

## THE RELATIONSHIP BETWEEN PLASMA SOLUBLE TNF-LIKE WEAK INDUCER OF APOPTOSIS LEVEL AND INFLAMMATORY MARKERS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Soluble TNF-like weak inducer of apoptosis (sTWEAK) is a member of the TNF super family with many biological activities. There is a limited number of studies on the role of sTWEAK in chronic kidney disease. We aimed in this study to examine the relation of sTWEAK with albuminuria and inflammatory markers in patients with type 2 diabetes mellitus (DM). One hundred and eighteen diabetic patients with varying levels of albuminuria were included. Group 1 comprised patients with albuminuria less than 30 mg/day, while Group 2 and Group 3 were composed of patients with albuminuria between 30-300 mg/day or more than 300 mg/day, respectively. Groups were compared for sTWEAK levels besides demographic, clinical and biochemical data. There was no difference between groups regarding sTWEAK and TNF- $\alpha$  levels. IL-1 levels in Group 1 were higher than in Group 3. hsCRP levels were significantly higher in Group 3 compared to other groups. Use of a renin angiotensin system blocker did not have any effect on sTWEAK, TNF- $\alpha$  and hsCRP levels, while IL-1 level was significantly lower in patients using a renin angiotensin blocker. A statistically significant positive correlation was detected between sTWEAK and IL-1 levels ( $r=0.245$ ;  $p=0.008$ ). The groups were found to be similar regarding sTWEAK and TNF- $\alpha$  level. This finding may be interpreted as there being no effect of proteinuria on sTWEAK levels. But the close correlation between proteinuria and IL-1, and between IL-1 and sTWEAK may be a clue for an indirect relationship. Lack of difference between groups regarding sTWEAK levels may be due to involvement of patients with GFR more than 60 ml/minute only.

Type 2 diabetes mellitus (DM) with its dramatically increased prevalence in the general population and increased cardiovascular morbidity and mortality is a serious clinical and financial burden for medical care. Diabetic nephropathy (DNP) is the most common cause of end-stage renal disease (1).

It is thought that the imbalance between proinflammatory and antiinflammatory cytokines plays an important role in the development of endothelial dysfunction, microalbuminuria and nephropathy (2, 3). Hatanaka et al. reported increased levels of proinflammatory cytokines like

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interleukin-1 (IL-1), tumor necrosis factor alpha (TNF- $\alpha$ ) and IL-8 in diabetic patients compared with the general population. TNF- $\alpha$  is a proinflammatory cytokine that increases endothelial adhesion molecules and IL-6 which in turn regulates C-reactive protein (CRP) (4). TNF- $\alpha$  directly increases the endothelial permeability and distorts glycocalyx. Satchell et al. found that TNF- $\alpha$  level in serum and renal interstitial tissue is increased before the onset of microalbuminuria (2).

Soluble TNF-like weak inducer of apoptosis (sTWEAK) is a member of the TNF super family with many biological activities including stimulation of angiogenesis, apoptosis, cellular proliferation, migration, cellular differentiation and growth, inflammation, wound healing and osteoclastogenesis (5, 6). There is a limited number of studies on the role of sTWEAK in chronic kidney disease and end stage renal disease. The expression of fibroblast growth factor inducible 14 (Fn14) which is the receptor of sTWEAK, increases during kidney injury and sTWEAK leads to proliferation of tubular cells and inflammation by way of stimulating chemokine release from renal cells (7). On the other hand, sTWEAK promotes apoptosis of mesangial and tubular cells in inflammatory conditions (7). sTWEAK-stimulated mesangial cells have been shown to secrete chemokines and express adhesion molecules (8).

The pathophysiological role of sTWEAK in chronic kidney disease is still not clear. Carrero et al. found lower sTWEAK levels in hemodialysis patients compared to healthy control subjects (9). In this study, higher sTWEAK levels were associated with worse survival. Güngör et al. reported a negative correlation between sTWEAK and carotid intima media thickness in chronic hemodialysis patients; and higher sTWEAK levels in patients with higher vascular calcification score (10). It was reported in a study carried out on 257 patients with stage 1-5 CKD that sTWEAK levels decrease with advancing stage of CKD and low sTWEAK level is associated with endothelial dysfunction and increased cardiovascular mortality (11). sTWEAK is regarded as a novel treatment target in kidney injury (7).

