Original Paper

Circulating α -Klotho Levels in Hemodialysis Patients and Their Relationship to Atherosclerosis

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Key Words

Hemodialysis • Soluble α -Klotho • Carotid intima-middle thickness • Atherosclerosis • Risk factor

Abstract

Background/Aims: Serum soluble Klotho (sKlotho) plays a role in cardiovascular disease in some populations. However, information regarding the effect of serum sKlotho on atherosclerosis in patients on maintenance hemodialysis (MHD) is limited. Therefore, we tested the level of serum sKlotho in MHD patients to investigate atherosclerosis disease and determine the relationship between sKlotho and atherosclerosis. **Methods:** Using cross-sectional research, anthropometric and laboratory data were collected for 330 MHD patients. The levels of serum sKlotho before dialysis were tested by enzyme-linked immunosorbent assay. Carotid intima-middle thickness (cIMT) and the number of carotid artery atherosclerotic plaques were measured by color Doppler ultrasonography. The risk factors of atherosclerosis were explored by multivariate logistic regression analysis. **Results:** Among 330 MHD patients, the average serum sKlotho was (379.93 ± 143.66) pg/ml. The level of serum sKlotho was positively related to hemoglobin ($P < 0.05$). It was negatively correlated with systolic pressure, pulse pressure, ultrafiltration volume, serum phosphorus, corrected serum calcium $\times$ phosphorus, high-sensitivity C-reactive protein (Hs-CRP), cIMT and carotid atherosclerotic plaque quantity and atherosclerosis ($P < 0.05$). Multivariate logistic regression analysis showed that age ($OR = 1.108$, 95% $CI = 1.067 \sim 1.151$, $P = 0.000$), dry weight ($OR = 1.050$, 95% $CI = 1.014 \sim 1.088$, $P = 0.007$), Hs-CRP ($OR = 1.126$, 95% $CI = 1.005 \sim 1.261$, $P = 0.041$), and serum sKlotho ($OR = 0.997$, 95% $CI = 0.995 \sim 1.000$, $P = 0.032$) were risk factors for atherosclerosis in MHD patients. **Conclusion:** Serum sKlotho was related to systolic pressure, pulse pressure, ultrafiltration volume, hemoglobin, serum phosphorus, corrected serum calcium $\times$ phosphorus, Hs-CRP,

increased cIMT, carotid atherosclerotic plaque quantity and atherosclerosis. Age, dry weight, Hs-CRP, and serum sKlotho were risk factors for atherosclerosis in MHD patients. Serum sKlotho may be a novel risk factor for atherosclerosis in MHD patients.

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Introduction

Klotho is an anti-aging protein predominantly expressed in the kidney, the parathyroid glands, and the choroid plexus of the brain [1]. It has two known human isoforms, including a single-pass transmembrane protein with a large extracellular domain (130 kDa, 1012 AA) and a secreted form of Klotho (62 kDa, 549 AA). The extracellular domain of Klotho is cleaved and released into extracellular fluid, including blood, urine, cerebrospinal fluid, and a secreted form of Klotho as a circulating substance to exert multiple systemic biological actions on distant organs. This cleaved extracellular domain of membrane Klotho and the secreted form of Klotho is referred to as soluble Klotho (sKlotho) in this chapter.

Cardiovascular disease (CVD) is the most important complication and the major cause of death in patients on maintenance hemodialysis (MHD), and the one of main mechanisms of CVD is atherosclerosis. Carotid atherosclerosis can be evaluated by ultrasound, which can represent the degree of systemic atherosclerosis. Carotid intima-media thickness (cIMT) is an independent predictor of carotid atherosclerosis [2], future cardiovascular risk [3], and long-term outcomes [4]. Recent research found that soluble α -Klotho (sKlotho) linearly decreased in chronic kidney disease stages 1 to 5 [5]. Semba RD et al. [6] reported that the higher plasma Klotho concentrations are independently associated with a lower likelihood of having CVD in community-dwelling adults. It is unclear whether or not serum sKlotho is involved in the occurrence and development of atherosclerosis in MHD patients. In this study, we aimed at testing the level of serum sKlotho in MHD patients, investigating atherosclerosis disease and determining the relationship between them.

