View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Via Medica Journals



Endokrynologia Polska
DOI: 10.5603/EP.a2016.0033
Tom/Volume 67; Numer/Number 6/2016
ISSN 0423–104X

# The role of circulating sTWEAK in the pathogenesis of Hashimoto's thyroiditis — a pilot study

## Rola krążącego sTWEAK w patogenezie choroby Hashimoto — badanie pilotażowe

***Mustafa Altay[1], İhsan Ateş[2], Fatma Meriç Yılmaz[3], Canan Topçuoğlu[3], Mustafa Kaplan[2]***

*[1]Keçiören Training and Research Hospital, Department of Endocrinology and Metabolism, Ankara, Turkey*
*[2]Ankara Numune Training and Research Hospital, Department of Internal Medicine, Ankara, Turkey*
*[3]Ankara Numune Training and Research Hospital, Department of Biochemistry, Ankara, Turkey*

## Abstract

**Introduction:** We aimed to investigate the role of sTWEAK in the pathogenesis of Hashimoto's thyroiditis, which is a chronic inflammatory autoimmune disease.

**Material and methods:** A total of 80 patients were included in the study, 60 of whom were newly diagnosed with Hashimoto's thyroiditis (20 patients in each of the euthyroid, subclinical hypothyroid, and overt hypothyroid subgroups), and 20 of whom were healthy volunteers. Thyroid function tests and autoantibodies were measured using the electro-chemiluminescence immunoassay method, and sTWEAK, IL-17A, IL-12, and TGF-$\beta$1 were measured using enzyme-linked immunosorbent assay method.

**Results:** The Hashimoto's Thyroiditis group had lower levels of sTWEAK and TGF-$\beta$1, but had higher levels of IL-12 and IL-17A as compared to the control group. Of these, only the difference between IL-17A levels reached statistical significance (2.1 pg/mL *vs.* 1.8 pg/mL, respectively; $p < 0.001$). While the levels of sTWEAK were similar in the control, euthyroid, and subclinical groups, the overt hypothyroidism group had lower level of sTWEAK than that of subclinical hypothyroidism (687.6 ± 153.3 pg/mL *vs.* 888.2 ± 374.4 pg/mL, respectively; $p = 0.03$). A negative correlation was determined between sTWEAK level and anti-TPO ($r = –0.533$, $p = 0.028$) and IL-17A ($r = –0.600$, $p = 0.005$) levels in the overt hypothyroidism group.

**Conclusions:** The reduced levels of sTWEAK with progression of Hashimoto's Thyroiditis and the significant correlation between the sTWEAK levels and anti-TPO found in this study suggest that sTWEAK plays an active role in chronic inflammation in the pathogenesis of Hashimoto's Thyroiditis and in the progression of autoimmunity. **(Endokrynol Pol 2016; 67 (6): 562–566)**

**Key words:** *Hashimoto's thyroiditis; IL-12; IL-17A; sTWEAK; TGF-β1*

## Streszczenie

**Wstęp:** Badanie przeprowadzono w celu ustalenia roli sTWEAK w patogenezie zapalenia tarczycy Hashimoto, przewlekłej zapalnej choroby autoimmunologicznej.

**Materiał i metody:** Do badania włączono łącznie 80 chorych, w tym 60 osób z nowo rozpoznaną chorobą Hashimoto (po 20 chorych w podgrupach z eutyreozą, subkliniczną niedoczynnością tarczycy i jawną niedoczynnością tarczycy) i 20 zdrowych ochotników. Badania czynności tarczycy oraz oznaczenia stężenia autoprzeciwciał przeprowadzono przy użyciu metod elektrochemiluminescencji, a stężenia sTWEAK, IL-17A, IL-12 i TGF-$\beta$1 oznaczono za pomocą testów enzymatycznych.

