NOTE

Significance of Thyroid Stimulating Antibody and Long Term Follow Up in Patients with Euthyroid Graves' Disease

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Abstract. We examined 13 patients with euthyroid Graves' disease suspected ophthalmologically, by comparing them with 20 patients with untreated Graves' disease and by following them up for 5 to 10 years. They had Graves' ophthalmopathy (NOSPECS class II~IV) without other ocular diseases, normal levels of serum thyroid hormones, and no previous history of Graves' disease. Proptosis in euthyroid Graves' disease was not significantly different from that in untreated Graves' disease. In 3 patients with euthyroid Graves' disease, TSH was suppressed. There was either no TSH response to TRH or it was low in 7 of 12 patients examined. The result of a T₃-suppression test was abnormal in 8 of 11 patients examined. Titers of serum TGHA, MCHA, TSH-binding inhibitory immunoglobulin (TBII), and thyroid stimulating antibody (TSAb) were significantly lower in patients with euthyroid Graves' disease compared than in patients with untreated Graves' disease. TSAb, however, was positive in 12 of 13 (92%) patients. In spite of positive TSAb, 9 of 13 patients with euthyroid Graves' disease had normal radioactive iodine uptake (RAIU). During the observation period, various abnormalities in thyroid function developed: persistent hyperthyroidism in 5 patients (38%), transient thyrotoxicosis in 2 (15%) and transient hypothyroidism in 1 (8%). We conclude that euthyroid Graves' disease is a subtype of Graves' disease that minimally develops thyrotoxicosis in spite of the existence of TSAb due to some mechanism inhibiting thyroid growth or stimulation, and that the measurement of TSAb provides a useful marker for the diagnosis of this disease.

Key words: Euthyroid Graves' disease, TSAb, Long term follow up

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ENDOCRINE or Graves' ophthalmopathy is a combination of symptoms and signs related to inflammation and vascular congestion in orbita and periorbita, which include various degrees of proptosis, lid retraction, extraocular muscle dysfunction and optic neuropathy [1]. It sometimes occurs without hyperthyroidism, mentioned as euthyroid Graves' disease.

Euthyroid Graves' disease or Graves' ophthalm-

opathy was first described as "an uncommon ophthalmic form of Graves' disease without goiter and hyperthyroidism" by Rundle and Wilson [2] in 1945. Its pathogenesis is not fully understood, but cellular and humoral immune response to orbital tissues have been described [3–11]. Werner [12] reported T₃-nonsuppressibility in patients with euthyroid Graves' disease and suggested its relation to thyroid function disorders. Furthermore, Salvi [13] and Weetman [14] reported that patients with euthyroid Graves' disease show certain abnormalities in the thyroid. Recently, auto-antibodies to several eye muscle membrane antigens were reported [15–18]. Antibody reactive with a 64 kDa eye muscle membrane antigen was detected in 71%

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of the patients with euthyroid Graves' disease [19].

On the other hand, Solomon *et al.* [20] proposed that ophthalmopathy was a single, "isolated" autoimmune disease in the study of 5 patients with euthyroid Graves' disease who showed T3-suppressibility, but neither antithyroid auto-antibodies nor LATSP activity, which indicated that there were two subgroups in Graves' disease: with and without ophthalmopathy. But our examination revealed that proptosis in Graves' disease showed a single normal distribution with a higher mean value than that in Hashimoto's disease and normal controls [21]. Furthermore, Bistriceanu *et al.* [22] reported that ultrasound exploration and CT scan of the orbital area, as well as the intraocular pressure values showed that occult changes existed in all patients with Graves' disease.

Pathogenesis of euthyroid Graves' disease is therefore thought to be related to a thyroid-associated auto-immune mechanism, but, it is very difficult to diagnose euthyroid Graves' disease, because there is no useful serological marker of Graves' ophthalmopathy, except for the antibody to the 64 kDa eye muscle membrane antigen. In this study, we endocrinologically examined 13 patients with ophthalmologically suspected euthyroid Graves' disease and compared the condition with untreated Graves' disease in order to classify the diagnosis and pathogenesis of euthyroid Graves' ophthalmopathy.

