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Relation between Sclerostin and Valvular Calcification in Hemodialysis Patients

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ABSTRACT

Introduction: Chronic kidney disease (CKD) is prevalent all over the world. Cardiovascular morbidity and mortality are high among CKD patients especially hemodialysis (HD) patients. Vascular calcifications are common among hemodialysis patients and its etiology is related to the derangements in mineral and bone metabolism. Sclerostin is a new glycoprotein that is involved in both vascular calcification and inhibition of bone formation in CKD patients.

Objective: The aim of the current study was to investigate the correlation between serum sclerostin level and valvular calcifications in End Stage Renal Disease (ESRD) patients.

Patients and methods: Aortic and mitral valve calcifications were assessed by echocardiography and serum sclerostin levels were measured by ELISA method in 80 ESRD patients on regular hemodialysis. Parathyroid hormone, serum phosphorus, serum calcium and serum alkaline phosphatase were also measured.

Results: Aortic valvular calcification was present in 58.8% of patients and mitral valvular calcification in 33.8% of patients. The mean level of sclerostin in our patients was 0.63 (SD 0.14) ng/ml. The mean parathyroid hormone was 521 pmol/l, the mean calcium level was 8.39 mg/dl while the mean phosphorus level was 5.5 mg/dl. There was no significant correlation between serum sclerostin levels and aortic valve or mitral valve calcification in our case series.

Conclusion: In ESRD patients receiving regular hemodialysis, serum sclerostin levels were not substantially associated with mitral valve or aortic valve calcification.

Keywords: Sclerostin, Valvular calcification, ESRD.

INTRODUCTION

The prevalence of chronic renal failure is approaching more than 10%, renal failure has different hazardous effects on public health [(1)].

Kidneys are involved in activation of vitamin D, regulation of calcium and phosphorus metabolism and hence affecting parathyroid hormone and bone metabolism, in CKD patients, serum phosphorus excretion is impaired while serum calcium is affected by the derangements in vitamin D and parathyroid hormone. These changes lead to bone disease and contribute to cardiovascular disease and anemia in chronic kidney disease (CKD) patients [(2-4)].

CKD patients show increased rate of cardiovascular complications, vascular calcifications (VC) secondary to abnormal mineral and bone metabolism is an important contributor to cardiovascular disease in CKD patients [(5.6)].

Osteocytes' wingless-related integration site beta-catenin pathway is crucial for bone development. The aberrant mineral metabolism and renal bone disease seen in CKD patients are caused by sclerostin, a recognized inhibitor of Wingless-related integration site beta-catenin (Wnt-b-catenin). Inhibitors of Wnt may be targeted with antibodies as a potential therapy for renal bone disease [(7)]. Sclerostin (Scl), which connects renal bone disease to VC, may help patients with chronic kidney disease forecast how their cardiovascular illness will progress [(8,9)].

Osteocytes are the main source of secretion of Scl. Sclerostin suppresses osteocyte function and enhances osteoblast apoptosis; these actions cause inhibition of bone formation and a decrease in bone mass [(9,10)].

In some studies, high sclerostin levels were associated with VC and coronary artery calcification (CAC) in CKD patients. It was found to be expressed in calcified vascular tissues, also its production by in vitro osteocytes was induced by calcifying medium and it was expressed in aortic extracts [(11-16)].

Sclerostin expression was discovered using immunohistochemistry in explanted aortic valves from hemodialysis patients [(17)].

In studies on CKD patients, there was positive correlation between sclerostin and vascular calcification [(6,8,9)], while, other studies did not show this association [(4,10)].

The aim of this work was to study the correlation between serum sclerostin level and valvular calcifications in End Stage Renal Disease (ESRD) patients.

PATIENTS AND METHODS

A total of 80 haemodialysis patients in the Dialysis Init of Al-Sahel Teaching Hospital were recruited in this study. Patients had four-hour sessions of hemodialysis three times per week.

Exclusion criteria were age younger than 18 years old, overt infection, malignancy, parathyroidectomy, previous renal transplantation or autoimmune rheumatic diseases.

A detailed history and thorough clinical examination were obtained from every patient in this study. Height, weight, waist and mid arm circumference were registered for all patients.

Laboratory investigations including serum Sclerostin (by ELISA), serum creatinine, urea, serum

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calcium, phosphorus, alkaline phosphatase, parathyroid hormone (PTH) and albumin were measured in all patients.

Sclerostin levels were measured using ELISA Kit, EIAab Science, Wuhan.

All patients were examined by echocardiography.

Ethical consent:

This study received ethical committee approval of Ain Shams University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 20 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test.

