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Clinical Research Article

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Clinical Course of Euthyroid Subjects With Positive TSH Receptor Antibody: How Often Does Graves' Disease Develop?

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Abstract

Background: Thyroid stimulating hormone receptor antibody (TRAb) is detected in the serum of patients with Graves' disease (GD). This study aims to investigate the prevalence of euthyroid individuals showing positive results for TRAb and to clarify the clinical course of thyroid function and TRAb levels in these subjects.

Objective: Subjects were female patients who newly visited our hospital for a screening test prior to fertility treatment and showed normal thyroid function and volume without nodules between 2014 and 2017. After excluding subjects with a history of thyroid disease, 5,622 subjects were analyzed.

Results: Forty-seven of the 5,622 subjects showed positive results for TRAb (reference range, <2.0 IU/L) at the initial visit. Median initial TRAb was 2.9 IU/L (range, 2.0-14.7 IU/L) and median follow-up was 18.3 months (range, 0-66.5 months). Six of the 47 subjects (12.8%) developed GD and median duration until development was 6.6 months (range, 1.2-13.2 months). Median TRAb values initially and at diagnosis of GD for those 6 patients were 3.7 IU/L (range, 2.7-5.1 IU/L) and 7.2 IU/L (range 3.6-21.4 IU/L), respectively. TRAb results turned negative for 20 of the 47 subjects but remained positive despite normal thyroid function in 13 of the 47 subjects.

Conclusion: GD developed over time in 12.8% of euthyroid young female patients showing positive TRAb within a median of 6.6 months. A positive result for TRAb itself did not mean development of GD, so other factors must be essential for the pathogenesis of GD.

Key Words: TSH receptor antibody, thyroid function

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Thyroid-stimulating hormone (TSH) receptor (TSHR) antibody (TRAb) is a specific antibody for Graves' disease (GD), and is usually detected in the serum of GD patients with a prevalence of about 90% to 100% depending on the methods of measurement [1-4]. Morshed et al. [5] showed 3 types of TRAb: TSHR-stimulating antibody (TSAb), TSHR-blocking antibody (TSBAb), and neutral antibody. Each of these shows a different degree of affinity for TSHR, inducing different degrees and types of thyroid dysfunction. Since TSAb shows the highest affinity to TSHR and initiates a second signaling via TSHR [5], most patients with GD show hyperthyroidism. However, we sometimes encounter cases that show positive TRAb with normal thyroid function, without a history of GD. Chailurkit et al [6] previously reported that in the male euthyroid population, the TRAb-positive rate was 6.5% and the TRAb value was related to the serum estrogen level. Furthermore, Chou et al [7] reported that among patients with euthyroid autoimmune thyroiditis, 21.6% showed positive results for TRAb, and no one developed hyperthyroidism within 57 months of follow-up. The clinical course of euthyroid individuals with TRAb-positive results is unclear. The aim of this study was thus to reveal the prevalence of TRAb-positive results among euthyroid fertile-age female population in Japan, and to clarify the clinical course for these subjects.

Materials and Methods

Subjects

Subjects were 12 455 women newly referred to our hospital by fertility clinics for a screening test prior to starting fertility treatment, between January 2014 and December 2017. Previous to the initial visit to our clinic, subjects were noted to have positive antithyroglobulin antibody (TgAb) and/or antithyroid peroxidase antibody (TPOAb) or an elevated TSH concentration (≧2.5 μIU/mL). Among these 12,455 females, 1,724 patients who showed thyroid dysfunction (either overt or subclinical dysfunction), 894 subjects with solid thyroid nodules detected on ultrasonography, and 2,093 subjects with irregular thyroid size (including either goiter or atrophic thyroid) were excluded from this study. Normal thyroid function was defined as free triiodothyronine (fT3), free thyroxine (fT4) and TSH were all within normal ranges, and normal thyroid size was defined as 6 to 18 mL. Another 2122 subjects with a history of thyroid disease were also excluded. After excluding these 6833 subjects, the remaining 5622 patients who did not meet any of the exclusion criteria were analyzed. As a retrospective study, data were obtained from a review of the medical records of these 5,622 patients. TSH, fT3, fT4, TRAb, TgAb, and TPOAb were tested on the initial visit for all subjects. As a follow-up, TSH, fT3, fT4, and TRAb were regularly measured every 1 to 6 months. GD was diagnosed based on both hyperthyroidism that consists of increased fT3 and fT4 levels and a suppressed TSH level that lasted for at least 2 months, and positive values for TRAb. This study was approved by the ethics committee of Ito Hospital and written informed consent was obtained from all participants.

