Wnt/β-catenin pathway proteins in end-stage renal disease

Hussein Kadhem Al-Hakeim
University of Kufa
Halah Nori Asad
Al Najaf Health Directorate, Higher Health Institute
Michael Maes (dr.michaelmaes@hotmail.com)
Chulalongkorn University

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Abstract

Background. Wnt-pathway proteins play a vital role in kidney development and defects in the Wnt-pathway are associated with kidney disorders. However, the knowledge on the role of Wnt/β-catenin pathway proteins in end-stage renal disease (ESRD) is limited.

Aim of the study. To delineate the association of ESRD and Wnt-proteins including the agonist

R-spondin 1, the transducer β-catenin and the antagonists Dickkopf-related protein 1 (DKK1) and sclerostin.

Methods. Serum Wnt-pathway proteins levels were measured by ELISA, while other biochemicals were measured spectrophotometrically in 60 ESRD patients and 30 normal controls.

Results. DKK1 and sclerostin were significantly higher in ESRD than in controls, and β-catenin and the catenin + R-Sponin-1 / DKK1 + sclerostin ratio, reflecting the ratio of agonist and transducer / antagonists (AT/ANTA), were significantly lower in ESRD. Logistic regression analysis showed that ESRD was significantly predicted by increased levels of DDK1 and sclerostin and lowered β-catenin ($p<0.001$). eGFR was significantly associated with DKK1 and sclerostin (inversely), β-catenin (positively) and the AT/ANTA ratio ($r=0.468$, $p<0.001$). DKK1 levels were significantly and positively correlated with urea, creatinine, and copper. DKK1 and sclerostin were inversely associated with hemoglobin and packet cell volume. Catenin was significantly negatively associated with copper, urea and creatinine.

Conclusion. Wnt/β-catenin pathway proteins show significant alterations in ESRD, indicating significantly increased levels of antagonists and, therefore, attenuated Wnt/β-catenin pathway activity. The latter is associated with lowered eGFR and increased serum copper levels. Wnt/β-catenin pathway proteins are possible drug targets to treat ESRD or its consequences.

Introduction

Acute kidney injury (AKI) is characterized by a reduction in kidney function, including decreased glomerular filtration rate (GFR) and subsequent kidney failure [1]. Kidney failure is considered when GFR is <15 ml/min per 1.73 m^2 body surface area and this condition may lead to end-stage renal disease (ESRD) [2]. ESRD is defined as irreversible decline in the kidney function, which is severe enough to be fatal in the absence of dialysis or kidney transplantation [3]. The adjusted prevalence of ESRD increased to a new high of 2,242 cases per million people in 2018 and at the end of 2018, there were 554,038 (70.7%) patients undergoing dialysis and 229,887 (29.3%) patients with a functioning kidney transplant [4]. ESRD is accompanied by many biochemical changes including in lipid and vitamins [5], cytokines [6, 7], trace elements including copper and zinc, [8, 9] and oxidative stress biomarkers [10, 11]. However, the knowledge on the Wnt/β-catenin pathway in ESRD is limited.

The Wnt/β-catenin signal transduction cascade governs various biological phenomena during growth and adult life [12]. Wnt proteins are a family of secreted lipid-modified glycoproteins with established roles in embryonic development and tissue homeostasis [13]. Alterations in Wnt pathway proteins are associated with developmental abnormalities and cancers. [14, 15] The pathways play a key role in kidney development [16] and nephron formation [17, 18]. Alterations in Wnt/β-catenin signaling is involved in congenital defects of the kidney and urinary tracts, renal carcinoma, obstructive nephropathy, chronic allograft nephropathy, diabetic nephropathy, polycystic kidney disease, focal and segmental glomerulosclerosis, and adriamycin nephropathy [20-23, 19, 24]. Most importantly, activation of the Wnt/β-catenin pathway plays a critical role in tubular repair and regeneration after AKI [25]. Nevertheless, there are no data on the association between AKI-associated ESRD and Wnt pathway proteins.

Endogenous antagonists of the Wnt/β-catenin pathway include *Dickkopf*-related protein 1 (DKK1) and sclerostin (SOST) [26]. DKK1, a glycoprotein and member of the Dickkopf family, interacts with low-density lipoprotein receptor-related protein (LRP)5/6 to antagonize Wnt/β-catenin signaling [27]. DKK1 is expressed in a number of tissues and cells including the kidneys, platelets, and prostate [28]. Sclerostin, by binding to LRP5/6, may inhibit the Wnt-β-catenin pathway [29]. Sclerostin expression is detected in proximal tubular cells [30] and in the kidney and articular chondrocytes [28]. Sclerostin levels are positively correlated with the presence of bone disease in pre-dialysis and hemodialysis CKD patients [31, 32]. In ESRD, serum sclerostin is associated with vascular calcification and predicts coronary and epigastric artery calcification [33]. R-spondin (RSPO) proteins are agonists of Wnt/β-catenin signaling [34] and attenuate DKK1-associated inhibition of the Wnt-pathway [12,35,36]. Moreover, RSPO stimulates Wnt-dependent stem cells to maintain tissue homeostasis [37]. RSPO1 treatment induces expression of the osteoblast differentiation marker genes, osteocalcin and osteoprotegerin, and increase alkaline phosphatase levels through stimulation of the Wnt/β-catenin signaling pathway [38].

