Nephrol Dial Transplant (2012) 27: 226–230
doi: 10.1093/ndt/gfr270
Advance Access publication 25 May 2011

Sclerostin serum levels correlate positively with bone mineral density and microarchitecture in haemodialysis patients

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Abstract

Background. Sclerostin is a soluble inhibitor of osteoblast function. Sclerostin is downregulated by the parathyroid hormone (PTH). Here, it was investigated whether sclerostin levels are influenced by intact (i) PTH and whether sclerostin is associated with bone turnover, microarchitecture and mass in dialysis patients.

Methods. Seventy-six haemodialysis patients and 45 healthy controls were included in this cross-sectional study. Sclerostin, Dickkopf-1 (DKK-1), intact parathyroid hormone (iPTH), vitamin D and markers of bone turnover were analysed. A subset of 37 dialysis patients had measurements of bone mineral density (BMD) using dual-energy X-ray absorptiometry and bone microarchitecture using high-resolution peripheral quantitative computed tomography.

Results. Dialysis patients had significantly higher sclerostin levels than controls (1257 pg/mL versus 415 pg/mL, $P < 0.001$). Significant correlations were found between sclerostin and gender ($R = 0.41$), iPTH ($R = -0.28$), 25-hydroxy-cholecalciferol ($R = 0.27$) and calcium ($R = 0.25$). Gender and iPTH remained significantly associated with sclerostin in a multivariate analysis. Sclerostin serum levels were positively associated with BMD at the lumbar spine ($R = 0.46$), femoral neck ($R = 0.36$) and distal radius ($R = 0.42$) and correlated positively mainly with trabecular structures such as trabecular density and number at the radius and tibia in dialysis patients. DKK-1 was related neither to bone measures nor to serologic parameters.

Conclusions. Considering that sclerostin is an inhibitor of bone formation, the observed positive correlations of serum sclerostin with BMD and bone volume were unexpected. Whether its increase in dialysis patients has direct pathogenetic relevance or is only a secondary phenomenon remains to be seen.

Keywords: dialysis; Dickkopf-1; HR-pQCT; renal osteodystrophy; sclerostin; Wnt-signaling

Introduction

Sclerostin and Dickkopf-1 (DKK-1) are soluble inhibitors of wnt/β-catenin (canonical) signalling, a pathway with major importance in bone biology [1]. Deletion or transcriptional attenuation of the sclerostin gene *(SOST)* has been identified as the underlying pathomechanism in patients with sclerosteosis and Van Buchem's disease, two conditions leading to increased bone mass [2, 3]. Knockout of the sclerostin gene or inhibition of sclerostin protein by an anti-sclerostin antibody results in a high bone mass phenotype in mice [4] and cynomolgus monkeys [5] and in massively increased bone formation in humans [6].

Several lines of evidence suggest that the effects of parathyroid hormone (PTH) on bone may be at least partly mediated by regulation of sclerostin expression. Exogenous administration of PTH results in downregulation of osteocytic sclerostin expression in mice [7]. This effect is probably mediated by the parathyroid hormone receptor 1 (PTHR1), as constitutive activation of PTHR1 in osteocytes attenuates sclerostin expression [8]. In humans, a significant inverse correlation between serum sclerostin levels and intact parathyroid hormone (iPTH) has been found [9]. This finding suggests that regulation of sclerostin by PTH is conserved between species.

So far, no deletions of *DKK-1* have been observed in humans, probably because this gene defect is lethal *in utero* [10]. Heterozygous deletion of the *DKK-1* gene leads to a high bone mass in mice [11]. Conversely, increased levels of DKK-1 are found in bone lesions of patients with multiple myeloma and are associated with bone erosions in patients with rheumatoid arthritis [12, 13].