**Wyniki:** W grupie osób z chorobą Hashimoto stężenia sTWEAK i TGF-$\beta$1 były niższe, a stężenia IL-12 i IL-17A wyższe niż w grupie kontrolnej. Jednak tylko różnice między stężeniami IL-17A osiągnęły poziom istotności statystycznej (odpowiednio 2,1 pg/ml *vs.* 1,8 pg/ml; $p < 0{,}001$). Podczas gdy stężenia sTWEAK były podobne w grupach kontrolnej, z eutyreozą i z subkliniczną niedoczynnością tarczycy, stężenia sTWEAK w grupie z jawną niedoczynnością tarczycy były niższe niż u osób z subkliniczną niedoczynnością tarczycy (odpowiednio 687,6 ± 153,3 pg/ml *vs.* 888,2 ± 374,4 pg/ml; $p = 0{,}03$). Stwierdzono ujemną korelację między stężeniem sTWEAK a stężeniami przeciwciał przeciw TPO ($r = –0{,}533$; $p = 0{,}028$) oraz IL-17A ($r = –0{,}600$; $p = 0.005$) w grupie z jawną niedoczynnością tarczycy.

**Wnioski:** Obniżanie się stężenia sTWEAK z progresją choroby Hashimoto oraz istotna korelacja między stężeniem sTWEAK a stężeniem przeciwciał przeciw TPO stwierdzone w tym badaniu wskazują, że sTWEAK odgrywa aktywną rolę w przewlekłym zapaleniu w patogenezie choroby Hashimoto, a także w progresji autoagresji. **(Endokrynol Pol 2016; 67 (6): 562–566)**

**Słowa kluczowe:** *zapalenie tarczycy Hashimoto; IL-12; IL-17A; sTWEAK; TGF-β1*

## Introduction

Hashimoto's thyroiditis (HT) is the most common organ-specific autoimmune disease and the most common cause of hypothyroidism. Many genetic and environmental factors are responsible for the aetiology in HT, and T cell-mediated autoimmunity plays an important role in its pathogenesis. CD4+ Th1 cells, through cytokines, induce CD8+ cytotoxic Th2 cells in the thyroid gland as a result of infiltration of T and B cells with

Mustafa Altay M.D., Keçiören Training and Research Hospital, Department of Endocrinology and Metabolism, Kuşcağız, 06010, Keçiören, Ankara, Turkey, phone: +90 312 356 90 00, fax: +90 312 356 90 03, e-mail: altay_mustafa@hotmail.com

reactivity of thyroid autoantigens and loss of immune tolerance, and initiate the apoptotic process, resulting in destruction of thyroid cells [1]. Th1 is dominant over autoimmunity in HT whereas Th 2 is often responsible for autoimmunity in Graves' disease. Th1 synthesises the proinflammatory cytokines (IL-2, IFN-$\gamma$, TNF, IL-1$\beta$, IL-12) and has a cytotoxic effect [2]. One subgroup of CD4 + T lymphocytes is Th3 cell, known as regulator T cell or Treg, which is responsible for regulation of adaptive immune system and synthesis of TGF-$\beta$1. TGF-$\beta$1 is a cytokine that inhibits the reproduction of T and B cells and the cytotoxicity of T cells [3]. TGF-$\beta$1 has a protective function from autoimmune thyroid disease. Another subgroup of T cells is the recently emerging IL-17, which is synthesised by Th17 and has a role in autoimmune diseases [4]. IL-17 and IL-12 have been shown to increase and TGF-$\beta$1 has been shown to reduce in HT and other several autoimmune diseases. Tumor necrosis factor like weak inducer of apoptosis (TWEAK), is a member of the tumor necrosis factor superfamily (TNFSF) and binds to fibroblast growth factor-inducible molecule 14 (Fn14) receptor and plays a role in cellular proliferation, migration, apoptosis, differentiation, angiogenesis, and inflammation via nuclear factor $\kappa$B (NF-$\kappa$B) pathway [5]. There are two active forms of TWEAK: membrane-bounded mTWEAK and its soluble form sTWEAK.

