74±0.31	1.70±0.41
FBG, mmol/l	5.00 (4.50, 5.82)	4.8 (4.25, 5.20)	4.96 (4.46, 6.10)	5.01 (4.60, 5.72)	5.15 (4.56, 6.43)
Hb, g/l	110.37±16.95	113.53±16.01	104.97±17.29	110.25±14.05	112.91±19.38
Ca, mmol/l	2.38±0.28	2.47±0.21	2.30±0.30	2.40±0.33	2.36±0.26
P, mmol/l	2.02±0.58	2.05±0.53	1.92±0.64	2.00±0.55	2.12±0.61
PTH, pg/ml	348 (150.75, 619.50)	394.00 (178.00, 893.00)	255.00 (91.48, 540.25)	451.00 (200.50, 612.50)	313.00 (118.00, 622.00)
TC, mmol/l	4.37±1.18	4.11±1.09	4.42±1.08	4.72±1.46	4.23±1.00
TG, mmol/l	1.85±1.32	1.84±1.59	1.57±1.00	1.75±1.00	2.23±1.56
LDL-C, mmol/l	2.36±0.93	2.04±0.93	2.43±0.82	2.61±1.03	2.36±0.86
HDL-C, mmol/l	1.06±0.45	0.99±0.58	1.03±0.29	1.18±0.43	1.03±0.43
Alb, g/l	39.23±4.76	39.39±3.60	39.25±6.37	38.98±5.13	39.30±3.52
HsCRP, mg/l	1.67 (1.48, 1.94)	1.97 (0.84, 6.07)	2.65 (0.98, 5.42)	1.22 (0.45, 1.83)	1.97 (0.88, 3.64)
AAC score	4.00 (0, 11.00)	11.00 (1.00, 16.00)	3.00 (0, 10.00)*	0 (0, 7.00)*	2.5 (0, 8.75)*
Drug class, %					
Calcitriol	43.4	56.2	37.5	42.2	37.5
ACEI/ARB	62.8	62.5	63.6	65.6	59.3
Phosphorus binders (calcium contained)	92.2	96.9	90.6	90.9	90.6

* Compared with group I $p < 0.01$. CVD = Cardiovascular disease; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; Ca = calcium; P = phosphate; PTH = parathyroid hormone; TC = total cholesterol; TG = triglyceride; LDL-C = low density lipoprotein cholesterol; HDL-C = high density lipoprotein cholesterol; Alb = albumin; hsCRP = high sensitivity C-reactive protein.

ROC Analysis

The areas under the curve (AUC) for sKl to diagnose severe vascular calcification was 0.746 (95% CI 0.612–0.880, p = 0.001), which showed that sKl concentration had a high accuracy for diagnosing abdominal aortic. A cutoff value of 265.39 pg/ml yielded to good sensitivity and specificity of abdominal severe calcification diagnosed by sKl concentration. The sensitivity and specificity were 88.5 and 56.2%, respectively (fig. 2).

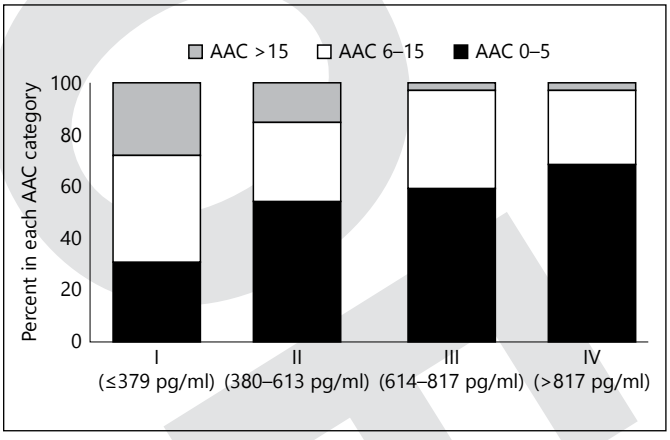

Fig. 1. Abdominal aorta calcification severity by serum Klotho quartiles: sKl was inversely correlated with the AAC score. Among patients with highest serum Klotho level, the incidence of severe calcification was the highest.