β-catenin, the key component of this signal pathway, also functions as a component of the cadherin complex, which controls cell-cell adhesion [39]. Wnt signaling leads to inhibition of β-catenin ubiquitination that normally occurs within the complex. Subsequently, the complex becomes saturated by the phosphorylated form of β-catenin, leading newly synthesized β-catenin to accumulate and translocate to the nucleus to activate target genes [40]. Consequently, β-catenin regulates the transcription of target genes by associating transcription factors, notably TCF (T cell factor) and LEF (lymphoid enhancer-binding factor) thereby controlling the differentiation and proliferation of cells [41]. The Wnt signaling pathway is a key factor in tissue growth and homeostasis [42] and is needed for nephron formation and kidney development [43-45]. β-catenin signaling is reactivated in fibrotic CKDs including obstructive nephropathy, diabetic nephropathy, adriamycin nephropathy, polycystic kidney disease, and chronic allograft nephropathy [20, 22, 23, 46].

The aim of the present study was to delineate the association of Wnt-protein antagonists DKK1 and sclerostin and the agonist R-spondin 1, and β-catenin in ESRD patients versus normal controls. The

specific hypothesis was that ESRD is accompanied by increased DKK1 and sclerostin and lowered β-catenin and R-spondin 1.

Subjects And Methods

Subjects

Sixty ESRD patients (30 male and 30 female) aged (15-55 years) participated in the current study. All of them had a previous AKI which progressed into renal failure and all were on continuous dialysis. The patients were recruited at the Dialysis Unit at Al-Hakeem General Hospital and Al-Sader Medical City in Najaf Governorate-Iraq during the period October 2020-December 2020. The assessment of patients was carried out by full medical history which considers the presence of any systemic disease. Patients with diabetes, liver diseases and heart diseases were excluded from the study. Serum C-Reactive Protein (CRP) concentrations were <6 mg/L (assayed using agglutination test) in all participants. The test was carried out to eliminate overt inflammation which causes changes in acute phase reactants. The patients were diagnosed by a senior physician according to the 10th revision of the International Statistical Classification of Diseases and Related Health Problems (2021 ICD-10-CM Diagnosis Code N18.6). All patients were on a continuous treatment with folic acid or iron and folate formula (Fefol®) in addition to calcium carbonate, Epoetin alfa (Eprex®), and heparin. The control group consisted of 30 healthy subjects (15 males and 15 females) without apparent physical illnesses. Their sex and age were matched with those of the patients. Written informed consent was obtained from the patients or their first-degree relatives. The protocol was approved by the Iraqi institutional review board (IRB) of the University of Kufa (543/2020), Kufa, Iraq. All participants provided written informed consent.

Measurements

Venous blood samples were taken from the participants in the morning between 8.00 a.m. and 9.00 a.m. after 12 hours fasting. Venous blood samples were collected into plain tubes. Samples were aliquoted and stored at −80 °C before assay. Serum was sampled before the hemodialysis session to assay all parameters. After separation, the sera were distributed into three new Eppendorf® tubes for further analysis. Serum sclerostin (SOST), R-Spondin 1 (RSPO), β-catenin (CAT), and DKK1 concentrations were measured using ELISA kits supplied by Melsin Medical Co., Ltd., Jilin, China. The interassay CV% of all the Wnt-pathway proteins were <15%. All measured concentrations of sclerostin (sensitivity < 0.1 ng/ml), R-spondin1 (sensitivity < 1 pg/mL), β-catenin (sensitivity < 1 pg/mL), and DKK1 (sensitivity < 1 ng/mL) were greater than the sensitivity of the assays. Consequently, we computed 3 z unit weighted composite scores, namely zDKK+zSOST (reflecting inhibitors of the Wnt pathway), zCAT+zRSPO (reflecting agonists / stimulators of the pathway), and z(zCAT+zRSPO) – z(zDKK+zSOST) (Wnt AT/ANTA ratio) reflecting net positive effects on the Wnt pathway). Hematological parameters were measured using a five-part differential *Mindray BC-5000* hematology analyzer (*Mindray* Medical

