Soluble Tumor Necrosis Factor Like Weak Inducer of Apoptosis and Vitamin D in Hemodialysis Patients: Relation to Carotid Intima-Media Thickness

Farahnaz Askarian¹ · Amir Ghorbanihaghjo¹ · Hassan Argani¹ · Davoud Sanajou¹ · Nima Nasehi² · Roya Askarian³ · Ravan Ahmadi¹ · Nadereh Rahtchizadeh¹

Received: 16 February 2017 / Accepted: 26 June 2017 / Published online: 22 July 2017
© Association of Clinical Biochemists of India 2017

Abstract Cardiovascular disease, as the leading cause of patient death with chronic kidney disease, could be predicted by carotid atherosclerosis. The aim of the present study was to evaluate a possible relationship between serum soluble tumor necrosis factor-like weak inducer of apoptosis (sTWEAK) and Vitamin D levels with mean right/left carotid intima-media thickness (cIMT), in the hemodialysis (HD) patients. In this cross-sectional study, serums were obtained from 50 stable chronic HD patients and 39 healthy controls. The serum levels of sTWEAK, Vitamin D, intact parathyroid hormone (iPTH) in both groups, and cIMT were determined in HD patients by

standard methods. Serum levels of sTWEAK were higher [808.8 (521.6–5032.4) pg/ml vs. 664.4 (487.4–2955.8) pg/ml ($p = 0.006$)] and Vitamin D levels were lower [13.4 (2.5–153) ng/ml vs. 27.8 (18.4–59.0) ng/ml ($p = 0.001$)] in the hemodialysis patients than in the healthy control. No important correlation was found between sTWEAK Vitamin D levels ($r = 0.010/p = 0.946$), and mean right ($r = -0.194/p = 0.178$) and left ($r = 0.061/p = 0.673$) cIMT in the HD patients. Our study shows that sTWEAK levels are elevated in HD patients. This elevation has no association with the cIMT.

Keywords Carotid intima-media thickness · Hemodialysis · sTWEAK · Vitamin D

✉ Nadereh Rahtchizadeh
rashtchizadeh@rocketmail.com

Farahnaz Askarian
askarianfa6@gmail.com

Amir Ghorbanihaghjo
ghorbaniamir@hotmail.com

Hassan Argani
hassanargani@gmail.com

Davoud Sanajou
davoodsanajoo@gmail.com

Nima Nasehi
nimanasehi@yahoo.com

Roya Askarian
royaaskarian@gmail.com

Ravan Ahmadi
ahmadi_ravan@yahoo.com

Introduction

Cardiovascular disease (CVD) is the main cause of mortality in chronic kidney disease (CKD) [1]. Although many traditional risk factors contribute to develop CVD, the role of non-traditional risks such as oxidative stresses could not be ignored in the patients with End Stage Renal Diseases (ESRD) [2]. Impaired kidney function predisposes patients to a low-grade chronic systemic inflammation [3], and it is well recognized that inflammation plays a significant role to develop atherosclerosis [4].

Tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK) is a member of the TNF superfamily and a multifunctional cytokine, and circulates through the plasma as a soluble protein (sTWEAK) with a molecular weight of 18 kD [5]. TWEAK has a wide tissue distribution [6], and is expressed and elevated following of many inflammatory states including kidney disease [7]. It has been shown that TWEAK is involved in atherosclerotic

¹ Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

² Department of Radiology, Bushehr University of Medical Sciences, Bushehr, Iran

³ Bushehr University of Medical Sciences, Bushehr, Iran

plaque development [8]. Furthermore TWEAK has a role in vascular remodeling, induces vascular inflammatory responses, and increases atherosclerotic lesion size [9].

Vitamin D formerly was known as an important factor for regulation of calcium phosphorus homeostasis and bone metabolism. However, a growing body of knowledge have also verified that Vitamin D has an important role in cardiovascular health [10]. Several studies demonstrated that the vast majority of hemodialysis patients have low levels of vitamin D [11]. Protective effect of Vitamin D on endothelial cells remodeling, and also prevention of inflammatory process inside atherosclerotic plaque have been documented [10].

