Indonesian Journal of Pharmacy
VOL 30 (2) 2019: 122 – 127 | RESEARCH ARTICLE

The Effect of Thionamide to TRH, TSH, IL-4, T-REG, and Anti-TPO in Graves' Disease

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Info Article

Submitted: 23-07-2019
Revised: 26-2-2019
Accepted: 13-3-2019

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ABSTRACT

The most common cause of hyperthyroidism is Graves' disease. TRH and TSH are hormonal factors that modulate and control thyroid function in Graves' disease. In the immunological aspect, Graves' disease is played by the role of T-reg, IL-4, and anti-TPO. Graves' disease treatment goal is to inhibit thyroid hormone secretion by administering thionamide. The evaluation of this treatment is its hormonal and immunological aspects. To describe the effect of thionamide on serum TRH, TSH, IL-4, T-reg, and anti-TPO levels in Graves' disease. This study is a clinical trial study in 25 study participants. All study participants were given thionamide, namely PTU 300mg for three months and blood samples were taken for laboratory tests. Serum TRH, TSH, IL-4, T-reg FOXP3, and anti-TPO levels were examined by ELISA. The mean levels at the beginning and after three months of therapy are: serum TRH 92.589pg/mL and 115.944pg/mL; serum TSH 0.041mU/L and 0.223mU/L; serum IL-4 19.759pg/mL and 23.040pg/mL; T-reg FOXP3 gene polymorphism 0.621ng/mL and 0.518 ng/mL; serum anti-TPO 2697.539pg/mL and 2604.710pg/mL. Increased levels of serum TRH and TSH levels were statistically significant. The change in serum IL-4, T-reg FOXP3 gene polymorphism, and anti-TPO levels were not statistically significant. The administration of thionamide in Graves' disease for three months will significantly decrease Wayne index and serum FT4 levels, increase serum TRH and TSH levels.

Keywords: TRH, TSH, T-reg, IL-4, anti-TPO

INTRODUCTION

The most common cause of hyper-thyroidism in the world is Graves' disease. Graves' disease occurs in 2 - 2.5% of women and 0.2 - 0.6% of men. The factors that play a role in Graves' disease are environment, genetic disorders, and autoimmune processes. Thyroid-stimulating hormone (TSH) is one of the links in a complex signaling network that modulates and controls thyroid growth and function in Graves' disease. TSH not only works at glands and thyroid function. TSH circulating in the tissues is controlled by thyrotropin-releasing hormone (TRH) levels and the feedback effect of thyroid hormone levels on the tissues. In the immunological aspect of Graves' disease, at higher level, is controlled by T-regulator (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). In addition to T-reg cells, B cells have role in humoral to produce several antibodies, one of them is anti-TPO (Lillevang-Johansen *et al.*, 2017; Wang *et al.*, 2012; Elvira *et al.,* 2017).

The stages of biosynthetic processes and secretions of thyroid hormones are stimulated by TSH. Thyroid hormone levels are controlled by TSH, which is produced by the anterior pituitary gland. TSH levels are regulated by TRH produced by the hypothalamus gland. Then, thyroid hormones will provide negative feedbacks to pituitary gland and hypothalamus (Davies *et al.*, 2011)

Graves' disease is not only explained by the theories of comparison Th1 and Th2. Lately

Indonesian J Pharm 30(2), 2019, 122-127 | DOI: 10.14499/indonesianjpharm30iss2pp122 indonesianjpharm.farmasi.ugm.ac.id

attention has been paid to T-reg cells. Initially T-reg cells are described as CD4 + suppressor cells. Later, regulatory T cells (T-reg) are known as subtypes of CD4 which has specificities in the presence of CD25 expression, where the expression of the Forkhead box P3 (FOXP3) molecule is a special marker from T-reg. CD4 + CD25 + Foxp3 + Natural T-reg is considered as the main component of T-reg cells and emerges from the thymus as fully differentiated cells. And the abnormality of the T-reg number and its function has an effect on several autoimmune diseases (Elvira *et al.*, 2017; Elvira *et al.*, 2016)

Interleukin-4 is the main cytokine that associated with autoimmune thyroid disease. IL-4 plays a main role in the process of differentiating naïve T cells into Th cells and increasing the expression of MCH class II in B cells, dendritic cells and macrophage cells. IL-4 stimulates the isotype of Immunoglobulin G3 (IgG3-SCS) secreting cell which is associated with the severity of Graves' disease. The increase in cytokine values in Graves' patients illustrate the activity and interplay of Th1 and Th2 which are compatible with long-term inflammation and the damage process of the thyroid gland (Decroli *et al.*, 2014).