Electronics Co., Shenzhen, *China*). Serum copper and zinc were measured spectrophotometrically using kits supplied by Spectrum Diagnostics Co. (Cairo, Egypt). Glucose, albumin, urea, and creatinine were measured spectrophotometrically by ready for use kits supplied by Biolabo® (Maizy, France). The Estimated GFR (eGFR) was calculated by using the Modification of Diet in Renal Disease Study equation [47]:

$$eGFR = 175 \times (S.Cr)^{-1.154} \times (Age)^{-0.203} \times 0.742 \text{ [if female]} \times 1.212 \text{ [if Black]}$$

Statistical Analysis

Kolmogorov-Smirnov test was used to examine the normality of distribution. Analysis of variance (ANOVA) was used to examine the between-group differences in scale variables. Statistical associations between categorical variables were evaluated by analysis of contingency tables (χ^2-test). Pearson's correlation coefficients (r) or Spearman's correlation coefficients (ρ, rho) were calculated to assess correlations between biomarkers. We transformed biomarkers into to Ln transformation to normalize the distribution as assessed using the Kolmogorov-Smirnov test. We employed multivariate generalized linear model (GLM) analysis to check the relationship among the biomarkers and the diagnosis (ESRD versus controls) while controlling for background variables including age, BMI, tobacco use disorder (TUD), and sex. Binary logistic regression analysis was used to delineate the most important explanatory variables that predict ESRD versus control as reference group. We used multiple regression analysis to assess the most significant Wnt proteins that are associated with eGFR. Power analysis showed that using an effect size of 0.3, alpha=0.05, power=0.8 and 2 groups the total sample size should be 90. All statistical analyses were performed using SPSS Statistics version 25 (2017), IBM-USA.

Results

Demographic data and Clinical data

The socio-demographic and clinical data of ESRD patients and healthy controls are presented in **Table 1.** There were no significant differences in age, sex ratio, marital status, TUD, family history, and rural/urban ratio between the study groups. ESRD patients have a significantly lower BMI and employment ratio than healthy controls. Our data showed results that are typical of the ESRD status, namely a significant increase in serum urea and creatinine and all patients show an eGFR indicative of ESRD. Serum copper was significantly increased, and serum zinc was significantly decreased in ESRD patients as compared with controls.

Differences in biomarkers between ESRD and healthy controls

Table 2 shows the results of the Wnt-pathway proteins and the three composite scores in both diagnostic groups while adjusting for age and sex. We found that there was a significant increase in DKK1 and sclerostin in ESRD patients as compared with healthy controls. We found a significant decrease in β-catenin, zCAT+zRSPO, and the Wnt AT/ANTA ratio in ESRD patients as compared with controls while the zDKK+zSOST composite score was significantly increased. **Figure 1** shows the Wnt-pathway protein profile (as z scores in a clustered bar graph) in ESRD patients versus healthy controls.

Table 3 shows the results of binary logistic regression analyses examining the best Wnt-pathway predictors of ESRD versus controls using an automatic stepwise method with biomarkers as explanatory variables while allowing for the effects of age, sex, BMI, and TUD. The regression analysis showed that ESRD was best predicted by increased levels of zDDK+zSOST combined with catenin ($\chi 2=25.233$, df=3, $p<0.001$, Nagelkerke=0.494) with an accuracy of 70.0%, sensitivity 68.3% and specificity 73.3%. Also, the Wnt AT/ANTA ratio was a significant predictor of ESRD ($\chi^2=27.526$, df=1, $p<0.001$, Nagelkerke $R^2=0.366$).

Intercorrelation matrix

In the total study group, there was a positive correlation between DKK1 and hemoglobin, PCV, urea, creatinine, eGFR (all inversely) and copper (positively). R-spondin-1 was significantly correlated with β-catenin. Sclerostin showed a significant positive correlation with blood urea, and negative correlations with hemoglobin, PCV, and eGFR. β-catenin was significantly and positively correlated with eGFR and negatively with urea, creatinine and copper. The Wnt AT/ANTA ratio showed a significant correlation with hemoglobin, PCV, and eGFR (positively), and urea, creatinine, and copper (inversely).

Results of multiple regression analyses

Table 5 shows different stepwise multiple regression analyses with eGFR and the Wnt-pathway proteins as dependent variables and other measured biomarkers as explanatory variables while allowing for the effects of age, sex, BMI, and TUD. Regression #1 shows that 27.7% of the variance in the eGFR was explained by the regression on β-catenin and the composite score zDKK+zSOST. **Figure 2** shows the partial regression plot of eGFR on the composite score reflecting antagonist activities. Regressions #2 shows that 6.0% in DKK1 can be explained by urea. Regression #3 shows that 10.4% of the variance in sclerostin was explained by hemoglobin concentrations. eGFR, copper, and PCV explained together 25.6% of the variance in β-catenin (regression #4). Regression #5 shows that 24.4% of the variance in the Wnt AT/ANTA ratio was explained by the combined effects of creatinine and copper. **Figure 3** shows the partial regression of this composite score on serum copper concentrations.

