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ORIGINAL ARTICLE

Influence of levothyroxine treatment on serum levels of soluble Fas (CD95) and Fas Ligand (CD95L) in chronic autoimmune hypothyroidism

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Abstract Fas/FasL-mediated apoptosis results in the destruction of thyrocytes in chronic autoimmune hypothyroidism (CAIH). In this study, we examined the serum levels of soluble Fas (sFas) and soluble sFas ligand (sFasL) in euthyroid patients with chronic autoimmune hypothyroidism, who were taking levothyroxine (euthyroid, LT4-CAIH), to investigate the possible role of thyroid hormone therapy in down-regulation of apoptotic factors. Fifty euthyroid patients with CAIH on levothyroxine (median of duration 36 months, range 6–228 months) were compared with 75 age- and sex-matched healthy individuals. Serum levels of soluble Fas and soluble Fas Ligand, autoantibodies to thyroid peroxide and thyroglobulin were measured using ELISA. Serum levels of sFas were significantly higher in the euthyroid, LT4-CAIH group [median 9.12 ng/ml, interquartile range (7.86–10.72 ng/ml)] than in the controls [6.11 ng/ml (5.60–6.81 ng/ml)] ($P < 0.0001$). Compared with controls [80.33 pg/ml (68.22–103.70 pg/ml)], the euthyroid, LT4-CAIH group [125.71 pg/ml (106.11–149.48 pg/ml)] had significantly higher levels of sFasL ($P < 0.0001$). In a chronological study, there was no significant correlation between sFas, sFasL, and the duration of levothyroxine therapy. In conclusion, normalization of serum sFas and sFasL levels cannot be achieved during levothyroxine treatment in patients with CAIH. It appears that levothyroxine therapy has no important effect on down-regulation of apoptotic factors in CAIH. Thus, like thyroid autoantibodies, monitoring of serum levels of sFas/sFasL is not indicated during thyroid hormone therapy.

Keywords Fas · FasL · Hypothyroidism · Thyroiditis · Immunity · Levothyroxine

Introduction

The Fas receptor (Fas, CD95, and APO-1) belongs to the subgroup of the tumor necrosis factor receptor (TNF-R) family that triggers apoptosis via activation by its natural ligand (FasL, CD95L) [1, 2]. The Fas/FasL system plays critical roles in immune system and maintaining thyroid tissue homeostasis, as well as in thyroid diseases [3, 4]. Increasing evidence supports that activation of the FasL-mediated apoptotic pathway by proinflammatory cytokines may be involved in the destruction of the thyroid follicular cells, leading to Hashimoto's thyroiditis (HT) [3, 5, 6].

Apart from its membrane-bound Fas receptor, Fas molecule is also detectable in soluble forms (sFas). sFas is an alternative spliced form of Fas lacking the transmembrane region [7]. It is thought that sFas protect target cells from Fas-mediated apoptosis via inhibiting Fas, FasL interaction [7, 8].

FasL is also detectable in a soluble form (sFasL) resulting from conversion of the membrane-bound FasL by a metalloproteinase [9]. sFasL is less cytotoxic than membrane-bound FasL; however, soluble FasL retains its capacity to interact with Fas, and restoration of its cytotoxic activity was achieved with the addition of cross-linking antibodies [10]. Thus, it is likely that sFasL induces apoptosis on Fas antigen-expressing thyrocytes.

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Accumulating evidence has emerged to support that apoptosis results in the destruction of thyrocytes in HT. It appears that the apoptotic pathway is initiated by Th1 cytokines, which overcome anti-apoptotic defense mechanisms of the thyrocytes [11].

As most thyroid hormone targets in plasma membranes and cytosol are involved in anti-apoptotic signalling pathways [12], anti-apoptotic effects of thyroid hormones have been under study. The function of thyroid hormones on the regulation of apoptosis seems to be cell type and species specific [13]. Multiple studies demonstrated anti-apoptotic effects for thyroid hormones; it has been reported that T3 down-regulates Fas and FasL expression and suppresses apoptosis in early placental extravillous trophoblasts in vitro [13]. An increase in apoptosis in the optic lobe of the chick embryo [14], inhibition of multilayer formation of the osteoblastic cell line, MC3T3-E1, by promoting apoptosis after T3 treatment [15] and thyroid hormone regulation of apoptosis induced by retinoic acid in promyeloleukemic HL-60 cells [16] were reported. The function of triiodo-L-thyronine as an important anti-apoptotic factor in mouse hepatocytes [12], inhibition of apoptotic potency by activating thyroid hormone receptors in mitochondria [17], activation of anti-apoptotic myeloid cell leukemia 1 (an anti-apoptotic member of the B-cell lymphoma-2 family) by triiodothyronine [18] have been shown. Resveratrol-induced gene expression and apoptosis were inhibited more than 50% by physiological concentration of thyroid hormone in papillary and follicular thyroid cancer cells [19]. It is also an anti-apoptotic in glioma cells [20].