Inverse correlation of serum parathyroid hormone (PTH) and deficiency of vitamin D in most ESKD patients have been shown in recent studies [12]. Hyperphosphatemia and high PTH levels have been known as risk factors for cardiovascular disease mortality in HD patients [13]. Elevated PTH level can lead to an increase in bone resorption and vascular calcification in chronic kidney diseases [14].

As we know carotid Intima-Media thickness (cIMT) is an independent predictor of coronary atherosclerosis in the hemodialysis patients [15], our hypothesis is that to evaluate any correlation between serum sTWEAK levels, Vitamin D deficiency, and cIMT in this group of patients.

Methods

This study was performed at the Department of Biochemistry, Tabriz University of Medical Sciences (TUMS). The study design was approved by the Ethics Committee of Tabriz University of Medical Sciences (cod: 5.4.582). All patients and controls gave written informed consent before participating.

The study groups comprised 39 healthy controls and 50 patients on hemodialysis. Sample size calculations were performed using the Power and Sample Size Calculation (PS) software, version 3.1.2. The calculation was based on information obtained from a pilot study for vitamin D. Thus, the sample size was estimated to be nearly 40 subjects per group, in order to achieve an alpha of 5% and a power of 80%.

Exclusion criteria for hemodialysis patients were: (1) a history of hormone therapy with PTH (2) liver disease, and (3) cardiovascular disease. All patients were stable and were under regular hemodialysis for at least 6 months (range, 6–84 months), for 4 h, 3 times per week.

In the present study, all hemodialysis patients were consuming active vitamin D3 (Calcitriol: 0.25 micrograms/day) and phosphate chelating agent routinely. Amlodipine, Furosemide, and Diltiazem were prescribed

for the treatment of hypertensive and also Atorvastatin for hyperlipidemic patients.

Blood samples were obtained from a peripheral vein after 12 h of overnight fasting, just prior to the beginning of hemodialysis in the hemodialysis group. Subsequently plasma and sera were separated within 30 min and the samples were kept frozen at -70°C until assays were carried out.

Estimation of Right and Left Common Carotid Artery Intima-Media Thickness

Common carotid artery (CCA) imaging was performed with an ultrasound device (Madison Prestige V20 South Korea) equipped with a 5–13 MHz linear probe and subjects was in su-pine position with a slight extension of the neck. IMT measurements of the right common carotid artery (RCCA) and left common carotid artery (LCCA) were obtained with ultrasound unit [16, 17]. Initially, a transversal scanning view of the CCA was performed in the longest extension possible from the base of the neck to the carotid bulb. At least three intima-media thickness (IMT) points were measured in the near and far walls in the most thickened area of each vessel. IMT is defined as a double-line pattern visualized on both walls of the common carotid artery (CCA) in a longitudinal view. Two parallel lines: lumen-intima and media-adventitia interfaces. Carotid IMT (cIMT) was evaluated at the wall of artery 3.5 cm proximal to its bifurcation. The left and right cIMT was calculated as the mean and maximal IMT of individual measurements at different points within the region of interest. A cIMT superior to 0.9 mm was considered to be an abnormal finding (Figs. 1 and 2) [18].

Enzyme-Linked Immunosorbent Assay

Serum concentrations of soluble Tumor necrosis factor-like weak inducer of apoptosis (sTWEAK) were measured using a human sTWEAK enzyme-linked immunosorbent assay (ELISA) kit in an ELISA plate reader (Eastbiopharm, China/Cat. No: CK-E11580, assay range: 10–4000 ng/ml, sensitivity: 5.51 ng/ml, intra-assay: CV < 10%, inter-assay: CV < 12%). Intact parathyroid hormone was measured by enzyme-linked immunosorbent assay (ELISA) (Immunodiagnostic Systems, Biomerica USA, Lot. No: 2535, assay range: 1.0–833 pg/ml, sensitivity 1.57 pg/ml, intra-assay: CV: 4.68%, inter-assay: CV: 3.2%). Vitamin D was measured by ELISA (Immunodiagnostic Systems, Bioactiva diagnostica, Germany, Lot. No: VDS 5047, assay range: 15–60 ng/ml, sensitivity: 0.67 ng/ml, intra-assay: CV: 3.87%, inter-assay: CV: 4.55%).