Proinflammatory cytokines, which are synthesised by Th1 lymphocytes, often play a role in the pathogenesis of autoimmune diseases. These proinflammatory cytokines increase the immunogenicity of antigenic structures in thyroid gland and similar tissues, which results in increased levels of autoantibody in IL-4, IL-5, and B lymphocytes. Because TWEAK is a subgroup of TNFSF that is synthesised by Th1 lymphocytes, we consider that TWEAK may play a role in increased autoimmune response. Indeed, a number of previous studies have shown that TWEAK is associated with the aetiopathogenesis of several autoimmune diseases (systemic lupus erythematosus [SLE], multiple sclerosis, type 1 diabetes mellitus, etc.) [6, 7].

However, it is not known whether TWEAK plays a role in the aetiopathogenesis of HT, which is an autoimmune disease. Therefore, we aimed to investigate the role of sTWEAK in the pathogenesis of HT and its relationship with IL-12, IL-17A, and TGF-$\beta$1.

## Material and methods

This study was conducted at the Internal Medicine Clinic of Ankara Numune Training and Research Hospital between April and June, 2015. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Local Ethics Research Committee. All subjects provided written, informed consent prior to participation in the study.

A total of 80 patients over the age of 18 years were included in the study: 60 patients newly diagnosed with HT, who did not receive any treatment, and 20 patients with no known disease as the healthy control group.

The exclusion criteria were other known systemic or metabolic diseases such as malignancy, infection, diabetes mellitus, atherosclerosis, and history of regular use of drugs.

Diagnosis of HT was established by increased serum levels of anti-thyroglobulin (anti-TG) and/or anti-thyroid peroxidase (anti-TPO) and/or the presence of heterogeneous echogenicity in the thyroid parenchyma demonstrated on the thyroid ultrasonography. Serum thyroid stimulating hormones (TSH), free thyroxine (fT4), anti-TG, and anti-TPO were measured for those to be included in the control group, and HT was eliminated by thyroid ultrasonography, which was performed separately.

### *Biochemical parameters*

After completing medical history taking and physical examination of the patient and control groups, serum samples were collected following a 12-hour fast, centrifuged, and kept at –80°C. Then sTWEAK, IL-17A, IL-12 and TGF-$\beta$1 were collectively measured.

Serum IL-17A concentrations were measured using a commercial ELISA kit (eBioscience, An Affymetrix Company, Austria). The limit of detection was 0.5 pg/ml. Intra and inter-assay precisions were 7.1% and 9.1%, respectively.

Serum IL-12p70 concentrations were measured using a commercial ELISA kit (eBioscience, An Affymetrix Company, Austria). The limit of detection was 2.1 pg/ml. Intra and inter-assay precisions were 3% and 4.8%, respectively.

Serum TGF-$\beta$1 concentrations were measured using a commercial ELISA kit (eBioscience, An Affymetrix Company, Austria). The limit of detection was 0.008 ng/mL. Intra and inter-assay precisions were 3.2% and 4.9%, respectively.

Serum sTWEAK concentrations were measured using a commercial ELISA kit (eBioscience, An Affymetrix Company, Austria). The limit of detection was 9.7 pg/ml. Intra and inter-assay precisions were 7.9% and 9.2%, respectively.

### *Statistical analysis*

The findings of this study were analysed with the Statistical Package for Social Sciences for Windows (SPSS v18) software. The conformity of continuous variables to normal distribution was tested with Kolmogorov-Smirnov test. The descriptive statistics of continuous

