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Correlation between Wnt Signalling Inhibitors (Dickkopf-1 & Sclerostin) and the Intimal Medial Thickness in Children on Maintenance Hemodialysis

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Abstract

Background: Wnt signalling inhibitors (Dickkopf-1 and Sclerostin) signalling play a role in vascular development and may contribute to calcification. **Aim:** To investigate the association between Dickkopf-1 and sclerostin serum concentrations in children undergoing maintenance hemodialysis with intimal medial thickness and peak systolic velocity of the main arteries. **Patients and Methods:** A study was conducted on 40 children undergoing maintenance hemodialysis and controls of the same age and sex. The study measured the initial medial thickness (IMT) and peak systolic velocity (PSV) of the main vessels (carotid, ulnar, and femoral). Dickkopf-1 and sclerostin serum levels in both groups were assessed, and a routine investigation was performed. **Results:** The findings indicate that the levels of serum Dickkopf-1 and Sclerostin were significantly higher in the hemodialysis group 2540.65 (2215.4 - 2909.2 pg/ml) and 1.17 (0.85 - 2.03 ng/ml)respectively (P = 0.001), compared to their control group it was 1110.45 (885.45 - 1527.65 pg/ml) and 0.28 (0.25 - 0.32 ng/ml)) respectively P = 0.001. Additionally, there was a significant increase in intima-media thickness (IMT) with a decrease in peak systolic velocity (PSV) in the main blood vessels, including the carotid, ulnar, and femoral arteries. A significant correlation was also observed between Dickkopf-1 and sclerostin levels and IMT of the carotid, ulnar, and femoral arteries. **Conclusion:** Wnt signalling inhibitors (Dickkopf-1 and Sclerostin) exert effects beyond the bone and significantly contribute to early vascular calcifica-

tion in pediatric patients undergoing maintenance hemodialysis.

Keywords

Wnt Signalling, Vascular Calcification, Hemodialysis, Children

1. Background

Patients with chronic kidney disease (CKD) have a higher likelihood of experiencing cardiovascular events. This increased risk cannot be solely attributed to traditional risk factors like age, hypertension, and diabetes. Other factors related to CKD and the chronic inflammatory state of 'uremia' may also contribute to this heightened risk [1] [2].

Premature arterial stiffening is primarily caused by disrupted calcium phosphate balance, which is critical in mediating these associations [3].

In chronic kidney disease (CKD), there is a risk of early-stage damage and calcification of blood vessels, which can quickly progress once dialysis is initiated [4] [5].

It's essential to boost our knowledge of the mechanisms involved, create reliable diagnostic methods, and establish effective prevention and treatment strategies. Recognising and addressing these risk factors is vital for reducing the risk of cardiovascular disease in patients [6] [7].

The Wnt signalling pathway plays a role in bone turnover. Calcifying smooth muscle cells is similar to osteoblasts, and increasing evidence suggests Wnt's involvement in vascular calcification. Different studies have identified various inducers, inhibitors, and regulatory proteins related to Wnt and other signalling pathways [8].

> "Dickkopf-1 and sclerostin are essential glycoproteins that play a pivotal role in regulating bone formation by counteracting Wnt/β-catenin signalling in osteoblasts and osteoclasts [9]."

Dickkopf-1, a secretory glycoprotein, can block the Wnt pathway by competitively binding to receptors (e.g., LRP5/6, Kremen) on the cell membrane [10].

Dickkopf-1 promotes inflammation in atherosclerotic plaques and is an atherogenic factor [11]. In a previous clinical study of patients with ACS, we found that DKK1 plasma levels were not only correlated with disease severity but also served as a prognostic predictor. Thus, DKK1 concentration may reflect the severity and stability of coronary atherosclerosis [12].

Sclerostin, a glycoprotein secreted by osteocytes, robustly binds to the transmembrane receptors of osteoblasts, LRP5/6, and effectively suppresses osteoblast proliferation, maturation, and differentiation. This inhibition results in the blockade of bone formation by interrupting the Wnt/β-catenin signalling pathway [13].

Vascular smooth muscle cells (VSMCs) line the walls of arteries and regulate blood pressure. During calcification, these cells can undergo a transformation

similar to that of bone-forming cells, much like mesenchymal stem cells transform into osteoblast cells during bone formation.

In atherosclerosis, the transformation of VSMCs into osteoblast-like cells leads to the expression of sclerostin, which inhibits the Wnt pathway [14] [15].

The published data highlight that the upregulation of Wnt components facilitates the initiation and progression of atherosclerosis, arterial remodelling, VSMC proliferation and phenotypic transition to the osteoblastic lineage in the arterial wall [16].

In most, but not all, cross-sectional observational studies, circulating sclerostin levels are positively associated with cardiovascular calcification [17] [18]. This may be due to variations in the study design, the unique populations and models studied, and the heterogeneous methods used.

We hypothesised that sclerostin and Dickkopf-1, which are Wnt antagonists in circulation, may contribute to vascular calcification in children undergoing maintenance hemodialysis.

2. Methods

This case-control study included 80 children. Of these, 40 were children with end-stage renal disease and were undergoing maintenance hemodialysis. The controls were of the same age and sex and were clinically healthy. These children were selected from the Pediatric Hemodialysis Unit and outpatient general clinic of AL-Zahraa Hospital, Al-Azhar University Cairo, Egypt. The age range of the patients was 4-16 years; 13 (32.5%) were female, and 27 (67.5%) were male, and they received dialysis three times a week for four hours per session at least three months at the time of the study [19]. A low-flux polysulfone dialyzer on the Fresenius 4008S was used for some patients, while a high-flux dialyzer was used on the Fresenius 5008 machine. The most common cause of end-stage renal disease was acquired in 15 patients (37.5%), followed by focal segmental glomerulosclerosis in 10 patients (25%), interstitial nephritis in 3 patients (7.5%), mesangial proliferative glomerulonephritis in 2 patients (5%), and congenital causes in 12 patients (30%), including vesicoureteral reflux in 5 patients (12.5%), multicystic dysplastic kidney in 5 patients (12.5%), and posterior urethral valve in 2 patients (5%). The cause was unknown in 11 cases (27.5%), whereas Joubert syndrome was responsible for 2 cases (5%). This study was conducted with the participation of the pediatric (nephrology and hemodialysis), clinical pathology, and radiology departments. Children with structural heart diseases were excluded from the study. All study populations were subjected to entire history taking, including aetiology, the onset of CKD, duration of hemodialysis, cardiac symptoms, medications, addition to routine and specific laboratory investigations, and radiological investigation that assessed the intimal medial thickness IMT and the peak systolic velocity (PSV)of the main arteries. Written consent was obtained from the participating parents in adherence to the ethical committee guidelines, Faculty of Medicine (for Girls), Cairo, AL-Azhar University, with

ID no. 2334.