Fig. 1 Control subject: IMT is measured as the distance between lumen-intima and media-adventitia (*green lines*) interfaces

Fig. 2 Patient subject: IMT is measured as the distance between lumen-intima and media-adventitia (*orange lines*) interfaces

Statistical Analysis

Numbers and their percentages were shown for categorical variables. The data were analyzed using the Student *t* test, Chi square test, Mann–Whitney *U* test, and the one-way analysis of variance. All statistical analyses were

performed using the SPSS software version18 (SPSS, Chicago, IL, USA). Results are expressed as median (minimum–maximum values), or mean $\pm$ SD, numbers and their percent when appropriate. Spearman's Correlation was used to examine the relationship between the variables and the $p < 0.05$ was considered significant.

Results

The demographic and biochemical data of the hemodialysis patients and healthy controls are summarized in Table 1. There was no significant difference in the mean age and sex distribution between the two study groups. The causes of ESRD were diabetic nephropathy in 17 patients (34%), hypertension in 26 (52%), chronic glomerulonephritis in 4 (8%), kidney stone disease in 2 (4%), and other or unknown causes in 1 (2%).

Serum sTWEAK levels were significantly higher in the hemodialysis patients than in the healthy control group [808.8 (521.6–5032.4) pg/ml vs. 664.4 (487.4–2955.8) pg/ml ($p = 0.006$)]. On the other hand Vitamin D levels were lower [13.4 (2.5–153.0) ng/ml vs. 27.8 (18.4–59.0) ng/ml ($p = 0.001$)] and iPTH levels were higher [171.0 (5.3–1716.2) pg/ml vs. 11.6 (8.6–17.9) pg/ml ($p = 0.001$)] in the hemodialysis patients.

In the subgroup analysis there was no significant difference in the serum sTWEAK levels between men and women in hemodialysis group [865.8 (533.1–5032.4) pg/ml vs. 785.3 (521.6–5032.2) pg/ml ($p = 0.467$)] and in the control group [675.1 (510.1–1523.6) pg/ml vs. 662.4

(487.4–2955.8) pg/ml ($p = 0.588$)], as has been shown in the Table 2. The serum levels of Vitamin D and iPTH, also was not different between men and the women in the hemodialysis group ($p = 0.109$ and $p = 0.371$, respectively).

Table 3 shows correlation coefficients. No significant correlation was seen between serum Vitamin D levels and serum sTWEAK levels ($r = 0.010$, $p = 0.946$) among hemodialysis patients. Additionally no significant correlation between serum sTWEAK and mean right/left cIMT ($r = -0.194/p = 0.178$ and $r = 0.061/p = 0.673$, respectively) was seen. The correlation between Vitamin D levels and mean right/left cIMT ($r = -0.096/p = 0.508$, $r = -0.008/p = 0.953$, respectively) was not also significant. There were no significant correlations with sTWEAK and iPTH levels in the patients and control group ($r = -0.035/p = 0.812$, $r = -0.056/p = 0.735$, respectively).

Discussion

Our findings demonstrated the four following results: (i) Serum sTWEAK levels were significantly higher in the HD patients than the controls. (ii) No significant correlation

