

The Prognostic Value of Thyroid Stimulating Immunoglobulin in the Management of Graves' Disease

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Research article

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Abstract

Background: The bioassay of thyroid-stimulating immunoglobulin (TSI) appears to be more sensitive than the commonly used TSH binding inhibition assay (TBII), also known as thyroid receptor antibody assay or TRAb.

Methods: An observational study was conducted to evaluate the prognostic value of TSI in clinic. Patients with different etiologies of thyrotoxicosis were enrolled to verify the diagnostic value of TSI.

Results: 77 Graves' disease (GD) patients who were treated with anti-thyroid drug (ATD) were in a continuous follow-up until 1 year after ATD discontinuation. Commercial kits of TSI (Thyretain™) and M22-TBII were used and compared. TSI was all negative in healthy controls, Hashimoto thyroiditis, subacute thyroiditis. TSI value was highest in untreated GD patients ($P < 0.001$). Under ATD treatment, TSI value decreased gradually. 21 patients had positive TSI at the end of treatment. According to clinical fate of patients with GD after withdrawal ATD, TSI value and positivity in patients with relapse were significantly higher than that reported in patients with remission ($P = 0.001$, $P < 0.001$). After adjustment for age, gender, initial TRAb, initial TSI, and TRAb at the end of treatment, the odds ratio (OR) of positive TSI for the risk of relapse was 33.271 (95% confidence interval [CI]: 4.741-233.458, $P < 0.001$) and OR of quantitative TSI was 1.009 (95% CI: 1.002–1.015, $P < 0.001$).

Conclusions: TSI is a good predictor of relapse in patients with GD subjected to ATD treatment. It might be safer to discontinue ATD when TSI and TRAb were both negative.

Background

Graves' disease (GD) is a common organ-specific autoimmune disease caused by the circulating antibodies to thyroid-stimulating hormone receptor (TSHR) [1]. Circulating TSHR antibodies (TRAb) is a group of heterogeneous antibodies that comprises TSHR-stimulating antibody (TSAb), TSHR blocking antibody (TBAb), and neutral or cleavage antibody. TSAb and TBAb exert different immunochemical properties through the recognition of different epitopes, although with some overlapping on TSHR [2–3]. And different concentration of TSAb and TBAb is responsible for the phenotype of Graves' disease in clinic. TRAb measured by TSH binding inhibition assays (TBII) is the most commonly indicator to differentiate the etiology of thyrotoxicosis when thyroid radioactive iodine uptake (RAIU) is unavailable or contraindicated [4–5]. However, TBII for TRAb measurement failed to distinguish between TSAb and TBAb. Bioassays that detect the increased or inhibited cAMP production in cell lines may overcome the limitation of TBII. A commercial kit Thyretain™ comprises Chinese hamster ovary (CHO) cells transfected with a chimeric TSHR, wherein a TBAb epitope on the C-terminal portion is replaced with 73 amino acid residues from the rat luteinizing hormone (LH) receptor (Mc4) that specifically detects TSAb without any interference from TBAb [6–8]. In theory, TSAb measurement should be more accurate in diagnosis of thyrotoxicosis.

Three effective treatment options are available for GD patients, including RAI therapy, anti-thyroid drugs (ATDs), and thyroidectomy [9]. ATDs are preferred by many patients and physicians, owing to the advantages of convenience and non-invasiveness. However, the relapse rate reported in GD patients was high after the discontinuation of ATDs [10–11]. Hence, an easy and specific indicator that predicts relapse in patients with GD is desirable. Studies suggested that younger age, smoking, male, thyroid gland size, and high titers of TRAb at the end of therapy were associated with high relapse rate after treatment with ATD [12–14]. Patients with high titers of TRAb after the discontinuation of medication may face the risk of experiencing new hyperthyroidism and GD relapse [15–16]. Around 20% of patients are known to experience relapse even with low titers of TRAb. Given specific detection of TSAbs, thyroid-stimulating immunoglobulin (TSI) bioassay may be more useful in the management of patients with thyrotoxicosis. However, few studies have focused on whether TSI can effectively predict the prognosis of GD treated with ATDs [17].

