Int Urol Nephrol
DOI 10.1007/s11255-017-1604-0

NEPHROLOGY – ORIGINAL PAPER

Radial artery sclerostin expression in chronic kidney disease stage 5 predialysis patients: a cross-sectional observational study

Hua Zhou[1] · Min Yang[1] · Min Li[1] · Li Cui[2]

Received: 25 December 2016 / Accepted: 20 April 2017

Abstract
Purpose Bone metabolism disorder is often associated with cardiovascular calcification in patients with chronic kidney disease (CKD). Sclerostin, a novel candidate protein, has been identified to be involved in the bone–vascular axis. The aims of the current investigation were to assess vessel sclerostin expression and its relationship with circulating sclerostin levels.
Methods A cross-sectional observational study was conducted from January 2012 to December 2014. Thirty-two predialysis patients with CKD stage 5 who received arteriovenous fistula (AVF) operations were enrolled in this study. Radial arteries were collected and paraffin-embedded during the AVF operation, followed by immunohistochemical staining for sclerostin expression. In addition, serum sclerostin levels were measured by the enzyme-linked immunosorbent assay.
Results The prevalence of positive sclerostin staining in the radial arteries was 56.25%. Sclerostin expression was localized in the artery media layer. Serum sclerostin levels in patients with positive sclerostin expression were much higher than in those with negative expression ($p = 0.018$). Multivariate logistic regression analyses including potential confounders as age, gender, systolic blood pressure (BP), diastolic BP, serum sclerostin, corrected calcium (Ca), phosphate (P), Ca × P product, alkaline phosphatase, intact parathyroid hormone, and estimated glomerular filtration rate showed that only serum sclerostin levels were closely related to vessel sclerostin expression ($p = 0.025$). The area under the curve of serum sclerostin levels for predicting positive vessel sclerostin expression was 0.742 with 61.1% sensitivity and 85.7% specificity ($p = 0.008$). The cutoff point for vessel sclerostin expression of serum sclerostin was 1591.53 pg/mL.
Conclusions Positive expression of sclerostin in the radial artery media layer was related to high serum sclerostin levels. Sclerostin may act as both a local and systemic regulator involved in vascular calcification.

Keywords Chronic kidney disease · Radial artery · Sclerostin · Vascular calcification

Introduction

Vascular calcification (VC), especially arteriosclerosis, is common in chronic kidney disease (CKD) patients, who have a high prevalence of cardiovascular morbidity and mortality [1]. Arteriosclerosis refers to calcification of the arterial media layer, resulting from the transformation of vascular smooth muscle cells (VSMCs) into an osteoblastic phenotype [2]. VC is an active regulatory process like bone metabolism [3] with the upregulation of osteoblastic genes [4]. Sclerostin, a major antagonist of the Wnt/β-catenin pathway [5], has been shown to be a novel candidate protein associated with the bone–vascular axis in the CKD population. Sclerostin is expressed in uremic calcified aortic valve tissue [6] and calcific uremic arteriolopathy skin lesions in vivo [7] and calcified VSMCs in vitro [8]; however, a recent study by Qureshi et al. [9] demonstrated that inferior epigastric arterial sclerostin mRNA and protein expression was low or absent in newly transplanted patients

✉ Li Cui
clturtle@163.com

[1] Department of Nephrology, The Third Affiliated Hospital of Soochow University, Changzhou, Jiangsu Province, China

[2] Department of Urology, The Third Affiliated Hospital of Soochow University, Changzhou, Jiangsu Province, China

Published online: 28 April 2017

and found no difference between calcified and non-calcified vessels. Thus, whether extraskeletal tissues express sclerostin is not known.

Additionally, previous studies have demonstrated that serum sclerostin levels are higher in patients with CKD than in healthy individuals [6, 10]. Some studies have shown a positive association between circulating sclerostin levels and VC [9, 11, 12], while other studies have shown a negative association [13–20] or no association at all [6] in patients with CKD or hemodialysis. Therefore, the relationship between serum sclerostin levels and VC is uncertain. Additionally, it is unknown whether sclerostin expression in tissues is correlated with serum sclerostin levels.

To our knowledge, there have been few reports about the vessel sclerostin expression in patients with CKD, as well as its relationship with circulating sclerostin levels. Regarding the hypothesis that sclerostin may act as both a local and systemic regulator of VC, this study aimed to investigate radial arterial sclerostin expression and serum sclerostin levels in predialysis patients with CKD stage 5 and to explore the probable mechanism of how sclerostin is involved in the bone–vascular axis.