Materials and Methods

Patients

An observational cross-sectional study in patients with MHD was conducted at the Department of Blood Purification of Beijing Chao-yang Hospital, Capital Medical University, and at the Department of Nephrology of Beijing Friendship Hospital, Capital Medical University, from March 2014 to October 2014. The criteria for enrollment were duration of dialysis >3 months, older than 18 years, in stable condition and voluntary participation in this study. Exclusion criteria included serious CVD (cardiac function level at III or IV by New York Heart Association standards), infectious diseases in the past two months, severe malnutrition, active liver diseases or advanced cancer.

All enrolled patients underwent hemodialysis 3 times per week, 4 hours per dialysis session. All patients used standard carbonate dialysis fluid, dialysate flow was 500 ml/min, and blood flow ranged from 200 to 350 ml/min. Dialysis liquid ingredients were as follows: sodium 138-140 mmol/L, potassium 2.5-3.0 mmol/L, calcium 1.25 or 1.75 mmol/L, and magnesium 0.5 mmol/L.

Methods

The recorded demographic data of the patients included age, sex, dialysis vintage, weight, primary kidney disease and complications, including diabetes, hypertension etc. Ultrafiltration was recorded the day blood samples were obtained. Pre-dialysis pressure was collected every two weeks, and the average was used to calculate the pulse pressure (pulse pressure = systolic pressure–diastolic pressure).

Laboratory data

All laboratory values, including hemoglobin, serum creatine (Cr), uric acid (UA), serum calcium, serum phosphorus (P), albumin (Alb), alkaline phosphatase (ALP), serum cholesterol (TC), triglyceride (TG), high

density lipoprotein (HDL), low density lipoprotein (LDL), fasting blood glucose (FBG), high-sensitivity C-reactive protein (Hs-CRP) and intact parathyroid hormone (iPTH) were measured by automated and standardized methods before hemodialysis. Blood samples were collected after an overnight fast. At the same time, supernatants were stored in aliquots at -80°C to measure serum sKlotho. When the serum albumin level was <4.0 g/dl, the serum calcium level was adjusted as follows: corrected serum calcium (cCa) level (mmol/L) = measured serum calcium level (mmol/L) + $[40 - \text{serum albumin level (g/L)}] \times 0.02$ mmol/L.

Soluble α -Klotho

Serum soluble klotho was measured by enzyme-linked immunosorbent assay (ELISA) kit (Immuno-Biological Laboratories Co., Ltd, Tokyo, Japan), as previously reported. Means $\pm$ standard deviation (SD) were $6.5 \pm 3.3\%$ for inter-assay and $3.6 \pm 1.5\%$ for intra-assay coefficients of variability.

Carotid ultrasonography

Carotid Doppler ultrasonography was performed by a unified standard. The operators were blinded about the history and laboratory findings of the patients. Choosing the side of non-intubation or non-arterial venous fistula, the transverse axis and longitudinal axis of the common carotid artery and the carotid bifurcation were gradually viewed from the neck of the patients in the supine position. The cIMT was defined as the distance between the leading edge of the first bright line and the leading edge of the second bright line. When an atherosclerotic plaque was present at the measurement site, it was included in the measurement of the cIMT. Plaques (defined as a protrusion into the lumen adding 50% to the thickness of the surrounding intima-media or maximal thickness of 1.5 mm in the carotid bifurcation or along the carotid arterial tree) were documented. According to the number of atherosclerotic plaques, patients were divided into four groups. They were assigned to the non-plaque group, small-plaque group (number of plaques = 1), multiple plaques group ($1 < \text{number} < 3$), and diffuse plaques group, respectively. According to the presence of cIMT thickening or atheromatous plaque, the patients were divided into an atherosclerosis group (thickening of the cIMT or having atherosclerotic plaque, AS group) and a non-atherosclerosis group (normal cIMT or no atherosclerotic plaque, non-AS group).

