The Relationship between Serum Sclerostin Levels and Bone Mineral Disorders and Vascular Calcification in Hemodialysis Patients

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Abstract

BACKGROUND: Sclerostin is produced by osteocytes and has been shown to down-regulate the synthesis of many markers of bone formation by osteogenic cells.

AIM: The aim of this study is to investigate the relationship between serum sclerostin levels and bone mineral disorders and vascular calcification in hemodialysis (HD) patients.

METHODS: This is a cross-sectional study of 70 patients with end-stage renal disease (ESRD) on regular HD for at least 6 months, Theodor Bilharz Research Institute, Giza, Egypt. Twenty-five subjects who matched the ages, genders, and demographics of the study patients were included as a control group. All patients and control groups included in the study underwent a full through history and clinical examination. Serum calcium, phosphorus, alkaline phosphatase (ALP), and intact PTH (iPTH) levels were measured. Serum sclerostin was measured by an ELISA. Bone mineral densitometry (BMD) measurements (g/cm²) were determined by dual-energy X-ray absorptiometry. CT scan was done to detect the presence or absence of vascular calcification and transthoracic echocardiogram to detect the presence or absence of valvular calcification.

RESULTS: The mean serum sclerostin levels was a statistically significant high in the HD patients when compared with the control group (156.8 ± 121.4 vs. 29.38 ± 0.84, p = 0.0001) and statistically significant high mean ALP in the HD patients when compared with the control group (147.2 ± 94.3 vs. 38.8 ± 23.4, p = 0.0001). The mean BMD was statistically significant low in the HD patients when compared with the controls (0.839 ± 0.086 g/cm² vs. 1.306 ± 0.153 g/cm², p = 0.0001). The mean serum sclerostin levels were statistically significant high in the HD patients with vascular and valvular calcification when compared with HD patients without calcification. Using spearman correlation coefficient analysis, there was statistically significant negative correlations between serum sclerostin levels and iPTH (r = −0.362, p = 0.0021), ALP (r = −0.301, p = 0.0114), and BMD (r = −0.469, p = 0.0278), and there was a statistically significant positive correlation between serum sclerostin levels and phosphate (r = 0.5629, p = 0.0001). Independent predictors of BMD in HD patients were determined using multi-variate regression analysis. Sclerostin levels, iPTH, ALP, and age were found to be independent predictors of BMD.

CONCLUSION: High sclerostin levels in patients with ESRD on HD were associated with high risk of vascular and valvular calcification and were independent predictors of low BMD in such population.

Introduction

Sclerostin is a 190-residue secreted glycoprotein that is predicted to contain a cysteine-knot motif and is a member of the DANT/Cerberus protein family [1]. Sclerostin is produced by osteocytes and has been shown to down-regulate the synthesis of many markers of bone formation by osteogenic cells [2], [3], [4], thereby indicating the importance of sclerostin in the regulation of bone formation.

Chronic kidney disease-mineral bone disorder (CKD-MBD) is defined by abnormalities in mineral and hormone metabolism that, decrease bone health and increase soft tissue calcification [5], [6]. Several studies have shown that these changes are associated with increased fracture rates and cardiovascular complications, including decreased vascular wall elasticity, vascular calcification, and left ventricular hypertrophy [7], [8].

Wnt/β-catenin signaling plays a key role in various biological processes, including cell proliferation, cell migration, and differentiation [9]. Wnt ligands bind to cell surface receptor complexes that are comprised of Frizzled and low-density lipoprotein receptor-related protein (LRP) family members [9], [10]. Sclerostin is a soluble Wnt inhibitor, Wnt ligands are blocked from binding the LRP-5/6-Frizzled receptor complex. Since Wnt signaling encompasses vascular development and endothelial cell specification as well as regulation of bone
modeling and remodeling, it appears prototypic for the crosstalk within the bone-vascular axis [11], [12], [13].

Taken together, the above findings are consistent with a hypothesis that increased serum Wnt antagonist levels induce low bone turnover, thereby indirectly increasing the propensity for vascular calcification. In suspect of this hypothesis, the serum sclerostin levels of hemodialysis (HD) patients have been found to be positively associated with coronary and aortic valve calcifications, and higher expression of sclerostin has been observed close to calcified areas in explanted aortic valves from dialysis patients beyond bone mineralization [14], [15]. Moreover, the results of a recent study of prevalent HD patients indicated that serum sclerostin is an independent predictor of mortality [16]. The aim of the present study was to investigate the association of circulating concentrations of sclerostin with MBD and vascular calcification in hemodialysis patients.

