ORIGINAL RESEARCH

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Lower plasma soluble TWEAK concentration in patients with newly diagnosed hypertension

Abstract

Purpose: To determine circulating levels of the soluble TNF-like weak inducer of apoptosis (sTWEAK) and its association with demographic and biochemical parameters in a young group of patients with newly diagnosed and never treated hypertension.

Methods: A total of 51 patients (mean age 21.7 ± 1.4 years, body mass index (BMI) 24.5 ± 1.6 kg/m²) with primary untreated hypertension, and 37 age- and BMI-matched healthy controls (mean age 22.5 ± 1.9 years, BMI 24.7 ± 1.5 kg/m²) were studied. Serums TWEAK and plasma asymmetrical dimethyl arginine (ADMA) levels were measured by EIA.

Results: In patients and controls, mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) were $149.8 \pm 5.65/93.4 \pm 3.4$ mmHg and $124.2 \pm 6.4/78.24 \pm 5.5$ mmHg, respectively. Serum sTWEAK levels were lower in the patient group (882.6 ± 228.9 µmol/L *vs.* 1060.2 ± 231.7 µmol/L, $p=0.001$), whereas plasma ADMA levels were higher (0.837 ± 0.34 µmol/L *vs.* 0.3176 ± 0.25 µmol/L, $p<0.001$). sTWEAK serum levels correlated with SBP ($r=-0.301$; $p=0.005$) and DBP ($r=-0.279$; $p=0.009$). Circulating plasma ADMA levels also correlated with SBP ($r=0.734$; $p<0.001$) and DBP ($r=0.733$; $p<0.001$).

Conclusion: Young patients with yet untreated primary hypertension have lower circulating serum sTWEAK level compared with healthy controls. Further research for possible associations among serum sTWEAK, endothelial dysfunction and other measures of atherosclerosis may be of benefit in order to better understand the pathophysiology of hypertension and to establish more effective treatment options.

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Hypertension is a major risk factor for atherosclerosis and cardiovascular disease (CVD) [1]. Various mechanisms have been suggested for hypertension related increases in the risk of CVD, with endothelial dysfunction (ED) and inflammation being the most studied conditions [1,2]. Vascular endothelium plays a pivotal role in the regulation of vascular tone and maintenance of cardiovascular homeostasis by releasing various vasoactive factors. Endothelial functions are influenced by systemic inflammation at the very early stages.

TNF-like weak inducer of apoptosis (TWEAK, TNFSF12) is one of the members of the TNF superfamily [3]. The human TWEAK gene encodes a 249-amino acid type II transmembrane glycoprotein (30 kD). TWEAK is a multifunctional cytokine, which regulates cell growth, migration and survival. Moreover, TWEAK activates the nuclear factor kappa B signaling pathway and induces expression of cell adhesion molecules and different proinflammatory cytokines [4,5]. Altered sTWEAK plasma levels are associated with aggravation of the ED and increased mortality risk [6]. Similarly, reduced sTWEAK levels have been reported in patients with subclinical atherosclerosis, which is accelerated in the presence of vascular inflammation [7,8].

Asymmetrical dimethyl arginine (ADMA) is a naturally occurring cellular product that is a competitive endogenous inhibitor of nitric oxide (NO) synthase [9]. Overproduced ADMA blocks the beneficial actions of NO on cellular functions. Recent studies have clearly shown that altered synthesis of ADMA is strongly associated with derangements of endothelial functions [20], making it a successful marker of ED especially for research purposes [11]. ED is an early, perhaps initiating, event in the pathogenesis of CVD and has been shown to be present in patients with essential hypertension [12].

In the present specifically designed study, we aimed to investigate serum sTWEAK levels [6,13], and determine the association with demographic characteristics and with ED, as determined by circulating ADMA measurement.

Materials and Methods

Patients and controls

A total of 51 young males (mean age: 21.7 ± 1.4 years) with newly diagnosed, primary and untreated hypertension were enrolled along with 37 age- and body mass index- (BMI) matched healthy controls (mean age: 22.5 ± 1.9 years). None of the patients or controls had family history of hypertension or diabetes mellitus. The secondary causes of hypertension were eliminated by medical history, physical examination and laboratory measurements where necessary. People with a his-

tory of metabolic or inflammatory disorders, dyslipidemia (low-density lipoprotein-LDL cholesterol levels >130 mg/dl, triglycerides >150 mg/dl), obesity (BMI >30 kg/m²), clinical evidence for CVD, rheumatic disease, renal failure, hepatic disease, thyroid dysfunction, concomitant medications (including herbal remedies and over-the-counter drugs) and regular alcohol or drug consumption were not included in the study. The local ethics committee of Gulhane School of Medicine approved the study protocol and written informed consent was obtained from each participant.

