ORIGINAL ARTICLE

Title

Sclerostin serum concentration in patients with predialysis CKD stage 3-5

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ABSTRACT

Introduction Sclerostin is a glycoprotein expressed by osteocytes and plays a role in bone turnover in the metabolism of the bone, by blocking the formation of a ligand with its receptor on the Wnt/β-catenin pathway and influencing the activity of osteoblasts and mineral and bone disturbances in CKD via the interaction between kidney, bone and vascular axis. The concentration of sclerostin will rise in patients with ESRD undergoing dialysis; however, it has not been reported yet in non-dialysis CKD patient stage 3-5. **Methods** This was an analytic cross-sectional study aimed to measure sclerostin concentration in non-dialysis patients with CKD stage 3-5. The sclerostin was measured using an enzyme-linked immunosorbent assay kit. CKD stages were diagnosed using the KDIGO-2012 criteria which measures the estimated GFR (eGFR) using CKD-EPI formulation. Fifty-six patients with CKD stage 3-5 were enrolled in this study. One way ANOVA comparative test followed with a post hoc analysis using Bonferroni test was used to analyze the data. **Results** The mean concentration level of serum sclerostin in this population was (79.7 ± 41.2) pmol/L, and in patients with CKD stage 3, CKD stage 4, and CKD stage 5 were 59.6 ± 28.5 pmol/L, 71.9 ± 42.2 pmol/L and 96.7 ± 39.8 pmol/L respectively. The comparative test of mean concentrations of the serum sclerostin between stages of CKD was statistically significant with a p=0.022. The post hoc analysis of serum sclerostin concentration between CKD stage 3 and CKD stage 5 had a significant difference with a mean of 37 pmol/L and p=0.037. **Conclusion**, The serum sclerostin concentration rises with the decline of kidney function in patients with pre-dialysis CKD stage 3-5.

Keywords: sclerostin, pre-dialysis CKD, eGFR, CKD-EPI

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INTRODUCTION

Chronic kidney disease (CKD) remains a major problem globally, with a steadily increasing prevalence currently affecting 8-16% of the world's population, and a big health burden in many countries.[1] The same applies to Indonesia; up to 12.5% of its population is affected with CKD, and the prevalence keeps increasing annually.[2] This fact is supported by the data provided by the Indonesian Renal Registry (IRR); the number of patients with end-stage renal disease (ESRD) receiving hemodialysis increased 300%, from 4977 new patients in 2007 to 21.050 patients in 2015. The cummulative incidence of new patients approximates to 23% annually in the past five years.[3]

Cardiovascular (CV) events are also a cause of 40% mortality and almost 50% of hospitalization. CKD patients have an increased risk for cardiovascular diseases (CVD up to 10 to 20 folds higher than the general population.[4,5] The high mortality rate of CVD in CKD patients is due to not only traditional risk factors but also the derangements of mineral and bone metabolism that occurs in CKD.[5,6]

Mineral and bone disorder is a complication frequently

found in CKD (CKD-MBD). It contributes to increased morbidity and mortality of CVD in CKD patients. Vascular calcification is commonly found with uremia and bone mineral disease state. Vascular calcification is more profound in CKD patients and is a strong predictor for poor CVD outcome in CKD patients.[7,8]

Sclerostin (Scl), a glycoprotein synthesized by osteocytes, works mainly by inhibiting Wnt-ß catenin canonical pathway. The activation of this pathway triggers osteoblasts activity through proliferation, maturation, differentiation, and formation of bone mass. Sclerostin has the potential to attenuate bone mineralization and plays an important role in the bone-vascular axis. Its concentration starts to increase in stage 3 CKD and significantly rises with the progression of renal disease, especially in ESRD patients receiving hemodialysis. The increase of sclerostin is associated with vascular calcification. [9,10] The concentration of sclerostin in pre-dialysis patients with CKD stage 3-5 has not been studied and reported, especially in Indonesia. This study aims to describe the serum sclerostin profile in patients with CKD stage 3-5 not on hemodialysis, as well as to find the correlation between the increase of sclerostin concentration with declining glomerular filtration rate (GFR).

