

The Relationship of Thyroid Stimulating Hormone Receptor Antibody (TRAb) Levels to Activity and Clinical Severity of Graves' Ophthalmopathy in RSUP Dr. Sardjito

Kharisma Wibawa Nurdin Putra¹, M. Robikhul Ikhsan², Vina Yanti Susanti²

¹Internal Medicine Specialist Program, Department of Internal Medicine, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada/Dr. Sardjito General Hospital

²Division of Endocrinology, Department of Internal Medicine, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada/Dr. Sardjito General Hospital

Corresponding author:

M. Robikhul Ikhsan, Department of Internal Medicine, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada – Dr. Sardjito Hospital, Yogyakarta, Indonesia. E-mail: m_robikhul@yahoo.co.id.

ABSTRACT

Background: Graves' disease (GD) is an autoimmune disease that is the most common cause of hyperthyroidism. Thyroid stimulating hormone receptor antibody (TRAb) is a specific biomarker for diagnosing GD. Graves' ophthalmopathy (GO) is the most common extrathyroid manifestation in Graves'. Each GO was assessed for activity and clinical severity for treatment.

Objectives: To determine the relationship between TRAb levels and GO clinical activity in Clinical Activity Score (CAS) classification and clinical severity of The European Group on Graves' Orbitopathy (EUGOGO).

Methods: This is a cross-sectional study, conducted from January to August 2021 at RSUP Dr. Sardjito Yogyakarta. 30 newly diagnosed Graves' patients or previously diagnosed with Graves' presented symptoms of hyperthyroidism were included. The patients had TRAb levels > 1.75 IU/L, and diagnosed with Graves' ophthalmopathy based on clinical criteria according to Bartley and Gorman and has not received steroid injections. Serum TRAb examination by electrochemiluminescence immunoassay (ECLIA) method. Clinical activity are assessed with CAS, while severity are assessed with EUGOGO criteria. The data are analyzed using Spearman correlation, multivariate analysis with linear regression and logistic regression.

Results: 30 study subjects mean age 35.43 years, female dominant (80%), median GD duration of 13 months. Median TRAb 8.15 IU/L, median CAS score 2 with 46.3% active. Mild severity 40% and moderate-severe 60%. Correlation of TRAb with CAS ($r=0.576$, $p=0.001$), EUGOGO ($r=0.535$, $p=0.002$). Multivariate analysis of TRAb with CAS ($B= 0.076$, $p=0.01$) while EUGOGO ($OR=1.198$, $p=0.045$)

Conclusion: TRAb is positively correlated with activity and clinical severity of OG.

Keywords: *Thyroid stimulating hormone receptor antibody (TRAb), Graves' Ophthalmopathy, Clinical Activity Score (CAS), The European Group on Graves' Orbitopathy (EUGOGO).*

ABSTRAK

Latar Belakang: Penyakit Graves (PG) suatu penyakit autoimun penyebab tersering hipertiroid. Thyroid stimulating hormone receptor antibody (TRAb) adalah biomarker spesifik untuk mendiagnosis PG. Oftalmopati Graves (OG) manifestasi tersering ekstratiroid pada Graves. Setiap OG dinilai aktivitas dan derajat keparahan klinis untuk tatalaksana.

Tujuan Penelitian: Mengetahui hubungan kadar TRAb terhadap aktivitas klinis OG berdasarkan klasifikasi Clinical Activity Score (CAS) dan derajat keparahan klinis berdasarkan The European Group on Graves' Orbitopathy (EUGOGO).

Metode Penelitian: Penelitian cross sectional, berlangsung pada bulan Januari sampai Agustus 2021 di RSUP Dr. Sardjito Yogyakarta. 30 pasien PG baru atau dalam pengobatan anti-tiroid yang memiliki gejala hipertiroid diinklusi. Pasien memiliki kadar TRAb > 1.75 IU/L, yang terdiagnosis OG menurut kriteria Bartley dan Gorman serta belum mendapatkan injeksi steroid. Pemeriksaan serum TRAb dengan metode electrochemiluminescence immunoassay (ECLIA). Penilaian aktivitas klinis CAS dan derajat keparahan EUGOGO. Uji analisis dengan korelasi Spearman. Analisis multivariat dengan regresi linier dan regresi logistik.