In this study, we compared the results of TSI bioassay and TRAb (using M22-TBII) to investigate the diagnostic value and prognostic value of TSI from a follow-up group of patients with GD that were subjected to ATD treatment.

Methods

Study population

Participants in our study were recruited from Ruijin Hospital affiliated to Shanghai Jiao-tong University Medical School since March 2013. Patients with new onset GD (first diagnosed and receiving no therapy) were recruited, subjected to ATD therapy and followed up. The diagnosis of GD was made according to the standard criteria including: clinical symptoms, increased serum concentrations of free thyroxine (FT4) and/or free triiodothyronine (FT3), decreased basal thyrotropin (TSH), positive TRAb or diffusely increased thyroid RAIU when available. Under ATD treatment, patients were consecutively followed up at the endocrinology outpatient clinic in Ruijin Hospital. ATD treatment was discontinued when thyroid hormone concentrations were within the normal range and serum TRAb was lower than 1.75 IU/L for twice in succession [5]. After discontinuation of ATD, GD patients were continued to follow up every 3 months. According to clinical fate of patients with GD after withdrawal ATD within 12 months, patients were divided into 2 groups, group without relapse and group with relapse. Patients were considered to have relapsed if FT4 level was above the normal range, TSH level was low, or TRAb level was above 1.75 IU/L within 1 year (The follow-up protocol was summarized in supplemental Fig. 1). Serum TRAb concentration and TSI were measured at the time of GD diagnosis (untreated), GD undertreatment, GD at the end of treatment, and after ATD withdrawal for 12 months.

To validate the diagnostic value of TSI bioassay in GD, we recruited healthy controls, Hashimoto thyroiditis and subacute thyroiditis. Healthy controls were subjects with no personal and family history of thyroid disease, normal thyroid ultrasound imaging, normal thyroid function and negative results for antibodies to thyroid peroxidase (TPOAb), thyroglobulin autoantibodies (TgAb), and TRAb. Hashimoto

thyroiditis was diagnosed based on as an increase of at least five-fold in the serum level of antibodies to TPOAb with or without TgAb, a heterogeneous hypoechoic pattern in thyroid ultrasound imaging, and when available, a decrease in thyroid RAIU. Subacute thyroiditis was diagnosed based on clinical findings of severe neck pain and fever, elevated erythrocyte sedimentation rates (ESR), and low thyroid RAIU. All patients with Hashimoto thyroiditis or subacute thyroiditis enrolled in our study were all with increased serum concentrations of FT4 and FT3, decreased basal TSH in serum. Thyroid function, TRAb and TSI were measured at diagnosis. Patients with Hashimoto thyroiditis, subacute thyroiditis and healthy control were not included in the follow up program. Exclusion criteria were as follows: younger than 18-year old, prior treatment with surgery or radiotherapy, pregnant and lactating women, and subjects with other autoimmune diseases.

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of the Ruijin Hospital, Shanghai Jiao Tong University School of Medicine. Written informed consent was obtained from each participant.

Thyroid function and antibody tests

Serum TSH, FT3, FT4, TPOAb and TgAb were measured by automated chemiluminescent immunoassays (Architect i2000SR; Abbott Laboratories, Chicago, IL). The laboratory reference ranges provided by the manufacturer were used in this study: TSH 0.35–4.94 μ IU/mL, FT4 9.01–19.04 pmol/L, FT3 2.63–5.70 pmol/L, TPOAb < 5.61 IU/ml, and TgAb < 4.11 IU/ml. Serum levels of TRAb were measured by electrochemiluminescence immunoassays (Cobas 601 analyzer, Roche Diagnostics) with a suggested cut-off value of 1.75 IU/L.

