



Correlation between Interleukin-4 Gene Promoter Polymorphisms with Thyrotropin Receptor Antibody and Transforming Growth Factor- β

Raveinal Raveinal^{1*}, Eryati Darwin², Eva Decroli³, Jamsari Jamsari⁴

¹Department of Internal Medicine, Allergic and Immunology Subdivision, Dr. M. Djamil General Hospital, Faculty of Medicine, Andalas University, Padang, Indonesia; ²Department of Histology and Immunology, Faculty of Medicine, Andalas University, Padang, Indonesia; ³Department of Internal Medicine, Endocrinology and Metabolic Subdivision, Dr. M. Djamil General Hospital, Faculty of Medicine, Andalas University, Padang, Indonesia; ⁴Department of Agriculture, Faculty of Agriculture, Andalas University, Padang, Indonesia.

Abstract

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***Correspondence:** Raveinal Raveinal, Dr. M. Djamil General Hospital, Faculty of Medicine, Andalas University, Padang, Indonesia.
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AIM: The aim of this study was to determine the correlation between interleukin-4 (IL-4) gene promoter polymorphisms with thyrotropin receptor antibody (TRAb) and transforming growth factor- β (TGF- β).

METHODS: This study was conducted from August 2015 until December 2015 in the internal medicine department in Dr. M. Djamil Hospital, Padang, West Sumatera, Indonesia. Graves' disease was confirmed by measuring free thyroxine, thyroid-stimulating hormone, and TRAb. We examined that IL-4 promoter gene polymorphism was examined with a polymerase chain reaction. Graves' disease serum patients will be used to check levels of TGF β and TRAb antibodies using the enzyme-linked immunoassay method.

RESULTS: There are 15 patients in this study. The average of age in patients group is 40.87 (11.23) years. The number of female patients in this study is more than male patients, with the percentage of women are 73.3%, and men are 26.7%. The sequencing examination on IL-4 gene promoter resulted in 2 single nucleotide polymorphism motifs, which are rs2243250 and rs2070847. The mean TRAb level in wild type and mutant group is 6.77 (5.73) IU/L and 4.66 (3.91) IU/L, respectively. The mean TGF- β levels in wild type and mutant group are 1168.89 (438.91) pg/mL and 1114.79 (296.02) pg/mL, respectively. Statistical tests showed no association between IL-4 gene promoter polymorphisms with TRAb and TGF- β levels ($p > 0.05$).

CONCLUSION: There is no correlation between IL-4 gene promoter polymorphisms with TRAb and TGF- β .

Introduction

The most common cause of hyperthyroidism is Graves' disease. Graves' disease is a hyperthyroid state characterized by diffuse enlargement of the thyroid gland due to immunological causes. The immunological process that underlies Graves' disease is the low clonal T cell regulator that functions to regulate the balance of T-helper (Th) 1 and Th2 cells in antibody production [1], [2], [3], [4], [5].

Interleukin (IL)-4 is part of cytokines that affect antibody production and allergic responses. Paschke *et al.* found various patterns of increased IL-2, IL-4, IL-10, and interferon- γ accumulation in thyroid surgery specimens from Graves patients. IL-4 is a cytokine produced by Th2 lymphocyte cells which have a role in the activation of B cells to form antibodies. IL-4 stimulates the number of isotypes of immunoglobulin

G-secreting cells (IgG3-SCs) associated with the severity of Graves' disease and thyrotropin receptor antibody (TRAb) levels, while also changing to IgG1 which can stimulate TRAb production effectively. Several studies have suggested a modest protective effect for the T allele of a promoter polymorphism in the IL-4 gene [6], [7], [8], [9], [10].

TRAb is an autoantibody that binds to thyroid-stimulating hormone (TSH) receptors on the thyroid gland so that excessive thyroid hormone production occurs. Transforming growth factor (TGF)- β is one of the growth factors in tissue regeneration and repair. TGF- β is produced by various types of cells, including T lymphocytes. Several previous studies have found that levels of TRAb and TGF- β increase in the disease of Graves [6], [8]. The aim of this study was to determine the correlation between IL-4 gene promoter polymorphisms with TRAb and TGF- β .

