

INTRODUCTION

Graves' disease is a thyroid autoimmune disease with several clinical manifestations such as hyperthyroidism, diffuse goiter, ophthalmopathy and with or without dermatopathy.⁽¹⁾ Incident of Graves' disease was about 40 cases/100.000 population per year and tend to increase annually. In Wickham study in the United Kingdom, the incidence was reported to be 100 – 200 cases per 100.000 population per year. The incidence in women reported more frequent than men. In Indonesia, incidence of hyperthyroidism caused by Graves' disease not yet known, but were reported about 44,44% - 48,93% from all of patients with thyroid disease.^(2,3,4) Graves' disease were the most common organ specific autoimmune disease and tend to increase through years. Genetic and environment factors thought to have a role in increasing of autoimmune disease, such as MHC-II expression, autoreactive B-cell activation and imbalance of Th1/Th2. Moreover, infection, stress, and excessive consumption of iodine were also suspected to trigger immune factor that cause Graves' disease.^(5,6)

Hyperthyroidism in Graves' disease patient cause by increasing of thyroid antibody which cause thyrotoxicosis. This antibody binds to thyroid stimulating hormone (TSH) receptor and mimics TSH activity in thyroid gland, known as TSH receptor antibody (TRAb). Antibody activation trigger increasing of thyroid hormone which cause hyperthyroidism manifestation in Graves' disease.^(7,8,9,10) TSH receptor antibody (TRAb) found 100% on Graves' disease compared to other thyroid antibody such as thyroperoxidase antibody (TPO-Ab) and thyroglobuline, so that increased of TRAb was one of diagnostic point in Graves' disease. Previous study also shown decreasing of TRAb in patients who already received anti thyroid medication, so it is often used as remission marker of disease activity.^(11,12)

Overactivity of thyroid hormone in Graves' disease may stimulate thyroid gland proliferation which then lead to enlargement of thyroid gland, known as goiter. WHO

classified thyroid gland enlargement into 3 (three) gradation : grade I (goitre only found from palpable); grade II (goitre is palpative and can be easily seen); and grade III (goitre is very big and retrosternal).⁽⁶⁾ Ling Chen (2008) shown that increasing of thyroid gland size related to severity and progresivity of the disease.⁽¹³⁾ Aim of this study was to investigated the association between TSH receptor antibody (TRAb) with goiter size.

MATERIAL AND METHODS

Study population

Blood samples were obtained from 30 patients with Graves' disease. Diagnosis of Graves' disease was based on the clinical manifestations found such as hyperthyroidism, diffuse goiter, ophtalmopathy and dermopathy with increasing of thyroid hormone (FT4) and decreasing of TSH level. Exclusion criteria were as follows : using anti-thyroid medications before the blood sample is checked. Patients were recruited from Metabolic Endocrinology Outpatient of Internal Medicine Department, M.Djamil Padang Hospital.

Methods

Serum blood of patients were collected about 3 cc to measure thyroid hormone (FT4), TSH and TRAb. Free thyroxin (FT4), TSH and TRAb were measured by using ELISA method. Size of goiter were measured using WHO classification which classified to 3 (three) gradation : I - goiter in normal posture of head it cannot be seen, only found when palpating; II - goiter is palpative and can be easily seen; III - goiter is very big and is retrosternal. Furthermore, thyroid ultrasonography were also performed to measure thyroid volume. Characteristic data such as age, sex were noted as baseline data.

Statistical analysis

Data analysis were determined by correlation analysis using SPSS version 20 (SPSS *Inc*). Pearson analysis were using when data normally distributed, or Spearman analysis when non-normally distributed. *Chi-square* test analysis were use to analyze categorical data.

RESULT

The aim of this study was to investigate the correlation between antibody receptor TSH (TRAb) and goiter size in Graves' disease patients. The study is cross sectional, composed of 30 patients of hyperthyroidism caused by Graves' disease. **Table 1** summarized baseline characteristic of this study, which is 66,7% patients were female and mean of age of this study were $40,7 \pm 14,81$ years, range from 19 to 74 years old.