Patients and Methods

Thirteen patients with euthyroid Graves' disease suspected ophthalmologically and 20 age and sex matched patients with untreated Graves' disease who visited our clinic, were examined. The criterion of euthyroid Graves' disease used in this study were based on the following findings: 1) Graves' ophthalmopathy without other known ocular disease diagnosed by ophthalmologists, 2) without a history of hyperthyroidism and 3) clinically euthyroid with normal levels of serum thyroid hormones. They first visited ophthalmologists and were then referred to our thyroid outpatient clinic. Ophthalmometric measurement was made with a single, accurately calibrated standard Hertel's ophthalmometer, and goiter was palpated by the same physician (N.A.) to avoid variations in the measurement. Orbital computerized tomography (CT)

was performed to examine extraocular muscle involvement. They had Graves' ophthalmopathy (NOSPECS class II~IV).

The serum level of FT₄ and FT₃ was measured with radioimmunoassay (RIA) kits, Free T₄ kit EIKEN, Eiken Immunochemical Laboratory, Tokyo, Japan and Amerlex-M Free T₃, Amersham Inc, UK, respectively. The serum level of TSH was measured with an immunoradiometric assay kit (SPAC-S TSH kit, Daiichi Radioisotope Ltd, Tokyo, Japan). TSH response to TRH was examined on 12 patients by intravenous administration of 500 µg TRH. T₃ suppression was tested on 11 patients, in which 50% suppression in 24-h thyroid radioiodine (131-I) uptake (RAIU) after the oral administration of 25 µg of T₃ every 8 h for 8 days is considered as normal suppressibility.

Anti-thyroid thyroglobulin antibody and anti-thyroid microsomal antibody were measured by hemagglutination assay (TGHA and MCHA, respectively) [23]. TBII was measured with a commercial kit (Baxter Travenol Co., Japan) which was originally developed by Shewring and Rees Smith [24]. TSAb activity was measured by the ability to induce cAMP release from rat thyroid cell strain FRTL-5 as previously described [25]. TSAb activity was expressed as follows:

$$\frac{\text{cAMP production by IgG from test serum}}{\text{cAMP production by IgG from normal pooled serum}} \times 100\%$$

Statistical methods

Student's *t* test and chi square test were used for the statistical analysis of the data.

Results

A familial history of thyroid diseases was seen in 4 of 13 patients (31%) with euthyroid Graves' disease and 7 of 20 patients (35%) with untreated Graves' disease. The difference was not significant. Eye signs in euthyroid Graves' disease and clinical data are shown in Table 1. Their ophthalmopathy is classified as NO SPECS classes II~IV. Nine patients consulted first for proptosis, 2 for diplopia, 1 for lid edema and 1 for lid retraction. Eye ball movement was disturbed in 8 patients at the upper lateral view. Orbital CT-scan revealed

Table 1. Clinical data of 13 patients with euthyroid Grave's disease

Case No.	Age	Sex	Chief complaint	Proptosis (mm)		Eyeball movement	Extraocular muscle enlargement (CT scan)
				r	l		
1	68	F	proptosis	23	22	normal	+ ^a
2	39	F	proptosis	20	19	abnormal	+
3	25	M	proptosis	17	19	abnormal	— ^b
4	40	F	proptosis	20	18	normal	—
5	49	M	lid edema	16	15	abnormal	+
6	31	F	double vision	19	21	abnormal	+
7	26	M	proptosis	17	20	abnormal	+
8	32	F	proptosis	18	21	normal	+
9	59	M	proptosis	18	19	abnormal	—
10	34	M	proptosis	21	14	normal	+
11	55	M	double vision	10	14	abnormal	+
12	32	M	proptosis	22	16	abnormal	+
13	32	F	lid retraction	18	18	normal	—

a: Enlargement of extraocular muscle confirmed by CT scan. b: No enlargement of extraocular muscle confirmed by CT scan.

Table 2. Thyroid function tests in 13 patients with euthyroid Grave's disease

Case No.	FT ₄ (ng/dl)	FT ₃ (pg/ml)	TSH (μU/ml)	T ₃ suppression test
1	1.0	3.1	0.9	nonsuppressible
2	1.0	2.8	<0.1	nonsuppressible
3	1.4	5.3	<0.1	nonsuppressible
4	1.3	5.1	2.6	suppressible
5	1.2	5.2	<0.1	nonsuppressible
6	1.2	3.9	2.1	nonsuppressible
7	1.4	3.5	0.4	nonsuppressible
8	1.4	3.3	4.1	nonsuppressible
9	1.1	4.4	8.7	N.D.*
10	1.3	5.3	0.7	suppressible
11	1.4	3.5	4.1	suppressible
12	1.3	3.6	1.9	N.D.
13	0.9	3.3	5.6	nonsuppressible
Normal ranges	0.8 ~ 1.4	2.8 ~ 5.8	0.4 ~ 5.6	

*: Not done.