Thyroid stimulating immunoglobulin bioassay

Serum was collected from each patient and stored in sterilization EP tubes at -80°C . After that, serum samples were transferred to laboratory for centralized measurements by skilled technicians who were unfamiliar with design of the study. TSI was measured with the Thyretain bioassay (Quidel, CA, USA) according to the manufacturer's instructions [18]. The results were reported as percentage of specimen-to-reference ratio (SRR%). SRR% values were calculated according to the following formula: $\text{SRR\%} = [\text{Average specimen relative light unit (RLU)} / \text{average reference control RLU}] \times 100$. The percentage coefficient variation (CV %) was calculated according to the formula: $\text{CV\%} = (\text{SD RLU specimen} / \text{Average Test RLU}) \times 100$. The assay cut-off for SRR% provided by the manufacture is 140%. All tests were performed in triplicate.

Statistical analysis

All statistics were analyzed using SPSS Statistics v19.0 (SPSS, Inc.) and P values < 0.05 were considered significant. We summarized demographic and laboratory characteristics as medians for continuous

variables, or numbers and percentages for categorical variables. Statistical difference was evaluated using Mann-Whitney U test for non-normally distributed variables. The Chi-square test or Fisher's exact test was used to test for categorical variables. Logistic regression analysis was carried out to evaluate the odds ratio (ORs) and 95% confidence interval (95% CIs) of relapse in quantitative and positive TSI group compared with negative TSI group.

Results

Comparison of TRAb and TSI in different etiologies of thyrotoxicosis

A total of 77 untreated GD patients were enrolled and followed up in our study. And a total of 36 Hashimoto thyroiditis, 17 subacute thyroiditis and 63 healthy control were also enrolled in our study to validate the diagnostic value of TSI. General characteristics of the study population was shown in Table 1. FT3, FT4 was highest and TSH was lowest in patients with untreated GD. TRAb titers and TSI value in participants were shown in Fig. 1. Both TRAb and TSI values were highest in the untreated GD than in healthy controls and patients with Hashimoto thyroiditis and subacute thyroiditis (all $P < 0.001$). The values of TSI were positive for patients with untreated GD (median SRR, 25% and 75% percentiles: 466.58%, 358.14%-547.60%). TSI values were all negative for patients with Hashimoto thyroiditis (median SRR, 25% and 75% percentiles: 48.68%, 40.12%-65.48%) and subacute thyroiditis (median SRR, 25% and 75% percentiles: 36.4%, 31.00%-45.85%) as well as the healthy control (median SRR, 25% and 75% percentiles: 57.95%, 49.97%-64.98%). TSI values gradually decreased with ATD treatment in GD patients (Fig. 1b and supplemental data). TSI value was (median SRR, 25% and 75% percentiles: 416.98%, 278.66%-543.23%) for GD patients undertreatment and (median SRR, 25% and 75% percentiles: 98.99%, 53.17%-139.86%) for GD patients at the end of treatment. TSI value for GD patients at the end of treatment was significantly lower than untreated GD patients ($P < 0.001$).