Discussion

The first major finding of the present study is that ESRD is accompanied by increased serum concentrations of *DKK1* and sclerostin *as compared with controls and that the levels of β-catenin were significantly lowered in ESRD. Previous findings showed that patients with* CKD have higher sclerostin levels than controls, with values progressively increasing across the CKD stages [48, 49]. Serum sclerostin levels start to increase from CKD stage III and progressively increase as CKD progresses to ESRD [31, 50]. It is worthy of note that urinary sclerostin excretion increased with declining eGFR, suggesting that increased serum sclerostin in CKD are not due to decreased renal elimination [30]. In another study, serum sclerostin, but not DKK1, was increased in the more advanced stages of CKD [48]. After renal transplantation, serum concentrations of sclerostin paralleled improvements in renal functions [51]. Fang et al. (2014) showed increased renal production of DKK1 and sclerostin and increased circulating DKK1 levels in a mouse model of CKD [52].

Inhibition of the Wnt pathway leads to degradation of β-catenin and reduction in its cytoplasmic levels [53]. The mechanistic explanation is that after binding of the inhibitors to the receptors, a*xin recruits casein kinase 1 to the multiprotein complex (β-catenin-axin-adenomatous polyposis coli (APC)-glycogen synthase kinase (GSK)-3β), causing priming of* β-catenin *and initiation of the* β-catenin *phosphorylation cascade performed by GSK-3β. Phosphorylated β-catenin is then recognized by β-transducin repeat-containing protein (β-TrCP) and degraded by the* ubiquitin proteasome system*, reducing the level of cytosolic* β-catenin [27, 54].

Mounting evidence indicates that secreted Wnt antagonists play an important role in the cross-talk between the kidneys, the vasculature, and the bone [52, 55]. Wnt signaling is involved in almost every aspect of embryonic growth and also regulates homeostatic self-renewal in a variety of adult tissues [56]. Findings show that in CKD, aberrant activation of the Wnt/β-catenin pathway is associated with proteinuria, renal function decline and kidney fibrosis [20-22, 46, 57, 58]. Our findings and previous findings that *a*ctivation of the Wnt/β-catenin pathway modulates tubular repair and regeneration after acute kidney injury [24] indicate that activation of this pathway may be a new drug target to treat AKI-related ESRD.

The second major finding of this study is the significant correlation between Wnt-pathway protein levels and the biochemical biomarkers of ESRD. Most importantly, eGFR is significantly and inversely associated with DKK1 and sclerostin, and positively with β-catenin and a composite score indicating the ratio between stimulators/antagonists of the Wnt-pathway. These results extend those of a previous study showing a negative association between GFR and sclerostin [32, 59]. In CKD patients, sclerostin was associated with indicants of inflammation and vascular damage [31, 50] and negatively correlated with histomorphometric parameters of bone turnover and osteoblast numbers [60]. The high sclerostin concentrations in patients receiving dialysis may be explained mainly by increased accumulation due to renal disorders [31].

Previous studies investigating the association between DKK1 and CKD yielded conflicting results with some reports observing no changes [32, 61] or increased DKK1 levels [52]. In CKD stage 5D, increased

DKK1 levels were associated with higher calcium, CRP, and blood platelets and lower PTH [48]. Of note, in our study, serum levels of DKK1 and sclerostin were unrelated pointing to different origin and different regulatory mechanisms [48]. Nevertheless, extraskeletal production of DKK1 may impact sclerostin functions as both factors bind to LRP5/6 with consequent inhibition of Wnt signaling [32].

Changes in these Wnt antagonists may have dire consequences on cardio-vascular disease and cause lowered blood supply to the kidneys which may further impact CKD/ESRD. Thus, DKK1 may contribute to atherosclerosis [62] and, additionally, play a role in the inflammatory interactions between platelets and endothelial cells [63]. Increased sclerostin levels are associated with adverse CKD outcomes including cardiovascular diseases [64] and all-cause mortality and cardiovascular events [65]. In long-term haemodialysis patients, circulating sclerostin, but not DKK1, was inversely associated with aortic calcification and future cardiovascular events [66]. Furthermore, CKD patients with sclerostin levels exceeding 0.748 ng/ml show an increased risk to impaired renal functions and vascular and coronary artery calcification [67, 68]. Also, another study reported that the increased sclerostin levels in CKD are associated with vascular lesions, inflammation, uremia and increased mortality [69].

Another major finding of this study is that ESRD patients show increased copper and lowered zinc levels as compared with healthy controls and that there is a significant inverse association between copper levels with β-catenin and the Wnt AT/ANTA ratio. Changes in those trace elements in CKD/ESRD were described in many [70-73], but not all studies [74]. The results indicate that hemodialysis patients are at increased risk of zinc deficiency [75, 76], which is linked to delayed wound healing, decreased immune functions, and increased infection susceptibility [77]. One determinant of increased plasma copper concentrations is perhaps serum creatinine [78]. It is interesting to note that patients with Wilson's disease, which is characterized by abnormal copper metabolism, show an abnormal β-catenin signaling pathway [79]. In other species (e.g., zebrafish), copper may suppress the Wnt signaling pathway [80].