Statistical analyses

The SPSS 17.0 statistics package for Windows was used. Continuous variables normally distributed were expressed as the mean $\pm$ standard deviation (SD), and we used Student's *t* tests for comparing the data of two groups or one-way ANOVA for comparison among more than two groups. Non-normally distributed variables were shown as median (P_{25} to P_{75}), and comparisons between the two groups were analyzed by Mann-Whitney *U* tests. Categorical variables were expressed as number or percentage for each item and used chi-square analysis. Spearman analysis was used for univariate correlations between sKlotho and other variables. Multivariate logistic regression analysis was used for related factors of atherosclerosis.

Results

Patient Characteristics

A total of 330 patients were screened for this study, including 179 males and 151 females. The mean age of enrolled dialysis patients was 63.43 ± 12.76 years. The median of dialysis duration was 69 (34, 117) months, and the range was from 5 to 373 months. The primary diseases of these maintenance hemodialysis patients were as follows: chronic glomerulonephritis, 136 (41.2%); diabetic renal disease, 54 (16.4%); hypertensive-related nephropathy, 42 (12.7%); chronic interstitial nephritis, 30 (9.1%); polycystic kidney, 24 (7.3%); and other, 44 (13.3%).

Serum sKlotho and Associated Variables

Mean sKlotho was (379.93 ± 143.66) pg/ml. It was positively related to hemoglobin and negatively related to systolic pressure, pulse pressure, ultrafiltration volume, serum

Table 1. Correlated analysis of sKlotho with other parameters in MHD patients. Abbreviations: DM, diabetes; Hb, hemoglobin; ALB, albumin; TC, serum cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; TG, triglyceride; Cr, serum creatine; UA, uric acid; FBG, fasting blood glucose; cCa, corrected serum calcium; P, serum phosphorus; cCa $\times$ P, calcium-phosphorus product; iPTH, intact parathyroid hormone; Hs-CRP, high-sensitivity C-reactive protein; SBP, systolic pressure; DBP, diastolic pressure; PP, pulse pressure; AS, atherosclerosis. ^acategorical variables; *P<0.05

Variables	r	P
Gender ^a	0.093	0.093
Age(years)	-0.062	0.327
Dialysis vintage(months)	0.100	0.071
primary diseases ^a	-0.029	0.594
DM ^a	-0.037	0.509
Weight(kg)	-0.042	0.461
Ultrafiltration volume(kg)	-0.114	0.048*
Hb(g/L)	0.126	0.023*
ALB(g/L)	0.091	0.102
TC(mmol/L)	-0.040	0.493
LDL(mmol/L)	-0.057	0.330
HDL(mmol/L)	0.065	0.265
TG(mmol/L)	-0.044	0.450
Cr(umol/L)	0.032	0.567
UA(mmol/L)	0.018	0.758
FBG(mmol/L)	0.000	0.998
cCa(mmol/L)	-0.088	0.117
P(mmol/L)	-0.144	0.010*
cCa $\times$ P(mg ² /dl ²)	-0.163	0.003*
iPTH (ng/dl)	-0.030	0.603
Hs-CRP(mg/L)	-0.350	0.000*
SBP(mmHg)	-0.187	0.001*
DBP(mmHg)	-0.044	0.449
PP(mmHg)	-0.182	0.001*
cIMT thickening ^a	-0.183	0.001*
The number of atherosclerotic plaque ^a	-0.146	0.011*
AS ^a	-0.153	0.005*

phosphorus, cCa $\times$ P, Hs-CRP, cIMT thickening, the number of atherosclerotic plaques, and atherosclerosis (Table 1, P<0.05). There were no significant relationships between sKlotho and sex, age, dialysis vintage, primary kidney disease, diabetes as a complication, weight, ALB, TC, LDL, HDL, TG, Cr, UA, FBG, cCa, iPTH, and diastolic pressure (Table 1, P>0.05).

