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Original Article

Soluble TWEAK and Cardiovascular Morbidity and Mortality in Chronic Kidney Disease Patients

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Abstract

Introduction: Cardiovascular disease (CVD) is a major cause of morbidity and mortality in chronic kidney patients (CKD). The aim of this study was to demonstrate the role of soluble tumor necrosis factor (TNF) weak inducer of apoptosis (sTWEAK) as a marker of cardiovascular morbidity and mortality in CKD patients.

Methods: The study included 75 CKD patients classified according to eGFR into three groups; group-1 included 15 patients with stage-1 CKD, group-2 included 30 patients with stage-2 and stage-3 CKD, and group-3 included 30 patients with stage-4 and stage-5 CKD. The three groups were compared to 20 matched controls. Interleukin-6 (IL-6) and sTWEAK were measured using ELISA and chemiluminescent techniques respectively. Carotid intima-media thickness (IMT) was also measured.

Results: We found that IL-6 showed significant difference between patient groups and controls, being highest in stage 4 and 5 CKD patients and lowest in controls. Soluble TWEAK showed significant difference between patient groups and controls, being lowest in stage 4 and 5 CKD patients and highest in controls. Soluble TWEAK level showed significant negative correlation with IL-6 ($r = -0.68$; $P<0.01$) and carotid IMT ($r = -0.95$; $P<0.01$). After two years follow up, nine out of 75 CKD patients developed ischemic heart disease (IHD). Two patients developed cerebrovascular stroke and another patient developed peripheral arterial disease. These patients had significantly lower levels of sTWEAK at baseline compared to other patients (160.5± 60.2 versus 274.8±90 pg/mL; $P < 0.05$).

Conclusion: Soluble TWEAK can be a novel biomarker of atherosclerosis and endothelial dysfunction as well as cardiovascular outcome in CKD patients.

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KeyWords: Cardiovascular morbidity; Cardiovascular mortality; CVD; IL-6; TWEAK

Introduction

Cardiovascular disease (CVD) represents a major cause of morbidity and mortality in chronic kidney disease patients (CKD) [1]. The progression to end stage renal disease (ESRD) is associated with increased incidence of adverse cardiovascular outcomes, which is responsible for increased mortality of CKD patients more than the progression of chronic kidney disease itself [2]. The mechanisms for the elevated CVD risk in CKD patients are complex and may involve changes in the heart and vasculature at earlier stages [3]. Hypertension, diabetes mellitus, dyslipidaemia, and premature atherosclerosis are major contributors to cardiovascular morbidity in CKD patients [4]. Endothelial dysfunction (ED) is the initial pathophysiologic step in the progression of vascular damage and premature atherosclerosis that precedes and leads to clinically manifest cardiovascular diseases [5]. ED is highly prevalent in patients with moderate to advanced chronic kidney disease and is linked to the elevated cardiovascular risk of this patient population [6]. The cause of ED is complex and involves dysregulation of multiple pathways. One of those could be mediated by the TNF-like weak inducer of apoptosis (TWEAK), a member of the TNF superfamily of cytokines [7]. TWEAK is a type II transmembrane glycoprotein (30 kD) that circulates in plasma in a soluble form (sTWEAK) with a molecular weight of 18 kD. Soluble TWEAK is generated by proteolytic cleavage of TWEAK, which is a member of the TNF super-family. In humans, TWEAK mRNA expression is abundant in a wide variety of tissues [8]. Soluble TWEAK increases the expression and secretion of various proteins involved in the inflammatory response, including prostaglandin E2; matrix metalloproteinase 1; IL-6 and IL-8 [9]. IL-6 is an inflammatory cytokine, which is secreted by a

variety of different cell types, including lymphoid and endothelial cells, fibroblasts, skeletal muscle, and adipose tissue. Circulating IL-6 levels correlate with premature atherosclerosis and ED [10].

TWEAK is widely expressed and can be found in pancreas, intestine, heart, brain, lung, ovary, the vasculature, skeletal muscle, liver, and kidney. Binding of TWEAK to its receptor, fibroblast growth factor inducible 14(Fn14), mediates multiple biologic effects such as cellular proliferation, migration, survival, differentiation, osteoclastogenesis, angiogenesis, and apoptosis [11]. In addition, TWEAK/Fn14 interactions have also been found to induce inflammation as they up regulate a number of chemokines, cytokines and adhesion molecules in various tissues [12]. While TWEAK and Fn14 genes are widely expressed, their expression level is dramatically elevated in the context of acute injury and disease [13]. Currently it is thought that TWEAK facilitates physiologic tissue repair and regeneration following acute injury, but in the setting of chronic inflammatory diseases the irregular expression of TWEAK is pathogenic [14].

Methods

This study included 75 patients, who were diagnosed as having CKD according to their estimated GFR (eGFR) and the presence of kidney injury as defined by National Kidney Foundation Kidney Disease Outcomes Quality Initiative Guidelines. Group-1included 15 patients who had stage 1 CKD, Group-2 included 30 patients who had stage 2 and 3 CKD, Group-3 included 30 patients who had stage 4 and 5 CKD. These groups were compared with 20 controls matched in age, sex and body mass index (BMI) with the 3 groups of patients. The patients were recruited from the outpatient clinic of nephrology, Cairo University and Cairo University hospitals. This study was performed from January 2008 to December 2010.

