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Serum sclerostin as a potential novel biomarker for heart valve calcification in patients with chronic kidney disease

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Abstract. – OBJECTIVE: To explore the correlation between change in sclerostin level and heart valve calcification in patients with chronic kidney disease (CKD) in stages 3-5, as well as the possible underlying mechanism, which could provide a clinical reference for the diagnosis and treatment of cardiovascular disease (CVD).

PATIENTS AND METHODS: 110 patients were divided into a healthy control group and three groups of patients with CKD stages 3, 4, and 5 according to CKD staging guidelines. Scr, BUN, AKP, TC, TG, HDL, LDL, Ca, Pi, and CRP were measured, and calcium-phosphate product (Ca×Pi) calculated. ELISA was used to measure the sclerostin level, and the estimated glomerular filtration rate (eGFR) was calculated by MDRD. Heart valve calcification was measured by a physician in the Cardiac Department of our hospital. The correlations between sclerostin-level change and heart valve calcification, as well as each index in CKD patients in stages 3–5, were analyzed.

RESULTS: Compared with the healthy control group, the serum Ca in CKD stage-3, stage-4, and stage-5 groups ($p < 0.05$) was reduced, and PTH was increased ($p < 0.05$). Blood Pi and Ca×Pi in the stage-4 and stage-5 groups were increased ($p < 0.05$). The serum sclerostin level increased with renal hypofunction in stage-3 CKD patients, and was significantly increased compared with that of the control group, reaching the highest level in the terminal stage ($p < 0.01$). Pearson correlation analysis indicated that serum sclerostin was negatively correlated with eGFR ($r = -0.91$, $p < 0.001$) and blood Ca ($r = -0.271$, $p < 0.001$), and positively correlated with SCr ($r = 0.608$, $p < 0.001$), blood Pi level ($r = 0.295$, $p < 0.001$), PTH ($r = 0.334$, $p < 0.001$), and Ca×Pi ($r = 0.275$, $p < 0.001$). The rate of heart valve calcification in the CKD patients in stage 5 was relatively high (11/30, 36.67%), and significantly higher than that in healthy controls (1/20, 5%; $p < 0.01$). Logistic regression analysis of heart valve calcification indicated that sclerostin was a risk factor for heart valve calcification in CKD patients in stages 3–5.

CONCLUSIONS: The sclerostin level gradually increased with renal hypofunction in CKD patients in stages 3–5, and the increase in serum sclerostin level in the CKD patients occurred earlier than the change in Pi and Ca×Pi. The risk of heart valve calcification in stage-5 CKD patients was significantly increased. Sclerostin is an independent risk factor for heart valve calcification in CKD patients.

Key Words:
Sclerostin, Chronic Kidney Disease, Heart Valve Calcification, Cardiovascular Disease.

Abbreviations

AKP: alkaline phosphatase; BMP: bone morphogenetic protein; BUN: blood urea nitrogen; Ca: blood calcium; CKD: chronic kidney disease; CRP: C-reactive protein; CVD: cardiovascular disease; ELISA: enzyme-linked immunosorbent assay; (e)GFR: (estimated) glomerular filtration rate; HDL: high-density lipoprotein; LDL: low-density lipoprotein; MBD: mineral and bone disorder; MDRD: Modification of Diet in Renal Disease equation; Pi: blood phosphate; (i)PTH: (intact) parathyroid hormone; SCr: serum creatinine; TC: total cholesterol; TG: triglyceride; TGF: transforming growth factor.

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Introduction

The incidence of chronic kidney disease (CKD) is increasing with lifestyle modifications and has become a common disease that threatens many people's lives. As one of the common complications of CKD, chronic kidney disease mineral and bone disorder (CKD-MBD) is closely related to the occurrence of cardiovascular disease (CVD)[1]. CKD patients will gradually show CKD-MBD after stage 3. Serum calcium will decrease along with renal function, but phosphate (Pi) and parathyroid hormone (PTH) will increase, leading to calcification in the soft tissues (cardiovascular and heart valve calcification)[2]. Sclerostin is one of the important proteins inhibiting osteoblast development during bone reconstitution[3] and is also involved in vascular calcification[4]. The increase in sclerostin level in CKD patients has a negative correlation with glomerular filtration[5]. The sclerostin level in CKD patients in stage 5 is 4- to 5-fold that of healthy people[6,7]. In this study, the correlation between the change in sclerostin level and heart valve calcification in patients was explored, as well as the possible underlying mechanism, with the aim of providing new ideas for the diagnosis and treatment of the disease and improvement of the mortality rate.