Methods

Study population

This cross-sectional observational study was conducted at the Department of Nephrology, the Third Affiliated Hospital of Soochow University, Changzhou, China, from January 2012 to December 2014. Thirty-two predialysis patients with CKD stage 5 who received arteriovenous fistula (AVF) operations for vascular access were enrolled. Patients with diabetes mellitus, severe infection, malignancy, or who were treated with vitamin D/phosphate binders were excluded. The study was approved by the Ethics Committee of the Third Affiliated Hospital of Soochow University, China. All participants provided written informed consent.

Study design

Fasting morning blood samples were centrifuged immediately after collection and stored at −80 °C until analysis. Radial arteries were collected during AVF operations with end-to-end anastomosis. All radial arteries were paraffin-embedded prior to immunohistochemical (IHC) staining for sclerostin (Abcam, Cambridge, UK). Data on demographics and clinical characteristics were collected at the time of enrollment before dialysis. Routine biochemical tests, such as serum creatinine (normal range 0.5–1.6 mg/dL), blood urea nitrogen (BUN) (normal range 4.8–23.2 mg/dL), uric acid (normal range 1.5–7.6 mg/dL), calcium (Ca) (normal range 8.0–11.6 mg/dL), phosphate (P) (normal range 2.2–5.0 mg/dL), alkaline phosphatase (AKP) (normal range 35–100 IU/L) (UniCel AU5800 immunoassay systems, Beckman Coulter, USA), and intact parathyroid hormone (iPTH) (normal range 12.2–87.2 pg/mL) (Beckman Coulter's UniCel DxI 800 immunoassay systems, Beckman Coulter, USA), were measured using standard laboratory methods. Serum sclerostin levels were measured by a commercially available enzyme-linked immunosorbent assay (TECO medical, Sissach, Switzerland) according to the manufacturer's instructions. The estimated glomerular filtration rate (eGFR) was determined with the CKD-EPI formula [21].

Statistical analyses

Statistical analyses were conducted using SPSS (version 19.0). Normally distributed data are represented as mean ± standard deviation. Student's t test was employed to compare the differences between two groups. The Chi-square test was applied to evaluate differences in prevalence. Multivariate logistic regression analyses were used to assess factors potentially related to vessel sclerostin expression after adjustment for age, gender, systolic blood pressure (BP), diastolic BP, serum sclerostin, corrected Ca, P, Ca × P product, AKP, iPTH, and eGFR. Receiver operating characteristic (ROC) areas under the curve (AUCs) and sensitivity/specificity for the cutoff point of serum sclerostin levels for discriminating positive vessel sclerostin expression were calculated. A $p < 0.05$ was considered to be statistically significant.

Results

Sclerostin expression in the radial arteries

A total of 32 predialysis patients with CKD stage 5 were recruited for this study. Nineteen patients had chronic glomerulonephritis, 2 had hypertensive nephrosclerosis, 1 had lupus nephritis, 1 had urinary tract obstruction, and 9 had unknown causes.

Among the radial arteries from the predialysis patients, 18 (56.25%) vessels had positive sclerostin staining, while the remaining 14 (43.75%) vessels had negative sclerostin staining. IHC staining showed that the sclerostin was localized in the artery media layer (Fig. 1).

Relationship between serum sclerostin levels and vessel sclerostin expression

As shown in Table 1, serum sclerostin levels in patients with positive sclerostin expression were much higher than in those with negative expression ($p = 0.018$). However, no obvious

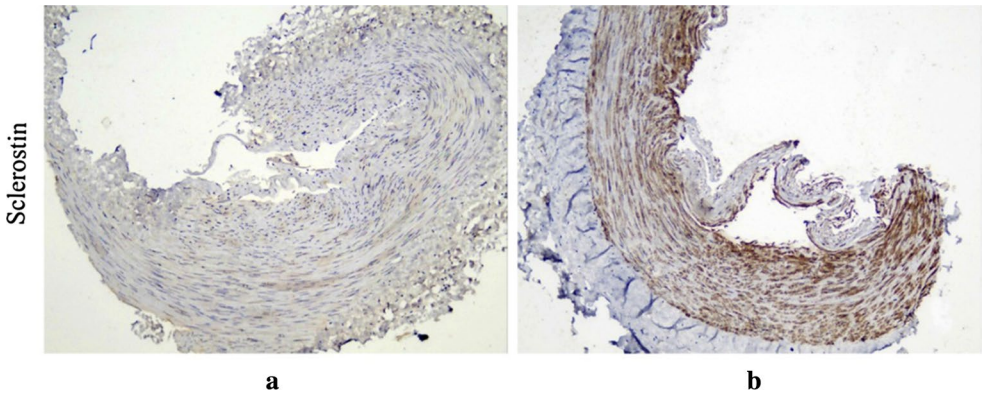

Fig. 1 Immunohistochemical staining of sclerostin in radial arteries from predialysis patients with CKD stage 5. **a** Negative expression of sclerostin in the artery media layer (×100); **b** positive expression of sclerostin in the artery media layer (×100)

