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Evaluation of Thyroid Function and Anti-thyroid Autoantibodies in Systemic Sclerosis

ILDIKÓ MOLNÁR[1], CSABA BALÁZS[1], ERZSÉBET SZABÓ[1] and LÁSZLÓ CZIRJÁK[2]

[1]*Kenézy Gyula County Hospital, Debrecen and* [2]*University Medical School of Debrecen, Hungary*

Parameters of thyroid metabolism, and the presence of anti-thyroid antibodies were investigated in 43 patients with systemic sclerosis. Anti-thyroid antibodies were detected in 14 cases. Elevated levels of anti-thyroglobulin antibodies were determined in 4 cases, anti-thyroid peroxidase (TPO) antibodies in 11, and anti-microsomal antibodies in 5. The detection of anti-TPO antibodies gave the most remarkable information about the presence of autoimmune thyroiditis. The patients with anti-TPO and/or reduced T_3 concentration tended to have secondary Sjögren's syndrome. Our results provide further evidence that anti-thyroid antibodies might be responsible for the remarkable appearance of autoimmune thyroiditis in systemic sclerosis. ***Key words: Anti-thyroid peroxidase antibody; Thyroid hormones.***

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L. Czirják, 3rd Department of Medicine, Univ. Med. School of Debrecen, Debrecen, H-4004 Hungary.

Progressive systemic sclerosis (PSS) is characterized by fibrotic, degenerative and inflammatory changes involving the skin and several internal organs (1–3). Previous studies have suggested that both the altered cellular immunity and the production of certain autoantibodies are involved in some of the pathologic processes in PSS. Antinuclear antibodies have been demonstrated in nearly 100% of patients' sera (1–3). Using the indirect immunofluorescence method, we have recently shown that anti-centromere antibody, which is characteristic of the CREST variant of PSS, is relatively rare among Hungarian PSS patients, whereas other autoantibody specificities, including anti-granulocyte, anti-platelet antibodies have already been shown in a relatively large proportion of cases (4).

Symptoms resembling those of the hypothyroid state were previously more frequently associated with PSS, causing problems in the differential diagnosis.

In the present study, anti-thyroglobulin (anti-Tg), anti-thyroid microsomal (anti-M), and anti-thyroid peroxidase (anti-TPO) antibodies were investigated in the sera of PSS patients. These antibodies have not previously been investigated in this disease. The hormone production by the thyroid gland was also evaluated simultaneously.

MATERIALS AND METHODS

Patients

Forty-three patients (2 men and 41 women) with progressive systemic sclerosis (PSS) were investigated according to a standard protocol (2). The mean age of the patients was 49 ± 11.6 years. Mean disease duration was 10.3 ± 7.6 years (Table I). Twelve of the patients showed symptoms of diffuse cutaneous systemic sclerosis (3) (Table I). Only one patient had an enlarged mass of thyroid gland. None of our cases exhibited signs of manifest clinical thyroid disease.

Twenty-two healthy volunteer controls were also investigated. Their mean age was 45 ± 9 years (from 31 to 62 years).

Methods

Preparation of human thyroglobulin (Tg), thyroid microsomal and peroxidase (TPO) fractions. Human Tg was purified from postmicrosomal saline extracts of toxic goitres by ammonium sulphate fractionation and sepharose 6B chromatography *ad modum* Hamada et al. (5). Human thyroid microsomal fraction was obtained from the 70,000 *g* pellet of thyroid homogenate after solubilization with deoxycholate in the 100,000 *g* supernatant fraction. Thyroid peroxidase was obtained from porcine thyroid tissue by Taurog et al., using solubilization with deoxycholote, digestion with trypsin and ionchanger chromatography (6).

Indirect enzyme-linked immunosorbent assay (ELISA). Anti-Tg, anti-M and anti-TPO antibodies were measured by ELISA technique. Indirect ELISA was performed by a modification of Voller's method, using antigen-coated Enzy-plate (Propylen GM, Pécs, Hungary) and 96-well plates (4, 7). The plates were coated with 100 µl of Tg, thyroid microsomal and TPO fractions (10–10 µg/ml) by overnight incubation at 4°C. 100 µl sera (diluted 1: 100) was added to the plates for 2 h at room temperature. Goat antihuman IgG antibodies conjugated with horseradish peroxidase (Human Institute, Budapest, Hungary) were chosen for detection of antibody binding (dilution 1: 1000). O-phenyldiamine was used as substrate. The results were given as an index consisting of the ratio of O.D. (optical density) triplicates of patients' samples to the mean O.D. of control samples. The contents of bound antibodies were measured in a semi-automatic ELISA reader at 492