**Table I.** *Demographical and biochemical features of controls and Hashimoto's Thyroiditis patients*

**Tabela I.** *Charakterystyka demograficzna i parametry biochemiczne osób z grupy kontrolnej i pacjentów z chorobą Hashimoto*

| Variables | Hashimoto's Thyroiditis | Control | p |
|---|---|---|---|
| | n (60) | n (20) | |
| Age (years) | 31.5 ± 8.8 | 32.7 ± 7.4 | 0.62 |
| Gender, n (f/m) | 53/7 | 17/3 | 0.23 |
| BMI [kg/m$^2$] | 24.1 ± 2.8 | 23.9 ± 2.5 | 0.82 |
| SBP [mm Hg] | 117 (90–185) | 110 (90–125) | 0.09 |
| DBP [mm Hg] | 69.5 (53–90) | 70 (60–80) | 0.61 |
| TSH [$\mu$IU/mL] | 7 (0.9–500) | 1.6 (0.9–3.5) | < 0.01* |
| fT3 [pg/mL] | 2.6 ± 0.8 | 2.8 ± 0.5 | 0.22 |
| fT4 [ng/dL] | 0.9 ± 0.3 | 1.2 ± 0.4 | 0.02* |
| Anti-TG [IU/mL] | 234.9 (10–7411) | 7.6 (2.4–26) | < 0.01* |
| Anti-TPO [IU/mL] | 168.5 (4.2–4578) | 3.9 (1–39) | < 0.01* |
| sTWEAK [pg/mL] | 780.4 ± 266.3 | 865.1 ± 218.5 | 0.23 |
| TGF-$\beta$1 [ng/mL] | 16.9 (0.4–205.8) | 29.4 (0.3–153.1) | 0.24 |
| IL-12 [pg/mL] | 3 (2.1–29.1) | 2.7 (1.9–4.2) | 0.07* |
| IL-17A [pg/mL] | 2.1 (1.1–10.7) | 1.8 (1.2–2.3) | < 0.01* |

*p < 0.05 shows statistical significance.
BMI — body mass index, SBP — systolic blood pressure, DBP — diastolic blood pressure, TSH — thyroid stimulating hormone, fT3 — triiodothyronin, fT4 — levothyroxine, Anti-TG — anti-thyroglobulin, anti-TPO — anti-thyroid peroxidase, sTWEAK — soluble tumour necrosis factor like weak inducer of apoptosis, TGF-$\beta$1 — transforming growth factor beta,1 IL-17A — interleukin 17A, IL-12 — interleukin 12

variables were expressed as mean ± SD or median (min-max). During the comparisons, Student t test for parametric and Mann-Whitney test for nonparametric variables was performed. In the subgroup analysis, the presence of a statistically significant differences between the groups in terms of continuous variables was examined with ANOVA for parametric and Kruskall Wallis test for non-parametric variables. Post hoc testing was performed where the overall significance of the ANOVA or Kruskall Wallis test was significant ($p < 0.05$). Pearson correlation analysis was used for correlation of the variables.

## Results

Table I shows the demographic characteristics of patient and control groups and the measurement results. Both of the groups had similar characteristics such as age, gender, and body mass index. The HT group had lower levels of sTWEAK and TGF-$\beta$1, but had higher levels of IL-12 and IL-17A as compared to the control group. Of these, only the difference between IL-17A levels reached statistical significance (2.1 pg/mL *vs.* 1.8 pg/mL, respectively; $p < 0.001$).

Table II shows in detail the levels of demographic and laboratory findings according to HT subgroups and control group. The level of sTWEAK was found to be similar in the control, euthyroid, and subclinical hypothyroidism groups. The subclinical hypothyroidism group had a higher level of sTWEAK than that of overt hypothyroidism (888.2 ± 374.4 pg/mL *vs.* 687.6 ± 153.3 pg/mL, respectively; $p = 0.03$). No significant differences were detected in TGF-$\beta$1 levels between groups. While the levels of IL-12 were similar in the control, euthyroid, and subclinical hypothyroidism groups, its level in the overt hypothyroidism group was higher than that of the euthyroid and control groups (3.6 pg/mL *vs.* 2.7 pg/mL *vs.* 2.7 pg/mL, respectively; $p < 0.01$). The level of IL-17A was higher in all HT subgroups than that of the control group; however, its level was higher in the subclinical and overt hypothyroidism groups than that of the euthyroid group among HT subgroups (control: 1.8 pg/mL, euthyroid: 2 pg/mL, subclinical hypothyroidism: 2.3 pg/mL, overt hypothyroidism: 2.3 pg/mL; $p < 0.01$).