Table 1
Clinical characteristics of the study population

	Healthy Control	Untreated GD	Hashimoto Thyroiditis	Subacute Thyroiditis
gender(M/F)	16/47	18/59	6/30	5/12
Age(years)	46.71 ± 13.57	41.20 ± 11.94	40.00 ± 12.79 ^a	43.94 ± 7.59
FT3(pmol/L)	4.01(3.71–4.36) bcd	18.63(12.58–29.31) a	5.65(4.47–7.69) ^{ab}	6.69(5.79–13.33) ^a
FT4(pmol/L)	12.63(11.85–13.72) bcd	32.95(27.91–40.41) a	18.10(15.18–22.34) ^{ab}	26.39(20.88–38.95) ^a
TSH(mIU/L)	1.98(1.18–2.47) bcd	0.0006(0.0001–0.0016) ^a	0.009(0.003–0.7865) ^{ab}	0.004(0.002–0.0255) ^a
TPOAb(IU/L)	0.26(0.09–0.48) b	356.69(90.61–1000) acd	167.10(14.44–679.44) abd	0.60(0.25–2.27) bc
TgAb(IU/L)	1.84(1.14–3.13) b	76.02(18.40–391.20) acd	51.61(14.17–660.03) ad	4.29(1.45–21.45) bc
TRAb(IU/L)	0.30(0.30–0.30) bcd	6.64(4.17–12.93) acd	0.30(0.30–0.88) ^b	0.44(0.30–0.54) b
TSI (SRR%)	57.95(49.97–64.98) ^b	466.58(358.14–547.60) acd	48.68(40.12–65.48) ^b	36.4(31.00–45.85) ^b
CV%	4.21(1.68–7.73)	3.33(1.49–6.18)	4.16(2.13–7.69)	2.35(1.37–6.48)
<p>TSI: thyroid-stimulating immunoglobulin; SRR: specimen-to reference ratio; CV: coefficient variation; SRR% and CV% were calculated according to the manufacturer's formula; SRR%= [Average specimen relative light unit (RLU) / average reference control RLU] × 100. CV%= (SD RLU specimen / Average Test RLU) × 100. compared to healthy control, ^aP<0.05; compared to untreated GD, ^bP<0.05; compared to Hashimoto thyroiditis, ^cP<0.05; compared to Subacute thyroiditis, ^dP<0.05; P value was calculated by Mann-Whitney U test.</p>				

The association of TSI with the risk of relapse in patients with GD

To investigate the association of TSI value with the risk of relapse in patients with GD, 77 patients were continued to follow up till discontinuation of ATD. 77 (18 males and 59 females) GD patients discontinued ATD treatment when serum TRAb was 1.75 IU/L or less for twice in succession and thyroid hormone concentrations were within the normal range. 70 (90.9%) patients were treated with methimazole and 7 (9.1%) patients treated with Propylthiouracil. A total of 21 (27.3%) patients were

positive for TSI measurement at the end of treatment and 19 (24.7%) patients showed relapse within 12 months after ATDs discontinuation.

According to clinical fate of patients with GD after withdrawal ATD within 12 months, patients were divided into 2 groups, group without relapse and group with relapse. Their clinical characteristics are summarized in Table 2. Age, thyroid function parameters at the end of treatment, including TSH, FT3, and FT4, showed no significant difference between two groups. In addition, no significant difference was observed in the TRAb level before treatment and at the end of treatment. The average ATDs therapy duration showed no significance difference between patients with relapse and patients with remission (for relapse patients was 15.14 ± 7.56 months and 16.62 ± 5.83 months for patients with remission, $P = 0.467$). However, TSI level at the end of treatment was significantly higher in relapse group ($P = 0.001$) but showed no significant difference from the value reported during diagnosis. Of 19 patients with relapse, 13 patients were positive for TSI at the end of treatment and 6 patients were negative for TSI. As shown in Fig. 2a, the relapse rate was significantly higher in the positive TSI group than in the negative TSI group (61.9% [13/21] versus 10.71% [6/56], respectively, $P < 0.001$). Based on the cutoff value of SRR 140%, Positive predictive value (PPV) and negative predictive value (NPV) for TSI in the relapse group was 61.9% (13/21) and 89.28% (50/56), respectively. As patients in our study were all TRAb negative (cutoff for TRAb < 1.75 IU/L) upon ATD discontinuation, PPV and NPV for TRAb in the relapse group was 0 (0/77) and 75.32% (58/77), respectively.