METHODS

This is a cross-sectional study done in May-June 2017. Data is collected from outpatient internal medicine and nephrology clinics from three hospitals in Bandung, West Java. The study collected data from 56 subjects with CKD stage 3-5, selected by consecutive sampling. All subjects have never had any dialysis treatment based on anamnesis, history of illness, and laboratory parameters. All subjects came to the clinics for diagnostic consultation and continuing previous treatment and were known to not have any acute condition that could affect the study. The protocol of the study was informed to all subjects regarding the risks and benefits of the study. Informed consent was acquired from all subjects.

Glomerular Filtration Rate

Stages of CKD were determined by the glomerular filtration rate according to the KDIGO 2012 criteria, and the estimated glomerular filtration rate is calculated using the CKD-EPI formula.

Blood Sample

After participants gave their informed consent, the blood sample was taken by trained nurses and then collected for biochemical parametric analysis. Each blood sample was centrifuged, divided into plasma and serum, and kept in a freezer of -80º C until all samples are collected. All biochemical parameters were then measured, which were urea, creatinine, fasting blood glucose, total cholesterol, and triglycerides, serum calcium, and phosphor. Serum sclerostin was measured using enzyme-linked immunosorbent assay kit.

Statistical Analysis

Data analysis were done with the software GraphPad Prism 7.0. Data is presented in mean values and standard deviations, and in median value, if the data were not normally distributed. Statistical difference of sclerostin concentration between each stage of CKD is measured using One Way ANOVA Test. Afterward, post hoc analysis was done using Bonferroni test to see whether a statistically significant difference exists between each stages' sclerostin concentration. The value of $p < 0.05$ is deemed to be statistically significant.

RESULT

Subject Characteristics

Data from a total of 56 subjects were obtained in this study. The subjects consisted of 32 male (57.1%), and the mean age of the participants was 56 years old (SD ± 13). Mean Body Mass Index (BMI) of the subjects were 23.3 kg/m^2 (SD ± 3.2). Other characteristics including anthropometric and biochemical values are described in Table 1.

CKD Stages

Based on CKD-EPI formula and K-DIGO 2012 criteria, the study population composed of 11 (19.6%) subjects with CKD stage 3, 22 (39.3%) subjects with CKD stage 4, and 23 (41.1%) subjects with CKD stage 5. (Table 1)

Biochemical Parameters

The median value of fasting blood glucose is 106 (60 – 282) mg/dL. The median value of serum cholesterol and triglyceride level are 187 (108 – 487) mg/dL and 144 (53 – 936) mg/dL, respectively. The total number of subjects with negative proteinuria is 7 (12.5%), and the rest of the subjects have +1 to +4 proteinuria. Diabetic kidney disease (DKD) were found to be the cause of kidney function decline in 24 (42%) subjects, hypertension in 17 (30.4%) subjects, chronic pyelonephritis in 10 (17.9%) subjects, and primary glomerulopathy in 5 (8.9%) subjects. A number of 44 (78.6%) subjects have hypertension as a comorbidity, with ACE-Inhibitor medication history in 43 (76.8%) subjects. The mean value of serum sclerostin in the study population is 79.7 (SD ±41.0) pmol/L. (Table 1)