Limitations of the study

The results of our study should be discussed with respect to its limitations. First, we performed a case-control study and, therefore, no firm causal inferences may be established. Second, it would have been more conclusive if we would have examined all receptors and agonists of Wnt-pathway in ESRD patients in a larger sample size.

Conclusions

In ESRD, the Wnt/β-catenin pathway may be inhibited because β-catenin levels are lowered and the levels of the antagonists sclerostin and DKK1 are increased. The Wnt activator/antagonist ratio is significantly and positively associated with eGFR, and inversely with urea, creatinine, and serum copper. Targeting the Wnt-pathway is a new potential drug target to treat CKD/ESRD or its consequences. There

is an urgent need to study the mechanism whereby increased copper levels may impact Wnt-pathway proteins and functions.

Declarations

Acknowledgment

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Conflict of interest

The authors have no conflict of interest with any commercial or other association in connection with the submitted article.

Author's contributions

All the contributing authors have participated in the preparation of the manuscript.

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Tables

Table 1. Socio-demographic, clinical and biomarker data of end-stage renal disease (ESRD) patients in comparison with healthy controls (HC).

Parameter	HC (n=30)	ESRD (n=60)	F/χ^2	df	p
Age (years)	32.0±9.2	33.1±11.9	0.20	1/88	0.653
BMI (kg/m^2)	25.04±2.47	22.84±3.68	8.74	1/88	0.004
Duration of illness (months)	-	28.30±33.25	-	-	-
Dialysis session / week	-	2.05±0.50	-	-	-
Last Dialysis (Day)	-	3.25±1.31	-	-	-
Total dialysis sessions	-	287.00±381.05	-	-	-
Age of onset (Year)	-	30.77±11.37	-	-	-
Sex (Female/Male)	15/15	30/30	0	1	1
Single/married	8/22	26/34	2.36	1	0.124
TUD (No/Yes)	28/2	57/3	FEPT	-	0.543
Urban/Rural	26/4	41/19	3.53	1	0.060
Employment (No/Yes)	3/27	57/3	65.03	1	<0.001
Family history (No/Yes)	30/0	58/2	-	-	-
Hemoglobin g/dl	13.39±1.42	8.11±1.71	182.73	1/88	<0.001
PCV %	41.23±4.27	25.23±5.07	191.22	1/88	<0.001
Glucose mg/dl	93.97±10.84	95.77±19.70	0.87	1/88	0.354
Urea mg/dl	29.07±6.45	170.40±55.78	628.46	1/88	<0.001
Creatinine mg/dl	0.72±0.24	8.04±2.44	276.33	1/88	<0.001
Zinc mg/l	0.73±0.26	0.55±0.29	6.27	1/88	0.014
Copper mg/l	0.81±0.14	1.51±0.75	22.92	1/88	<0.001
eGFR* ml/minute	129.85±63.30	7.80±3.54	224.24	1/88	<0.001

*BMI: body mass index, TUD: tobacco use disorder, eGFR: estimated glomerular filtration rate, PCV: packed cell volume, FETP: Fisher's exact probability test. *Processed in Ln transformation*

Table 2. Wnt/β-catenin pathway biomarkers in end-stage renal disease (ESRD) and healthy controls (HC)

Variables	HC (n=30)	ESRD (n=60)	F	df	p
DKK1 (ng/ml)	18.68±10.95	26.33±16.66	7.18	1/88	0.009
R-Spondin (pg/ml)	240.50±110.99	216.13±72.02	1.30	1/88	0.258
Sclerostin (ng/ml)	3.60±1.67	4.43±1.73	6.66	1/88	0.011
β-Catenin (pg/ml)	114.81±66.40	77.12±39.44	11.395	1/88	0.001
zDKK+zSOST	-0.492±0.981	0.246±0.923	12.297	1/88	0.001
zCAT+zRSPO	0.369±0.975	-0.184±0.968	6.51	1/88	0.012
Wnt AT/ANTA ratio	0.861±1.370	-0.431±1.075	23.97	1/88	<0.001

DKK1: Dickkopf-related protein 1, SOST: sclerostin, RSPO: R-spondin-1, CAT: β-catenin, Wnt AT/ANTA: agonist+transducer/antagonist ratio.

Table 3. Results of binary logistic regression with end-stage renal disease (ESRD) as the dependent variable and healthy controls (HC) as reference group and biomarkers as explanatory variables.