Patients and Methods

Study Subjects and Grouping

Following the definition and staging in the Clinical Practice Guide for Chronic Kidney Disease (2010), CKD is defined as: (1) renal damage over 3 months with or without decreased glomerular filtration rate (GFR), kidney damage, i.e., abnormality in structure or function showing abnormal pathological morphology or positive indices (including abnormal blood or urine components or imaging examination); and (2) GFR< 60 mL/min•1.73 m^2 for ≥ 3 months with or without kidney damage. Ninety CKD patients (average age 60.6±11.99 years) receiving treatment in our hospital from March to June 2016 were enrolled and divided into three groups: stage-3 group (*n*=30, male 14, female 16, average age 59.13±12.21 years), stage-4 group (*n*=30, male 15, female 15, average age 62.47±10.87 years), and stage-5 group (*n*=30, male 13, female 17, average age 62.33±12.71 years). There were 33 patients with diabetic nephropathy (36.7%), 8 patients with hypertensive nephropathy (8.9%), 31 patients with glomerulonephritis (34.4%), 7 patients with chronic interstitial nephritis (7.8%), and 11 patients with unknown causes (12.2%). At the same time, 20 healthy volunteers visiting our physical examination center were enrolled as a control group. Everyone received routine blood and urine tests, biochemical tests, chest radiography, and B-scan ultrasound. The age range was 29–73 years with average age 54.7±14.9 years. Exclusion criteria: (1) patients showing cardiopulmonary dysfunction, infection, surgery, and trauma within 1 month; (2) patients with autoimmunity, tumor, familial hyperlipidemia, primary hyperthyroidism, skeletal disease, or administration of hormone and immunosuppressors; (3) except for patients undergoing hemodialysis using heparin, patients who received long-term vitamin D3, phosphate binder, statins, or anticoagulants; (4) patients with acute renal failure or transient decrease in GFR, or with possibility of reversal; (5) patients who were pregnant, in a lactation period, had psychological problems, or did not want to participate in the trial. The study granted Ethical approval by the Yantai Yuhuangding Hospital Ethics Committee and was performed in accordance with the guidelines of the Helsinki Declaration.

Data Collection

Collection of Specimens

The patients in the healthy control group and experimental groups were fasted for 10 h before blood collection, and upper limb venous blood was taken. The venous blood of the patients receiving hemodialysis was taken before hemodialysis and collected into pro-coagulation tubes. The blood was centrifuged at 3,000 rpm for 10 min. The serum (2 mL) was collected in an ER tube and stored at -80°C. Repeated freezing and thawing was avoided to improve the effectiveness.

Detection of Specimens

(1) Routine biochemical indices, including serum creatinine, urea nitrogen, alkaline phosphatase (AKP), total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), calcium (Ca), and Pi, were detected by a Bayer ADVIA 1650 device. (2) A Beckman Array 360 System was used to detect C-reactive protein (CRP). (3) Intact PTH (iPTH) was measured by isotope chemiluminescence. (4)

Enzyme-linked immunosorbent assay (ELISA) was used to measure the sclerostin level. The interval between test time and blood collection time was longer than 1 week.

Detection of Heart Valve Calcification

Heart valve calcification of CKD patients was detected by ultrasonic echocardiography (IE33, Philips, Amsterdam, The Netherlands). Diagnostic criteria for heart valve calcification consisted of one, or more than one, cuspid valve with bright echo in the aortic valve and a mitral valve (ring) larger than 1 mm, based on which the sensitivity and specificity of valve calcification were 76% and 89-94%, respectively.

Statistical Analysis

SPSS 19.0 (IBM Corp., IBM SPSS Statistics for Windows, Armonk, NY, USA) was used to analyze the data. The one-sample Kilmogorov–Smirnov test was used to analyze normal distribution. The measurement data meeting normal distribution were expressed as mean ± SD, and those not meeting the distribution were expressed as median (P25, P75)M(Q). An independent-samples *t*-test was used for comparison between groups, and analysis of variance was used for comparison among groups. Pearson correlation analysis was used to analyze the linear correlation between sclerostin level and each index. Binary linear regression was used to analyze the correlations of sclerostin with each index. Logistic regression analysis was used to analyze the independent risk factors for heart valve calcification. $p < 0.05$ was considered statistically significant.

