

THE PROFILE OF THYROTROPIN-RELEASING HORMONE, T-REGULATOR, AND INTERLEUKIN-4 OF UNTREATED GRAVES' DISEASE IN INDONESIA

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ABSTRACT

Objective: This study was conducted to the profile of hormonal and immunological aspects, which is thyrotropin-releasing hormone (TRH), T-regulator (T-reg) Forkhead box P3 (FOXP3) gene polymorphism, and interleukin-4 (IL-4) of untreated Graves' disease with hyperthyroid in M Djamil General Hospital Padang, Indonesia.

Methods: A cross-sectional study was conducted to observe Graves' disease with hyperthyroid in the Internal Medicine Department in Dr. M. Djamil Hospital, Padang, West Sumatera, Indonesia. Graves' disease was confirmed by measuring free thyroxine (FT4), thyroid-stimulating hormone (TSH), and antithyroid peroxidase (anti-TPO). TRH, T-reg FOXP3 gene polymorphism, and IL-4 were examined with an enzyme-linked immunosorbent assay method.

Results: Twenty-five subjects with Graves' disease included in this study with an average of age is 27.48 (5.6) years. Mean Wayne index is 23.44 (1.4), mean serum FT4 level is 55.55 (17.98) pmol/l, median serum TSH level is 0.041 (0.004–0.053) mU/l, and mean anti-TPO level is 2697.539 (479.72) pg/ml. Median serum TRH level is 92.589 (24.843–253.186) pg/ml, mean T-reg FOXP3 gene polymorphism is 0.621 (0.23) ng/ml, and mean serum IL-4 level is 19.759 (7.03) pg/ml).

Conclusion: Graves' disease with hyperthyroid has median serum TRH level 92.589 (24.843-253.186) pg/ml and high levels of T-reg FOXP3 gene polymorphism and IL-4.

Keywords: Thyrotropin-releasing hormone, T-regulator, Interleukin-4, Graves' disease.

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INTRODUCTION

Hyperthyroidism is the tissue response to the excessive metabolic effects of thyroid hormone. It is found in 0.8–1.3% of populations worldwide. In Indonesia, the prevalence of hyperthyroidism reaches 6.9%. Hyperthyroidism can be caused by excessive stimulation of thyroid-stimulating hormone (TSH) receptors, autonomic secretion of thyroid hormones, damage to thyroid follicles, and secretion of thyroid hormones from extrathyroidal tissues. Hyperthyroidism is mostly caused by Graves' disease which stimulates the excessive activity of the thyroid gland through its receptors. The aspects that play a role in Graves' disease are hormonal and immunological aspects [1-4].

The hormonal factors are TSH and thyrotropin-releasing hormone (TRH). TSH is one of the links in a complex signaling network that modulates and controls thyroid growth and function in Graves' disease. TSH circulating in the tissues is controlled by TRH levels and the feedback effect of thyroid hormone levels on the tissues. The TRH effect is initiated by binding of peptides with G protein-coupled receptor (GPCR) on the thyrotropic plasma membrane. TRH secretion is influenced by thyroid hormones through a negative feedback mechanism in the hypothalamic-pituitary-thyroid axis [5,6].

In the immunological aspects of Graves' disease, at a higher level, it is controlled by T-regulator (T-reg) cells. Forkhead box P3 (FOXP3) is a major regulatory factor for T-reg cells. In the next stage, the T-reg will differentiate T-helper (Th) cells, which is will produce various inflammatory cytokines, including interleukin-4 (IL-4). IL-4 stimulates isotype-secreting cells immunoglobulin G3 (IgG3-SCS) which are associated with the severity of Graves' disease. In addition to T-reg cells, B cells have a role in humoral to produce several antibodies, one

of them is antithyroid peroxidase (anti-TPO). Measuring anti-TPO can be used as a predictive marker in diagnosing patients with autoimmune thyroid disorders. [6-9].

Due to the important role of these two aspects in Graves' disease, this study was conducted to gather the levels of both aspects in patients with Graves' disease who have not received any hyperthyroid therapy.