**Methods**

**Study population**

This is a cross-sectional study of 70 patients with end-stage renal disease (ESRD) on regular HD for at least 6 months in the Nephrology department, Theodor Bilharz Research Institute, Cairo, Egypt. All patients received three, 4-h dialysis sessions/week. Patients with congestive heart failure, malignancy and sepsis, and/or liver, autoimmune, and chronic inflammatory diseases were excluded from the study.

Twenty-five subjects who matched the ages, genders, and demographics of the study patients were included as a control group.

All patients and control groups included in the study underwent a full through history and clinical examination. Demographic and clinical characteristics, including age, gender, body mass index (BMI), blood pressure (BP), duration of dialysis, and etiology of ESRD, were recorded.

Nineteen HD patients received quadruple antihypertensive medications (angiotensin converting enzyme inhibitors [ACEI] or angiotensin receptor blockers [ARBs], calcium channel blocker [amlodipine], beta blocker [atenolol] and vasodilators [hydralazine]), 17 received triple antihypertensive medications (ACEI or ARBs, amlodipine and atenolol), 14 received two antihypertensive medications (ACEI or ARBs and amlodipine), 13 received monotherapy (ACEI or ARBs or amlodipine or hydralazine) and 7 HD patients did not receive antihypertensive medications. Patients were prescribed treatments including CaCO₃ (21 patients), sevelamer-HCl (18 patients), calcitriol (46 patients), cinacalcet (16 patients), antplatelet agents (34 patients), warfarin (3 patients), and erythropoietin (43 patients).

**Laboratory parameters**

**Blood sample collection**

Fasting blood samples were collected in tubes from the patients and control groups after proper disinfection. The tubes were centrifuged at 4000 rpm (10 min) to obtain plasma and serum. The plasma and serum samples were kept at −80°C until analysis of sclerostin. Routine examinations included complete blood picture using tripotassium EDTA-based anticoagulated blood samples (Sysmex K-1000 auto analyzer, Block Scientific, USA) within 30 min of sampling [17], kidney function tests (serum creatinine, urea, sodium and potassium, and uric acid), random blood sugar, total cholesterol, low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), and triglycerides (TGs) using standard methods. Intact PTH (iPTH) level was measured by a radioimmunometric assay (Scantibodies, Santee, CA): normal range is 14–66 pg/ml; intra- and inter-assay coefficients of variation are <5 and <7%, respectively. Blood chemistry measurements for calcium, phosphorus alkaline phosphatase (ALP) were done using automated techniques. C-reactive protein (C-RP) was measured using a BN2 model nephelometer (Dade-Behring, Germany) [18].

Serum bone alkaline phosphatase (BAP) was measured, as a marker of bone formation, using an enzyme immunoassay kit (Alkphase-B; Metra Biosystem) [19]. The assay detected 0.7–140 U/L of BAP. The intra- and inter-assay coefficients of variation were 2.3 and 3.1%, respectively [20].

**Measurement of serum sclerostin**

Sclerostin was measured by an ELISA. 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 at 4°C overnight. Plates were washed with 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 microliters 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,
Waltham, MA). Color development was achieved with the tetramethylbenzidine substrate system (Chemicon-Millipore, Billerica, MA). Serial dilutions of recombinant human sclerostin (1406-ST; R&D Systems) were used to establish a standard curve. Normal values in 44 healthy volunteer’s age 19–76 years are between 131 and 1156 pg/ml; intra- and inter-assay coefficients of variation are 7.5 and 6.3%, respectively [21].

Bone mineral densitometry (BMD) measurements

BMD measurements (g/cm²) were determined for the anteroposterior lumbar spine (L1–L4) and mean of proximal right and left femur (total and subregions) by dual-energy X-ray absorptiometry, using LUNAR Prodigy Model (Lunar Corp., Madison, WI, USA) according to standard protocol. Quality-control procedures were carried out in accordance with the manufacturer’s recommendations as described previously [22]. BMD values were classified according to the WHO criteria: a T-score between _1 and _2.5 is indicative of osteopenia, whereas a T-score of _2.5 and below reflects osteoporosis, and a T-score of _1.0 and above is considered normal [23].