So far, the effects of thyroid hormones on sFas and sFasL levels have not been studied in patients with chronic autoimmune hypothyroidism (CAIH). In this study, we examined the serum levels of sFas and sFasL in euthyroid patients with chronic autoimmune hypothyroidism on levothyroxine (euthyroid, LT4-CAIH) to investigate the possible role of thyroid hormone therapy in down-regulation of apoptotic factors in these patients.

Materials and methods

Subjects

The subjects of our study were 67 consecutive patients (mean age ± SE 31.03 ± 1.51 years, 59 women, 8 men) with chronic autoimmune hypothyroidism on levothyroxine therapy examined at the endocrine clinic of Bushehr University of Medical Sciences. The diagnosis of chronic autoimmune hypothyroidism (CAIH) was based on biochemical evidence of primary hypothyroidism or present treatment with levothyroxine in combination with the presence of antibodies against thyroid peroxidase or thyroglobulin [21], and consisted of those with hypothyroidism with (Hashimoto's thyroiditis) or without (atrophic thyroiditis) goiter.

Grading of goiter was based according to the modified World Health Organization recommendation in 1994 [22]: "Grade 0: No palpable or visible goiter; Grade 1: A goiter that is palpable, but not visible when the neck is in the normal position; Grade 2: A swelling in the neck that is clearly visible when the neck is in a normal position and is consistent with an enlarged thyroid when the neck is palpated."

A total of 50 patients with CAIH were euthyroid, on levothyroxine therapy (euthyroid, LT4-CAIH). Although, the remaining 17 patients with CAIH were on levothyroxine therapy, they had no steady-state T4 and TSH concentrations (hypothyroid, LT4-CAIH). For comparison, 75 healthy individuals (mean age ± SE 28.86 ± 1.02 years, 72 women, 3 men) with normal thyroid function, no goiter, and negative history of autoimmune diseases were also investigated.

The study was approved by the medical-ethical committee of Bushehr University of Medical Sciences.

Laboratory measurements

Venous blood was obtained from all the patients and healthy controls. All the sera were kept frozen at −70°C until used.

For the detection of sFas or sFasL in samples, a commercially available ELISA (Quantikine, R&D Systems, Minneapolis, USA) was used according to the manufacturer's instructions. In brief, after rinsing, microplate wells (coated with a murine monoclonal antibody against Fas or FasL) were blocked with the assay diluents. Then, standard (recombinant human Fas/Fc chimera in a buffered protein base or recombinant human FasL in a buffer) or serum sample was added to each well and incubated for 2 h at room temperature. After washing, polyclonal antibodies against Fas or FasL, conjugated to horseradish peroxidase, was added to each well and incubated for 2 h at room temperature. After washing, substrate solution containing stabilized hydrogen peroxide and horseradish stabilized chromogen was added to each well and incubated for 30 min at room temperature. The reaction was stopped with stop solution (2 N sulfuric acid). The optical density of each well was determined at 540–570 nm within 30 min. The minimum detectable dose of sFas was typically less than 20 pg/ml, with an intra-assay coefficient of variation of 4.6% and inter-assay coefficient of variation of 2.9%. The minimum detectable dose of sFasL was 2.66 pg/ml, with an intra-assay coefficient of variation of 5.4% and inter-assay coefficient of variation of 6.4%.

For the determination of TSH and for the measurements of autoantibodies to thyroid peroxide and thyroglobulin,

commercially available TSH ELISA kits (Pishtaz Teb Zaman, Tehran, Iran) and microplate enzyme immunoassays (Monobind, Inc., California, USA) were used. The reference interval for TSH assay was 0.32–5.2 mIU/l. The TgAb ELISA and TPOAb ELISA had a sensitivity of 5 IU/ml and 1.5 IU/ml, respectively.

