Serum Sclerostin and Adverse Outcomes in Nondialyzed Chronic Kidney Disease Patients

Mehmet Kanbay, Dimitrie Siriopol, Mutlu Saglam, Yasemin Gulcan Kurt, Mahmut Gok, Hakki Cetinkaya, Murat Karaman, Hilmi Umut Unal, Yusuf Oguz, Sebahattin Sari, Tayfun Eyileten, David Goldsmith, Abdulgaffar Vural, Gabriel Veisa, Adrian Covic, and Mahmut Ilker Yilmaz

Department of Medicine, Division of Nephrology, Istanbul Medeniyet University School of Medicine, Istanbul, Turkey 34000; Department of Nephrology (D.S., G.V., A.C.), Gr. T.Popa University of Medicine and Pharmacy, Iasi, Romania 700115; Departments of Radiology (M.S, S.S.), Biochemistry (Y.G.K.), and Nephrology (M.G., H.C., M.I.Y., H.U.U., Y.O., T.E., A.V.), Gülhane School of Medicine, Ankara, Turkey 06018; Department of Nephrology (D.G.), Guy's Hospital, King's Health Partners, London, United Kingdom SE1 7EH, and Department of Medicine (M.K.), Division of Nephrology University School of Medicine, Instanbul, Turkey 34450

Background: The chronic kidney disease (CKD)-mineral and bone disorder (MBD) syndrome is an important contributor to the CKD-associated cardiovascular disease and high mortality rates. Sclerostin, a protein synthesized in osteocytes, is a potent downregulator of bone metabolism and a novel candidate for the bone-vascular axis in CKD patients. We tested whether serum sclerostin values are predictive for all-cause mortality and cardiovascular events (CVEs) in a CKD population.

Methods: Serum sclerostin was obtained from 173 CKD (stage 3–5) and 47 control patients, and its concentration was correlated with estimated glomerular filtration rate and to mineral and vascular abnormalities that are present in the CKD evolution. All-cause mortality and CVEs were also analyzed in relation to serum sclerostin values.

Results: Patients with CKD showed higher sclerostin levels (median 63.5 pmol/L vs 52 pmol/L, $P < .001$) than controls, with values progressively higher across the CKD stages. In univariate analysis, serum sclerostin concentrations were correlated with gender, estimated glomerular filtration rate, flow-mediated dilatation, and endothelium-independent vasodilatation as markers of endothelial dysfunction and with different serum CKD-MBD–associated parameters. However, in multivariate analysis, only gender, fibroblast growth factor-23, phosphate, flow-mediated dilatation, and cholesterol remained significantly associated with sclerostin levels. During the observational period, there were 19 deaths and 50 CVEs. In survival analysis, different sclerostin levels were associated with all-cause mortality and CVEs in these patients.

Conclusions: This is the first study that shows that serum sclerostin values are associated, even after multiple adjustments, with fatal and nonfatal CVEs in a nondialyzed CKD population. **(*J Clin Endocrinol Metab* 99: E1854–E1861, 2014)**

The increased cardiovascular risk observed in patients with chronic kidney disease (CKD) is multifactorial and is due partly to pathophysiological processes specific to chronic kidney disease that make prevention of cardiovascular disease by standard interventions directed at single traditional risk factors difficult (1). As kidney function worsens, there is a progressive alteration of mineral homeostasis, with a disruption of normal serum and tissue

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Abbreviations: ALP, alkaline phosphatase; BP, blood pressure; CI, confidence interval; CKD, chronic kidney disease; CVE, cardiovascular event; eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor-23; FMD, flow-mediated dilatation; HD, hemodialysis; HOMA-IR, homeostasis model assessment of insulin resistance; HR, hazard ratio; hsCRP, high-sensitivity C-reactive protein; iPTH, intact PTH; MBD, mineral and bone disorder; NMD, nitroglycerine-mediated dilatation.

concentrations of phosphorus and calcium and changes in circulating levels of hormones. The CKD-mineral and bone disorder (CKD-MBD) syndrome is an extremely important complication of kidney diseases and is present early in the evolution of CKD (2, 3). It describes the complex bone and mineral abnormalities that occur in CKD and is an important contributor to the CKD-associated cardiovascular disease and high mortality rates (4, 5).

Sclerostin (22.5 kDa), a protein synthesized in osteocytes, acts as an inhibitor of the Wnt coreceptor low density lipoprotein receptor-related protein5/6. Wnt pathway activation promotes the osteoblast cell lineage by controlling proliferation, maturation, terminal differentiation, and bone formation (6). Sclerostin is a potent downregulator of bone metabolism, thus being a novel candidate for this bone-vascular axis in CKD patients.

Serum sclerostin levels increase with the progression of CKD (7–9) and are associated with bone mass (10, 11), various histomorphometric parameters of bone turnover (12), and cardiovascular calcification (13, 14). Recently, Viaene et al (15) showed that in hemodialysis (HD) patients, high circulating sclerostin levels are associated with improved survival.

The aim of this study was to evaluate in a nondialyzed CKD population the relationship between sclerostin, different parameters of CKD-MBD, and endothelial function and to test its ability in predicting future cardiovascular events (CVEs) and mortality.