Table 1 Characteristics of predialysis patients with CKD stage 5 in positive and negative sclerostin expression groups

Variables	Positive expression ($n = 18$)	Negative expression ($n = 14$)	p
Age (years)	53.78 ± 9.23	48.93 ± 10.26	0.170
Gender (male)	9 (50.00%)	6 (42.86%)	0.688
Current smoking	1 (5.56%)	0	0.370
Systolic BP (mmHg)	160.72 ± 25.65	155.00 ± 24.58	0.529
Diastolic BP (mmHg)	90.44 ± 13.35	88.36 ± 16.91	0.699
Corrected Ca (mg/dL)	10.29 ± 1.13	10.09 ± 1.15	0.638
P (mg/dL)	5.19 ± 1.35	5.71 ± 1.88	0.370
Ca × P product (mg^2/dL^2)	53.04 ± 13.58	56.89 ± 16.94	0.481
AKP (IU/L)	94.83 ± 41.71	84.43 ± 27.05	0.425
Log (iPTH) (pg/mL)	8.00 ± 1.18	7.58 ± 1.83	0.433
Sclerostin (pg/mL)	1508.83 ± 451.90	1140.41 ± 352.46	0.018
BUN (mg/dL)	82.15 ± 28.93	73.05 ± 34.28	0.422
Creatinine (mg/dL)	9.64 ± 2.43	10.00 ± 3.36	0.725
Uric acid (mg/dL)	7.39 ± 2.31	7.27 ± 2.47	0.888
eGFR (mL/min·1.73 m^2)	5.29 ± 2.22	5.29 ± 2.00	0.991

differences were found in other variables such as age, gender, current smoking status, BP, corrected Ca, P, Ca × P product, AKP, iPTH, and eGFR between the two groups.

Next, the univariate and multivariate logistic regression analyses were used to further evaluate factors potentially related to positive vessel sclerostin expression. The results showed that serum sclerostin level was the only factor associated with positive vessel sclerostin expression ($p = 0.025$; Table 2).

Furthermore, ROC curve analysis showed that the cutoff point of serum sclerostin levels to discriminate patients with and without vessel sclerostin expression was 1591.53 pg/mL with 61.1% sensitivity and 85.7% specificity (Fig. 2). The AUCs of the serum sclerostin level for predicting positive vessel sclerostin expression were 0.742 (95% confidence interval (CI) = 0.557–0.880, $p = 0.008$).

Discussion

The major findings of this study were that over half of our sample of predialysis patients had positive sclerostin staining in their radial arteries, and positive vessel sclerostin expression was independently associated with serum sclerostin levels.

This study, for the first time, showed that sclerostin was expressed in over half of radial arteries in predialysis patients with CKD stage 5. IHC staining suggested that sclerostin was localized in the artery media layer, which appeared to be the same area as calcification. This may be indirect evidence implicating sclerostin in VC. Previously, the differentiation of VSMCs into osteoblast-like cells with the expression of osteogenic proteins like sclerostin was associated with media calcification [4].

Table 2 Univariate and multivariate logistic regression analyses between vessel sclerostin expression and clinical characteristics

Variables	Univariate 95% CI	*p*	Multivariate 95% CI	*p*
Age	1.055 (0.977, 1.139)	0.169	–	0.538
Gender	0.750 (0.184, 3.057)	0.688	–	0.958
Systolic BP	1.010 (0.980, 1.040)	0.517	–	0.425
Diastolic BP	1.010 (0.962, 1.061)	0.689	–	0.685
Sclerostin	1.002 (1.000, 1.004)	0.025	1.002 (1.000, 1.004)	0.025
Corrected Ca	1.171 (0.620, 2.215)	0.626	–	0.781
P	0.807 (0.510, 1.278)	0.361	–	0.614
Ca × P product	0.982 (0.936, 1.031)	0.468		0.685
AKP	1.009 (0.987, 1.031)	0.419	–	0.694
Log (iPTH)	1.224 (0.744, 2.012)	0.426	–	0.602
eGFR	0.998 (0.712, 1.400)	0.991	–	0.872

