**THYROID-STIMULATION HORMONE - RECEPTOR ANTIBODIES AS A PREDICTOR OF THYROSUPPRESSIVE DRUG THERAPY OUTCOME IN GRAVES’ DISEASE PATIENTS**

**Antitestele na receptore tiroidnog stimulisanog hormona kao prediktor ishoda medikamentne tirosupresivne terapije kod pacijenata sa Grejvsovom bolešću**

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**Summary**

**Introduction.** Graves’ disease is autoimmune hyperthyroidism caused by pathological stimulation of thyroid-stimulation hormone – receptor antibodies. The decision on changing the therapy can be made on time by determining the prognostic factors of thyrosuppressive drug therapy outcome. The aim of the study was to determine the significance of thyroid-stimulation hormone - receptor antibodies level on the prediction of therapy outcome. **Material and Methods.** The study was prospective and involved 106 drug-treated patients with newly diagnosed Graves’ disease. Thyroid-stimulation hormone - receptor antibodies level was measured at the beginning of therapy, during therapy and 12 months after it had been introduced. **Results.** No statistically significant difference in the level of thyroid-stimulation hormone - receptor antibodies was found at the beginning of disease and 12 months after the introduction of thyrosuppressive drug therapy among the patients who had been in remission and those who had not. Regardless of the outcome, thyroid-stimulation hormone - receptor antibodies level significantly decreased in all patients 12 months after the therapy had been introduced. **Conclusion.** The level of thyroid-stimulation hormone - receptor antibodies at the beginning of disease and 12 months after the introduction of therapy cannot predict the outcome of thyrosuppressive drug therapy. **Key words:** Graves Disease; Receptors; Thyrotropin-Releasing Hormone; Antibodies; Treatment Outcome; Antithyroid Agents; Remission Induction; Recurrence; Prognosis

**Introduction**

Graves’ disease (GD) is an organ-specific autoimmune disorder characterized by hyperthyroidism with diffuse goiter and a possible presence of extra-thyroid manifestations such as eye changes (thyroid ophthalmopathy), skin change (dermopathy) and fingertips change (acropathy) [1, 2]. According to some estimation, GD is present in up to 1% of the population. In areas with adequate iodine supply, Graves’ disease is the most frequent type of hyperthyroidism [1].

Etiology of GD is multifactorial: environmental factors, immune aberrations and minimal changes in the target organ probably interact with each other within the genetic predisposition. Among other things, vitamin D receptor gene polymorphism is associated with an increased risk for the occurrence of autoimmune thyroid disease. Possible external factors which play a role in the occurrence of disease are some of infectious agents.
The presence of antibodies to the thyroid-stimulation hormone (TSH) – receptor of thyroid (TRAb) is of great pathogenic importance for the development and persistence of autoimmune hypothyroidism. Using their own stimulatory effect upon binding to the TSH – receptor, these antibodies cause increased synthesis and secretion of thyroid hormones. GD patients may have stimulating, blocking and even neutral antibodies in the serum, and the clinical picture is a consequence of their relative potency and dominance [1, 2].

According to the reports on the achievements of long-term remission in patients treated with medical thyroid suppressive therapy (MTT), the percent of treated patients range from 14 – 80% [1, 2]. The overall recurrence rate of GD in patients treated with MTT after cessation of treatment is about 30 – 50% [5, 6].

Consensus about the TRAb importance, regarding the prediction of GD relapse and remission has not been reached yet. Studies conducted so far differ among themselves not only by the type of TRAb essay methodology applied but by the study design as well, some of them being retrospective and others prospective. They also differ in time when TRAb was tested during the disease. In addition, population genetics and iodine status could affect the results of studies because of different geographic areas where those studies were conducted [7].

The aim of this study was to determine the significance of TRAb level for predicting remission occurrence during thyr sopressive drug therapy. The hypothesis is that TRAb level at the beginning of the disease and during MTT is a predictive factor for the outcome of therapy.