Table I. *Clinical profile of patients with systemic sclerosis*

Total number of cases	43
Age (mean yr)	49 ± 11.6
Onset of disease (mean, yr)	38.4 ± 11.2
Females	41
Number of patients with "Proximal" scleroderma	31
Diffuse scleroderma	12
Esophageal dysfunction	22
Secondary Sjögren's syndrome	15
Polymyositis	8
Pulmonary manifestation	35
Subcutaneous calcinosis	3
Teleangiectasia	26
Skin involvement on trunk	17
Severe hand deformity	17
Exposure to chemicals (7)	12
Cardiac manifestation	10
Positive ANA test (on HEp-2)	40
Anticentromere antibody	4
Nucleolar + diffuse granular staining	18
Antimitochondrial antibody	6

Table II. *The levels of thyroid hormones, T3-uptake and TSH receptor antibodies in the 43 patients with PSS*

	Nos. of patients with levels		
	Decreased	Normal	Increased
T_3-uptake	2	37	4
Levels of T3 (nmol/l)	20	23	0
Levels of T4 (nmol/l)	0	37	6
FT4 index	0	41	2
Levels of TSH (mU/l)	0	35	8

Normal serum levels: T3-uptake: 0.8–1.15; T3 (nmol/l): 1.2–3.0; T4 (nmol/l): 52–154; FT4 index: 50–160; TSH (mU/l): 0.6–3.8.

nm. The background level was subtracted. When the O. D. of the given sample exceeded the simultaneously measured control value + 2 SD, the result was regarded as constituting positive antibody.

Determination of the levels of thyroid hormone and TSH receptor antibodies. The concentrations of thyroid hormones were determined with radio-immunoassays (T3-RIA (normal 1.2–3.0 nmol/l), T4-RIA (normal 52–154 nmol/l) (MTA Isotope Institute, Budapest)). The investigations of the levels of TSH hormone and TSH receptor antibodies were performed with Byk Mallinckrodt (Steinberg, Germany) (normal 0.6–3.8 mU/l) and TRAK (normal < 14 U/l) (Henning, Berlin, Germany) kits respectively. T3-uptake (normal 0.8–1.15) was measured by a test developed in our laboratory.

Statistical analysis

Linear regression analysis was performed for the evaluation of ELISA index values and TSH hormone levels.

RESULTS

Reduced T3 concentrations (mean value 0.95 ± 0.24 nmol/l, range 0.05–1.19 nmol/l) were found in 20 patients. An elevated TSH level (mean value 6.78 ± 2.56 mU/l, range 3.89–10.09 mU/l) was demonstrated in 8 cases. A polyclonal increase in IgG concentration was associated with an increased TSH level in 5 patients. Six patients with PSS exhibited an increased T4 concentration without any sign of hyperthyroidism (Table II). Ten out of the 15 patients with secondary Sjögren's syndrome showed a reduced T3 concentration (Table III). Anti-thyroid antibodies were detected in 14 cases. Anti-Tg was demonstrated in 4 cases, anti-M in 5 cases and anti-TPO in 11 of our sera (Fig. 1).

Three patients with increased TSH showed anti-Tg, anti-M, or anti-TPO. In those 8 patients who had an elevated THS level, a correlation was found between the presence of anti-Tg, anti-M, and anti-TPO (linear regression analysis *r*-values were 0.6032, 0.6059 and 0.7289, respectively). Of the 8 patients with an increased TSH concentration, a total of 7 cases of pulmonary fibrosis and 5 of hypo-hyperpigmentation were detected (data not shown). No correlation was found between the normal TSH hormone values and antibody findings.

Two patients were found with anti-M antibody positivity but with no anti-TPO present. The linear regression analysis *r*-values among the anti-thyroid antibodies were as follows: anti-M/anti-TPO: $r = 0.402$; anti-Tg/anti-TPO: $r = 0.4642$, and anti-Tg/anti-M: $r = 0.415$.

Patients with anti-TPO tended to belong to the category with symptoms of secondary Sjögren's syndrome. Either their mean age or their age at the onset of disease tended to be older (Table IV).

No positive case was found in the TRAK assay.

DISCUSSION

The association between PSS and hypothyroidism is well documented (8). D'Angelo et al. reported thyroid gland fibrosis in 24% of autopsied cases (9), while another study reported histologic evidence of severe thyroid fibrosis in 14% (10). Thyroid hormone studies showed a mild associated myxedema state in PSS (11). It has recently been shown that anti-Tg and anti-M antibodies are present in 15% of euthyroid scleroderma patients, while 50% of patients with chemical hypothyroidism exhibit anti-thyroid autoantibodies (12). Anti-TPO antibodies have not previously been investigated in PSS.