Atherosclerosis by carotid ultrasonography

Among the 330 MHD patients, the presence of a thickened cIMT was 46.7% (154 cases) and a normal cIMT was 53.3% (176 cases). The non-plaque group had 94 cases (28.4%), the small-plaque group had 61 cases (18.4%), the multiple plaques group had 64 cases (19.4%), and the diffuse plaques group had 111 cases (33.8%) by carotid ultrasound. The presence of atherosclerosis was 57.3% (189 cases). Fig. 1 presents the box plots of sKlotho by the different groups according to carotid ultrasonography.

Fig. 1. The box plots of serum sKlotho by the different groups according to carotid ultrasonography. (a) Serum sKlotho was significantly higher in those with non-thickened cIMT than in those thickened cIMT (P<0.05). (b) Serum sKlotho was different by the group of the number of atherosclerotic plaques (P=0.05). (c) Serum sKlotho was significantly higher in non-atherosclerosis group than atherosclerosis group (P<0.05).

Table 2. Comparison of clinical characteristics between patients with and without atherosclerosis. Abbreviations: HD, hemodialysis; DM, diabetes; SBP, systolic pressure; DBP, diastolic pressure; PP, pulse pressure; UV, Ultrafiltration volume; ALB, albumin; Hb, hemoglobin; TC, serum cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; TG, triglyceride; Cr, serum creatine; UA, uric acid; FBG, fasting blood glucose; cCa, corrected serum calcium; P, serum phosphorus; cCa $\times$ P, calcium-phosphorus product; ALP, alkaline phosphatase; iPTH, intact parathyroid hormone; Hs-CRP, high-sensitivity C-reactive protein; ^acategorical variables, *P<0.05

