TSH receptor antibodies for confirming the diagnosis and prediction of remission duration, in newly diagnosed Graves' disease patients

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Abstract

Antibodies to TSH receptors (TRAb) have great pathogenetic importance in the development and maintenance of hyperthyroidism in Graves' disease (GD). Measurement of their serum level could be of diagnostic and prognostic importance in autoimmune hyperthyroidism and in monitoring the efficiency of thyrosuppressive drug (TSD) treatment in GD. *The aim* of our study was to assess the sensitivity (SN), specificity (SP), positive predictive value (PPV) and negative predictive value (NPV) of the TRAb assay in confirming the diagnosis and to define its prognostic value for remission duration in GD patients at the end of TSD treatment with thionamide derivatives, propylthiouracil or methimazole. *We studied* 99 patients, 65 female, 34 male, 18-76 years old; 52 GD patients and 47 as control group. We have studied frequency of relapses and remission duration in GD patients by a 2nd generation serum TRAb commercial kit. *Our results* showed that, the SN, SP, PPV and NPV of the TRAb test were 100%, 97%, 98% and 100%, respectively. Remission duration after TSD treatment was longer and relapses were fewer in GD patients with lower levels of TRAb before ($P<0.01$) and at the end of TSD treatment ($P<0.05$). *In conclusion*, our results suggest that serum TRAb is very sensitive and specific for confirming the diagnosis of GD. TRAb levels at the beginning of TSD treatment, above 5IU/L gives 18% greater chance, and above 15IU/L, 36% greater chance for remission, shorter than 6 months.

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Introduction

Graves' disease (GD) is defined as hyperthyroidism with diffuse goiter caused by immunogenic disturbances. The reported incidence of GD disease is up to 80/100 000 per year in women and 8/100 000 per year in men [1]. In regions with adequate iodine supply, GD is the most frequent type of hyperthyroidism [1].

There are many subpopulations of TSH receptor antibodies in GD patients, each with different biological activity such as stimulation of TSH receptor, inhibition of TSH binding or stimulation of thyroid growth. Radio receptor assays (RRA), measure immunoglobulin, which inhibits TSH binding on TSH receptors (TBII). Thus, using these tests it is not possible to confirm thyro-stimulating activity of antibodies.

Antibodies on thyroid gland TSH receptors (TRAb) have significant pathogenic importance for the development and maintenance of autoimmune hyperthyroidism as in GD. TRAb relate to a great number of TSH activities: iodine accumulation, synthesis and release of iodo-thyronines, synthesis of DNA and cells growth [2-6]. The incidence of TRAb in the sera of the patients with GD, according to different reports varies between 70%-100% at the time of diagnosis [7-13].

The level of TRAb is a useful parameter in prognosis and monitoring thyro-suppressive drug (TSD) efficiency in GD [7]. The lack of TRAb level normalization during TSD treatment could be associated with persisting gland hyperactivity. In patients with a longstanding increased TRAb level (non responders), stable remission is not likely to occur. In these cases it is reasonable to choose ablative treatment: thyroidectomy or radioiodine. Increased levels of these auto-antibodies in GD in remission, preceded with great probability relapse and in healthy persons may precede the development of GD [7].

Nevertheless, there are different aspects about the usefulness of diagnostic and clinical importance of TRAb measurements. Most of the new reports show the clinical value of TRAb in diagnosis and monitoring TSD treatment in GD, while some older reports underscore their value, especially when the first choice treatment is radioiodine [14-22].

We assessed of the value of TRAb in clinical practice, in terms of confirming the diagnosis, in terms of remission duration and in frequency of relapses in GD patients.

Patients and methods

We studied 52 consecutive, newly diagnosed GD patients , 45 females and 7 males, with average age 41.95±12.80 years and 47 controls without clinical and biochemical signs of thyroid disease, 27 males and 20 females with average age of 63.04±8.67 years. All patients were from Timok region of Serbia and were receiving sufficient iodine supply from iodinated salt. Diagnosis of the thyroid status of each patient was made clinically and by the levels of thyroid hormones, iodine-131 (^{131}I)-uptake test (RAIU), technetium-99m pertechnetate ($^{99m}TcO_4^-$) uptake test (TcU) and by thyroid gland scintigraphy.