The mean serum concentration of free T4 (fT4) was found increase significantly ($74,28 \pm 61,1$ nmol/L), with reference value was 9-20 nmol/L. Mean serum concentration of TSH were decrease significantly with $0,1 \pm 0,09$ IU/ml (normal reference 0,25-5 IU/ml). Increasing of TRAb and lower serum of TSH values were found to be 100% as shown in **table 1**.

Table 1. Baseline characteristic of Graves' disease patients.

	Mean ± SD (n =30)	P value
Gender (male:female)	3:7	
Age (years)	40,67 ± 14,81	> 0,05
< 30	9 (30%)	
30 – 39	6 (20%)	
> 40	15 (50%)	
Goitre Gradation		
Grade I	7 (23,3%)	
Grade II	12 (40%)	
Grade III	11 (36,7%)	
Goitre Volume (ml)	6,58 ± 4,77	> 0,05
< 10 ml	24 (80%)	
10-20 ml	5 (16,7%)	
> 20 ml	1 (3,3%)	
FT4 (nmol/L)	74,28 ± 61,1	> 0,05
TSH (IU/ml)	0,1 ± 0,09	> 0,05
TRAb (pg/ml)	5,63 ± 3,72	> 0,05

FT4, free thyroxine; TSH, thyroid stimulating hormone; TRAb, thyroid stimulating hormone receptor antibody.

Presence of thyroid antibody (TRAb)

Mean serum concentration of TRAb was significantly higher than normal limit ($p > 0,05$). The reference value of TRAb was $> 0,1$ pg/ml for positive Graves' disease.

Goiter gradation and volume

Gradation of goiter in this study were using WHO classification, divided to three (3) grade, grade I (goiter only found by palpable), grade II (goiter is palpative and easily seen) and grade III (goiter easily seen from distance). From this study, gradation of goiter found the

most in grade II (40%) than grade I, III (23,3% and 36,7% respectively). Size of goiter were also measured using ultrasonography by adding left and right goiter lobus volume, and in this study mean of goiter volume were $6,58 \pm 4,77$ ml with 80% of goiter volume was less than 10% (**table 1**). Only 1 case were found > 20 ml (3,3%).

Correlation of thyroid antibody and goiter size

As shown in figure 1 below, we found there is no correlation between thyroid antibody and goiter volume ($r = 0,63$; $p\text{-value} = 0,09$). We are also found there was no significant correlation between TRAb and gradation volume by using Chi-square test analysis ($r = 0,39$). Figure 2 described that TRAb level decreasing with advanced of goiter gradation.