Renal osteodystrophy (ROD) is part of the mineral and bone disorder (MBD) found in patients with chronic kidney disease (CKD). It is associated with high fracture risk and vascular calcification, which both lead to increased morbidity and mortality [14, 15]. ROD can roughly be divided into increased or decreased bone turnover (high or low turnover

ROD) [16]. Recently, high levels of sclerostin were demonstrated to be associated with decreased bone turnover and osteoblast number in dialysis patients [17]. Sclerostin was superior to iPTH in predicting bone turnover state. Whether the effects of sclerostin on bone turnover also translate into clinically significant changes in bone mass and microarchitecture, however, remains unknown.

The aim of the present study was therefore to identify associations between sclerostin or DKK-1 serum levels and PTH, bone density and structure in dialysis patients.

Materials and methods

Study participants

The study was reviewed and approved by the local ethics committee and was conducted in accordance with the Helsinki Declaration. Informed consent was obtained from all participants.

Dialysis patients were on chronic treatment with intermittent haemodialysis or haemodiafiltration thrice weekly at the Medical University Vienna, Austria. Of 84 dialysis patients who were contacted, 76 (all Caucasian) agreed to participate in the study. In a subset of 37 dialysis patients, who had similar demographic characteristics as the whole study population (age 60 ± 14 years; time on dialysis: 4.4 ± 3.6 years; gender distribution: 54% male), high-resolution peripheral quantitative computed tomography (HR-pQCT) and dual-energy X-ray absorptiometry (DXA) measurements were performed. Serum samples were collected at the beginning of the dialysis session, after a 3-day dialysis-free interval, and then stored at −80°C until further analysis

Sex- and age-matched healthy controls were recruited via the Department of Clinical Pharmacology and compensated for the expenditure of time. All controls were required to have normal serum creatinine (<1.2 mg/dL) and iPTH serum levels (<65 pg/mL). Subjects with a history of pathologic fracture, established or suspected osteoporosis, past or present bisphosphonate therapy or known derangements of calcium homeostasis were excluded. Of 56 age-matched volunteers who were screened, 11 subjects were excluded because of elevated serum creatinine levels or iPTH or underlying medical conditions.

Sclerostin and DKK-1 measurements

Sclerostin was measured by an enzyme-linked immunosorbent assay (ELISA) as described previously [17]. Briefly, microtiter plates (MaxiSorp; Nunc-Thermo Fisher scientific, Waltham, MA) were coated with 100 µL of monoclonal anti-sclerostin antibody (# MAB1406; R& D Systems, Minneapolis, MN) at a concentration of 2 µg/mL in carbonate buffer (pH 9.6) and were incubated overnight at 4°C. Plates were washed with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (Sigma-Aldrich, Vienna, Austria) and blocked with PBS containing 0.05% Tween and 1% human serum albumin (Sigma-Aldrich). Fifty microliter of serum was loaded per well, incubated overnight at 4°C, washed and incubated for 1 h at 37°C followed by incubation at 4°C for 1 h with a biotinylated polyclonal anti-sclerostin antibody (# BAF 1406; R& D Systems) diluted to a concentration of 0.5 µg/mL in dilution buffer. Wells were washed with PBS/Tween, followed by the addition of 100 µL of a 1:20 000 dilution of streptavidin–horseradish peroxidase (Endogen-Thermo Fisher Scientific). Color development was achieved using a TMB substrate system (Chemicon-Millipore, Billerica, MA). Serial dilutions of recombinant human sclerostin (#1406-ST; R&D Systems) were used to establish a standard curve. The intraassay and interassay coefficient of variation was 7.5 and 6.3%, respectively. DKK-1 was measured using a commercially available ELISA (# BI-20412; Biomedica, Vienna, Austria) according to the manufacturer's instructions.