Statistical analysis

Normal distribution of the data was controlled with Kolmogorov–Smirnov test. Probability values <5% were considered statistically significant. The significance of the difference in the results between the two groups was determined by chi-square analysis using 2 × 2 contingency tables. A two-tailed *t* test was used to compare the values across groups in the presence of a normal distribution. Significant differences were assessed with the Mann–Whitney U test in the absence of a normal distribution. Pearson's correlation was used to assess the strength of association between parameters analyzed. Spearman correlation was used to make comparisons between the different parameters that were not normally distributed. All the statistical analyses were performed using the PASW Statistics GradPack 18 (SPSS Inc., Chicago, IL).

Results

Table 1 shows the demographic and basic characteristics of patients with CAIH [(euthyroid, 50 subjects) + (hypothyroid, 17 subjects)] and euthyroid patients with CAIH on levothroxine (euthyroid, LT4-CAIH group, 50 subjects) compared to the control group (75 subjects). There was no difference in the mean age between euthyroid, LT4-CAIH, and control groups. As expected, euthyroid patients with LT4-CAIH had increased TPOAb, TgAb and T4 levels.

The results of serum sFas and sFasL levels are shown as median and interquartile ranges in Figs. 1 and 2, respectively. Serum levels of sFas were significantly higher in euthyroid, LT4-CAIH [median 9.12 ng/ml, interquartile range (7.86–10.72 ng/ml)] than in the control group [6.11 ng/ml (5.60–6.81 ng/ml)]. Compared to the controls, the euthyroid, LT4-CAIH group had significantly higher levels of sFasL [125.71 pg/ml (106.11–149.48 pg/ml)]

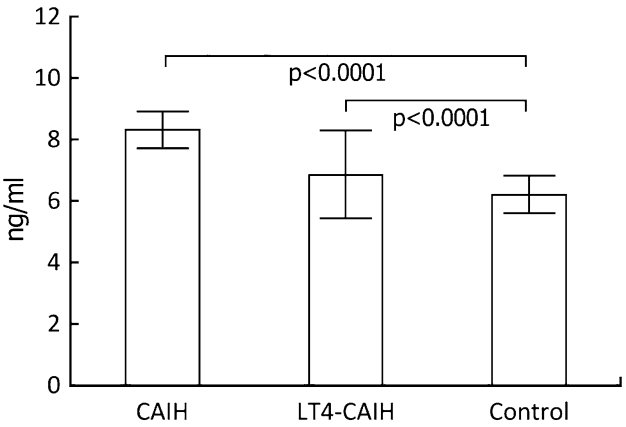

Fig. 1 The median and interquartile ranges of the levels of serum sFas in patients with chronic autoimmune hypothyroidism (CAIH, $n = 67$), euthyroid patients with chronic autoimmune hypothyroidism on levothroxine (LT4-CAIH group, $n = 50$) and age- and sex-matched controls ($n = 75$). CAIH group includes all the patients with chronic autoimmune hypothyroidism on levothyroxine [(euthyroid, 50 subjects) + (hypothyroid, 17 subjects)]

Table 1 The basic characteristics of patients with chronic autoimmune hypothyroidism (CAIH) and euthyroid patients with CIAH on levothyroxine (Euthyroid, LT4-CAIH group) compared to the control group

	CAIH[a] ($n = 67$)	Euthyroid, LT4-CAIH ($n = 50$)	Control ($n = 75$)
Age (years)	31.03 (1.51)[b]	32.36 (1.75)	28.86 (1.02)
Sex (female)	88.1%	90.0%	96.0%
Goiter[c]			
Grade 0	47.8%	46.0%	100.0%
Grade 1	22.4%	24.0%	0.0%
Grade 2	29.9%	30.0%	0.0%
T4 (μg/dl)	10.56 (0.35)	11.35 (0.31)*	10.00 (0.19)
TSH (mIU/l)	10.66 (2.90)[€]	1.71 (0.18)	1.81 (0.11)
TPOAb (IU/ml)	356.20 (32.46)*	314.69 (36.10)*	26.37 (10.11)
TgAb (IU/ml)	847.07 (117.61)*	856.68 (137.28)*	99.47 (9.08)

[a] This group includes all the patients with chronic autoimmune hypothyroidism on levothyroxine [(euthyroid, 50 subjects) + (hypothyroid, 17 subjects)]