Qualitative data were represented as frequencies and relative percentages. Chi square test ($\chi 2$) and Fisher's exact test to calculate difference between two or more groups of qualitative variables. Quantitative data were expressed as mean and standard deviation (SD). Independent samples t-test was used to compare between two independent groups of normally distributed variables (parametric data). Analysis of correlation (using Pearson's method): to determine how strongly two quantitative variables are associated. The size and direction (positive or negative) of the linear relationship between two variables are expressed by the correlation coefficient, which is symbolically indicated by the letter "r". P value ≤ 0.05 was considered significant.

RESULTS

Demographic data and duration of dialysis of patients are summarized in Table 1.

Table (1): Description of the sample age and duration of dialysis.

Quantitative variable		Mean ± SD		Range
Age		47.91 ± 10.64		21 - 60
Duration of dialysis		6.48 ± 3.45		1 - 17
Qualitative variable		**N**		**%**
Sex	Female	34		42.5%
	Male	46		57.5%
Virology	Negative	55		68.8%
	Positive	25		31.3%

Laboratory investigations for all patients are shown in **Table 2**.

Table (2): Description of the sample lab data.

Variable	Mean ± SD
Sclerostin (ng/ml)	0.63 ± 0.14
Ca (mg/dl)	8.39 ± 1.12
Po4 (mg/dl)	5.59 ± 1.21
PTH (mg/dl)	521.41 ± 28.66
Alk phosphatase	125.79 ± 30.02
Albumin (g/dL)	3.54 ± 0.45
Cr	9.29 ± 1.88
Urea (mg/dL)	137.16 ± 27.48

About 58.8% of patients have aortic valve calcification, while 33.8% of patients have mitral valve calcification (**Table 3**).

Table (3): Description of the sample valve calcification.

Variable		N	%
Aortic calc.	No	33	41.3%
	Yes	47	58.8%
Mitral calc.	No	53	66.3%
	Yes	27	33.8%
Valve calcification	No	27	33.8%
	Yes	53	66.3%

Sclerostin levels did not significantly differ between patients with and without aortic valve calcification in either group of patients (table 4).

Table (4): Comparison between those with and without aortic valve calcification regarding sclerostin level.

Sclerostin		N	Mean	SD	t test	
					P value	sig.
Aortic calc.	No	33	0.62	0.07	0.713	NS
	Yes	47	0.64	0.14		

Sclerostin levels did not significantly differ between patients with and without mitral valve calcification in either group of patients (**Table 5**).

Table (5): Comparison between those with and without mitral valve calcification regarding sclerostin level.

Sclerostin		N	Mean	SD	t test	
					P value	sig.
Mitral calc.	No	53	0.61	0.07	0.100	NS
	Yes	27	0.68	0.15		

Regarding sclerostin levels, there was no discernible difference between patients with and without valve calcification (**Table 6**).

Table (6): Comparison between those with and without valve calcification regarding sclerostin level.

Sclerostin		N	Mean	SD	t test	
					P value	sig.
valve calcification	No	27	0.61	0.07	0.461	NS
	Yes	53	0.64	0.14		

Sclerostin level and hepatitis C virus (HCV) status did not significantly correlate with one another (Table 7).

Table (7): Comparison between those with positive and negative virology regarding sclerostin level.

Sclerostin		N	Mean	SD	t test	
					P value	sig.
HCV	Negative	55	0.62	0.08	0.331	NS
	Positive	25	0.66	0.16		

There was no connection between sclerostin levels and laboratory tests that was particularly noteworthy (**Table 8**).

Table (8): Correlation between sclerostin level and age, duration and lab results.

N = 80		Age	Duration of dialysis	Ca	PO4	Ca * PO4	Pth	Alk. Ph.	Albumin	Cr	Urea
Sclerostin	r	0.156	0.002	0.029	-0.011	0.002	-0.137	0.012	-0.190	0.073	0.051
	p	0.168	0.984	0.796	0.926	0.985	0.226	0.919	0.092	0.519	0.651

DISCUSSION

Cardiac calcifications are common among hemodialysis patients. Valvular calcifications are important factors contributing to the high cardiovascular complications among hemodialysis patients [18].

Numerous proteins and pathways had been identified to be related to vascular calcification processes; Klotho-FGF-23 axis, Osteoprotegerin and Rank L/ Rank axis, bone morphogenic proteins, Fetuin-A, matrix gla-protein [19].

Sclerostin, one of the most new proteins implicated in bone vascular cross talk, blocks the Wingless-related integration site beta-catenin pathway [20].

Sclerostin inhibits bone formation, increases bone resorption and it has been found in cardiac valves and blood vessels calcifications [13,20,21].

In our study, calcification was documented in 66.2% of our HD patients, aortic calcification in 58.8% and mitral calcification in 33.8%.