Methods

This study was conducted from August 2015 until December 2015 in the internal medicine department in Dr. M. Djamil Hospital, Padang, West Sumatera, Indonesia. This study involved 15 patients with Graves' disease. Graves' disease was confirmed by measuring free thyroxine (FT4), TSH, and TRAb. Patients with other autoimmune hyperthyroid diseases, chronic infection, and malignancy were excluded. All blood samples have taken from these study participants for laboratory tests. All patients have provided signed consent. This research has received an ethical approval from the Ethics Committee of the Medical Faculty of Andalas University.

We examined that IL-4 promoter gene polymorphism was examined with polymerase chain reaction (PCR). Graves' disease serum patients will be used to check levels of TGF β and TRAb antibodies using the enzyme linked immunoassay method.

Results

There are 15 patients in this study. The average of age in patients group is 40.87 (11.23) years. The number of female patients in this study is more than male patients, with the percentage of women are 73.3% and men are 26.7%.

Mean FT4 level is 77.89 (72.77) pmol/l, mean serum TSH level is 0.07 (0.09) UIU/l, and mean TRAb level is 5.23 (4.35) IU/l. The reference range for these parameters is as follows: FT4 9 – 23 pmol/l, TSH 0.35 – 5.5 UIU/l, and TRAb <0.9 IU/l. Research's subject characteristics are shown in Table 1.

Table 1: Baseline characteristics

Characteristics (n=15)	Mean (SD)	n (%)
Average Age (yo)	40.87 (11.23)	
Sex		4 (26.7%)
Male		11 (73.3%)
Female		
IL-4 (pg/ml)	27.59 (12.61)	
IL-10 (pg/ml)	1.15 (1.06)	
TGF- β (pg/ml)	1,129.21 (323.24)	
TNF- α (pg/ml)	61.89 (9.54)	
FT4 (pmol/l)	77.89 (72.77)	
TSH (UIU/l)	0.07 (0.09)	
TRAb (IU/l)	5.23 (4.35)	
Goiter size (cm)	6.18 (3.91)	

Sequencing examination on IL-4 gene promoter resulted in 2 single nucleotide polymorphism (SNP) motifs, which are rs2243250 and rs2070847. The allele that found in these SNP is CC (wild type), TT (mutant homozygote), and CT (mutant heterozygote). PCR results of direct DNA sequencing methods for SNP rs2243250 and rs2070847 in samples and controls are shown in Table 2.

Table 2: SNP motifs frequency in patients and controls

SNP motifs	Allele	Allele frequency				p
		Patient		Control		
		f	%	f	%	
rs2243250	CC	4	26.7	4	26.7	0.693
	TT	7	46.6	5	33.3	
	CT	4	26.7	6	40	
rs2070847	CC	4	26.7	2	13.3	0.311
	TT	7	46.6	5	33.3	
	CT	4	26.7	8	53.4	

From Table 3, it can be seen that in the group that experienced mutations, the mean TRAb level was 6.77 (5.73) IU/L. This figure is higher than the average TRAb level in the group that did not experience mutations, which is 4.66 (3.91) IU/L. Statistical tests showed no relationship between IL-4 gene promoter polymorphisms and TRAb levels ($p > 0.05$).

Table 3: Correlation between interleukin-4 gene promoter polymorphisms SNP rs2243250 and rs2070847 with TRAb

Type	Frequency	Mean (SD)(IU/L)	p
Wild type	4	6.77 (5.73)	0.514
Mutant	11	4.66 (3.91)	

From Table 4, it can be seen that the mean TGF- β levels in the mutated group are 1168.89 (438.91) pg/mL. In the group that did not experience mutations, the average TGF- β level was 1114.79 (296.02) pg/mL. Statistical tests showed no association between IL-4 gene promoter polymorphisms and TGF- β levels ($p > 0.05$).

Table 4. Correlation between interleukin-4 gene promoter polymorphisms SNP rs2243250 and rs2070847 with TGF- β

Type	Frequency	Mean (SD) (pg/mL)	p
Wild type	4	1,168.89 (438.91)	0.786
Mutant	11	1,114.79 (296.02)	

Discussion

Laurberg *et al.* (2014) conducted a study of 208 Graves patients in Denmark with the aim to see the relationship of serum TRAb levels to the manifestations of Graves' disease. The mean age of patients in the study was 45 years with an age range of 35–53 years. The average age of this study sample is smaller than the study conducted by Laurberg. The average age obtained in this study is in accordance with the Indonesian Society of Endocrinology Task Force on Thyroid Disease in 2012 which states that Graves' disease appeared more frequently in the third and fourth decades. This statement is consistent with the basic characteristics of patients with Graves' disease in other studies in different countries [5], [11].