Laboratory investigations

Sampling

Blood samples were collected during the mid-week dialysis session after a 12-hour overnight fast. The blood was drawn into three tubes using a vacuum system: one with EDTA and two gel vacuum tubes. An EDTA tube was used to analyze the CBC on the automated cell counter Swelab alpha. In contrast, one gel tube was used to measure urea, creatinine, calcium, phosphorous, potassium, cholesterol, and triglyceride levels using a Cobas C311 chemistry autoanalyzer (Roche Diagnostics).

The second gel tube was centrifuged, and the serum was separated into two tubes. After careful labelling, it was stored at −20 until the DKK and Sclerostin assays.

Dickkopf levels were measured in the serum using ELISA with Fine Test kits. Samples were added to wells coated with a specific antibody, followed by incubation with biotin-labelled antibodies and an HRP conjugate. TMB substrate was added to create a blue colour in wells containing Dickkopf.1. After stopping the reaction with sulfuric acid and measuring the optical density at 450 nm; the results were calculated using a standard curve (https://www.fn-test.com/product/em0067/).

Quantitative determination of the serum sclerostin concentration was performed using ELISA. The kits were delivered by Glory Bioscience (www. glory-bioscience.com). In a microtiter plate, samples were added to wells coated with antibodies specific to sclerostin (SOST), incubated with SOST antibody and labelled HRP to form an antibody-antigen-enzyme-antibody complex. The wells were washed to remove non-specific complexes, and TMB substrate solution was added. The TMB substrate became blue in the HRP enzyme-catalyzed wells. The reaction was terminated by adding a stop solution, and the colour change was measured at a wavelength of 450 nm. The concentration of SOST in the samples was determined by comparing the (OD)cal density O.D. of the samples to the standard curve.

Radiological investigations:

Doppler ultrasound was used to measure the intimal medial thickness and peak systolic velocity of the main arteries, including the carotid, femoral, and ulnar arteries, using our university hospital's "Esaote MyLab 50 Xvision" apparatus.

Intimal medial thickness assessment:

The inner lining thickness of the carotid, femoral, and ulnar arteries was measured using grey-scale ultrasound with a 7.5 MHz probe. Patients were examined while lying down with their heads slightly tilted to the side for carotid measurements. For femoral measurements, the groin and abdominal area were exposed. The intima-media thickness was determined as the distance between the leading edge of the lumen-intima interface and the media-adventitia interface on the artery's far wall [20].

Peak systolic velocity (PSV)

The peak systolic velocity (PSV) was measured using grayscale ultrasound with a 7.5 MHz probe. Patients were examined while lying down. The groin area was evaluated for femoral PSV, the neck was tilted to one side for carotid PSV, and the ulnar artery of the upper limb was assessed. Gel was applied to the examination area, and femoral arteries were examined longitudinally using a 7.5 MHz probe. Doppler examination included B-mode, colour mode, pulse wave, and peak systolic velocity measurements.

Statistical analysis

Data were collected, revised, coded, and entered into the Statistical Package for Social Science (IBM SPSS) version 27 by IBM in the USA. Qualitative data were presented as numbers and percentages. In contrast, quantitative data were presented as mean, standard deviations, and ranges when their distribution was parametric and as median with interquartile ranges(IQR) when their distribution was nonparametric. The comparison between two groups with qualitative data was done using the Chi-square test and Fisher exact test instead of the Chi-square test when the expected count in any cell was less than 5.

The comparison between two independent groups with quantitative data was done using an Independent t-test when the data were parametric and a Mann-Whitney test when the data were non-parametric. Spearman correlation coefficients were used to assess the relation between two studied parameters in the same group.

When the data were nonparametric, **Spearman correlation coefficients** were used to assess the relation between two studied parameters in the same group.

The receiver operating characteristic (ROC) curve was used to determine the best cut-off point based on sensitivity and specificity. Regarding probability values, it was explicitly stated that $P > 0.05$ was considered non-significant, while $P < 0.05$ was deemed significant.

The results

Table 1 compares patients and their controls, revealing a significant increase in systolic and diastolic blood pressure, cholesterol, triglyceride, urea, creatinine, PTH, phosphate, and CRP levels in the hemodialysis group compared to their controls. Additionally, platelet counts were higher in the patients than in their controls. The same table shows a significant increase in sclerostin and DKK1 in the hemodialysis group compared to their controls. Meanwhile, there was a substantial decrease in the haemoglobin, weight, and height z score in the patients when compared to their controls.

Table 2 shows that the most common causes of CKD in the patient's group are acquired in 15 (37.5%), congenital in 12 (30%), and unknown in 11 (27.5), followed by hereditary causes in 2 (5%).

Table 3 compares the two groups (hemodialysis patients and controls) regarding the intima-media thickness (IMT) and peak systolic velocity (PSV) of the main arteries. IMT of the carotid, ulnar, and femoral arteries was significantly higher

Table 1. The clinical and laboratory data of the patients and their corresponding control group.