Hemodialysis patients have a high incidence of valve calcification with broad variations in frequency; this difference in frequency may be brought on by varied patient features and various valvular calcification detection techniques [22].

The prevalence of vascular and valve calcification in HD patients ranged from 71% to 80% in a meta-analysis of 12 study [23], while another research indicated a prevalence of 75% [24].

In the last few years, many studies were published illustrating the eelation between serum Sclerostin and Vascular calcification. In our study, the highest Sclerostin level was 1.65 ng\ml and the lowest sclerostin level was 0.41 ng\ml and the correlation between serum Sclerostin level and valvular calcification was not statistically significant.

Aortic valve calcification (AVC) and sclerostin were significantly correlated in a recent research of hemodialysis patients. They also discovered that sclerostin was expressed in the tissue of the aortic valve. However, there was no association with CAC [17]. This lack of a positive relation between sclerostin and CAC cannot explained and in conflict with the results of **Morena *et al.*** [16] study.

Qureshi *et al.* [6] a single center cohort study found that Sclerostin level is a predictor of VC and CAC in HD patients.

Koos *et al.* [13] demonstrated in a different research the significance of elevated sclerostin levels in the AVC process in non-uremic patients.

In vitro study, calcification was induced in human vascular smooth muscle cell. It was found that Wingless-related integration site beta-catenin plays a role in the process of calcification [25]. So we can conclude from this in vitro study that inhibition of Wingless-related integration site beta-catenin may have protective actions against calcifications, hence the increased Scl in cases of VC observed in many studies may be a compensatory mechanism against VC.

On the other hand, many studies had been published proving absence of statistically significant relation between Scl levels and VC.

Kanbay *et al.* [23]**,** had reviewed twelve studies of Scl in HD patients did not find a significant correlation between Scl and VC in nine of twelve studies on HD patients. **NasrAllah *et al.*** [26], found that sclerostin levels were correlated with improved bone density and lesser VC in HD Egyptian patients. **Register et al.** [27]

investigated 450 diabetic African Americans and found that calcification of the carotid artery was negatively associated with serum Sclerostin.

In another study high Scl levels were correlated to AC but in multivariate analysis this correlation became inverse and lower Scl levels were predictors of AC [(12)].

From physiological point of view, Sclerostin is Wingless-related integration site beta-catenin inhibitor so it can decrease new bone deposition by inhibiting the osteoblasts differentiation and down-regulation of bone genesis markers [(28)]. The pathogenesis of VC shares similarities to physiologic bone process [(29)], so sclerostin is expected to act as an inhibitor to VC as well.

So the correlation between serum Scl levels and VC may be due the compensatory action of Sclerostin to equate for the VC occurrence which is caused by other pathogenetic methods [(23)].

Kramann *et al.* [(30)] and **Koos *et al.*** [(13)] claimed that Scl is spilled from calcified vascular tissue to the circulation; **Qureshi *et al.*** contradicted that point of view as they biopsied inferior epigastric arteries in the newly transplanted patients and they discovered that the expression of m RNA of Scl was not significantly different between patients who did not have VC and patients who had VC. As a result, they suggested that osteocytes are the primary source of circulating Scl [(6)].

In our study we found no statistically significant association between Scl levels and age, sex or HD duration. Variations of patients group regarding age, HD duration, comorbid conditions, and the discrepancy of the studied areas of calcification (cardiac valves, coronary arteries, large arteries) and the different methodology for measuring serum Scl may be responsible for the confliction results of the studies.

In conclusion, serum Scl levels were not significantly correlated to mitral valve or aortic valve calcification in ESRD patients on regular hemodialysis.

