

Effect of levothyroxine treatment on clinical symptoms and serum cytokine levels in euthyroid patients with chronic idiopathic urticaria and thyroid autoimmunity

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Summary

Background. Screening for thyroid autoimmunity in patients with chronic idiopathic urticaria (CIU) is generally recommended. However, there are not yet sufficient data as to whether levothyroxine treatment is beneficial for the clinical symptoms of CIU in patients with thyroid autoimmunity.

Aim. We investigated the effect of levothyroxine treatment on clinical symptoms and serum tumour necrosis factor (TNF)- α , interleukin (IL)-10 and interferon (IFN)- γ levels in euthyroid patients with CIU and thyroid autoimmunity.

Methods. In total, 15 patients with CIU and positive thyroid autoantibodies were randomized to receive either levothyroxine plus 5 mg/day desloratadine (suppression group, $n = 8$) or 5 mg/day desloratadine alone (control group, $n = 7$) for 12 weeks. Clinical symptoms of CIU, thyroid hormone levels, thyroid antibodies and serum cytokine levels were assessed at baseline and after the treatment.

Results. There were significant improvements in pruritus score and severity of weals in both groups compared with baseline values, but when the two groups were compared, there was no significant difference in the patients' clinical symptoms. Thyroid antibody titres were not different according to intragroup and intergroup analysis. In the suppression group, serum IFN- γ and TNF- α levels were increased after treatment with levothyroxine compared with baseline values and there was a borderline statistical significance ($P = 0.05$ for both).

Conclusions. These results suggest that levothyroxine treatment is not a reasonable option in euthyroid patients with CIU and thyroid autoimmunity. Augmentation of cytokine production after levothyroxine treatment seems to be related to the immunomodulatory effects of TSH-suppressive treatment.

Introduction

Chronic urticaria is defined as a history of ≥ 6 -weeks of widespread, transient, itchy cutaneous swellings, with individual lesions lasting < 24 h.¹ Chronic urticaria is idiopathic (CIU) in 60% and autoimmune in 40% of patients.² Thyroid autoimmunity has been

reported to be associated with both, although it is more prevalent in the autoimmune group.^{3–5} Screening for thyroid autoimmunity and thyroid function in patients with CIU is recommended.⁶ It has been reported in some open-labelled studies that clinical remission was obtained with levothyroxine treatment.^{7–9} However, the study populations in those studies consisted of both euthyroid and hypothyroid patients, and there was no control or placebo group,^{8,9} or the number of patients was very small.⁷ It is unclear if levothyroxine treatment is beneficial, especially in euthyroid patients with CIU and thyroid autoimmunity.

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There is an inflammatory process in patients with CIU characterized by increased tumour necrosis factor (TNF)- α , interleukin (IL)-10 and chemokine production.¹⁰ Modulation of the immune response by thyroid hormones has been well documented. On the other hand, several cytokines are able to modulate the secretion of hormones, such as thyroid-stimulating hormone (TSH), by the hypothalamus and pituitary.¹¹ These considerations have led to the hypothesis that levothyroxine treatment might possibly affect the expression and secretion of cytokines that play a role in pathogenesis of CIU associated with thyroid autoimmunity.

In this study, we investigated the clinical response to levothyroxine treatment in euthyroid patients with CIU and thyroid autoimmunity, and compared their results with those of controls. We also analysed serum TNF- α , IL-10 and interferon (IFN)- γ levels before and after treatment with levothyroxine.

Methods

Informed consent was obtained from all participants and the study was performed in accordance with the Declaration of Helsinki and with the approval of the local ethics committee.

Patients and study design

This study was planned as a prospective open-labelled randomized trial. CIU was defined as widespread short-lived (< 24 h) weals occurring daily or almost daily for at least 6 weeks, where a predominant physical cause had been excluded. Patients were recruited from the dermatology outpatient clinic of a tertiary referral centre. The inclusion criteria were age 18–55 years, euthyroid status, presence of thyroid antibodies and use of antihistamine treatment for at least 1 month without clinical response. Exclusion criteria were use of any medication for CIU (except antihistamines) or of immunomodulatory drugs; presence of cardiovascular, gastrointestinal, hepatic, renal, rheumatological, neoplastic, infectious, immunological or bone diseases (such as osteoporosis); and pregnancy.