Hasil Penelitian: 30 subjek penelitian usia rerata 35.43 tahun, dominan wanita (80%), median durasi PG 13 bulan. Median TRAb 8.15 IU/L, skor median CAS 2 dengan 46.3% kondisi aktif. Derajat keparahan ringan 40% dan sedang-berat 60%. Korelasi TRAb dengan CAS ($r=0.576$, $p=0,001$), EUGOGO ($r=0.535$, $p=0,002$). Multivariat analisis TRAb dengan CAS ($B= 0.076$, $p=0,01$) sedangkan EUGOGO ($OR=1.198$, $p=0.045$).

Kesimpulan: TRAb berkorelasi positif terhadap aktivitas dan derajat keparahan klinis OG.

Kata kunci: Thyroid stimulating hormone receptor antibody (TRAb), Oftalmopati Graves, Clinical Activity Score (CAS), The European Group on Graves' Orbitopathy (EUGOGO).

INTRODUCTION

Graves' disease (GD) is an autoimmune disease caused by the interaction of genetic and environmental factors.¹ This disorder is the most common cause of hyperthyroidism. The annual incidence is 20 to 50 cases per 100,000 people. Incidence increases between the ages of 30 and 50 but can occur at any age.²

Thyroid stimulating hormone receptor antibody (TRAb) is an antibody that is a specific biomarker for diagnosing GD.³ This antibody increases in GD and mimics the effect of thyroid stimulating hormone (TSH) causing hyperthyroidism.⁴ The sensitivity and specificity of TRAb in diagnosing GD are 97% and 98%.⁵ TRAb examination is important in differentiating GD from other causes of hyperthyroidism. This examination also has therapeutic and prognostic implications in GD.⁴

Graves' ophthalmopathy (GO) is the most common extrathyroid manifestation in GD.⁶ Graves' ophthalmopathy occurs in approximately 25% of GD patients. The

estimated incidence of GO in the general population is 16 in women and 3 in men per 100,000 people per year, with severity occurring in 3-5% of cases.⁷ There is an autoimmune complex with an inflammatory process, subsequent tissue remodeling, and fibrosis in the orbit. Graves' ophthalmopathy progression causes proptosis, strabismus, corneal ulceration, and even optic nerve entrapment which can lead to blindness.⁸

Graves' ophthalmopathy assessment can be done based on clinical symptoms and signs, Mourits et al in 1989 distinguished active and inactive GO conditions with a Clinical Activity Score (CAS).⁹ The European Group on Graves Orbitopathy (EUGOGO) recommends that each patient with GO be assessed for clinical grade and severity.⁶ If clinical assessment is not possible, imaging studies are required, and computed tomographic scans can be used to assess the presence of GO.¹⁰

The relationship between TRAb levels and GO is still not known with certainty.^{11,12} Several studies have shown an association

between GO and serum TRAb levels. Another study stated that this antibody is useful for detecting the tendency of GD to become GO.^{13,14} The main independent and significant risk factors for GO were high baseline FT4 concentrations, TRAb, stress, and smoking.¹⁵ Higher TRAb levels were found in GD with GO than in GD without GO.¹⁶ Graves' disease with moderate-to-severe GO untreated, TRAb levels correlated with clinical grade GO.¹⁴

METHODS

A cross-sectional study to examine the relationship between TRAb levels and activity and clinical severity of Graves' ophthalmopathy. The research was conducted at the Endocrine Clinic of Internal Medicine and the Eye Clinic of RSUP Dr. Sardjito, Yogyakarta from January 2021 to August 2021. The subjects of the study were all Graves' patients with ophthalmopathy at Dr. RSUP Dr. Sardjito, who had been given information about the study and signed the consent form with inclusion criteria, namely: 1) Patients newly diagnosed with Graves' or previously diagnosed with Graves' with symptoms of hyperthyroidism, whether on medication or not on hyperthyroid medication with TRAb levels > 1.75 IU/L, 2)

Patient was diagnosed with Graves' ophthalmopathy based on clinical criteria according to Bartley and Gorman and 3) Age 18 years or above. The study exclusion criteria were: 1) Graves' patients who had a history of thyroid ablation, 2) Graves' patients who had a history of thyroid surgery, and 3) Graves' ophthalmopathy patients who had received steroid injection treatment.

RESULTS

There were 30 subjects, with a mean age of 35.47 years. Most of the patients were female (80.0%). The mean duration of hyperthyroidism was 13 months (2-120 months). A total of 3 (10%) subjects consumed cigarettes. The median level of TSH was 0.01 μ IU/ml (0.005-5.54) and FT4 1.83 ng/dL (0.81-7.77). Median of TRAb levels were 8.15 IU/L (1.83-40.0). There were 1 (3.3%) DM patients and 5 (16.7%) subjects with hypertension. A total of 26 (86.7%) subjects used anti-hyperthyroid drugs thiamazole and 4 (13.3%) subjects used PTU. The median score of CAS ophthalmopathy was 2 (1-7). GO condition is active 46.3% and inactive 53.7%. The EUGOGO score was scored as mild 12 (40%), moderate-severe 18 (60%) and 0 subjects as sight-threatening.