Variable	Control group No. = 40 Mean ± SD	Patients group No. = 40 Mean ± SD	Test value	P-value
Age (years)	10.60 ± 2.98	11.98 ± 3.39	1.929	0.057
SBP (mmHg)	101.75 ± 7.89	131.0 ± 13.92	−11.560	0.001
DBP (mmHg)	61.38 ± 6.20	83.63 ± 9.13	−12.753	0.001
TLCX 10^3/UL	7.72 ± 1.30	6.61 ± 2.31	2.655	0.01
RBCsX 10^6/UL	4.74 ± 0.37	3.51 ± 0.63	10.669	0.001
Hb(gm/dl)	12.00 ± 0.86	9.46 ± 1.85	7.911	0.001
Hct%	36.46 ± 2.50	28.61 ± 5.75	7.922	0.001
Platelet 10^3/UL	±79.96224	291.78 ± 54.88	4.377	0.001
Ca(mg/dl)	9.61 ± 2.31	8.46 ± 2.55	1.543	0.127
Ph(mg/dl)	3.51 ± 0.63	5.44 ± 2.18	−3.100	0.003
Cholesterol mg/dl	101.95 ± 11.79	168.93 ± 32.41	−12.281	0.001
Triglyceride mg/dl	83.38 ± 17.44	175.33 ± 46.46	−11.718	0.001
Urea mg/dl	6.97 ± 26.43	36.26 ± 113.9	112.97	0.001
Variable	Median (IQR)	Median (IQR)	Test value	p
Z-score: Wt	0.29 (−0.48 - 1.30)	−0.56 (−1.00 - 0.22)	3.137	0.002
Z-score: Ht	0.55 (−0.18 - 1.13)	−0.43 (−1.24 - 0.30)	4.043	0.001
Z-score: BMI	−0.32 (−0.82 - 1.13)	−0.18 (−0.68 - 0.25)	0.720	0.474
CRP (mg/l)	3 (2 - 4)	12.5 (8 - 24)	−6.664	0.001
Creatinine(md/dl)	0.4 (0.25 - 0.50)	2.1 (1.65 - 3.20)	−7.704	0.001
PTH (pg/ml)	54.5 (44.5 - 63.0)	342.5 (173.5 - 714)	−7.430	0.001
Sclerostin (ng/ml)	0.28 (0.25 - 0.32)	1.17 (0.85 - 2.03)	−7.701	0.001
Dickkopf-1 (pg/ml).	1100 (880 - 1537.8)	2540.65 (2215.4 - 2909.2)	6.805	0.001

SBP: systolic blood pressure; DBP: diastolic blood pressure; PTH: parathyroid hormone.

Table 2. Etiology of CKD of the study patients.

1ry lesion of CKD	No.40 No. (%)
Congenital	12 (30%)
Reflux nephropathy	5 (12.5%)
Posterior urethral valve	2 (5%)
Multicyclic dysplastic kidney	5 (12.5%)
Hereditary	2 (5%)
Joubert syndrome	2 (5%)

Continued

Acquired	15 (37.5%)
Focal segmental glomerulosclerosis	10 (25%)
Masango proliferative glomerulonephritis	2 (5%)
Interstitial nephritis	3 (7.5%)
Unknown	11 (27.5%)

Table 3. Comparison of IMT & PSV of the main arteries between the patient group & control group.

IMT (cm)	Control group No. = 40 Mean ± SD	Patients group No. = 40 Mean ± SD	t-test	P-value
Carotid	0.05 ± 0.01	0.06 ± 0.02	−2.992	0.004
Ulnar	0.04 ± 0.01	0.05 ± 0.01	−3.800	0.001
Femoral	0.05 ± 0.01	0.07 ± 0.01	−6.616	0.001
Mean PSV cm/sec				
Carotid	52.8 ± 8.16	47.35 ± 4.81	3.641	0.001
Ulnar	43.53 ± 6.26	38.8 ± 6.36	3.349	0.0003
Femoral	60 ± 8.54	57.43 ± 9.88	1.247	0.10806

IMT: intimal medial thickness; PSV: peak systolic velocity.

in the hemodialysis group than in the control group. Additionally, the PSV in the carotid and ulnar arteries was considerably lower in the hemodialysis group than in the control group.

Table 4 shows that sclerostin was significantly correlated with blood pressure, platelet count, PTH, cholesterol, triglyceride, and CRP levels. Moreover, sclerostin was found to have a significant correlation with the intima-media thickness (IMT) of the carotid, ulnar, and femoral arteries. However, there was a significant negative correlation between sclerostin level and height, serum calcium level, and peak systolic velocity (PSV) of the main arteries.

Similarly, the same table also reveals a significant correlation between Dickkopf-1. and age, blood pressure, platelets, cholesterol, triglycerides, and PTH levels. Additionally, it showed a significant correlation with the IMT of the main vessels' carotid, ulnar, and femoral arteries, with a negative correlation with the height and PSV of the main arteries.

Table 5 displays the optimal cutoff point, sensitivity, and specificity of sclerostin and Dickkopf-1 in predicting cardiovascular risk in children undergoing maintenance hemodialysis. The sensitivity and specificity were 100.0%, 87.50%, 100.0%, and 97%, respectively.

Figure 1 depicts the cIMT of one patient group in the study, with a carotid intimal medial thickness of 0.04 cm.

Table 4. Sclerostin and Dickkopf-1 level correlation with the study groups' clinical, laboratory and radiological data

Variable	Sclerostin (ng/ml)		Dickkopf-1 (pg/ml)	
	r	p-value	r	p-value
Sclerostin (ng/ml)	1.000		0.574	0.001
Dickkopf-1	0.574	0.001		
Age	0.298	0.062	0.349	0.027
Ht (cm)	−0.486	0.001	−0.350	0.027
BMI	0.077	0.636	0.035	0.830
SBP (mmHg)	0.253	0.115	0.415	0.008
DBP (mmHg)	0.425	0.006	0.346	0.029
TLCX 10^3/UL	−0.082	0.615	−0.009	0.954
RBCsX 10^6/UL	0.194	0.230	0.135	0.408
Hb (gm/dl)	0.185	0.252	0.171	0.291
Hct %	0.179	0.270	0.057	0.727
Platelet 10^3 /UL	0.440	0.004	0.362	0.022
Urea (mg/dl)	−0.226	0.161	−0.182	0.261
Creatinine (mg/dl)	−0.189	0.243	−0.163	0.316
Ca (mg/dl)	−0.581	0.001	−0.311	0.051
Ph (mg/dl)	−0.140	0.389	−0.125	0.441
ALP (U/L)	−0.131	0.420	0.071	0.664
PTH (pg/ml)	0.631	0.001	0.406	0.009
Cholesterol (mg/dl)	0.423	0.007	0.093	0.570
Triglyceride (mg/dl)	0.514	0.001	0.246	0.126
CRP (mg/l)	0.628	0.001	0.378	0.016
Carotid IMT (cm)	0.663	0.001	0.516	0.001
Ulnar IMT (cm)	0.437	0.005	0.319	0.045
Femoral IMT (cm)	0.410	0.009	0.355	0.024

IMT: intimal medial thickness; BMI: body mass index; PTH: parathyroid hormone: ALP: alkaline phosphatase.