Exclusion Criteria were: the presence of ischemic heart disease (IHD) at initial presentation, the presence of diabetes mellitus and current smoking. All patients were subjected to complete history taking and clinical examination, measurement of BMI, and laboratory measurements. Seven milliliters of blood were drawn from each patient after fasting for 12 hours, distributed and aliquoted. Hemoglobin was measured using an EDTA tube. Two serum aliquots were frozen at -20C for the analysis of TWEAK and IL6. One serum aliquot was immediately used for the determination of total cholesterol (TC), triglycerides (TGD), calcium, phosphorus, albumin and serum creatinine. Estimated GFR was calculated according to the Modification of Diet in Renal Disease formula (MDRD) as defined by Levey *et al* [15]. Interleukin (IL-6) assay was done with a solid-phase, enzyme-labeled, chemiluminescent sequential immunometric assay (Immulite 2000; DPC DIPESA S.A., Madrid, Spain)[16]. Serum concentrations of sTWEAK were determined with a sandwich ELISA technique (Bender Med Systems, Vienna, Austria) [17], where sTWEAK was sandwiched between an anti-TWEAK polyclonal coating antibody and a biotin-conjugated polyclonal anti-TWEAK antibody. Twenty-four hour urinary protein measurements were performed at baseline. ECG and echocardiography were performed at the start of the study and after two years follow up. Carotid intima media thickness was measured using a high resolution scanning device.

Analysis of data was done using SPSS (version 12). Values were expressed as means ± SD. Unpaired t-test and one way ANOVA were used to compare two or more groups, respectively. Correlation coefficient was used to rank variables against each other. P value <0.05 was considered significant.

Results

Table-1 summarizes the demographic and clinical data of the patients in the three study groups. All the three groups were comparable in these respects. Table-2 compares the laboratory data obtained in the three study groups. As expected Group-3 patients (CKD-4 and CKD-5) had significantly lower mean serum calcium, lower mean hemoglobin and higher mean serum phosphorus levels compared to Group-1 patients (CKD-1). The mean carotid IMT was significantly higher in Groups 3 and 4 compared to Group-1 patients. The mean sTWEAK levels were significantly higher in the control group compared to patients in groups 2 and 3, while IL-6 levels were significantly lower in the control group compared to all the three test groups (Table-3).

Soluble TWEAK had statistically-significant negative correlation with systolic blood pressure ($r = -0.82$; $P<0.01$), diastolic blood pressure ($r = -0.71$; $P<0.01$), age ($r = -0.75$; $P<0.01$), TC ($r = -0.56$; $P<0.01$), TGD levels ($r = -0.40$; $P<0.05$), BMI ($r = -0.66$; $P<0.01$), carotid IMT ($r = -0.95$; $P<0.01$) and IL-6 ($r = -0.68$; $P<0.01$). No significant correlation was found with serum calcium, serum phosphorus, hemoglobin levels, serum albumin, or the degree of proteinuria ($P>0.05$).

After two years of follow up, nine CKD patients out of 75 developed IHD, two of whom died from myocardial infarction. Two patients developed cerebrovascular stroke and another patient developed peripheral arterial disease. These patients had significantly lower level of sTWEAK (160.5± 60.2 pg/mL) at baseline compared to other patients (274.8±90 pg/mL).

Table 1: Demographic and clinical data of studied CKD patients

Variable	Group 1	Group 2	Group 3	P value
Age	49.6±6.1	49.8±3.3	51±6.4	>0.05
Gender (males/females)	12/3	25/5	26/4	>0.05
Systolic blood pressure*	133.5±6	134±8.3	135.4±9	>0.05
Diastolic blood pressure	85±4.5	85.9±6.3	86.9±6	>0.05
BMI (kg/m^2)	27.2±1.8	26.4±2.1	25.5±1.5	>0.05
Etiology of CKD				
Glomerulonephritis	4	9	10	>0.05
Interstitial nephritis	4	7	6	
ADPKD**	3	6	4	
Hypertension	4	7	7	
Unknown	0	1	3	

* Blood pressure was controlled by angiotensin converting enzyme inhibitors, angiotensin receptor blockers, beta blockers, calcium channel blockers, or centrally acting antihypertensives.
**Autosomal dominant polycyctic kidney disease.

Discussion

Chronic kidney disease (CKD) is strongly associated with cardiovascular disease (CVD), which may account for 50% of all deaths in this patient population [18]. Irrespective of the cause of renal disease, there is firm evidence that a chronic proinflammatory state and progressive atherosclerosis coexist in patients with CKD and that inflammation contributes to cardiovascular morbidity and mortality [19]. This inflammatory phenomenon is frequently observed even before the initiation of renal replacement therapy [20]. In recent years, a number of new circulating biomarkers of atherosclerosis-associated cardiovascular risk have been identified in the general population.