We aimed in this study to examine the relationship of sTWEAK with albuminuria and inflammatory

markers in patients with type 2 DM.

## MATERIALS AND METHODS

This is a cross-sectional study performed with patients having varying stages of diabetic nephropathy. The study was approved by the local ethics committee, and written informed consent was obtained from all patients. The diagnosis of DM was made according to the criteria of the American Diabetes Association. Patients under 18 and over 70 years of age, those with renal disease other than DNP, patients with temporary renal dysfunction, any systemic infectious or inflammatory disease, acute vascular event, autoimmune diseases, glomerular filtration rate less than 60 ml/minute, viral hepatitis, malignancy, advanced cardiac or respiratory disease were excluded from the study.

The demographic data (age, gender) and physical findings [(height, weight, body mass index (BMI), waist circumference, and hip circumference), duration of DM, and the medications that the patients were using at that time were recorded for all patients.

Laboratory analysis: Serum and plasma samples were obtained from patients after 12 hours of fasting. Glucose, HbA<sub>1c</sub>, urea, creatinin, uric acid, sodium (Na), potassium (K), calcium (Ca), phosphorus (P), total protein, albumin, parathyroid hormone (PTH), total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, triglyceride, aspartate transaminase (AST), alanine transaminase (ALT), high sensitivity C-reactive protein (hsCRP), hemoglobin (Hb), hematocrit, total leukocyte count, mean corpuscular volume (MCV), thrombocyte count, iron, total iron binding capacity (TIBC), transferrin saturation, ferritin, TNF- $\alpha$ , IL-1 and sTWEAK levels were measured. Glomerular filtration rate was estimated by CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula (12). Proteinuria and microalbuminuria was calculated by dividing the corresponding levels by creatinin level in spot urine samples, and they were regarded as acceptable if two measurements performed within the previous three months were concordant.

Biochemical parameters were measured by appropriate techniques using Architect c1600 machine. HbA<sub>1c</sub> level was studied by high performance liquid chromatography via TOSOH C7 analyzer. Haematological parameters were studied using HORIBO ABX pentra dx 120 machine. sTWEAK levels were measured by enzyme linked immunosorbent assay (ELISA) using Bender Med System (Vienna, Austria) kit. Human TNF- $\alpha$  and IL-1 levels were studied by ELISA method using BIOTEK EL 50 and BIOTEK EL 800 machines, respectively.

Patients were grouped according to their albuminuria. Group 1 comprised patients with albuminuria less than 30 mg/day, while Group 2 and Group 3 were composed of patients with albuminuria between 30-300 mg/day or more than 300 mg/day, respectively.

Groups were compared regarding demographic, clinical and laboratory parameters.

Statistical analyses were performed with SPSS (Statistical Package for Social Sciences) package program for Windows, version 16.0. Numerical values were expressed as mean±standard deviation (SD). Median, minimum and maximum values were used for variables not with normal distribution. Comparisons of groups for parameters with normal distribution were performed with Student's *t*-test or ANOVA. Comparisons of qualitative data were performed with a *chi*-squared test. Kruskal Wallis-H variant analysis was used for variables with abnormal distribution. The Pearson product moment correlation or the Spearman rank correlation was used to evaluate correlations. Post-hoc comparisons were performed by Tukey HSD. Statistical significance was defined by  $p < 0.05$ . Multivariate analysis was performed with linear regression (stepwise method).

## RESULTS

A total of 118 patients were involved in the study. The mean age and male/female ratio were  $53.47 \pm 9.07$  years and 51/67. The mean age of patients in Group 1 ( $n=41$ ), Group 2 ( $n=39$ ) and Group 3 ( $n=38$ ) were  $53.02 \pm 7.75$  years,  $54.28 \pm 10.63$  years and  $53.10 \pm 8.83$  years, respectively. The corresponding female/male ratios in order were 24/17, 19/20 and 24/14. There was no significant difference between the groups regarding age ( $p=0.792$ ) and gender ( $p=0.70$ ).