Results

Comparison of Basic Information and Laboratory Indices Between Healthy Control Group and Patients in Stages 3-5

No statistical significance was found in the sex ratio and age between the healthy group and patients in stages 3-5 ($p > 0.05$). Compared with the healthy control group, estimated GFR (eGFR) in the patients was significantly decreased, but creatinine and urea were significantly increased ($p < 0.05$). Moreover, in the CKD patients, blood Ca, cholesterol, HDL, and LDL were decreased, while Pi and Ca×Pi were increased. The patients in stage 5 showed significantly increased AKP ($p < 0.05$). Compared with the healthy controls, PTH and sclerostin levels of the patients were significantly increased ($p < 0.05$), as shown in Table I.

Correlation Between Sclerostin and Laboratory Indices

Serum sclerostin showed negative correlations with GFR and blood Ca, and positive correlations with serum creatinine, Pi, iPTH, and Ca×Pi ($p < 0.05$) (Table II and Figures 1-4).

Table I. Clinical and biological characteristics of the patients.

Item	Health control group	CKD stage 3	CKD stage 4	CKD stage 5
Age	55.7 ± 11.72	59.13 ± 11.16	63.63 ± 9.75	60.33 ± 12.7
Sex	20 (10/10)	30 (15/15)	30 (16/14)	30 (13/17)
eGFR (ml/min•1.73 m^2)	114.62 ± 11.72	45.68 ± 6.79[a]	21.42 ± 4.04[a,b]	7.73 ± 2.57[a,b,c]
Creatinine (mmol/L)	65.3 ± 12.41	132.2 ± 27.77[a]	254.77 ± 51.16[a,b]	659.4 ± 265.63[a,b,c]
Urea (mmol/L)	5.28 ± 2.09	9.56 ± 2.99[a]	18.27 ± 6.76[a,b]	29.94 ± 20.77[a,b,c]
Alkaline phosphatase	70.45 ± 19.71	77.8 ± 21.74	80.7 ± 23.92	101.97 ± 46.14[a,b,c]
Blood calcium (mmol/L)	2.27 ± 0.19	2.14 ± 0.12[a]	2.08 ± 0.15[a]	1.97 ± 0.24[a,b,c]
Blood phosphorus (mmol/L)	1.09 ± 0.15	1.17 ± 0.24	1.36 ± 0.27[a,b]	1.94 ± 0.83[a,b,c]
Ca×Pi (mg^2/dl^2)	30.3 ± 3.68	30.36 ± 6.34	34.47 ± 7.02[a]	45.41 ± 13.73[a,b,c]
Cholesterol (mmol/L)	6.4 ± 3.2	5.22 ± 1.07[a]	5.0 ± 1.31[a]	4.56 ± 1.12[a,b]
Triglyceride (mmol/L)	1.94 ± 1.16	1.65 ± 0.89	1.31 ± 0.36a	1.4 ± 0.87
HDL (mmol/L)	1.4 ± 0.35	1.12 ± 0.18[a]	1.02 ± 0.37[a]	0.9 ± 0.33[a,b]
LDL (mmol/L)	4.2 ± 1.6	3.24 ± 0.7[a]	3.16 ± 1.04[a]	2.7 ± 0.91[a,b]
CRP (mg/L)	3.17	3.17 (3.17~3.72)	3.76 (3.17~5.9)[a,b]	5.37 (3.17~10.73[a,b]
iPTH (mmol/L)	22.6 (20.66~23.81)	45.31 (36.61~62.42)[a]	73.27 (46.75~112.95)[a,b]	199.35 (81.22~343.48[a,b,c]
Sclerostin (pmol/L)	33.11 ± 4.35	72.84 ± 9.14[a]	81.62 ± 5.72[a,b]	118.79 ± 10.34[a,b,c]

Compared with the control group, [a]$p < 0.05$; compared with stage 3, [b]$p < 0.05$; compared with stage 4, [c]$p < 0.05$.

Table II. Correlation between sclerostin and laboratory indices.

		eGFR	Blood calcium	Creatinine	Blood phosphorus	iPTH	CaxPi
Sclerostin	r	-0.91	-0.271	0.608	0.295	0.334	0.275
	p	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

Heart Valve Calcification in The Healthy Control Group and CKD Patients

Among the 20 healthy volunteers, only one had heart valve calcification, a specific rate of 5%. In the patient groups, 4 of 30 patients (13.33%) from the stage-3 group, 6 of 30 patients (20%) from the stage-4 group, and 11 of 30 patients (36.67%) from the stage-5 group had heart valve calcification ($p < 0.05$). The rate in the stage-5 group was the highest and significantly higher than that of the control group ($p < 0.01$), as shown in Table III.