Table 1. Characteristics of research subjects

| | | Mean±SD or Median (min – max) | N | % |
|-------------------------------|-------------------|----------------------------------|----|-------|
| Age (years) | | 35.43 ± 10.62 | | |
| Gender | Male | | 6 | 20.0% |
| | Female | | 24 | 80.0% |
| Hyperthyroid duration (month) | | 13 (2 – 120) | | |
| Smoking | Yes | | 3 | 10.0% |
| | No | | 27 | 90.0% |
| TSH (μIU/ml) | | 0.010 (0.005 – 5.54) | | |
| FT4 (ng/dL) | | 1.83 (0.81 – 7.77) | | |
| TRAb (IU/L) | | 8.15 (1.83 – 40.0) | | |
| DM | Yes | | 1 | 3.3% |
| | No | | 29 | 96.7% |
| HT | Yes | | 5 | 16.7% |
| | No | | 25 | 83.3% |
| Thyroid Drugs | Thiamazole | | 26 | 86.7% |
| | PTU | | 4 | 13.3% |
| CAS | | 2 (1 – 7) | | |
| | Active (CAS ≥3) | | 14 | 46.3% |
| | Inactive (CAS <3) | | 16 | 53.7% |
| EUGOGO | Mild | | 12 | 40.0% |
| | Moderate-Severe | | 18 | 60.0% |
| | Sight-Threatening | | 0 | 0% |
| Steroid | Yes | | 18 | 60.0% |
| | No | | 12 | 40.0% |

TRAb was correlated with the clinical degree of CAS using the Spearman correlation. The result of the correlation between TRAb and CAS was statistically significant, namely $p=0.001$ ($p<0.05$). The correlation coefficient is positive and the plot points to the top right indicate the higher the

TRAb, the higher the CAS. The results of the correlation $r = 0.576$, with a moderate category closeness, namely the correlation coefficient is in the interval 0.4-0.599. The following figure shows the correlation between TRAb and CAS.

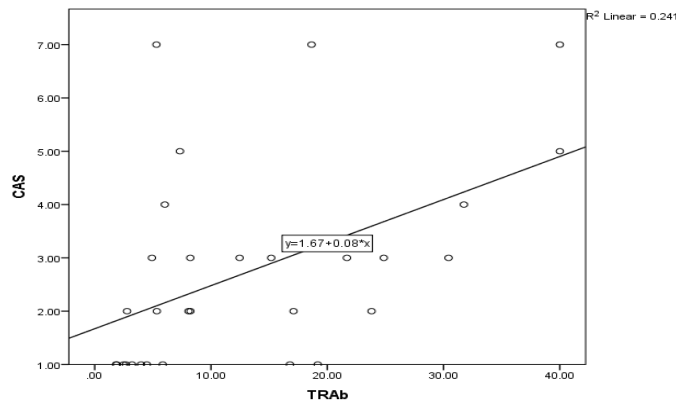


Figure 1. Relationship of TRAb with CAS

Multivariate analysis was performed with linear regression of several variables using CAS. Multivariate analysis showed that TRAb had a significant effect on CAS $p=0.01$ with a regression coefficient of 0.76, meaning that every 1 IU/L increase in TRAb would increase CAS 0.76 units. Variables outside the duration of hyperthyroidism, TSH, and hypertension were not statistically significant ($p>0.05$) (Table 4).

TRAb levels were correlated with the severity of the EUGOGO with the Spearman analysis test. The results of the relationship between TRAb and EUGOGO were statistically significant, namely $p=0.002$ ($p<0.05$). The correlation coefficient is positive with $r = 0.535$, and the closeness of the medium category, namely the correlation coefficient is in the interval 0.4 - 0.599. Figure 2 shows the correlation between TRAb and CAS.