DM duration was  $8.24 \pm 7.06$  years in Group 1,  $9.10 \pm 6.97$  years in Group 2 and  $11.34 \pm 8.99$  years in Group 3 ( $p=0.254$ ). The mean BMI of patients in Groups 1, 2 and 3 were  $29.6 \pm 5.2$  kg/m<sup>2</sup>,  $31.9 \pm 5.6$  kg/m<sup>2</sup> and  $30.7 \pm 4.8$  kg/m<sup>2</sup>, respectively ( $p=0.131$ ). Waist circumference/hip circumference ratio was lower in Group 1 ( $0.93 \pm 0.071$ ) than in Group 2 ( $0.97 \pm 0.044$ ) ( $p=0.012$ ). This ratio was  $0.94 \pm 0.06$  in Group 3, and was similar with other groups.

The medications that the patients were using are presented in Figs. 1 and 2. The groups were similar regarding use of angiotensin converting enzyme inhibitors ( $p=0.07$ ), angiotensin receptor blockers ( $p=0.224$ ), other antihypertensives including calcium channel blockers, beta blockers and diuretics ( $p=0.265$ ), acetylsalicylic acid ( $p=0.511$ ), acarbose ( $p=0.474$ ), glitazone ( $p=0.550$ ), insulin secretagogues ( $p=0.093$ ), sulfonylureas ( $p=0.114$ ) and other oral antidiabetic drugs ( $p=0.997$ ). The number of patients using metformin was significantly lower in Group 3 compared to the other groups ( $p < 0.001$ ). Insulin use was most frequent in Group 3 and least in Group 1.

Hematological and biochemical analysis results of the patients are presented in Tables I and II, respectively. Hematocrit level was significantly lower in Group 3 compared to Group 2 ( $p=0.034$ ). Albumin level was lower in Group 3 than in Group 1 ( $p=0.006$ ) and Group 2 ( $p=0.018$ ). Serum creatinine and triglyceride levels were higher in Group 3 compared to Group 1 ( $p=0.025$  and  $p=0.036$ ). Groups were not different regarding other hematological and biochemical parameters. Albuminuria level in

**Table I.** Hemotological paratemeters of the patients.

	Group 1	Group 2	Group 3	P
Hemoglobin (g/dl)	$13.51 \pm 1.35$	$13.56 \pm 1.31$	$12.89 \pm 1.39$	0.056
Hematocrit (%)	$40.83 \pm 3.50$	$41.36 \pm 3.78$	$39.20 \pm 3.92$	<b>0.034*</b>
Mean corpuscular volume (fl)	$86.4 \pm 5.03$	$85.5 \pm 4.0$	$86.4 \pm 5.4$	0.656
Leukocyte (/mm <sup>3</sup> )	$8.07 \pm 1.95$	$7.99 \pm 1.72$	$8.82 \pm 2.42$	0.155
Thrombocyte (x1000/mm <sup>3</sup> )	$280 \pm 84$	$279 \pm 51$	$288 \pm 82$	0.838
Trasferrin saturation (%)	$20.04 \pm 8.05$	$20.01 \pm 8.37$	$18.72 \pm 7.60$	0.733
Ferritin (ng/ml)	$53.8 \pm 40.7$	$62.5 \pm 46.4$	$47.2 \pm 39.6$	0.334

\* The statistically significant difference was between Group 2 and Group 3.