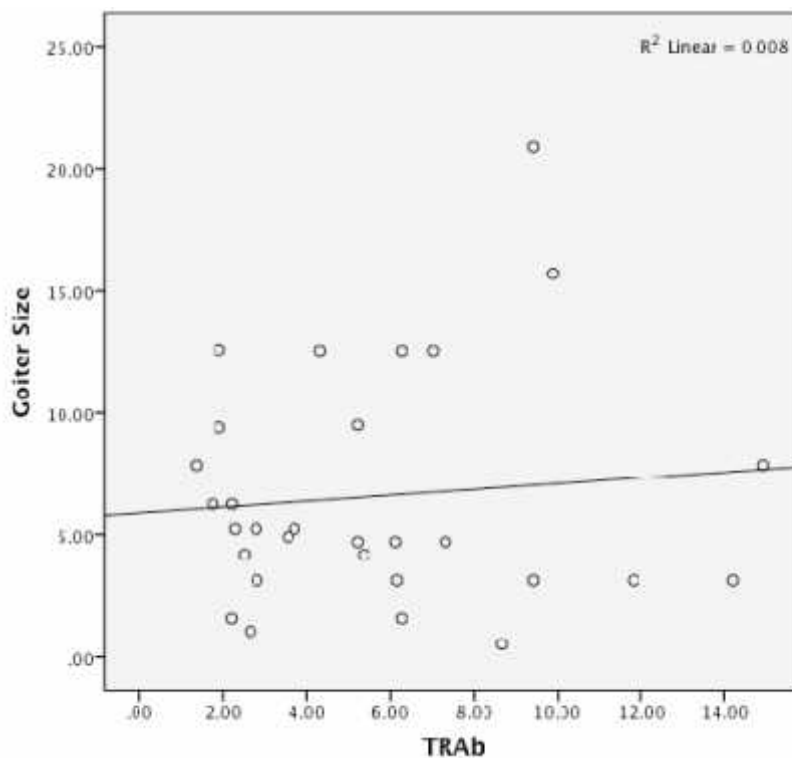


Fig.1 Correlation between size of goiter with thyroid antibody (TRAb) in Graves' disease patients.

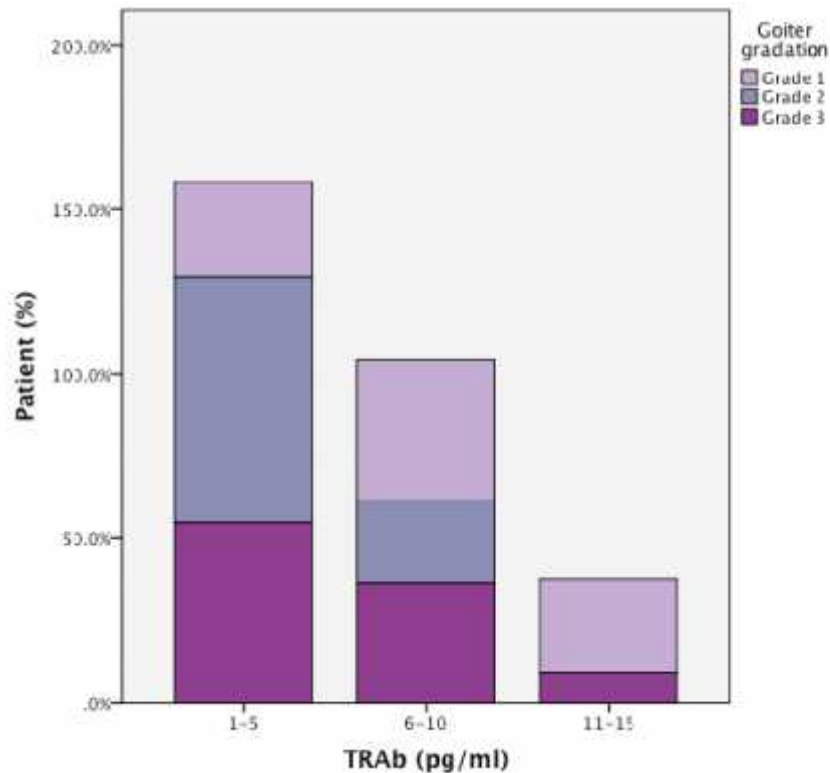


Fig 2. Goiter gradation as a function of TRAb (Grade I ; Grade II ; Grade III)

DISCUSSION

Gender and Age.

Based on gender, female patients were found more than male with percentage 66,7 : 33,3. Similar result was also found in Marina (2010) with 90% samples were female, and only 10 % were male patients. Hartono (2010), Mao (2011) and Zhu (2012) were also shown similar results with our study.^(7,10,14) This phenomenon was initially ascribed to hormonal differences, estrogens exacerbate and androgens inhibit immune responses, which influenced by gender.⁽¹⁵⁾

In this study, onset age of Graves' disease varied from puberty to old age, with range of age from 19 to 74 years old, and mostly found in the age over 40 years (50%) and the lowest between 30-39 years old (20%). Marina (2010) studies shown the same result with our

study that 50% of patients were over 40 years old. Mao (2011) shown that mean of age of 77 patient of Graves' disease were $41,1 \pm 12,7$ years old.^(7,10)

Correlation of thyroid antibody (TRAb) and goiter size.