Table 2
Clinical data of Graves' disease patients with or without relapse at the end of treatment

	with relapse	without relapse	<i>P</i> value
Gender(M/F)	5/14	13/45	0.462
Age (years)	41.86 ± 12.61	43.50 ± 12.64	0.860
FT3 (pmol/L)	4.13(3.98–4.80)	4.11(3.84–4.46)	0.211
FT4 (pmol/L)	13.35(12.64–14.85)	13.31(12.75–14.66)	0.655
TSH (mIU/L)	1.37(0.45–1.87)	1.57(1.09–2.18)	0.127
TRAb (IU/L)	0.70(0.51–0.97)	0.61(0.38–0.87)	0.136
TRAb at diagnosis (IU/L)	5.30(3.60-10.13)	7.06(3.72–16.19)	0.331
TSI (SRR%)	216.54(112.06-377.04)	98.99(65.06-128.51)	0.001
TSI at diagnosis (SRR%)	491.9(369.90-589.70)	461.55(336.33-549.08)	0.446
CV%	5.74(2.71–9.46)	4.70(1.30–7.41)	0.312
Positive TSI (n)	13	8	< 0.001
Negative TSI (n)	6	50	
Duration of ATD treatment (months)	15.14 ± 7.56	16.62 ± 5.83	0.467
TSI: thyroid-stimulating immunoglobulin; SRR: specimen-to reference ratio; CV%: coefficient variation; SRR% and CV% were calculated according to the manufacturer's formula; SRR%= [Average specimen relative light unit (RLU) / average reference control RLU] × 100. CV%= (SD RLU specimen / Average Test RLU) × 100. <i>P</i> value was calculated by Mann-Whitney U test.			

To analyze the longitudinal TSI measurements in patients with relapse or with remission, we plotted TSI values at the time of ATD withdrawal and 1 year after ATD withdrawal (Fig. 2b) and found that TSI values significantly decreased only in remission patients. On the contrary, TSI values increased in patients with relapse.

Logistic regression analysis of quantitative or positive TSI and relapse

After adjustment for age, gender, TRAb at diagnosis, TSI at diagnosis, and TRAb at the end of treatment, the odds ratio (OR) of TSI positivity (SRR > 140%) at the end of treatment for the risk of relapse was 33.271 (95% CI: 4.741-233.458, *P* < 0.001) and OR of quantitative TSI was 1.009 (95% CI: 1.002–1.015, *P* = 0.008) (Table 3).

Table 3

Association of TSI measurement with relapse in Graves' patients treated with anti-thyroid drugs

	OR (95%CI)	<i>P</i>	OR (95%CI)	<i>P</i>
Age	0.983(0.921–1.036)	0.517	0.964(0.906–1.026)	0.252
Gender (Male = 1)	0.397(0.092–1.707)	0.215	0.315(0.062–1.612)	0.166
TSI at diagnosis	0.999(0.994–1.005)	0.773	0.996(0.989–1.002)	0.183
TRAb at diagnosis	0.982(0.91–1.059)	0.636	0.966(0.886–1.054)	0.443
TRAb at the end of treatment	3.029(0.542–16.933)	0.207	3.685(0.574–23.648)	0.169
TSI at the end of treatment	1.009(1.002–1.015)	0.008	/	/
TSI positivity (SRR > 140%) at the end of treatment	/	/	33.271(4.741-233.458)	< 0.001
TSI: thyroid-stimulating immunoglobulin; SRR: specimen-to reference ratio; OR: odds ratio; 95% CI: 95% confidence interval.				

Discussion

In this study, we screened patients with common etiologies of thyrotoxicosis and demonstrated that TSI exhibits high sensitivity and specificity in the management of patients with GD. We found that TSI were all positive in patients with GD, whereas TSI were all negative in Hashimoto thyroiditis, subacute thyroiditis and healthy controls. The accurate diagnostic value of TSI in GD was also reported in other studies [2, 8, 18–19]. Thus, TSI could be used in differential diagnosis of the etiology of thyrotoxicosis.