Table 2. Multivariate Linear Regression against CAS

| | B | <i>p</i> | 95.0% CI | |
|-----------------------|--------|----------|-------------|-------------|
| | | | Lower Bound | Upper Bound |
| (Constant) | 5.191 | .012* | 1.245 | 9.138 |
| TRAb | .076 | .010* | .020 | .131 |
| Hyperthyroid duration | -.017 | .104 | -.038 | .004 |
| TSH | -.129 | .643 | -.697 | .439 |
| HT | -1.590 | .095 | -3.480 | .300 |

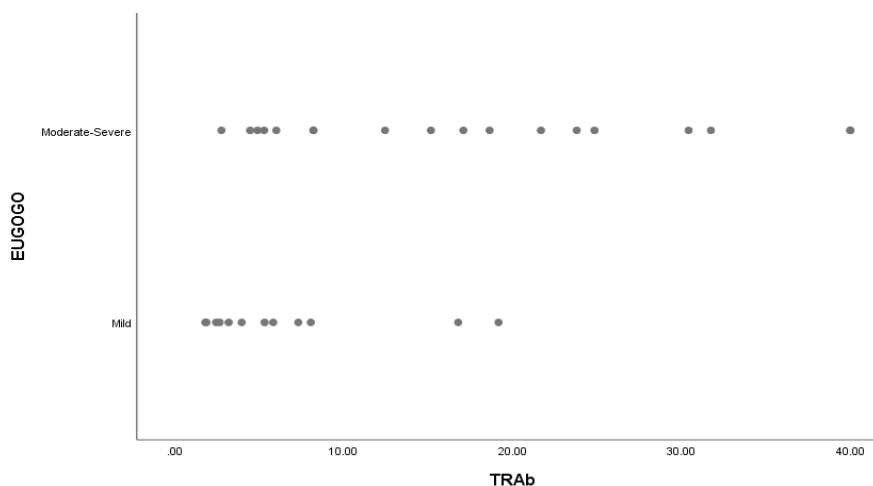


Figure 2. Relationship of TRAb with EUGOGO

Multivariate analysis was performed on the data using EUGOGO. The results of multivariate analysis showed that TRAb had a significant effect on EUGOGO $p=0.045$ with OR=1.198. Variable FT4 did not show a

statistically significant effect $p=0.108$. But clinically (OR>2) every 1 ng/dL increase in FT4 risked moderate-severe EUGOGO 6.245 times.

Table 3. Multivariate Logistic Regression against EUGOGO

| | <i>p</i> | OR | 95.0% CI | |
|----------|----------|-------|-------------|-------------|
| | | | Lower Bound | Upper Bound |
| TRAb | .045* | 1.198 | 1.004 | 1.429 |
| FT4 | .108 | 6.245 | .671 | 58.136 |
| Constant | .049 | .009 | | |

DISCUSSION

This study subjects had a mean age of 35.43 years, including 24 (80%) women and 6 (20%) men, maintaining a ratio of 1:4, aligning with previous theories suggesting that Graves' disease typically manifests between the ages of 20 and 50, with a higher prevalence in women than in men. Some data indicate that the lifetime risk in women and men is 3% and 0.5%.¹⁷ Hypertension in this study was found in 5 (16.7%) subjects. Thyroid hormones play an important role in cardiovascular homeostasis, including regulation of cardiac contractility, electrophysiological function, and cardiac structure.¹⁸ Hyperthyroidism increases cardiac output by increasing heart rate, contractility, cardiac preload, and decreasing peripheral vascular resistance, resulting in hypertension.¹⁹

Hyperthyroidism is defined as a TSH level below the normal value with an increase

in T3 level and/or an increase in FT4.²⁰ In this study, the average TSH level was 0.01 μ IU/ml (0.005 – 5.54) and FT4 1.83 ng/dL (0.81 – 7.77). A permanent cure for Graves' hyperthyroidism is possible although at a low rate of about 27% after 20 years²¹. In this study the length of time the subjects had hyperthyroidism was between 2 and 120 months, all subjects were on anti-thyroid medication. Methimazole is recommended for use in almost all GD, except in the first trimester of pregnancy where PTU is used, in thyroid storm conditions, and in patients who do not respond to methimazole.²⁰ In this study, the most commonly used anti-thyroid drug was methimazole (86.7% patients).

