Subclinical Graves' disease as a cause of subnormal TSH levels in euthyroid subjects

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ABSTRACT. In order to elucidate causes of subclinical thyrotoxicosis, we reviewed records of thyroid function tests obtained in our hospital between 1990 and 1992, showing normal thyroid hormones and subnormal TSH levels, and analyzed underlying clinical conditions of the patients. Of 186 patients with normal T_4 and/or free T_4 and normal T_3 and/or free T_3 but subnormal TSH (<0.1 mU/l) levels in serum, 150 were under treatment with antithyroid drugs for hyperthyroid Graves' disease or with thyroid hormones for hypothyroidism. Twelve were in remission after treatment for Graves' disease, and 4 had destructive thyroiditis. Of the remaining 20 patients, 4 had autonomously functioning thyroid nodule (AFTN), 9 had euthyroid ophthalmic Graves' disease (EOG), and 7 had diffuse goiter without apparent ophthalmopathy (DG). When thyroid stimulating antibodies (TSAb) were measured in the last 3 groups of the patients, they were detected in none with AFTN but in all patients with EOG and DG. These 7 DG patients without ophthalmopathy had a clinical feature showing unstable thyroid functions, changeable to euthyroidism, overt hyperthyroidism and even hypothyroidism during follow-up. In conclusion, TSAb measurement is useful for detection of subclinical Graves' disease in euthyroid subjects with subnormal TSH levels in serum.
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INTRODUCTION

Since the development of sensitive immunometric assays of TSH, subclinical thyrotoxicosis with low serum TSH but normal thyroid hormone concentrations has easily been identified without performing TRH test. Slight excess of thyroid hormone production causes decreased output of TSH from the pituitary, thereby resting the thyroid gland and normalizing serum thyroid hormone levels. Since this condition may be at increased risk of developing true thyrotoxicosis, clarification of causes of subclinical thyrotoxicosis is clinically important. It is well known that subclinical thyrotoxicosis develops in patients with autonomously functioning thyroid nodule (AFTN), euthyroid ophthalmic Graves' disease (EOG), treated Graves' disease, and also in those taking excessive doses of thyroid hormone (1-5). In an effort to review the records of thyroid function tests obtained in our hospital during the past 3 years (1990-1992) and to assess whether there is any discrepancy between TSH and thyroid hormone levels, we could identify 7 patients with low serum TSH (<0.1 mU/l) and normal thyroid hormone concentrations but without apparent ophthalmopathy, all of whom had thyroid stimulating antibodies (TSAb) in serum.

Key-words: Subclinical thyrotoxicosis, TSH, TSH-receptor antibodies (TRAb), subclinical Graves' disease, euthyroid ophthalmic Graves' disease, autonomously functioning thyroid nodule (AFTN),TSH-binding inhibitor immunoglobulins (TBII), thyroid stimulating antibodies (TSAb).

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MATERIALS AND METHODS

Patients

Twenty-two thousand five hundred and fifty-one patients who visited Kyoto University Hospital and underwent blood tests for T_4 or free T_4, T_3 or free T_3 and TSH from the beginning of 1990 to the end of 1992 were studied. Among them 186 (0.82%) showed serum TSH concentrations lower than 0.1 mU/l in spite of normal T_4 or free T_4 and T_3 or free T_3.

The diagnosis of hyperthyroid Graves' disease was made on the basis of diffuse goiter, hyperthyroid symptoms and signs, elevated thyroid hormone levels, increased ^{99m}Tc thyroid uptake, and detectable TSH binding inhibitor immunoglobulins (TBII) and/or TSAb activities. EOG was defined as ophthalmopathy of Graves' disease in euthyroid subjects, showing several ophthalmic symptoms corresponding to Class II-IV in the American Thyroid Association Classification (6). The diagnosis of AFTN was made on the basis of presence of a functioning nodule with suppressed extranodular parenchyma on ^{123}I or ^{99m}Tc thyroid scintigram.

TBII assay

TBII activity was measured by radioreceptor assay using ^{125}I-labeled bovine TSH (^{125}I-TSH) and solubilized porcine thyroid membranes, as previously described (7) with a minor modification (8). The receptor fractions were preincubated with the patient's serum for 120 min before addition of ^{125}I-TSH. The results were expressed as follows: (1-specific ^{125}I-TSH binding in the presence of patient's serum/specific ^{125}I-TSH binding in the presence of normal serum) x 100%. The normal range (mean±2SD) determined in 53 norrnal subjects was -11.9% to 11.0%.