Measurements of bone turnover markers, vitamin D status, intact PTH and electrolytes

Bone alkaline phosphatase (bAP) was measured using an immunosorbent enzyme-linked assay (Metra Biosystems, Behring Diagnostic, Eschborn, Germany). Osteocalcin (OC), c-telopeptide pyridinoline cross-links of type I collagen (CTX, CrossLaps) and 25-hydroxy-cholecalciferol (25-OH-D) were measured by the use of electro-chemiluminescence (Modular and Elecsys Systems, Roche, Switzerland). Calcitriol (1,25-$(OH)_2$-D) was analysed after chromatographic separation by radioimmunoassay (DiaSorin). iPTH was measured using a 'second generation' assay (Elecsys PTH intact, Roche, Switzerland). Normal ranges were the following: bAP—men: 6–30 ng/mL and women: 6–26 ng/mL; OC—men: 14–34 ng/mL and women: 14–46 ng/mL; iPTH—15–65 pg/mL; CTX—men: 0.08–0.35 ng/mL and women: 0.09–0.44 ng/mL; 25-OH-D—28–107 nmol/L; 1,25-$(OH)_2$-D—25–66 pg/mL; calcium—2.2–2.65 mmol/L; phosphate: 0.91–1.45 mmol/L. All measurements were performed according to Good Laboratory Practice (GLP) standards.

High-resolution peripheral quantitative computed tomography

Trabecular and cortical microarchitecture was determined by HR-pQCT at the distal radius and tibia, as reported previously by Boutroy *et al.* (XtremeCT; Scanco Medical AG, Bruetisellen, Switzerland) [18]. Briefly, 110 CT slices were obtained at each site, from which a 9-mm long 3D-volume was reconstructed. A threshold-based algorithm distinguished trabecular structures from cortical bone. The data were converted into histomorphometric parameters as described by Laib *et al.* [19]. Cortical thickness [(Ct.Th (mm)] was defined as the mean cortical volume divided by the outer surface. Trabecular density [Dtrab (mg HA/cm^3)], cortical density [Dcort (mg HA/cm^3)] and total density [Dtot (mg HA/cm^3)] were determined as mean densities within each region. The bone volume fraction (BV/TV [%]) was calculated by relating trabecular density to an arbitrary fully mineralized bone block of 1200 mgHA/cm^3. To correct for partial volume effects, a thickness-independent structure extraction was employed for measurement of trabecular number (TbN/mm) [20]. Trabecular thickness (TbTh) was calculated as BV/TV/TbN. Intra-individual distribution of separation (Tb1/NSD), which is a measure of the heterogeneity of trabecular structure, was determined as the standard deviation of the Tb1/NSD.

Bone mineral density by DXA

Areal bone mineral density (aBMD) measurements were performed with dual-energy X-ray absorptiometry on a QDR-4500 scanner (Hologic, Waltham, MA), using the manufacturer's recommended standard procedures for the posteroanterior lumbar spine at L_1–L_4, and the proximal femur. The aBMD is given in gram per square centimeter.

Statistical analysis

Data are presented as means ± SDs unless indicated otherwise. Positively skewed parameters were log-transformed. Comparisons between two groups were performed using the Mann–Whitney *U*-test. Associations were analysed using the Pearson's correlation analysis, followed by linear regression analysis. *P*-values <0.05 were considered statistically significant. Statistical analysis was performed using the PASW 18 software (SPSS Inc., Chicago, IL).

Results

Sclerostin and DKK-1 serum levels in dialysis patients and healthy controls

Demographic data of dialysis patients are given in Table 1. Forty-five sex- and age-matched healthy volunteers (57% male; age 57 ± 8 years; s-creatinine 0.9 ± 0.1 mg/dL) served as controls.

Serum levels of iPTH, calcium, phosphate, vitamin D status and serologic markers of bone turnover in dialysis patients and controls are shown in Table 2. The mean sclerostin level was 3-fold higher in dialysis patients than in healthy controls. Sclerostin levels were significantly higher in male than in female dialysis patients (1532 ± 1067 pg/mL versus 837 ± 829 pg/mL; $P < 0.0001$). There was a trend for higher sclerostin levels in healthy men (449 ± 259 pg/mL) than in women (368 ± 263 pg/mL), which did not reach statistical significance. No statistically significant differences in DKK-1 serum levels were found between dialysis patients and healthy controls, male and female healthy controls or male and female dialysis patients.