MDCT scan to detect vascular calcification and transthoracic echocardiogram

Vascular calcification was evaluated using MDCT scan to detect the presence or absence of vascular calcification along the whole course of aorta on the thorax, abdomen if present; and transthoracic echocardiogram to detect the presence or absence of valvular calcification.

Statistical analysis

Data were presented as mean ± SD. Or number of cases and percentages. Comparisons between variables in the study groups were performed using unpaired two-tailed Student’s t-tests (MedCalc Statistical Software). Correlation between sclerostin levels and various variables was done using Spearman correlation coefficient analysis (MedCalc Statistical Software). Independent predictors of BMD in HD patients were determined using multi-variate regression analysis (MedCalc Statistical Software). p < 0.05 was considered statistically significant.

Results

The baseline demographic and clinical characteristics of the study population are shown in Table 1. The mean age of the patients was 54.9 ± 14.46 years, and the mean dialysis duration was 65.6 ± 36.8 months. The main causes of ESRD were diabetes mellitus (DM), hypertension, glomerulonephritis, obstructive uropathy, and adult polycystic kidney disease.

Laboratory parameters of the study population are shown in Table 2. When compared with the control group, HD patients had higher levels of serum creatinine, phosphorus, and PTH. There was a statistically significant low mean value of 25-OH Vitamin D₃ in HD patients when compared with the controls (44.56 ± 17.35 vs. 55.76 ± 23.75 respectively, p = 0.0141).

Table 2: Laboratory parameters of hemodialysis patients and control group

| Variables | HD (n = 70) | Control (n = 25) | p-value |
|-----------|------------|-----------------|---------|
| S. creatinine (mg/dl) | 8.3 ± 1.8 | 8.95 ± 0.60 | 0.0001 |
| S. calcium (mg/dl) | 8.8 ± 0.9 | 9.3 ± 0.60 | 0.1046 |
| S. phosphorus (mg/dl) | 5.7 ± 1.1 | 3.63 ± 0.55 | 0.0001 |
| S. PTH (pg/ml) | 269.87 ± 63.76 | 34.7 ± 4.68 | 0.0001 |
| T.cholesterol (mg/dl) | 154.5 ± 17.8 | 119.7 ± 11.9 | 0.0001 |
| HDL-C (mg/dl) | 126.1 ± 30.2 | 98.8 ± 24.4 | 0.0037 |
| T.sclerostin (pmol/L) | 156.8 ± 121.4 | 290.38 ± 0.84 | 0.0001 |
| Total ALP | 147.2 ± 94 | 338.8 ± 23.4 | 0.0001 |
| S. BAP (U/L) | 119.2 ± 23.2 | 88.9 ± 19.1 | 0.0001 |
| Vit. D₃ (IU/L) | 11.4 ± 1.5 | 14.5 ± 1.20 | 0.0001 |
| S. glucose (mg/dl) | 127.8 ± 25.7 | 118.5 ± 20.6 | 0.0001 |
| S. phosphorus (mg/dl) | 5.7 ± 1.1 | 3.63 ± 0.55 | 0.0001 |
| S. calcium (mg/dl) | 8.8 ± 0.9 | 9.3 ± 0.60 | 0.1046 |
| S. creatinine (mg/dl) | 8.3 ± 1.8 | 8.95 ± 0.60 | 0.0001 |
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| HDL-C (mg/dl) | 126.1 ± 30.2 | 98.8 ± 24.4 | 0.0037 |
| S. BAP (U/L) | 119.2 ± 23.2 | 88.9 ± 19.1 | 0.0001 |

The mean serum sclerostin levels was a statistically significant high in the HD patients when compared with the control group (156.8 ± 121.4 vs. 29.38 ± 0.84, p = 0.0001) and statistically significant high mean ALP in the HD patients when compared with the control group (147.2 ± 94.3 vs. 38.8 ± 23.4, p = 0.0001). The mean BMD was statistically
significant low in the HD patients when compared with the controls (0.839 ± 0.086 g/m² vs. 1.306 ± 0.153 g/m² respectively, p = 0.0001).

The mean serum sclerostin levels were statistically significant high in the HD patients with vascular and valvular calcification when compared with HD patients without calcification.

The mean values of serum sclerostin were higher in male HD patients than female HD patients and high in patients with type 11 DM when compared with non-diabetic patients.