TSAb assay

TSAb activity was measured using the method previously developed in our laboratory (9). FRTL-5 thyroid cells ($1x10^6$ cells/well), kindly provided by Dr. L.D. Kohn (NIH, Bethesda, MD, USA), were incubated with crude immunoglobulin fractions precipitated from 250 ul serum with 15% polyethylene glycol (no. 4000) and dissolved in 300 ul Hank's medium without NaCl containing 15 g/l BSA, 20 mmol/l HEPES, and 0.5 mmol/l 3-isobutyl-1-methylxanthine. The cAMP released into the medium during the 2h incubation period at 37 C was measured in duplicate by RIA. The results were expressed as the percentage of cAMP compared with the mean of values for 10 normal subjects. The normal range (mean±2SD) determined in 31 normal subjects was 55-145%. All individual serum samples were tested in a single assay run for the measurement of TSAb as well as TBII activities.

Serum thyroid hormone and other measurements

Serum T_4, T_3, free T_4 and free T_3 concentrations were determined in duplicate by RIA using commercially available kits (T_4 and T_3, Dinabot Radioisotope Laboratories, Tokyo, Japan; free T_4 and free T_3, Amersham International Ltd., Aylesbury, Buckinghamshire, United Kingdom). The normal range (mean±2SD) were as follows: T_4, 77.2-167.3 nmol/l; T_3, 1.38-2.92 nmol/l; free T_4, 10.3-27.0 pmol/l; and free T_3, 3.4-8.6 pmol/l. Serum TSH concentrations were determined by the second generation immunoradiometric assay (RIAGNOST hTSH Kit, Hoechst Japan, Tokyo, Japan; normal range, 0.3-3.9 mU/l; the lowest detectable level, 0.04 mU/l). Antithyroglobulin and antimicrosomal antibody titers were determined by using commercially available kits (Fujizoki, Inc., Tokyo, Japan; normal range, <100 for both assays).

^{99m}Tc thyroid uptake was measured 30 min after the iv injection of 148 MBq ^{99m}Tc-pertechnetate. The normal range was 0.4-3.0%. ^{123}I thyroid uptake was measured 3 h and 24 h after oral administration of 3.7 MBq Na^{123}I. The normal range was 7-35% at 24 h.

RESULTS

Of our 186 patients with suppressed TSH but normal T_4 or free T_4 and T_3 or free T_3 levels, 79 (42.5%) were taking thyroid hormones. All 72 patients, except 7 with secondary hypothyroidism due to pituitary insufficiency, appeared to have iatrogenic subclinical thyrotoxicosis caused by excessive treatment. Among them, 32 had been totally (n=30) or subtotally (n=2) thyroidectomized for thyroid cancer and had confirmed or suspected metastatic disease, and the remaining 40 (27 with Hashimoto's thyroiditis and 13 with adenomatous goiter or adenoma) were on excessive doses of thyroid hormones for achieving regression of proven or apparently benign nodules or diffuse goiter.

Seventy-one (38.2 %) of the 186 patients were treated with antithyroid drugs for overt thyrotoxicosis due to Graves' disease and their subclinical thyrotoxicosis could be explained by delayed recovery of the pituitary-thyroid axis or inadequate antithyroid effect on the activated thyroid.

Twelve and 4 of the remaining 36 patients who were in remission after antithyroid drug treatment for overt thyrotoxicosis due to Graves' disease and who had destructive thyroiditis, respectively, exhibited subnormal TSH levels. When our 12 patients in remission were followed, 1 developed overt thyrotoxicosis in 12 months, and 6 became euthyroid with normal TSH in 7-28 months. The remaining 20 patients had untreated, endogeneous subclinical thyrotoxicosis. This group included 4 patients with AFTN, 9 with EOG, and 7 with diffuse goiter who did not present clinical symptoms of Graves' ophthalmopathy such as exophthalmos, soft tissue involvement and ophthalmoplegia.

After the classification of our 186 patients, our interest was focused on the last 7 patients with diffuse goiter and without ophthalmopathy. All these patients appeared euthyroid with normal pulse rate and with neither tremor nor moist skin. They had diffuse goiter with a transverse diameter of 4-7 cm (Table 1). ^{99m}Tc thyroid uptake was not suppressed by a 7-days treatment with T_3 (75 ug/day) to the value less than half the pretreatment value in 3 patients tested (10). Thyroid scintigram revealed homogeneous uptake in all except 2 patients. Patient 2 showed presence of functioning lesions. Patient 6 showed nonhomogeneous uptake. Ultrasonography revealed diffuse goiter in all patients. Patient 2's mother also had Graves' disease. None of the remaining 6 patients had a family history of thyroid diseases. These patients were not taking any medication known to affect thyroid function and the measurement of TSH as well as thyroid hormone concentrations.