Graves' disease is a Th2 mediated disease with mild lymphocytic infiltration of the thyroid and induce activation of auto-reactive B cells that secrete thyrotropin receptor (TSH-R) stimulating antibodies (TRAb), which cause hyperthyroidism.^(15,16) Enlargement of thyroid (goiter), ophthalmopathy, and or dermopathy were found as manifestation in Graves' disease patient which is caused by occurrence of hyperthyroid.^(17,18)

The aim of this study was to correlate between thyroid antibody and goiter size, which were described as goiter gradation and volume. Goiter gradation of this study mostly found in grade II (40%), followed by grade III (36,7%) and grade I (23,3%). Ling Chen (2008) was found that from 154 Graves' disease patient, grade II also found the most, followed by grade III and I. Marina (2010) studied 25 patients, also found grade II the most (60%), followed by grade I and III. Vos (2009) from 263 patients shown different result with grade I found at most.^(12,13)

Thyroid volume through ultrasonography result shown that mean of thyroid volume of Graves' disease patient on this study were $6,58 \pm 4,77$ ml, with size < 10 ml were found at most. Different from Klatka (2014) that shown mean of thyroid volume were 24,14 ml, with size range between 10,47 – 35,5 ml. Remission or relapse may occur in Graves' patients with large thyroid gland.^(18,19)

The hyperactive of the thyroid gland is due to the presence of TRAb, and these are now known to recognize and activate the thyroid stimulating hormone receptor (TSHR). These TRAb increase the growth and the function of the thyroid follicular cells leading to the excessive production of thyroid hormones (both T3 and T4) and symptoms of tachycardia,

anxiety and weight loss among others.⁽⁸⁾ As seen in results, TRAb on this study were found increase between 1,36 – 14,93 IU/l with mean $5,63 \pm 5,21$ IU/l. Skowronck (2013) study showed increased TRAb in 30 patients with Graves' with range between 7 – 462 IU/l. Klatka (2014) study showed almost the same result as our research with mean $7,63 \pm 4,62$ IU/l.^(19,20,21) While Zhu (2012) showed mean of TRAb $24,21 \pm 6,06$ IU/l.⁽¹⁴⁾ This difference result may occurred due to difference in number of sample and characteristic of studied subjects such as age and method of analysis.

On this study, main finding of our study is that volume goiter and gradation is not related to the TRAb (TSHR antibody) level ($p > 0,05$) as seen in figure 1. However, as seen in figure 2, we can see clearly that TRAb level decreased with advanced of goiter gradation, which described that the more of TRAb level didn't correlate with enlargement of thyroid. Previous study by Ertek (2010) also shown no correlation between goiter volume and thyroid antibody in Graves' disease patients, but TSH levels was significantly correlated with thyroid antibodies ($p = 0.0000$ for TRAb), which is not described in our study.^(20,21) Laurberg (2014) found different result that there was correlation between goiter size and TRAb level ($r = 0.25$, $p < 0.001$).⁽²²⁾

Contribution of genetic and environment factors to Graves' disease such as iodine consumption, drugs, infection, smoking and stress may influenced level of TRAb.⁽²³⁾ Complex interaction between these factors may alter central and peripheral immune tolerance that affect to goiter size and level of TRAb.^(24,25) Limitation of this study is that we are not measure iodine consumption in our study; thus we cannot see if there is one of this factor contribute to our result. Research of T-cell role in Graves' disease, such as T regulator and cytokines begin to grow. In Graves' disease, both Th1 mediating cell mediated immunity and Th2 mediating humoral immunity occur. Studies have shown that active inflammatory phase of Graves' disease is associated with a prevalent Th1-mediated immune response, which

switches to a Th2 phenotype in long standing Graves' disease. Cytokine expression profiles in sera and thyroid tissues from Graves' disease patients indicate a mixed Th1/Th2 status at any time. Further studies, it's necessary to examine the role of cytokines that provide by T-cell on Graves' disease.^(26,27)

CONCLUSION

There is no correlation between goiter gradation and volume to TRAb serum levels. Further studies, the interaction between genetic and environmental factors such as iodine consumption, infection, smoking and stress should be investigated considering influence of these factors in development of the disease.

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