Discussion

Mineral bone disease (MBD), which includes arterial calcification, is a common complication in CKD, especially ESRD patients. Vascular calcification presents a closely relation to poor outcome, includes cardiovascular

Table 2. Association of Klotho with moderate-severe AAC

	Klotho quartiles, Klotho range, pg/ml				sKl continuous per SD (485.72 pg/ml) greater
	I <379 (n = 32)	II 379–613 (n = 33)	III 613–817 (n = 32)	IV >817 (n = 32)	
n with AAC score ≥5/total, %	22/32	15/33	10/32	13/32	60/129
Age and sex adjusted	1	0.328 (0.109–0.987) p = 0.047	0.445 (0.260–0.762) p = 0.003	0.623 (0.428–0.907) p = 0.013	0.656 (0.434–0.993) p = 0.046
Lifestyle adjusted*	1	0.372 (0.012–10.428) p = 0.112	0.440 (0.244–0.793) p = 0.006	0.626 (0.413–0.950) p = 0.028	0.638 (0.414–0.983) p = 0.042
Fully adjusted**	1	0.677 (0.150–3.059) p = 0.612	0.436 (0.204–0.931) p = 0.032	0.559 (0.331–0.945) p = 0.030	0.629 (0.413–0.959) p = 0.031

* Adjusted for age, sex, current smoking, medical use. ** Adjusted for lifestyle model (*) plus dialysis duration, total cholesterol, triglycerides, albumin, hsCRP, calcium, phosphorus, parathyroid hormone.

death in hemodialysis patients. To our knowledge, this may be the first study in observing the relationship between sKl and vascular calcification in MHD patients. The results showed that the sKl concentration had a negative correlation with abdominal aortic calcification. The decrease of sKl concentration can increase the risk of vascular calcification in MHD patients.

Klotho, was originally identified as an aging suppressor and encoding 130 kDa of Klotho protein, is widely expressed in the kidney, parathyroid and brain. It is thought to be part of a newly described bone-kidney endocrine axis to maintain phosphorus and vitamin D balance [2, 3], anti-aging and prolong life cycle [8, 9]. Klotho has a relationship with vascular calcification in CKD and directly inhibits vascular calcification and smooth muscle cell differentiation [5]. The knockout Klotho gene animal and CKD patients show a wide range of vascular calcification, and the expression of Klotho by genetic engineering transfer Klotho gene into animal model can effectively inhibit vascular calcification [5, 10]. This study presents evidence that not only membrane-bound Klotho, but also sKl might have an effect on aortic calcification pathogenesis in MHD patients. Serum sKl might impact? The risk of CVD in MHD patients.

Abdominal aortic calcification score increases in patients are closely related to CVD events [11, 12]. Previous studies have shown that the incidence of coronary artery calcification was up to 94.6% in patients with moderate-to-severe calcification in abdominal aorta, and furthermore, 78.4% of them were severe coronary artery calcification. The incidence of cardiovascular events was significantly higher in patients with severe than mild calcification of the abdominal aorta [13]. In this study,

Table 3. Multivariate logistic regression analysis of abdominal aorta severe calcification in MHD patients

Associated factors	p	OR	95% CI
Klotho	0.005	3.559	1.453–8.717
Smoking	0.006	5.065	1.583–16.206
Age (20)	0.432	1.337	0.648–2.757
Sex (woman)	0.456	0.549	0.113–2.661