According to Kahaly *et al.* (2018), Graves' disease is more common in women than men and has a population prevalence of 1–1.5%. A higher percentage of events in women than men was found in studies conducted by several researchers and other research reports on Graves' disease, which is an autoimmune disorder [12].

Boot states that the diagnostic level of TRAb for Graves' disease is more than 1.7 IU/L. In this study, the average TRAb level was 5.23 (4.35) IU/L. This means that all the samples in this study were laboratory-suffering from Graves' disease. TRAb is an autoantibody that binds to TSH receptors on the thyroid gland so that excessive thyroid hormone production occurs [13]. TRAb levels in this study were found to be lower than the studies conducted by Bell in 2018. Bell *et al.* conducted a study by evaluating the diagnosis of Graves' disease using TRAb. The average TRAb level obtained was 11.48 (1.46) IU/L [14].

Research conducted by Ylli *et al.* in Albania and Chen *et al.* in China who conducted a study of patients with Graves' disease also obtained mean results of higher TRAb levels compared to this study, which was 8.89 (3, respectively, 71) IU/L and 17.15 (12.88) IU/L [15], [16].

Hunt *et al.* conducted research on the relationship between IL-4-590C/T polymorphisms and autoimmune thyroid disease. This study found that the IL-4-590C/T genotype had a protective effect on the development of autoimmune thyroid disease, especially Graves' disease. However, in this study, no significant polymorphism (including IL-4-590 polymorphism) was detected with the age of onset, size of thyroid gland, thyroid peroxidase titer or thyroglobulin antibody, thyroid eye disease, or the presence of other autoimmune diseases [17].

In this study, there was no correlation between IL-4 gene polymorphisms and TRAb levels. In the group that did not experience a gene mutation, the average TRAb value was 6.77, while in the group that experienced a gene mutation, the average TRAb value was 4.66. From the analysis of the data, it was found that there was no correlation between IL-4 gene mutations (polymorphisms) with TRAb levels of Graves' patients.

Khalilzadeh *et al.* (2009) investigated the relationship between IL-4, IL-10, and TGFβ polymorphisms in Graves' disease. The number of samples of 247 people consisting of 107 patients with Graves' disease and 140 controls healthy patients. The results are Graves' disease more often found in the IL-4-1098G allele and GG genotype. In 68 Graves' disease patients, there was an increase in TGF-β in the allele + 869C with CC genotype. Therefore, there is a very significant relationship between polymorphisms of anti-inflammatory cytokine genes and Graves' disease [18].

In this study, there was no correlation between IL-4 gene polymorphisms and TGF-β levels. In the wild type group, the mean TGF-β value was 1168.89 while in the group that had a gene mutation, the mean TGF-β value was 1114.79. From the analysis of the data, it was found that there was no correlation between IL-4 gene mutations polymorphisms with TGF-β levels in Graves' patients with $p = 0.786$.

In this study, the average levels of TGF-β Graves' disease were found in 1129.21 (323.24) pg/ml, while the research conducted by Elvira *et al.* about the relationship between polymorphism of the T-Regulator FOXP3 gene promoter with TGF-β in Graves' disease patients found that the average levels TGF-β in Graves' disease were higher than in the control group which was 1030.01 (277.64) ng/ml, while the control group was 889.72 (37.86) ng/ml [19].

Kotajima *et al.* (2010) conducted a study on the effect of thyroid hormone and TGF-β on the concentration of cystatin C. This study was conducted in patients with Graves' disease and hypothyroidism who were never given drugs. In this study, they found a very significant increase in TGF-β level in Graves' disease compared to the control group, whereas hypothyroid patients TGF-β level were lower than in the control group. In the control group consists of 25 samples, TGF-β level is 11.7 (3.6) ng/ml, while Graves' disease consists of 33 patients, TGF-β level is 18.3 (5.1) ng/ml, hypothyroid patients consist of 8 patients, and TGF-β level is 8.8 (4.2) ng/ml [20].

Conclusion

It can be concluded that with a limited number of subjects in this study, there is no correlation between IL-4 gene promoter polymorphisms with TRAb and TGF-β.

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