In total, 15 patients with CIU (14 women, 1 man; mean $\pm$ SD age 37.9 ± 14.7 ; mean duration of CIU 21.2 ± 25.5 months) were enrolled in the study. There was no significant difference between the two groups with respect to age, gender and laboratory parameters at baseline.

After inclusion, patients were randomized to the suppression (eight women) or control (six women, one

man) group after a 2-week washout period. All patients were treated with H1 receptor-blocking medication (desloratadine 5 mg/day). Patients in the suppression group were treated with increasing doses of levothyroxine until suppression of TSH level < 0.5 μ IU/mL. After reaching the target TSH level, levothyroxine treatment was given at the same dosage during the study period. The control group was followed for 12 weeks, although patients in the suppression group were followed for 12 weeks after reaching the target TSH level. At the beginning and end of the study, blood samples were taken from all patients in the morning, after fasting for 12 h. TSH, free thyroxine (FT4), free triiodothyronine (FT3), antithyroglobulin (anti-TG) and antithyroperoxidase (anti-TPO) antibodies, C4, total eosinophil count and erythrocyte sedimentation rate (ESR) were measured. IFN- γ , TNF- α and IL-10 levels were analysed from blood cultures.

Improvement in CIU symptoms was assessed clinically by the Urticaria Activity Score (UAS). Patient self-evaluation of each symptom was recorded by rating the severity of weals and daily and nocturnal pruritus on a 4-point scale (0 = absent, 1 = mild, 2 = moderate, 3 = severe). Patients in the suppression group were asked to record the severity of symptoms after their TSH levels reached < 0.5 μ IU/mL. The severity of weals was evaluated according to their number (0 = absent, 1 = 1–6, 2 = 7–12, 3 $\geq$ 12) and size (0 = absent, 1 $\leq$ 1.25 cm, 2 = 1.25–2.50 cm, 3 $\geq$ 2.50 cm). The scores for size and number of weals were calculated and the median was taken for statistical analysis.

Measurements

Serum FT3, FT4, TSH, anti-TPO and anti-TG antibody levels were measured by automated luminescent immunoassays (Abbott Laboratories, Abbott Park, IL, USA). The mean coefficient of variation was < 10% for all these assays. The normal ranges for thyroid function tests were: 1.71–3.71 pg/mL for FT3, 0.7–1.48 ng/dL for FT4, 0.35–4.94 mIU/mL for TSH, 0–5.6 IU/mL for anti-TPO antibodies and 0–4.11 IU/mL for anti-TG antibodies.

For measuring cytokine production, whole blood was collected into vacuum tubes (Vacutainer; Becton Dickinson, Franklin Lakes, NJ, USA) containing ethylene diamine tetraacetic acid (EDTA). Peripheral blood was diluted as 1 : 1 with complete RPMI 1640 medium (Biochrom Ag, Berlin, Germany) supplemented with 1 mmol/L sodium pyruvate, 1% minimum essential medium, nonessential amino acids and vitamins, 2 mmol/L L-glutamine, 100 U/mL penicillin, 100 μ g/mL

streptomycin and 50 µmol/L 2-mercaptoethanol (all from Gibco BRL, Basel, Switzerland) and 10% heat-inactivated fetal calf serum. Peripheral blood was either unstimulated or stimulated with phytohaemagglutinin (L-9017; Sigma, Ronkonkoma, NY, USA) (3 µg/mL) in a 48-well plate and incubated at 37 °C in a humidified 5% CO₂ incubator. Supernatants were harvested on day 2 and kept frozen (–20 °C) until use for cytokine detection. Production of IFN-γ, IL-10 and TNF-α was evaluated with commercially available ELISA kits (Bio-source, Camarillo, CA, USA) according to the manufacturers' instructions with sensitivities of 4 pg/mL, 5 pg/mL and 4 pg/mL, respectively.

Statistical analysis

The χ^2 test was used for clinical data variables. Comparisons between groups were performed using the Mann–Whitney test, the Wilcoxon signed-rank test was used for within-group comparisons, and correlation analysis was performed using the Pearson coefficient test. The data were analysed using SPSS for Windows (version 11.5; SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered significant. Data are expressed as mean $\pm$ SD.