**Table II.** Biochemical data of the patients.

	Group 1	Group 2	Group 3	P
Glucose (mg/dl)	184±79	190±90	213±88	0.301
HbA <sub>1c</sub> (%)	8.16±2.12	8.50±1.93	9.08±1.96	0.124
Urea (mg/dl)	32.14±7.25	31.0±9.2	36.2±14.6	0.082
Creatinin (mg/dl)	0.72±0.14	0.78±0.16	0.84±0.28	<b>0.033**</b>
eGFR (ml/minute)	100±14	96±15	89±25	<b>0.033**</b>
Uric acid (mg/dl)	4.8±1.5	4.83±1.12	5.31±1.81	0.252
Sodium (mmol/L)	139±3	138±3	138±4	0.592
Potassium (mmol/L)	4.57±0.43	4.51±0.41	4.66±0.55	0.358
Calcium (mg/dl)	9.76±0.55	9.75±0.54	9.67±0.49	0.715
Phosphorus (mg/dl)	3.49±0.60	3.69±0.41	3.64±0.55	0.235
Parathyroid hormone (pg/mL)	50.2±21.0	53.5±20.1	57.9±33.5	0.456
Total protein(gr/dl)	7.38±0.39	7.50±0.42	7.27±0.44	0.058
Albumin (gr/dl)	4.34±0.25	4.31±0.35	4.10±0.42	<b>0.004***</b>
Alanine transaminase (U/L)	27.8±15.5	24.5±14.1	29.9±25.9	0.460
Aspartate transaminase (U/L)	24.3±16.0	22.4±9.3	24.0±10.2	0.771
Total cholesterol (mg/dl)	205±58	220±39	222±63	0.333
LDL-cholesterol (mg/dl)	126±47	136±35	136±51	0.519
HDL-cholesterol (mg/dl)	44±10	43±10	42±10	0.558
Triglyceride (mg/dl)	170±84	212±101	258±238	<b>0.047**</b>

\* Statistical significance was between Group 2 and Group 3. \*\* Statistical significance was between Group 1 and Group 3. \*\*\* Statistical significance was between Group 1 and Group 3 ( $p=0.006$ ) and between Group 2 and Group 3 ( $p=0.018$ ).

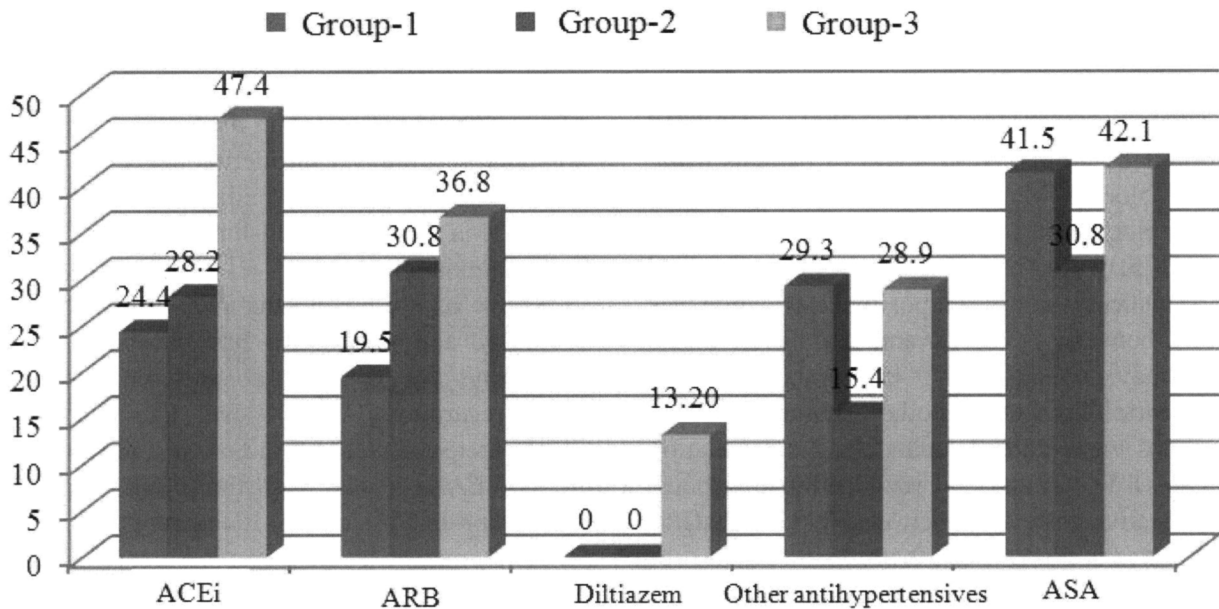
**Table III.** sTWEAK, IL-1, TNF- $\alpha$  and hsCRP levels of the groups.