When we examined the relation between demographic, clinical, and laboratory parameters in the HT group we determined a positive correlation between IL-12 level and anti-TPO ($r = 0.496$, $p < 0.001$) level. A positive correlation was determined between sTWEAK level and TGF-$\beta$1 level ($r = 0.278$, $p = 0.032$). In the HT subgroup analysis, we determined a negative correlation between sTWEAK level and anti-TPO ($r = –0.533$, $p = 0.028$), IL-17A ($r = –0.600$, $p = 0.005$) levels in overt hypothyroidism group.

## Discussion

In the present study, the overt hypothyroidism group had a low sTWEAK level as compared to the subclinical hypothyroidism group. The level of sTWEAK was similar in the control, euthyroid, and subclinical hypothyroidism groups. There was a negative correlation between anti-TPO and IL-17A in the overt hypothyroidism group. As far as we know, this study is the first to determine the level of sTWEAK in HT and to investigate its relationship with thyroid autoantibodies.

Soluble TWEAK is a multifunctional cytokine that induces apoptosis in one cell while activating cell reproduction in another cell. It induces many cellular response and regulates inflammatory pathways depending on the cell type and micro environment [8]. The biological activity of sTWEAK may vary by onset period of inflammation. After acute tissue injury, sTWEAK induces progenitor cells and enables tissue regeneration, and alters tissue repair by inhibiting differentiation of the same progenitor cells following

**Table II.** *Comparison of subgroups of the HT patients and controls*
**Tabela II.** *Porównanie podgrup pacjentów z chorobą Hashimoto i osób z grupy kontrolnej*

| Variables | Control | Euthyroid | Subclinical hypothyroidism | Overt hypothyroidism | p |
|---|---|---|---|---|---|
| | n (20) | n (20) | n (20) | n (20) | |
| Age (years) | 35.3 ± 7.5 | 32.5 ± 8.2 | 30.9 ± 9.9 | 31.9 ± 8.2 | 0.91 |
| Gender, n (f/m) | 2/18 | 1/19 | 2/18 | 3/17 | 0.24 |
| BMI [kg/m$^2$] | 24.6 ± 2.3 | 23.6 ± 2 | 25.1 ± 3 | 24 ± 3.3 | 0.62 |
| SBP [mm Hg] | 110 (90–125) | 118.5 (100–128) | 110 (90–132) | 116 (90–135) | 0.08 |
| DBP [mm Hg] | 70 (60–80) | 62.5 (56–87) | 70 (53–90) | 72.5 (60–85) | 0.02[a, d, e] |
| TSH [$\mu$IU/mL] | 1.6 (0.9–3.5) | 2.1 (0.9–4.2) | 7.1 (4.6–14.5) | 33.3 (9.6–207.6) | 0.01[a, b, c, d, e, f] |
| fT3 [pg/mL] | 2.8 ± 0.5 | 2.8 ± 0.4 | 3 ± 0.4 | 2.2 ± 0.8 | < 0.01[b, d, f] |
| fT4 [ng/dL] | 1.2 ± 0.5 | 1.2 ± 0.1 | 1.1 ± 0.1 | 0.6 ± 0.1 | < 0.01[c] |
| Anti–TG [IU/mL] | 7.6 (2.4–26) | 207.1 (16.7–1538) | 219 (10–3910) | 341 (59.4–7411) | < 0.01[a, b, c] |
| Anti–TPO [IU/mL] | 3.9 (1–39) | 43.1 (5–443) | 119 (4–9578) | 515.5 (6–7977) | < 0.01[a, b, c, d, e, f] |
| sTWEAK [pg/mL] | 838.5 ± 236.5 | 771.4 ± 215.4 | 888.2 ± 374.4 | 687.6 ± 153.3 | 0.04[f] |
| TGF–$\beta$1 [ng/mL] | 29.4 (0.3–153.1) | 7.3 (0.5–104.6) | 27 (0.6–205.8) | 15.7 (0.5–97.5) | 0.24 |
| IL–12 [pg/mL] | 2.7 (1.9–4.2) | 2.7 (2.2–5.8) | 3.1 (2.1–29.1) | 3.6 (2.4–7.6) | < 0.01[c, e] |
| IL–17A [pg/mL] | 1.8 (1.2–2.3) | 2 (1.1–2.7) | 2.3 (1.6–10.7) | 2.4 (1.5–7.8) | < 0.01[a, b, c, d, e] |

