CMJ Original Research December 2016, Volume: 38, Number: 4
Cumhuriyet Medical Journal 315-321
http://dx.doi.org/10.7197/cmj.v38i4.5000185086

The effect of Vitamin D treatment on thyroid function and the levels of thyroid autoantibodies, TNF-α, IL-6, IL-1β in patients with autoimmune thyroiditis

Otoimmun tiroiditi bulunan hastalarda D vitamini tedavisinin tiroid fonksiyonları ve tiroid otoantikorları, TNF-α, IL-6, IL-1β düzeyleri üzerine etkisi

Fettah Acıbucu[1], H. Sebila Dokmetas[2], Fatih Kılıclı[2], Cem Celik[3], Mustafa Aydemir[4]

[1]Sivas Numune Hospital, Department of Endocrinology, Sivas, Turkey
[2]Medipol University Medical School, Department of Endocrinology, Istanbul, Turkey
[3]Cumhuriyet University Medical School, Departments of Microbiology, Sivas, Turkey
[4]Afyon State Hospital, Department of Endocrinology, Afyon, Turkey
Corresponding author: Hatice Sebila Dökmetaş, Medipol Üniversitesi Tıp Fakültesi, Endokrinoloji ve Metabolizma Hastalıkları Bilim Dalı, İstanbul, Turkey
E-mail: sebiladokmetas@hotmail.com
Received/Accepted: April 11, 2016 / August 28, 2016
Conflict of interest: There is not a conflict of interest.

SUMMARY

Objective*:* To investigate the relationship between autoimmune thyroid disease and vitamin D treatment.

Method: Fifty four (54) patients with both vitamin D deficiency and newly diagnosed euthyroid Hashimoto's thyroiditis (HT) were recruited for this study. The patients were given intramuscular administration of cholecalciferol at a dose of 300,000 IU/month for 3 months. At the time of diagnoses and after the treatment of vitamin D, free T3 (FT3), free T4 (FT4), thyroid stimulating hormone (TSH), antithyroid peroxidase (anti-TPO), antithyroglobulin (anti-TG), 25 (OH) D3, parathormone (PTH), calcium (Ca), phosphorus (P) and alkaline phosphatase (ALP) levels were measured in all patients; TNF-a, IL-6 and IL-1ß levels were measured in only 43 patients.

Results: A statistically significant difference ($p<0.05$) was observed between the pre and post treatment FT4, TSH, antiTPO, antiTG, PTH and ALP levels. After the treatment of vitamin D, a statistically significant increase was found in 25 (OH) D3 and FT4 levels, and a significant decrease was found in TSH, antiTPO, antiTG, PTH and ALP levels, whereas no significant difference was noted in FT3, Ca, P, TNF- a, IL-6 and IL-1ß levels. Further, levels of vitamin D were not correlated with FT3, FT4, TSH, antiTPO, antiTG, TNF-a, IL-6 and IL-1ß levels ($p>0.05$).

Conclusions: For patients with both vitamin D deficiency and newly diagnosed HT, treatment of vitamin D had a positive effect on the thyroid antigenicity and thyroid function.

Keywords: Autoimmune thyroid disease, Hashimoto's thyroiditis, Vitamin D deficiency

ÖZET

Amaç: D vitamini tedavisinin otoimmun tiroid hastalığı ile ilişkisini araştırdık.

Yöntem: Çalışmaya D vitamini yetersizliği ve beraberinde yeni tanı alan Hashimoto tiroiditi olan (HT) 54 hasta alındı. Hastalara 3 ay süreyle ayda bir 300.000 IU kolekalsiferol intramuskuler olarak verildi. Tanı esnasında ve D vitamini tedavisi sonrasında serbest T3(FT3), serbest T4 (FT4), tiroid

stimulan hormon (TSH), anti tiroid peroksidaz (TPO) ve anti tiroglobulin (TG), 25(OH)D3, parathormon (PTH), kalsiyum (Ca), fosfor (P) ve alkalen fosfataz (ALP) seviyeleri ve yalnız 43 hastada TNF- α, IL-6, IL-1b seviyeleri ölçüldü.