Spearman correlation coefficient analysis of serum sclerostin levels with MBD markers in HD patients demonstrated statistically significant negative correlations between serum sclerostin levels and iPTH (r = –0.362, 95% CI for r –0.550–0.139, p = 0.0021; Figure 1), ALP (r = –0.301, 95% CI for r –0.500–0.0706, p = 0.0114; Figure 2), and BMD (r = –0.469, 95% CI for r –0.508–0.0298, p = 0.0278; Figure 3); there was a statistically significant positive correlation between serum sclerostin levels and phosphate and age (r = 0.5829, 95% CI for r 0.4031–0.7193, p = 0.0001; Figure 4).

Discussion

The present study demonstrated that mean values of serum sclerostin levels were significantly high in HD patients when compared with the control group and that serum sclerostin levels correlated negatively with iPTH, ALP and BMD and positively with Ps.

Sclerostin is mainly secreted by osteocytes, and it decreases bone formation by inhibiting the terminal differentiation of osteoblasts and promoting
their apoptosis. Sclerostin blocks Wnt signaling pathway in osteoblasts by binding to LRP-5/6 receptors [13]. Wnt signaling and bone morphogenetic protein are involved in osteoblastogenesis and bone formation [24]. Sclerostin is also expressed in several non-skeletal tissues, especially in the vasculature, but whether this is a cause or a consequence of vascular calcification is yet to be determined [25].

CKD-MBD is a syndrome of bone disorders and is associated with disturbances in calcium and phosphorus homeostasis in association with hyperparathyroidism [26]. PTH is known to bind directly to cells of the osteoblast/osteocyte lineage and promote increased RANKL expression, which leads to osteoclast activation.

In our work, mean value of serum sclerostin levels was significantly high in HD patients when compared with the control group. The molecular size of sclerostin is approximately 22.5 kDa, and filtered through glomeruli and reabsorbed by renal tubular cells in a normal kidney. A decrease in glomerular filtration rate (GFR) and/or increased sclerostin production by osteocytes in CKD patients may lead to high serum sclerostin due to accumulation of sclerostin in the serum. There is a controversy for the mechanism involved in increased serum sclerostin levels in CKD patients. For example, Cejka et al. reported that renal elimination of sclerostin increased regardless decreased renal function and urinary sclerostin excretion increased with declining GFR [27]. Furthermore, increased extraskeletal production of sclerostin may be one of the causes of its high serum levels. For example, Roforth et al. reported that bone mRNA levels did not increase in older people regardless of their high serum sclerostin levels [28]. Circulating sclerostin levels have been found to be increased in several cohorts of CKD patients. Cejka et al. were the first to report finding increased serum sclerostin levels in a cross-sectional study of dialysis patients [29], and their finding has been validated by other studies in ESRD patients [25], [21], [30], [31], [32], [33]. Pelletier et al. reported that higher serum sclerostin levels were starting at CKD Stage 3 [31]. However, the degree to which serum sclerostin levels reflect changes in expression versus accumulation in individuals with impaired renal function is not fully understood. A previous study examining the local expression of sclerostin across stages of CKD revealed that highest osteocyte expression occurred at initial stages of the disease [34]. Although this study examined the number of sclerostin-positive osteocytes rather than absolute protein levels, the resulting data suggest that sclerostin accumulation in the serum is at least partially due to increased osteocyte production. Moreover, the rapid restoration of serum sclerostin to the normal range post-transplant suggests that decreased renal clearance may also be responsible for accumulation at least in late stages [35].

The present study demonstrated that serum sclerostin levels correlated negatively with iPTH and correlated positively with phosphate. Our results are consistent with many previous study. Delanaye et al. reported finding that plasma sclerostin levels in HD patients were positively associated with their phosphate levels and negatively associated with their PTH levels [36]. A recent study demonstrated an increased serum sclerostin levels and that serum sclerostin were closely associated with serum phosphate and FGF23 levels and treatment with Vitamin D in HD patients with low serum PTH levels [37]. Furthermore, several previous studies showed a significant and negative correlation between serum sclerostin levels and serum iPTH in non-CKD [38], [39] and HD patients [29], [21].