	Group 1	Group 2	Group 3	P
sTWEAK (pg/ml)	640±153	611±150	633±189	0.696
IL-1 (pg/ml)	36±50	23±28	19±28	<b>0.049</b>
TNF- $\alpha$ (pg/ml)	18±23	21±57.05	22±33	0.620
hsCRP (mg/dl)	0.45±0.41	0.47±0.36	0.93±1.14	<b>0.012</b>

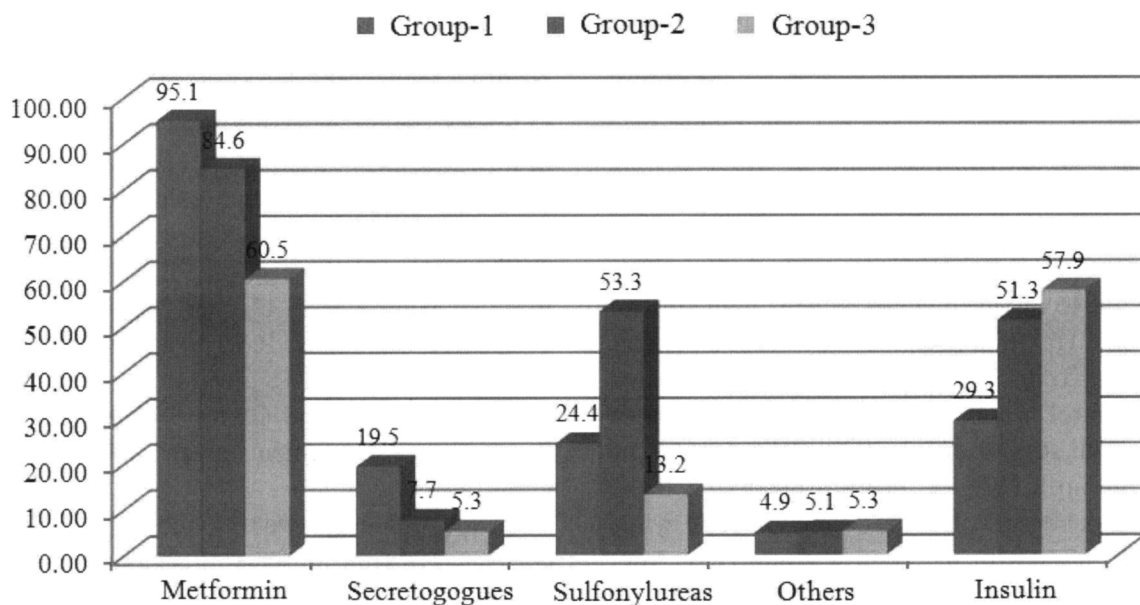
Group 3 (513±270 mg/day) was significantly higher than in Group 1 (10.01±9.42 mg/day) and Group 2 (86.9±62.38 mg/day) ( $p<0.001$ ).

The mean sTWEAK, IL-1, TNF- $\alpha$  and hsCRP levels are presented in Table III. There was no

difference between groups regarding sTWEAK ( $p=0.696$ ) and TNF- $\alpha$  ( $p=0.620$ ) levels. IL-1 levels in Group 1 (36.99±50.65 pg/ml) were higher than in Group 3 (19.03±28.82 pg/ml) ( $p=0.018$ ). hsCRP levels were significantly higher in Group 3 compared



**Fig. 1.** Antihypertensive medications and acetylsalicylic acid used by the patients. The most common antihypertensive agents were renin-angiotensin-aldosterone system blockers in all groups.



**Fig. 2.** Antidiabetic medications used by the patients. In Group 1, the most common antidiabetic agents were metformin, insulin, sulfonylureas and secretagogues with descending order of frequency. With increasing stage of nephropathy, rate of metformin use decreased while use of insulin increased.

to other groups. Male and female patients had similar levels of sTWEAK ( $p=0.612$ ), IL-1 ( $p=0.188$ ) and TNF- $\alpha$  ( $p=0.672$ ).

When patients using a renin angiotensin system

(RAS) blocker ( $n=67$ ) were compared with those not using any RAS blocker ( $n=51$ ), sTWEAK, TNF- $\alpha$  and hsCRP levels were similar while IL-1 level was significantly lower in patients using an

RAS blocker ( $19 \pm 32$  pg/ml vs  $35 \pm 43$  pg/ml). When this comparison was made between the groups, no relationship was detected between RAS blocker use and levels of sTWEAK, TNF- $\alpha$ , IL-1 and hsCRP in Groups 2 and 3. TNF- $\alpha$  levels of patients using an RAS blocker were lower than those not using an RAS blocker in Group 1 ( $10.72 \pm 10.90$  pg/ml vs  $23.91 \pm 28.51$  pg/ml;  $p=0.028$ ).