Bulgular: Tedavi öncesi ve sonrası FT4, TSH, antiTPO, antiTG, PTH ve ALP seviyeleri açısından arada istatistiksel olarak anlamlı fark vardı ($p<0.05$). D vitamini tedavi sonrasında 25 (OH) D3 ve FT4 seviyelerinde istatiksel olarak önemli bir artış olurken TSH, antiTPO, antiTG, PTH ve ALP seviyelerinde ise önemli bir düşüş gözlendi. FT3, Ca, P, TNF- α, IL-6 ve IL-1b açısından önemli bir fark yoktu. D vitamini seviyesi ile FT3, FT4, TSH, antiTPO, antiTG, TNF- α, IL-6 ve IL-1b arasında bir korelasyon yoktu.

Sonuç: D vitamini eksikliği ve yeni tanı HT'i olan hastalarda D vitamini tedavisinin tiroid antijenitesi ve tiroid fonksiyonu üzerine olumlu yönde etkisi vardır.

Anahtar sözcükler: Otoimmun tiroid hastalığı, Hashimoto tiroiditi, D vitamini yetersizliği

INTRODUCTION

Hashimoto's thyroiditis (HT) is the most widespread autoimmune thyroid disorder, with a prevalence rate of 18%, independent of age and gender[1]. HT is characterized by a gradual destruction and fibrosis of the thyroid parenchyma following lymphocyte infiltration of the thyroid tissue and has a biochemical feature of the presence of antibodies directed against the two major thyroid antibodies, thyroid peroxidase (TPO) and thyroglobulin (TG)[2]. Antithyroid peroxidase (antiTPO) and antithyroglobulin (antiTG) antibodies may result in antibody-dependent cell damage in the thyroid cells. The activation of the apoptotic pathway through T-cell mediated cytotoxicity may be another mechanism in the pathogenesis of HT. Thyroid autoantibodies, which show a positive antiTPO in >90% of patients and a positive antiTG in 80% of the patients, play an important role in the diagnosis of thyroid autoimmunity[2,3]. The clinical range of autoimmune thyroid disease varies from the presence of the thyroid antibodies alone to severe thyroid dysfunction. Following the euthyroid phase, the disease often gradually progresses to subclinical hypothyroidism and overt hypothyroidism[4].

The main form of circulating vitamin D, 25(OH) D3, is the most commonly used form measured in the laboratory to determine vitamin D levels[5]. Vitamin D possesses immunomodulatory properties, and deficiency is associated with the development of autoimmune diseases[6]. Vitamin D supplementation has been shown to decrease the prevalence of autoimmune diseases, such as type 1 diabetes mellitus (DM) and multiple sclerosis (MS)[7-9]. Furthermore, vitamin D treatment is beneficial against autoimmune diseases and has also been demonstrated to reduce the activity of MS and psoriatic arthritis and to lower C-reactive protein levels in rheumatoid arthritis[10-12]. Although autoimmune thyroid disease is more common than type 1 diabetes and multiple sclerosis, the effect of 25(OH) D deficiency on autoimmune thyroid disease has been evaluated in only a limited amount of studies.

In the literature, the effects of vitamin D treatment on thyroid functions, autoantibodies and proinflammatory cytokines have not been studied. In this study, the effects of vitamin D treatment on thyroid functions, thyroid autoantibodies and proinflammatory cytokines that trigger cell damage, such as tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6) and interleukin-1 beta (IL-1β), in patients with vitamin D deficiency and autoimmune thyroiditis were investigated.

MATERIAL AND METHODS

This study included 54 patients (males: 8; females: 46) diagnosed with autoimmune thyroid disease and vitamin D deficiency at our university hospital. The study was performed according to the approval of the University Ethics Committee. For patients included in the study, the diagnosis of autoimmune thyroid disease was established by the elevated levels of serum Anti-TPO(>5.61IU/mL) and/or Anti-TG (> 4.11IU/ml) antibodies. The

diagnosis of vitamin D deficiency was established by serum 25(OH) D3 levels of ≤20 ng/ml. All of the patients included in the study were newly diagnosed patients who did not need thyroid hormone replacement therapy. Exclusion criteria were patients with any acute disease or chronic disease, patients on medications shown to affect the metabolism of vitamin D or patients requiring thyroid hormone replacement therapy. The patients were given intramuscular administration of cholecalciferol at a dose of 300,000 IU/month for 3 months.

Free T3 (FT3), free T4 (FT4), thyroid stimulating hormone (TSH), anti-TPO, anti-TG, 25(OH)D3, parathormone (PTH), calcium (Ca), phosphorus (P) and alkaline phosphatase (ALP) levels were measured in all patients, and TNF-α, IL-6 and IL-1β levels were measured in only 43 patients. These tests were conducted twice: a) upon diagnosis pretreatment and b) following treatment with vitamin D (one month following the last course of drug treatment).