Correlations between sclerostin, DKK-1, iPTH, markers of bone turnover and demographics

On bivariate analysis, sclerostin was positively associated with gender ($R = 0.41$, $P < 0.001$), 25(OH)D_3 ($R = 0.27$, $P < 0.05$) and calcium ($R = 0.25$, $P < 0.05$) and inversely associated with iPTH ($R = -0.28$, $P < 0.05$). Sclerostin levels did not correlate with bAP, OC, CTX, age or body mass index (BMI). No significant correlations were found for DKK-1 in dialysis patients. In healthy controls, neither sclerostin nor DKK-1 correlated significantly with any laboratory parameter.

On multivariate analysis with iPTH, calcium, 25(OH)D_3 and gender as independent variables and sclerostin as the dependent variable, there was a significant association registered for iPTH ($\beta = -0.24$, $P < 0.05$) and gender ($\beta = 0.41$, $P < 0.001$).

Association between sclerostin, DKK-1 and bone mineral density and microarchitecture

Sclerostin levels correlated significantly with bone mineral density (BMD) at the femoral neck, the lumbar spine and the radius (Table 3). A significant association was also found between femoral neck BMD and the BMI (Table 3). Neither DKK-1 nor any other serologic parameter correlated significantly with BMD at any site. Sclerostin was found to be highly correlated with parameters of bone microarchitecture measured by HR-pQCT (Table 4). At both sites, tibia and radius, there was a significant positive correlation between sclerostin and BV/TV and Dtrab. There were significant positive correlations between sclerostin and TbN at the radius and Dtot, CtTh and TbTh at the tibia. A multivariate analysis was performed for each HR-pQCT parameter significantly correlating with sclerostin serum levels, including, gender, age and BMI as further independent variables (Table 5). All HR-pQCT parameters remained significantly correlated with sclerostin serum levels, whereas age and BMI correlated only with cortical parameters of the tibia and gender correlated only with parameters of the radius in multivariate analysis.

Table 1. Demographic characteristics of haemodialysis patients[a]

n	76
Age (years)	62 ± 16
Men (%)	60
Women (%)	40
Dialysis (years)	3.6 ± 4.5
BMI (kg/m^2)	25.9 ± 5.6
Diabetes mellitus (%)	29
Previous transplantations (%)	26
Calcium supplement (%)	30
Active vitamin D treatment (%)	34
Calcimimetic (%)	15
Bicarbonate (mmol/L)	24 ± 2.5

[a]Percentages or mean ± SD values are given.

Discussion

Three major phenomena were observed in the present study: firstly, sclerostin serum levels are increased in patients with end-stage renal disease. Secondly, high levels of sclerostin correlate with increased BMD in dialysis patients. Thirdly, higher sclerostin levels correlate with better bone microarchitecture in this population.

The cause for the high sclerostin levels in dialysis patients is unknown. Due to its molecular mass of 22KD, sclerostin might be cleared by the kidney and thus accumulate in the presence of renal failure. So far, there is no data on the metabolism of sclerostin. As sclerostin expression is decreased by mechanical loading of the skeleton [21], low physical activity, which is often found in patients with renal failure, might add to the elevation of sclerostin serum levels. This association has been noted before in immobilized patients [22]. Furthermore, in uraemia, the skeleton is partially resistant to the actions of PTH. PTH resistance is explained by PTH receptor downregulation and accumulation of potentially antagonistic PTH fragments. However, other mechanisms for the skeletal resistance to PTH in CKD must also be taken into consideration [16, 23]. Whatever the exact mechanism, the reduced effect of PTH on bone might decrease inhibition of the *SOST* gene, which would stimulate the expression of sclerostin. Even high levels of PTH may then be unable to suppress sclerostin synthesis. However, DKK-1 synthesis is similarly downregulated by PTH [24], and PTH resistance should therefore also increase DKK-1 serum levels. The absence of such an increase makes an influence of PTH resistance on sclerostin or DKK-1 synthesis unlikely. Since DKK-1 levels were similar between controls and dialysis patients, DKK-1 seems not do be metabolized by the kidney. Furthermore, no correlations were found between DKK-1 serum levels and PTH or markers of bone turnover. Hence, DKK-1 does not seem to influence bone status in end-stage renal disease.