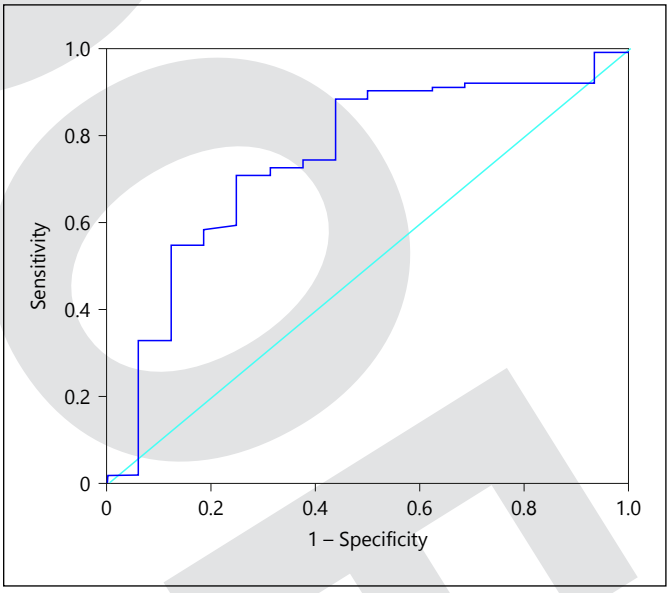

Fig. 2. The role of serum Klotho in predicting the presence of severe vascular calcification. The receiver operating characteristic curve illustrates serum Klotho. Areas under the curves are 0.746 for serum Klotho.

patients with sKl in the lower quartile had significantly higher risk of moderate-to-severe abdominal aortic calcification than those in higher quartile one, even adjusted for age and sex, lifestyle factors, biochemical indicators. These results suggested that the low level of sKl was an independent risk factor for abdominal aortic calcification, even without or with very few traditional risk factors leading to vascular calcification in MHD patients.

Logistic regression analysis also showed that sKl is an independent risk factor for the MHD patients with severe calcification of the abdominal aorta. sKl indeed played an important role in the incidence and development of abdominal aortic calcification. The mechanism for the decrease in Klotho concentration and vascular calcification is not clear, and phosphorus excretion disorders may be the possible mechanism. It has been confirmed that decreasing Klotho concentration reduces phosphorus excretion and leads to elevated serum phosphate levels [14]. To date, the mechanism by which Klotho protects against vascular calcification have focused on its role as an obligate co-factor for fibroblast growth factor 23 (FGF23) signaling in regulating phosphate and vitamin D metabolism. However, emerging evidence suggests that Klotho may also exert direct effects on VSMC and act as a novel circulating inhibitor of vascular calcification. Animals lacking Klotho showed upregulated expression of the phosphate transporters Pit1/2 and the key osteogenic transcription factor Runx2. This suggested that in the absence of Klotho, upregulation of Pit1/2 increases Pi transport into VSMCs and this drives osteogenic conversion, a key event in the calcification process [15]. In our study, there were no significant differences of serum phosphate levels in AAC groups, and the level of sKl is higher in patients with little calcification. It may indicate sKl directly inhibits the Pit1/2 activity and prevents VSMC differentiation [5].

Klotho may hold potential in terms of its role as a biomarker. Fall in plasma or urinary Klotho can be one of the earliest abnormalities in CKD from a variety of causes. The most promising data thus far is that urinary Klotho is reduced in very early stage CKD (stages 1 and 2) and is progressively lowered with declining eGFR [5]. In this study, a low level of sKl was shown to be a vital sign of severe vascular calcification. Although FGF23 level was not measured, we postulated the sKl level decreasing may be also related to FGF receptor down regulation in ESRD patients. FGF23 was considered to be closely related to the membrane type Klotho, which was a common receptor of FGF23 and regulated of phosphorus and vitamin D. Klotho and FGFR1/3 expression are downregulated when human aorta derived-SMCs are incubated with high phosphate and calcium in CKD. De-differentiation of SMC and loss of the ability to respond to FGF23 may result from downregulation of Klotho-FGFR1/3 expression in the arteries [16]. Further, the impact of FGF23 on vascular calcification and endothelial response was evaluated in bovine vascular smooth muscle cells (bVSMC) and in a murine ex vivo model of endothelial function, respectively. FGF23 did not modify calcification in bVSMCs or dilatory, contractile and structural properties in mice arterial specimen ex vivo [17]. As a portion of sKl was sheared from the extracellular domain of membrane Klotho, sKl level may in some degree reflect the FGF23 level.

Several limitations should be acknowledged. First, this is a cross-sectional study. Although observing that sKl had a strong negative correlation with vascular calcification, whether sKl has a relationship with CVD and mortality in hemodialysis patients needs to be further studied. Second, as we didn't measure the FGF23 level, the pathway through which sKl contributed to vascular calcification can't be elaborated. Further in vivo and in vitro studies are needed. At last, we used a plain film to assess the AAC score in this study because of its convenience and because it was suggested by KDIGO guideline. However, computer tomography scanning might be more accurate to detect the vascular calcification, and make the relationship between vascular calcification and sKl clearer than before. Further studies are needed to validate the postulation.