In our study, elevated levels of anti-thyroid antibodies were found in 14 patients, predominantly anti-TPO antibodies (in 11 cases). Anti-M antibodies are commonly found in autoimmune thyroiditis and their presence correlates closely with lymphocyte infiltration of thyroid gland (13). Despite intensive investigations, the nature of M-antigen remained unknown for almost three decades. Recently, immunoprecipitation and immunoblotting studies have revealed that the thyroid M antigen is a poorly glycosylated protein of 100–107 kD (14). It was found that this protein is in fact TPO (15). Ludgate et al. (16) characterized two epitope domains of TPO, named C2 and C21 (17), which were detected in an ELISA by 65% and 52% of Hashimoto's sera. These data show the heterogeneity of anti-M/anti-TPO antibodies. This heterogeneity of anti-TPO could partly explain our observation of a

Table III. *Comparison of patients with reduced and normal T3 levels*

	The levels of T_3 were	
	Normal	Reduced T3
Number of cases	23	20
Diffuse SSc	7	5
Sjögren's syndrome	5	10
Polyclonal increase in IgG	0	3

Table IV. *Presence of thyroid peroxidase antibody in the patients with PSS.*

	Patients with anti-thyroid peroxidase antibody	
	Positive	Negative
Number of cases	11	32
Diffuse SSc	3	9
Sjögren's syndrome	6	9
Cryoglobulinemia	4	13
Mean age	53.6 ± 10.2	47.5 ± 11.6
Age at onset	42.7 ± 9.1	36.8 ± 11.5

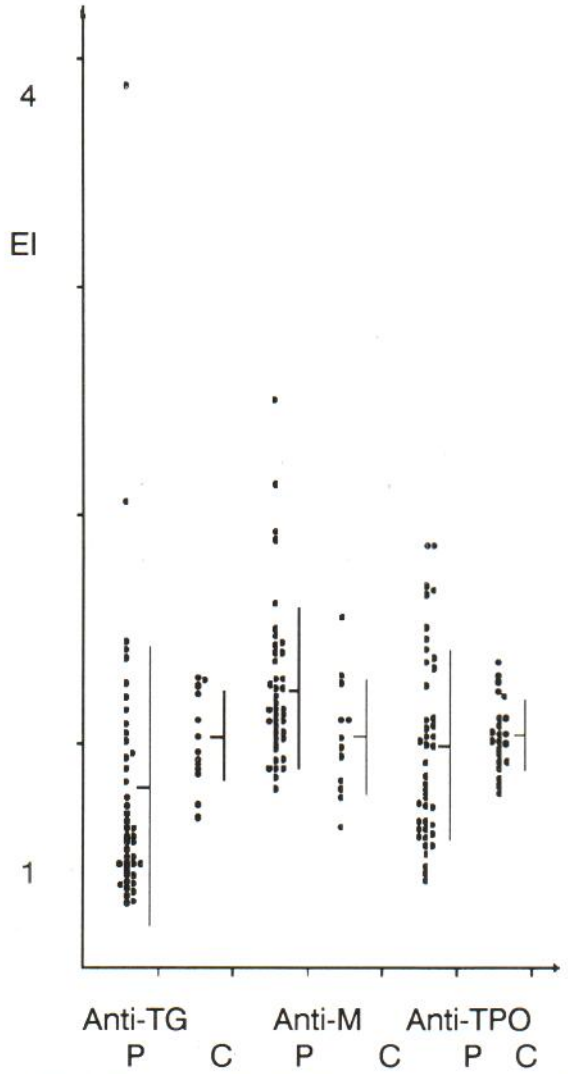

Fig. 1. Anti-thyroglobulin, anti-microsomal and anti-thyroid peroxidase antibodies in 43 patients with systemic sclerosis. Abbreviations used:. P: patients ($n = 43$), C: controls. The ELISA index (EI) was calculated: Anti-thyroglobulin antibodies: PSS: 0.79 ± 0.61, controls group (12): 1 ± 0.2. Anti-microsomal antibodies: PSS: 1.21 ± 0.35, controls (12): 1 ± 0.25. Anti-thyroid peroxidase antibodies: PSS: 0.95 ± 0.41, controls (22) 1 ± 0.15.

higher incidence of autoantibodies to TPO than those to M antigen. Another possibility is that after the purification procedure, TPO should give a high concentration of various epitopes (C2 and C21) of TPO, which would give the ELISA technique an enhanced detection limit. Admittedly M antigen fraction might have been contaminated with Tg of low concentration, which could also have been responsible for this observation.

Anti-thyroid antibodies can occur in association with connective tissue disorders such as SLE (systemic lupus erythematosus), rheumatoid arthritis, and Sjögren's syndrome (17).

The precise mechanism of anti-TPO action is obscure. It seems likely that inhibition of the biosynthesis of thyroid hormones is of importance (18). It is noteworthy that our patients with anti-TPO and/or reduced concentrations of T_3 tended to belong to the category with symptoms of secondary Sjögren's syndrome. The increased concentration of anti-TPO suggests that common determinant(s) in different organs may be involved in the production of anti-TPO in PSS.

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