aControl vs. Euthyroid, bControl vs. subclinical Hypothyroidism, cControl vs. Overt hypothyroidism, dEuthyroid vs. Subclinical hypothyroidism, eEuthyroid vs. Overt hypothyroidism, fSubclinical hypothyroidism vs. Overt hypothyroidism.
BMI — body mass index, SBP — systolic blood pressure, DBP — diastolic blood pressure, TSH — thyroid stimulating hormone, fT3 — triiodothyronin, fT4 — levothyroxine, Anti-TG — anti-thyroglobulin, anti-TPO — anti-thyroid peroxidase, sTWEAK — soluble tumor necrosis factor like weak inducer of apoptosis, TGF-$\beta$1 — transforming growth factor beta,1 IL-17A — interleukin 17A, IL-12 — interleukin 12

chronic injury [9]. A large part of data increasingly highlight the contribution of sTWEAK/Fn14 pathway to inflammation in autoimmune diseases [10]. Literature has no studies investigating the relationship between the serum sTWEAK and HT, an autoimmune disease. In evaluation of studies examining the association of other autoimmune diseases with sTWEAK, a study by Albert Einstein College of Medicine on SLE and healthy control group reported that SLE patients had relatively lower levels of sTWEAK than those of a healthy control group [11]. Similarly, a study by Noa Schwartz et al. identified lower levels of sTWEAK in SLE patients as compared to a healthy control group [11]. In addition, many studies with animal and human models indicate that sTWEAK might be associated with the aetiopathogenesis of multiple sclerosis [12]. Furthermore, since sTWEAK has been shown to be associated with the pathophysiology of rheumatoid arthritis and SLE, recent studies have focused on the effect of anti-TWEAK therapies on the prognosis of the disease in such diseases

In this study, the level of sTWEAK was found to be similar in the control, euthyroid, and subclinical hypothyroidism groups. The overt hypothyroidism group had lower level of sTWEAK as compared to the subclinical hypothyroidism group. Normally, because TWEAK is a proinflammatory cytokine, we expect the level of TWEAK to increase in inflammatory events. However, this study detected the level of sTWEAK to be low. Low serum level of sTWEAK in HT might have resulted from the increased Fn-14 level, which bound to sTWEAK and decreased the serum level of sTWEAK in chronic inflammation [13], or the increased level of CD 163, a scavenger receptor, which causes sTWEAK to be destructed by inflammatory macrophages in the case of inflammation [14]. A result of this study that supports the possible explanations provided above is that the level of IL-17A, a proinflammatory cytokine, increased from the control group to the overt hypothyroidism group. A negative correlation found between sTWEAK and IL-17A strongly supports our hypothesis.

The lower level of sTWEAK found in the overt hypothyroidism group than that of the subclinical hypothyroidism group had several possible causes. The first one might be the significant insufficiency of thyroid hormone, the second one might be the excessive chronic inflammation, and the most important one might be the increased autoimmune response. Lack of correlation between the thyroid function test and sTWEAK in the correlation analysis refutes our first hypothesis. As the pathogenesis of HT has a chronic inflammatory process, the expected finding is that the level of sTWEAK, a proinflammatory cytokine, varied with the progression of the disease. Our second hypothesis

is therefore acceptable. We consider that the primary cause of variation of the level of sTWEAK as compared to subclinical hypothyroidism could be the increased autoimmune response in the overt hypothyroidism group. Our hypothesis is strongly supported by the higher level of anti-TPO in the overt hypothyroidism group compared to all other groups, and by the strong correlation found between the level of sTWEAK and anti-TPO in the overt hypothyroidism group.