Risk Factor Analysis of Heart Valve Calcification of CKD Patients

The CKD patients with heart valve calcification in stages 3-5 and those without calcification were divided into two groups to compare their differences. The results indicated that eGFR, AKP, Ca, Pi, urea, creatinine, TC, LDL, and sclerostin were statistically significant parameters ($p < 0.05$) (Table IV).

The results of logistic regression analysis of valve calcification indicated that eGFR, Pi, sclerostin, and urea were independent risk factors influencing valve calcification, using valve calcification as dependent variable and differences between groups as independent variables (Table V).

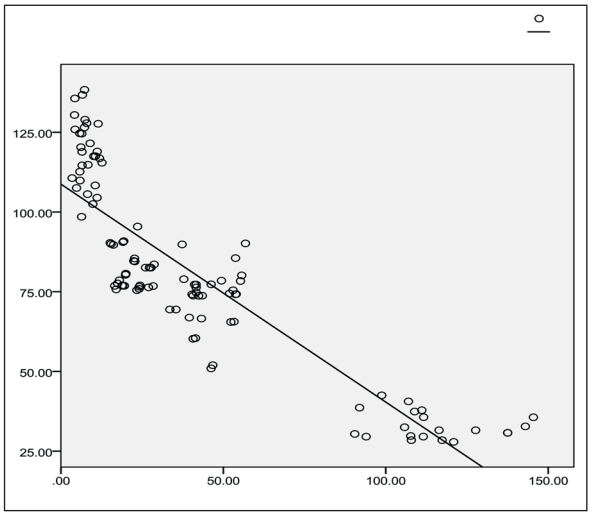

Figure 1. Serum sclerostin correlation with eGFR.

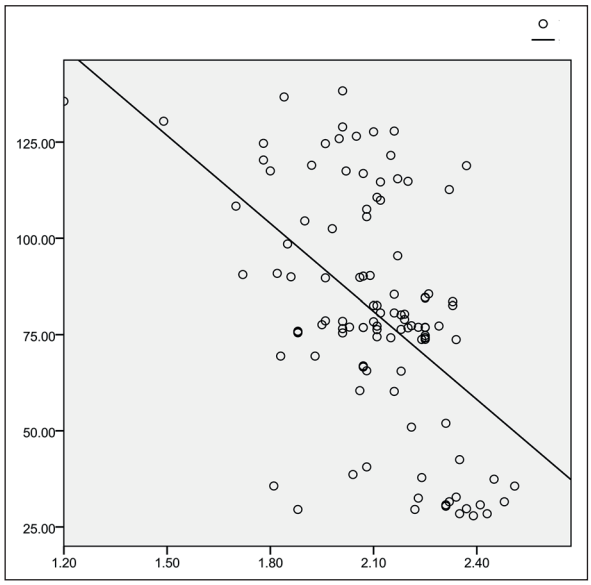

Figure 2. Serum sclerostin correlation with calcium.

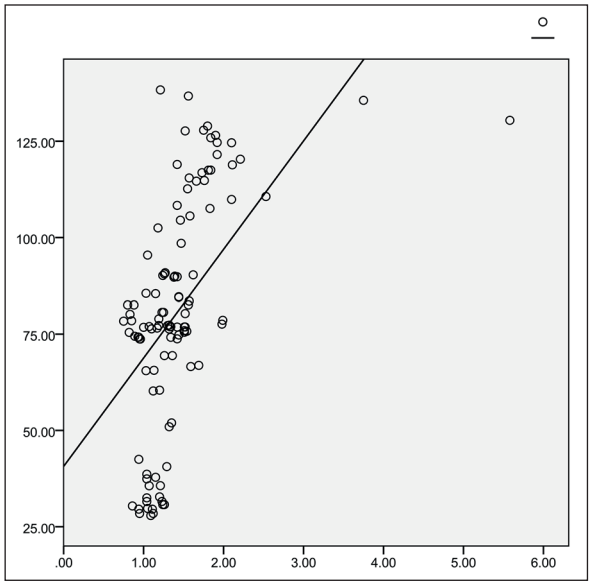

Figure 3. Serum sclerostin correlation with blood phosphorus.