Material and Methods

This prospective study included 106 patients with newly diagnosed GD who started treatment with MTT. After the diagnosis of hyperthyroidism had been made according to anamnestic data, clinical examination, signs of hypermetabolism, suppressed TSH level and increased free fraction levels of serum thyroid hormones, TRAb level was determined in all patients. TRAb was measured by radio receptor method (DYNO test TRAK human Brahms Diagnostica GMBH) with normal values up to 1.5 IU/L. MTT was given using the process of titration. TRAb level was determined 6 and 12 months after the beginning of therapy. According to the response to thyrosuppressive drug therapy, the patients were divided into two groups: group A – patients who achieved remission, and group B – patients who did not achieve remission, non-responders, whereby the remission meant a clinical and biochemical euthyroid state which was maintained at least 12 months after MTT had been discontinued. TRAb level was expressed as the mean value ± SD, median, as well as minimum and maximum value (Table 1). T-test for two independent samples and analysis of variance of repeated measures were used to test differences between groups. The tests were done using transformed data. ROC analysis (Receiver Operating Characteristic curve analysis) was made in order to determine a specific TRAb level for the prediction of remission. The data are shown in tables and graphs.

Results

Out of 106 respondents, 21 (19.81%) were male and 85 (80.19%) were female. The average age of all the respondents was 44.27 ± 15.35 years (age range 14 – 74). The total follow-up of patients lasted 45 months. The average duration of MTT was 21.20 ± 14.01 (the median being 17 months), and the average follow-up after the therapy had been discontinued was 28.38 ± 27.21 (the median being 24 months).

Having been diagnosed, all our respondents underwent MTT titration regimen. Most of the patients were treated with methimazole (79%), while some of them (18%) were treated with propiltiouracil. Other patients (3%) started the treatment with methimazole and then continued with propiltiouracil, mainly because of some allergic manifestation to methimazole.

The average TRAb level in our patients at the beginning of disease was 11.42 U/L. Six months after the introduction of MTT, the average TRAb level in our patients was 5.9 U/L, and 12 months after the introduction of MTT, the average TRAb level was 2.3 U/L as shown in Graph 1.

Table 1. Structure of the patients according to thyrosuppressive drug therapy outcome 12 months after the introduction of therapy

<table>
<thead>
<tr>
<th>Outcome/Isход</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Remission/Remisija</td>
<td>62</td>
<td>58.5</td>
</tr>
<tr>
<td>B – Non-responder/Bez remisije</td>
<td>44</td>
<td>41.5</td>
</tr>
<tr>
<td>Total/Ukupno</td>
<td>106</td>
<td>100</td>
</tr>
</tbody>
</table>
Graph 1. Mean TRAb level in patients. TRAb 1 – at the beginning of therapy; TRAb 2 – 6 months on therapy; TRAb 3 – 12 months after the introduction of therapy; y axis – U/L

**Graph 2.** Shows average TRAb level in patients in remission and in non-responders at the beginning of disease and 6 and 12 months after the introduction of therapy.

According to the statistical analysis of transformed data obtained by t-test for two independent samples, no statistically significant difference was found between the non-responders and the patients in remission regarding TRAb level at the beginning of disease (t=0.450; p=0.654), 6 months after the introduction of therapy (t=0.816; p=0.419) and 12 months after the introduction of therapy (t=1.467; p=0.146).

The analysis of variances of repeated measures showed a highly statistically significant difference among these three measurements in the whole group of patients (F = 69.264; p< 0.001; Eta2part = 0.596), but no statistically significant difference in the change of TRAb level during the follow-up between the non-responders and the patients in remission (F=0.870; p=0.395; Eta2part = 0.018).

There was a statistically significant difference in TRAB level at the beginning of disease and 12 months after the introduction of therapy in the patients in remission (p < 0.001) as well as in the non-responders (p < 0.001).

In predicting the therapy outcome, receiver operating characteristic curve (ROC) analysis was used to test TRAb sensitivity and specificity at the beginning of disease, 6 months and 12 months after the introduction of therapy. **Graph 3** shows ROC analysis for TRAb level at the beginning of disease and the therapy outcome. **Graphs 4 and 5** show ROC
ROC analysis for TRAb level 6 months after the introduction of therapy and the therapy outcome. It is obvious that the surface under the curve in ROC analysis for TRAb level at the beginning of disease and 6 months after the introduction of therapy was small, and that TRAb level at the beginning of disease and 6 months after the introduction of therapy did not have any significance in predicting the outcome of therapy. The surface under the curve was small (0.532; $p = 0.577$ and 0.446; $p = 0.510$ respectively.