Treatment with TSD was performed by administering an initial dose of 40mg/day methimazole (MMI) or 300mg/day of propylthiouracil (PTU). Doses were gradually decreased and discontinued when the patients were clinically and biochemically euthyroid for at least 3 months with the minimum maintenance dose (MMI 5mg/day or PTU 25mg/day). TRAb was measured at the beginning of TSD treatment and when planning discontinuation of treatment according to previously mentioned criterion.

Free thyroid hormones, triiodothyronine (FT3) and thyroxine (FT4) were measured by competitive luminescent immunoassay and dissociation-enhanced lanthanide fluorescence immunoassay (LIA-FT4 Brahms Diagnostica GMBH, Germany and DELFIA FT3 Wallac Oy, Turku, Finland). The range of normal values was 9.25-25pmol/L for FT4 and 3.5-9.0pmol/L for FT3. TSH was measured by ultrasensitive DELFIA method (DELFIA, h-TSH Ultra Wallac Oy, Turku, Finland) with reference range of 0.1-4mU/L. TRAb was measured by radio receptor method (DYNO test TRAK human, Brahms Diagnostica GMBH, Germany) with normal values up to 1IU/L. Rank level of R1 (2-4IU/L), R2 (5-15IU/L) and R3 (>15IU/L) was considered as positive. The principle of RRA assay for measuring TRAb is as follows: TRAb from the patient's sera binds on the recombinant human TSH receptor on coated test tubes. Then, bovine ^{125}I-TSH is added and binds on the remaining free TSH receptors on the coated tubes. Bound TSH is measured in the gamma counter and this measurement is inversely proportional to the quantity of TRAb in the tested sample. TRAb concentration is calculated from a standard curve of samples with a known TRAb concentration. The biological activity (stimulatory or inhibitory effect on the TSH receptor) of such measured TRAb is not known.

RAIU was measured after 3h and/or 24h of oral application of 0.37–0.74MBq Na ^{131}I by a scintillation probe with NaI (Tl) crystal, 3X3 inches and a divergent collimator of a rectilinear scanner (Pho/Dot Scanner, Nuclear Chicago, USA). Reference range of RAIU was 8%-20% for 3h and 20%-45% for 24h. Thyroid scintigraphy, that confirmed the diagnosis was performed 15-20min after the intravenous (i.v.) injection of 37-74MBq of $^{99m}TcO_4^-$. A gamma camera (Siemens Diacam, Germany) was used. A short ^{99m}Tc uptake test (TcU) was done on a gamma camera (PHO/gamma IV Searle Radiographics, USA) with a LPP collimator as follows: after the i.v. injection of about 50-74MBq of $^{99m}TcO_4^-$, radioactivity over the thyroid gland was continuously measured for 5min. HISTO program was used: from the region of interest over the thyroid gland and a curve activity/time was plotted. TcU was calculated from the ratio of the activity rate increment expressed in impulses per sec and the activity over a limit stop A, which divides the end of "vascular " phase from the beginning of an early accumulation of the radionuclide. The increment is calculated in the linear part of the curve marked by limit stop A, at a limit stop B put at the end of the linear increment, more often for about 3min. Calculations were done by multiplying the product of the increment and the activity over A by 1000. All values over 5 were considered increased and less than 1 decreased.

Statistical analysis

The results were expressed in tables and Box-plot graphics. The age of the patients, TSD treatment duration and remission duration for all groups for those values with variation coefficient greater than 30% were expressed as a mean value ± SD or median (Me). SN, SP, PPV and NPV for TRAb were calculated using the usual formulas. The TRAb at the beginning of TSD treatment and at the end of treatment were analyzed by Wilcoxon Signed Ranks Test and expressed with Box-plot graphics.

Logistic regression was used to calculate crude OR for prognostic factors of remission duration. Statistical significance was set as $P<0.05$ using statistical software SPSS (version 15).