Table 1 - *Clinical data in 7 patients with subclinical thyrotoxicosis having diffuse goiter but no ophthalmopathy and serial changes in thyroid functions and thyroid-related antibodies during the follow-up period without medication.*

Patient	Sex age	Date	T_4 (nmol/l)	T_3 (nmol/l)	FreeT_4 (pmol/l)	FreeT_3 (pmol/l)	TSH (mU/l)	anti -Tg	anti -M	TBII (%)	TSAb (%)	Complaints at first visit	Goiter (size*)	$^{99m}Tc(^{123}I)$ uptake (%)
1	F	9-1991	127.3	1.78	15.6	—	<0.04	(-)	1600	21.4	324	anorexia	diffuse	5.2
	22	11-1991	106.7	1.67	12.7	—	0.35	—	—	-1.8	310	excessive sweating	(4.5 cm)	
2	F	12-1990	129.9	2.56	20.6	7.2	<0.04	—	—	—	—	cold intolerance	diffuse	0.6 before T3
	49	11-1991	—	—	19.3	7.8	<0.04	(-)	(-)	8.9	592		(4.2 cm)	1.0 after T3
		5-1992	104.2	1.86	18.0	5.4	0.33	(-)	(-)	12.0	398			
		3-1993	—	—	15.4	5.8	0.30	(-)	(-)	8.5	350			
		11-1994	—	—	21.8	5.8	<0.04	—	—	13.0	456			
3	F	8-1991	96.5	2.33	17.0	6.6	<0.04	(-)	3200	34.9	874	general fatigue	diffuse	1.2 before T3
	47	11-1991	66.9	1.33	—	5.8	0.93	—	—	32.7	538		(4.2 cm)	0.7 after T3
		1-1992	59.2	1.50	—	7.2	2.47	(-)	3200	14.6	358			
		10-1992	83.6	2.27	—	—	<0.04	—	—	57.5	656			
		1-1993	65.6	1.58	12.9	4.6	0.05	—	—	57.2	572			
		4-1993	—	—	30.9	10.4	<0.04	100	1600	70.1	814			
4	F	8-1990	120.9	2.59	19.3	6.9	<0.04	(-)	(-)	3.0	240	discomfort in the neck	diffuse	0.4 before T3
	25	10-1990	—	—	15.4	5.8	3.9	—	—	-7.8	252	(6 months postpartum)	(4.5 cm)	0.5 after T3
5	F	9-1990	95.2	2.38	15.4	7.1	<0.04	100	1600	4.1	669	anterior neck swelling	diffuse	(29.9 at 3h)
	25	12-1990	—	—	12.9	5.4	0.63	—	—	5.3	—	(6 months postpartum)	(4.2 cm)	(60.4 at 24h)
		1-1994	—	2.72	20.8	—	<0.04	—	—	8.3	108			
		6-1994	—	1.54	10.4	—	2.3	100	100	—	—			
6	F	7-1989	115.7	2.76	15.4	—	<0.04	1600	409600	32.5	704	palpitation	diffuse	3.4
	27	1-1990	—	—	20.6	6.9	<0.04	400	409600	24.2	526		(7.0 cm)	
		4-1990	146.6	2.15	21.9	—	<0.04	—	—	16.0	—			
		1-1992	154.3	3.38	—	12.1	<0.04	1600	102400	17.1	—			
		1-1993	88.7	1.69	—	—	<0.04	—	—	—	—			
		6-1994	—	—	15.6	6.1	0.50	400	25600	9.1	—			
		7-1995	—	—	14.3	5.3	0.55	—	—	—	—			
7	F	5-1991	—	—	18.0	7.4	0.4	(-)	6400	26.7	1549	anterior neck swelling	diffuse	7.0
	67	1-1992	117.0	2.16	15.0	6.7	<0.04	100	102400	74.1	11327		(4.0 cm)	
		3-1992	—	—	14.0	6.7	1.3	—	—	34.2	10578			
		4-1993	—	—	5.0	5.1	35.3	—	—	55.0	—			
		5-1994	—	—	15.2	6.0	1.8	100	25600	13.6	—			
		9-1995	—	—	18.9	5.7	1.4	—	—	—	—			
Normal range			77.2~ 167.3	1.38~ 2.92	10.3~ 27.0	3.4~ 8.6	0.3~ 3.9	<100	<100	-11.9~ +11.0	55~ 145			