Blanco-Colio *et al* [21] suggested that sTWEAK is one of the potential novel biomarkers of atherosclerosis. In this study, we measured sTWEAK and IL-6 level in patients with different stages of CKD and controls, and we found that sTWEAK was significantly different between groups, being lowest in stage 4 and 5 CKD patients, and highest in controls. IL-6 was also significantly different between groups, being highest in stage 4 and 5 CKD patients, and lowest in controls.

Yilmaz *et al* [22] measured sTWEAK in non-dialysis CKD patients and found that sTWEAK gradually decreased parallel to increased CKD stages and this decrease was parallel to the reduction in eGFR. Also, Meier *et al* [23] reported that sTWEAK plasma levels decrease with impaired renal function and are associated with the aggravation of the endothelial dysfunction and the mortality risk. In this study, we found that sTWEAK had significant negative correlation with both systolic and diastolic BP, age, TC, TGD levels, BMI, carotid IMT, and IL-6. These data suggest that sTWEAK may play a role in endothelial dysfunction (ED) and atherogenesis, being inversely correlated with traditional atherogenic risk factors and an inflammatory cytokine (IL6) that plays an important role in ED and atherosclerosis. It is also indicated in this study that sTWEAK can predict cardiovascular outcome in CKD patients as it had strong negative correlation with carotid IMT and the patients who developed IHD on follow up had significantly lower levels of sTWEAK at baseline. Yilmaz *et al* [22] also reported that sTWEAK can be a marker of cardiovascular outcome in CKD patients. Although carrero *et al* [3] also found a reduction in sTWEAK levels in CKD patients compared to healthy controls, their observations contradicted with our findings in that the worst prognosis was for those with high sTWEAK levels. This contradiction may be due to the different type of studied patients, as their study was conducted on HD patients with more prominent fluctuations in their clinical conditions and surely, biochemical markers.

Table 2: Laboratory data and carotid intima media thickness (IMT) of studied CKD patients

Variable	Group 1	Group 2	Group 3	P value
Total cholesterol (mg/dL)	188±23.5	190.5±28	188.6±33.7	>0.05
Triglycerides (mg/dL)	156.4±13.5	157±13.2	157±11	>0.05
Serum calcium (mg/dL)	9.9±0.5	8.2±0.6	7.2±0.8	<0.01
Serum phosphorus (mg/dL)	4.5±0.4	5.9±0.7	6.8±1.4	<0.01
Serum albumin (g/dL)	3.8±0.2	3.7±0.3	3.9±0.1	>0.05
24 hours urinary proteins (g/day)	1.5±0.5	1.1±0.3	0.7±0.3	<0.01
Hemoglobin (g/dL)	13.5±1.4	11.2±1	8.9±0.9	<0.01
Carotid IMT (mm)	0.5±0.01	0.71±0.06	0.92±0.1	<0.01

Table 3: sTWEAK and IL-6 level in studied CKD patients and controls

Variable	Group 1	Group 2	Group 3	Controls	P value
sTWEAK(pg/mL)	380.2±50.5	290.6±50.8	160.8±70.8	450±64.4	<0.01
IL-6(pg/mL)	4.2±1.8	7.1±2	11±2.1	2.1±1	<0.01

Blanco-Colio *et al* [21] analyzed supernatants obtained from cultured human carotid plaques and healthy arteries and found that sTWEAK levels were decreased in carotid plaque supernatants. Subsequent measurement of sTWEAK in plasma showed a reduced concentration in subjects with carotid stenosis compared with healthy subjects. Furthermore, they found that sTWEAK concentrations were negatively correlated with the carotid intima-media thickness in asymptomatic subjects, and reported that sTWEAK can be an index of subclinical atherosclerosis.

Studies are directed towards the discovery of the cause of decreased sTWEAK levels as a provoking factor of atherogenesis. It was found that in animal models the pathologic effects of sTWEAK were mediated by the binding with its receptor Fn14. Fn14 expression is practically absent in healthy human aortic wall but is highly increased under pathologic conditions. A pro-inflammatory environment increases Fn14 expression, and also allows CD163 to sequester and degrade sTWEAK by acting as a scavenger receptor, thus preventing Fn14 binding [24].

On the basis of these explanations we can speculate that the reduction in sTWEAK concentrations across CKD stages could potentially reflect either of these two processes. King [25] reported that administration of TWEAK in experimental animals resulted in formation of extensive atherosclerotic lesions, which were prevented by pretreatment with anti-TWEAK antibody. Muñoz-García *et al* [26] also reported that in experimental mice, administration of TWEAK aggravated macrophage and chemokine expression in atherosclerotic plaques and in renal lesions.

A possible explanation of this apparent contradiction is that endogenous TWEAK participates in the atherogenic process. Recent data suggest that TWEAK is also a ligand for the scavenger receptor, CD163. Differences observed between studies could be attributed to TWEAK/CD163-mediated effects, suggesting that TWEAK may mediate some of its effects through receptors other than Fn14 [25].

Conclusion

Soluble TWEAK levels are significantly lower in CKD patients compared to controls. Soluble TWEAK can be a novel biomarker of atherosclerosis and endothelial dysfunction as well as cardiovascular outcome in CKD patients.

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