In addition, the analysis of TSI level after medication in a follow-up group of patients with GD revealed the correlation between TSI and relapse. Several studies have been performed to investigate if TSI may predict relapse or remission after ATD therapy and better sensitivity and specificity has been reported. In a retrospective study conducted in 92 patients with GD that were treated with ATD in Korea, M22-TBII and Mc4-TSAb titer and positivity were significantly higher than in those with remission [20]. Based on the manufacturer's cutoff value (1.75 IU/L for M22-TBII and 140% for Mc4-TSAb), two assays showed similar sensitivities, specificities, NPV, and PPV. However, higher sensitivity (85%) and PPV (69%) were reported with a higher Mc4-TSAb cutoff value (230%) than with the best M22-TBII cutoff value. In another 5-year follow-up prospective study conducted in 55 patients with GD, TSI (Thyretain) has a trend toward improved negative predictive value (82.6 vs. 81.8 and 76.9%) and sensitivity (80 vs. 77.8 and 70%) comparing Mc4, and M22 assays, respectively [17]. Prior to the availability of the commercial kit for Mc4-TSAb bioassay, TRAb measurement at the end of ATD therapy was considered as a useful indicator of relapse rate in patients with GD. Thus, ATD was recommended to be discontinued if TSH and TRAb levels decreased to the normal reference range reported in the recent clinical guidelines [5]. However, the predictive value of TSI in patients that discontinued ATD and displayed negative TRAb value has not

been documented. In our study, we followed up patients with GD until 1 year after ATD withdrawal and analyzed TSI performance in patients with relapse. TSI value and positivity at the end of treatment was significantly higher in patients with relapse than in patients with remission. TSI showed a predictive value of relapse in GD group, with 61.9% PPV and 89.28% NPV when TRAb has lost of its positive predictive value. Our data suggest that the clinical fate of relapse or remission in patients with GD after the withdrawal of ATD therapy may be predicted from the trend of TSI values, as confirmed from the logistic regression analysis. After adjustment for age, gender, TRAb at diagnosis, TSI at diagnosis, and TRAb at the end of treatment, we found that quantitative TSI and TSI positivity are independent predictors of relapse in patients with GD. These results showed that about 20% GD patients with negative TRAb turn into relapse, and ATD treatment should be prolonged since the majority of these patients with positive TSI.

Through the expression of Mc4 TSHR chimera in CHO cells, TSI (ThyretainTM) was reported to be more specific in detecting TSAb and may detect lower titers of TRAb. The analytical performance of TSI and TBII assays by serial dilution of certain sera and found that TSI was positive at a dilution of 1:300, suggesting that TSI bioassay may detect lower levels of TSHR autoantibodies [21]. The presence of TSI may serve as the best predictor of relapse or remission in patients with GD following ATD therapy withdrawal. A minority of patients with positive TSI values showed no relapse. We evaluated the tendency of TSI and found that the TSI level would gradually decrease in these patients. However, the TSI level significantly increased in relapsed patients with positive TSI values. In other words, withdrawal of ATD when TSI was negative would significantly reduce the rate of relapse in patients with GD.

The strengthen of our study was that we investigated TSI measurement in the management of GD patients. It was a longitudinal observation study. All GD patients were subjected with ATD treatment and were in continuous follow-up. The limitation was that the follow-up time after withdraw ATD treatment was only 12 months.

Conclusion

In conclusion, our study validated the diagnostic value in different etiologies of thyrotoxicosis and the prognostic value of TSI bioassay in patients with GD that were subjected to ATD therapy. We demonstrated that TSI exerted advantages in the management of GD and serves as a good predictor of relapse in patients with GD treated with ATDs. Withdraw ATD when TSI was negative could significantly reduce the risk of relapse.

