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Research Article

THE ROLE OF INTERLEUKIN-4 GENE PROMOTER POLYMORPHISMS IN GRAVES' DISEASE

RAVEINAL RAVEINAL[1], ERYATI DARWIN[2], EVA DECROLI[3], JAMSARI JAMSARI[4]

[1]Department of Internal Medicine, Allergic and Immunology Subdivision, Dr. M. Djamil General Hospital/Faculty of Medicine, Andalas University, Padang, Indonesia. [2]Department of Histology and Immunology, Faculty of Medicine, Andalas University, Padang, Indonesia. [3]Department of Internal Medicine, Endocrinology and Metabolic Subdivision, Dr. M. Djamil General Hospital/Faculty of Medicine, Andalas University, Padang, Indonesia. [4]Department of Agriculture, Faculty of Agriculture, Andalas University, Padang-Indonesia. Email: raveinal_pdg@yahoo.co.id

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ABSTRACT

Objective: This study was conducted to prove the role of interleukin-4 gene promoter polymorphisms in Graves' disease patients in M Djamil General Hospital Padang, Indonesia.

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 and 15 normal subjects. We examined that IL-4 promoter gene polymorphism was examined with PCR.

Results: Sequencing examination on IL-4 gene promoter resulted in 2 Single Nucleotide Polymorphism (SNP) motifs, which is rs2243250 and rs2070847. IL-4 SNP gene promoter polymorphisms rs2243250 and rs2070847 were found in both patient and control groups. TT is homozygous SNP polymorphisms. CT is heterozygous SNP polymorphisms. CC is wild type or no mutation SNP polymorphisms. Based on statistical tests, no difference in rs2243250 and rs2070847 SNP polymorphisms was found between patient and control group ($p > 0.05$).

Conclusion: This study observed no difference in interleukin-4 gene promoter polymorphism between Graves' disease patients and control group.

Keywords: IL-4, Gene promoter, Polymorphism, Graves' disease.

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INTRODUCTION

Graves' disease is a hyperthyroid state characterized by diffuse enlargement of thyroid gland due to immunological causes. The immunological process that underlies Graves' disease is the low clonal T-cell regulator. The T-cell regulator function is to regulate the balance of T-helper 1 (Th1) and T-helper 2 (Th2) cells in antibody production [1-5].

There are several studies linking the genetic role of Graves' disease. There are various genes related to autoimmune thyroid disease. These genes control immune responses such as major histocompatibility complex genes, T cell receptor genes, and antibody genes and genes that encode autoantigen targets in autoimmune thyroid disease. Recent studies have found that promoter gene polymorphisms are associated with Graves' disease [6-11].

Cytokines are crucial in the inflammatory response. Genes associated with these cytokines are strong candidates to be cause of autoimmune thyroid disease. Cytokine related genes include interleukin (IL)-1a, IL-1b, IL-1 receptor antagonists, IL-1 receptors, IL-4 receptors, IL-6, IL-10, and tumor growth factor-β (TGF-β). Research shows that the variant of IL-4 is closely related to the development of autoimmune thyroid disease, especially Graves' disease. Variation of the IL-4 gene may be the key to autoimmune thyroid disease or other organ-specific autoimmune diseases [9,12].

Due to the important role of interleukin-4 gene promoter polymorphisms, this study was conducted to prove the role of this gene in Graves' disease.

MATERIALS AND 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 and 15 normal subjects. Graves' disease was confirmed by measuring free thyroxine (FT4), thyroid-stimulating hormone (TSH), and thyrotropin receptor antibody (TRAb). Patients with other autoimmune hyperthyroid diseases, chronic infection, and malignancy were excluded from the study. All blood samples have taken from these study participants for laboratory tests. All patients have provided a signed consent. This research has received an ethical approval from the Ethics Committee of Medical Faculty of Andalas University.

Examination methods

We examined that IL-4 promoter gene polymorphism was examined with PCR.

Statistical analysis

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

RESULTS

There are 15 patients and 15 controls in this study. 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 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. Research's subject characteristics are shown in Table 1.

Sequencing examination on IL-4 gene promoter resulted in 2 Single Nucleotide Polymorphism (SNP) motifs, which is rs2243250 and rs2070847. PCR results of direct DNA sequencing methods for SNP

rs2243250 and rs2070847 in samples and controls are shown in Table 2.

Table 2 showed that IL-4 SNP gene promoter polymorphisms rs2243250 and rs2070847 were found in both patient and control groups. TT is homozygous SNP polymorphisms. CT is heterozygous SNP polymorphisms. CC is wild type or no mutation SNP polymorphisms. Based on statistical tests, no difference in rs2243250 and rs2070847 SNP polymorphisms was found between patient and control group ($p > 0.05$).