Anti-thyroid peroxidase antibodies (anti-TPO) are antibodies that bind to transmembrane proteins in tyrosite which are involved in the synthesis of thyroid hormones. Anti-TPO is also known as an microsomal anti-thyroid antibodies which is an important examination in autoimmune thyroid disease because it is found to be positive in more than 80% of patients with Graves' disease. There is some laboratory examination to describe hyperthyroid in Graves' disease, such as: Total T3, Total T4, FT3 and FT4. FT4 is an important laboratory examination because FT4 have longer half-life and not influenced by serum albumin levels. In addition, Wayne index that represented the symptoms and clinical manifestation including vital sign of Graves' disease is also important in assessing the patient's performance (Davies *et al.*, 2011).

In general, the goal of treating hyperthyroidism in Graves' disease is to inhibit thyroid hormone secretion. One of the effective treatment for hyperthyroidism is antithyroid agents, which is thionamide. Antithyroid treatment consists of initial and maintenance therapy. Initial therapy is given until serum FT4 levels are normal. Initial therapy ranges from 4 to 12 weeks. After achieving normal serum FT4 levels, maintenance therapy begins by reducing the dose of thionamide by 50% (Davies *et al.*, 2011; Elvira *et al.*, 2016; Decroli *et al.*, 2014).

The Wayne index, serum FT4 and TSH levels are evaluated in the treatment of Graves' disease. Meanwhile, TRH levels have not been evaluated. The effect of therapy on Graves' disease should also influence the factors that play a role in immunological aspects, such as T-reg, IL-4, and anti-TPO. In this study, we want to see the effect of thionamide on levels of TRH, TSH, IL-4, T-reg, and anti-TPO in Graves' disease (Davies *et al.*, 2011; Elvira *et al.*, 2016; Decroli *et al.*, 2014).

MATERIAL AND METHODS

This study is a clinical trial study with pre and post treatment to the study sample. This study involved 25 patients with Graves' disease who had not received prior treatment and who controlled to the metabolic endocrine clinic at the RSUP Dr. M. Djamil Padang who has signed the informed consent. Pregnant patients, allergic to thionamide, and Graves' relapse were excluded. All study participants were given initial therapy for three months of thionamide antithyroid treatment, PTU 300mg. All blood samples have taken from this study participants for laboratory tests at the beginning and at the end of initial therapy. We examined variables like serum TRH, TSH, IL-4, T-reg FOXP3 gene polymorphism, and anti-TPO levels. This research has received an ethical approval from the Ethics Committee of Medical Faculty of Andalas University.

Methods

Serum TRH, TSH, IL-4, T-reg FOXP3, and anti-TPO levels were examined with enzyme-linked immunosorbent assay techniques (ELISA) method. The variables examinations were using Elabscience Biotechnology experimental kits.

There are several steps in this examination process. Add 100μL sample each well. Incubate for 90 minutes at 37°C. Remove the liquid. Add 100μL Biotinylated Detection Ab. Incubate for one hour at 37°C. Aspirate and wash 3 times. Add 100μL HRP Conjugate. Incubate for 30min at 37°C. Aspirate and wash 5 times. Add 90μL Substrate Reagent. Incubate for 15min at 37°C. Add 50μL Stop Solution. Read at 450nm immediately. And then, calculate the results.