Table 1. Clinical and biochemical characteristics of subjects

	TOTAL n=56	CKD Stage Stage-3 n=11	Stage-4 n=22	Stage-5 n=23	*p-value*
Gender, n (%)					
Male	32 (57.1)	6 (54.5)	11 (50.0)	15 (65.2)	0.577
Female	24 (42.9)	5 (45.5)	11 (50.0)	8 (34.8)	
Age (years)					
Mean ± SD	56 ± 13	52 ± 15	59 ± 13	55 ± 11	0.317
BMI (SD), n (%)					
Mean ± SD)	23.3 ± 3.2	23.6 ± 3.7	23.6 ± 3.0	22.9 ± 3.2	0.705
FBG					
Median (range)	106 (60 – 282)	99 (74 – 167)	127 (60 – 282)	97 (71 – 266)	0.222
Total Cholesterol					
Median (range)	187 (108 – 487)	221 (156 – 471)	178 (108 – 329)	194 (112 – 487)	0.126
Triglyceride					
Median (range)	144 (53 – 936)	143 (82 – 590)	143 (78 – 936)	144 (53 – 342)	0.892
Proteinuria, n (%)					
Negative	7 (12.5)	2 (18.2)	4 (18.2)	1 (4.3)	**0.037***
+1	10 (17.9)	5 (45.5)	3 (13.6)	2 (8.7)	
+2	18 (32.1)	2 (18.2)	10 (45.5)	6 (26.1)	
+3	20 (35.7)	2 (18.2)	5 (22.7)	13 (56.5)	
+4	1 (1.8)	0 (0.0)	0 (0.0)	1 (4.3)	
Etiology, n (%)					
Hypertension	17 (30.4)	2 (18.2)	8 (36.4)	7 (30.4)	0.283
Diabetes	24 (42.9)	3 (27.3)	8 (36.4)	13 (56.5)	
PNC	10 (17.9)	4 (36.4)	4 (18.2)	2 (8.7)	
GNP	5 (8.9)	2 (18.2)	2 (9.1)	1 (4.3)	
Comorbidity, n (%)					
Hypertension	44 (78.6)	10 (90.9)	13 (59.1)	21 (91.3)	**0.017***
Smoking	4 (7.1)	1 (9.1)	0 (0.0)	3 (13.0)	0.227
Drug, n (%)					
ACE	43 (76.8)	9 (81.8)	15 (68.2)	19 (82.6)	0.471
Statin	13 (23.2)	2 (18.2)	8 (36.4)	3 (13.0)	0.163
Aspilet	10 (17.9)	1 (9.1)	4 (18.2)	5 (21.7)	0.666
Sclerostin (pmol/L) Mean ± SD	79.7 ± 41.0	59.6 ± 28.5	71.9 ± 42.2	96.7 ± 39.8	**0.022***

SD=Standard Deviation, [a]Chi Square test, [b]One Way ANOVA-test, [c]Kruskall Wallis test, *p<0.05

Table 2. Post Hoc analysis on serum sclerostin levels between stages

Characteristic	Difference	*p-value*
Stage-3 vs Stage-4	-12.3 (-47.8 – 72.3)	0.999
Stage-3 vs Stage-5	-37.0 (-72.3 – (-1.8))	**0.037***
Stage-4 vs Stage-5	-24.8 (-53.4 – 3.9)	0.112

Analysis using Bonferroni test, * p<0,05

Sclerostin Serum Concentration in Pre-Dialysis CKD Stages 3-5

The mean value of serum sclerostin in the study population is 79.7 (SD ±41.4) pmol/L. When subjects are divided into groups according to their CKD stages 3-5, the mean serum concentration of sclerostin was 59.6 (SD ± 28.5) pmol/L, 71.9 (SD ± 42.2) pmol/L, and 96.7 (SD ± 39.8) pmol/L, respectively. Comparative test using One Way ANOVA test showed a statistically significant difference of serum sclerostin concentration between subjects with CKD stage 3, CKD stage 4, and CKD stage 5 with p = 0.022. (Table 1).

To further analyze the difference of serum sclerostin concentration between each group, a post hoc analysis using Bonferroni test showed that serum sclerostin concentration is significantly different between subjects with CKD stage 3 and subjects with CKD stage 5, with the mean difference of 37 pmol/L (p=0.037). (Table 2, Figure 1)