Dichotomy	Explanatory variables	B	S.E.	Wald	df	p	OR	95% CI for OR
ESRD vs. HC	β-catenin	-2.358	0.615	14.714	1	<0.001	0.095	0.028-0.316
	zDKK+zSOST	2.139	0.588	13.217	1	<0.001	8.491	2.680-26.902
ESRD vs. HC	Wnt AT/ANTA ratio	-1.646	0.476	11.929	1	0.001	0.193	0.076-0.491

DKK1: Dickkopf-related protein 1, SOST: sclerostin, RSPO: R-spondin-1, CAT: β-catenin, OR: odd ratio, CI: confidence interval, Wnt AT/ANTA: agonist + transducer / antagonist ratio.

Table 4. Intercorrelation matrix between the Wnt-pathway proteins and the measured biomarkers.

Parameter	DKK1	RSPO	SOST	β-Catenin	Wnt AT/ANTA ratio
Hemoglobin	-0.229*	0.004	-0.323**	0.169	0.342**
PCV	-0.226*	0.001	-0.316**	0.170	0.337**
Glucose	0.017	-0.053	0.058	0.124	-0.007
Urea	0.245*	-0.114	0.222*	-0.382**	-0.436**
Creatinine	0.233*	-0.151	0.199	-0.394**	-0.439**
Zinc	0.008	0.083	-0.020	0.114	0.088
Copper	0.240*	-0.055	0.148	-0.348**	-0.359**
DKK1	1	0.016	0.190	-0.027	-
RSPO	0.016	1	0.187	0.636**	-
SOST	0.19	0.187	1	0.169	-
β-Catenin	-0.027	0.636**	0.169	1	-
eGFR	-0.265*	0.149	-0.241*	0.379**	0.468**

DKK1: Dickkopf-related protein 1, SOST: sclerostin, RSPO: R-spondin-1, CAT: β-catenin, and PCV: packed cell volume.

Wnt AT/ANTA ratio: agonist + transducer / antagonist ratio; **p<0.05 (2-tailed); **p<0.01 (2-tailed)*

Table 5. Results of multiple regression analysis with Wnt-pathway proteins and eGFR and other biomarkers as dependent variables.

Regression	Explanatory variables	β	t	p	F model	df	p	R^2
#1. eGFR	**Model**				16.65	2/87	<0.001	0.277
	B-Catenin	0.413	4.51	<0.001				
	DKK+SOST	-0.366	-4.00	<0.001				
#2. DKK1	**Model**				5.62	1/88	0.020	0.060
	Urea	0.245	2.37	0.020				
#3. SOST	**Model**				10.25	1/88	0.002	0.104
	Hb	-0.323	-3.20	0.002				
#4. B-catenin	**Model**				9.85	3/86	<0.001	0.256
	eGFR	0.625	3.94	<0.001				
	Copper	-0.275	-2.63	0.010				
	PCV	-0.452	-2.85	0.006				
#5. Wnt AT/ANTA ratio	**Model**				14.05	2/87	<0.001	0.244
	Creatnine	-0.360	-3.65	<0.001				
	Copper	-0.239	-2.42	0.018				

DKK1: Dickkopf-related protein 1, SOST: sclerostin, RSPO: R-spondin-1, CAT: β-catenin, Hb: hemoglobin, eGFR: estimated glomerular filtration rate, PCV: packed cell volume, Wnt AT/ANTA: agonist + transducer / antagonist ratio