Another cytokine is IL-17A, which is synthesised by Th17 and has a role in proinflammation with many cytokines. Recently, the association of IL-17A with autoimmune diseases has often been underlined. In studies conducted with HT, Graves disease, and healthy control groups, HT had significantly higher levels of IL-17A than those of Graves and healthy control groups [15-18]. Dapeng Li et al. reported negative correlation between the IL-17A level and the extent of hypothyroidism in HT. In the same study, increased levels of IL-17A both in thyroid gland and in serum suggested that IL-17A played an active role in the pathogenesis of HT [15]. Data from our study support the role of IL-17A in HT.

A previous study reported that euthyroid and overt hypothyroidism with HT had higher levels of IL-12 compared to the control group, and the hypothyroidism group had higher IL-12 levels compared to the euthyroid group within the HT group [19]. Another study reported that the euthyroid HT group had significantly higher levels of IL-12 than those of a healthy control group [20]. In our study, the levels of IL-12 were found to be similar to those in the studies above in the HT group and subgroup analyses. Th1 is said to play a dominant role in inflammation of HT [2]. Increased level of IL-12, synthesised by Th1, in the HT group of our study is consistent with that hypothesis.

In studies conducted with TGF-$\beta$1, TGF-$\beta$1 level was significantly lower in patients with HT compared to the healthy control group, and its level was reported to decrease further towards late phase of disease [21–23]. In fact, it is not surprising to identify the TGF-$\beta$1 level to be low in HT, because the immunosuppressive effect of TGF-$\beta$1 is known well. Absence or low level of TGF-$\beta$1 contributes to activation and differentiation of T cells and development of autoimmune diseases by initiating apoptosis [22]. We found TGF-$\beta$1 levels to be lower in the HT group than those of the healthy control group, but this was not statistically significant. This might be related to our patients in the HT group, who were only at the onset phase of the disease.

The critical limitations of this study are the insufficient number of cases and controls. In addition, the second major limitation of our study was that fn14 and CD163 levels were not determined, which began to increase with TWEAK levels in the pro-inflammatory process and acted on the reduced level of circulating TWEAK.

As a result the reduced level of sTWEAK with HT progression and significant relationship between sTWEAK level and anti-TPO found in our study suggest that sTWEAK played an active role in chronic inflammation in the pathogenesis of HT and in the progression of autoimmunity. Further studies are needed to clearly understand the active role of sTWEAK in autoimmune process in HT, which is an autoimmune thyroiditis.