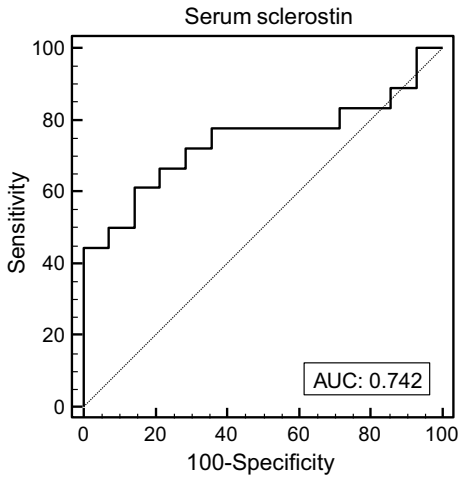

Fig. 2 Receiver operating characteristic (ROC) analysis of vessel positive sclerostin expression with respect to serum sclerostin levels

Furthermore, our study found that high serum sclerostin levels were strongly correlated with positive vessel sclerostin expression. Serum sclerostin level was a relatively reliable predictor to discriminate vessel sclerostin expression. As previously reported, bone marrow plasma and circulating sclerostin levels are correlated, and a major source of sclerostin is the osteocytes in bone in patients without CKD [22]. The elevated circulating sclerostin in patients with CKD results not from reduced excretion but from increased secretion in bone [23]. A study by Qureshi et al. [9] found that the vascular wall is not the primary source of circulating sclerostin. However, Zhu et al. [8] reported increased expression of sclerostin during calcification in VSMCs, which suggests that production of sclerostin in the vasculature may be another source of circulating sclerostin. Our study shows a positive relationship between serum sclerostin levels and vessel sclerostin expression. Thus, we speculate that higher serum sclerostin levels in patients with CKD may be partly attributed to the production of sclerostin in the vasculature. It is well known that circulating sclerostin levels are elevated in the early stages of CKD and are even higher in end stage renal disease [10, 23]. Sclerostin is an antagonist of the Wnt/β-catenin signaling pathway and downregulates bone metabolism [5]. Sclerostin may also be an inhibitor of VC with similar mechanism acting on bone. We speculate that serum sclerostin is elevated as a systemic regulator prior to the mineralization of the vascular wall. And the upregulation of sclerostin expression in the vessels may be a local regulatory factor that protects against VC together with systemic regulation. As a review by Kanbay et al. [24] reported, most studies have shown an inverse association between circulating sclerostin levels and VC, which further support the protective role of sclerostin against VC. However, the causality and mechanism of sclerostin action currently remains unclear.

Sclerostin could be an important mediator between the bone and vascular axis in patients with CKD. High serum sclerostin levels may be partly attributed to production in the vasculature that further inhibits VC as a systemic regulator. On the other hand, the upregulation of sclerostin expression in vessels may act as a local mechanism to prevent VC. Future studies are needed to confirm the hypothesis that sclerostin overexpression in the circulation and vessels protects against VC.

The novelty of the current study lies in comparing IHC staining of vessels with peripheral circulating sclerostin levels. Limitations of this study include the lack of co-staining of histological calcification along with sclerostin, the lack of clinical parameters measuring arteriosclerosis, no data on vascular mapping before fistula surgery, the relatively small sample size, and the lack of normal arteries as controls. Finally, the cross-sectional design does not allow for the establishment of a cause-effect relationship.

Conclusions

In conclusion, we found that 56.25% of predialysis patients with CKD stage 5 showed positive expression of sclerostin in the radial artery media layer. Positive vessel sclerostin

expression was related to high serum sclerostin levels. Sclerostin may be one of the key markers that inhibit VC by both local and systemic mechanisms.