Table 5. The best cut-off points for sensitivity and specificity of sclerostin and Dickkopf-1 in predicting cardiovascular risk in children on maintenance hemodialysis.

Parameters	Cut off point	AUC	Sensitivity	Specificity	+PV	−PV
Sclerostin (ng/ml)	>0.38	1.000	100.0%	100.0%	100.0%	100.0%
Dickkopf-1 (pg/ml)	>1851	0.945	87.50%	97.44%	97.2%	88.4%

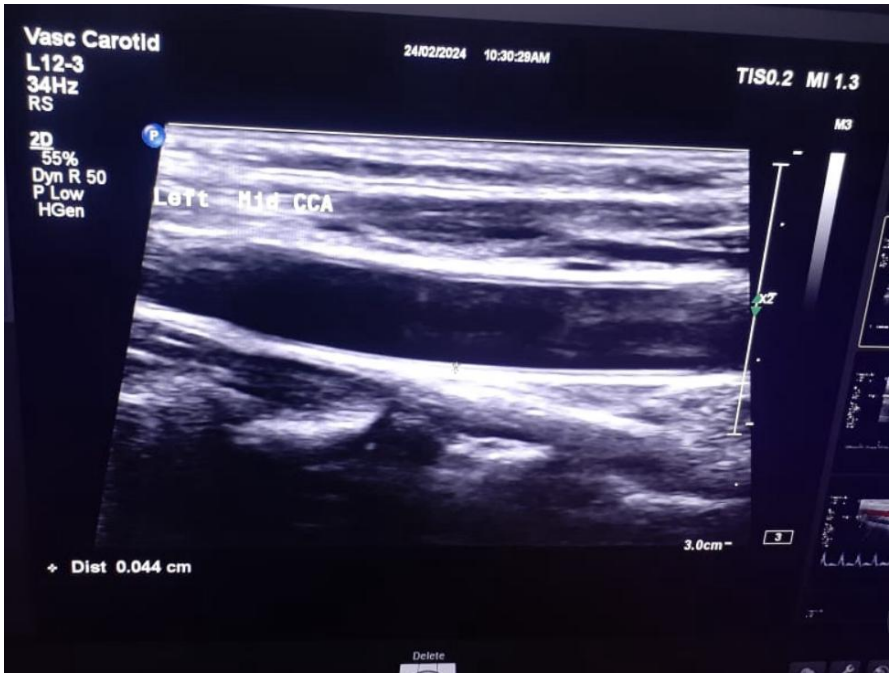

Figure 1. Carotid intimal medial thickness in one of the study patient group.

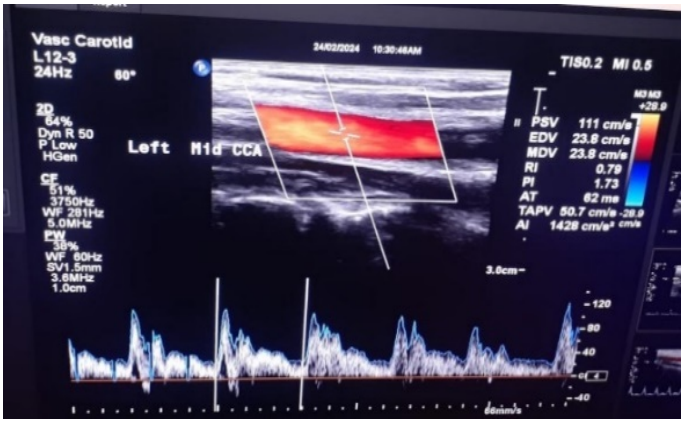

Figure 2. The mean PSV of the carotid artery in one of the study patient group.

Figure 2 shows that one of the study patients' groups, PSV, is 50 cm/sec.

Figure 3 shows a significant positive correlation between carotid IMT and sclerostin serum levels.

Figure 4 shows a significant positive correlation between the carotid IMT and Dickkopf-1.

Figure 5 shows the specificity and sensitivity of sclerostin and Dickkopf-1 Serum levels in predicting cardiovascular risk in pediatric patients on regular hemodialysis: 100.0%, 87.50%, 100.0%, and 97%, respectively.

3. Discussion

This case-control study is the first to describe the impact of sclerostin and Dickkopf-1 on vascular calcification (VC) in children undergoing hemodialysis by

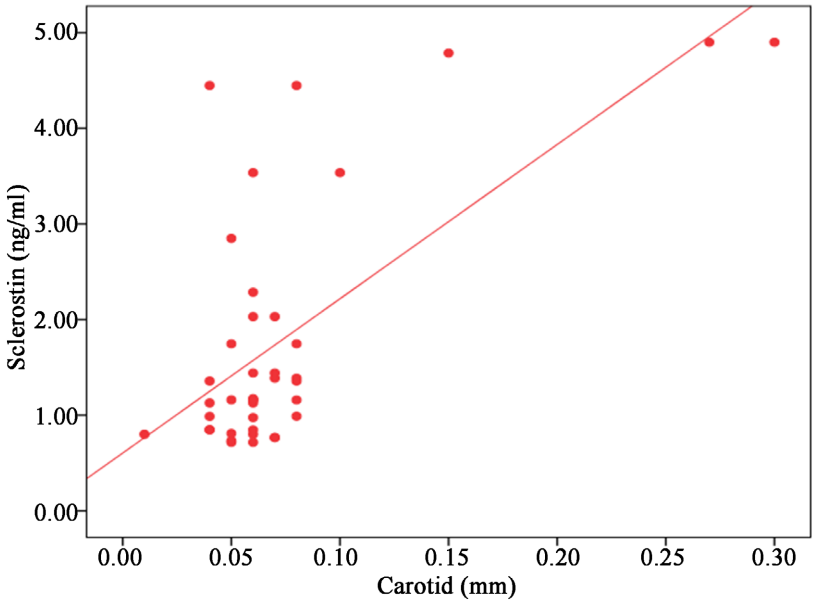

Figure 3. Correlation between the carotid IMT and sclerostin serum level.