However, ROC analysis for TRAb level 12 months after the introduction of therapy showed that the surface under the curve was larger and $p$ value was near the conventional level of significance ($p = 0.068$).

Discussion

According to the therapy outcome, the our respondents are divided into those who achieved remission and non–responders (41.5%). The rate of remission ranges from 14 to 80% according to the published data [1, 2, 5, 6, 8–15]. A great number of studies show that at least 30 – 60% of the patients with GD relapse into disease within two years after MTT discontinuation; however, early prognostic parameters that would indicate long–term remission have not been determined so far [11–15].

The study published by Peixoto et al. revealed that a high initial TRAb level was associated with a reduced rate of remission in 46 patients with GD who were treated with MTT for 12 months and then followed up for 12 months after the discontinuation of drug [16].

In their study, Jonas et al. included 37 patients treated with thiamazole for 12 months to observe clinical and biochemical parameters of thyroid status after 1, 3, 6, 9 and 12 months from the introduction of therapy. The follow-up period after withdrawal of therapy was about 27 months. Out of the total number of patients, 32% had relapse of hyperthyroidism, usually 8 months after withdrawal of therapy. It was noticed that the patients with TRAb levels higher than 14 IU/L after 3 months therapy, and those with TRAb levels higher than 8 IU/L after 6 months of therapy had relapse more often than those patients with lower TRAb levels, with sensitivity of 50% and specificity of 92% and 96% respectively. The authors have concluded that TRAb level in the early phase of thyrosuppressive therapy may be helpful in choosing an adequate therapy for GD, opting for more radical treatment with radioiodine or surgical treatment [12].
The median of TRAb level was about 11 IU/L in our patients, the range going from marginally positive to maximal 155 IU/L. During MTT, TRAb level decreased progressively and differed statistically significantly from the levels measured at the beginning of disease and 6 months after the introduction of MTT. Six months after the therapy had been introduced, the median TRAb level was about 6 IU/L, almost half of the initial value. The maximum measured TRAb value was 145.9 IU/L and 155.7 IU/L in the patients in remission and the non-responders, respectively. At the beginning of disease, the median of TRAb level did not differ significantly in the patients in remission and the non-responders, the value being about 11 IU/L. Six months after the introduction of therapy, the median of TRAb did not differ significantly in the patients in remission and the non-responders, the value being about 6 IU/L for both groups. Six months after the introduction of therapy, the maximum measured value was higher in the patients in remission (73.7 IU/L) than in the non-responders (46.2 IU/L).

In a prospective randomized clinical study, 47.7% of 218 patients with GD had a relapse of disease within two years after withdrawal of MTT. The patients who showed positive TRAb after 12 months of therapy had a higher relapse rate than TRAb negative patients [13].

Quadbeck et al. were determining thyroid status in 96 patients four weeks after discontinuation of thyrosuppressive drug therapy. The relapse rate was being assessed throughout the post-therapeutic two-year follow-up period. During the follow-up period, relapse of disease occurred in 49% of patients. Mean TRAb level at the end of therapy in the group of patients with relapse was significantly higher than in those who were in remission. Using cut off value of 1.5 IU/L, both the positive predictive value and the negative predictive value were low, being 49% and 54%, respectively (the specificity being 14%); however, taking 10 IU/L as a cut off values, the positive predictive value and the negative predictive value were improved to 83% and 62%, respectively (the specificity being 92%). Other factors such as age, sex, thyroid volume, smoking and the presence of ophtalmopathy did not have any influence on the relapse rate [11].

Okamoto et al. investigated the therapy outcome in 71 patients. The therapy was discontinued after euthyroid state had been achieved and maintained first during 6 months with a daily methimazole (MMI) dose of 5 mg, or 50 mg of propylthiouracil (PTU) and then during the next 3 months with the same dose but every other day. The follow-up continued one year after the therapy had been discontinued. During the follow-up, 37% of the patients had relapse. Mean TRAb level at the end of therapy in the group with relapse was significantly higher. All patients who had the same TRAb value or higher than 3 IU/L achieved relapse during the follow-up [15].