Statistical analysis was carried out by comparing serum TRH, TSH, IL-4, T-reg FOXP3 gene polymorphism, and anti-TPO levels at the beginning and at the end of three months therapy of thionamide. Paired t-test was used to analyze

Table I. Baseline Characteristics

Characteristics (n=25)	Mean (SD)	Median	n (%)
Average Age (yo)	**27.48 (5.6)**		
Sex			
Male			**1 (4%)**
Female			**24 (96%)**
Heart Rate (beats/minute)	**110.40 (6.63)**		
Systolic Blood Pressure (mmHg)	**139.48 (6.63)**		
Diastolic Blood Pressure (mmHg)	**63.48 (8.64)**		
Pulse Pressure (mmHg)	**76.00 (11.77)**		
Temperature (^{0}C)	**37.62 (0.51)**		
Wayne Index	**23.44 (1.4)**		
FT4 (pmol/L)	**55.55 (17.98)**		
TRH (mU/L)		**92.589**	
TSH (pg/mC)		**0.041**	
T-reg FOXP3 gene polymorphism (ng/mL)	**0.621 (0.23)**		
IL-4 (pg/mL)	**19.759 (7.03)**		
Anti-TPO (pg/mL)	**2697.539 (479.72)**		

Table II. Changes in vital signs at initial and after three months of thionamide therapy

Variable	Mean		p
	Initial (n=25)	After three months (n=25)	
Heart Rate (beats/minute)	110.40 (6.63)	82.12 (5.07)	0.001
Systolic Blood Pressure (mmHg)	139.48 (6.63)	121.12 (5.39)	0.001
Diastolic Blood Pressure (mmHg)	63.48 (8.64)	77.32 (6.38)	0.001
Pulse Pressure (mmHg)	76.00 (11.77)	43.80 (9.55)	0.001
Temperature (^{0}C)	37.62 (0.51)	36.87 (0.28)	0.069

the differences of before and after the three months therapy. A value of $p < 0.05$ was considered as significant.

RESULT AND DISCUSSION

The baseline characteristics of this study (Table I). From the study, the range of patients' age are 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 percentage of women are 96% and men are 4%. In this study, only one from the men patients was attended at the study. The average of age from this study's sample is smaller than some other studies. The average of age was obtained in this study is appropriate with The Indonesian Society of Endocrinology Task Force on Thyroid Diseases (2012) which states that Graves' disease appears more frequently at the third and fourth of decades (The Indonesian Society of Endocrinology, 2012; Voskuhl, 2011).

The higher percentage of women than men was also found by Voskuhl (2011). Voskuhl (2011) states that women are more prone to suffer from autoimmune disorders. This is in accordance with Ngo *et al* (2014) which states that autoimmune disorders are more common in women. There are several studies that gave the prevalence of autoimmune disorders in several countries. Carle *et al* (2011), Gaujoux *et al* (2006), Guo *et al* (2013), and Phitayakorn *et al* (2013) reported that Graves' disease was more prevalent among women in the United States, France, Denmark, and China. The Indonesian Society of Endocrinology Task Force on Thyroid Diseases (2012) states that the ratio of women to men with Graves' disease is 8:1 (The Indonesian Society of Endocrinology, 2012; Voskuhl, 2011; Ngo *et al.*, 2014).

Changes in vital signs, Wayne index and serum FT4 levels at initial and after three months of thionamide therapy (Table II and Table III).

Table III. Changes in Wayne index and FT4 serum levels at initial and after three months of thionamide therapy

Variable	Mean		p
	Initial (n=25)	After three months (n=25)	
Wayne index	23.44 (1.42)	11.80 (1.47)	0.001
FT4 Serum (pmol/L)	55.55 (17.98)	9.44 (2.67)	0.001

Table IV. Changes in TRH and TSH serum levels at initial and after three months of thionamide therapy

Variable	Median		p
	Initial (n=25)	After three months (n=25)	
TRH Serum (pg/mL)	92.589	115.944	0.001
TSH Serum (mU/L)	0.041	0.223	0.001

Tabel V. Changes in serum T-reg FOXP3 polymorphism, IL-4 and anti-TPO levels at initial and after three months of thionamide therapy

Variable	Mean		p
	Initial (n=25)	After three months (n=25)	
T-reg FOXP3 gene polymorphism (ng/mL)	0.621 (0.23)	0.518 (0.25)	0.124
IL-4 (pg/mL)	19.759 (7.03)	23.040 (7.35)	0.150
Anti-TPO (pg/mL)	2697.539 (479.72)	2604.710 (458.80)	0.361

Heart rate, systolic blood pressure, and pulse pressure are decreased after initial thionamide therapy. But, there is no significant changes in temperature. The decreased of Wayne index and serum FT4 levels are statistically significant. These showed that initial therapy of thionamide can improve the patient's performance. Davies *et al* (2011) also found that initial therapy of thionamide will decreased nervousness, palpitation and heart rate, increased strength, and weight gain. The decreased of these parameters are important to evaluate the outcome of therapy (Davies *et al.*, 2011).