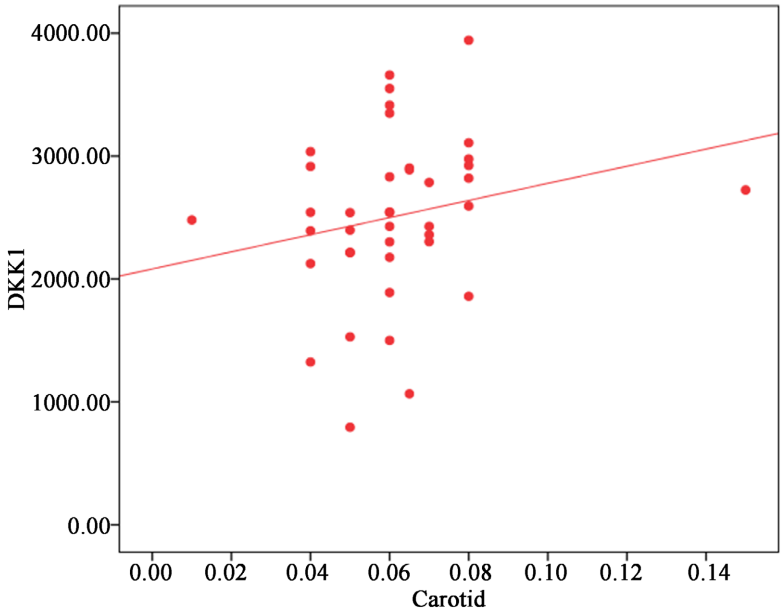

Figure 4. Correlation between the carotid IMT and Dickkopf-1 (DKK1).

investigating the correlation between sclerostin and Dickkopf-1 and intima-media thickness (IMT), peak systolic velocity of the main arteries, and traditional calcification markers.

The current study found that children undergoing maintenance hemodialysis showed significantly increased levels of sclerostin and Dickkopf-1 in their serum. As chronic kidney disease progresses, plasma sclerostin levels rise, reaching the highest levels in HD patients [21]. However, the role of serum sclerostin and Dickkopf-1 in patients with CKD varies across different studies. Some investigations have shown positive correlations, while others have suggested no or even

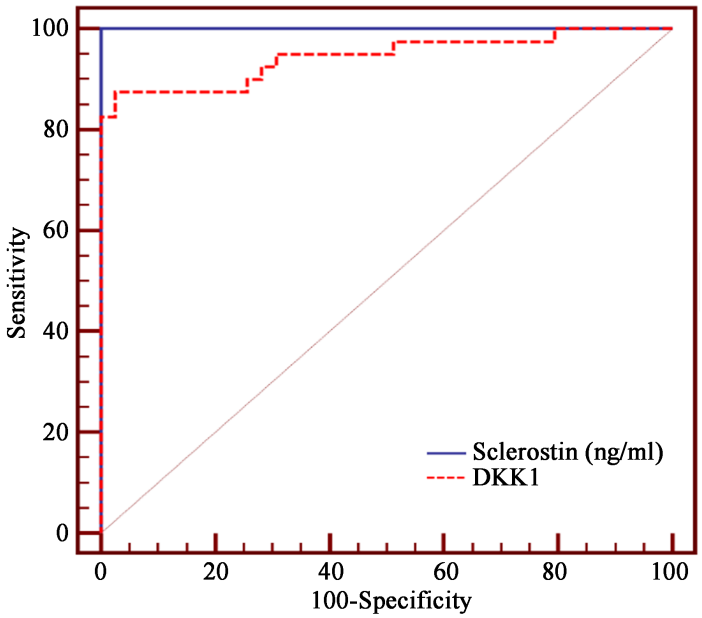

Figure 5. ROC curve for sclerostin and Dickkopf-1in predicting cardiovascular risk in children on maintenance hemodialysis.

negative correlations [16].

The current study recorded a significant increase in the IMT of the main arteries and carotid, ulnar, and brachial arteries in children undergoing hemodialysis. The thickness was notably higher in the hemodialysis group than in the control group.

Vascular calcification (VC) is the bone-like material forming in blood vessels due to inflammatory factors. However, the specific mechanisms behind VC in patients with chronic kidney disease (CKD) are not fully understood yet. One known mechanism involves transforming vascular smooth muscle cells (VSMCs) into cells similar to bone tissue within the arteries [22] [23].

Furthermore, atherosclerosis, a chronic inflammatory condition, significantly contributes to cardiovascular diseases by causing plaque rupture and thrombosis, as emphasised [24] [25]. Exciting research has found a significant positive correlation between Sclerostin and Dickkopf-1 levels and the thickness of the carotid, ulnar, and brachial arteries in hemodialysis patients. This study suggests that sclerostin may directly affect atherosclerosis through biological mechanisms related to Wnt signalling. Previous research has linked Wnt signalling to the development of atherosclerosis. [26]. However, as Wnt inhibitors, sclerostin and Dickkopf-1 are predicted to protect against atherosclerosis instead of increasing its risk. Although sclerostin and Dickkopf-1 inhibit skeletal mineralization, this is secondary to their effect on bone formation and osteoblast function and represents an entirely distinct process of vascular calcification [27] [28].

