

ORIGINAL

Kinetic analyses of changes in serum TSH receptor antibody values after total thyroidectomy in patients with Graves' disease

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Abstract. We often recommend total thyroidectomy for patients with Graves' disease who wish to have a child in the near future in order to prevent fetal or neonatal hyperthyroidism, especially if the patients' serum thyrotropin receptor antibody (TRAb) values are high. The aim of this study was to analyze changes in serum TRAb values using a quantitative third-generation assay after total thyroidectomy and the half-lives of serum TRAb values to estimate the postoperative time needed to achieve the safe TRAb value for mothers. We retrospectively examined the records of 45 Graves' disease patients who underwent a total thyroidectomy and had high serum TRAb values. We also evaluated factors that prolonged the postoperative reduction of serum TRAb values. The serum TRAb values decreased rapidly in most of the patients, especially within the early postoperative (3-month) period. The presence of Graves' ophthalmopathy (GO) ($p=0.001$), smoking ($p=0.004$), and serum thyroglobulin values > 0.5 ng/mL at postoperative 12 months ($p=0.039$) were significantly associated with prolonged half-lives of the serum TRAb values. The median TRAb value half-life was 93.5 days in the patients without GO or smoking, 162.5 days in the patients with GO or smoking, and 357.4 days in the patients with both GO and smoking. Our findings indicate that using the half-life of patients' serum TRAb values determined by this third-generation assay would be effective to evaluate the reduction of serum TRAb values after total thyroidectomy and to estimate the postoperative time needed to achieve the maternal safe value.

Key words: Graves' disease, Thyrotropin receptor antibody (TRAb), Total thyroidectomy, TRAb half-life, Graves' ophthalmopathy (GO)

FETAL or neonatal hyperthyroidism may arise in the setting of maternal Graves' disease resulting from the transport of stimulating thyrotropin receptor antibodies (TRAb) across the placenta after the end of the second trimester [1–6]. The prevalence of fetal or neonatal hyperthyroidism among all women with active Graves' hyperthyroidism or a history of it is 1%–5% [1], and although this prevalence is relatively low, this possibly life-threatening situation for the fetus or infant must be avoided. Therefore, for women with high serum TRAb values who are planning to become pregnant, the reduction of their serum TRAb values to safe levels

before conception is recommended.

We reported that the postoperative decrease in serum TRAb values in patients with Graves' disease who underwent a total thyroidectomy was more rapid than that in patients who underwent a subtotal thyroidectomy [7]. Thus, for patients with Graves' disease who wish to have a child in the near future, we currently recommend a total thyroidectomy, especially if the patient's serum TRAb values are significantly high.

TRAb activities have long been expressed as the percentage of inhibition of the binding of labeled thyroid-stimulating hormone (TSH) to TSH receptor, and our previous study also used percentage activities [7]. With that method, high TRAb values reach a peak at $> 80\%$ – 90% , and there is no dose-response relationship in this range [8]. This presents limitations in kinetic analyses of postoperative changes in the TRAb values.

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A quantitative third-generation assay system for TRAb became available and is now used widely in clinical settings [9]. The present study is the first report on serum TRAb values expressed as IU/L after thyroidectomy; this revised expression of TRAb values enabled us to precisely analyze the postoperative changes in serum TRAb values.

The aim of the present study was to analyze short-term changes in serum TRAb values measured with the third-generation assay system after total thyroidectomy in order to estimate the postoperative time needed for achieving a safe value. We also investigated the factors associated with difficulties in achieving a reduction of the postoperative serum TRAb values.

Materials and Methods

Patients

We retrospectively examined the changes of serum TRAb values and the half-lives of serum TRAb values in 45 Graves' patients who underwent a total thyroidectomy between January and December 2010 at Kuma Hospital. The patients underwent blood tests preoperatively and at 1, 3, 6, and 12 months postoperatively. Patients with normal preoperative TRAb values were excluded. To exclude the effect of glucocorticoids on the reduction of TRAb, we also excluded patients who received a glucocorticoid after surgery. Patients were considered to have active Graves' ophthalmopathy (GO) when they had a clinical activity score (CAS) of 3 or more [10] or exhibited inflammation on magnetic resonance imaging (MRI), and were prescribed an anti-inflammatory treatment by an ophthalmologist.

When patients met the anesthesiologist before surgery and when patients were hospitalized, their smoking status was checked in an interview.