Variables	All (n=330)	non-AS group (n=141)	AS group (n=189)	t/ χ^2 /z	P
Male [n (%)]	179 (54.2%)	66 (46.8%)	113 (59.8%)	5.482	0.025*
Age ($\bar{x} \pm s$, ys)	56.43 $\pm$ 12.76	49.02 $\pm$ 12.32	62.05 $\pm$ 9.92	-10.588	0.000*
HD vintage [M(P ₂₅ ,P ₇₅),M]	69(34, 117)	69(34, 111)	66(33, 121)	-0.443	0.657
DM [n (%)]	73(22.1%)	24(17.0%)	49(25.9%)	3.717	0.036*
primary diseases ^a	-	-	-	17.946	0.021*
SBP($\bar{x} \pm s$, mmHg)	141.22 $\pm$ 19.02	138.55 $\pm$ 17.99	143.06 $\pm$ 19.53	-2.043	0.042*
DBP($\bar{x} \pm s$, mmHg)	78.84 $\pm$ 12.42	81.63 $\pm$ 11.13	76.92 $\pm$ 12.91	3.303	0.001*
PP($\bar{x} \pm s$, mmHg)	62.32 $\pm$ 16.10	56.66 $\pm$ 14.29	66.54 $\pm$ 16.11	-5.764	0.000*
Weight	61.65	59.50	64.00	-3.151	0.020*
[M(P ₂₅ ,P ₇₅), kg]	(53.50, 70.00)	(51.50, 66.50)	(55.75, 71.80)		
UV ($\bar{x} \pm s$, kg)	3.54 $\pm$ 0.91	3.56 $\pm$ 0.94	3.51 $\pm$ 0.89	0.396	0.692
ALB($\bar{x} \pm s$, g/L)	37.95 $\pm$ 4.31	38.05 $\pm$ 4.45	37.88 $\pm$ 4.21	0.353	0.724
Hb($\bar{x} \pm s$, g/L)	115.59 $\pm$ 14.68	114.23 $\pm$ 15.10	116.60 $\pm$ 14.32	-1.439	0.151
TC($\bar{x} \pm s$, mmol/L)	4.07 $\pm$ 0.92	3.96 $\pm$ 0.86	4.14 $\pm$ 0.96	-1.608	0.109
LDL($\bar{x} \pm s$, mmol/L)	2.24 $\pm$ 0.72	2.08 $\pm$ 0.66	2.35 $\pm$ 0.74	-3.169	0.002*
HDL($\bar{x} \pm s$, mmol/L)	1.08 $\pm$ 0.32	1.11 $\pm$ 0.34	1.06 $\pm$ 0.30	1.297	0.196
TG($\bar{x} \pm s$, mmol/L)	1.73 $\pm$ 1.23	1.64 $\pm$ 1.18	1.79 $\pm$ 1.26	-1.060	0.290
Cr($\bar{x} \pm s$, μ mol/L)	870.94 $\pm$ 203.81	927.91 $\pm$ 219.41	828.83 $\pm$ 180.83	4.422	0.000*
UA($\bar{x} \pm s$, μ mol/L)	397.27 $\pm$ 80.62	412.99 $\pm$ 86.29	386.99 $\pm$ 75.17	2.741	0.006*
FBG	5.22	5.05	5.35	-2.216	0.027*
[M(P ₂₅ ,P ₇₅),mmol/L]	(4.39, 6.84)	(4.22, 6.64)	(4.52, 6.99)		
cCa($\bar{x} \pm s$, mmol/L)	2.43 $\pm$ 0.35	2.32 $\pm$ 0.31	2.52 $\pm$ 0.34	-2.208	0.028*
P($\bar{x} \pm s$, mmol/L)	1.78 $\pm$ 0.52	1.79 $\pm$ 0.55	1.78 $\pm$ 0.49	0.230	0.818
cCa $\times$ P($\bar{x} \pm s$,mg ² /dl ²)	54.97 $\pm$ 18.64	54.25 $\pm$ 19.47	55.49 $\pm$ 18.03	-0.587	0.558
ALP	85.0	89.5	81.5	-1.763	0.078
[M(P ₂₅ ,P ₇₅),U/L]	(69.0, 114.0)	(71.0, 122.5)	(68.0, 108.3)		
iPTH					
[M(P ₂₅ ,P ₇₅),ng/dl]	223.90 (101.75, 368.85)	232.90 (120.00, 413.95)	204.25 (92.15, 341.28)	-1.295	0.195
Hs-CRP	2.32	2.09	4.65	-5.045	0.000*
[M(P ₂₅ ,P ₇₅),mg/L]	(1.16, 6.23)	(0.84, 4.62)	(2.22, 8.68)		
sKlotho($\bar{x} \pm s$, pg/ml)	379.93 $\pm$ 143.66	405.86 $\pm$ 147.67	360.59 $\pm$ 137.83	2.863	0.004*

The Related Factors of Atherosclerosis

Table 2 lists the clinical characteristics and biochemical data in different groups. There were significant differences in age, sex, primary kidney disease, diabetes as a complication, systolic pressure, diastolic pressure, pulse pressure, weight, LDL, Cr, UA, FBG, cCa, Hs-CRP, and sKlotho in the atherosclerosis group compared to the non-atherosclerosis group (P<0.05). There were no significant differences in dialysis vintage, ultrafiltration volume, Alb, Hb, TC, HDL, TG, P, cCa $\times$ P, ALP, and iPTH (P>0.05).

With atherosclerosis as the dependent variable, and age, sex, dialysis vintage, primary kidney disease, diabetes as a complication, systolic pressure, diastolic pressure, weight, Alb, LDL, Cr, UA, FBG, cCa, P, Hs-CRP, iPTH and sKlotho as covariates, the binary classification logistic multivariate regression analysis showed that age, weight, Hs-CRP and sKlotho were the risk factors of atherosclerosis in MHD patients (Table 3, P<0.05).