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REFERENCES

1. **Ketteler M, Elder G, Evenepoel P (2015):** Revisiting KDIGO clinical practice guideline on chronic kidney disease mineral and bone disorder: a commentary from a Kidney Disease: improving Global Outcomes controversies conference. Kidney Int., 87:502-28.
2. **Fukagawa M, Komaba H (2017):** Chronic Kidney Disease-Mineral and Bone Disorder in Asia. Kidney Dis (Basel), 3(1):1-7.
3. **Brandenburg V, D'Haese P, Deck A *et al.* (2016):** From skeletal to cardiovascular disease in 12 steps-the evolution of sclerostin as a major player in CKD-MBD. Pediatr Nephrol., 31(2):195-206.
4. **Yang C, Chang Z, Chau Y *et al.* (2015):** Circulating Wnt/βcatenin signalling inhibitors and uraemic vascular calcifications. Nephrol Dial Transplant., 30(8):1356-63.
5. **Claes K, Heye S, Bammens B *et al.* (2013):** Aortic calcifications and arterial stiffness as predictors of cardiovascular events in incident renal transplant recipients. Transpl Int., 26(10):973-81.
6. **Qureshi A, 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):1356-64.
7. **Evenepoel P, D'Haese P, Brandenburg V (2015):** Sclerostin and DKK1: new players in renal bone and vascular disease. Kidney Int., 88(2):235-40.
8. **Lee Y, Ng H, Chiu T, *et al.* (2016):** Association of bone-derived biomarkers with vascular calcification in chronic hemodialysis patients. Clin Chim Acta., 452:38-43.
9. **Lv W, Guan L, Zhang Y *et al.* (2016):** Sclerostin as a new key factor in vascular calcification in chronic kidney disease stages 3 and 4. Int Urol Nephrol., 48(12):2043-50.
10. **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):181-90.
11. **Zhu D, Mackenzie N, Millan J *et al.* (2011):** The appearance and modulation of osteocyte marker expression during calcification of vascular smooth muscle cells. PLoS One, 6(5):e19595. doi: 10.1371/journal.pone.0019595.
12. **Claes K, Viaene L, Heye S *et al.* (2013):** Sclerostin: another vascular calcification inhibitor? J Clin Endocrinol Metab., 98(8):3221-8.
13. **Koos R, Brandenburg V, Mahnken A *et al.* (2013):** Sclerostin as a potential novel biomarker for aortic valve calcification: an in vivo and ex-vivo study. J Heart Valve Dis., 22(3):317-25.
14. **Didangelos A, Yin X, Mandal K *et al.* (2010):** Proteomics characterization of extracellular space components in the human aorta. Mol Cell Proteom MCP., 9(9):2048-62.
15. **Wang X, Yuan L, Zhang J *et al.* (2017):** Serum sclerostin values are associated with abdominal aortic calcification and predict cardiovascular events in patients with chronic kidney disease stages 3-5D. Nephrology, 22(4):286-92.
16. **Morena M, Jaussent I, Dupuy A *et al.* (2015):** Osteoprotegerin and sclerostin in chronic kidney disease prior to dialysis: potential partners in vascular calcifications. Nephrol Dial Transplant., 30(8):1345-56.
17. **Brandenburg V, Kramann R, Koos R *et al.* (2013):** Relationship between sclerostin and cardiovascular calcification in hemodialysis patients: a cross-sectional study. BMC Nephrol., 14:1-10.
18. **Vervloet M, Cozzolino M (2017):** Vascular calcification in chronic kidney disease: different bricks in the wall? Kidney Int., 91(4):808-17.
19. **Leopold J (2015):** Vascular calcification: Mechanisms of vascular smooth muscle cell calcification. Trends Cardiovasc Med., 25(4):267-74.
20. **Asamiya Y, Tsuchiya K, Nitta K (2016):** Role of sclerostin in the pathogenesis of chronic kidney disease-mineral bone disorder. Renal Replacement Therapy, (2):1-8.

21. **Catalano A, Bellone F, Morabito N *et al.* (2020):** Sclerostin and Vascular Pathophysiology. Int J Mol Sci., 21(13):4779. doi: 10.3390/ijms21134779
22. **Rroji M, Seferi S, Cafka M *et al.* (2014):** Is residual renal function and better phosphate control in peritoneal dialysis an answer for the lower prevalence of valve calcification compared to hemodialysis patients?. Int Urol Nephrol., 46:175-82.
23. **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):2029-42.
24. **Ikee R, Honda K, Ishioka K *et al.* (2010):** Differences in associated factors between aortic and mitral valve calcification in hemodialysis. Hypertens Res., (33):622-6.
25. **Martínez-Moreno J, Muñoz-Castañeda J, Herencia C *et al.* (2012):** In vascular smooth muscle cells paricalcitol prevents phosphate-induced Wnt/β-catenin activation. Am J Physiol Renal Physiol., 303(8):1136-44.
26. **NasrAllah M, Nassef A, Elshaboni T *et al.* (2016):** Comparing different calcification scores to detect outcomes in chronic kidney disease patients with vascular calcification. Int J Cardiol., 220:884-9.
27. **Register T, Hruska K, Divers J *et al.* (2014):** 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., 99(1):315-21.
28. **Williams B (2014):** Insights into the mechanisms of sclerostin action in regulating bone mass accrual. J Bone Miner Res., 29(1):24-8.
29. **Smith E (2016):** Vascular Calcification in Uremia: New-Age Concepts about an Old-Age Problem. Methods Mol Biol., 1397:175-208.
30. **Kramann R, Kunter U, Brandenburg V *et al.* (2013):** Osteogenesis of heterotopically transplanted mesenchymal stromal cells in rat models of chronic kidney disease. J Bone Miner Res., 28(12):2523-34.