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

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ABSTRACT

Background: 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 evaluationof this treatment is its hormonal and immunological aspects.**Objective:**To describe the effect of thionamide on serum TRH, TSH, IL-4, T-reg, and anti-TPO levels in Graves' disease. **Methodology:**This study is a clinical trial study in 25 study participants. All study participants were given thionamide, namely PTU 300 mg for three months and blood samples were taken for laboratory tests. Serum TRH, TSH, IL-4, and anti-TPO levels were examined by ELISA. The T-reg FOXP3 gene polymorphism was examined by PCR.**Results:**The mean levels at the beginning and after three months of therapy are:serum TRH 92.589 pg/ml and 115.944 pg/ml; serum TSH 0.041 mU/l and 0.223 mU/l; serum IL-4 19.759 pg/ml and 23.040 pg/ml; T-reg FOXP3 gene polymorphism 0.621 ng/ml and 0.518 ng/ml; serum anti-TPO 2697.539 pg/ml and 2604710 pg/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 levelswere not statistically significant.**Conclusion:**The administration of thionamide in Graves' disease for three months will significantly increase serum TRH and TSH levels and not have a significant effect on serum IL-4, T-reg FOXP3 gene polymorphism, and anti-TPO levels.

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

INTRODUCTION

The most common cause of hyperthyroidism 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 humoralto produce several antibodies, one of them is anti-TPO[1],[2],[3].

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 [4].

Graves' disease is not only explained by the theories of comparison Th1 and Th2. Lately 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[3],[5].

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 [6].

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 [4].

In general, the goal of treating hyperthyroidism in Graves' disease is to inhibit thyroid hormone secretion. In the treatment of Graves' disease, the levels of thyroid hormone and TSH are evaluated. 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 [4],[5],[6].

METHODOLOGY

Materials

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 300 mg. 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, serum TSH, serum IL-4, T-reg FOXP3 gene polymorphism, and serum anti-TPO. This research has received an ethical approval from the Ethics Committee of Medical Faculty of Andalas University.

Methods

TRH serum, TSH serum, IL-4 serum, and anti-TPO serum were examined with enzyme-linked immunosorbent assay techniques (ELISA). The FOXP3 T-reg gene polymorphism was examined using the polymerase chain reaction (PCR) method.

Statistical analysis was carried out by comparing TRH serum levels, TSH serum, IL-4 serum, T-reg FOXP3 gene polymorphism, and anti-TPO serum at the beginning and at the end of three months therapy of thioanamide. Paired t-test was used to analyze 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 are shown in table 1. 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 [7],[8].

Table 1. Baseline Characteristics

Characteristics (n=25)	**Mean (SD)**	**Median**	**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)**		
TRH (mU/l)		**92,589**	
TSH (pg/ml)		**0,041**	
T-reg FOXP3gene polymorphism (ng/ml)	**0,621 (0,23)**		
IL-4 (pg/ml)	**19,759 (7,03)**		
Anti-TPO (pg/ml)	**2697,539 (479,72)**		

The higher percentage of womenthan 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 [7],[8],[9].

Table 2. Changes in TRH and TSH serum levels at initial and after three months of thionamidetherapy

Variable	**Median**		**p**
	Initial (n=25)	**After three months (n=25)**	
TRHSerum(pg/ml)	92,589	115,944	0,001
TSHSerum(mU/l)	0,041	0,223	0,001

Changes in TRH and TSH serum levels at initial and after three months of thionamide therapy can be seen in table 2. Median of TSH serum level before administration of thionamide in this study was 0.041 mU/l. After giving thionamide for three months, the median of TSH serum level was 0.223 mU/l. There was an increase in TSH serum 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 TSH serum levels of zebrafish after the administration of thionamide [10].

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) and Guissouma et al (2002).[11],[12].

Tabel 3. Changes in T-reg FOXP3 polymorphism serum, IL4 serum and anti-TPO serum 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

Changes in T-reg FOXP3 polymorphism serum, IL4 serum and anti-TPO serum levels at initial and after three months of thionamide therapycan be seen in table 3. 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 0.518 ng/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 19.759 pg/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 [6], [13].

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 [3],[6],[14].

CONCLUSION

The administration of thionamide in Graves' disease for three months will significantly increase TRH and TSH serum levels and doesn't have a significant effect on the T-regFOXP3 gene polymorphism, IL-4 serum levels, and anti-TPO serum.

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