Table 3. Multiple regression analysis for atherosclerosis. Abbreviations: DM, diabetes; PD, primary diseases; SBP, systolic pressure; DBP, diastolic pressure; LDL, low density lipoprotein; Cr, serum creatine; UA, uric acid; FBG, fasting blood glucose; cCa, corrected serum calcium; Hs-CRP: high-sensitivity C-reactive protein; sKlotho, serum soluble Klotho. OR: odds ratio; 95%CI: 95% confidence interval. Model 1 was adjusted age. Model 2 was adjusted Model 1 + gender and hemodialysis vintage. Model 3 was adjusted for Model 2 + DM, PD, SBP, DBP, weight, Alb, LDL, Cr, UA, FBG, cCa, P, Hs-CRP, iPTH and sKlotho. *P<0.05

Parameter	Unadjusted			Model 1			Adjusted Model 2			Model 3		
	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
Gender	0.604	0.388-0.938	0.025*	0.304	0.174-0.531	0.000*	1.200	1.056-1.363	0.005*	0.367	0.128-1.049	0.061
Age	1.111	1.082-1.141	0.000*	-	-	-	1.128	1.095-1.161	0.000*	0.108	1.067-1.151	0.000*
DM	1.759	1.018-3.038	0.043*	1.352	0.735-2.487	0.332	1.190	0.617-2.294	0.603	0.261	0.057-1.200	0.084
PD	1.039	0.937-1.152	0.465	1.001	0.997-1.005	0.664	1.087	0.955-1.237	0.207	1.347	0.983-1.847	0.064
SBP	1.012	1.000-1.024	0.057	1.010	0.996-1.025	0.171	1.006	0.991-1.021	0.449	1.028	0.981-1.078	0.251
DBP	0.968	0.949-0.987	0.001*	0.999	0.977-1.023	0.955	0.994	0.970-1.018	0.619	1.007	0.926-1.095	0.869
Weight	1.027	1.007-1.047	0.007*	1.041	1.017-1.065	0.001*	1.026	1.001-1.053	0.048*	1.050	1.014-1.088	0.007*
LDL	1.742	1.203-2.522	0.003*	1.358	0.927-1.990	0.117	1.746	1.119-2.723	0.014*	1.015	0.285-3.613	0.982
Cr	0.997	0.996-0.999	0.000*	1.000	0.999-1.002	0.859	0.999	0.997-1.000	0.138	0.998	0.994-1.002	0.285
UA	0.996	0.993-0.999	0.009*	1.002	0.998-1.006	0.390	1.001	0.997-1.006	0.477	1.003	0.995-1.010	0.497
FBG	1.084	0.992-1.185	0.075	1.043	0.948-1.147	0.388	1.012	0.913-1.123	0.816	1.088	0.872-1.358	0.457
cCa	2.348	1.105-4.990	0.027*	1.760	0.734-4.221	0.205	3.009	1.168-7.750	0.023*	1.287	0.117-14.225	0.837
Hs-CRP	1.115	1.020-1.217	0.016*	1.109	1.002-1.227	0.045*	1.119	1.010-1.239	0.031*	1.126	1.005-1.261	0.041*
sKlotho	0.998	0.996-0.999	0.007*	0.998	0.996-0.999	0.031*	0.998	0.996-0.999	0.039*	0.997	0.995-1.000	0.032*

Discussion

Semba RD et al. [6] found that the median (25th, 75th percentiles) sKlotho level was 676 (530, 819, respectively) pg/ml and that sKlotho was correlated with age, HDL cholesterol, and C-reactive protein in a population-based sample of adults residing in Italy. The studies indicated that sKlotho levels continuously declined with progressive degrees of severity of renal insufficiency and were negatively correlated to the stage of chronic renal disease [5, 7-9]. Recently, a Japanese study showed the sKlotho level was 430 (386, 540) pg/ml in MHD patients [10]. The sKlotho level was lower in our study (379.93±143.66 pg/ml) than in the foreign study, which may be associated with the lower renal function of patients when they start dialysis in China.

In our study, the prevalence of thickened cIMT was 46.7%, and the prevalence of atherosclerosis was 57.3%; these findings were consistent with previous investigations [11, 12]. As the traditional factor of atherosclerosis, the incidence of atherosclerosis increased with age. It also plays an important role in MHD patients in this study. At the same time, this study indicated that weight was also a correlation factor of atherosclerosis, and the incidence of atherosclerosis increased by 5.0% as weight increased by 1 kg.