Acknowledgements This study was funded by Youth Talent Programme of Changzhou Government Healthy Service (No. QN201402). The authors thank Zhong Xue for making substantial contributions to data collection.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Russo D, Corrao S, Battaglia Y et al (2011) Progression of coronary artery calcification and cardiac events in patients with chronic renal disease not receiving dialysis. Kidney Int 80(1):112–118
2. Gauthier-Bastien A, Ung RV, Larivière R et al (2014) Vascular remodeling and media calcification increases arterial stiffness in chronic kidney disease. Clin Exp Hypertens 36(3):173–180
3. Davies MR, Hruska KA (2001) Pathophysiological mechanisms of vascular calcification in end-stage renal disease. Pathophysiological mechanisms of vascular calcification in end-stage renal disease. Kidney Int 60(2):472–479
4. Demer LL, Tintut Y (2008) Vascular calcification: pathobiology of a multifaceted disease. Circulation 117(22):2938–2948
5. Gaudio A, Privitera F, Battaglia K et al (2012) Sclerostin levels associated with inhibition of the Wnt/β-catenin signaling and reduced bone turnover in type 2diabetes mellitus. J Clin Endocrinol Metab 97(10):3744–3750
6. Brandenburg VM, Kramann R, Koos R et al (2013) Relationship between sclerostin and cardiovascular calcification in hemodialysis patients: a cross-sectional study. BMC Nephrol 14:219
7. Kramann R, Brandenburg VM, Schurgers LJ et al (2013) Novel insights into osteogenesis and matrix remodelling associated with calcific uraemic arteriolopathy. Nephrol Dial Transplant 28:856–868
8. Zhu D, Mackenzie NC, Millán JL et al (2011) The appearance and modulation of osteocyte marker expression during calcification of vascular smooth muscle cells. PLoS ONE 6(5):e19595
9. Qureshi AR, Olauson H, Witasp A et al (2015) Increased circulating sclerostin levels in end-stage renal disease predict biopsy-verified vascular medial calcification and coronary artery calcification. Kidney Int 88(6):1356–1364
10. Kanbay M, Siriopol D, Saglam M et al (2014) Serum sclerostin and adverse outcomes in nondialyzed chronic kidney disease patients. J Clin Endocrinol Metab 99(10):E1854–E1861
11. Morena M, Jaussent I, Dupuy AM et al (2015) Osteoprotegerin and sclerostin in chronic kidney disease prior to dialysis: potential partners in vascular calcifications. Nephrol Dial Transplant 30(8):1345–1356
12. Pelletier S, Confavreux CB, Haesebaert J et al (2015) Serum sclerostin: the missing link in the bone-vessel cross-talk in hemodialysis patients? Osteoporos Int 26(8):2165–2174
13. Claes KJ, Viaene L, Heye S et al (2013) Sclerostin: another vascular calcification inhibitor? J Clin Endocrinol Metab 98(8):3221–3228
14. Balcı M, Kırkpantur A, Turkvatan A et al (2015) Sclerostin as a new key player in arteriovenous fistula calcification. Herz 40(2):289–297
15. Kirkpantur A, Balci M, Turkvatan A et al (2015) Serum sclerostin levels, arteriovenous fistula calcification and 2-years all-cause mortality in prevalent hemodialysis patients. Nefrologia 36(1):24–32
16. Delanaye P, Krzesinski JM, Warling X et al (2014) Clinical and biological determinants of sclerostin plasma concentration in hemodialysis patients. Nephron Clin Pract 128(1–2):127–134
17. Yang CY, Chang ZF, Chau YP et al (2015) Circulating Wnt/β-catenin signalling inhibitors and uraemic vascular calcifications. Nephrol Dial Transplant 30(8):1356–1363
18. Evenepoel P, Goffin E, Meijers B et al (2015) Sclerostin serum levels and vascular calcification progression in prevalent renal transplant recipients. J Clin Endocrinol Metab 100(12):4669–4676
19. Lee YT, Ng HY, Chiu TT et al (2016) Association of bone-derived biomarkers with vascular calcification in chronic hemodialysis patients. Clin Chim Acta 452:38–43
20. Jean G, Chazot C, Bresson E et al (2016) High serum sclerostin levels are associated with a better outcome in haemodialysis patients. Nephron 132(3):181–190
21. Levey AS, Stevens LA, Schmid CH et al (2009) A new equation to estimate glomerular filtration rate. Ann Intern Med 150(9):604–612
22. Drake MT, Srinivasan B, Mödder UI et al (2010) Effects of parathyroid hormone treatment on circulating sclerostin levels in postmenopausal women. J Clin Endocrinol Metab 95(11):5056–5062
23. Cejka D, Marculescu R, Kozakowski N et al (2014) Renal elimination of sclerostin increases with declining kidney function. J Clin Endocrinol Metab 99(1):248–255
24. Kanbay M, Solak Y, Siriopol D et al (2016) Sclerostin, cardiovascular disease and mortality: a systematic review and meta-analysis. Int Urol Nephrol 48(12):2029–2042