A statistically significant positive correlation was detected between sTWEAK and IL-1 levels in the whole study population ( $r=0.455$ ;  $p<0.001$ ). There was no correlation between other parameters. When the groups were studied separately, the correlation between sTWEAK and IL-1 remained in all groups, and a negative correlation between IL-1 and hsCRP was detected in Group 3 ( $r=-0.400$ ;  $p=0.026$ ).

## DISCUSSION

In the present study we aimed to evaluate changes in sTWEAK levels in varying stages of diabetic nephropathy, and its relationship with other inflammatory markers. The groups were found to be similar regarding sTWEAK ( $p=0.696$ ) and TNF- $\alpha$  ( $p=0.616$ ). IL-1 levels in Group 1 was higher than in Group 3 ( $p=0.018$ ). If patients in Group 1 were considered as the control group and the others as groups with DNP, similarity between groups regarding sTWEAK is an interesting finding. This may be due to involvement of patients with GFR of more than 60 ml/min. In a study in which 75 patients with stage 1-5 chronic kidney disease were compared with a control group, IL-6 levels were highest in patients with stage 4 and 5 CKD, and lowest in the control group. sTWEAK levels were found to be lowest in patients with stage-4 and -5 CKD while they were highest in the control group (13). After two years of follow-up, patients who had an acute ischemic vascular disease were found to have lower sTWEAK levels (14). It may therefore be concluded that sTWEAK level is directly related with the stage of CKD, especially in later stages.

The mean GFR level was lower in Group 3 compared to Group 1. Patients in Group 3 had higher triglyceride, creatinine and hsCRP levels, and lower albumin levels. Considering the stage of diabetic nephropathy, these differences are expected, and the

groups can be regarded as homogeneously distributed.

We detected a positive correlation between sTWEAK and IL-1 levels ( $r=0.245$ ;  $p=0.008$ ). This relationship was detected in all three groups when examined separately. Considering the central role of IL-1 in inflammation, this finding supports the idea that there is a close relationship between sTWEAK and inflammation.

As far as we know, the relationship between sTWEAK and proteinuria has not been studied previously. We detected no difference between groups regarding sTWEAK level. This finding may be interpreted as there being no effect of proteinuria on sTWEAK levels. But the close correlation between proteinuria and IL-1, and between IL-1 and sTWEAK may be a clue for an indirect relationship. Previous studies reported that sTWEAK levels are correlated with GFR, so lack of difference between sTWEAK levels of the groups in our study may be due to involvement of patients with GFR more than 60 ml/min only.

Yilmaz et al. studied 108 diabetic patients with stage-1 CKD treated with valsartan, amlodipine or both. They detected a significant increase in sTWEAK levels in association with improvement in flow mediated vasodilation (14). We detected no difference between patients using ( $n=67$ ) and not using ( $n=51$ ) an RAS blocker regarding sTWEAK, TNF- $\alpha$  and hsCRP levels, while IL-1 level was lower in those using a RAS blocker. When groups were considered separately, RAS blockers did not have any effect on sTWEAK, TNF- $\alpha$ , IL-1 and hsCRP levels in Group 2 and Group 3. The mean TNF- $\alpha$  level was lower in patients given an RAS blocker in Group 1.

sTWEAK levels were found to be negatively correlated with the presence of DM, smoking history, systolic and diastolic blood pressure, fasting plasma glucose, HbA<sub>1c</sub> and IL-6 in type-1 diabetic patients (15). We detected no relationship between sTWEAK levels and fasting plasma glucose and HbA<sub>1c</sub> levels in any of the study groups. It is difficult to comment on the effect of DM on sTWEAK levels because of lack of a non-diabetic control group and the effective anti-diabetic treatment with good glucose regulation in the involved patients.