Laboratory methods

TSH, fT3, and fT4 were measured using electrochemiluminescence immunoassay kits (ECLusys TSH, ECLusys fT3, and ECLusys fT4, respectively; Roche Diagnostics, Basel, Switzerland). Reference ranges from the manufacturers were as follows: TSH, 0.2 to 4.5 mIU/L; fT3, 2.2 to 4.3 pg/mL; and fT4, 0.8 to 1.6 ng/dL. TRAb was measured using an ECLusys TRAb electrochemiluminescence immunoassay kit (RRID: AB_2801453) (Roche Diagnostics; normal range, <2.0 IU/L). TgAb and TPOAb were measured using TgAb and TPOAb radio-immunoassay kits (Roche Diagnostics; normal range, TgAb ≦ 40 IU/mL and TPOAb ≦ 28 IU/mL). The same devices were used for measuring TSH, fT3, fT4, TRAb, TgAb, and TPOAb throughout the observation period, so reference ranges for these values remained the same. Thyroid volume was estimated ultrasonographically by measuring the length, width, and depth of each lobe of the thyroid gland in millimeters and then calculating the volume according to the following formula [8]: thyroid volume = (0.7365 × right lobe length × width × depth + 0 .7412 × left lobe length × width × depth) – 0.55.

Statistical analysis

Baseline parameters and collected data were analyzed using JMP version 14.0 software (SAS Institute, Cary, NC, USA). The Wilcoxon rank-sum test was applied to compare parameters between subjects, and paired *t*-tests were used to compare initial TRAb values and onset of GD. Fisher's exact test was performed to compare the prevalences of TgAb and TPOAb positivity between subjects with and without GD. Statistical significance was defined for values of $P < 0.05$.

Results

Characteristics of subjects with positive TRAb on initial visit and clinical course

Among the 5,622 participants, 47 subjects (0.84%) showed a positive result for TRAb on the initial visit. Detailed data

are shown in Table 1. Median age for these 47 subjects was 39 years (range, 28-46 years), and median follow-up was 18.3 months (range, 0-66.5 months). Six of the 47 participants did not show up to a second examination. As a result, only the initial data were available for those 6 subjects. In addition, TRAb value was only measured on the initial visit in 2 of the 47 participants, so while thyroid function parameters were available afterwards, subsequent TRAb values were not. None of the 47 subjects were tested for TSAb. Twenty-six of the 47 participants received treatment with levothyroxine, even though thyroid function was within normal range. Median values of initial TSH, fT3, and fT4 were 1.63 μIU/L (range, 0.22-3.95 μIU/L), 2.8 pg/mL (range, 2.3-3.5 pg/mL), and 1.16 ng/dL (range, 0.82-1.52 ng/dL), respectively, and median TRAb value was 2.8 IU/L (range, 2.0-14.7 IU/L).

Among the 41 subjects for whom clinical course could be followed-up, 6 patients developed GD, 13 subjects remained positive for TRAb, 20 subjects showed a turn to negative results for TRAb, and TRAb levels were unavailable in 2 subjects, although thyroid function remained euthyroid for the entire follow-up period. TRAb values of the 41 subjects are shown in Figure 1.

Characteristics of subjects who developed GD

Six of the 47 TRAb-positive subjects developed GD during follow-up. Detailed data for these 6 patients are shown in Table 2. Median age was 36.5 years (range, 29-41 years). Median follow-up was 41.6 months (range, 4.9-66.5 months), and median duration to development of GD was 6.7 months (range, 1.2-13.2 months). Median TRAb values initially and at the diagnosis of GD were 3.7 IU/L (range, 2.7-5.1 IU/L) and 7.2 IU/L (range, 3.6-21.4 IU/L), respectively. Values of TRAb were significantly higher at the time of GD diagnosis than on the initial visit (P = 0.04). Conversely, comparing initial values of TRAb between subjects who did and did not develop GD revealed no significant differences (P = 0.06). Likewise, no significant differences in initial thyroid volume (P = 0.12) or positive ratio of TgAb and/or TPOAb (P = 0.63) were evident between subjects who did and did not develop GD.

Three of the 6 patients who developed GD delivered during follow-up (Table 2). During gestation, 2 of these 3 patients were treated with propylthiouracil (PTU), and the remaining 1 patient was treated with potassium iodide. Two of the 3 patients were able to withdraw from medication during gestation: 1 patient treated with PTU ceased pharmacotherapy from 25 weeks of gestation, and the other treated with potassium iodide ceased pharmacotherapy from 24 weeks of gestation. One patient needed to continue PTU throughout the course of gestation. Thyroid function was controlled to within the normal range during the gestational period in all 3 patients, and TRAb values turned negative in 2 patients. As perinatal complications, 2 patients experienced proteinuria, and the remaining 1 patient experienced postpartum hypertension. None of the neonates showed any signs of neonatal thyroid dysfunction. Two of these 3 mothers experienced increased TRAb after delivery, and the remaining mother developed relapse of GD at 5 months postpartum.