Blood tests included TRAb, thyroglobulin antibody (TgAb), and antithyroid peroxidase antibody (TPOAb) as well as free T4 (FT₄), free T3 (FT₃) and TSH. These measurements were done during the patients' hospital visits. The thyroglobulin (Tg) concentration was measured at 1 month after surgery and again at 12 months after surgery. When there were deficiencies in any of these laboratory values, they were measured again using blood samples frozen and stored at -20°C .

We calculated the half-life of TRAb using the logarithmic conversion TRAb value; three or more points of valid data were needed to do so. When the TRAb values were below the detectable level or scaled out,

the data point was excluded from this calculation. We also analyzed factors that might influence the half-life of the TRAb values.

The study was approved by the Ethical Committee of Kuma Hospital.

Laboratory measurements

The TSH, FT₄, and FT₃ concentrations were measured using chemiluminescent immunoassays (Architect TSH, Architect FT₄, and Architect FT₃ respectively; Abbott Japan, Tokyo). Normal ranges were 0.3–5.0 mIU/L for TSH, 0.7–1.6 ng/dL for FT₄, and 1.7–3.7 pg/mL for FT₃. The serum levels of TRAb, TgAb, TPOAb, Tg were measured using an electrochemiluminescence immunoassay (ECLusys 2010; Roche Diagnostics Japan, Tokyo; normal range: <1.9 IU/L for TRAb, <39.9 IU/mL for TgAb, <27.9 IU/mL for TPOAb, and <35 ng/mL for Tg).

Statistical analysis

Differences between the reduction rate at 3 months after surgery and that at 3–12 months after surgery were analyzed by Wilcoxon signed-rank test. To assess significant factors correlated with the half-life of the serum TRAb values, we used Spearman's rank correlation coefficient. These analyses were performed using StatFlex version 6 software (Artech, Osaka, Japan). Differences were considered significant at $p < 0.05$.

Results

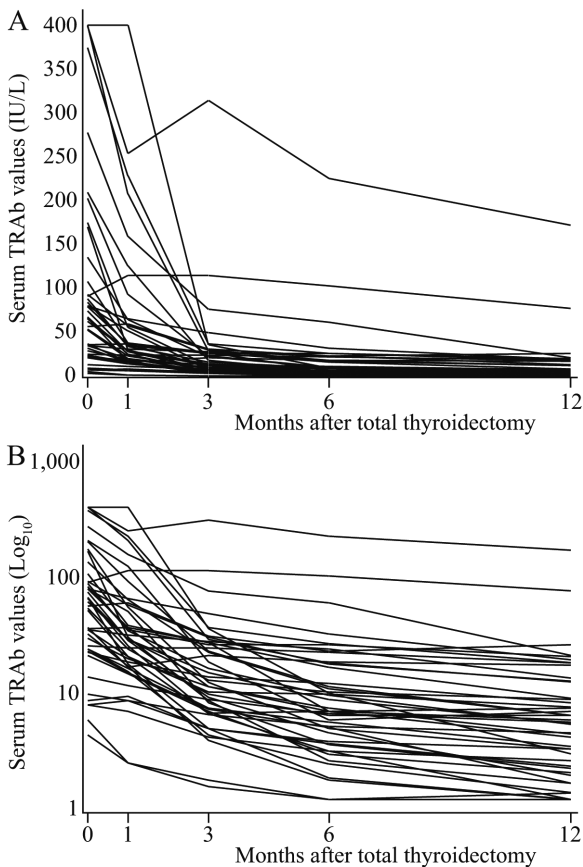
The characteristics of the 45 patients are summarized in Table 1. The median serum value of preoperative TRAb was 63.5 IU/L (range 5.6–400.0). Postoperatively the serum TRAb values decreased rapidly in most of the patients (Fig. 1). The slopes were steep in the early postoperative period of time of surgery to 3 months after surgery (the early postoperative period) and gradual in the later period of 3 to 12 months after surgery (the later period). The median reduction rate of TRAb values in the early postoperative period was 72.6% (range 8.1%–93.3%), which was significantly higher than that in the later period: 56.3% (range 6.1%–86.9%) ($p = 0.005$).