Fig. 1. The extent of proptosis in 13 patients with euthyroid Graves' disease and 20 patients with untreated Graves' disease. Open circle, left eye; Closed circle, right eye.

extra-ocular muscle enlargement in 9 cases. Goiter was not seen in any of the cases. Proptosis in euthyroid Graves' disease was not significantly different to that in untreated Graves' disease (Fig. 1).

Thyroid function test results are shown in Table 2. Serum FT₄ and FT₃ levels are in the normal range as the definition of euthyroid Graves' disease. Serum TSH levels, however, are low in 3 patients (case nos. 2, 3 and 5) and high in one (case no. 9). These patients were classified as euthyroid Graves' disease because they were clinically eu-

thyroid and their FT_4 and FT_3 levels were normal. T_3 -suppressibility was abnormal in 8 of 11 examined. There was no TSH response to TRH or it was low in 7 of 12 patients examined (Fig. 2). Thus, 10 of 13 (76.9%) patients with euthyroid Graves' disease revealed thyroid dysfunction.

In euthyroid Graves' disease, either TGHA or MCHA was positive in 4 patients and the titers of both were significantly lower compared than those

of untreated Graves' disease (Fig. 3A). TBII was positive in only 4 of 13 patients (31%) with euthyroid Graves' disease and significantly lower than that in the patients with untreated Graves' disease (Fig. 3B). TSAb, however, was positive in 12 of 13 patients with euthyroid Graves' disease, though the titer was significantly low compared with that in the patients with untreated Graves' disease (Fig. 3C). No correlation was seen between TSAb and TBII, TSH response to TRH, or T_3 suppressibility. Moreover, RAIU was not related to TSAb (data not shown).

The clinical course of 13 patients with euthyroid Graves' disease was followed up for 5 to 10 years. Five patients (case nos. 2, 3, 5 and 13) developed persistent hyperthyroidism, 2 (case nos. 1 and 7) developed transient hyperthyroidism, one (case no. 9) developed transient hypothyroidism, and the other 5 patients remain euthyroid during

Fig. 2. TSH response to TRH in 12 examined patients with euthyroid Graves' disease. The meshed area indicates the normal range of response.

Table 3. Clinical courses of 13 patients with euthyroid Graves' disease

Clinical course	Number
Persistent hyperthyroidism	5 (38%)
Transient hyperthyroidism	2 (16%)
Transient hypothyroidism	1 (8%)
Euthyroid	5 (38%)

Fig. 3. Thirteen patients with euthyroid Graves' disease and 20 patients with untreated Graves' disease. A: The titer of TGHA and MCHA, open circle and closed circle indicate TGHA and MCHA, respectively. B: The TBII values. C: The TSAb values. The meshed area indicates the normal range.

the observation period (Table 3). In the patients who developed persistent hyperthyroidism, TBII and TSAb were significantly higher than in other patients, and the class of their ophthalmopathy not significantly changed (data not shown).

Discussion

In this study, we demonstrated the significance of TSAb in the diagnosis of euthyroid Graves' disease and the importance of long term follow up of patients. The features of ophthalmopathy in euthyroid Graves' disease were almost the same to those in untreated Graves' disease and there was no significant difference between euthyroid Graves' disease and untreated Graves' disease in the degree of proptosis.

Almost all the patients with euthyroid Graves' disease suspected ophthalmologically had abnormalities in thyroid function and/or in antithyroid auto-antibodies [26]. In combination with TRH test, T₃-suppression test, TBII, TGHA and MCHA, we found abnormalities in the thyroid in 10 patients (77%), and more in 12 patients (92%) when TSAb was examined. The positive rate and the titers of TGHA, MCHA or TBII were significantly lower than in untreated Graves' disease. TSAb, however, was detected as frequently as in untreated Graves' disease, although the titer was significantly lower. TSAb was therefore a good indicator to use in the diagnosis of euthyroid Graves' disease, and should be measured in patients with proptosis, diplopia and lid disturbance of unknown origin. Three (case nos. 2, 3 and 5) of 13 patients who had suppressed TSH and developed active Graves' disease, may not be classified as euthyroid Graves' disease.