DISCUSSION

In this study, the age range between 31 and 40 years and female sex is the most group who experiences Graves' disease. This is consistent with the American Thyroid Association data which explain that although Graves' disease can occur in all ages, it is more common in women than men in the 7–8:1 ratio. Like most other autoimmune diseases, female sex is the group that most often experiences autoimmune disease. It is suspected the role of the hormone estrogen in stimulating antibody and autoantibody production through B cells. Estrogen also increases levels of IL-4, IL-10, and TGF-β and expression of CD80 and FOXP3 [13-15].

A study of the relationship of rs2243250 polymorphism with Graves' disease in Caucasian populations shows controversial results and requires further study in other populations. Many studies analyze the relationship between IL-4 polymorphisms and autoimmune diseases. However, the results of these studies are inconsistent and inconclusive [16].

A meta-analysis study conducted by Shen *et al*. assessed the relationship between IL-4 gene polymorphisms and the risk of thyroid autoimmune disease. The results of this study indicate that there is a proven relationship in the entire population except in a small subgroup of Asia and Caucasians. This study also found an association between the polymorphisms of the IL-4 gene rs2243250, rs2070874, and rs2243289 with Graves' disease [16].

Heward *et al*. investigated the polymorphism of the −590 C/T promoter gene in the IL-4 gene. They reported that there was no significant difference in the frequency of alleles between the group with Graves' disease (14.3%) and the control group (12%). This study also showed that there was no difference in genotype frequency between groups with Graves' disease (26%) compared to the control group (20.3%) [17].

Meanwhile, research conducted by Hunt *et al.* reported that there was no difference in IL-4 gene polymorphism between the group with Graves' disease (6%) and the control group (14%). This study states that the T alleles polymorphism of −590 C/T in the IL-4 promoter gene is a protective factor against Graves' disease in the United Kingdom. This is indicated by a decrease in the frequency of CT genotypes observed in patients with Grave's disease when compared with control subjects [12].

Several case–control studies showed evidence of a correlation between IL-4 intron-3 promoter polymorphisms and various diseases, such as rheumatoid arthritis, respiratory infections in children, and early dental infections [18]. In a study conducted by Zhu *et al*., they found that genetic variants of the IL-3, IL-4, IL-5, IL-9, and IL-13 genes were determinants of the possibility of Graves' disease in Chinese populations [19]. Meanwhile, a study by Nakkuntod *et al*. in Thailand reported that the allele frequency of −589T did not differ between Graves' disease patient and control groups (69% vs. 69.3%). This study concluded that gene polymorphisms could not be used as genetic markers of disease susceptibility Grave in Thailand population [20].

In this study, polymorphisms were seen in genes with SNP motifs rs2243250 and rs2070847. In SNP rs2243250, the most alleles were TT (58.3%) in the patient group and CT alleles (60%) in the control group, while the SNP rs2070847 obtained that the most alleles were CC (66.7%) in the patient group and CT alleles (66.7%) in the control group.

There was no significant difference in the IL-4 gene polymorphism between the control group and the Graves' disease group, with p value of 0.693 at SNP rs2243250 and $p = 0.311$ at SNP rs2070874. This is in line with other researches that also did not find differences in genotype frequency between the Graves' disease and the control group [12,17,21-23].

Table 1: Baseline characteristics

Characteristics (n=15)	Mean (SD)	n (%)
Average age (yo)	40.87 (11.23)	
Sex		
Male		4 (26.7)
Female		11 (73.3)
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)	

Table 2: IL-4 promoter gene polymorphisms in Graves' disease

SNP motifs	Allele	Allele frequency				p
		Patient		Control		
		F	%	f	%	
rs2243250	**CC**	4	50	4	50	0.693
	TT	7	58.3	5	41.7	
	CT	4	40	6	60	
rs2070847	**CC**	4	66.7	2	33.3	0.311
	TT	7	58.3	5	41.7	
	CT	4	33.3	8	66.7	

CONCLUSION

This study observed no difference in interleukin-4 gene promoter polymorphism between Graves' disease patients and control group, suggesting that this polymorphism does not play a role in the genetic susceptibility in Graves' disease.

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AUTHORS' CONTRIBUTIONS

Raveinal concepted the research, provided the methods, collected the data, and authored the manuscript. Eryati Darwin and Eva Decroli managed the literature searches and interpreted the data. Jamsari performed the statistical analysis.