Table 1. Characteristics of subjects (n = 47) with positive TRAb on initial visit

Characteristics	Median (range)	Percentage
Age (years)	39 (28-46)	
Observation period (months)	39.9 (0-66.5)	
TSH (μIU/L)	1.63 (0.22-3.95)	
fT3 (pg/mL)	2.8 (2.3-3.5)	
fT4 (ng/dL)	1.16 (0.82-1.52)	
Thyroid volume (mL)	14.1 (6.5-17.8)	
TRAb (IU/L)	2.8 (2.0-14.7)	
TgAb (positive ratio)		63.8
TPOAb (positive ratio)		44.7
TgAb and/or TPOAb (positive ratio)		68.1

Characteristics of subjects who remained positive for TRAb

Thirteen of the 47 subjects remained positive for TRAb during follow-up, although thyroid function remained euthyroid. Median age was 37 years (range, 30-44 years), and median duration of follow-up was 12.6 months (range, 1.1-42.9 months). Median value of initial TRAb was 3.0 IU/L (range, 2.1-14.7 IU/L), and median value for the entire duration of follow-up was 2.6 IU/L (range, 0.8-14.7 IU/L). TRAb values during follow-up fluctuated below and above normal limits in 2 patients, with the lowest value of 0.8 IU/L counted as a negative result for TRAb. However, all 13 subjects were confirmed to show positive results for TRAb at the final measurement. Eight of the 13 subjects (61.5 %) showed positive results for TgAb and/or TPOAb.

Characteristics of subjects in whom TRAb turned negative

Twenty of the 47 patients (42.6%) showed TRAb turn negative during follow-up. Median age for these 20 patients was 40 years (range, 28-45 years), and median TRAb value was 2.7 IU/L (range, 2.0-8.8 IU/L). Median follow-up was 27.1 months (range, 3.3-64.7 months), and median duration to TRAb turning negative was 7.4 months (range,

2.1-41.7 months). Thyroid function of those 20 subjects remained euthyroid for the entire duration of follow-up. Fifteen of those 20 subjects (75.0%) showed positive results for TgAb and/or TPOAb.

Discussion

This study showed the clinical course of euthyroid patients with positive TRAb among fertile-age women in Japan. The overall prevalence of TRAb positivity was 0.84% in our study, although only 12.8% of positive subjects developed GD. Since testing for TRAb is usually performed when hyperthyroidism due to GD is suspected, some reports have investigated the prevalence of positive TRAb in euthyroid populations, showing highly variable results such as 21.6% and 0% [7,9]. Previous studies have targeted euthyroid subjects diagnosed with autoimmune thyroid disease, and 1 study [9] used a testing kit that was less sensitive than the current kit. Such factors may have contributed to different prevalences compared to our study. One possible mechanism leading to a euthyroid status despite TRAb-positive findings might be differences in the bioactivity and affinity of antibodies. TSAb is known to initiate a signaling cascade in thyrocytes and induce hyperthyroidism, whereas TSBAb induces hypothyroidism by blocking stimulation from TSH. Since none of the patients in the present investigation showed thyroid dysfunction on the initial visit, the activity of TSAb and TSBAb were suggested to be relatively balanced. Another possible mechanism underlying euthyroid status was the coexistence of chronic thyroiditis. Due to lymphocyte infiltration, thyroid tissue gradually degenerates and may be unable to fully respond to stimulation signals from TRAb via TSHR. As the prevalence of positive TgAb and/or TPOAb did not differ significantly between patients who did and did not develop GD in this study, this hypothesis seems relatively unlikely.

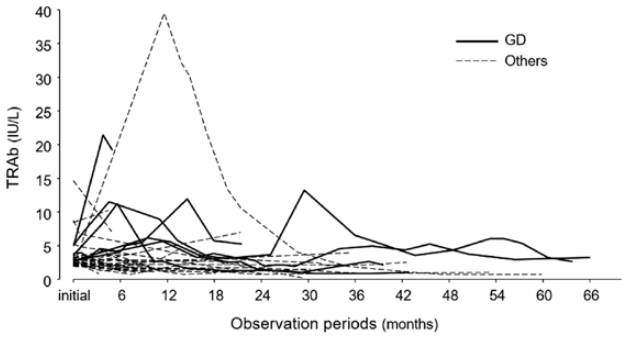

Figure 1. Clinical course of TRAb values in all subjects. Red line shows TRAb in subjects who developed GD. Blue line shows remaining euthyroid subjects. Eight subjects underwent TRAb testing just once, at the initial visit.