Contrary to the above mentioned results, a study with 129 patients found the positive predictive value and negative predictive value of only 55% and 62%, respectively for the prediction of relapse when TRAb cut off value was 1.5 IU/ml. The authors concluded that despite high diagnostic sensitivity and specificity of “TRAK human” assay, its predictive value for relapse of hyperthyroidism was not increased when measuring was done at the end of thyrosuppressive drug therapy [17].

One of rarely performed studies on this issue in our country reported high TRAb values in the group of non-responders after the first month of therapy, while the responders had a significantly lower TRAb level. The authors believe that the decision regarding treatment can be reached by obtaining either the positive or negative response to drug therapy, measured by the level of TRab [18].

One of our earlier studies, which had followed the number of relapses and duration of remission in drug treated patients with GD, revealed that the duration of remission was longer and the relapse rate was lower in the patients with lower initial TRAb level and lower TRAb level at the end of the therapy. The initial TRAb level exceeding 5 IU/L increases the chance for remission shorter than 6 months by 18%, whereas the TRAb level exceeding 15 IU/L at the end of therapy makes this chance higher by 36% [19].

Persistently elevated TRAb values are associated with both a difficult clinical course and a low remission rate of hyperthyroidism, as it has been found in a study conducted by Eckstein et al. The authors have therefore suggested TRAb value cut off to be a prognostic factor during 6, 12 and 18 months of drug therapy as the basis for making an early decision on the final therapy [20].

Apart from the role of TRAb played in predicting the outcome of MTT, some authors emphasize the significance of TRAb in decision making regarding duration of the therapy as well as in the assessment of clinical course of thyroid ophtalmopathy [21]. Discontinuation of thyrosuppressive drug therapy is recommended when the normal concentration of free thyroxine and TSH is achieved and maintained during a certain duration of therapy with 5 mg of methimazole every other day, the so called minimum maintenance dose (MMD). The achieved rate of remission was 86.9% for 6 months, 73.8% for one year and 68.2% for two years after the discontinuation of MTT. The remission rate was higher in the patients with longer duration of MMD therapy, and significantly higher in the patients with MMD therapy for 19 months and longer than in those patients with MMD therapy for 6 months or shorter. The remission rate was also significantly lower in TRAb positive patients than in TRAb negative ones at the time of the discontinuation of therapy. It is recommended not to discontinue thyrosuppressive drug therapy in TRAb positive patients who have been using MMD therapy for 6 months or shorter [22].

Twelve months after the introduction of therapy, the median of TRAb level in our patients was about 2 IU/L, the maximum measured value being about 45 IU/L. Twelve months after the introduction of therapy, the median of TRAb level in the patients in remis-
A small number of patients with autoimmune thyroid disease can experience changes in the types of TSH receptor antibodies, from thyroid stimulating antibody (TSAb) into thyroid blocking antibody (TBAb) and vice versa with the consequent changes in the thyroid function. During thyrosuppressive drug therapy, TSAb level falls causing predominance of TBAb. On the contrary, TSAb development during tyroxine substitution therapy can be sufficient to annul the inhibitory effect of TBAb. This conversion can be one of the reasons which diminish the importance of TRAb for predicting remission and relapse [2].

Recent research has been focused on discovering drugs such as low molecular weight ligands which, by binding to a transmembrane allosteric pocket of TSH receptors, could block the pathological activation of receptor caused by TSH receptor antibodies [24–26].

**Conclusion**

Level of antibodies to thyroid-stimulation hormone receptors at the beginning of disease and 12 months after the start of therapy cannot predict the outcome of thyrosuppressive drug therapy. Persistently elevated level of antibodies to thyroid-stimulation hormone receptors for more than 12 months after the introduction of therapy could be a late predictive factor for an adverse outcome of thyrosuppressive drug therapy and could be clinically significant in making an early decision on changing the type of therapy.

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**References**

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