Table 1 Baseline characteristics of the study population

Characteristic	Hemodialysis group (n = 50)	Control group (n = 39)	<i>p</i> value*
Age (years), (mean $\pm$ SD)	52.06 $\pm$ 14.93	48.44 $\pm$ 8.48	0.18 ^a
Sex (male/female), n	29/21	20/19	0.67 ^b
Underlying diagnoses			
Diabetic nephropathy	17 (34%)		
Chronic glomerulonephritis	4 (8%)		
Hypertension	26 (52%)		
Kidney stone	2 (4%)		
Unknown etiology	1 (2%)		
Calcium, mg/dl, median (min–max)	9.1 (6.2–10.4)	9.2 (8.9–9.6)	0.083 ^c
Phosphorus, mg/ml, median (min–max)	4.6 (2.0–8.6)	3.6 (3.4–4.0)	0.001 ^c
ALP, IU/L, median (min–max)	306.5 (92.0–3860.0)	155.0 (114.0–192.0)	0.001 ^c
Vitamin D, ng/ml, median (min–max)	13.4 (2.5–153.0)	27.8 (18.4–59.0)	0.001 ^c
iPTH, pg/ml, median (min–max)	171.0 (5.3–1716.2)	11.6 (8.6–17.9)	0.001 ^c
sTWEAK, pg/ml, median (min–max)	808.8 (521.6–5032.4)	664.4 (487.4–2955.8)	0.006 ^c
cIMT			
IMT right carotid mean of max, mm, (mean $\pm$ SD)	0.85 $\pm$ 0.15	0.77 $\pm$ 0.09	0.002 ^a
IMT right carotid mean of mean, mm, (mean $\pm$ SD)	0.74 $\pm$ 0.13	0.68 $\pm$ 0.083	0.013 ^a
IMT left carotid mean of max, mm, (mean $\pm$ SD)	0.80 $\pm$ 0.10	0.74 $\pm$ 0.061	0.001 ^a
IMT left carotid mean of mean, mm, (mean $\pm$ SD)	0.71 $\pm$ 0.13	0.63 $\pm$ 0.060	0.001

* Comparison of Hemodialysis group VS. Control group

ALP alkaline phosphatase; cIMT carotid intima-media thickness; iPTH intact parathyroid hormone; sTWEAK soluble tumor necrosis factor (TNF)-like weak inducer of apoptosis

^a Independent-sample *t* test

^b Chi square test

^c Mann–Whitney *U* test

Table 2 Serum levels of Vitamin D, sTWEAK, and intact parathyroid hormone (iPTH) in men and women of hemodialysis and control groups

Parameter	Men	Women	<i>p</i> value*
sTWEAK, pg/ml			
Hemodialysis group	865.8 (533.1–5032.4) (n = 29)	785.3 (521.6–5032.2) (n = 21)	0.467
Control group	675.1 (510.1–1523.6) (n = 20)	662.4 (487.4–2955.8) (n = 19)	0.588
Vitamin D, ng/mL			
Hemodialysis group	19.6 (2.5–51.7)	13.0 (2.6–153.0)	0.109
Control group	29.4 (18.4–59.0)	27.3 (22.4–36.7)	0.368
iPTH, pg/ml			
Hemodialysis group	154.4 (8.2–1716.2)	339.7 (5.3–1012.7)	0.371
Control group	12.1 (8.8–15.5)	11.0 (8.6–17.9)	0.391

iPTH intact parathyroid hormone; *sTWEAK* soluble tumor necrosis factor (TNF)-like weak inducer of apoptosis. Values are reported as median (min–max)

* Mann-Whitney *U* test

Table 3 Correlations with serum sTWEAK concentrations in the two subgroups

	Hemodialysis group		Control group	
	<i>r</i>	<i>p</i> value	<i>r</i>	<i>p</i> value
Age, years	−0.103	0.477	0.083	0.616
Calcium, mg/dl	0.221	0.123	0.034	0.837
Phosphorus, mg/dl	0.068	0.637	0.030	0.855
ALP, IU/L	−0.229	0.109	−0.263	0.106
Vitamin D, ng/ml	0.010	0.946	−0.196	0.232
iPTH, pg/dl	−0.035	0.812	−0.056	0.735
IMT right carotid mean of max, mm	−0.166	0.249	0.124	0.452
IMT right carotid mean of mean, mm	−0.194	0.178	0.210	0.200
IMT left carotid mean of max, mm	0.023	0.875	0.025	0.881
IMT left carotid mean of mean, mm	0.061	0.673	0.062	0.706

ALP alkaline phosphatase; *cIMT* carotid intima-media thickness; *iPTH* intact parathyroid hormone; *mm* millimeter; *SD* standard deviation; *sTWEAK* soluble tumor necrosis factor (TNF)-like weak inducer of apoptosis. Spearman's test was used to determine correlations

was observed with high serum sTWEAK levels and low serum Vitamin D levels. (iii) No significant correlation was seen with high serum sTWEAK levels and mean right/left cIMT. (iv) No significant correlation was seen with low serum Vitamin D levels and mean right/left cIMT.