In the present study, TBII and TSAb were detectable in 4 and 12 of 13 patients with euthyroid Graves' disease, respectively. In previous reports, the incidence of TSAb was 43–90% [27, 28]. The reason for this discrepancy is not clear, but the difference in sensitivity of the TBII and TSAb assays or the difference in disease activity may be involved. The TSAb value was related neither to the severity of the ophthalmopathy nor to the future development of thyrotoxicosis in this study (data not shown). Furthermore, RAIU was high in only 4 of 13 patients (31%) with euthyroid Graves' disease and no relation was observed between

TSAb and RAIU.

Antibody to 64 kDa eye muscle membrane protein [15–19] may generally be difficult to examine. For other serological markers such as soluble intercellular adhesion molecule-1 [29] and serum interleukin-2 receptor [30], the positive rate is thought to be low, especially in Hashimoto disease.

Though the pathogenesis of Graves' ophthalmopathy is still unclear, many investigators have proposed an autoimmune mechanism [29–40]. As the histological evidence of autoimmunity, lymphocyte and macrophage infiltration and peptide factors such as IL-1 α , TNF α , IFN γ , TGF β , PDGF and IGF-I, were detected in retroorbital tissues [41–44]. Anti-thyroglobulin antibody cross-reacted with human orbital tissue [45, 46]. Wang *et al.* [47] and Dillon *et al.* [48] suggested antibody dependent cytotoxicity acting on human eye muscle cells. The stimulating type antibody TSAb is considered to play an important role in the development of thyroid dysfunction in autoimmune thyroid disease [49] and euthyroid Graves' disease [27]. Recently, Heufelder [50] and Feliciello [51] indicated that TSH receptor exists in retroocular fibroblasts. Anti-TSH receptor antibodies, especially TSAb may therefore be concerned with Graves' ophthalmopathy. TSAb titers in the patients who later developed Graves' disease became higher at the onset of Graves' disease, but the severity of Graves' ophthalmopathy was not changed in spite of the increase in TSAb. TSAb may therefore not be the main cause of Graves' ophthalmopathy but TSAb is measured as the activity stimulating thyroid cells and may therefore not be the uniform anti-TSH receptor antibody. The antibody that really causes Graves' ophthalmopathy may be included in TSAb in our assay system.

In our study, goiter was not seen even in the patients with a slight increase in serum TSH. One possibility is that TSAb is transiently positive and so not enough for the development of thyrotoxicosis and thyroid enlargement. Another possibility is that the thyroid reserve is diminished. However, cytotoxic auto-immune mechanisms are not considered to be responsible for the thyroid reserve diminution, because lymphocytic infiltration in the thyroid gland examined by ultrasonography was not always seen in euthyroid Graves' disease patients (data not shown). Finally, we speculate that there is a mechanism which inhibits production of thy-

roid hormone and thyroid growth or that TSAb detected in euthyroid Graves' disease is inactive in human thyroid cells. Wiersinga *et al.* [52] reported there was time lag between the onset of thyroid-associated ophthalmopathy and that of Graves' disease. Eight of 13 patients with euthyroid Graves' disease developed thyroid dysfunction, so the inhibition may diminish and release from the inhibition may allow the development of thyroid dysfunction. Transient hyperthyroidism and hypothyroidism were developed in two and one patients, respectively. These patients were thought to have developed destructive thyroiditis. Drexhage *et al.* [53], who identified thyroid-growth stimulating antibody (TGI) by nucleic-acid cytophotometry, also reported growth inhibiting antibody, which may be one of the candidates.

We conclude that 1) euthyroid Graves' disease is a subtype of Graves' disease with hormonally

and endocrinologically euthyroid features, 2) abnormalities in thyroid function and/or in antithyroid autoantibodies are detected, and especially TSAb is a beneficial marker for the diagnosis of this disease, and 3) long term follow up is important because of the later development of thyroid dysfunction.

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