Figures

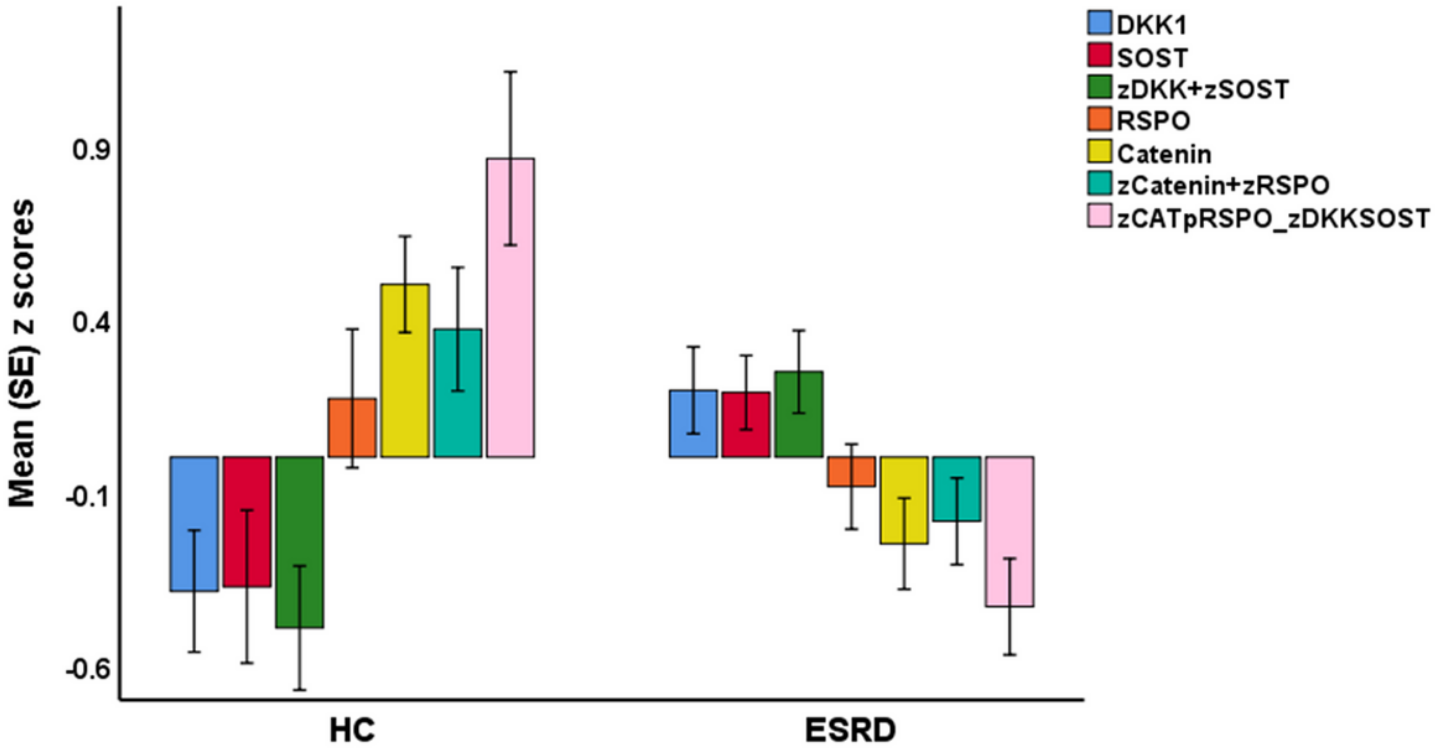

Figure 1

Measurements of Wnt-pathway proteins Dickkopf-related protein 1 (DKK1), sclerostin (SOST), R-spondin-1 (RSPO), β-catenin (Catenin), and the composite scores made form the levels of the proteins expressed as mean (±SE) in end stage renal disease (ESRD) and healthy control groups. All values are shown as z scores.