As for the first time by Blanco-Colio et al., sTWEAK level is decreased in the patients with subclinical atherosclerosis and this low level has an inverse correlation with cIMT [19]. Moreover has been reported that the sTWEAK level decreases with progression of the degree of CKD and reached to the minimum in the stage 5 CKD [20, 21]. Valdivielso et al. [21] demonstrated also, a negative correlation of sTWEAK level with cIMT in CKD patients. In 4-year prospective multicenter cohort study among 1058 CKD patients, sTWEAK level was convincingly lower in CKD stage 5 Dialysis group than CKD stage 3 group. In this study sTWEAK level negatively correlated with the degree of cIMT [22]. Our results, however, demonstrated a significantly increased sTWEAK level in

the HD patients than the healthy controls, although no correlation was seen with sTWEAK level and mean right/left cIMT. Our different finding compared to the literature is thought that the elevation in sTWEAK concentration in our study would be consequent of up-regulation of the TWEAK and its receptor Fn14 expression secondary to tissue injury and inflammatory states in the kidney diseases [23–25]. Accordingly the elevation seen in sTWEAK level seems to be plausible, considering that CKD is a chronic inflammatory state [3]. Nonetheless the reason for declined sTWEAK level seen in the previous studies is not clear and it may be attributed to concurrent Fn14 and CD 136 increase that leads to sTWEAK tissue retention and degradation and subsequently reduced sTWEAK levels in serum [26, 27].

We found that no significant difference in sTWEAK concentration between men and women in the hemodialysis group [865.8 (533.1–5032.4) ng/ml vs. 785.3 (521.6–5032.2) ng/ml (*p* = 0.467)]. Contrary to

our results, Kralisch et al. [28], reported that sTWEAK levels were significantly higher in females ($520.36 \pm 227.66 \mu\text{g/l}$) compared with males ($431.41 \pm 185.14 \mu\text{g/l}$) ($p < 0.05$) when chronic dialysis and control groups were analyzed together. This difference may be the result of differences in underlying conditions of study population, considering that the majority of patients and controls in the mentioned study had type 2 diabetes mellitus.

Yadav et al. [29], demonstrated a significant and independent association between low Vitamin D concentrations and the degree of cIMT in stage 4 and 5 non-dialysis CKD patients. This finding is in accord with those obtained by Drechsler et al. ($n = 1108$) [11], that documented a strong association between Vitamin D deficiency and cardiovascular events in diabetic hemodialysis patients. On the contrary other studies could not show a link between serum Vitamin D levels and the degree of cIMT in different stages of CKD patients [30, 31]. In favor of these results, we could not document a correlation between decreased serum Vitamin D levels and mean right/left cIMT in hemodialysis patients. In support of our results, Vitamin D supplementation have failed to lower cardiovascular events in an interventional studies [32]. As high prevalence of atherosclerotic coronary artery calcification in the CKD patients has multifactor, including high serum phosphorus, Klotho and FGF23 [33], the role of vitamin D deficiency alone would be disappeared.

Our study has several limitations which should be considered with precautions: first, relatively small sample size may have reduced the statistical power of the study. Second, serum Fn14 and CD 163 have not been measured which could give some insight into the cause of sTWEAK elevation in our study population. Third, other factors including hsCRP, IL-6, FGF23, and α -klotho have not been considered which could potentially be confounding.

Conclusion

Serum sTWEAK levels are significantly increased in hemodialysis patients, although it lacks a significant correlation with the mean right/left cIMT.

Conflict of interest The authors declare they have no competing interests or conflicts of interest associated with this manuscript.

Ethical standards The ethics committee at the Tabriz University of Medical Sciences reviewed and approved the present study, in compliance with the Declaration of Helsinki.

Informed consent Informed consent was obtained from all participants.