Calcium, phosphorus and alkaline phosphatase studies were conducted using spectrophotometric method on automathic analyzer of Beckman Coulter LX20, and PTH measurements were conducted with Cobas e 601 (Roche Hitachi), using enzyme immunoassay technique. FT3, FT4 and TSH were measured with Architect i 2000 SR device using enzyme immunoassay method. AntiTPO, 25OHD3 and antiTG studies were also measured with Architect i 2000 SR device using chemiluminescent microparticle immunoassay technique.

Blood samples of the 43 patients were collected at diagnosis and then again 1 month after the treatment (one month following the treatment). These samples were centrifuged in Hettich Universal 32R centrifuge at 4000 rpm for 5 minutes. Using 1.4 ml Eppendorf tubes, serum samples were taken and stored at -80°C. During the study, all samples were brought to room temperature and TNF-α, IL-6 and IL-1β levels were measured at our hospital's microbiology laboratories.

IL-6, TNF-α and IL-1β levels were measured with Grifols' TRITURUS® ELISA System - a completely automated ELISA immunoassay analyzer- using the Sandwich ELISA method with bioscience platinum test kits.

The data were analyzed using SPSS version 14.00. Wilcoxon signed rank test and correlation analysis was performed. Our data are presented as arithmetic mean ± standard deviation.

RESULTS

The mean age of the all patients was 32.5±9.40 years. Pre-treatment and post-treatment laboratory values of the patients are shown in table 1.

Following treatment, a statistically significant increase was observed in the Free T4 and 25 (OH) D3 levels (Table 1). A statistically significant decrease was observed in Anti-TPO, Anti-TG, TSH, PTH and ALP levels following treatment (Table 1). Before treatment, the minimum Anti-TPO level was 7.47 IU/ml, the maximum level was 990 IU/ml and the median level was 213.98 IU/ml. For Anti-TG minimum, median and maximum levels were 4.62 IU/ml, 57.43IU/ml and 990 IU/ml respectively (Figure 1). No statistically significant difference was found between the pre-treatment and post-treatment levels for Free T3, calcium, phosphorus, TNF- α, IL-6 and IL-1β (Table 1).

Lastly, no significant statistical correlation was found between 25(OH)D3 levels and FT3, FT4, TSH, antiTPO, antiTG, PTH, Ca, P, ALP, TNF- α, IL-6 and IL-1β in pre-treatment and post-treatment periods

Table 1. Pre and post-treatment laboratory values of patients treated with Vit D.

	Pre-treatment Mean±SD	Post-treatment Mean±SD	
FT3(1.71-3.71pg/ml)	2.86±0.26	2.89±0.28	p=0.494
FT4(0.70-1.48ng/dl)	0.99±0.15	1.10±0.13	p=0.001
TSH(0.35-4.94µIU/ml)	3.75±2.36	2.53±1.87	p=0.001
AntiTPO(0-5.61IU/ml)	325.51±330.81	149.95±158.19	p=0.001
AntiTG(0-4.11IU/ml)	190.45±272.90	79.89±101.48	p=0.001
25(OH)D3(ng/ml)	10.08±4.38	48.52±15.21	p=0.001
PTH(15-68.3pg/ml)	48.59±18.02	33.36±12.17	p=0.001
Ca(8.6-10.2mg/dl)	9.19±0.41	9.27±0.44	p=0.271
P(2.5-4.5mg/dl)	3.26±0.61	3.34±0.51	p=0.327
ALP(45-129U/L)	62.42±18.12	60.03±13.64	p=0.023
TNF- α(ng/ml)	0.02±0.06	0.02±0.01	p=0.317
IL-6(ng/ml)	0.001±0.003	0.002±0.002	p=0.160
IL-1β (pg/ml)	4.01±0.36	4.13±0.61	p=0.300

FT3: Free T3; FT4: Free T4; TSH: Thyroid Stimulating Hormone; PTH: Parathormone; Ca: Calcium; P: Phosphorus; ALP: Alkaline phosphatase; TNF-α: Tumor necrosis factor-alpha; IL-6: Interleukin-6; IL-1β: Interleukin-1 beta