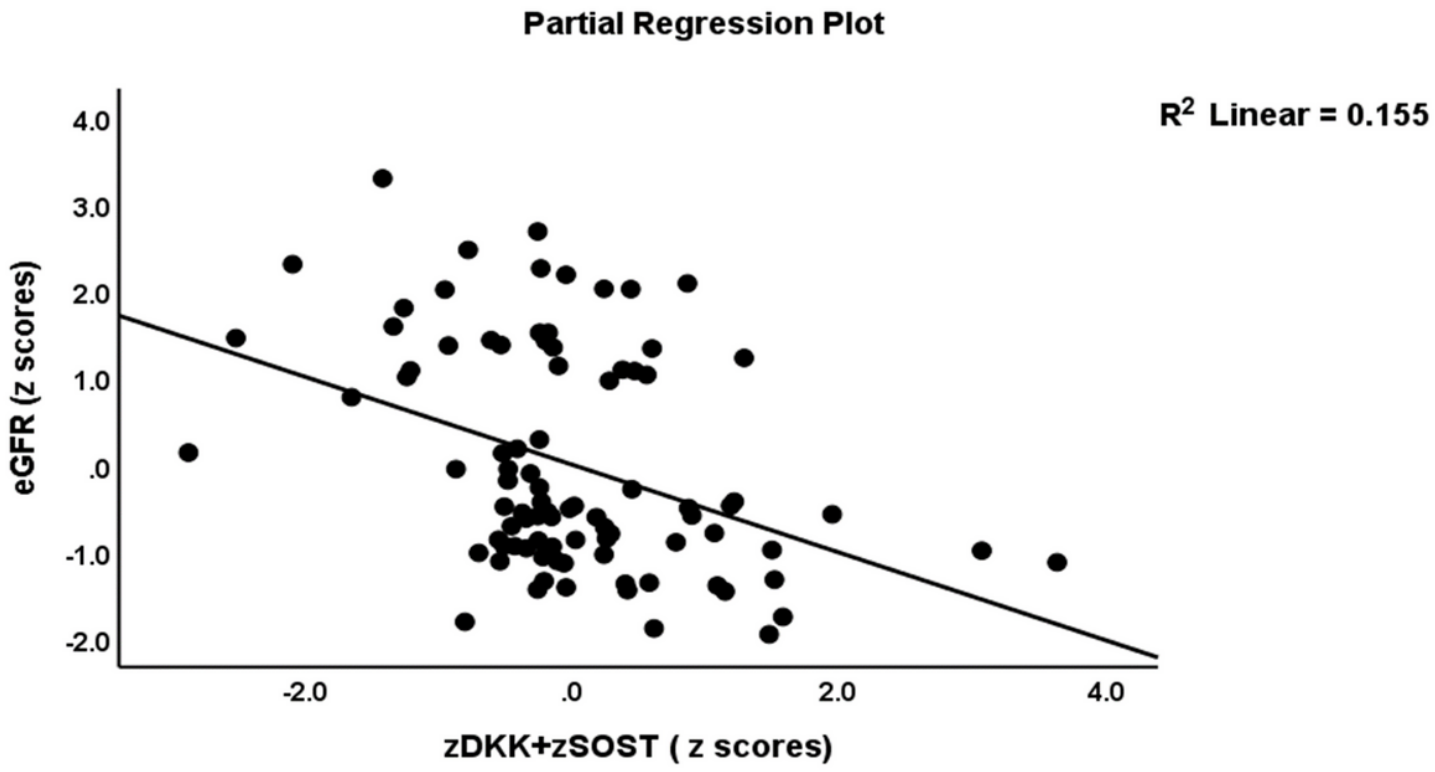

Figure 2

Partial regression of the estimated glomerular filtration rate and z-score of the composite built from Dickkopf-related protein-1 (DKK) and sclerostin (SOST).

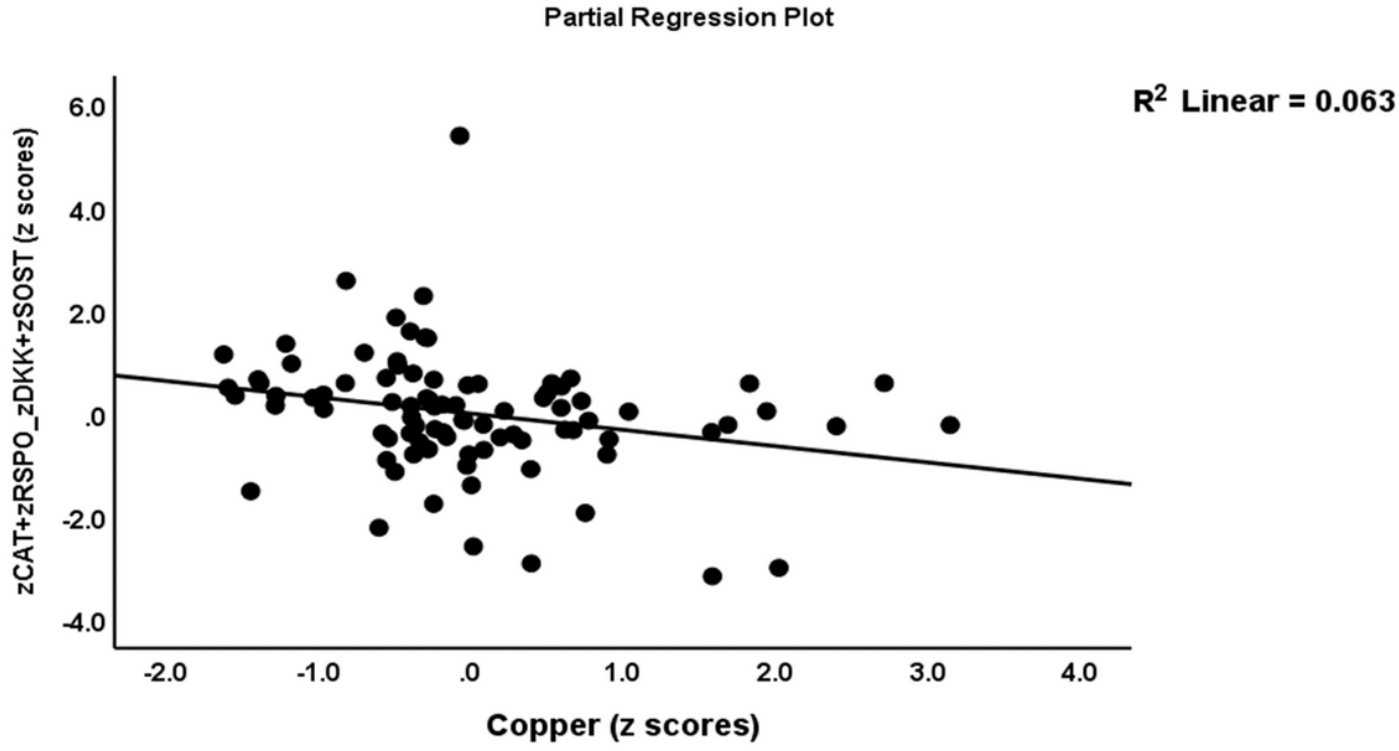

Figure 3

Partial regression of the z-score of the composite of four Wnt proteins (zCATpRSPO_zDKKSOST) on the serum copper concentrations.