Our study showed that there was a significant negative correlation between serum sclerostin levels and BMD. These findings are consistent with the hypothesis that, as would be expected of a negative regulator of bone formation, higher serum sclerostin levels promote low bone turnover, which leads to loss of bone mass over time. Our results are consistent with many previous studies. A cross-sectional study of 60 dialysis patients showed that their serum sclerostin levels were inversely correlated with the patients’ bone formation rates [32]. A subsequent prospective study of 81 dialysis patients found that higher sclerostin serum levels predicted greater loss of bone mass over a 1-year period [33]. However, in contrary to our results, Cejka et al. [29] observed positive correlations of serum sclerostin with BMD and considering that sclerostin is an inhibitor of bone formation, the observed results were unexpected. Whether its increase in dialysis patients has direct pathogenetic relevance or is only a secondary phenomenon remains to be seen.

In this work, we founded that the mean values of serum sclerostin were high in HD patients with vascular and valvular calcification when compared with HD patients without calcification. Recent study findings indicate that sclerostin is involved in vascular disease. An in vitro and rodent study by Zhu et al. could show that sclerostin is upregulated in experimental models of vascular calcification [40]. We extend these findings for a potential linkage between serum sclerostin levels and vascular and valvular calcification in humans with ESRD. Our results are consistent with recent human study results indicating an association of sclerostin expression with non-uremic aortic valve calcification [41] and Brandenburg et al. [14] found a strong association of sclerostin with calcifying aortic heart valve disease in hemodialysis patients and sclerostin is locally produced in aortic valve tissue adjacent to areas of calcification. Therefore, sclerostin appears to be a promising future research target in CKD-MBD offering potential therapeutic perspectives [42], [43].

In the present study, serum sclerostin levels were high in male HD patients when compared with female HD patients and were significantly correlated with age. Furthermore, serum sclerostin levels were high in diabetic HD patients when compared with...
non-diabetic HD patients. Our results are consistent with many previous studies. Serum sclerostin levels were significantly correlated with age and were higher in male than female patients with Stage 3b and 4 CKD [44]. Larger bone mass in males, hormonal effect (a role of estrogen in reducing sclerostin levels), skeletal remodeling and imbalances in vascular remodeling with aging in males might be responsible for the observed differences [45], [46], [47]. In non-CKD patients, serum sclerostin levels have been reported to be higher in males, in patients with higher age, and in patients with Type 2 diabetes [39], [45], [46], [48], [49]. In another study of HD patients, serum sclerostin was also higher in males than females. However, age was not associated significantly with serum sclerostin levels, and serum sclerostin levels were not different between patients with and without diabetes [32].

**Conclusion**

This study demonstrated significant high serum sclerostin levels in HD patients and high serum sclerostin levels were associated with high risk of vascular and valvular calcification and were independent predictors of low BMD in such population.

**References**

1. Winkler DG, Sutherland MK, Geoghegan JC, Yu C, Hayes T, Skobier JE, et al. Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. EMBO J. 2003;22(23):6267-76. https://doi.org/10.1093/emboj/cdg599.
PMID:14633986

2. van Bezooijen RL, Roelen BA, Visser A, van der Wee-Pals L, de Witte E, Karperien M, et al. Sclerostin is an osteocyte-expressed negative regulator of bone formation, but not a classical BMP antagonist. J Exp Med. 2004;199(6):805-14. https://doi.org/10.1084/jem.20031454.
PMID:15024046

3. Poole KE, van Bezooijen RL, Loveridge N, Hamersma H, Papapoulos SE, Lohik CW, et al. Sclerostin is a delayed secreted product of osteocytes that inhibits bone formation. FASEB J. 2005;19(13):1842-4. https://doi.org/10.1096/fj.05-4221fje.
PMID:16123173

4. Moe S, Druke T, Cunningham J, Goodman W, Martin K, Olgaard K, et al. Kidney disease: Improving global outcomes (KDIGO): Definition, evaluation, and classification of renal osteodystrophy: A position statement from kidney disease: Improving global outcomes (KDIGO). Kidney Int. 2006;69(11):1945-53. https://doi.org/10.1038/sj.ki.5000414.
PMID:16641930

5. Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease-mineral and bone disorder (CKD-MBD). Kidney Int Suppl. 2009;113:S1-130. https://doi.org/10.1038/ki.2009.188.
PMID:19644521

6. Cannata-Andia JB, Roan-Garcia P, Hruska K. The connections between vascular calcification and bone health. Nephrol Dial Transplant. 2011;26(11):3429-36. https://doi.org/10.1093/ndt/gfr591.
PMID:22039012

7. Nitta K. Vascular calcification in patients with chronic kidney disease. Ther Apher Dial. 2011;15(6):513-21. https://doi.org/10.1111/j.1744-9987.2011.00979.x.
PMID:22107687