Patients and Methods

Patients and study design

Between November 2010 and August 2013, 289 patients were referred to the Renal Unit of the Gülhane School of Medicine Medical Center, Ankara, Turkey, for suspected or manifest renal failure. All patients included in the study were previously diagnosed as having CKD according to National Kidney Foundation K/DOQI Guidelines (16) and were followed in our unit for at least 3 months to exclude acute kidney injury. By protocol, and to minimize any confounding effects of conditions that may influence CKD-MBD assessment at baseline, 49 patients who were taking phosphate binders (n = 19) or supplemental vitamin D pills (n = 30) were excluded from this observational cohort. Otherwise, other exclusion criteria including acute infections and unwillingness to participate in the study were applied (n = 30). In addition 37 eligible patients withdrew informed consent. Stages of CKD were determined using estimated glomerular filtration rates (eGFRs) that were estimated at inclusion according to the Modification of Diet in Renal Disease Equation (17). In total, 173 patients were included in the final analysis: 60 patients with stage 3, 55 patients with stage 4, and 58 patients with stage 5 CKD. None of the patients in stage 5 CKD were on dialysis. Some of the subjects that we enrolled were included in our previous study (18).

We further recruited in the control group 47 patients, matched to the CKD patients for age and gender, from our outpatient department whose primary reason for referral did not include diabetes or cardiovascular or renal disease.

All included patients were followed up for time-to-event analysis until occurrence of fatal or nonfatal CVEs. Information on fatal and nonfatal CVEs including death, stroke, and myocardial infarction were obtained from the Gülhane School of Medicine Medical Center registries. Death certificates and hospital records were reviewed by 3 of the investigators unaware of baseline parameters. If information could not be obtained, the patient was assumed to be lost to follow-up starting from the date of the last actual visit.

The Gülhane School of Medicine ethical committee approved the study protocol, and all patients were included in the study after signing informed consent forms.

Arterial blood pressure (BP) was determined in the morning in all patients by a physician by 3 consecutive measurements, each after a 15-minute resting period, with the mean values calculated for systolic and diastolic pressure.

Biochemical analyses

All blood samples were obtained from patients in the morning, after 12 hours of fasting, for measurement of serum albumin, high-sensitivity C-reactive protein (hsCRP), and fasting plasma glucose, total serum cholesterol, triglyceride, and high- and low-density lipoprotein cholesterol. The serum basal insulin value was determined by the coated tube method (Diagnostic Products Corp). The homeostasis model assessment of insulin resistance (HOMA-IR) was computed using the following formula: HOMA-IR = fasting plasma glucose (milligrams per deciliter) × immunoreactive insulin (micro-international units per milliliter)/405 (19). Proteinuria was quantified using 24-hour timed urine collection.

Serum sclerostin was measured using the quantitative sandwich ELISA method developed by Biomedica. In our laboratory, 2 samples of known concentrations were tested 6 times to assess intra-assay variability (4%), and 2 samples of known concentrations were tested in 3 assays to assess interassay variability (3%). Sclerostin measurements are reported throughout in picomoles per liter, with the lower limit of detection less than 10 pmol/L.

Intact fibroblast growth factor-23 (FGF23) was measured using an ELISA according to the manufacturer's protocol (Kainos Laboratories International). This second-generation, 2-site, monoclonal antibody ELISA has previously been shown to recognize the biologically active intact FGF23. The Kainos intact FGF23 assay has a lower limit of detection of 3 pg/mL and intra-assay and interassay coefficients of variation of less than 5%. The calculated overall intra-assay coefficient of variation was 2.5%, and the calculated overall interassay coefficient of variation was 2.8%. We measured all samples in duplicate.

To measure 25-hydroxyvitamin D, we used HPLC kits following the manufacturer's instructions (ImmuChrom GmbH). Quantification of 25-hydroxyvitamin D_3 was made by anHPLC system with UV (264 nm) detector (Thermo Electron). The intra-assay coefficient of variation was 0.9% to 2.9%, the calculated interassay coefficient of variation was 1.7% to 3.9%, and recovery was 91%. Intact PTH (iPTH) was measured by immunoradiometric assay, using a commercial kit (Immulite intact PTH) from Diagnostic Products Corp with a sensitivity of 1 pg/mL.

Assessment of endothelial function

Endothelium-dependent vasodilatation (flow-mediated dilatation [FMD]) and endothelium-independent vasodilatation (nitroglycerine-mediated dilatation [NMD]) of the brachial artery were assessed noninvasively, using high-resolution ultrasound as described by Celermajer et al (20). The method for the vascular assessment met the criteria established by the International Brachial Artery Reactivity Task Force (21). Measurements were made by a single observer using an ATL 5000 ultrasound system (Advanced Technology Laboratories Inc., Bothell, WA.) with a 12-Mhz probe. The maximum FMD and NMD dilatation diameters were calculated as the average of the three consecutive maximum diameter measurements. The FMD and NMD were then calculated as the percent change in diameter compared with baseline resting diameters. The intra-observer coefficient of variation for FMD was 5.9%.