References

- Karohl C, Raggi P. Approach to cardiovascular disease prevention in patients with chronic kidney disease. *Curr Treat Options Cardiovasc Med*. 2012;14(4):391–413.
- Liu M, Li X, Lu L, Cao Y, Sun R, Chen S, et al. Cardiovascular disease and its relationship with chronic kidney disease. *Eur Rev Med Pharmacol Sci*. 2014;18(19):2918–26.
- Stenvinkel P, Wanner C, Metzger T, Heimbürger O, Mallamaci F, Tripepi G, et al. Inflammation and outcome in end-stage renal failure: does female gender constitute a survival advantage? *Kidney Int*. 2002;62(5):1791–8.
- Yin K, Agrawal DK. Vitamin D and inflammatory diseases. *J Inflamm Res*. 2014;7:69–87.
- Winkles JA. The TWEAK–Fn14 cytokine–receptor axis: discovery, biology and therapeutic targeting. *Nat Rev Drug Discov*. 2008;7(5):411–25.
- Du M, Liang Z, Xu X, Li J, Ai H, Zhang J, et al. Molecular cloning, phylogenetic analysis, tissue distribution, and expression of the cytokine TWEAK in zebrafish (*Danio rerio*). *Biochem Genet*. 2012;50(3):318–24.
- Sanz AB, Izquierdo MC, Sanchez-Niño MD, Ucero AC, Egido J, Ruiz-Ortega M, et al. TWEAK and the progression of renal disease: clinical translation. *Nephrol Dial Transplant*. 2014;29(suppl 1):i54–62.
- Blanco-Colio LM. TWEAK/Fn14 axis: a promising target for the treatment of cardiovascular diseases. 2. 2014.
- Muñoz-García B, Moreno JA, López-Franco O, Sanz AB, Martín-Ventura JL, Blanco J, et al. Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) enhances vascular and renal damage induced by hyperlipidemic diet in ApoE-knockout mice. *Arterioscler Thromb Vasc Biol*. 2009;29(12):2061–8.
- Kassi E, Adamopoulos C, Basdra EK, Papavassiliou AG. Role of vitamin D in atherosclerosis. *Circulation*. 2013;128(23):2517–31.
- Drechsler C, Pilz S, Obermayer-Pietsch B, Verduijn M, Tomaschitz A, Krane V, et al. Vitamin D deficiency is associated with sudden cardiac death, combined cardiovascular events, and mortality in haemodialysis patients. *Eur Heart J*. 2010;31(18):2253–61.
- Zheng J-Q, Hou Y-C, Zheng C-M, Lu C-L, Liu W-C, Wu C-C, et al. Cholecalciferol additively reduces serum parathyroid hormone and increases vitamin D and cathelicidin levels in paricalcitol-treated secondary hyperparathyroid hemodialysis patients. *Nutrients*. 2016;8(11):708.
- Rashtchizadeh N, Ghorbanihaghjo A, Argani H, Mahmoudi Meimand S, Safa J, Vatankhahan H, et al. Serum receptor activator of nuclear factor- κ B ligand, osteoprotegerin, and intact parathyroid hormone in hemodialysis and renal transplant patients. *Ther Apher Dial*. 2012;16(6):600–4.
- Hawley CM, Holt SG. Parathyroid hormone targets in chronic kidney disease and managing severe hyperparathyroidism. *Nephrology*. 2017;22(S2):47–50.
- Bots ML, Baldassarre D, Simon A, De Groot E, O'Leary DH, Riley W, et al. Carotid intima-media thickness and coronary atherosclerosis: weak or strong relations? *Eur Heart J*. 2007;28(4):398–406.
- Dogan S, Duivenvoorden R, Grobbee DE, Kastelein JJ, Shear CL, Evans GW, et al. Ultrasound protocols to measure carotid intima-media thickness in trials; comparison of reproducibility, rate of progression, and effect of intervention in subjects with familial hypercholesterolemia and subjects with mixed dyslipidemia. *Ann Med*. 2010;42(6):447–64.
- Zwiebel WJ, Pellerito JS. Introduction to vascular ultrasonography. Philadelphia: Elsevier Saunders; 2005.