Surprisingly, and in contrast to our expectations, patients with higher sclerostin serum levels also had a higher BMD measured by DXA. Furthermore, trabecular bone volume and trabecular BMD were positively associated

Table 2. Intact PTH, serologic bone markers, sclerostin and DKK-1 in haemodialysis patients ($n = 76$) and healthy controls ($n = 46$)[a]

	iPTH (pg/mL)	OC (ng/mL)	bAP (U/L)	CTX (ng/mL)	25 (OH)D_3 (nmol/L)	1,25 (OH)$_2D_3$ (pmol/L)	Calcium (Ca) (mmol/L)	Phosphate (PO_4) (mmol/L)	Sclerostin (pg/mL)	DKK-1 (pmol/L)
HD (mean ± SD)	238 ± 250	258 ± 278	31 ± 17	2.1 ± 1.2	44 ± 20	11 ± 8	2.2 ± 0.2	1.8 ± 0.5	1257 ± 1032	102 ± 68
Control (mean ± SD)	47 ± 7[b]	20 ± 6[b]	18 ± 4[b]	0.25 ± 0.1[b]	54 ± 15	47 ± 16[b]	2.5 ± 0.1[b]	1.1 ± 0.1[b]	415 ± 227[b]	106 ± 45

[a]1,25(OH)$_2D_3$, calcitriol.
[b]Significant difference between the groups.

Table 3. Bivariate correlation analysis between BMD and sclerostin, DKK-1, iPTH, dialysis vintage, age, BMI and serologic markers of bone turnover in dialysis patients ($n = 37$)[a]

	Femoral neck (g/cm^2)		Lumbar spine (g/cm^2)		Radius (g/cm^2)	
	R	P	*R*	P	*R*	P
Duration of dialysis	0.01	n.s.	0.15	n.s.	−0.28	n.s.
Age	−0.02	n.s.	−0.15	n.s.	−0.19	n.s.
BMI	0.40	0.02	0.28	n.s.	0.35	n.s.
Sclerostin	0.36	0.04	0.46	0.006	0.42	0.04
DKK-1	0.06	n.s.	−0.09	n.s.	−0.23	n.s.
iPTH	−0.19	n.s.	−0.14	n.s.	0.07	n.s.
Osteocalcin	−0.27	n.s.	−0.25	n.s.	0.09	n.s.
CTX	−0.27	n.s.	−0.20	n.s.	0.13	n.s.
bAP	−0.22	n.s.	−0.20	n.s.	−0.16	n.s.

[a]n.s., not significant.

Table 4. Bivariate correlation analysis between sclerostin and different parameters of HR-pQCT, according to anatomical site in dialysis patients ($n = 37$)

	Sclerostin	
	R	P
Radius		
Dtot	0.19	n.s.
Dcort	−0.09	n.s.
Ct.Th	0.64	n.s.
Dtrab	0.50	0.002
BV/TV	0.50	0.002
TbN	0.51	0.002
TbTh	0.33	0.06
Tb1/NSD	−0.21	n.s.
Tibia		
Dtot	0.41	0.01
Dcort	0.13	n.s.
Ct.Th	0.33	0.049
Dtrab	0.48	0.003
BV/TV	0.47	0.003
TbN	0.32	0.06
TbTh	0.50	0.002
Tb1/NSD	−0.05	n.s.

Table 5. Multivariate analysis between HR-pQCT as the dependent and sclerostin, age, gender and BMI as independent variables in dialysis patients ($n = 37$)[a]

	Sclerostin		Age		Gender		BMI	
	β	P	β	P	β	P	β	P
Radius								
Dtrab	0.37	0.04	−0.08	n.s.	−0.32	0.06	0.08	n.s.
BV/TV	0.38	0.04	−0.09	n.s.	−0.31	0.07	0.07	n.s.
TbN	0.38	0.03	−0.17	n.s.	−0.37	0.02	0.19	n.s.
Tibia								
Dtot	0.51	0.01	−0.30	0.08	0.06	n.s.	0.37	0.03
Ct.Th	0.41	0.02	−0.50	0.004	−0.10	n.s.	0.38	0.02
Dtrab	0.43	0.02	0.09	n.s.	−0.06	n.s.	0.27	n.s.
BV/TV	0.42	0.02	0.07	n.s.	−0.05	n.s.	0.28	n.s.
TbTh	0.61	<0.01	−0.07	n.s.	0.22	n.s.	0.13	n.s.