[b] Values are means (standard errors)

[c] Grading of goiter was based according to the modified World Health Organization recommendation in 1994

[€] $P = 0.002$ in comparison to the control group

* $P < 0.0001$ in comparison to the control group

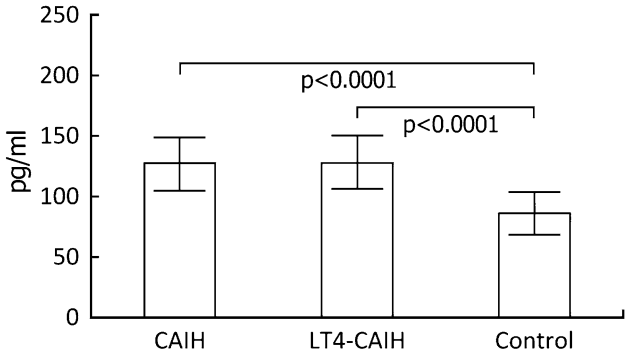

Fig. 2 The median and interquartile ranges of the levels of serum sFas Ligand in patients with chronic autoimmune hypothyroidism (CAIH, $n = 67$), euthyroid patients with chronic autoimmune hypothyroidism on levothroxine (LT4-CAIH group, $n = 50$) and age- and sex-matched controls ($n = 75$). CAIH group includes all the patients with chronic autoimmune hypothyroidism on levothyroxine [(euthyroid, 50 subjects) + (hypothyroid, 17 subjects)]

(Fig. 2). Significant differences for sFas and sFasL could be seen when all CAIH (euthyroid and hypothyroid subjects) were compared with controls (Figs. 1 and 2).

There were no significant differences in sFas or sFasL concentrations between euthyroid patients with CAIH and the total group of CAIH ($P > 0.05$).

There were significant positive correlations between TPOAb and sFas ($r = 0.45$, $P < 0.0001$), TgAb and sFas ($r = 0.41$, $P < 0.0001$), TPOAb and sFasL ($r = 0.49$, $P < 0.0001$), TgAb and sFasL ($r = 0.36$, $P < 0.0001$), sFas and sFasL ($r = 0.48$, $P < 0.0001$) in CAIH.

There was a positive correlation between age and sFas ($r = 0.29$, $P = 0.04$) in the euthyroid, LT4-CAIH group; however, there were no correlations between age, TgAb, TPOAb, sFas, and sFasL.

The median duration of treatment with levothyroxine was 36 months (range 6–228 months). In a chronological study, there was no significant correlation between sFas, sFasL, and the duration of levothyroxine therapy.

Discussion

In this study, we evaluated serum sFas, sFasL in euthyroid patients with CAIH on levothyroxine (euthyroid, LT4-CAIH group). We found that serum levels of sFas or sFasL levels were higher in the euthyroid, LT4-CAIH group than in the age- and sex-matched healthy controls.

The effects of treatment on sFas, sFasL, and apoptotic were investigated in Graves' disease (GD) [23, 24, 26, 27]. Compared with normal subjects, serum sFas was decreased in patients with GD in remission [23]. The levels of sFas/sFasL were lower in euthyroid GD than in untreated hyperthyroid GD patients [25]. A reduction in sFas levels was reported in GD when they became euthyroid after anti-thyroid therapy [24]. The concentration of sFas and sFasL increased to 50% after 131I-therapy in GD [26]. Serum sFas levels were introduced as an accurate index of the outcome of steroid pulse treatment in thyroid-associated ophthalmopathy [28].

So far, the effects of treatment on sFas and sFasL levels have not been studied in CAIH. In our study, sFas and sFasL levels were not normalized in euthyroid patients with CAIH on levothyroxine (euthyroid, LT4-CAIH group). Thus, we may conclude that levothyroxine therapy has not a role in down-regulation of apoptotic factor in patients with CAIH.

As a major limitation of our study, serum sFas and sFasl levels had not been measured in a pre-treatment state; therefore, a reduction of apoptotic factor is a possibility after levothyroxine treatment. However, we did not find a negative correlation between sFas or sFasL levels and duration of levothyroxine therapy. There was also no significant difference in sFas or sFasL levels between levothyroxine-treated and non-treated patients with CAIH. However, we acknowledge that the cross-sectional design of our study could not determine if there is a mechanistic direct relationship between the sFas/sFasL system and levothyroxine therapy. Thus, a longitudinal study would be far more informative.