Results

TRAb average levels as well as rank levels in newly diagnosed GD patients at the beginning of the TSD treatment and of the control group are shown in Tables 1 and 2. The SN, SP, PPV and NPV of TRAb test were 100%, 97%, 98% and 100%, respectively. At the beginning of newly diagnosed disease, all patients had well increased TRAb. At the end of treatment 52% of GD patients had low levels of TRAb.

Table 1. *Number of GD patients and average level of TRAb according to the rank levels at the beginning of the TSD treatment*

	TRAb (IU/L)			
	R0 (<2)	R1 (2-4)	R2 (5-15)	R3 (>15)
N(%)	0(0.0)	4(7.6)	24(46.2)	24(46.2)
Range		2.1-4	4.3-14.6	15.6-113
Mean±SD		2.85±0.82	8.85±3.36	39.38±29.58
Median		2.65	8.35	25.1

R0: rank 0, TRAb<2IU/L; R1: rank 1, TRAb 2-4 IU/L; R2: rank 2, TRAb 5-15 IU/l; R3: rank 3, TRAb>15 IU/L; N: number of patients; SD: standard deviation; Median: median values.

Table 2. *Number of subjects of the control group and average level of TRAb according to the rank levels*

	R0 (<2)	R1 (2-4)
N	46	1
Range	0.1 – 0.7	2.5
Mean± SD	0.41±0.19	
Median	0.4	

N: number of patients; SD: standard deviation.; Median: median values; R0: rank 0, TRAb < 2IU/L; R1: rank 1, TRAb 2-4 IU/L.

Figure 1 shows the median value of TRAb at the beginning of TSD treatment and at the end of treatment. TRAb level before TSD treatment was significantly higher than after TSD treatment (Wilcoxon Z=4.123; P=0.001).

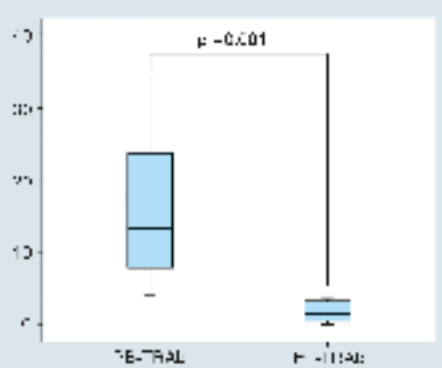
There was highly significant difference in remission duration in GD patients in relation to the rank level of TRAb at the beginning of TSD treatment (P<0.01) and low at the end of treatment (P<0.05). We also found highly significant difference in the number of relapses in GD patients in relation to the rank level of TRAb at the beginning of TSD treatment (P<0.01) and at the end of treatment (P<0.01). There were no significant differences in remission duration and in the number of relapses related to TRAb according to gender.

In univariate logistic regression, TRAb level greater than 2 IU/ml is statistically significant risk factor (OR=1.476; 95% CI=0.976-1.859; P=0.008) for shorter remission.

Comparing with TRAb rank 1 at the beginning of TSD treatment, rank 2 gives 18% (OR=1.182; 95% CI=0.142-9.827; P=0.04), and rank 3 two times greater chances (OR=2.000; 95% CI=0.236-16.928; P=0.03), for remission shorter than 6 months (Table 3).

Discussion

In GD patients, TRAb and RAIU are typically increased. On the other hand, decreased TRAb and low RAIU are present in almost all patients with transitory, destructive thyroiditis such as subacute thyroiditis (SAT) and painless thyroiditis (PT) [23-25]. It was found that TRAb level is much higher in GD than in hyperthyroidism of Hashimoto's disease, while thyroid peroxidase antibodies (TPOAb) and thyroglobuline antibodies (TGAb) are much higher in Hashimoto's disease than in GD. Clinical diagnosis of disseminated thyroid autonomy (DTA) can be made only by excluding GD [26, 27]. In both diseases, autoimmune hyperthyroidism and autonomous hyperthyroidism with disseminated pattern, scintigraphic findings are the same and in the absence of clinical signs of thyroid ophtalmopathy, positive TRAb can confirm autoimmune hyperthyroidism [27]. The use of a new generation of RRA (DYNO test TRAK human Brahms Diagnostica GMBH, Germany) shows that about one fifth of the patients previously classified as disseminated thyroid autonomy are TRAb positive, suggesting the presence of autoimmune hyperthyroidism [26, 17]. High incidence of TRAb in multinodular toxic goiter (MTG) could reflect overlapping of GD and MTG in some patients [26-28].