8. Krishnan V, Bryant HU, Macdougald OA. Regulation of bone mass by Wnt signaling. J Clin Invest. 2006;116(5):1202-9. https://doi.org/10.1172/JCI28551.
PMID:16670761

9. Baron R, Kreissel M. WNT signaling in bone homeostasis and disease: From human mutations to treatments. Nat Med. 2013;19(2):179-92. https://doi.org/10.1038/nm.3074.
PMID:23389618

10. Johnson ML, Kamel MA. The Wnt signaling pathway and bone metabolism. Curr Opin Rheumatol. 2007;19(4):376-82. https://doi.org/10.1097/BOR.0b013e32816e06f9.
PMID:17551370

11. Dejana E. The role of Wnt signaling in physiological and pathological angiogenesis. Circ Res. 2010;107(8):943-52. https://doi.org/10.1161/CIRCRESAHA.110.223750.
PMID:20947863

12. Vervoel MG, Massy ZA, Brandenburg VM, Mazzaferrro S, Cozzolino M, Urena-Torres P, et al. Bone: A new endocrine organ at the heart of chronic kidney disease and mineral and bone disorders. Lancet Diabetes Endocrinol. 2014;2(5):427-36. https://doi.org/10.1016/S2213-8587(14)70059-2.
PMID:24792556

13. Williams BO. Insights into the mechanisms of sclerostin action in regulating bone mass accrual. J Bone Miner Res. 2014;29(1):24-8. https://doi.org/10.1002/jbmr.2154.
PMID:24285419

14. Brandenburg VM, Kramann R, Koos R, Kruger T, Schurgers L, Muhlenbruch G, et al. Relationship between sclerostin and cardiovascular calcification in hemodialysis patients: A cross-sectional study. BMC Nephrol. 2013;14:219. https://doi.org/10.1186/1471-2369-14-219.
PMID:24112318

15. Brandenburg VM, D’Haese P, Deck A, Mekahli D, Meijers B, Neven E, et al. From skeletal to cardiovascular disease in 12 steps—the evolution of sclerostin as a major player in CKD-MBD. Pediatr Nephrol. 2016;31(2):195-206. https://doi.org/10.1007/s00467-015-3069-7.
PMID:25735207

16. Goncalves FL, Elias RM, dos Reis LM, Graciolli FG, Zampieri FG, Cozzolino M, et al. Serum sclerostin is an independent predictor of mortality in hemodialysis patients. BMC Nephrol. 2014;15:190. https://doi.org/10.1186/1471-2369-15-190.
PMID:25465028

17. Spencer K. Analytical reviews in clinical biochemistry: The estimation of creatinine. Ann Clin Biochem. 1986;23(1):1-25. https://doi.org/10.1177/00045632862300101.
PMID:3532908

18. Zein N, Ganaiza C, Kushner I. Significance of serum C-reactive protein elevation in patients with systemic lupus erythematosus. Arthritis Rheum. 1979;22(1):7-12. https://doi.org/10.1002/art.1780220102.
PMID:103559

19. Okuno S, Inaba M, Kitatani K, Ishimura E, Yamakawa T,
Nishizawa Y. Serum levels of C-terminal telopeptide of Type I collagen: A useful new marker of cortical bone loss in hemodialysis patients. Osteoporos Int. 2005;16(5):501-9. https://doi.org/10.1007/s00198-004-1712-4
PMid:15309383

20. Kumeda Y, Inaba M, Tahara H, Kurioka Y, Ishikawa T, Morii H, et al. Persistent increase in bone turnover in Graves’ patients with subclinical hyperthyroidism. J Clin Endocrinol Metab. 2000;85(11):4157-61. https://doi.org/10.1210/jcem.85.11.6979
PMid:11095447

21. Cejka D, Herberth J, Brancsum AJ, Fardo DW, Monier-Faugere MC, Dianna D, et al. Sclerostin and dickkopf-1 in renal osteodystrophy. Clin J Am Soc Nephrol. 2011;6(4):877-82. https://doi.org/10.2215/CJN.06550810
PMid:21164019

22. Ardawi MS, Maimani AA, Bahksh TA, Rouzi AA, Qari MH, Radaddi RM. Reference intervals of biochemical bone turnover markers for Saudi Arabian women: A cross-sectional study. Bone. 2010;47(4):804-14. https://doi.org/10.1016/j.bone.2010.07.017
PMid:20659600

23. World Health Organization. Assessment of Fracture Risk and Its Application to Screening for Postmenopausal Osteoporosis. Technical Report Series No. 843. Geneva: World Health Organization; 1994.