When the results of Fas/FasL system in serum are analyzed, two limitations should be considered. First, we measured soluble forms of Fas and FasL in serum, and it is unclear to what extent this correlates to local these apoptotic factors production or action within the thyroid tissue. Second, Fas and FasL are not thyroid-specific and are produced by various nonthyroidal tissues [29].

Autoimmunity may be present many years before the diagnosis of autoimmune diseases. Thus, as a limitation, a negative history of autoimmunity in the control group may not be completely reliable to exclude an on-going asymptomatic autoimmune process.

Scant data are available in the medical literature on the serum levels of sFas and sFasL in hypothyroidism [23, 30, 31]. However, serum levels of sFas were investigated in patients with autoimmune thyroid diseases (AITD) [23, 30, 32]. It was suggested that serum sFas plays a role in the pathogenesis of GD [24]. Increased sFas was detected in hyperthyroidism independent of underlying thyroid diseases [33]. It was introduced as a useful marker for evaluation of immunological processes [34], inflammatory process activity [35], or useful index to gauge the status of convalescence [28] in Graves' ophthalmopathy. Recently, increased sFas in patients with multinodular goiter was reported [36].

Serum sFasL levels were also investigated in AITD as a candidate marker for evaluating disease aggression or regression in GD [25]. A high sFasL plasma concentration in patients with benign and malignant thyroid tumors was found [37].

Overall, we found higher circulating levels of sFas and sfasL in patients with CAIH than controls. There are a few contradictory studies [23, 30, 31] about soluble forms of Fas and FasL in apoptosis of thyroid cells. Shimaoka et al. [23] reported that sFas was decreased in euthyroid patients with HT, but was normal in hypothyroid patients with HT. No significant changes were reported in patients with subclinical HT or clinical HT, when compared with control subjects [30]. However, consistent with our findings, Myśliwiec et al. [31] showed levels of sFas were higher in patients with euthyroid and hypothyroid HT compared to the controls.

Like the study of Myśliwiec et al. [31], high levels of sFas in our study reflect the intensity of Fas and FasL-mediated apoptosis and enhanced release of Fas mRNA variant. Based on this hypothesis, we also found high levels of FasL in the CAIH group compared to the controls. However, in the only two existing reports regarding serum sFasL in HT, Myśliwiec et al. [31] found no significant differences in sFaL concentrations and Fountoulakis et al. [30] reported that the measurements of sFasL were below the sensitivity level of the assay in both controls and patients.

Another point of interest that emerged from our study is that sFas and sFasL levels were positively correlated with TPOAb and TgAb. Alternatively, this finding highlights close apoptotic pathways' immune interactions. These interactions need further research to examine the interrelationship between apoptotic pathways and immune system in thyroid diseases.

One of the major hallmarks of AITD is production of antibodies to thyroid peroxide and thyroglobin [38]. These serum thyroid antibodies concentrations do not decrease with levothyroxine therapy, even 24 months after initiation of treatment [39]. According to our findings, like autoantibodies, normalization of sFas and sFasL levels cannot be achieved during levothyroxine treatment in patients with CAIH.

Although the cross-sectional design of the current study obscures the real alteration of sFas and sFasL from pre-treatment with levothyroxine therapy, it appears that treatment with this drug cannot normalize sFas or sFasL concentrations and, like thyroid autoantibodies, monitoring of serum levels of sFas and sFasL is not indicated during thyroid hormone therapy.