PB-TRAb- RAb level at the beginning of TSD treatment;
ET-TRAb-TRAb level at the end of treatment; y axis-level of TRAb in IU/L.

Figure 1. Level of TRAb at the beginning of TSD treatment and at the end of treatment.

Table 3. *Prognostic factors analysis for remission duration (Binar logistic regression)*

Factor	OR	95%CI	P
BT – TRAb (IU/L)			
[<2]	1		
>2	1.476	0.976-1.859	0.008
BT - TRAB rank			
[1]	1		
2	1.182	0.142-9.827	0.04
3	2.000	0.236-16.928	0.03
Goiter			
[No]	1		
Dif1	2.250	1.179-13.967	0.384
Dif2	16.000	1.093-234.248	0.043
Ophthalmopathy			
[No]	1		
Yes	1.653	0.534-5.112	0.383

[]-Reference category; BT-beginning of TSD treatment; Dif1-palpatory small diffuse goiter; Dif2- palpatory bigger diffuse goiter; Ophthalmopathy No-absence of clinical signs of ophtalmopathy; Ophthalmopathy Yes-presence of clinical signs of ophthalmopathy of various degrees.

We realize that our control group consisted of much older subjects and more males than the GD group, which may have induced lower normal levels for TRAb. On the other hand our results are really based on comparing the levels of TRAb in the same GD patients before and during TSD.

Our results support the view that TRAb measurements have excellent SN, SP, PPV and NPV for confirming the diagnosis of GD. The fact that diffuse goiter is present in more than one third of our patients with non immune hyperthyroidism, and that clinical signs of ophthalmopathy are not present in almost two thirds of our GD patients indicates the usefulness of TRAb assay as a specific diagnostic test for GD.

There have been continuous efforts to identify the clinical signs of GD which could predict remission and relapse after TSD withdrawal [14, 15]. Reports show long term remission in 14%-80% of treated GD patients [17, 29-35]. The total relapse rate in GD patients treated with TSD is 30%-50% [29, 36-39]. Forty-four percent of our GD patients were in remission after at least 6 months of follow-up. The rest had relapses. Most of

the patients with relapses had one to two relapses of the disease and were treated by TSD. The number of relapses was significantly greater in GD patients with higher TRAb levels at the beginning of the disease and at the end of TSD treatment (P<0.01 for both). According to others, TRAb level is a useful parameter in monitoring TSD treatment efficiency in GD [38]. High initial levels of TRab are a bad prognostic factor [37]. Low TRAb levels at the end of TSD treatment are significantly associated with a higher risk for relapse [36-41]. We found a shorter remission duration in patients with higher TRAb at the end of TSD treatment (P<0.05). TRAb levels higher than 2 IU/ml is a statistically significant risk factor for a shorter duration of remission.

At the beginning of TSD treatment, comparing TRAb rank 1, with rank 2 gives 18% and with rank 3 gives twice greater chance, for remission shorter than 6 months.

Some show a correlation between TRAb concentration and the size of thyroid gland [42]. In our GD patients we haven't found such a correlation at the beginning of the disease, but goiter size was an independent risk factor for shorter remission. Clinical signs of ophthalmopathy were more frequent in patients with higher levels of TRAb at the beginning of the disease as others have reported, and yields greater risk for shorter remission [43- 46].

In conclusion, our results suggest that serum TRAb is very sensitive and specific for confirming the diagnosis of GD. TRAb level at the beginning of TSD treatment, above 5IU/L gives 18% greater possibility, and above 15IU/L, twice that possibility for remission shorter than 6 months.

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