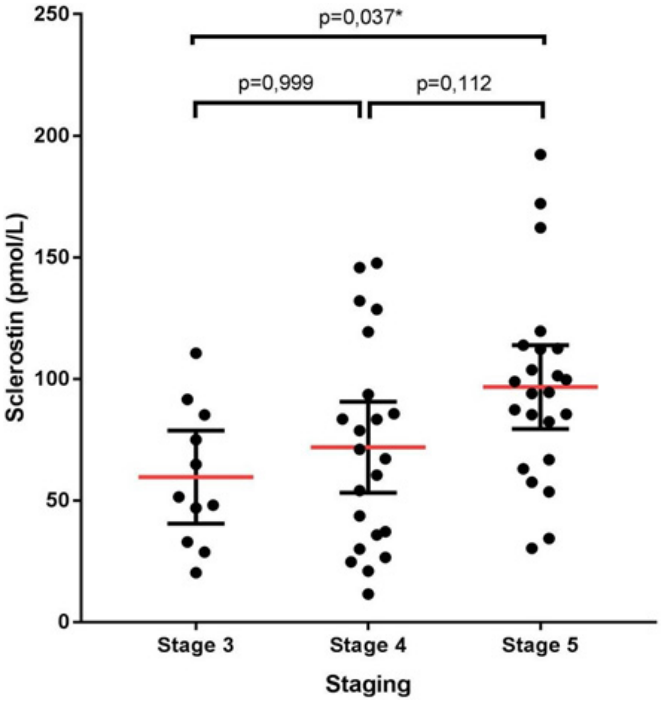

Figure 1. Scatter Plot of sclerostin serum levels between CKD stages.

DISCUSSION

Chronic kidney disease results in multiple systemic complications. The incident and prevalence of complication will increase with the decline of glomerular filtration rate (GFR). Clinical signs and symptoms of the complication will also be more profound in accordance with the progression of the disease into stage 3 – 4. The most notable complications include anemia, anorexia with progressive malnutrition, metabolic disturbance of calcium, phosphor, and mineral-regulating hormones such as calcitriol (1,25(OH)2D3) and parathyroid hormone (PTH), and dysfunction in sodium, potassium, water, and acid-base homeostasis.[2,12,13]

The disruption of mineral homeostasis resulted in calcium and phosphor imbalance and leads to hormonal imbalance. This imbalance includes the improper level of circulating PTH, 25-hydroxyvitamin D (25(OH) D), 1,25-dihydroxy vitamin D (1.25(OH)2D) and other vitamin D metabolites, FGF-23, and growth hormone (GH).[7,14]

The consequences of such disruptions in mineral balance and hyperparathyroidism (HPT) manifested in bone metabolism disorder. This contributes to the high concentration of calcium and phosphor in the systemic circulation. Other than bone malformation, soft tissue (extra-skeletal) calcification also occur due to these abnormalities, such as in the skin and subcutaneous tissue, the cornea and conjunctiva, skeletal muscles, lungs, gastrointestinal tract, and cardiovascular system.[8,9,14,15]

Calcification in the cardiovascular tissues affects the cardiac muscle, cardiac electrical conductive system, and the cardiac valve. Vascular stiffness leads to higher systolic blood pressure, pulse wave velocity (PWV), and increased left ventricular mass. These are markers for CV risk in both general population and CKD population.[8,16]

Extra-skeletal calcification in patients with CKD is more pronounced, progressive, systemic, and complicated compared to the non-CKD population. Vascular calcification is not only related to a number of traditional CV risks such as age, hypertension, and type 2 diabetes but also to non-traditional risk factors such as abnormal mineral metabolism.[17,18]

A study conducted by Wilson et al on 2515 subjects in the Framingham Heart Study showed vascular calcification is a predictor for coronary heart disease, heart failure, and mortality. The risk of CVD and mortality is directly proportional to the degree of calcification. The presence of vascular calcification is also an independent predictor for CV morbidity and mortality.[19]

The osteocyte is the main source of many important glycoproteins, such as FGF-23 and sclerostin. Both serve the purpose as the main key signal of molecular transduction with the effect of negative regulation against signal Wnt/ß-catenin in the bone.[10,11,20,21]