Sclerostin and Dickkopf-1 inhibit the canonical Wnt signalling pathway, which controls cellular proliferation and differentiation and regulates bone formation, immune responses [19], atherosclerosis, and vascular calcification [29]

[30].

The presence of sclerostin in vascular tissue suggests it might directly affect vascular calcification and atherosclerosis, consistent with previous observations [31] [32].

Canonical Wnt signalling differentiates progenitor and vascular smooth muscle cells in vascular calcification into osteo/chondrogenic phenotypes [33]. Both dickkopf-1 and sclerostin [34] [35] reduce the expression of runt-related transcription factor 2, which is crucial for osteogenic differentiation [36].

Numerous studies have unequivocally established a direct positive correlation between serum sclerostin concentration and the severity of aortic valve, coronary, or aortic calcification across diverse populations, encompassing patients with end-stage renal disease (ESRD), less severe CKD, healthy men, and postmenopausal women [37]-[38].

In a study of rheumatoid arthritis patients, a clear association was found between serum sclerostin levels and the severity of aortic calcification. However, no association was observed with the severity of coronary artery calcification [39].

It was established that there is an inverse association between serum sclerostin concentration and the severity of abdominal aortic calcification in patients with ESRD [40]. Di M *et al.* found that overexpression of Dickkopf-1 results in enlarged and destabilised atherosclerotic lesions and increased apoptosis in aortic and carotid plaque [41].

The current study showed a significant decrease in the main arteries' peak systolic velocity (PSV). Pulse-wave velocity (PWV) is an unquestionably reliable indicator of arterial stiffness. It is widely acknowledged as an independent predictor of cardiovascular events and mortality in patients with chronic kidney disease (CKD) who require hemodialysis [42].

This study found that the decrease in PWV was significantly correlated with the levels of sclerostin and Dickkopf-1. Previous studies have shown a negative relationship between circulating Dickkopf-1 and arterial stiffness in patients with CKD [43]. The current study reported a significant association of sclerostin and Dickkopf-1 with systolic and diastolic blood pressure, which could be explained by arterial sclerosis and reduced PSV in these patients. Thickened cIMT is strongly associated with incident hypertension risk [44].

We observed a significant correlation between Dickkopf-1 and sclerostin and CRP and platelets. Dickkopf-1 is linked to inflammation, platelet activation, and endothelial dysfunction, and it plays a crucial role in the early stages of atherosclerosis. In an inflammatory environment within the atherosclerotic plaques, activated platelets release Dickkopf-1. This inhibits the Wnt/β-catenin pathway in endothelial cells and contributes to the atherosclerotic process [45].

Our analysis revealed a significant correlation between sclerostin, cholesterol, and triglycerides. However, we did not examine any other lipid profiles. In terms of the potential role of abnormal lipid metabolism in mediating the connection

between sclerostin and cardiovascular disease, a previous study also discovered a positive link between sclerostin and LDL cholesterol [46], which is consistent with our current findings. This may represent another mechanism through which sclerostin influences CVD risk.

4. Limitations of the Study

Not many studies focus on this issue in the pediatric age group.

5. Conclusion

The current study implicates sclerostin and Dickkopf-1 in vascular calcification, and they are highly independent early predictors of vascular calcification in children on maintenance hemodialysis.

6. Recommendation

Further research is required to comprehend the roles of Dickkopf-1 and sclerostin in the pathophysiology of vascular calcification.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Mitsnefes, M.M. (2012) Cardiovascular Disease in Children with Chronic Kidney Disease. *Journal of the American Society of Nephrology*, **23**, 578-585. https://doi.org/10.1681/asn.2011111115

[2] Georgianos, P.I., Sarafidis, P.A. and Lasaridis, A.N. (2015) Arterial Stiffness: A Novel Cardiovascular Risk Factor in Kidney Disease Patients. *Current Vascular Pharmacology*, **13**, 229-238. https://doi.org/10.2174/15701611113119990147

[3] Memmos, E., Sarafidis, P., Pateinakis, P., Tsiantoulas, A., Faitatzidou, D., Giamalis, P., *et al.* (2019) Soluble Klotho Is Associated with Mortality and Cardiovascular Events in Hemodialysis. *BMC Nephrology*, **20**, Article No. 217. https://doi.org/10.1186/s12882-019-1391-1

[4] Chavers, B.M., Molony, J.T., Solid, C.A., Rheault, M.N. and Collins, A.J. (2015) One-Year Mortality Rates in US Children with End-Stage Renal Disease. *American Journal of Nephrology*, **41**, 121-128. https://doi.org/10.1159/000380828

[5] Guérin, A.P., Pannier, B., Marchais, S.J. and London, G.M. (2008) Arterial Structure and Function in End-Stage Renal Disease. *Current Hypertension Reports*, **10**, 107-111. https://doi.org/10.1007/s11906-008-0021-2

[6] Shroff, R.C., Donald, A.E., Hiorns, M.P., Watson, A., Feather, S., Milford, D., *et al.* (2007) Mineral Metabolism and Vascular Damage in Children on Dialysis. *Journal of the American Society of Nephrology*, **18**, 2996-3003. https://doi.org/10.1681/asn.2006121397

[7] Litwin, M., Wu, E., Jourdan, C., Trelewicz, J., Niemirska, A., Fahr, K., *et al.* (2005) Altered Morphologic Properties of Large Arteries in Children with Chronic Renal Failure and after Renal Transplantation. *Journal of the American Society of Nephrology*, **16**, 1494-1500. https://doi.org/10.1681/asn.2004110932

[8] Schaefer, F., Doyon, A., Azukaitis, K., Bayazit, A., Canpolat, N., Duzova, A., *et al.* (2016) Cardiovascular Phenotypes in Children with CKD: The 4C Study. *Clinical Journal of the American Society of Nephrology*, **12**, 19-28. https://doi.org/10.2215/cjn.01090216

[9] Shroff, R.C., McNair, R., Figg, N., Skepper, J.N., Schurgers, L., Gupta, A., *et al.* (2008) Dialysis Accelerates Medial Vascular Calcification in Part by Triggering Smooth Muscle Cell Apoptosis. *Circulation*, **118**, 1748-1757. https://doi.org/10.1161/circulationaha.108.783738