Patient 3 had low TBG concentration at 11.2 mg/l. Patient 1 and Patient 4 could not be followed after 11-1991 and 10-1990, respectively. Patient 3 has been treated with methimazole since 4-1993.
*transverse diameter. — not determined. Tg: thyroglobulin M: thyroid microsomal antigen

When these patients were followed without medication, their thyroid functions were unstable. Serial changes of the thyroid status are summarized as follows: Patient 1, subclinical thyrotoxicosis/normal TSH; Patient 2, subclinical thyrotoxicosis/normal TSH/subclinical thyrotoxicosis; Patient 3, subclinical thyrotoxicosis/normal TSH/subclinical thyrotoxicosis/overt thyrotoxicosis; Patient 4, subclinical thyrotoxicosis/normal TSH; Patient 5, subclinical thyrotoxicosis /normal TSH/subclinical thyrotoxicosis/normal TSH; Patient 6, subclinical thyrotoxicosis/overt thyrotoxicosis(T_3 toxicosis)/ subclinical thyrotoxicosis/normal TSH; Patient 7, normal TSH/subclinical thyrotoxicosis/normal TSH/overt hypothyroidism/normal TSH (Table 1).

Interestingly, TSAb were detected in all 7 patients. On the other hand, TBII were detected in 4 patients at the time of subclinical thyrotoxicosis. During follow-up, changes in TBII did (Patient 1 and 3) or did not (Patient 2,4 and 5) correlate and those in TSAb did (Patient 2, 3 and 7) or did not (Patient 1 and 4) correlate with the thyroid functions (Table 1). In Patient 7, there was an episode of hypothyroidism, when TBII were transiently elevated (55.0 %), suggesting the preferential production of blocking-type TSH-receptor antibodies.

Table 2 demonstrates that TBII and TSAb were detected in 5 and all 9 patients with EOG, but neither were detected in 4 patients with AFTN. Among the 9 patients with EOG, 7 could be followed without medication. Serum TSH concentrations were normalized in 4 patients 14, 16, 29 and 60 months after the blood test. In one patient, normalization of serum TSH in 5 months was followed by development of subclinical thyrotoxicosis again in 12 months and the repeated normalization of TSH in 31 months. Two patients developed mild but transient overt thyrotoxicosis in 6 and 8 months.

Table 2 - *TBII and TSAb activities in patients with euthyroid ophthalmic Graves' disease (a) and AFTN(b).*

	Patient	Sex	Age	TBII (%)	TSAb (%)
(a)	1	F	46	7.3	242
	2	F	27	8.6	1212
	3	F	56	18.0	297
	4	M	68	32.9	219
	5	F	45	15.3	180
	6	F	20	-6.8	198
	7	F	54	19.9	174
	8	M	66	-2.8	296
	9	M	62	35.9	1346
(b)	1	F	46	5.3	124
	2	F	72	1.0	123
	3	F	57	7.8	120
	4	F	72	1.5	77
normal range				-11.9	55-
				+11.0	145

DISCUSSION

The measurement of serum TSH concentrations by the sensitive immunometric assay is believed to be the first-line test for evaluation of thyroid functions. Because of amplified and sensitive responses of TSH to changes in serum thyroid hormone levels, there exists a clinical state of subclinical thyrotoxicosis or hypothyroidism with reduced or elevated serum TSH, respectively, but normal thyroid hormone levels. In a group composed of both inpatients and outpatients with suppressed TSH levels, 17% had this conditions from causes other than thyrotoxicosis, including central hypothyroidism, nonthyroidal illness (NTI), psychiatric illness and medication, according to Ehrmann et al. (2).

In those who were taking thyroid hormones or antithyroid drugs, subclinical thyrotoxicosis developes depending on the dosage, as shown in a majority of our patients (150/186). In patients with Graves' disease after treatment with antithyroid drugs, subclinical thyrotoxicosis does (11) or does not (12) suggest a high risk of relapse. Our patients showed rather low rate of relapse.

Our greatest interest in this study is to elucidate the role of TSH receptor antibodies in the cause of endogeneous subclinical thyrotoxicosis. AFTN has been known to be a cause of subclinical thyrotoxicosis (1, 2, 5, 13). Overt or subclinical thyrotoxicosis due to AFTN has recently been recognized to be the result from continuous activation of adenylate cyclase due to mutations involving the transmembrane domain of the TSH receptor (14). Neither TBII nor TSAb were detected in our patients with AFTN, indicating that TSH receptor antibodies do not play a pathogenetic role in this condition (15).