We calculated the median half-lives of the patients' serum TRAb, TgAb, and TPOAb values after surgery: 125.0, 110.4, and 112.2 days, respectively. There were no significant correlations among these half-lives in the patients. The analyses for factors that might influ-

Table 1 Clinical characteristics of the 45 Graves' patients who underwent a total thyroidectomy

Age	39.2 ± 13.4
Sex	Male 4 (8.9%) / Female 41 (91.1%)
Moderate to severe GO	Yes 7 (15.6%) / No 38 (84.4%)
Smoking	Yes 17 (37.8%) / No 28 (62.2%)
Resection weight of thyroid (g)	111.4 [28.3–333.1]
TRAb (IU/L) ^a	63.5 [5.6–400]
TgAb (IU/mL) ^a	475.2 [28–4,000]
TPOAb (IU/mL) ^a	394.7 [16–600]
TSH (mIU/L) ^a	0.003 [0.003–17.9]
FT4 (ng/dL) ^a	0.9 [0.4–2.2]
FT3 (pg/mL) ^a	3.5 [1.9–15.8]
Tg 1M (ng/mL) ^b	0.5 [0.5–187.5]
Tg 12M (ng/mL) ^b	0.5 [0.5–18.8]

^a Preoperative values are shown as median [range]; Values <0.003, <16, <28, >400, >600, and >4,000 were calculated as 0.003, 16, 28, 400, 600, and 4,000, respectively. ^b Tg 1M meant the median [range] Tg value in 1 mo. after surgery, and Tg 12M meant the median [range] Tg value in 12 mos. after surgery. The values < 0.5 were calculated as 0.5.

**Fig. 1** The changes of serum TRAb values after total thyroidectomy in 45 Graves' patients. TRAb values are shown in A and B (log₁₀). Undetectable levels <1.3 and >400 are shown as 1.3 and 400, respectively.

ence the half-life of TRAb revealed that the presence of active GO ($p=0.001$), smoking ($p=0.004$), and serum Tg value >0.5 ng/mL at 12 months postoperatively ($p=0.039$) were significant factors (Table 2). However, the weight of the resected thyroid and the preoperative TRAb values were not significantly correlated with the half-life of TRAb.

Based on this result, we divided the 45 patients into three groups with the presence of active GO and smoking status: GO (–) Smoking (–), $n=24$; GO (+) Smoking (–) or GO (–) Smoking (+), $n=18$; and GO (+) Smoking (+), $n=3$. We then compared the half-life of TRAb among these groups. The half-lives of serum TRAb values were 93.5 days (range 46.3–318.5) in the GO (–) Smoking (–) group, 162.5 days (range 56.9–1014.2) in the GO (+) Smoking (–) or GO (–) Smoking (+) group, and 357.4 ± 193.5 days in the GO (+) Smoking (+) group. The group of GO (–) Smoking (–) had significantly shorter TRAb half-lives compared to those of the other two groups (Fig. 2).

Since there were only two patients classified as GO (+) Smoking (–), we analyzed them in the same group as the GO (–) Smoking (+) patients. Even considering these two groups separately, the results were similar. Thus, the presence of active GO and smoking are risk factors for slowing the reduction of postoperative serum TRAb.

We examined 41 patients whose preoperative TRAb values were >10 IU/L, and we calculated the rate of achieving a serum TRAb level <10 IU/L after surgery. The achievement rate of this cut-off value was 70.7% at 12 months after surgery among these 41 patients. The TRAb half-life was significantly shorter in the patients who achieved the cut-off value than in the patients who did not: 97.4 days (range 47.2–348.9) vs. 252.4 days (range 60.5–1014.2), $p=0.003$ by Mann-Whitney U-test. On the other hand, the preoperative TRAb values were not significantly different between the patients who achieved the cut-off value and the patients who did not: 65.2 IU/L (range 11.0–361.9) vs. 88.5 IU/L (range 26.3–400.0), $p=0.065$ by Mann-Whitney U-test.

Among the 17 patients with one or both of the two risk factors mentioned above, *i.e.*, active GO and/or smoking, the rate of achieving a serum TRAb level <10 IU/L after surgery was lower (52.9%). By contrast, in the 24 patients without either of these risk factors, the achievement rate was higher (83.3%) (Fig. 3).

Table 2 Factors' correlations with the half-life of TRAb value

	<i>r</i>	<i>p</i> -value
Age	-0.275	0.065
Sex (Male: 1, Female: 0)	0.176	0.241
Moderate to severe GO (Yes: 1, No: 0)	0.477	0.001
Smoking (Yes: 1, No: 0)	0.422	0.004
Resection weight of thyroid (g)	-0.157	0.298
Preoperative TRAb (IU/L)	-0.080	0.596
Tg 1M (ng/mL) ^a	0.047	0.759
Tg 12M (ng/mL) ^a	0.324	0.039

p-values were obtained using Spearman's rank correlation coefficient. *p*-values <0.05 were accepted as significant and are shown in bold. ^aTg 1M = the Tg value at 1 month after surgery, and Tg 12M = the Tg value at 12 months after surgery. Values <0.5 were calculated as 0.5.