In summary, the level of sKl in MHD patients with abdominal aortic calcification is an independent risk factor in patients with severe abdominal aortic calcification. Low sKl concentrations may contribute to severe calcification of abdominal aorta in MHD patients. Further study of sKl in vascular calcification and CVD are needed, which is expected to regulate the expression, to reduce or delay the complications of hemodialysis patients, to improve the patient's survival and quality of life.

Acknowledgment

This study was supported by the National Nature Science Foundation Grant of China (81170687) and Grand Science and Technology Project from Shanghai Science and Technology Committee (08dz1900501); the National Key Technology R&D Program (2011BAI10B08).

Disclosure Statement

None of the authors had any conflict of interest to declare.

References

1 Razzaque MS: The FGF23 – Klotho axis: endocrine regulation of phosphate homeostasis. Nat Rev Endocrinol 2009;5:611–619.
2 Hu MC, Zhang J, Shi M, et al: Klotho is a phosphaturic hormone: in vivo evidence. J Am Soc Nephrol 2006;17:105A.
3 Razzaque MS, Lanske B: The emerging role of the fibroblast growth factor-23 – klotho axis in renal regulation of phosphate homeostasis. J Endocrinol 2007;194:1–10.
4 Hu MC, Kuro-o M, Moe OW: Secreted Klotho and chronic kidney disease. Adv Exp Med Biol 2012;728:126–157.
5 Hu MC, Shi M, Zhang J, et al: Klotho deficiency causes vascular calcification in chronic kidney disease. J Am Soc Nephrol 2011;22: 124–136.
6 Kauppila LI, Polak JF, Cupples LA, et al: New indices to classify location, severity and progression of calcific lesions in the abdominal aorta: a 25-year follow-up study. Atherosclerosis 1997;132:245–250.
7 Verbeke F, Van Biesen W, Honkanen E, et al: Prognostic value of aortic stiffness and calcification for cardiovascular events and mortality in dialysis patients: outcome of the calcification outcome in renal disease (CORD) study. Clin J Am Soc Nephrol 2011;6:153–159.
8 Kurosu H, Yamamoto M, Clark JD, et al: Suppression of aging in mice by the hormone Klotho. Science 2005;309:1829–1833.
9 Kuro-o M, Matsumura Y, Aizawa H, et al: Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature 1997; 390:45–51.
10 Nagai R, Saito Y, Ohyama Y, et al: Endothelial dysfunction in the Klotho mouse and downregulation of Klotho gene expression in various animal models of vascular and metabolic diseases. Cell Mol Life Sci 2000;57:738–746.
11 Wilson PW, Kauppila LI, O'Donnell CJ, et al: abdominal aortic calcific deposits are an important predictor of vascular morbidity and mortality. Circulation 2001;103:1529–1534.
12 Bolland MJ, Wang TK, van Pelt NC, et al: Abdominal aortic calcification on vertebral morphometry images predicts incident myocardial infarction. J Bone Miner Res 2010;25:505–512.
13 Virmani R, Burke AP, Farb A: Plaque morphology in sudden coronary death. Cardiologia 1998;43:267–271.
14 John GB, Cheng CY, Kuro-o M: Role of Klotho in aging, phosphate metabolism, and CKD. Am J Kidney Dis 2011;58:127–134.
15 Abedin M, Tintut Y, Demer LL: Vascular calcification: mechanisms and clinical ramifications. Arterioscler Thromb Vasc Biol 2004;24: 1161–1170.
16 Lim K, Lu TS, Zehnder D, et al: Development of Klotho-FGFR1/3 dependent resistance to FGF-23 in human aortic smooth muscle cells exposed to calcifying stress. J Am Soc Nephrol 2010;21:140A.
17 Lindberg K, Olauson H, Amin R, et al: Arterial klotho expression and FGF23 effects on vascular calcification and function. PLoS One 2013;8:e60658.