A reference range for sTWEAK level has not

been reported in the literature. The kit that was used in our study was tried on random healthy subjects. The measurement range and the mean level were defined as 426-925pg/ml and 608pg/ml, respectively. The manufacturer warned that these values may change with the population studied, therefore, it is not possible to say whether sTWEAK values determined in our study groups are within normal limits or not. Normoalbuminuric patients (Group 1) were used as the control group. This would omit a possible interaction between sTWEAK levels and the presence of DM, as all patients were diabetic.

In conclusion, normoalbuminuric, microalbuminuric and macroalbuminuric diabetic patients with reserved renal function have similar levels of sTWEAK and associated inflammatory markers. There is need for further studies on this subject in patients with kidney disease of other etiologies.

## REFERENCES

1. [http://www.usrds.org/2013/view/v2\\_01.aspx](http://www.usrds.org/2013/view/v2_01.aspx).
2. Satchell SC, Tooke JE. What is the mechanism of microalbuminuria in diabetes: a role for the glomerular endothelium? *Diabetologia* 2008; 51(5):714-25.
3. Fichtlscherer S, Breuer S, Heeschen C, Dimmeler S, Zeiher AM. Interleukin-10 serum levels and systemic endothelial vasoreactivity in patients with coronary artery disease. *J Am Coll Cardiol*. 2004; 44(1):44-49.
4. Hatanaka E, Monteagudo PT, Marrocos MS, Campa A. Neutrophils and monocytes as potentially important sources of proinflammatory cytokines in diabetes. *Clin Exp Immunol* 2006; 146(3):443-47.
5. Lynch CN, Wang YC, Lund JK, Chen YW, Leal JA, Wiley SR. TWEAK induces angiogenesis and proliferation of endothelial cells. *J Biol Chem* 1999; 274(13):8455-59.
6. Polek TC, Talpaz M, Darnay BG, Spivak-Kroizman T. TWEAK mediates signal transduction and differentiation of RAW264.7 cells in the absence of Fn14/sTweakR. Evidence for a second TWEAK receptor. *J Biol Chem* 2003; 278(34):32317-23.
7. Sanz AB, Sanchez-Niño MD, Ortiz A. TWEAK, a multifunctional cytokine in kidney injury. *Kidney Int* 2011; 80(7):708-18.
8. Campbell S, Burkly LC, Gao HX, et al. Proinflammatory effects of TWEAK/Fn14 interactions in glomerular mesangial cells. *J Immunol* 2006; 176(3):1889-98.
9. Carrero JJ, Ortiz A, Qureshi AR, et al. Additive effects of soluble TWEAK and inflammation on mortality in hemodialysis patients. *Clin J Am Soc Nephrol* 2009; 4(1):110-8.
10. Gungor O, Kircelli F, Asci G, et al. Soluble TWEAK level: is it a marker for cardiovascular disease in long-term hemodialysis patients? *J Nephrol* 2013; 26(1):136-43.
11. Yilmaz MI, Sonmez A, Ortiz A, et al. Soluble TWEAK and PTX3 in nondialysis CKD patients: impact on endothelial dysfunction and cardiovascular outcomes. *Clin J Am Soc Nephrol* 2011; 6(4):785-92.
12. Levey AS, Stevens LA, Schmid CH, et al. CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; 150(9):604-12.
13. Hassan SB, El-demery AB, Ahmed AI, Abukhalil RE. Soluble TWEAK and cardiovascular morbidity and mortality in chronic kidney disease patients. *Arab J Nephrol Transplant* 2012; 5(1):27-32.
14. Yilmaz MI, Carrero JJ, Martín-Ventura JL, et al. Combined therapy with renin-angiotensin system and calcium channel blockers in type 2 diabetic hypertensive patients with proteinuria: effects on soluble TWEAK, PTX3, and flow-mediated dilation. *Clin J Am Soc Nephrol* 2010; 5(7):1174-81.
15. Llauradó G, González-Clemente JM, Maymó-Masip E, Subías D, Vendrell J, Chacón MR. Serum levels of TWEAK and scavenger receptor CD163 in type 1 diabetes mellitus: relationship with cardiovascular risk factors. A case-control study. *PLoS One* 2012; 7(8): e43919.