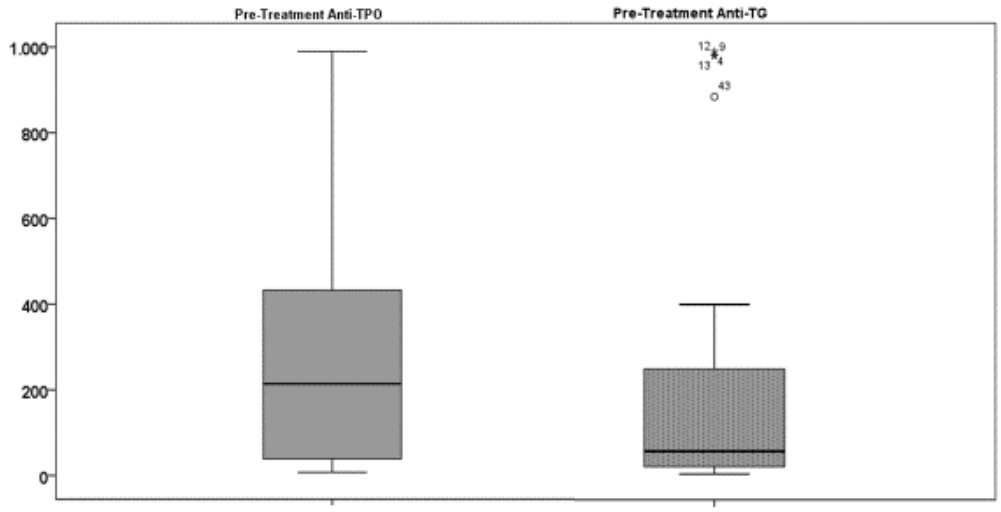

Figure 1: Pre treatment serum levels of Anti-TPO and Anti-TG in patients.

DISCUSSION

In this study, we identified the FT3, FT4, TSH, antiTPO, antiTG, 25 (OH) D3, PTH, Ca, P and ALP levels in 54 patients diagnosed with autoimmune thyroid disease, according to antiTPO and antiTG antibody levels, and the accompanying vitamin D deficiency at diagnosis and 3 months after the treatment with vitamin D. Further, we examined the effects of vitamin D therapy on these parameters. After the treatment of vitamin D, a statistically significant increase was found in 25 (OH) D3 and FT4 levels, and a significant decrease was found in TSH, antiTPO, antiTG, PTH and ALP levels, whereas no significant difference was noted in FT3,

Ca and P levels. The increase in FT4 levels and the decrease in TSH, antiTPO and antiTG levels suggest that vitamin D therapy might have a positive effect on thyroid antigenicity and thyroid function in autoimmune thyroid disease.

Vitamin D plays an important role in the metabolism of calcium. In addition, a significant relationship has been demonstrated between vitamin D and immunity. In vitro studies have shown the immunomodulatory effects of 1.25(OH)2D3 on T cells[5,13,14]. Deficiencies in vitamin D and vitamin D receptors may cause an increase in Th1-mediated immune response. The active form of vitamin D therapy has proven to be useful in autoimmune diseases by inhibiting the development and function of Th1 cells[15,16]. Vitamin D can inhibit the autoimmune process at different stages of HT, reduce cytokine production from Th1 cells and invoke cytokine-induced antigenicity of the thyroid's Th1 cells. The biologically active form of vitamin D potentially reduces the formation of thyroid antibodies reacting with the thyroid antigens[5,17]. Vitamin D levels were found to be statistically significantly lower than age- and sex-matched controls in patients with HT. No significant difference, however, was found between euthyroid, subclinical hypothyroidism and hypothyroidism groups of HT in terms of vitamin D levels. Therefore, it was concluded that there may be a correlation between vitamin D deficiency and HT. No investigation was conducted to determine if vitamin D deficiency has any effect on the progression of the thyroid cell damage in the pathogenesis of HT [5]. Another study found lower levels of vitamin D in patients with HT versus the control patients. A relationship has also been found between vitamin D deficiency and the presence of anti-thyroid antibodies and TSH levels[18]. In another study, no significant role for vitamin D was discovered in the pathogenesis of HT[19]. In the public-based research project conducted by Goswami et al, a significant inverse correlation between 25(OH)D and antiTPO levels were shown[20]. The effect of vitamin D treatment on thyroid functions and antibody levels was not evaluated in this study[20]. We investigated the effect of vitamin D treatment on HT and have shown that it has a positive effect, however, we were not able to find any statistically significant correlation between 25(OH)D and TSH, antiTPO and antiTG levels. Of the 642 subjects who participated in the study performed by Goswami et al, 21% of them had antiTPO antibody positivity[20]. The reason for the different results can be attributed to the limited number of patients we had as well as the high antibody titers of the patients included in the study.