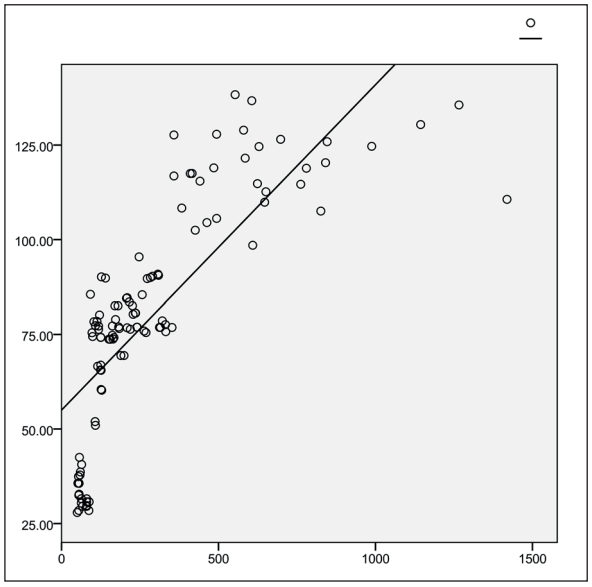

Figure 4. Serum sclerostin correlation with creatinine.

Discussion

CVD is the leading cause of death arising from chronic failure of renal function[8]. Vascular calcification is a possible reason for the high incidence of CVD and fatality in CKD patients. Epidemiological surveys show that patients with CKD in stage 1 frequently have CVD and that the incidence increases with development of the CKD[9]. Currently, computed tomography (spiral CT or electron beam CT) is the gold standard to evaluate cardiovascular calcification. However, as it is very expensive, radiation is not avoidable, and it is not universally available, its widespread application has been limited. In the KDIGO guideline (2009), ultrasonic echocardiography is suggested as a method of calcification detection to replace the radioactive approach[10]. Sclerostin is coded by the sclerosteosis (SOST) gene. It is composed of 213 amino acids secreted by osteocytes. Apart from osteocytes, sclerostin is also expressed in the heart, liver, pancreas, and especially the kidney. The Wnt/β-catenin signaling pathway can influence osteogenesis effects by regulating osteoblast differentiation and specific gene expression, enhancing the activity of osteoblasts and improving formation and mineralization of the bone matrix[11]. Sclerostin inhibits the Wnt/β-catenin signaling pathway, reducing osteoblast and bone formation[12,13]. In the TGF-β/BMP pathway, another signal transduction pathway affecting bone formation, sclerostin, inhibits osteoblast differentiation and bone formation by competitively binding the bone morphogenetic protein (BMP) receptor[14]. It has been found[15,16] that in patients with vascular calcification, sclerostin is also expressed in the calcified tissues, suggesting that sclerostin may participate in the regulation of vascular calcification[15]. In patients with CKD, sclerostin has been proved to participate in vascular and heart valve calcification[17]. Sclerostin is closely associated with cardiovascular-related death and all-cause mortality of CKD patients. In this study, we found that the sclerostin level in patients with heart valve calcification was increased compared with those without calcification. Sclerostin is thus a risk factor for heart valve calcification. Given the results of previous studies, the increase in sclerostin in early-stage CKD patients could protect against vascular calcification. Once the calcification occurs, the calcified tissues may express sclerostin and further increase the sclerostin concentration[17,18]. The sclerostin level in CKD patients showed a negative correlation with GFR, and gradually increased alongside CKD stage development, the level in the terminal stage being about 3- to 4- fold that in the control group. It has been reported that in patients undergoing hemodialysis (and peritoneal dialysis), the high sclerostin level was negatively correlated with risk of cardiovascular death and all-cause mortality. Sclerostin can thus be used to evaluate hemodialysis. A few small cohort studies assessed the serum sclerostin level. In

Table III. Correlation between CKD staging and heart valve calcification (Fisher x^2-test).

	Heart valve calcification			
Group	No	Yes	χ^2	*p*
Control group	19	1	8.21	0.031
CKD3 26	4			
CKD4 24	6			
CKD5 19	11			

Table IV. Comparison of indices between valve calcification group and non-valve calcification group (mean ± SD) [M(Q)].