Statistical analysis

All statistical analyses were performed using SPSS version 15.0 (SPSS Inc) statistical package. Nonnormally distributed variables are expressed as median (range) and normally distributed variables as mean ± SD, as appropriate. A P value $< .05$ was considered to be statistically significant. Between-group comparisons were assessed for nominal variables with the χ^2 test, by Mann-Whitney U or Kruskal-Wallis test for nonnormally distributed variables, and by independent t test or one-way ANOVA for normally distributed variables. Spearman's rank correlation was used to determine correlations between paired variables. Backward stepwise multivariate regression analysis was used to assess the predictors for sclerostin. Survival and time-to-event analysis of cardiovascular outcomes were done using Kaplan-Meier with log-rank test used to compare groups and Cox proportional hazards model, including adjustment for potential confounding factors. For multivariable Cox analysis, the backward stepwise method was applied. We included in the model all univariate predictors for mortality and used a removal probability of .05. Data are presented in the form of hazard ratios (HRs) and 95% confidence intervals (CIs). We performed bootstrapping validation when needed to avoid the problem of overfitting due to the low number of incident outcomes.

Results

Characteristics of the study population

Our study included 173 patients (median age 47, IQR 38–59 years, 49.7% males) with CKD and 47 controls (non-CKD, nonhypertensive, and nondiabetic). Primary renal disease was diabetes-associated nephropathy (26%), hypertension (19.1%), chronic glomerulonephritis (16.8%), polycystic kidney disease (6.4%), reflux nephropathy (6.4%), and unknown etiology (25.4%).

As expected, patients with CKD had higher systolic BP and hsCRP values and lower albumin levels, altered mineral metabolism (as determined by lower serum calcium and vitamin D and higher phosphate, iPTH, and FGF23), and endothelial function (as evaluated by FMD and NMD) (see Table 1) compared with controls. Different demographic, clinical, and biological characteristics of the study population across stages of CKD are shown in Supplemental Table 1.

CKD patients had significantly higher sclerostin values than controls (median 63.5 vs 52.0 pmol/L, $P < .001$; mean difference 17.7, 95% CI 13.9–21.4 pmol/L).

There was no difference in sclerostin levels between diabetic and nondiabetic patients (median 73.0 [range = 54.2–87.2] pmol/L vs 60.6 [range = 55.3–72.1] pmol/L, $P = .11$), or between male and female patients (median 63.2 [range = 54.7–88.8] pmol/L vs 63.5 [range = 55.7–73.0] pmol/L, $P = .28$). Importantly, sclerostin values significantly increased across the 3 CKD stages ($P < .001$; see Figure 1 and Supplemental Table 1). We further divided the CKD patients into 2 subgroups according to the sclerostin median value (63.5 pmol/L). Patients with sclerostin levels above the median had significantly higher CRP, glucose, FGF23, iPTH, alkaline phosphatase (ALP), and phosphate and lower calcium, eGFR, FMD, and NDM values (see Table 1).

Correlations of serum sclerostin with demographic, clinical, and biological parameters

In univariate analysis, we identified eGFR, phosphate, and iPTH as the best significant correlates for serum sclerostin levels. In multivariate linear regression analysis, including all the univariate predictors of sclerostin, only FGF23, phosphate, cholesterol, FMD, and gender maintained an independent association (R^2 of the model = 0.42) with sclerostin values (Table 2).

All-cause mortality and CVEs according to sclerostin levels

The mean follow-up period was 26 (range = 23–31) months. During this period, 19 patients died, 11 due to cardiovascular causes. Cardiovascular mortality was defined as death due to coronary heart disease (n = 8), sudden death (n = 1), or stroke (n = 4). A total of 39 nonfatal CVEs took place during the follow-up period. These included stroke (n = 12), myocardial infarction (n = 23), and peripheral vascular disease (n = 4).

Using sclerostin as a continuous variable, we found in univariate Cox analysis that its levels are associated with all-cause mortality (HR = 1.03, 95% CI 1.01–1.04, $P = .003$) and CVEs (HR = 1.03, 95% CI 1.02–1.04, $P < .001$). After adjustment for all univariate predictors, sclerostin levels remained significantly associated only with CVEs (see Supplemental Table 2).

There were 5 deaths and 7 CVEs in the group of patients with sclerostin levels below the median, whereas in the other group, there were 14 deaths and 43 CVEs. Kaplan-Meier curves show significantly higher all-cause mortality

Table 1. Demographic Characteristics, Biochemical Parameters, and Vascular Assessments of the Study Population[a]