HT, a complex disease associated with genetic and environmental factors, is defined as a cell-mediated immunity disorder caused by a genetic defect in suppressor T-cell functions[5]. Proinflammatory cytokines, such as TNF-α and IL1, can affect immunity-related apoptosis. TNF-α and IL1 in combination with IFN-γ have been shown to induce apoptosis in thyroid cell culture studies[21-23]. The mechanism of apoptosis in HT seems to be associated with the stimulation of cytokines secreted from local lymphocytes[23]. Studies have shown an association between TNF-α and HT as well as between TNF-α, IL-6 and anti-TPO[24-26]. Moreover, a study investigating the relationship between dosing of levothyroxine and cytokines, such as IL-6, IL-1β and TNF-α, showed a positive linear correlation between the replacement dose of levothyroxine and IL-6 and TNF-α, while no correlation was found with IL-1β. Furthermore, a negative correlation was found between IL-6 and serum T3 and T3/T4 ratios, and no correlation was found with TNF-α. This has been shown to be related with the inhibition of hepatic 5'-monodeiodinase by IL-6 as in nonthyroidal diseases. In addition, a positive correlation was found between IL-6 and TNF-α[27].

Proinflammatory cytokines, such as IL-6, IL-1β and TNF-α, can increase cell damage via its local secretion from immune cells and also accelerate the progression of the disease. As vitamin D

can affect the secretion of proinflammatory cytokines from immune cells, vitamin D deficiency can increase the secretion of proinflammatory cytokines, such as IL-6, IL-1β, and TNF-α and thereby increase cell damage in patients with HT[28]. In this study, we investigated serum TNF-α, IL-6 and IL-1β levels of 43 patients before and after vitamin D treatment to discover if vitamin D treatment can have a positive impact on HT via this mechanism. In most patients included in this study, the pre-treatment and post-treatment serum TNF-α, IL-6 and IL-1β levels were too low to be detected and also had no statistically significant difference. Our results revealed that vitamin D deficiency and autoimmune thyroid disease had no effect on systemic levels of inflammatory cytokines, such as TNF-α, IL-6, and IL-1β. However, these cytokines can be released from immune cells at the tissue level and their secretion can be decreased with vitamin D treatment. The positive effect we found in our study using vitamin D treatment in patients with autoimmune thyroid disease may have occurred in this way, by other cytokines or by other mechanisms that were not evaluated in this study.

In our study, vitamin D treatment was observed to have a positive effect on the thyroid antigenicity and thyroid function. Vitamin D treatment could slow or prevent this progress in patients with both HT and vitamin D deficiency. We recommend that when diagnosing patients with HT, vitamin D levels should be evaluated, and in the case that any deficiency is seen proper treatment should be administered.

REFERENCES

1. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA et al. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab 2002; 87: 489-99
2. Zaletel K. Determinants of thyroid autoantibody production in Hashimoto's thyroiditis. Expert Rev Clin Immunol 2007; 3: 217-23
3. McLachlan SM., Rapoport B. Why measure thyroglobulin autoantibodies rather than thyroid peroxidase autoantibodies? Thyroid 2004; 14: 510-20.
4. Vanderpump MP, Tunbridge WM, French JM, Appleton D, Bates D, Clark F et al. The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. Clin Endocrinol 1995; 43: 55-68.
5. Tamer G, Arik S, Tamer I, Coksert D. Relative vitamin D insufficiency in Hashimoto's thyroiditis. Thyroid 2011; 21: 891-6.
6. Toubi E, Shoenfeld Y. The role of vitamin D in regulating immune responses. Isr Med Assoc J 2010; 12: 174-5.
7. Zipitis CS, Akobeng AK. Vitamin D supplementation in early childhood and risk of type 1 diabetes: a systematic review and meta-analysis. Arch Dis Child 2008; 93: 512–7.
8. Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA 2006; 296: 2832-8.
9. Hypponen E, Laara E, Reunanen A, Jarvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet 2001; 358: 1500-3.
10. Andjelkovic Z, Vojinovic J, Pejnovic N, Popovic M, Dujic A, Mitrovic D et al. Disease modifying and immunomodulatory effects of high dose 1 alpha (OH) D3 in rheumatoid arthritis patients. Clin Exp Rheumatol 1999; 17: 453-6.
11. Gaal J, Lakos G, Szodoray P, Kiss J, Horvath I, Horkay E et al. Immunological and clinical effects of alphacalcidol in patients with psoriatic arthropathy: results of an open, follow-up pilot study. Acta Derm Venereol 2009; 89: 140-4.
12. Goldberg P, Fleming MC, Picard EH. Multiple sclerosis: decreased relapse rate through dietary supplementation