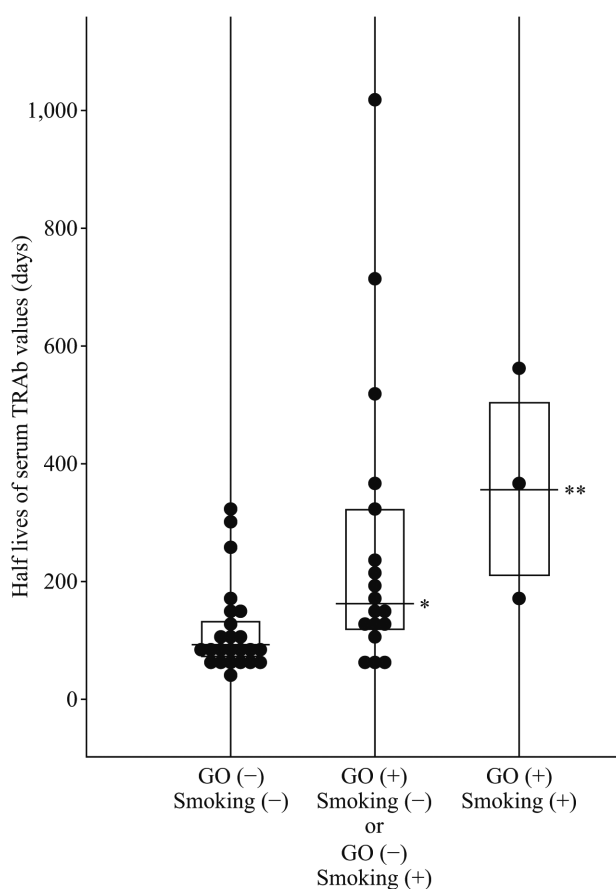


Fig. 2 Relationship between the half-life of the TRAb values and the presence of active GO or smoking status. The median half-lives of serum TRAb values were 93.5 days (range 46.3–318.5) in the GO (-) Smoking (-) group, 162.5 days (range 56.9–1014.2) in the GO (+) Smoking (-) or GO (-) Smoking (+) group, and 357.4 days in the GO (+) Smoking (+) group. * Significantly longer than that of the GO (-) Smoking (-) group ($p < 0.01$). ** Significantly longer than that of the GO (-) Smoking(-) group ($p < 0.05$).

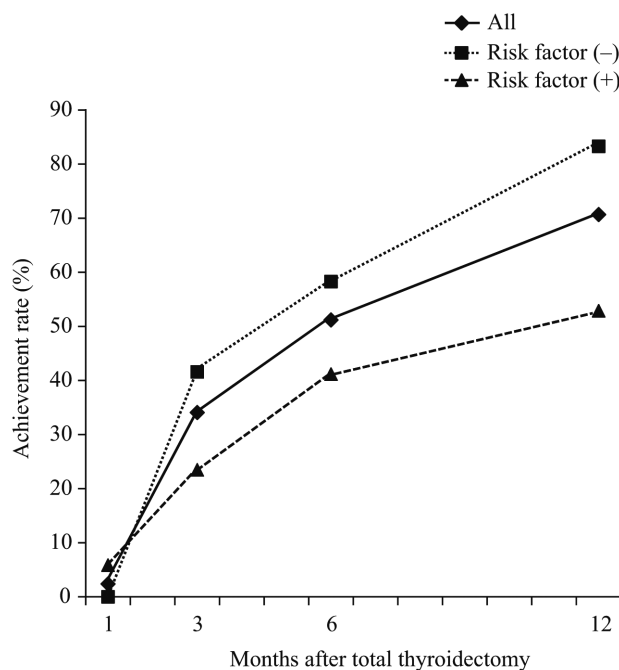


Fig. 3 The rates of achieving a serum TRAb value < 10 IU/L after surgery. The subjects were 41 patients whose preoperative TRAb values were > 10 IU/L. The rate of achieving this cut-off value was 2.4% at 1 mo., 34.1% at 3 mos., 51.2% at 6 mos., and 70.7% at 12 mos. after surgery among all 41 patients. Risk factor (-): The 24 patients without active GO or smoking. The achievement rate was 0% at 1 mo., 41.6% at 3 mos., 58.3% at 6 mos., and 83.3% at 12 mos. after surgery. Risk factor (+): The 17 patients with active GO and/or smoking. The achievement rate was 5.9% at 1 mo., 23.5% at 3 mos., 41.2% at 6 mos., and 52.9% at 12 mos. after surgery.