Changes in serum TRH and TSH levels at initial and after three months of thionamide therapy (Table IV). Median of serum TSH level before administration of thionamide in this study was 0.041 mU/l. After giving thionamide for three months, the median of serum TSH level was 0.223 mU/L. There was an increase in serum TSH level after administration of thionamide for three months, statistically significant. This result is supported by Schimdt and Braunbeck (2011) who stated that zebrafish who get thionamide will experience thyroid gland hyperplasia and increased blood vessel flow in the thyroid gland tissue. Immunohistological staining at cells which are producing TSH in the pituitary shows a significant increase. This indicates that an increase occurs in serum TSH levels of zebrafish after the administration of thionamide (Schmidt *et al.*, 2011).

Median serum TRH level before administration of thionamide in this study was 92.589 pg/mL. After giving thionamide for three months, the median serum TRH level was 115.944 pg/mL There was an increase in TRH levels after the administration of thionamide for three months, which is statistically significant. Propylthiouracil is effective in reducing thyroid hormone levels back to normal with the initial therapy for three months. This decrease in thyroid hormone levels will be followed not only by increased in TSH levels, but also by an increase in TRH levels through the hypothalamic-pituitary-thyroid axis. (Alkemade, 2015; Guissouma *et al.*, 2002).

Changes in serum T-reg FOXP3 polymorphism serum, IL-4 and anti-TPO levels at initial and after three months of thionamide therapy (Table V). The mean T-reg FOXP3 polymorphism before administration of thionamide in this study was 0.621 ng/ml. After giving thionamide for three months, the average T-reg FOXP3 polymorphism in this study was 0.518ng/mL. The change in FOXP3 T-reg polymorphism statistically was not significant.

The mean serum IL-4 level before administration of thionamide in this study was 19.759pg/mL. After giving thionamide for three months, the mean serum IL-4 level in this study was 23,040 pg/mL. This change in IL-4 levels statistically was not significant. The mean serum anti-TPO level before administration of thionamide in this study was 2,697,539 pg/mL. After giving thionamide for three months, the mean serum anti-TPO level in this study was 2,604,710 pg/mL. This change in the level of anti-TPO statistically was not significant.

Humar *et al* (2008) explained that thionamide is an antithyroid drug with immunomodulating effects. This was proven in his research that showed thionamide which inhibited the synthesis of proinflammatory cytokines TNF-α and interferon-λ. Decroli *et al* (2014) found that administration of thionamide for one year would reduce IL-4 levels. Levels of IL-4 were found to drop dramatically in the first six months of thionamide therapy. However, the influences of thionamide on T-reg and anti-TPO has not been widely discussed (Decroli *et al.*, 2014; Humar *et al.*, 2008).

This study shows that the administration of initial therapy of thionamide for three months will improve Graves' disease hormonally, which are normal levels of FT4, significantly increased TSH and TRH serum levels. In this study, there were no significant changes in the FOXP3 T-reg gene polymorphism, IL-4 levels, and anti-TPO serum. It explains that the importance to continue the administration of thionamide in Graves' disease to see its immunological effects (Elvira *et al.,* 2017; Decroli *et al.*, 2014; Lauberg *et al.*, 2014).

CONCLUSION

The administration of thionamide in Graves' disease for three months will significantly decrease Wayne index and serum FT4 levels, increase serum TRH and TSH levels.

ACKNOWLEDGEMENT

This research was funded by Andalas University (UNAND) through LPPM. This research included in *Klaster Riset Penelitian Percepatan ke Guru Besar – Penelitian Dasar Unggulan* (KRP2GB-PDU) UNAND 2018.

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