	Controls	CKD Patients	P^b	Sclerostin <63.5 pmol/L	Sclerostin ≥63.5 pmol/L	P^c
n	47	173		87	86	
Age, y	49.0 (47.0–59.0)	47.0 (38.0–59.0)	.34	46.0 (38.0–60.0)	49.5 (38.0–58.3)	.61
Male, n (%)	21 (44.7%)	86 (49.7%)	0.62	43 (49.4%)	43 (50.0%)	1.00
Diabetes, n (%)	-	45 (26.0%)	NA	17 (19.5%)	28 (32.6%)	.06
Smoking, n (%)	18 (38.3%)	80 (46.2%)	.41	39 (44.8%)	41 (47.7%)	.76
Hypertensive, n (%)	-	33 (19.1%)	NA	14 (16.1%)	19 (22.1%)	.34
BMI, kg/m^2	26.0 (24.5–26.5)	25.7 (24.0–27.5)	.90	26.0 (24.0–28.0)	25.0 (23.5–27.0)	.14
Albumin, g/dL	4.3 (4.1–4.6)	4.0 (3.8–4.4)	**<.001**	4.0 (3.8–4.4)	4.1 (3.7–4.4)	.82
hsCRP, mg/L	2.0 (1.7–3.0)	18.0 (13.0–25.0)	**<.001**	17.0 (12.0–21.0)	21.0 (14.0–27.0)	**.001**
Proteinuria, g/d		1.4 (0.8–1.9)	NA	1.3 (0.8–1.8)	1.5 (0.8–2.2)	.28
Cholesterol, mg/dL	198.0 (188.0–208.0)	198.0 (186.0–210.0)	.61	200.0 (195.0–219.0)	198.0 (183.8–208.3)	.09
HDL-cholesterol, mg/dL	45.0 (41.0–49.0)	45.0 (40.0–49.0)	.31	45.0 (40.0–49.0)	44.0 (38.0–49.0)	.08
LDL-cholesterol, mg/dL	125.0 (120.0–131.0)	125.0 (116.0–136.0)	.50	128.0 (120.0–137.0)	124.0 (110.8–134.3)	.33
Triglycerides, mg/dL	142.0 (136.0–152.0)	142.0 (131.0–155.0)	.69	141.0 (131.0–154.0)	142.0 (129.8–158.0)	.53
SBP, mm Hg	129.0 (125.0–133.0)	133.0 (127.0–138.0)	**.003**	133.0 (124.0–137.0)	129.0 (133.0–138.3)	0.20
DBP, mm Hg	83.0 (81.0–86.0)	83.0 (81.0–88.0)	.14	83.0 (81.0–88.0)	83.0 (81.0–86.3)	.69
HOMA-IR, μIU/mL	1.3 (1.2–1.6)	1.5 (1.3–2.1)	**<.001**	1.5 (1.3–1.9)	1.6 (1.4–2.2)	.08
Glucose, mg/dL	85.0 (78.0–90.0)	90.0 (81.0–101.0)	**.002**	88.0 (81.0–96.0)	92.0 (82.8–104.3)	**.01**
FGF23, rU/mL	30.3 (25.8–34.5)	44.5 (34.5–66.0)	**<.001**	38.0 (33.2–56.8)	53.6 (36.1–68.1)	**.002**
Vitamin D, nmol/L	54.6 ± 7.8	45.4 ± 10.9	**.001**	45.4 ± 9.89	45.4 ± 12.1	.99
PTH, pg/mL	42.0 (33.0–52.0)	154.0 (129.5–231.0)	**<.001**	142.0 (120.0–165.0)	192.0 (151.5–245.5)	**<.001**
ALP, U/L	52.0 (44.0–113.0)	78.0 (49.0–119.5)	**.03**	66.0 (46.0–112.0)	90.5 (54.8–129.8)	**.002**
Calcium, mg/dL	9.2 (8.7–9.6)	8.2 (7.9–8.5)	**<.001**	8.2 (7.9–8.7)	8.0 (7.9–8.3)	**.024**
Phosphate, mg/dL	3.8 (3.4–4.1)	5.0 (4.5–7.4)	**<.001**	4.8 (4.1–5.2)	6.0 (4.9–7.3)	**<.001**
FMD, %	8.5 (8.3–9.2)	6.4 (5.7–7.4)	**<.001**	7.0 (6.4–7.4)	5.9 (5.2–6.4)	**<.001**
NMD, %	13.4 (12.8–13.7)	12.8 (12.1–13.2)	**<.001**	12.9 (12.6–13.2)	12.6 (12.0–13.0)	**.001**
Sclerostin, pmol/L	52.0 (49.0–54.9)	63.5 (55.3–78.9)	**<.001**	55.3 (50.3–58.5)	78.9 (69.0–97.9)	**<.001**
eGFR, ml/min/1.73m^2	116.0 (113.0–119.0)	23.0 (4.0–38.0)	**<.001**	27.0 (21.0–47.0)	15.0 (3.0–27.0)	**<.001**

Abbreviations: BMI, body mass index; DBP, diastolic BP; HDL, high-density lipoprotein; hsCRP, high-sensitive C reactive protein; LDL, low-density lipoprotein; NA, not applicable; SBP, systolic BP.

[a] Data are expressed as mean ± SD or median (range) as appropriate. Bold values are statistically significant.

[b] Comparison between controls and CKD patients.

[c] Comparison between groups.

and CVE rates in CKD patients with serum sclerostin levels above the median (see Figure 2). In unadjusted Cox regression, patients with sclerostin values ≥63.5 pmol/L had an HR for death of 3.19 (95% CI 1.15–8.89, $P = .03$) and for CVEs of 7.74 (95% CI 3.47–17.24, $P < .001$), respectively (see Table 3). However, in multivariate Cox analysis, sclerostin levels above the median again remained significantly associated only with CVEs (HR = 7.25, 95% CI 3.25–16.19, $P < .001$), independently of eGFR, markers of endothelial dysfunction, or other CKD-MBD parameters.

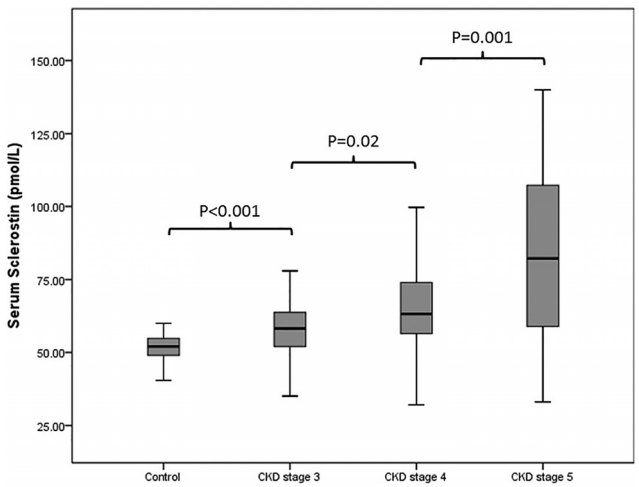

Figure 1. Serum sclerostin values (picomoles per liter) in the control group and across the CKD population.