Discussion

This study, using a third-generation TRAb assay that is more informative than the conventional second-generation assay, demonstrated that total thyroidectomy was effective from the viewpoint of reducing serum TRAb values, a result which is consistent with that of our previous study [7] and reports by other groups [11–13]. The serum TRAb value was found to be reduced rapidly within the first 3 months as opposed to 3–12 months after surgery. These results were also consistent with those of our previous report [7].

Based on these past and present findings, it appears that the rapid disappearance of TRAb in the early post-operative period may be induced mainly by the removal of intrathyroidal B lymphocytes [12, 13]. In the later period (3–12 months post-surgery), the slow reduction

of TRAb that might be produced by lymphocytes outside the thyroid might affect the reduction of the TRAb value. Peripheral memory lymphocytes may continue to produce TRAb [14].

Several studies reported that the serum TRAb values usually decreased during pregnancy [3, 15–17]. As a possible mechanism of this, Amino *et al.* suggested that Th2-dependent humoral immunity is suppressed in human pregnancy [16]. However, in some cases including those after radioiodine therapy, the high serum TRAb values might not decrease during pregnancy, resulting in fetal hyperthyroidism [18, 19]. In light of these findings, surgery is considered the most secure treatment to reduce serum TRAb values in Graves' disease patients, and our present results support this therapeutic strategy.

It is thought that fetuses and neonates are susceptible to thyrotoxicosis if circulating levels of TRAb are approx. five times the upper limit of the normal range [1, 20–22] or > 10 IU/L [15]. We therefore used 10 IU/L as the cut-off value for TRAb in the present study. Our evaluation of the 41 patients whose preoperative TRAb values were > 10 IU/L suggested that the rate of achieving this cut-off value is more closely associated with TRAb's half-life than the preoperative TRAb value.

Our investigation of factors that may influence the high serum values of TRAb after total thyroidectomy revealed a number of interesting results. The presence of active GO, smoking, and Tg values > 0.5 ng/mL at 12 months after total thyroidectomy were shown to be risk factors for the prolongation of TRAb's half-life. The median half-life of the TRAb values was approx. 3 months in the patients without active GO or smoking and approx. 1 year in the patients with both active GO and smoking.

Several investigators reported that persistent high serum TRAb values were associated with a severe course of GO [23–27]. Although the TSH receptor antigen is considered to exist in the orbital adipose tissues and extra-ocular muscles, the precise pathogenetic link remains unclear. When patients have active GO, TRAb might be produced outside of the thyroid. From this viewpoint, the treatment of GO is also advisable when it is necessary to reduce a patient's TRAb value immediately.

It was reported that smoking increases both the relapse rate in patients with Graves' disease [28] and the risk of the progression of GO [29–31]. Quadbeck *et al.* [28] reported that smokers had significantly

higher serum TRAb values than those of non-smokers, which suggests that smoking modifies the immunological consequences in patients with Graves' disease. Although the biological mechanisms by which smoking contributes to the high serum level of TRAb are not yet clear, some reports suggested that smoking might influence cytokine secretion [32–34]. Moreover, the present study's patients with active GO and/or smoking had significantly longer TRAb half-lives compared to the patients with neither of these features. These data suggest that patients who want to become pregnant after undergoing a total thyroidectomy should quit smoking—especially patients with GO—and this should be carried out strictly.

Our study showed that the presence of Tg > 0.5 ng/mL after total thyroidectomy is associated with the prolongation of the half-life of serum TRAb values. Although TgAb also showed some influence, the continued presence of Tg in serum at 12 months after surgery indicated the presence of residual thyroid tissue. These results suggest that the persistent production of TRAb was caused by the remnant of thyroid antigen and auto-reactive lymphocytes. Nart *et al.* reported that the B cells infiltrating the thyroid follicles were the major source of TRAb [12], and De Bellis *et al.* reported that thyroidectomy seemed to lead to the disappearance of the antibodies due to the removal of thyroid auto-antigens and T and B autoreactive lymphocytes [13]. Thus, in a total thyroidectomy all thyroid tissue must be carefully removed.

In conclusion, our present findings indicate that measuring the half-life of serum TRAb values using a quantitative third-generation assay would be useful to evaluate the reduction of serum TRAb values after total thyroidectomy and to estimate the postoperative time needed to achieve the maternal safe value. The half-life of serum TRAb values also clarified the risk factors that impede the reduction of serum TRAb values. Our findings suggest that when patients with high TRAb values after total thyroidectomy wish to have children in the immediate or near future, it is necessary to consider a strategy such as GO treatment and/or non-smoking adherence to achieve the maternal safe TRAb value.

Disclosure

None of the authors have any potential conflicts of interest associated with this research.

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