Definition of Hypertension

Blood pressure was measured with an appropriate arm cuff and a mercury column sphygmomanometer after a resting period of at least 5 min from the right arm. The same physician performed all measurements, and the mean of the two sitting measurements on different days was used as the systolic blood pressure (SBP) and diastolic blood pressure (DBP) of the patients and controls. As a routine practice for the diagnosis of hypertension in the youth in our clinic, 24-hour ambulatory blood pressure was monitored in all patients and controls. Hypertension was defined as SBP greater than 140 mmHg or DBP greater than 90 mmHg on repeated manual measurements.

Demographic and Biochemical Parameters

Weight (in kilograms) and height (in centimeters) were measured and BMI was calculated as $\text{body weight}/\text{height}^2$ (kg/m²). Waist circumference was measured around the line in the middle of the lower rib and the anterior superior iliac spine. For biochemical analyses, all blood samples were drawn in the morning after at least 10 h of fasting. The samples were promptly centrifuged and the plasma and serum were separated and stored at -80°C . All plasma samples were run in the same assay. Fasting plasma glucose, total cholesterol (TC), triglyceride and high-density lipoprotein cholesterol (HDL-C) levels were measured by the enzymatic colorimetric method with an Olympus AU2700 auto analyzer using reagents from Olympus Diagnostics (GmbH, Hamburg, Germany). Low-density lipoprotein cholesterol (LDL-C) was calculated by Friedewald's Formula [14]. ADMA was measured in plasma by EIA (ADMA direct ELISA Kit, Lot Nr. K7828, Immundiagnostik AG, Bensheim, Germany) (minimum detectable concentration = 0.05 $\mu\text{mol/L}$). sTWEAK was measured in serum by ELISA kit in duplicate (Bender MedSystems, Lot Nr. BMS2006INST, Vienna, Austria). The calculated overall intra-assay and inter-assay coefficient of variation for sTWEAK 7.9%

and 9.2%, respectively (minimum detectable concentration = 9.7 pg/ml). ELISA measurements were carried out using Bio-Tek Synergy HT plate reader (Biotek Instruments Inc., Winooski, VT, USA).

Statistical analysis

SPSS 15.0 statistical package was used for the statistical analyses. Results are reported as mean $\pm$ SD. The Kolmogorov Smirnov test was used to determine the distribution characteristics of variables and Levene's test was used to evaluate the equality of variance. Differences between groups were tested for significance by independent samples: *t*-test as appropriate. The relationship between variables was analyzed by Pearson correlation. The analysis of covariance (ANCOVA) was performed to adjust the parameters according to the other dependent vari-

ables. Differences and correlations were considered significant at $p < 0.05$.

Results

Demographic characteristics and biochemical results of the study groups are shown in Table 1. Patients and the controls were matched for age and BMI. Mean SBP and DBP were 149.8 ± 5.65 and 93.4 ± 3.4 mmHg in the patient group. Because the work was designed to study increased blood pressure that cannot be described as long standing or chronic, the participants were enrolled only when they were young but had sustained elevations in both systolic and diastolic pressures. A control group, consisting of healthy subjects, had a mean SBP of 124.2 ± 6.4 mmHg and DBP of 78.2 ± 5.5 mmHg. Fasting plasma glucose, serum total cholesterol, triglyceride, HDL-

TABLE 1. Demographics, anthropometrics, baseline characteristics and laboratory results of the hypertensive patients and normotensive controls.