18. Mancini GJ. Carotid intima-media thickness as a measure of vascular target organ damage. *Curr Hypertens Rep.* 2000;2(1):71–7.
19. Blanco-Colio LM, Martín-Ventura JL, Muñoz-García B, Orbe J, Páramo JA, Michel J-B, et al. Identification of soluble tumor necrosis factor-like weak inducer of apoptosis (sTWEAK) as a possible biomarker of subclinical atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2007;27(4):916–22.
20. Yilmaz MI, Carrero JJ, Ortiz A, Martín-Ventura JL, Sonmez A, Saglam M, et al. Soluble TWEAK plasma levels as a novel biomarker of endothelial function in patients with chronic kidney disease. *Clin J Am Soc Nephrol.* 2009;4(11):1716–23.
21. Valdivielso JM, Coll B, Martín-Ventura JL, Moreno JA, Egido J, Fernández E, et al. Soluble TWEAK is associated with atherosclerotic burden in patients with chronic kidney disease. *J Nephrol.* 2013;26(6):1105–13.
22. Fernández-Laso V, Sastre C, Valdivielso JM, Betriu A, Fernández E, Egido J et al. Soluble TWEAK and major adverse cardiovascular events in patients with CKD. *Clin J Am Soc Nephrol.* 2016;CJN. 07900715.
23. Yepes M, Brown SA, Moore EG, Smith EP, Lawrence DA, Winkles JA. A soluble Fn14-Fc decoy receptor reduces infarct volume in a murine model of cerebral ischemia. *Am J Pathol.* 2005;166(2):511–20.
24. Sharif MN, Campanholle G, Nagiec EE, Wang J, Syed J, O'Neil SP, et al. Soluble Fn14 is detected and elevated in mouse and human kidney disease. *PLoS ONE.* 2016;11(5):e0155368.
25. Wilhelm A, Shepherd EL, Amatucci A, Munir M, Reynolds G, Humphreys E, et al. Interaction of TWEAK with Fn14 leads to the progression of fibrotic liver disease by directly modulating hepatic stellate cell proliferation. *J Pathol.* 2016;239(1):109–21.
26. Axelsson J, Møller HJ, Witasp A, Qureshi AR, Carrero JJ, Heimbürger O, et al. Changes in fat mass correlate with changes in soluble sCD163, a marker of mature macrophages, in patients with CKD. *Am J Kidney Dis.* 2006;48(6):916–25.
27. Muñoz-García B, Martín-Ventura JL, Martínez E, Sánchez S, Hernández G, Ortega L, et al. Fn14 is upregulated in cytokine-stimulated vascular smooth muscle cells and is expressed in human carotid atherosclerotic plaques modulation by atorvastatin. *Stroke.* 2006;37(8):2044–53.
28. Kralisch S, Ziegelmeier M, Bachmann A, Seeger J, Lössner U, Blüher M, et al. Serum levels of the atherosclerosis biomarker sTWEAK are decreased in type 2 diabetes and end-stage renal disease. *Atherosclerosis.* 2008;199(2):440–4.
29. Yadav AK, Banerjee D, Lal A, Jha V. Vitamin D deficiency, CD4 + CD28null cells and accelerated atherosclerosis in chronic kidney disease. *Nephrology.* 2012;17(6):575–81.
30. Gracia M, Betriu A, Martínez-Alonso M, Arroyo D, Abajo M, Fernández E, et al. Predictors of subclinical atheromatosis progression over 2 years in patients with different stages of CKD. *Clin J Am Soc Nephrol.* 2016;11(2):287–96.
31. Ng Y-M, Lim S-K, Kang P-S, Kadir KAA, Tai M-LS. Association between serum 25-hydroxyvitamin D levels and carotid atherosclerosis in chronic kidney disease patients. *BMC Nephrol.* 2016;17(1):151.
32. Salekzamani S, Babil AS, Mehralizadeh H, Jafarabadi MA, Ghezel A, Gargari BP. The effects of vitamin D supplementation on proatherogenic inflammatory markers and carotid intima media thickness in subjects with metabolic syndrome: a randomized double-blind placebo-controlled clinical trial. *Endocrine.* 2017;57(1):51–59.
33. Yamada S, Giachelli CM. Vascular calcification in CKD-MBD: roles for phosphate, FGF23, and Klotho. *Bone.* 2016.