It has been reported that some EOG patients develop subclinical thyrotoxicosis (5, 13, 16-18). Our EOG patients with subclinical thyrotoxicosis all had detectable TSAb in serum. Such a high incidence of TSAb has been reported previously (9, 10, 15, 18). In comparison with TSAb, TBII were negative or weakly positive. It is conceivable that TSH receptor antibodies of these patients were not potent enough to cause overt thyrotoxicosis, as discussed previously (10, 18).

Some patients with multinodular goiter are known to display subnormal TSH levels (1, 19, 20). Although nonhomogeneous distribution and localized in-

creased uptake of ^{99m}Tc were observed in 2 of our 7 subclinically thyrotoxic patients with diffusely enlarged goiter and without apparent ophthalmopathy, there were no discrete nodules on ultrasono-graphy. All of these 7 patients with diffuse goiter were TSAb-positive. High or T_3-nonsuppressible thyroid uptake suggests that TSAb were active in vivo, although the second episode of subclinical thyrotoxicosis in patient 5 could be explained by destructive thyroiditis (4 months postpartum) in view of negative TSAb. This group seems pathogenetically similar to that of EOG patients in the sense that stimulating-type TSH receptor antibodies were responsible for the mild activation of the thyroid but not so potent enough to cause overt thyrotoxicosis. Thus, these patients could be diagnosed as having subclinical Graves' disease without apparent ophthalmopathy.

The similar cases of subclinical thyrotoxicosis have been reported. Seven patients with unknown underlying disease reported by Ross et al. (1) and 15 patients with probable multinodular goiter reported by Scott et al. (21) might have subclinical Graves' disease, but these authors did not measure TSAb activities. Smyth et al. (22), using a highly sensitive cytochemical bioassay, measured thyroid stimulating activities of immunoglobulins in euthyroid women, and detected TSAb in 9 of 11 with multinodular goiter and 2 of 2 with diffuse goiter, who showed impairment in TRH resposiveness. The relationship between thyroid nodules and thyroid stimulators such as TSH receptor antibodies has been suggested by other investigators as well. Brown et al. (23) reported that TBII were detected in 19 of 37 patients with non-toxic and toxic multinodular goiter. Kreim et al. (15), measuring cAMP produced in cultured thyroid cells, detected TSAb in 11 of 26 patients (42%) with toxic multinodular goiter. Scintigraphic hot or warm lesions were observed in approximately half of EOG patients known to have TSAb in serum (18). Thus, it is conceivable that stimulating-type TSH receptor antibodies activate only more responsive follicles, resulting in the formation of functioning lesions and multiple nodules in such patients during a long course of illness (15, 24). The presence of scintigraphic functioning lesions in one of our patients could also be explained by the same mechanism.

This is the first paper in which the causes of subclinical endogeneous thyrotoxicosis were analyzed in a group of euthyroid patients selected from the list of laboratory data including TSH determined by the sensitive immunometric assay. Presumably due to the low prevalence of autonomously functioning thyroid nodule(s) in the area with high iodine intake like Japan (5, 25), a majority of the patients with endogeneously subclinical thyrotoxicosis had subclinical Graves' disease (16/20, the remaining 4 with AFTN) irrespectively of the presence of ophthalmopathy, supporting the usefulness of the measurement of TSH-receptor antibodies especially TSAb.

Changes in TBII and/or TSAb during follow-up were considered responsible for the unstable thyroid function in most of the patients. Our TSAb-positive 7 patients with subclinical thyrotoxicosis are similar to the 9 TBII-positive euthyroid relatives of patients with Graves' disease reported by Tamai et al. (26). They were subclinically hyperthyroid with small diffuse or unpalpable goiter. Of their 9 patients, 7 with detectable TSAb developed over thyrotoxicosis during follow-up, in contrast to a rather low frequency of occurrence of overt thyrotoxicosis (2 of 5 patients with follow-up period of >20 months) in our cases. The difference is unexplicable, but some genetic factors might be involved. Our EOG patients also displayed unstable thyroid functions during follow-up, in agreement with the observation by Tamai et al. (17).

In conclusion, 1) euthyroid patients with subnormal TSH levels should be tested for TSAb to detect subclinical Graves' disease and 2) thyroid functions of such patients should be carefully monitored.

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