Results

All patients completed the study. There was no significant change in serum levels of FT3, FT4, anti-TG antibodies, anti-TPO antibodies, C4, total eosinophil count and ESR according to the intragroup and intergroup analysis at the end of the study (Table 1). Serum TSH level was suppressed in the suppression group compared with the control group at the end of the study ($P = 0.03$). The mean levothyroxine dosage was 137 ± 40 µg/day (range: 100–225 µg/day) in the

suppression group, and TSH suppression was achieved in 35.7 ± 13.1 days (range: 22–62 days) after the beginning of the treatment. We did not observe any adverse effects of levothyroxine treatment during the study period. After treatment with levothyroxine, serum IFN-γ and TNF-α levels were increased in the suppression group and there was borderline significance in both parameters compared with baseline values ($P = 0.05$ and $P = 0.05$, respectively). However, when the two groups were compared, percentage changes in serum IFN-γ and TNF-α levels were not different ($P = 0.54$ and $P = 0.96$, respectively). There was also no meaningful change in serum IL-10 levels in the intragroup and intergroup analysis (Table 1).

Weekly mean UAS of groups are shown in Fig. 1. There were significant improvements in pruritus score and severity of weals in both groups compared with baseline values, but when the two groups were compared, there was no significant difference in the patients' clinical symptoms (Table 1).

Discussion

In this study, we did not find any significant improvement in clinical symptoms of euthyroid patients treated with levothyroxine plus desloratadine compared with patients treated with desloratadine alone.

In line with our results, Kandeel *et al.*¹² also found that most patients continued to have relapses of urticaria with no change in the course of their disease after treatment with thyroid hormone, regardless of presence of hypothyroidism. However, in some studies, it was reported that exogenous thyroid hormone treatment led to significant improvement in patients with CIU.^{7–9} Aversano *et al.*⁹ treated 16 patients with levothyroxine and found that symptoms of CIU decreased

Table 1 Thyroid hormone, antibody and cytokine levels and clinical parameters of patients at baseline and 12 weeks.

	Suppression group			Control group			
	Baseline	Week 12	<i>P</i>	Baseline	Week 12	<i>P</i>	<i>P</i> *
FT3, pg/mL	3.21 $\pm$ 0.50	3.24 $\pm$ 0.47	0.78	3.19 $\pm$ 0.26	2.81 $\pm$ 0.42	0.09	0.15
FT4, ng/dL	1.05 $\pm$ 0.10	1.27 $\pm$ 0.27	0.07	1.12 $\pm$ 0.22	1.00 $\pm$ 0.10	0.08	0.09
TSH, µIU/mL	1.73 $\pm$ 0.95	0.52 $\pm$ 0.73	0.03	2.14 $\pm$ 0.81	1.66 $\pm$ 0.46	0.18	0.03
Anti-TPO, IU/mL	313.3 $\pm$ 687.6	288.9 $\pm$ 399.9	0.61	101.1 $\pm$ 136.8	221.8 $\pm$ 274.2	0.12	0.15
Anti-TG, IU/mL	592.3 $\pm$ 1204.3	626.8 $\pm$ 1187.3	0.21	130.2 $\pm$ 224.8	264.1 $\pm$ 671.7	0.50	0.54
IFN-γ, pg/mL	139.4 $\pm$ 294.9	880.9 $\pm$ 1173.7	0.05	541.0 $\pm$ 671.3	896.4 $\pm$ 585.5	0.40	0.54
IL-10, pg/mL	1.0 $\pm$ 0.00	8.3 $\pm$ 14.3	0.10	4.8 $\pm$ 7.6	14.6 $\pm$ 16.0	0.24	0.70
TNF-α, pg/mL	7.5 $\pm$ 10.9	176.3 $\pm$ 268.5	0.05	138.7 $\pm$ 258.3	188.5 $\pm$ 168.3	0.40	0.96
Pruritus score	1.7 $\pm$ 0.4	0.5 $\pm$ 0.3	0.01	1.5 $\pm$ 0.9	0.5 $\pm$ 0.5	0.02	0.78
Weals score	1.6 $\pm$ 0.5	0.7 $\pm$ 0.6	0.01	1.4 $\pm$ 0.9	0.6 $\pm$ 0.8	0.03	0.46

FT4, free thyroxine; FT3, free triiodothyronine; IFN, interferon; IL, interleukin; TNF, tumour necrosis factor; TG, thyroglobulin antibodies; TPO, thyroperoxidase; TSH, thyroid-stimulating hormone. *Percentage change of parameters in the suppression group vs. control group.