Abbreviations

GD: Graves' disease; TSHR: thyroid-stimulating hormone receptor; TRAb: TSHR antibodies; TSAb: TSHR-stimulating antibody; TBAb: TSHR blocking antibody; RAIU: radioactive iodine uptake; TSH binding inhibition assays (TBII); CHO: Chinese hamster ovary; TSI: thyroid-stimulating immunoglobulin; ATD: anti-thyroid drugs; FT4: free thyroxine; FT3: free triiodothyronine; TSH: thyrotropin; TPOAb: antibodies to

thyroid peroxidase; TgAb: thyroglobulin autoantibodies; ESR: erythrocyte sedimentation rates; SRR: specimen-to reference ratio; RLU: relative light unit; CV: coefficient variation; OR: odds ratio; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value

Declarations

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of review board of Ruijin Hospital affiliated to Shanghai Jiao Tong University Medical School and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from each participant.

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Authors' contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Xinxin Chen, Yulin Zhou, Mengxi Zhou and Yicheng Qi. Patient recruitment were performed by Weiqing Wang, Guang Ning and Shu wang. The first draft of the manuscript was written by Xinxin Chen and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no conflict of interest.

Declaration of conflict interests

None.

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Figures

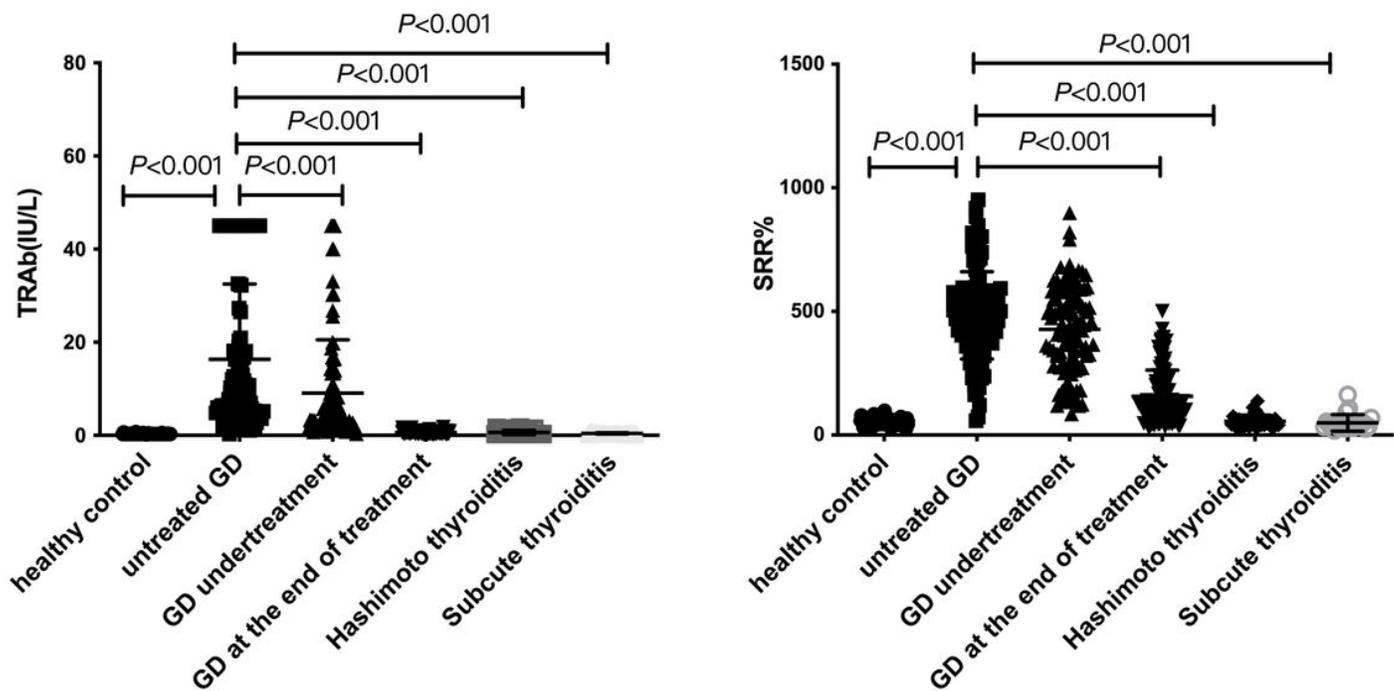


Figure 1

Comparison analysis of TSH binding inhibition assay and thyroid-stimulating immunoglobulin (TSI) in healthy control and different etiologies of thyrotoxicosis. According to thyroid function and treatment, patients were divided into untreated GD group (new onset), GD undertreatment group and GD at the end of treatment. Fig.1a showed TRAb titers by means of TSH binding inhibition assay and Fig.1b showed thyroid-stimulating immunoglobulin (TSI) values in healthy control and different etiology of thyrotoxicosis. P values were calculated by a Mann–Whitney U test.

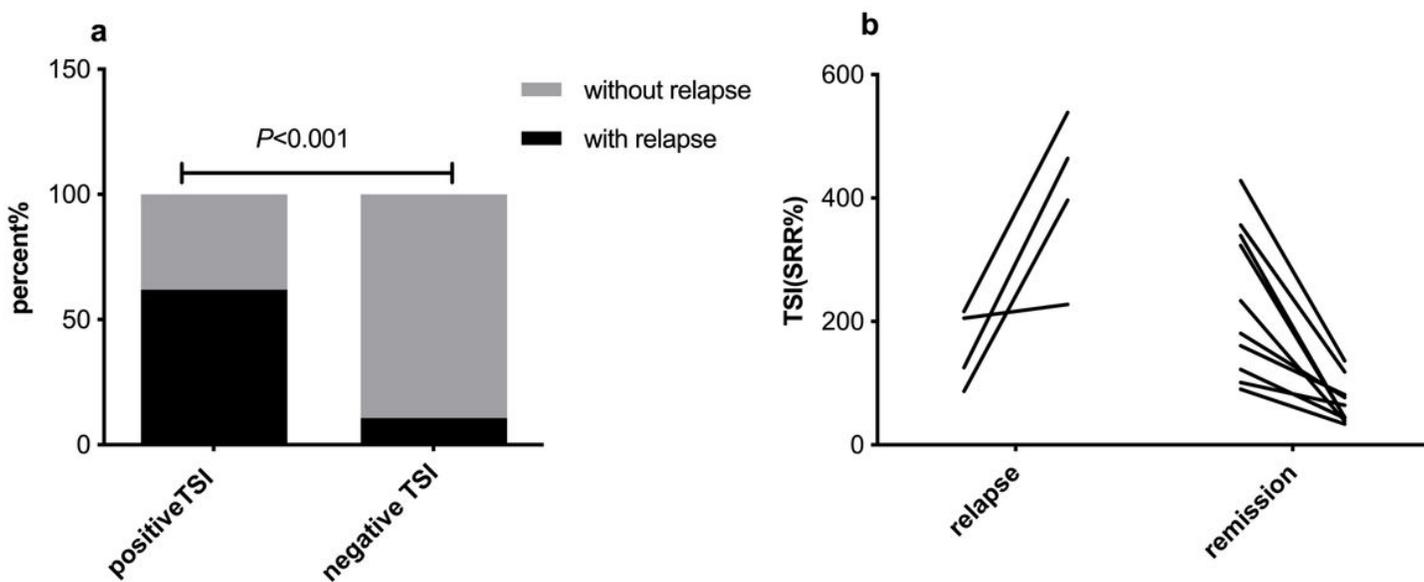


Figure 2

Higher relapse rate in positive thyroid-stimulating immunoglobulin (TSI) group at the end of anti-thyroid treatment in patients with Graves' disease Fig.2a showed relapse rate in positive and negative thyroid-stimulating immunoglobulin (TSI) group respectively. P value was calculated by a Chi-square test; Fig. 2b plotted the change of TSI from the end of treatment to one year after stopping medication. in Graves' disease patients with relapse (n=4) or with remission(n=11) at the end of treatment.

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