Item	Valve calcification	Non-valve calcification
Case	21	69
Age	62.67 ± 11.39	60.54 ± 11.32
Sex (male/female)	11/10	33/36
eGFR (ml/min•1.73 m^2)	19.07 ± 17.26*	26.73 ± 15.93
Alkaline phosphatase (IU/L)	97.67 ± 51.65*	83.52 ± 25.98
Calcium (mmol/l)	1.98 ± 0.27*	2.1 ± 0.15
Phosphorus (mmol/l)	1.74 ± 1.07*	1.41 ± 0.36
Ca×Pi (mg^2/dl^2)	39.66 ± 17.5	35.97 ± 8.8
Urea (mmol/l)	29.2 ± 26.24*	16.23 ± 7.6
Creatinine (mmol/l)	461.1 ± 318*	314.61 ± 252.74
TC (mmol/l)	4.4 ± 0.78*	5.1 ± 1.25
TG (mmol/l)	1.35 ± 0.78	1.53 ± 0.77
HDL (mmol/l)	1.04 ± 0.37	1.03 ± 0.23
LDL (mmol/l)	2.58 ± 0.68*	3.2 ± 0.93
CRP (mg/L)	4.28(3.17~8.64)	3.17 (3.17~4.73)
PTH (mmol/L)	120.9 (40.08~332.65)*	53.11 (28.27~83.25)
Sclerostin (pmol/L)	107.56 ± 21.29*	86.07 ± 19.43

Compared with non-heart valve calcification group, *$p < 0.05$.

a study of kidney dialysis, the sclerostin levels of the patients were increased[19]. This has been proved by subsequent multiple small-sample studies including hemodialysis patients[20-22] and chronic renal disease patients without hemodialysis[17,20,23]. These results[24] indicated that the sclerostin level gradually increased along with a decrease in GFR. From stage 3 onward, the serum sclerostin began to show a negative correlation with GFR and blood calcium, and a positive correlation with serum creatinine, blood Pi level, and Ca×Pi. Generally serum sclerostin is related to bone metabolism, but whether the increase in bone expression in patients with nephropathy or whether chronic accumulation of sclerostin in these patients determines the serum sclerostin concentration is still unclear. It has been reported that the sclerostin expression in mice and in human and mouse vascular calcification in renal injury could increase the circulatory sclerostin. After kidney transplantation the sclerostin rapidly recovered to the normal level, suggesting that the glomerular clearance rate was decreased, thus playing the main role in the accumulation at the terminal stage[25]]. The reason for the slight increase in sclerostin level needs further exploration. It has been reported that mitral valve calcification is an independent risk factor for all-cause mortality in maintenance hemodialysis patients. The mortality risk of patients with two-valve calcification is 2-fold that of those without calcification[26]. This was a cross-sectional study, and the severity of heart valve calcification was not detected. Thus, the long-term prognosis cannot be evaluated. For a better explanation of the correlation between sclerostin and the high mortality risk of patients with chronic renal failure, larger sample sizes and multicentric trials are warranted.

Conclusions

In this study, the sclerostin level gradually increased with renal hypofunction in the patients in CKD stages 3–5, and the increase in serum sclerostin level in CKD patients occurred earlier than the change in Pi and Ca×Pi. It was speculated

Table V. Logistic regression analysis of heart valve calcification.

Item	B	SE	Wald	df	Sig	Exp (B)
Phosphorus	4.08	1.9	4.6	1	0.3	59.3
eGFR	-0.1	0.41	5.7	1	0.02	0.9
Urea	-0.27	0.09	9.4	1	0.02	0.77
Sclerostin	-0.14	0.5	1.17	1	0.28	0.56

that the increase might be related to the decrease in GFR and accumulation in the body. The risk of heart valve calcification in CKD patients in stage 5 was significantly increased. Sclerostin is a risk factor for heart valve calcification of CKD patients. Serum sclerostin can thus be used as an early-stage indicator to predict heart valve calcification in CKD patients.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Ethics Approval and Consent to Participate

The study was granted ethical approval by the Yantai Yuhuangding Hospital Ethics Committee and was performed in accordance with the guidelines of the Helsinki Declaration.

Availability of Data and Materials

The datasets and materials supporting the conclusions of this article are included within the article.

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Authors' Contribution

YQ J performed the experiments, found references and drafted the manuscript. LN G and JL analyzed the data and wrote the manuscript. PY Y and XQ S participated in the ELISA, blood tests and ZW S analysis. WL designed the study, modified the manuscript. GP Y and CR guided the above work. All authors read and approved the final manuscript.

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