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References

1. S. Nagata, P. Golstein, Science **267**, 1449–1456 (1995)
2. A. Strasser, P.J. Jost, S. Nagata, Immunity **30**, 180–192 (2009)
3. G. Stassi, A. Zeuner, D. Di Liberto et al., Clin. Immunol. **21**, 19–23 (2001)
4. M. Andrikoula, A. Tsatsoulis, Eur. J. Endocrinol. **144**, 561–568 (2001)
5. L.J. Hammond, F.F. Palazzo, M. Shattock et al., Thyroid **11**, 919–927 (2001)
6. L.J. Hammond, M.W. Lowdell, P.G. Cerrano et al., J. Pathol. **182**, 138–144 (1997)
7. J. Cheng, T. Zhou, C. Liu et al., Science **263**, 1759–1762 (1994)
8. T. Suda, H. Hashimoto, M. Tanaka et al., J. Exp. Med. **186**, 2045–2050 (1997)
9. N. Kayagaki, A. Kawasaki, T. Ebata, J. Exp. Med. **182**, 1777–1783 (1995)
10. P. Schneider, N. Holler, J.L. Bodmer et al., J. Exp. Med. **187**, 1205–1213 (1998)
11. S.H. Wang, J.R. Baker, Thyroid **17**, 975–979 (2007)
12. O.A. Sukocheva, D.O. Carpenter, J. Endocrinol. **191**, 447–458 (2006)
13. J.B. Laoag-Fernandez, H. Matsuo, H. Murakoshi et al., J. Clin. Endocrinol. Metab. **89**, 4069–4077 (2004)
14. M. Ghorbel, I. Seugnet, A.M. Ableitner et al., Neurosci Lett. **231**, 127–130 (1997)
15. F. Varga, E. Luegmayr, N. Fratzl-Zelman et al., J. Endocrinol. **160**, 57–65 (1999)
16. M. Hara, S. Suzuki, J. Mori et al., Thyroid **10**, 1023–1034 (2000)
17. N. Saelim, D. Holstein, E.S. Chocron et al., Apoptosis **12**, 1781–1794 (2007)
18. M. Pietrzak, M. Puzianowska-Kuznicka, J. Mol. Endocrinol. **41**, 177–186 (2008)
19. H.Y. Lin, H.Y. Tang, A. Shih et al., Steroids **72**, 180–187 (2007)
20. H.Y. Lin, H.Y. Tang, T. Keating et al., Carcinogen **29**, 62–69 (2008)
21. T.H. Brix, K.O. Kyvik, L. Hegedüs, J. Clin. Endocrinol. Metab. **85**, 536–539 (2000)
22. World Health Organization, United Nations Children's Fund, International Council for Control of Iodine Deficiency Disorders, *Assessment of the Iodine Deficiency Disorders and Monitoring their Elimination.* (World Health Organization, Geneva, 2001), WHO document WHO/NHD/01.1
23. Y. Shimaoka, Y. Hidaka, M. Okumura et al., Thyroid **8**, 43–47 (1998)
24. Y. Hiromatsu, T. Bednarczuk, E. Soyejima et al., Thyroid **9**, 341–345 (1999)
25. C.Y. Wang, W.B. Zhong, T.C. Chang et al., Metabolism **51**, 769–773 (2002)
26. F. Rogowski, A. Parfieńczyk, A. Sopotyk et al., Nucl. Med. Rev. Cent. East Eur. **7**, 117–122 (2004)
27. W. Zhao, B.L. Gao, M. Tian et al., J. Clin. Invest. Med. **32**, E158–E165 (2009)
28. K. Maeda, Y. Ohara, M. Hashimoto, Clin. Ophthalmol. **2**, 609–612 (2008)
29. L. Xerri, E. Devilard, J. Hassoun et al., Mol. Pathol. **50**, 87–91 (1997)
30. S. Fountoulakis, G. Vartholomatos, N. Kolaitis et al., Eur. J. Endocrinol. **158**, 853–859 (2008)
31. J. Myśliwiec, M. Okota, A. Nikołajuk, Adv. Med. Sci. **51**, 119–122 (2006)
32. J. Mysliwiec, M. Oklota, A. Nikolajuk et al., Immunol. Invest. **36**, 247–257 (2007)
33. J. Feldkamp, E. Pascher, M. Schott et al., Clin. Endocrinol. Metab. **86**, 4250–4253 (2001)
34. K. Ohtsuka, M. Hashimoto, Br. J. Ophthalmol. **84**, 103–106 (2000)
35. J. Myśliwiec, A. Kretowski, A. Stepień et al., Pol. Merkur. Lekarski. **17**, 368–370 (2004)
36. M. Andrikoula, N. Kolaitis, G. Vartholomatos et al., Immunol. Invest. **38**, 398–407 (2009)

37. K. Kolomecki, P. Maciaszczyk, H. Stepien et al., Bratisl. Lek. Listy. **106**, 297–300 (2005)
38. B. Rapoport, S.M. McLachlan, J. Clin. Invest. **108**, 1253–1259 (2001)
39. L. Hegedüs, J.M. Hansen, U. Feldt-Rasmussen, Clin. Endocrinol. **35**, 235–238 (1991)