In addition to these two traditional factors, we found that Hs-CRP and sKlotho were non-traditional factors in accelerating atherosclerosis in MHD patients. The incidence of atherosclerosis increased by 12.6% as Hs-CRP increased by 1 mg/L. The incidence of atherosclerosis declined by 0.3% as the sKlotho level increased 1 pg/ml. Hs-CRP is a non-specific marker for the acute phase of inflammation of the liver and a pro-inflammatory cytokine that is associated with the occurrence, evolution and development of atherosclerosis. It plays the same role in MHD patients.

As a newly discovered protein with a variety of biological effects, sKlotho participates in various pathophysiological changes, including regulating calcium and phosphorus metabolism, reducing oxidative stress and inflammation, improving cardiac hypertrophy and ventricular remodeling, and improving endothelial cell function [13]. Atherosclerosis is considered to be vascular endothelium damage because of a variety of factors and results in chronic inflammation in the arterial wall. Based on several studies [1, 14-18], we considered whether sKlotho prevented atherosclerosis and reduced CVD by multiple protective effects on endothelial cells. First, sKlotho plays a key role in regulating the nitric oxide activity of endothelial cells. Studies with α -Klotho knockout mice display attenuation of endothelium-dependent vascular protection along with decreased aortic and arteriolar vasodilation as a

result of reduced nitric oxide (NO) production [19]. Rescue treatment with *in vivo* α -Klotho delivery resulted in the reversal of endothelial dysfunction, a reduction of elevated blood pressure, and the prevention of arterial wall hypertrophy and perivascular fibrosis [14]. Six et al. [20] demonstrated that, although phosphate, sKlotho and FGF23 separately stimulated aorta contraction, sKlotho mitigated the effects of phosphate and FGF23 on contractility via increased NO production, thereby protecting the vessel, to some extent, against the potentially noxious effects of high phosphate or FGF23 concentrations. Second, sKlotho can maintain the integrity of endothelial cells and protect vascular permeability. Calcium ion signals regulate nitric oxide production and release and endothelial cell permeability, proliferation, migration and apoptosis [21, 22]. Classical transient receptor potential channel-1 (TRPC1) is involved in calcium influx-mediated vascular endothelial growth factor (VEGF) [23, 24]. sKlotho maintains endothelial cell integrity by combining with TRPC1 and VEGF receptor-2. The combination of the three complexes produces calcium influx by VEGF stimulation [25]. Third, Maekawa Y et al. [26] found that Klotho suppressed TNF- α -induced expression of adhesion molecules and NF- κ B activation *in vitro*. In mice, knockout of Klotho induces ischemic-mediated angiogenesis [27, 28], increased expression of plasminogen activator inhibitor-1 [29], dysfunction of the sinus node and sudden cardiac death [30].

Our study has several important limitations. First, this study was cross-sectional research; we did not make longitudinal observations concerning serum sKlotho and atherosclerosis. Second, fibroblast growth factor 23 and vitamin D levels were not measured, and the relationship between serum sKlotho, fibroblast growth factor 23, and vitamin D was not a focus of this study. Last, we neglected the history of CVD and the use of statins and their influence on cIMT and atherosclerosis.

Conclusion

Our study found that serum sKlotho was related with systolic pressure, pulse pressure, ultrafiltration volume, Hb, P, corrected Ca $\times$ P, Hs-CRP, increased cIMT, carotid atherosclerotic plaque quantity and atherosclerosis in hemodialysis patients. Age, dry weight, Hs-CRP, and serum sKlotho were risk factors for atherosclerosis. This study is the first to provide clinical evidence of the association between serum sKlotho and atherosclerosis in hemodialysis patients. Serum sKlotho may participate in the pathophysiological process of atherosclerosis in MHD patients.

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Disclosure Statement

The authors declare they have no conflicts of interest.

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