with calcium, magnesium and vitamin D. Med Hypotheses 1986; 21: 193-200.

13. Arnson Y, Amital H, Shoenfeld Y. Vitamin D and auto-immunity: new aetiological and therapeutic considerations. Ann Rheum Dis 2007; 66: 1137-42.

14. Cantorna MT, Mahon BD. Mounting evidence for vitamin D as an environmental factor affecting autoimmune disease prevalence. Exp Biol Med 2004; 229: 1136-42.

15. Cantorna MT, Zhu Y, Froicu M, Wittke A. Vitamin D status, 1,25-dihydroxyvitamin D3, and the immune system. Am J Clin Nutr 2004; 80: 1717-20.

16. Cantorna MT. Vitamin D and autoimmunity: is vitamin D status an environmental factor affecting autoimmune disease prevalence? Proc Soc Exp Biol Med 2000; 223: 230-3.

17. Jackson IMD, Hennessey JV. Thyroiditis. In: Becker KL (ed) Principles and Practice of Endocrinology and Metabo-lism, 3rd edition. Lippincott Williams & Wilkins, Philadel-phia, 2001; 456-9.

18. Kivity S, Agmon-Levin N, Zisappl M, Shapira Y, Nagy EV, Danko K et al. Vitamin D and autoimmune thyroid diseases. Cell Mol Immunol 2011; 8: 243-7.

19. Effraimidis G, Badenhoop K, Tijssen JG, Wiersinga WM. Vitamin D deficiency is not associated with early stages of thyroid autoimmunity. Eur J Endocrinol 2012;.167: 43-8.

20. Goswami R, Marwaha RK, Gupta N, Tandon N, Sreenivas V, Tomar N et al. Prevalence of vitamin D deficiency and its relationship with thyroid autoimmunity in Asian Indians: a community-based survey. Br J Nutr. 2009; 102: 382-6.

21. Bretz JD, Rymaszewski M, Arscott PL, Myc A, Ain KB, Thompson NW et al. TRAIL death pathway expression and induction in thyroid follicular cells. J Biol Chem 1999; 274: 23627-32.

22. Bretz JD, Baker Jr JR. Apoptosis and autoimmune thyroid disease: following a TRAIL to thyroid destruction? Clin Endocrinol 2001; 55: 1-11.

23. Zaletel K, Gaberšček S. Hashimoto's Thyroiditis: From Genes to the Disease. Curr Genomics 2011; 12: 576-88.

24. Inoue N, Watanabe M, Nanba T, Wada M, Akamizu T, Iwatani Y. Involvement of functional polymorphisms in the TNFA gene in the pathogenesis of autoimmune thyroid diseases and production of anti-thyrotropin receptor antibody. Clin Exp Immunol 2009; 156: 199-204.

25. Karanikas G, Schuetz M, Wahl K, Paul M, Kontur S, Pietschmann P et al. Relation of anti-TPO autoantibody titer and T lymphocyte cytokine production patterns in Hashimoto's thyroiditis. Clin Endocrinol 2005; 63: 191-6.

26. Nielsen CH, Brix TH, Leslie RG, Hegedüs L. A role for autoantibodies in enhancement of proinflammatory cytokineresponses to a self-antigen, thyroid peroxidise. Clin Immunol 2009; 133: 118-27.

27. Papanas N, Papazoglou D, Papatheodorou K, Antonoglou C, Kotsiou S, Maltezos E. Thyroxine replacement dose in patients with Hashimoto disease: a potential role for interleukin-6. Cytokine 2006; 35: 166-70.

28. Guillot X, Semerano L, Saidenberg-Kermanac'h N, Falgarone G, Boissier MC. Vitamin D and inflammation. Joint Bone Spine 2010; 77: 552-7.