[10] Shroff, R.C., McNair, R., Skepper, J.N., Figg, N., Schurgers, L.J., Deanfield, J., *et al.* (2010) Chronic Mineral Dysregulation Promotes Vascular Smooth Muscle Cell Adaptation and Extracellular Matrix Calcification. *Journal of the American Society of Nephrology*, **21**, 103-112. https://doi.org/10.1681/asn.2009060640

[11] Shroff, R., Long, D.A. and Shanahan, C. (2013) Mechanistic Insights into Vascular Calcification in CKD. *Journal of the American Society of Nephrology*, **24**, 179-189. https://doi.org/10.1681/asn.2011121191

[12] Mitsnefes, M.M. (2008) Cardiovascular Complications of Pediatric Chronic Kidney Disease. *Pediatric Nephrology*, **23**, 27-39. https://doi.org/10.1007/s00467-006-0359-0

[13] Baron, R. and Kneissel, M. (2013) WNT Signaling in Bone Homeostasis and Disease: From Human Mutations to Treatments. *Nature Medicine*, **19**, 179-192. https://doi.org/10.1038/nm.3074

[14] Monroe, D.G., McGee-Lawrence, M.E., Oursler, M.J. and Westendorf, J.J. (2012) Update on Wnt Signaling in Bone Cell Biology and Bone Disease. *Gene*, **492**, 1-18. https://doi.org/10.1016/j.gene.2011.10.044

[15] Evenepoel, P., D'Haese, P. and Brandenburg, V. (2015) Sclerostin and DKK1: New Players in Renal Bone and Vascular Disease. *Kidney International*, **88**, 235-240. https://doi.org/10.1038/ki.2015.156

[16] Cejka, D., Herberth, J., Branscum, A.J., Fardo, D.W., Monier-Faugere, M., Diarra, D., *et al.* (2011) Sclerostin and Dickkopf-1 in Renal Osteodystrophy. *Clinical Journal of the American Society of Nephrology*, **6**, 877-882. https://doi.org/10.2215/cjn.06550810

[17] Golledge, J. and Thanigaimani, S. (2022) Role of Sclerostin in Cardiovascular Disease. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **42**, e187-e202. https://doi.org/10.1161/atvbaha.122.317635

[18] Krishna, S.M., Seto, S., Jose, R.J., Li, J., Morton, S.K., Biros, E., *et al.* (2017) Wnt Signaling Pathway Inhibitor Sclerostin Inhibits Angiotensin Ii-Induced Aortic Aneurysm and Atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **37**, 553-566. https://doi.org/10.1161/atvbaha.116.308723

[19] Kidney Disease: Improving Global Outcomes (KDIGO) ANCA Vasculitis Work Group (2024) KDIGO 2024 Clinical Practice Guideline for the Management of Antineutrophil Cytoplasmic Antibody (ANCA)-Associated Vasculitis. *Kidney International*, **105**, S71-S116. https://doi.org/10.1016/j.kint.2023.10.008

[20] Jayanthi, K.B., Rajasekaran, C., Madian, N. and Thyagarajah, K. (2017). Analysis on Various Segmentation Techniques—IMT Measurement of Common Carotid Artery. *TENCON* 2017-2017 *IEEE Region* 10 *Conference*, Penang, 5-8 November 2017, 2435-2440. https://doi.org/10.1109/tencon.2017.8228270

[21] Claes, K.J., Viaene, L., Heye, S., Meijers, B., d'Haese, P. and Evenepoel, P. (2013) Sclerostin: Another Vascular Calcification Inhibitor? *The Journal of Clinical Endo-*

crinology & Metabolism, **98**, 3221-3228. https://doi.org/10.1210/jc.2013-1521

[22] Evenepoel, P., Goffin, E., Meijers, B., Kanaan, N., Bammens, B., Coche, E., *et al.* (2015) Sclerostin Serum Levels and Vascular Calcification Progression in Prevalent Renal Transplant Recipients. *The Journal of Clinical Endocrinology & Metabolism*, **100**, 4669-4676. https://doi.org/10.1210/jc.2015-3056

[23] Speer, M.Y., Yang, H., Brabb, T., Leaf, E., Look, A., Lin, W., *et al.* (2009) Smooth Muscle Cells Give Rise to Osteochondrogenic Precursors and Chondrocytes in Calcifying Arteries. *Circulation Research*, **104**, 733-741. https://doi.org/10.1161/circresaha.108.183053

[24] Lusis, A.J. (2000) Atherosclerosis. *Nature*, **407**, 233-241. https://doi.org/10.1038/35025203

[25] Herrington, W., Lacey, B., Sherliker, P., Armitage, J. and Lewington, S. (2016) Epidemiology of Atherosclerosis and the Potential to Reduce the Global Burden of Atherothrombotic Disease. *Circulation Research*, **118**, 535-546. https://doi.org/10.1161/circresaha.115.307611

[26] Clevers, H. (2006) Wnt/β-Catenin Signaling in Development and Disease. *Cell*, **127**, 469-480. https://doi.org/10.1016/j.cell.2006.10.018

[27] Catalano, A., Bellone, F., Morabito, N. and Corica, F. (2020) Sclerostin and Vascular Pathophysiology. *International Journal of Molecular Sciences*, **21**, Article No. 4779. https://doi.org/10.3390/ijms21134779

[28] Koos, R., Brandenburg, V., Mahnken, A.H., *et al.* (2013) Sclerostin as a Potential Novel Biomarker for Aortic Valve Calcification: An *in-vivo* and *ex-vivo* Study. *The Journal of Heart Valve Disease*, **22**, 317-325.