As a supplementary sensitivity analysis, we further evaluated the predictive power of median sclerostin value for all-cause mortality and CVEs in each CKD stage. In every CKD stage, a sclerostin value above the median increases the risk for CVEs, but not for all-cause mortality (see Supplemental Figures 1–3).

Finally, we performed a receiver operating characteristic curve analysis and found a better cutoff for sclerostin as survival predictor in this CKD population. A value of sclerostin >69 pmol/L had a sensitivity of 67.5% and a specificity of 73.7%, with positive predictive value of 95.4% and a negative predictive value of 21.9%. The group with a sclerostin value >69 pmol/L remained, even after multiple adjustments and bootstrapping validation,

Table 2. Univariate and Multivariate Associates of Sclerostin as a Continuous Variable in Nondialysis CKD Patients

Parameters	ρ or β	*P*
Univariate ρ		
hsCRP, mg/L	0.29	<.001
FGF 23, rU/mL	0.32	<.001
iPTH, pg/ml	0.37	<.001
ALP, U/L	0.23	.003
Phosphate, mg/dL	0.47	<.001
FMD, %	−0.52	<.001
NMD, %	−0.27	<.001
eGFR, ml/min/1.73 m^2	−0.41	<.001
Cholesterol, mg/dL	−0.15	.04
Gender (1 = male, 2 = female)	−0.15	.04
Multivariate β		
FGF23, rU/mL	0.25	.002
FMD, %	−5.22	.001
Phosphate, mg/dL	4.94	<.001
Cholesterol, mg/dL	−0.14	.03
Gender (1 = male, 2 = female)	−5.72	.03

Table 3. Univariate and Multivariate Cox Analysis Predicting All-Cause Mortality and Cardiovascular Events Using Sclerostin Median (63.5 pmol/L) Groups

Parameters	HR (95% CI)	*P*
Fatal events (n = 19)		
Univariate analysis		
Diabetes (yes/no)	5.35 (2.07–13.82)	.001
FMD, %	0.47 (0.29–0.76)	.002
DBP, mm Hg	0.89 (0.81–0.99)	.04
Glucose, mg/dL	1.01 (1.00–1.02)	.01
Phosphate, mg/dL	1.42 (1.07–1.88)	.02
iPTH, pg/mL	1.01 (1.00–1.01)	.02
Sclerostin median groups	3.19 (1.15–8.89)	.03
Multivariate analysis		
Diabetes (yes/no)	4.54 (1.73–11.89)	.002
FMD, %	0.49 (0.29–0.83)	.008
DBP, mmHg	0.87 (0.76–0.99)	.03
CVEs (n = 50)		
Univariate analysis		
Diabetes (yes/no)	2.75 (1.56–4.83)	<.001
FMD, %	0.50 (0.38–0.67)	<.001
NMD, %	0.66 (0.48–0.92)	.02
SBP, mm Hg	1.03 (1.00–1.06)	.04
Glucose, mg/dL	1.01 (1.00–1.01)	.03
Hypertension (yes/no)	2.81 (1.57–5.03)	<.001
Phosphate, mg/dL	1.44 (1.20–1.71)	<.001
iPTH, pg/mL	1.01 (1.00–1.01)	.04
FGF23, rU/mL	1.03 (1.01–1.04)	<.001
eGFR, ml/min/1.73m^2	0.98 (0.96–0.99)	.004
Sclerostin median groups	7.74 (3.47–17.24)	<.001
Multivariate analysis		
Diabetes (yes/no)	2.37 (1.35–4.18)	.003
Sclerostin median groups	7.25 (3.25–16.19)	<.001

Abbreviations: DBP, diastolic BP; SBP, systolic BP.

associated with all-cause mortality (see Supplemental Table 3). Forcing eGFR, age, or proteinuria into the model didn't affect the final results.

Discussion

Our study shows that sclerostin levels are associated with markers of CKD-MBD and vascular function in a CKD stage 3 to 5 population. Most importantly, we demonstrated for the first time that in a predialysis CKD cohort, serum sclerostin values are associated, even after multiple adjustments, with fatal and nonfatal CVEs.