[a]Only HR-pQCT parameters yielding significant correlations with sclerostin serum levels were included in the multivariate analysis. β = corrected correlation coefficient.

with sclerostin based on HRp-QCT measurements. Patients with higher DXA-BMD also had a better quality of trabecular bone, indicating that the increased BMD was predominantly attributable to the trabecular compartment. This finding contradicts current knowledge of sclerostin function derived from patients with sclerosteosis/Van Buchem's disease, murine sclerostin knock-out models as well as the effects of the treatment with an anti-sclerostin antibody in rodents, primates and humans [2–4, 6, 25]. It also contradicts the results on low BMD and bone volume in mice overexpressing sclerostin [26]. However, two groups reported a positive correlation between BMD and sclerostin messenger RNA levels extracted from bone biopsy specimens of postmenopausal women and osteoporotic men [27, 28]. Moreover, Mödder *et al.* [29] recently found that sclerostin serum levels were positively associated with total body bone mineral content, lumbar BMD and microarchitectural parameters of the spine, femoral neck and radius in a large study encompassing >650 subjects, which is in line with the findings presented here. The reason for this positive association between sclerostin and BMD and bone structure is unknown. A possible explanation could be that serum levels of sclerostin, which is produced by osteocytes [30], reflect osteocyte number. A higher bone mass would result in more osteocytes and therefore higher sclerostin levels. This could also explain the higher sclerostin levels found in male compared to female dialysis patients, but this gender difference could also result from an influence of sex hormones on sclerostin production. On the other hand, sclerostin serum levels were reported to increase with age, irrespective of bone mass, and were associated with bone mass only in older individuals, but not in subjects under the age of 40 years [29]. This argues against the hypothesis that sclerostin serum levels are merely a function of osteocyte number but suggests a mechanism related to aging. A more speculative explanation could be that high sclerostin levels lead to decreased osteoclast activity and bone resorption. This could result from downregulation of β-catenin, a protein which is thought to be necessary for PTH-mediated osteoclast activation via the OPG/RANKL/RANK system, as proposed by Romero *et al.* [31]. Indeed, reduced osteoblast activity in dialysis patients with high sclerostin levels has been recently demonstrated [17]. Sclerostin was associated with decreased activation frequency, bone formation rate and the number of osteoblasts on bone surface. Since osteoblast activity is a prerequisite for bone resorption by osteoclasts, a reduction of bone formation consequently also reduces bone resorption. These findings might therefore further support the hypothesis that sclerostin counteracts PTH-induced high turnover ROD and possibly protects from excessive bone resorption.

The study has several limitations. The number of patients is rather small and selection bias might be present. This could explain the lack of associations between age or BMI and bone volume. Furthermore, due to its cross-sectional design, a causal relationship cannot be stated. Whether sclerostin truly protects from bone loss in dialysis patients remains to be determined in larger and prospective studies.

Taken together, these findings highlight an important difference between sclerostin as a target for therapeutic intervention and sclerostin serum levels as marker for bone biology. Although an anti-sclerostin antibody showed promising results in a Phase I study and is being tested further [6], it is currently unclear whether endogenous sclerostin levels influence the response to this type of treatment in the general population and even more so in patients with ROD.

In conclusion, the finding of high sclerostin levels in dialysis patients with higher bone volume and density was unexpected and, regarding previous publications on the effect of sclerostin on bone, is counterintuitive. However, whether sclerostin has a true protective effect or the high serum levels are merely a secondary phenomenon remains to be determined.

Conflict of interest statement. None declared.

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Received for publication: 17.9.10; Accepted in revised form: 18.4.11