Sclerostin, an inhibitor for Wnt signal pathway, is expressed by most mature osteocytes nested in the bone matrix. Sclerostin is a glycoprotein product (22kDa) from SOST gene composed of 190-residue secreted glycoprotein which contains cysteine-knot motive and

is a part of DAN/cerebrus protein family. A proportion of sclerostin is secreted to the systemic circulation, which affects the vascular smooth muscles.[10,20,22]

Sclerostin plays a role in cross-talk interaction between the bone and the vascular system. Vascular smooth muscle cells (VSMCs) plays a dominant role in medial vascular calcification and can go on a phenotype transition either as an osteoblast or as a chondrocyte in a calcification process environment. This axis also involves bone proteins which regulate the vascular calcification process and vascular proteins that are also involved in bone metabolism regulation.[10,22,23,24]

Sclerostin in CKD

Both pre and post dialysis CKD patients have increased sclerostin serum concentration.[10,11,20] A study by Tumbiah et al on pre-dialysis CKD showed that bone mineral density (BMD) was related to sclerostin level in CKD stage 3b and 4.[25] Pilot study by Pelletier et al studied the relationship between kidney function and sclerostin serum levels in adult CKD patients.[26] Sclerostin levels commonly increase in CKD stage 3. Glomerular filtration rate (GFR), gender, dan serum phosphate level were found to be related to sclerostin levels in these patients.

Another study by Wei et al on CKD stage 3-4 showed that sclerostin serum concentrations were negatively correlated with renal function, and its increase is positively correlated with the prevalence of vascular calcification.[27]

A study by Cejka et al on ESRD patients with dialysis proved that the accumulation of sclerostin contributed to PTH resistance and calcitriol deficiency.[28] This study found that sclerostin and intact parathyroid hormone (iPTH) was negatively correlated on CKD patients with dialysis. A study conducted by Brandenburg et al showed that the risk of coronary artery and aortic calcification was 60% and 40% respectively.[29] This study also proved strong relations between increased sclerostin levels and the prevalence of calcification.[29,30]

Other studies also analyzing sclerostin levels in CKD pre and post dialysis patients showed increased sclerostin levels were associated with multiple parameters such as calcification, cardiovascular survival, and mortality. All of them were related to increased sclerostin accumulation due to decreased renal function.[31,35]

In this study, sclerostin levels rose in proportion with the decline of renal function, and each stage of CKD had significantly high levels of sclerostin with $p = 0.022$ (Table 2). When each stage was compared to each other, subjects with pre-dialysis CKD stage 3 and CKD stage 5 were found to have significantly different sclerostin levels (Table 3) with a mean difference of 37 pmol/L ($p = 0.037$). Subjects in the pre-dialysis CKD stage 5 groups are dominant of the male gender (65%) with the mean age of 55 years old.

A number of epidemiological studies in the past and the ones that are still in progress studied the association between circulating sclerostin and CVD in CKD population, especially the ones undergoing dialysis. These studies give an understanding of the underlying mechanism of mineral metabolism, specifically the role of sclerostin in CKD-MBD.[9,16,25,26,29] It is important to know in advance the abnormal bone and vascular metabolism, a non-traditional CVD risk factor in CKD population. The disruption leads to stiffness and calcification of soft tissues including vascular systems and results in the increase of morbidity and mortality in CVD.[31,35]

This study is a pilot study in Indonesia especially in pre-dialysis CKD patients that study sclerostin as an anti-anabolic protein involved in the bone-vascular axis. The limitation of this study that it is a cross-sectional study and cannot elaborate the causality between the two variables, having a relatively small number of subjects (56 patients), and can only describe the difference of sclerostin levels between each CKD patients' groups. However, this study can be a starting point for further researches in the topic of CKD-MBD and its association with sclerostin in pre-dialysis CKD patients.

In summary, it is shown that there was a significant association between the increase of serum sclerostin level with the decline of kidney function in pre-dialysis CKD stage 3-5 patients. It is important to further analyze the role of sclerostin as anti-anabolic protein in bone metabolism and its impact on vascular calcification through the kidney-bone-vascular axis.

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