Sclerostin, the product of the *SOST* gene in osteocytes, is a glycoprotein that inhibits osteoblast and bone formation. Sclerostin acts as an inhibitor of the Wnt coreceptor low density lipoprotein receptor-related protein 5/6. The

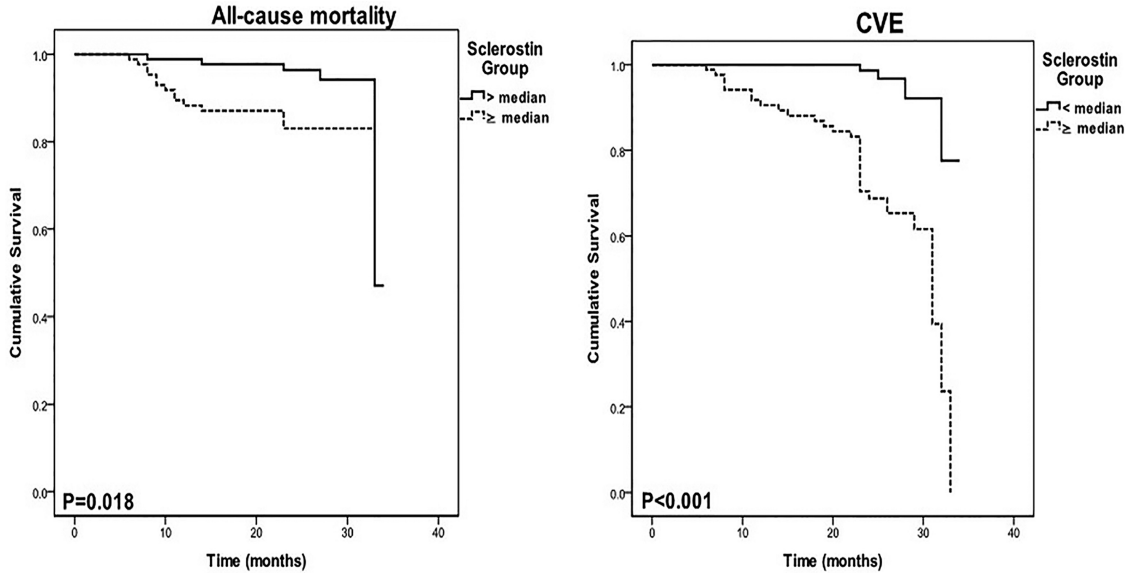

Figure 2. Kaplan-Meier analysis for all-cause mortality and CVE according to median (63.5 pmol/L) sclerostin groups. CVE represents both fatal and nonfatal CVEs.

canonical Wnt pathway has a bone-anabolic and anticatabolic effect. Wnt signaling inhibits bone resorption and upregulates osteoprotegerin, which binds and inhibits the receptor activator of nuclear factor-κB ligand.

The sclerostin levels from our control group were similar to other non-CKD studied populations (22, 23). Our study confirms previous findings that show increased levels of sclerostin in CKD patients (11, 14, 24). Recently, it has been established that these levels are not the result of reduced renal clearance, because urinary sclerostin excretion increases with declining eGFR (9). Sabbagh et al (7) suggests that an increase in osteocyte production is most likely the primary event that leads to the elevation in serum sclerostin levels observed in CKD patients.

It has been shown, both in non-CKD (25) and CKD (11, 12) patients, that sclerostin levels are associated with PTH values. In contrast with these aforementioned studies, after multiple adjustments, in our cohort, sclerostin and PTH levels were not related. There are several potential explanations for this lack of correlation. First, in our CKD population, iPTH levels were relatively low. Second, there are important methodological differences among the published studies regarding the type of CKD population and of sclerostin assays. Third, and possibly the most important hypothesis, there could be PTH-independent mechanisms for sclerostin regulation.

In line with this last assumption, the present study shows that sclerostin is correlated with FGF23 and phosphate. Recently, it was hypothesized that CKD-MBD has an impact on the Wnt pathway and that phosphorus and FGF-23 could be additional modulators of sclerostin expression (8). The same group also showed in an animal model that a high-phosphate diet was associated with an increase in both serum and bone sclerostin levels (26). These corroborated results strengthen the possible role of phosphorus and FGF23 in sclerostin regulation through a PTH-independent mechanism (8).

Numerous data now implicate the Wnt pathway in metabolic disease (27, 28). It has been shown that high levels of sclerostin are associated with hyperlipidemia in animal models (29) and non-CKD patients (30). Our results are in agreement with the findings of Claes et al (13) who found an inverse relationship between cholesterol and sclerostin levels in CKD patients. Osteocytes are formed during the terminal differentiation of bone-forming osteoblasts (30), and serum cholesterol has inhibitory effects on this process (31). Whether this negative correlation between sclerostin, as a marker of osteocyte number and activation (11), and cholesterol levels in CKD implies an increased transformation of osteoblasts or a negative feedback response to the increased number of osteoblasts in CKD patients needs further investigation.

It is now well established that in CKD patients, vascular calcifications are common and related to worse outcomes (32). Previous studies have described different relationships between sclerostin and vascular disease. Some of them have observed that higher sclerostin levels are associated with carotid intima thickness (33), aortic calcification (14, 34, 35), or pulse wave velocity (35). Increased sclerostin expression was associated with calcification of aortic valves (14) or cultured mouse vascular smooth muscle cells (33, 36). In contrast with the aforementioned studies, an inverse relationship between sclerostin levels and vascular calcification was noted in diabetic African American (37) or CKD (12) patients. Whether these different outcomes are the result of subject selection, differences in population ancestry or different competing factors used in multivariable analysis remains unknown.