[29] Chae, W. and Bothwell, A.L.M. (2018) Canonical and Non-Canonical Wnt Signaling in Immune Cells. *Trends in Immunology*, **39**, 830-847. https://doi.org/10.1016/j.it.2018.08.006

[30] Foulquier, S., Daskalopoulos, E.P., Lluri, G., Hermans, K.C.M., Deb, A. and Blankesteijn, W.M. (2017) WNT Signaling in Cardiac and Vascular Disease. *Pharmacological Reviews*, **70**, 68-141. https://doi.org/10.1124/pr.117.013896

[31] Sage, A.P., Tintut, Y. and Demer, L.L. (2010) Regulatory Mechanisms in Vascular Calcification. *Nature Reviews Cardiology*, **7**, 528-536. https://doi.org/10.1038/nrcardio.2010.115

[32] Shao, J., Cai, J. and Towler, D.A. (2006) Molecular Mechanisms of Vascular Calcification. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **26**, 1423-1430. https://doi.org/10.1161/01.atv.0000220441.42041.20

[33] Cai, T., Sun, D., Duan, Y., Wen, P., Dai, C., Yang, J., *et al.* (2016) WNT/β-Catenin Signaling Promotes VSMCs to Osteogenic Transdifferentiation and Calcification through Directly Modulating Runx2 Gene Expression. *Experimental Cell Research*, **345**, 206-217. https://doi.org/10.1016/j.yexcr.2016.06.007

[34] M. McArthur, K., M. Kay, A., A. Mosier, J., N. Grant, J., A. Stewart, J. and LaShan Simpson, C. (2017) Manipulating the Plasticity of Smooth Muscle Cells to Regulate Vascular Calcification. *AIMS Cell and Tissue Engineering*, **1**, 165-179. https://doi.org/10.3934/celltissue.2017.3.165

[35] Nguyen-Yamamoto, L., Tanaka, K., St-Arnaud, R. and Goltzman, D. (2019) Vitamin D-Regulated Osteocytic Sclerostin and BMP2 Modulate Uremic Extraskeletal Calcification. *JCI Insight*, **4**, Article 126467. https://doi.org/10.1172/jci.insight.126467

[36] Mori, H., Torii, S., Kutyna, M., Sakamoto, A., Finn, A.V. and Virmani, R. (2018) Coronary Artery Calcification and Its Progression. *JACC: Cardiovascular Imaging*,

11, 127-142. https://doi.org/10.1016/j.jcmg.2017.10.012

[37] Li, M., Zhou, H., Yang, M. and Xing, C. (2018) Relationship between Serum Sclerostin, Vascular Sclerostin Expression and Vascular Calcification Assessed by Different Methods in ESRD Patients Eligible for Renal Transplantation: A Cross-Sectional Study. *International Urology and Nephrology*, **51**, 311-323. https://doi.org/10.1007/s11255-018-2033-4

[38] Qureshi, A.R., Olauson, H., Witasp, A., Haarhaus, M., Brandenburg, V., Wernerson, A., *et al.* (2015) Increased Circulating Sclerostin Levels in End-Stage Renal Disease Predict Biopsy-Verified Vascular Medial Calcification and Coronary Artery Calcification. *Kidney International*, **88**, 1356-1364. https://doi.org/10.1038/ki.2015.194

[39] Pelletier, S., Confavreux, C.B., Haesebaert, J., Guebre-Egziabher, F., Bacchetta, J., Carlier, M.-C., *et al.* (2015) Serum Sclerostin: The Missing Link in the Bone-Vessel Cross-Talk in Hemodialysis Patients? *Osteoporosis International*, **26**, 2165-2174. https://doi.org/10.1007/s00198-015-3127-9

[40] Paccou, J., Mentaverri, R., Renard, C., Liabeuf, S., Fardellone, P., Massy, Z.A., *et al.* (2014) The Relationships between Serum Sclerostin, Bone Mineral Density, and Vascular Calcification in Rheumatoid Arthritis. *The Journal of Clinical Endocrinology & Metabolism*, **99**, 4740-4748. https://doi.org/10.1210/jc.2014-2327

[41] Lee, Y., Ng, H., Chiu, T.T., Li, L., Pei, S., Kuo, W., *et al.* (2016) Association of Bone-Derived Biomarkers with Vascular Calcification in Chronic Hemodialysis Patients. *Clinica Chimica Acta*, **452**, 38-43. https://doi.org/10.1016/j.cca.2015.10.031

[42] Thambiah, S., Roplekar, R., Manghat, P., Fogelman, I., Fraser, W.D., Goldsmith, D., *et al.* (2012) Circulating Sclerostin and Dickkopf-1 (DKK1) in Predialysis Chronic Kidney Disease (CKD): Relationship with Bone Density and Arterial Stiffness. *Calcified Tissue International*, **90**, 473-480. https://doi.org/10.1007/s00223-012-9595-4

[43] Sarafidis, P.A., Loutradis, C., Karpetas, A., Tzanis, G., Piperidou, A., Koutroumpas, G., *et al.* (2017) Ambulatory Pulse Wave Velocity Is a Stronger Predictor of Cardiovascular Events and All-Cause Mortality than Office and Ambulatory Blood Pressure in Hemodialysis Patients. *Hypertension*, **70**, 148-157. https://doi.org/10.1161/hypertensionaha.117.09023

[44] Ueland, T., Otterdal, K., Lekva, T., Halvorsen, B., Gabrielsen, A., Sandberg, W.J., *et al.* (2009) Dickkopf-1 Enhances Inflammatory Interaction between Platelets and Endothelial Cells and Shows Increased Expression in Atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **29**, 1228-1234. https://doi.org/10.1161/atvbaha.109.189761

[45] Mikheev, A.M., Mikheeva, S.A., Maxwell, J., Rivo, J.V., Rostomily, R., Swisshelm, K., *et al.* (2007) Dickkopf-1 Mediated Tumor Suppression in Human Breast Carcinoma Cells. *Breast Cancer Research and Treatment*, **112**, 263-273. https://doi.org/10.1007/s10549-007-9867-2

[46] Urano, T., Shiraki, M., Ouchi, Y. and Inoue, S. (2012) Association of Circulating Sclerostin Levels with Fat Mass and Metabolic Disease—Related Markers in Japanese Postmenopausal Women. *The Journal of Clinical Endocrinology & Metabolism*, **97**, E1473-E1477. https://doi.org/10.1210/jc.2012-1218