24. Canalis E. Wnt signaling in osteoporosis: Mechanisms and novel therapeutic approaches. Nat Rev Endocrinol. 2013;9(10):575-83. https://doi.org/10.1038/nrendo.2013.154
PMid:23938284

25. Vlaes K, Behets GJ, Vlaes K, Meijers B, Blocki F, Brandenburg V, et al. Sclerostin: Another bone-related protein related to all-cause mortality in haemodialysis? Nephrol Dial Transplant. 2013;28(12):3024-30. https://doi.org/10.1093/ndt/gft039
PMid:23605174

26. Moothr RN, Moe S. Recent advances in the noninvasive diagnosis of renal osteodystrophy. Kidney Int. 2013;84(5):886-94. https://doi.org/10.1038/ki.2013.254
PMid:23802194

27. Cejka D, Marculescu R, Kozakowski N, Pischke M, Reiter T, Gessl A, et al. Renal elimination of sclerostin increases PMid:11095447

28. Delanaye P, Krzesinski JM, Warling X, Moonen M, Smelten N, Medart L, et al. Clinical and biological determinants of sclerostin plasma concentration in hemodialysis patients. Nephron Clin Pract. 2014;128(1-2):127-34. https://doi.org/10.1159/000366449
PMid:25377055

29. Brandenburg V, et al. Targeted deletion of the sclerostin gene in mice results in increased bone formation and bone strength. J Bone Miner Res. 2008;23(6):860-9. https://doi.org/10.1359/jbmr.080216
PMid:18269310

30. Ishimura E, Okuno S, Ichi M, Norimine K, Yamakawa T, Shoji S, et al. Relationship between serum sclerostin, bone metabolism markers, and bone mineral density in maintenance hemodialysis patients. J Clin Endocrinol Metab. 2014;99(11):4315-20. https://doi.org/10.1210/jc.2014-2372
PMid:25093620

31. Sheehan HR, Araki Y, Takahashi K, Zhang J, Kohno T, et al. Targeted deletion of the sclerostin gene in mice results in increased bone formation and bone strength. J Bone Miner Res. 2008;23(6):860-9. https://doi.org/10.1359/jbmr.080216
PMid:18269310

32. Ke HZ, Richards WG, Li X, Omnisky MS. Sclerostin and dickkopf-1 as therapeutic targets in bone diseases. Endocr Rev.

Abdallah et al. Sclerostin in hemodialysis patients

2012;33(5):747-83. https://doi.org/10.1210/er.2011-1060
PMid:22723594

44. Thambiah S, Roplekar R, Manghat P, Fogelman I, Fraser WD, Goldsmith D, 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(6):473-80. https://doi.org/10.1007/s00223-012-9595-4
PMid:22527202

45. Mödder UI, Hoey KA, Amin S, McCready LK, Achenbach SJ, Riggs BL, et al. Relation of age, gender, and bone mass to circulating sclerostin levels in women and men. J Bone Miner Res. 2011;26(2):373-9. https://doi.org/10.1002/jbmr.217
PMid:20721932

46. Jean G, Chazot C. Sclerostin in CKD-MBD: One more paradoxical bone protein? Nephrol Dial Transplant. 2013;28(12):2932-5. https://doi.org/10.1093/ndt/gft222
PMid:24030835

47. Kirmani S, Amin S, McCready LK, Atkinson EJ, Melton LJ 3rd, Müller R, et al. Sclerostin levels during growth in children. Osteoporos Int. 2012;23:1123-130. https://doi.org/10.1007/s00198-011-1669-z
PMid:21617991

48. Amrein K, Amrein S, Drexler C, Dimai HP, Dobnig H, Pfeifer K, et al. Sclerostin and its association with physical activity, age, gender, body composition, and bone mineral content in healthy adults. J Clin Endocrinol Metab. 2012;97(1):148-54. https://doi.org/10.1210/jc.2011-2152
PMid:21994959

49. Register TC, Hruska KA, Divers J, Bowden DW, Palmer ND, 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(1):315-21. https://doi.org/10.1210/jc.2013-3168
PMid:24178795