GD developed during follow-up in 12.8% of our subjects, representing a markedly higher prevalence than the 0% that Chou et al reported [7]. According to Davies et al [5], 3 potential categories of factors may cause susceptibility to TSHR autoimmunity: (1) external factors, such as infection, trauma, stress, iodine intake, and irradiation; (2) internal factors, such as thyroid autoantibodies, sex steroids, pregnancy, and microchimerism; and (3) genetic susceptibilities, such as HLA class II genes, CTLA-4, CD40, and thyroglobulin. As our subjects were all women showing positive results for TRAb, the

Table 2. Characteristics of patients who developed Graves' disease

	Case no.					
Characteristics	1	2	3	4	5	6
Age (years)	38	35	40	29	30	41
Duration to development of GD (months)	11.5	1.2	13.2	3.7	9.6	3.8
Total follow-up (months)	66.5	64.3	21.6	43.2	39.9	4.9
Thyroid volume (mL)	16.4	17.6	15.1	14.1	11.3	17.3
Treatment	KI	PTU	KI	PTU	KI	KI
TRAb value (initial) (IU/L)	3.8	5.1	2.7	3.6	2.7	5.0
TRAb value (onset of GD) (IU/L)	5.6	3.6	11.9	8.2	6.1	21.4
TgAb value (IU/L) (reference range:≧40)	10	152.9	69.6	18	197.9	270
TPOAb value (IU/mL) (reference range: ≧28)	12.7	5.0	36.6	6.5	71.5	144
Delivery (Yes/ No)	Yes	Yes	No	Yes	No	No
TRAb (IU/L) (gestational period)	Decreased	Turned negative	—	Turned negative	—	—
TRAb (IU/L) (post-delivery)	Increased	Increased	—	Remained negative	—	—
Perinatal complications	Hypertension	Proteinuria	—	Proteinuria	—	—
Treatment withdrawal	from 25 weeks	from 24 weeks	—	Continued	—	—
Relapse	5 months postpartum	—	—	—	—	—

Abbreviations: KI, potassium iodide; PTU, propylthiouracil.

condition of internal factors was regarded as equal. We then assumed that external or genetic factors played roles in the development of GD. Moreover, Morshed et al [10] showed that neutral antibody activates multiple signaling cascades in thyrocytes via TSHR and induces cell apoptosis, which may represent a key mechanism in the initiation of inflammatory processes by immune responses. Accordingly, we also presumed that the neutral antibodies induced continuous apoptosis of thyrocytes in patients, resulting in the development of GD.

In our study, TRAb turned negative during follow-up for 42.6% of subjects. A report from Takasu et al [11] showed TSBAb in patients with hypothyroidism disappeared over time. They assumed that this was because thyrocytes somehow ceased or reduced production of TSBAb, but the actual mechanisms underlying the disappearance of TRAb have not yet been revealed. Our study did not check which type of TRAb was present in subjects, but TSHR antibodies could have somehow disappeared over time.

A key limitation in this study was that we should have measured values for each of TSAb, TSBAb, and neutral antibody in all subjects to clarify which TRAb activity was the highest and allow the prediction of thyroid dysfunction. However, thyroid function in subjects was stable and the subjects were unwilling to undergo detailed but invasive testing, so those tests were not performed. Moreover, when diagnosing GD, confirmation of diffuse high uptake using radioiodine (^{123}I) would have been preferable. However, since all subjects constantly had a possibility of becoming pregnant, performing such testing would have been ethically unfeasible. Instead, we confirmed that all patients with GD continued to show hyperthyroidism for at least 2 months and that none experienced hypothyroidism as would be expected to occur following the course of painless thyroiditis. Furthermore, our study targeted only fertile-age women referred by a fertility clinic, so the population sample showed selection bias from the start. In addition, some medications used for fertility treatments could have induced or influenced thyroid dysfunction. Further analysis in men and younger and older subjects is needed.

Conclusion

The overall prevalence of TRAb positivity among fertile-age women in Japan was 0.84%. Among euthyroid young female subjects with positive results for TRAb, 12.8% developed GD within a median of 6.6 months. Positive TRAb itself did not imply development of GD, so other factors must play key roles in the development of GD.

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Additional Information

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Data Availability: Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

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