METHODS

This was a cross-sectional study conducted in the Internal Medicine Department in Dr. M. Djamil Hospital, Padang, West Sumatera, Indonesia. This study was conducted from April 2018 until July 2018. This study involved 25 patients with Graves' disease who had not received prior treatment and admitted to the metabolic, endocrine clinic at the RSUP Dr. M. Djamil Padang. Graves' disease was confirmed by measuring free thyroxine (FT4), TSH, and anti-TPO. Pregnant patients and Graves' relapse were excluded. All blood samples have taken from these study participants for laboratory tests. All patients have provided signed consent. This research has received ethical approval from the Ethics Committee of the Medical Faculty of Andalas University.

Examination methods

We examined serum TRH, T-reg FOXP3 gene polymorphism, and IL-4 levels. All variables were examined with enzyme-linked immunosorbent assay techniques method (Elabscience Biotechnology).

Statistical analysis

Categorical scale data were written in frequencies and percentages, while interval data or ratio scale was written in mean (standard deviation) or median (min-max).

RESULTS

There are 25 samples in this study. Patients' age in this study ranged from 17 to 33 years old. From the study, the range of patients' age is from 17 to 33 years, with the average of age is 27.48 (5.6) years. The number of female patients in this study is more than male patients, with the percentage of women are 96% and men are 4%. In this study, only one from the men patients was attended at the study. All of the patients are Graves' disease with hyperthyroid.

Mean Wayne index is 23.44 (1.4), mean serum FT4 level is 55.55 (17.98) pmol/l, median serum TSH level is 0.041 (0.004–0.053) mU/l, and mean anti-TPO level is 2697.539 (479.72) pg/ml. Median serum TRH level is 92.589 (24.843–253.186) pg/ml, mean T-reg FOXP3 gene polymorphism is 0.621 (0.23) ng/ml, and mean serum IL-4 level is 19.759 (7.03) pg/ml. Research's subject characteristics can be seen in Table 1.

DISCUSSION

There were 25 subjects with Graves' disease treatment naïve included in this study. The average age of this study is smaller than the study by Laurberg *et al.*, which is 45 years old. Liu *et al.* obtained the average age of their study of 33.8 years old. The average age obtained in this study is in accordance with the Indonesian Society of Endocrinology Task Force on Thyroid Diseases which states that Graves' disease appears more frequently in the third and fourth decades. This statement also fits the basic characteristics of patients with Graves' disease in other studies in different countries [4,10,11].

In this study, there was only one male patient (4%). Molnar *et al.* obtained a percentage of a sample of women in the study of 86.5%. Calissendorff and Falhammar obtained all the samples as women. Voskuhl stated that women are more prone to suffer from autoimmune disorders. Being a woman is a greater risk factor than genetic or environmental risk factors that have been discovered to date [12-14].

Mean Wayne index is 23.44 (1.4). This indicates that the study sample was clinical hyperthyroid patients. In the study by Sabit *et al.*, mean Wayne index is 31.6. The sensitivity of the Wayne index in diagnosing hyperthyroidism is 86.9%, while the specificity is 96.6% [15,16].

Mean serum FT4 level in this study is 55.55 (17.98) pmol/l. This serum FT4 level was higher than the study by Choi and Yoo, who obtained serum FT4 levels before the initial treatment of 48.9 (21.9) pmol/l. Molnar *et al.* reported lower serum FT4 levels than this study and Choi and Yoo, which was 30.86 (39.54) pmol/l [12,17].

Median serum TSH levels in this study were not much different from the mean serum TSH levels in other studies. Santos *et al.* conducted a study of 100 Graves' disease patients and obtained the average TSH level was 0.04 (0.1) mU/l. Azizi *et al.* conducted a study of 85 Graves' disease patients who were divided into 34 patients who would receive methimazole and 51 patients who would receive radioiodine therapy

in Iran. In patients receiving antithyroid drugs, the serum TSH level before therapy is 0.012 (0.001) mU/l. While patients who will receive radioiodine, TSH level before therapy is 0.014 (0.001) mU/l [18,19].