## References


1. Mikos H, Mikos M, Obara-Moszynska M et al. The role of the immune system and cytokines involved in the pathogenesis of autoimmune thyroid disease (AITD). Endokrynol Pol 2014; 65: 150–155.
2. Berger A. Th1 and Th2 responses: what are they? BMJ 2000; 321: 424.
3. Cerwenka A, Swain S. TGF-beta1: immunosuppressant and viability factor for T lymphocytes. Microbes Infect, 1999; 1: 1291–1296.
4. Ruggeri RM, Saitta S, Cristani M et al. Serum interleukin-23 (IL-23) is increased in Hashimoto's thyroiditis. Endocr J 2014; 61: 359–363.
5. Ren MY, Sui SJ. The role of TWEAK/Fn14 in cardiac remodeling. Mol Biol Rep 2012; 39: 9971–9977.
6. Sonmez A, Haymana C, Aydogdu A et al. Endothelial dysfunction, insulin resistance and inflammation in congenital hypogonadism, and the effect of testosterone replacement. Endocr J 2015; 62: 605–613.
7. Zheng TS, Burkly LC.No end in site: TWEAK/Fn14 activation and autoimmunity associated- end-organ pathologies. J Leukoc Biol. 2008; 84: 338–347.
8. Ates I, Özkayar N, Akyel F et al., The relationship between asymptomatic organ damage, and serum soluble tumor necrosis factor-like weak inducer of apoptosis (sTWEAK) and Interleukin-17A (IL-17A) levels in non-diabetic hypertensive patients. BMC nephrology, 2014; 15: 159.
9. Burkly LC, Michaelson JS, Hahm K et al. TWEAKing tissue remodeling by a multifunctional cytokine: role of TWEAK/Fn14 pathway in health and disease. Cytokine 2007; 40: 1–16.
10. Bertin D, Stephan D, Khrestchatisky M et al. Is TWEAK a Biomarker for Autoimmune/Chronic Inflammatory Diseases? Front Immunol 2013; 4: 489.
11. Schwartz N, Rubinstein T, Burkly LC et al. Urinary TWEAK as a biomarker of lupus nephritis: a multicenter cohort study. Arthritis Res Ther 2009; 11: R143.
12. Nazeri A, Heydarpour P, Sadaghiani S et al. A further TWEAK to multiple sclerosis pathophysiology. Mol Neurobiol 2014; 49: 78–87.
13. Vendrell J, Chacon MR. TWEAK: A New Player in Obesity and Diabetes. Front Immunol 2013; 4: 488.
14. Moreno JA, Munoz-Garcia B, Martin-Ventura JL et al. The CD163-expressing macrophages recognize and internalize TWEAK: potential consequences in atherosclerosis. Atherosclerosis 2009; 207: 103–110.
15. Li D, Cai W, Gu R et al. Th17 cell plays a role in the pathogenesis of Hashimoto's thyroiditis in patients. Clin Immunol 2013; 149: 411–420.
16. Figueroa-Vega N, Alfonso-Perez M, Benedicto I et al. Increased circulating pro-inflammatory cytokines and Th17 lymphocytes in Hashimoto's thyroiditis. J Clin Endocrinol Metab 2010; 95: 953–962.
17. Bossowski A, Moniuszko M, Idzkowska E et al. Evaluation of CD4+CD161+CD196+ and CD4+IL-17+ Th17 cells in the peripheral blood of young patients with Hashimoto's thyroiditis and Graves' disease. Pediatr Endocrinol Diabetes Metab 2012; 18: 89–95.
18. Wang S, Baidoo SE, Liu Y et al. T cell-derived leptin contributes to increased frequency of T helper type 17 cells in female patients with Hashimoto's thyroiditis. Clin Exp Immunol 2013; 171: 63–68.
19. Hidaka Y, Okumura M, Fukata S et al., Increased serum concentration of interleukin-12 in patients with silent thyroiditis and Graves' disease. Thyroid 1999; 9: 149–153.
20. Phenekos C, Vryonidou A, Gritzapis AD et al. Th1 and Th2 serum cytokine profiles characterize patients with Hashimoto's thyroiditis (Th1) and Graves' disease (Th2). Neuroimmunomodulation 2004; 11: 209–213.
21. Akinci B, Comlekci A, Yener S et al. Hashimoto's thyroiditis, but not treatment of hypothyroidism, is associated with altered TGF-beta1 levels. Arch Med Res 2008; 39: 397–401.
22. Vural P, Degirmencioglu S, Erden S et al. The relationship between transforming growth factor-beta1, vascular endothelial growth factor, nitric oxide and Hashimoto's thyroiditis. Int Immunopharmacol 2009; 9: 212–215.
23. Manolova I, Gerenova J, Ivanova M. Serum levels of transforming growth factor-beta1 (TGF-beta1) in patients with systemic lupus erythematosus and Hashimoto's thyroiditis. Eur Cytokine Netw 2013; 24: 69–74.