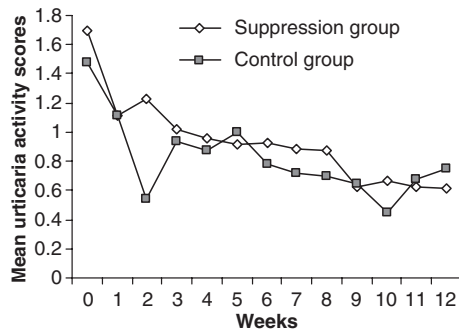


Figure 1 Mean urticaria activity scores of patients in the suppression and control groups during the study period.

significantly with levothyroxine treatment in 80% of patients. Both euthyroid and hypothyroid patients were included in that study; clinical improvement was evident in all hypothyroid patients but it was uncertain in euthyroid patients. In the study of Gaig *et al.*,⁸ 18 patients were treated with levothyroxine if hypothyroidism or normal thyroid functions were present, and levothyroxine treatment was successful in 15 of those patients. In our study, all of the patients treated with desloratadine with or without levothyroxine were euthyroid. Moreover, there was no control or placebo group in these previously mentioned studies,^{7–9} thus a placebo effect of levothyroxine treatment could not be excluded. In the present study, compared with baseline values, an improvement was seen in clinical symptoms of patients treated with levothyroxine plus desloratadine treatment. However, when compared with controls, there was no significant difference between the two groups, suggesting that the improvement seen in CIU with levothyroxine treatment might be a placebo effect or the effect of antihistamine treatment.

We are only beginning to understand the immunological basis of chronic urticaria, but there is an extensive literature base describing an autoimmune basis in a significantly large population.^{1,2,4,13} The most direct evidence of an autoimmune process in some patients with CIU is the demonstration of functional IgG autoantibodies to the alpha subunit of the high-affinity IgE receptor or to IgE itself.¹³ O'Donnell *et al.*¹⁴ reported that patients with positive anti-TPO antibodies were more likely to have histamine-releasing autoantibodies as predicted by positive autologous intradermal serum skin testing and positive release *in vitro* of histamine from donor basophilic leucocytes. It is possible that cellular immunity initiated in the thyroid gland could trigger the development of urticarial lesions via cytokines released by T lymphocytes.

The cytokine profile of patients with chronic urticaria has also been examined.¹⁵ TNF- α was previously shown to be augmented in serum and biopsy specimens of patients with CIU.^{16,17} It was also found that TNF- α , IL-10, IL-1 β and IL-6 production was augmented, whereas IFN- γ was reduced in patients with CIU compared with controls.^{10,18} In the present study, after suppressive thyroxine treatment, TNF- α and IFN- γ levels were increased in the suppression group compared with baseline values, and there was borderline significance ($P = 0.05$). TSH suppression is able to produce considerable changes in the immune response especially in humoral immunity.^{19,20} Botella-Carretero *et al.*²¹ found that patients receiving TSH-suppressive doses of thyroxine presented with increased levels of serum IL-2 receptor and IL-18. The percentage of natural killer (NK) cells in peripheral blood was also higher in these patients. The authors concluded that thyroxine treatment modulates cell-mediated immune response via increasing serum IL-18 levels and numbers of NK cells. In line with our results, Papanas *et al.*²² found that thyroxine dosage was positively correlated with serum TNF- α level in euthyroid patients with total thyroidectomy. TNF- α , which is a chemotactic agent for several cell types, stimulates cell-mediated immune responses and interacts with IFN- γ in a synergistic fashion.²³ Alterations in the serum concentration of TNF- α and IFN- γ in the suppression group seem to be related to the stimulant effect of levothyroxine treatment on cell-mediated immune response, independent from the course of urticaria.

In conclusion, levothyroxine treatment did not result in any significant improvement in clinical symptoms of patients with CIU and thyroid autoimmunity. Augmentation in cytokine production after levothyroxine treatment seems to be related to the immunomodulatory effect of TSH-suppressive levothyroxine treatment.

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