As many as 50% of GO are generally mild and about 3–7% of GO patients are visually threatening due to corneal exposure or compression optic neuropathy.^{22,23} The prevalence of active GO according to

research by Lat et al in the Philippines was 8% with 85% having mild disease.²⁴ In this study, the condition of active GO (CAS 3) was 14 (46.7%) and inactive (CAS <3) 16 (53.3%). The severity of EUGOGO was 18 (60%), moderate 12 (40%), and 0 subjects were visually threatening. The prevalence is not in accordance with previous studies, perhaps the assessment of the selected subjects using clinical GO assessment, so that the subjects who entered this study were already in a severe GO condition and few subjects. The incidence of GO is much higher when assisted by imaging modalities such as computed tomography scan (CT-scan), magnetic resonance imaging (MRI), ultrasonography (US), and color doppler imaging (CDI) because it can identify extraocular muscle involvement at a stage where there are no clinical signs of GO.²⁵

The American Thyroid Association (ATA) in 2016, recommended TRAb examination in addition to radio-iodine uptake and thyroid scanning to accurately assess Graves' diagnosis.²⁰ TRAb examination usually uses the electrochemical luminescence immunoassays (ECLIA) binding technique, namely the thyrotropin binding inhibitory immunoglobulin (TBII) test) 3rd generation automatic.²⁶ This automated TRAb test has 100% sensitivity and 98.7% specificity in diagnosing Graves'.²⁷ Subjects were selected based on clinical symptoms of hyperthyroidism with high TRAb levels (>

1.75 IU/L) to rule out the cause of hyperthyroidism other than Graves'. The mean value of TRAb subjects was 8.15 (1-53-40.0).

Analysis of TRAb levels clinical activity of GO with CAS found a significant correlation, both with bivariate analysis ($r=0.579$, $p=0.001$) and multivariate linear regression analysis ($p=0.01$), this correlation is in the moderate category, which is between 0.4- 0.599. These results are in line with several other studies, that TRAb is correlated with the severity and clinical activity of GO.^{14,26} High levels of TRAb in the early phase of GO can predict a poor response to treatment.¹³ A study of 90 GO patients, the association of TRAb with CAS, found a significant correlation between TRAb and CAS 28. In a study of 163 adult Graves' patients in the Philippines increased TRAb was associated with the degree of CAS activity (OR 1.03, $p=0.002$).²⁴

The study showed that TSH-R mRNA and protein were present in orbital tissue and orbital fibroblasts of GO patients, and TSH-R expression was higher in GO orbital tissue compared to normal orbital adipose tissue.^{29,30} Furthermore, the positive correlation between TSH-R mRNA levels in orbital tissue and clinical activity in GO, suggests a role for TSH-R in the pathogenesis of GO.^{31,32} In this regard, TSH-R autoantibodies (TRAb) are believed to affect orbital tissue, leading to adipogenesis and

ultimately inflammation.³³ Other studies agree with the central role of TSH-R in the pathogenesis of GO. Primarily involved in the early stages of GO, where orbital fibroadipose tissue correlates with serum TRAb levels, and thus with GO activity.³⁴ In this study, the TRAb levels of subjects who received high-dose intravenous steroids after data collection were 13.8 IU/L (2.78-40.0 IU/L) while subjects who did not receive high-dose intravenous steroids were 4.66 IU/L (1.8-23.81 IU/L). Statistically, there was a significant difference with $p=0.016$. Inflammatory mediators also affect the degree of activity and severity of GO. In a study of 18 patients with GO and 15 healthy controls, serum levels of IL-6, IL-4, and IL-13 were assessed before and after administration of 3 doses of intravenous corticosteroid followed by oral prednisone for 6 months. The most significant finding was a decrease in IL-6 levels after 2 weeks and continued after 6 months in 8 of 18 patients.³⁵

A correlation was found between TRAb level and GO severity according to EUGOGO. The results of bivariate analysis were obtained with a statistically significant value of $p=0.002$ with a correlation of $r=0.535$. Likewise in the multivariate logistic regression analysis, TRAb affected the severity of EUGOGO ($p=0.045$, OR 1.19). This is consistent with a previous study in 163 adult Graves' patients in the Philippines, that an increase in TRAb was associated with the

severity of Graves' ophthalmopathy EUGOGO (OR 1.02, $p=0.007$).²⁴ In an Italian study of 90 untreated GO patients, the increase in TRAb was not correlated to the severity of NOSPECS. This may be because OG tends to correlate more strongly with autoimmunity than with GO severity, where the severity of OG persists for a long period even though ocular disease does not have active inflammation.^{28,36}

CONCLUSION

Thyroid stimulating hormone receptor antibody (TRAb) correlates with the degree of ophthalmopathy activity in the Graves' Clinical Activity Score (CAS) and the severity of the European Group on Graves' Orbitopathy (EUGOGO). This finding supports that TRAb plays a role in Graves' ophthalmopathy. This study can provide guidance in diagnosis and managing Graves' patients with ophthalmopathy.

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