Mean anti-TPO in this study is 2697.539 (479.72) pg/ml. Anti-TPO antibodies from healthy subjects did not block TPO activity or interfere with the blocking activity of anti-TPO antibodies from Graves' disease, while anti-TPO antibodies from Graves' disease can fix complement, destroy thyrocytes, and act as competitive inhibitors of enzymatic activity [20].

Median serum TRH level was higher than the average TRH level obtained by Lombardi *et al.* in 1978 in Italy. Lombardi *et al.* reported average serum TRH levels 30.8 (10.5) pg/ml in normal adult men and 33.2 (11.9) pg/ml in normal adult women. However, the study did not report serum TRH levels in patients with Graves' disease [21].

In hyperthyroid patients, the thyroid hormone is stronger in inhibiting the production of TSH in the pituitary gland than the production of TRH in the hypothalamus. Graves' disease patients with hyperthyroidism show no TRH-induced response. This concludes that increased levels of circulating thyroid hormone in the blood have a strong inhibitory effect on TSH secretion from the pituitary [19,22].

Synthesis of TRH and TSH is inhibited at the level of transcription by thyroid hormones. Thyroid hormones also inhibit post-translational modification of TSH and TSH secretion. Furthermore, the thyroid hormone also modulates TSH expression by disrupting the TRH receptor in the pituitary. Although TRH stimulates TSH synthesis and secretion, negative feedback by the thyroid hormone in the pituitary remains the most important regulator of serum TSH levels. The effect of thyroid hormone in negative feedback on the hypothalamic-pituitary-thyroid axis is mediated largely by TR. The physiological set point for the synthesis and secretion of TSH is determined by the balance between the positive input of TRH and the strong negative influence of thyroid hormone [22,23].

The mean T-reg FOXP3 gene polymorphism in this study is 0.621 (0.23) ng/ml. T-reg appears to be crucial in the pathogenesis in Graves' disease. This level is higher than normal levels. Elvira and Darwin examined Graves' disease patients and obtained a T-reg FOXP3 gene polymorphism level of 23.51 (15.7) pg/ml. T-reg has a central role in the prevention of Graves' disease development. The expression of FOXP3 is crucial for T-reg development and function in regulating the balance of the immune system [8,9].

The mean IL-4 levels in this study were found to be higher than normal levels. This level is also higher than IL-4 levels in other studies of Graves' disease. Decroli *et al.* conducted a study in patients with Graves' disease. They found that IL-4 level before the patient received therapy was 12.23 (5.74) pg/ml. The elevated serum cytokines of Graves' disease reflect the activation and interplay of mixed Th1 and Th2 cells [7,24-27].

Table 1: Baseline characteristics

Characteristics (n=25)	Mean (SD)	Median (min-max)	n (%)
Average age (yo)	27.48 (5.6)		
Sex			
Male			1 (4)
Female			24 (96)
Wayne index	23.44 (1.4)		
FT4 (pmol/l)	55.55 (17.98)		
TSH (pg/ml)		0.041 (0.004–0.053)	
Anti-TPO (pg/ml)	2697.539 (479.72)		
TRH (mU/l)		92.589 (24.843–253.186)	
T-reg FOXP3 gene polymorphism (ng/ml)	0.621 (0.23)		
IL-4 (pg/ml)	19.759 (7.03)		

TRH: Thyrotropin-releasing hormone, T-reg: T-regulator, IL-4: Interleukin, anti-TPO: Antithyroid peroxidase, FOXP3: Forkhead box P3

CONCLUSION

Graves' disease with hyperthyroid has median serum TRH level 92.589 (24.843–253.186) pg/ml and high levels of T-reg FOXP3 gene polymorphism and IL-4.

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AUTHORS' CONTRIBUTION

Decroli *et al.* conceived the research, provided the methods, collected the data, and authored the manuscript. Dwitya Elvira collected and analyzed the data. Dinda Aprilia collected and analyzed the data.

CONFLICTS OF INTEREST

All the authors declare that they have no conflicts of interest in publishing this research article.

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