Parameter	Patients n=51	Controls n=37	<i>p</i>
Age	21.7 $\pm$ 1.4	22.5 $\pm$ 1.9	0.031
Height (cm)	175.7 $\pm$ 6.2	177 $\pm$ 5.2	0.304
Weight (kg)	75.8 $\pm$ 7.3	77.6 $\pm$ 6.7	0.227
BMI (kg/m ²)	24.5 $\pm$ 1.6	24.8 $\pm$ 1.5	0.458
Waist circumference (cm)	96.6 $\pm$ 2.4	97.6 $\pm$ 2.2	0.068
Systolic blood pressure (mmHg)	149.8 $\pm$ 5.7	124.2 $\pm$ 6.4	<0.001
Diastolic blood pressure (mmHg)	93.4 $\pm$ 3.4	78.2 $\pm$ 5.6	<0.001
Mean arterial pressure (mmHg)	112.2 $\pm$ 3.4	93.6 $\pm$ 5.1	<0.001
Hemoglobin (g/dl)	14.9 $\pm$ 1.2	15.4 $\pm$ 0.9	0.57
MCV (fL)	89.0 $\pm$ 3.6	88.4 $\pm$ 4.7	0.202
MPV (fL)	7.6 $\pm$ 0.9	7.4 $\pm$ 0.6	0.402
WBC ($\times 10^6/\mu\text{L}$)	6.5 $\pm$ 1.6	6.7 $\pm$ 1.9	0.919
Fasting plasma glucose (mg/dl)	86.8 $\pm$ 5.7	85.4 $\pm$ 7.5	0.314
Urea (mg/dl)	13.4 $\pm$ 5.2	13.4 $\pm$ 3.3	0.63
Creatinine (mg/dl)	0.91 $\pm$ 0.11	0.99 $\pm$ 0.13	0.365
Uric acid (mg/dl)	5.2 $\pm$ 1.4	5.1 $\pm$ 1.1	0.055
Total cholesterol (mg/dl)	150.6 $\pm$ 18.1	152.3 $\pm$ 14.6	0.643
Triglyceride (mg/dl)	85.1 $\pm$ 18.7	90.3 $\pm$ 14	0.158
HDL-cholesterol (mg/dl)	41.6 $\pm$ 9.2	40.6 $\pm$ 7.1	0.586
LDL-cholesterol (mg/dl)	92 $\pm$ 15.4	93.6 $\pm$ 12.1	0.607
AST (U/L)	23.1 $\pm$ 9.0	24.8 $\pm$ 8.8	0.938
ALT (U/L)	31.2 $\pm$ 12.1	30.9 $\pm$ 9.7	0.796
sTWEAK ($\mu\text{mol/L}$)	882.6 $\pm$ 228.9	1060.2 $\pm$ 231.7	0.001
ADMA ($\mu\text{mol/L}$)	0.837 $\pm$ 0.34	0.317 $\pm$ 0.025	<0.001

BMI: Body Mass Index, MCV: Mean Corpuscular Volume, MPV: Mean Platelet Volume, WBC: White Blood Cell, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, sTWEAK: TNF-like weak inducer of apoptosis, ADMA: Asymmetrical dimethyl arginine.

FIGURE 1. Comparison of serum sTWEAK levels between hypertensive patients and the normotensive control group.

FIGURE 2. Comparison of plasma ADMA level between hypertensive patients and the normotensive control group.

cholesterol, LDL-cholesterol, urea, creatinine, uric acid, aspartate aminotransferase, alanine aminotransferase and hemoglobin levels, white blood cell counts, mean corpuscular volume and mean platelet volumes were similar.

Mean circulating sTWEAK levels were significantly lower in the patient group compared to the control group (882.6 ± 228.9 vs. 1060.18 ± 231.7 $\mu\text{mol/L}$, $p=0.001$) (Figure 1). On the other hand, mean plasma ADMA levels were significantly higher in the patient group (0.837 ± 0.34 vs. 0.317 ± 0.25 $\mu\text{mol/L}$, $p<0.001$) (Figure 2).

SBP and DBP correlated negatively with the serum sTWEAK level ($r=-0.301$, $p=0.005$, and $r=-0.279$, $p=0.009$, respectively). There was a strong, significant positive correlation between SBP, DBP and plasma ADMA level ($r=0.734$, $p<0.001$, and $r=0.733$, $p<0.001$, respectively). After adjustment for age, BMI, waist circumference, total cholesterol, triglyceride and fasting plasma glucose, the strength of the association of SBP to sTWEAK and ADMA levels did not reduce ($p=0.048$ and $p<0.001$, respectively). No correlation was found between serum sTWEAK and plasma ADMA levels.

Discussion

The current study is the first to examine serum sTWEAK and plasma ADMA levels together in young patients with a recent diagnosis of hypertension. All patients were male and a group of appropriate healthy controls was specifically formed. Such a scenario has the advantage of observing the alterations in circulating hormone or protein levels due mostly to in-

creased blood pressure but not to chronic systemic changes. Serum levels of several cytokines or adipokines in people with hypertension, dyslipidemia or obesity have been reported to change according to age associated comorbidities or gender [15].