Our study shows an independent relationship with FMD, a known marker of endothelial dysfunction. Additionally, we also found that higher levels of sclerostin are predictive for fatal and nonfatal CVEs and even mortality. Recently, and in contrast with our results, Viaene et al (15) showed that higher levels of sclerostin are associated with improved survival in HD patients. However, although a trend toward worse survival in patients with sclerostin values below the median was observed in univariate analysis, this conclusion was drawn only after adjustments for age and gender. The authors explained their findings by the known suppressor effect of sclerostin on bone-specific alkaline phosphatase, an inducer of vascular calcification in CKD (38) and a biomarker associated with mortality in HD (39, 40). By comparison, our study was done in CKD patients not on dialysis and without any vitamin supplementation, and thus the 2 populations probably had different renal osseous pathologies. Although direct bone histomorphometry was not performed in either of these studies, this hypothesis is supported by the differences observed in serum vitamin D, PTH, or calcium levels. Because most of the CKD patients die before needing renal replacement therapy, it is known that those on dialysis probably have special unidentified characteristics, which could also account for the different findings between these 2 studies. Another possible explanation is that, like other markers of CKD-MBD, serum sclerostin levels could have a U-shaped effect on mortality.

Our study has several limitations and strengths. Like any observational study, no direct inferences could be made between serum sclerostin levels and all-cause mortality and CVE in this CKD population. Second, as mentioned before, we didn't perform bone density or histomorphometry assessment and vascular calcification analysis and as such couldn't evaluate the impact of these anomalies on sclerostin levels and on our outcomes. Third,

although the difference in sclerostin levels between controls and CKD patients is rather small, it has statistical significance. Because, like phosphorus or other hormonal (vitamin D, FGF23, and PTH) levels, more abnormal sclerostin values are proper to later CKD stages, this small difference could be the result of a diluting effect of having included also patients with near-normal renal function. Fourth, our sample size and number of outcomes were relatively small. However, we used a holistic approach, analyzing serum sclerostin levels in association with markers of endothelial dysfunction, inflammation, or CKD-MBD.

Conclusions

This is the first study that demonstrates an increased incidence in mortality and CVE in the presence of higher serum sclerostin levels in CKD nondialyzed patients. The exact role of sclerostin on the bone-vascular axis in CKD and whether it represents only a marker or is actually an effector needs further evaluation.

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Address all correspondence and requests for reprints to: Mehmet Kanbay, MD, Department of Medicine, Division of Nephrology, Koc University School of Medicine, Istanbul, 34450 Turkey. E-mail: drkanbay@yahoo.com .or mkanbay@ku.edu.tr

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References

1. **Gansevoort RT, Correa-Rotter R, Hemmelgarn BR, et al.** Chronic kidney disease and cardiovascular risk: epidemiology, mechanisms, and prevention. *Lancet*. 2013;382:339–352.
2. **Moe SM, Radcliffe JS, White KE, et al.** The pathophysiology of early-stage chronic kidney disease-mineral bone disorder (CKD-MBD) and response to phosphate binders in the rat. *J Bone Miner Res*. 2011;26:2672–2681.
3. **Fang Y, Ginsberg C, Sugatani T, Monier-Faugere MC, Malluche H, Hruska KA.** Early chronic kidney disease-mineral bone disorder stimulates vascular calcification. *Kidney Int*. 2014;85:142–150.
4. **Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY.** Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *The N Engl J Med*. 2004;351:1296–1305.
5. **Kestenbaum B, Sampson JN, Rudser KD, et al.** Serum phosphate levels and mortality risk among people with chronic kidney disease. *J Am Soc Nephrol*. 2005;16:520–528.
6. **Baron R, Rawadi G.** Wnt signaling and the regulation of bone mass. *Curr Osteoporos Rep*. 2007;5:73–80.
7. **Sabbagh Y, Graciolli FG, O'Brien S, et al.** Repression of osteocyte Wnt/β-catenin signaling is an early event in the progression of renal osteodystrophy. *J Bone Miner Res*. 2012;27:1757–1772.
8. **de Oliveira RB, Graciolli FG, dos Reis LM, et al.** Disturbances of Wnt/β-catenin pathway and energy metabolism in early CKD: effect of phosphate binders. *Nephrol Dial Transplant*. 2013;28:2510–2517.
9. **Cejka D, Marculescu R, Kozakowski N, et al.** Renal elimination of sclerostin increases with declining kidney function. *J Clin Endocrinol Metab*. 2014;99:248–255.
10. **Thambiah S, Roplekar R, Manghat P, et al.** Circulating sclerostin and dickkopf-1 (dkk1) in predialysis chronic kidney disease (CKD): relationship with bone density and arterial stiffness. *Calcif Tissue Int*. 2012;90:473–480.
11. **Cejka D, Jäger-Lansky A, Kieweg H, et al.** Sclerostin serum levels correlate positively with bone mineral density and microarchitecture in haemodialysis patients. *Nephrol Dial Transplant*. 2012;27:226–230.
12. **Cejka D, Herberth J, Branscum AJ, Fardo DW, et al.** Sclerostin and dickkopf-1 in renal osteodystrophy. *Clin J Am Soc Nephrol*. 2011;6:877–882.
13. **Claes KJ, Viaene L, Heye S, Meijers B, d'Haese P, Evenepoel P.** Sclerostin: another vascular calcification inhibitor? *J Clin Endocrinol Metab*. 2013;98:3221–3228.
14. **Brandenburg VM, Kramann R, Koos R, et al.** Relationship between sclerostin and cardiovascular calcification in hemodialysis patients: a cross-sectional study. *BMC Nephrol*. 2013;14:219.
15. **Viaene L, Behets GJ, Claes K, et al.** Sclerostin: another bone-related protein related to all-cause mortality in haemodialysis? *Nephrol Dial Transplant*. 2013;28:3024–3030.
16. **National Kidney Foundation.** K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis*. 2002;39:S1–S266.
17. **Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D.** A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of diet in renal disease study group. *Ann Intern Med*. 1999;130:461–470.
18. **Solak Y, Yilmaz MI, Saglam M, et al.** Mean corpuscular volume is associated with endothelial dysfunction and predicts composite cardiovascular events in patients with chronic kidney disease. *Nephrology (Carlton)*. 2013;18:728–735.
19. **Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC.** Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28:412–419.
20. **Celermajer DS, Sorensen KE, Gooch VM, et al.** Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet*. 1992;340:1111–1115.
21. **Corretti MC, Anderson TJ, Benjamin EJ, et al.** Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol*. 2002;39:257–265.
22. **Durosier C, van Lierop A, Ferrari S, Chevalley T, Papapoulos S, Rizzoli R.** Association of circulating sclerostin with bone mineral mass, microstructure, and turnover biochemical markers in healthy elderly men and women. *J Clin Endocrinol Metab*. 2013;98:3873–3883.
23. **Yamamoto M, Yamauchi M, Sugimoto T.** Elevated sclerostin levels are associated with vertebral fractures in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab*. 2013;98:4030–4037.