In the present study, although the decreased serum TWEAK levels were accompanied by an increase in circulating plasma ADMA levels, a statistical correlation between the two alterations could not be detected. A high plasma ADMA level is accepted as an indicator of ED [11], which can ideally be identified by flow mediated dilation (FMD) [16-18]. Increased circulating ADMA in people with hypertension was previously reported by Böger [19]. Therefore, the present results simply suggest that dysregulated synthesis and secretion of sTWEAK is not involved in the mechanism of impaired endothelial functions. Whether different results could be observed if ED was measured by FMD is unclear. In a recent study, treatment with anti-hypertensive medication was found to improve FMD and normalize proteinuria, pentraxin3 (PTX3) and sTWEAK in diabetic chronic kidney disease stage 1 patients with hypertension [20]. The newly diagnosed hypertensives in the present study provide the advantage of studying "null" hypertensives – patients who had no drug treatment before – and allows for the observation of early changes in serum chemical markers, although the duration of the inflammation is not known.

Dysfunction of the vascular endothelium plays an important role in initiation and progression of atherosclerosis [21]. The most important endothelium-derived relaxing factor is NO, which may be released after stimulation by endogenous

and pharmacologic agonists and physical stimuli such as flow-mediated shear stress [22-24]. It is well known that endothelium-dependent vasodilation is impaired in forearm vasculature of hypertensive patients, and is responsible for the increase in vascular resistance and the vascular structural changes [25]. It seems that ED contributes to the pathogenesis of hypertension by altering the balance between vasodilator and vasoconstrictor forces on the vasculature. This abnormality, probably multifactorial, may be related to a diminished NO bioavailability that follows a reduction of NO synthesis and/or its increased inactivation by oxidative stress. Thus, in patients with hypertension and other cardiovascular risk factors, ED characterized by decreased endothelium-dependent vasodilation and proinflammatory, proliferative and procoagulatory factors that promote atherosclerosis may be considered the initial modification [1].

In accordance with the previous studies that reported high ADMA levels in middle-aged hypertensives [26,27], increased plasma ADMA levels were found in our young hypertensive group compared with matched controls. Circulating ADMA levels, as a novel cardiovascular risk factor, are increased in patients with systemic atherosclerosis, hypercholesterolemia, essential hypertension and end-stage renal failure [28]. These diseases are linked to an impairment of the NO-pathway and endothelial functions [11]. Several prospective studies, in which cardiovascular disease surrogate endpoints were used, supplied evidence of a pathophysiological role of ADMA in the pathogenesis of vascular dysfunction and cardiovascular disease in humans. Moreover, high plasma ADMA level was found to be associated with carotid atherosclerosis in a study with healthy human subjects [29]. The present study shows that circulating ADMA is increased in the very early stages of clinically established hypertension. This may be one explanation of the fact that a high ADMA blood level is a strong predictor of future CVD.

Circulating sTWEAK levels have been shown to be reduced in some chronic conditions. Lower serum sTWEAK has been reported in subjects with subclinical atherosclerosis, diabetics and chronic kidney disease patients [8,20,30]. Moreover in chronic kidney disease, serum sTWEAK appeared to be negatively associated with ED, and to all-cause and cardiovascular mortality in subjects undergoing hemodialysis [6,31]. In patients with chronic heart failure, a reduced serum sTWEAK level was a predictor of an adverse prognosis [32,33]. These findings may be explained, at least in part, by the presence of a newly discovered scavenger receptor of sTWEAK, CD163, which can sequester and degrade sTWEAK under proinflammatory conditions [34]. Expression of CD163 has also been

shown to be upregulated in chronic kidney disease patients [35]. These data suggest that one reason for reduced sTWEAK serum level in our young patients with recent onset primary hypertension may be the scavenging effect of CD163. On the other hand, our study showed that alterations in the synthesis and secretion of TWEAK protein occurs, in contrast to chronic conditions stated above, very early in the period of blood pressure increase.

There are a couple of limitations in the present study. First, the number of patient population is too small to represent all hypertensive patients, although this was not the primary aim of the study. Second, to make further comments on the possible role of sTWEAK in the mechanism of hypertension, data related to other measures of insulin sensitivity, inflammation and ED such as HOMA calculation, hs-CRP and FMD test, would have to be measured.

In conclusion, young patients with newly diagnosed and never treated primary hypertension have lower level of circulating sTWEAK compared to healthy controls. Further evaluation of the associations among TWEAK, ED and other measures of atherosclerosis may be of benefit in order to better understand the pathophysiology of hypertension and to establish more effective treatment options.

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