24. **Seifert ME, de las Fuentes L, Rothstein M, et al.** Effects of phosphate binder therapy on vascular stiffness in early-stage chronic kidney disease. *Am J Nephrol*. 2013;38:158–167.
25. **Garnero P, Sornay-Rendu E, Munoz F, Borel O, Chapurlat RD.** Association of serum sclerostin with bone mineral density, bone turnover, steroid and parathyroid hormones, and fracture risk in postmenopausal women: the OFELY study. *Osteoporos Int*. 2013; 24:489–494.
26. **Ferreira J, Ferrari GO, Neves KR, et al.** Effects of dietary phosphate on adynamic bone disease in rats with chronic kidney disease–role of sclerostin? *PLoS One*. 2013;8(11):e79721.
27. **Grant SF, Thorleifsson G, Reynisdottir I, et al.** Variant of transcription factor 7-like 2 (tcf7l2) gene confers risk of type 2 diabetes. *Nat Genet*. 2006;38:320–323.
28. **Christodoulides C, Lagathu C, Sethi JK, Vidal-Puig A.** Adipogenesis and Wnt signalling. *Trends Endocrinol Metab*. 2009;20:16–24.
29. **Magoori K, Kang MJ, Ito MR, et al.** Severe hypercholesterolemia, impaired fat tolerance, and advanced atherosclerosis in mice lacking both low density lipoprotein receptor-related protein 5 and apolipoprotein e. *J Biol Chem*. 2003;278:11331–11336.
30. **Kogianni G, Noble BS.** The biology of osteocytes. *Curr Osteoporos Rep*. 2007;5:81–86.
31. **Parhami F, Jackson SM, Tintut Y, et al.** Atherogenic diet and minimally oxidized low density lipoprotein inhibit osteogenic and promote adipogenic differentiation of marrow stromal cells. *J Bone Miner Res*. 1999;14:2067–2078.
32. **Moe S, Drüeke T, Cunningham J, et al.** Definition, evaluation, and classification of renal osteodystrophy: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int*. 2006;69:1945–1953.
33. **Zhu D, Mackenzie NC, Millan JL, Farquharson C, MacRae VE.** The appearance and modulation of osteocyte marker expression during calcification of vascular smooth muscle cells. *PloS One*. 2011;6: e19595.
34. **Koos R, Brandenburg V, Mahnken AH, et al.** Sclerostin as a potential novel biomarker for aortic valve calcification: an in-vivo and ex-vivo study. *J Heart Valve Dis*. 2013;22:317–325.
35. **Hampson G, Edwards S, Conroy S, Blake GM, Fogelman I, Frost ML.** The relationship between inhibitors of the wnt signalling pathway (dickkopf-1(dkk1) and sclerostin), bone mineral density, vascular calcification and arterial stiffness in post-menopausal women. *Bone*. 2013;56:42–47.
36. **Didangelos A, Yin X, Mandal K, Baumert M, Jahangiri M, Mayr M.** Proteomics characterization of extracellular space components in the human aorta. *Mol Cell Proteomics*. 2010;9:2048–2062.
37. **Register TC, Hruska KA, Divers J, et al.** Sclerostin is positively associated with bone mineral density in men and women and negatively associated with carotid calcified atherosclerotic plaque in men from the african american-diabetes heart study. *J Clin Endocrinol Metab*. 2014;99:315–321.
38. **Lomashvili KA, Garg P, Narisawa S, Millan JL, O'Neill WC.** Upregulation of alkaline phosphatase and pyrophosphate hydrolysis: potential mechanism for uremic vascular calcification. *Kidney Int*. 2008;73:1024–1030.
39. **Taliercio JJ, Schold JD, Simon JF, et al.** Prognostic importance of serum alkaline phosphatase in CKD stages 3–4 in a clinical population. *Am J Kidney Dis*. 2013;62:703–710.
40. **Kovesdy CP, Ureche V, Lu JL, Kalantar-Zadeh K.** Outcome predictability of serum alkaline phosphatase in men with pre-dialysis CKD. *Nephrol Dial Transplant*. 2010;25:3003–3011.