CONFLICTS OF INTEREST

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

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REFERENCES

1. Decroli E, Elvira D, Aprilia A. The profile of thyrotropin-releasing hormone, T-regulator, and interleukin-4 of untreated Graves' disease in

Indonesia. Asian J Pharm Clin Res 2020;13:57-9.
2. Davies T, Laurberg P, Bahn R. Hyperthyroid disorders. In: Melmed S, Polonsky K, Larsen R, Kronenberg H, editros. Williams Textbook of Endocrinology. 13th ed. Philadelphia, PA: Elsevier; 2011. p. 369-415.
3. Lillevang-Johansen M, Abrahamsen B, Jorgensen H, Brix T, Hegedus L. Excess mortality in treated and untreated hyperthyroidism is related to cumulative periods of low serum TSH. J Clin Endocrinol Metab 2017;102:2301-9.
4. Liu J, Fu J, Xu Y, Wang G. Antithyroid drug therapy for Graves' disease and implications for recurrence. Int J Endocrinol 2017;2017:3813540.
5. The Indonesian Society of Endocrinology Task Force on Thyroid Diseases. Indonesian clinical practice guidelines for hyperthyroidism. J ASEAN Fed Endocr Soc 2012;27:34.
6. Wang PW, Chen IY, Juo SH, His E, Liu RT, Hsieh CJ. Genotype and phenotype predictors of relapse of Graves' disease after antithyroid drug withdrawal. Eur Thyroid J 2012;1:251-8.
7. Decroli E, Elvira D, Aprilia A. The effect of thionamide to TRH, TSH, IL-4, T-reg, and anti-TPO in Graves' disease. Indones J Pharm 2019;30:122-7.
8. Decroli E, Manaf A, Syahbuddin S. Immunologic and hormonal effects of prophylthiouracil treatment using maintenance dose in Graves' disease patients. Acta Med Indones 2014;46:314-9.
9. Elvira D, Darwin E. Role of pro-inflammatory and regulatory cytokines in pathogenesis of Graves' disease in association with autoantibody thyroid and regulatory FoxP3 T-cells. Int J Med Health Sci 2017;11:69-72.
10. Elvira D. The role of T-regulatory expression in autoimmune thyroid disease and its association with thyroid antibody. J Autoimmune Disord 2016;2:19.
11. Tomer Y, Davies TF. Searching for the autoimmune thyroid disease susceptibility genes: From gene mapping to gene function. Endocr Rev 2003;24:694-717.
12. Hunt PJ, Marshall SE, Weetman AP, Bell JI, Wass JA, Welsh KI. Cytokine gene polymorphisms in autoimmune thyroid disease. J Clin Endocrinol Metab 2000;85:1984-8.
13. Fairweather D, Frisancho-Kiss S, Rose NR. Sex differences in autoimmune disease from a pathological perspective. Am J Pathol 2008;173:600-9.
14. Ngo ST, Steyn FJ, McCombe PA. Gender differences in autoimmune disease. Front Neuroendocrinol 2014;35:347-69.
15. Khan D, Ahmed SA. The immune system is a natural target for estrogen action: Opposing effects of estrogen in two prototypical autoimmune diseases. Front Immunol 2016;6:635.
16. Shen X, Yan X, Xie B, Xu D, Wang K, Zhu J, *et al*. Genetic variants of interleukin-4 gene in autoimmune thyroid diseases: An updated meta-analysis. Autoimmunity 2015;48:129-35.
17. Heward JM, Nithiyananthan R, Allahabadia A, Gibson S, Franklyn A, Gough S. No association of an interleukin 4 gene promoter polymorphism with Graves' disease in the United Kingdom. J Clin Endocrinol Metab 2001;86:3861-3.
18. Mitchell RE, Hassan M, Burton BR, Britton G, Hill EV, Verhagen J, *et al*. IL-4 enhances IL-10 production in Th1 cells: Implications for Th1 and Th2 regulation. Sci Rep 2017;7:11315.
19. Zhu W, Liu N, Zhao Y, Jia H, Cui B, Ning G. Association analysis of polymorphisms in IL-3, IL-4, IL-5, IL-9, and IL-13 with Graves' disease. J Endocrinol Invest 2010;33751-5.
20. Nakkuntod J, Wongsurawat T, Charoenwongse P, Snabboon T, Sridarma V, Hirankarn N, *et al*. No association between an interleukin 4 gene promoter (-589) polymorphism and Graves' disease in Thai patients. J Med Assoc Thai 2004;87:123-8.
21. Sarkar D, Chakraborty A, Bhattacharya C, Singh LH, Chandra AK. Exploration of goitrogenic/antithyroidal potentiality of bamboo-shoots in relation to thiourea. Int J Pharm Pharm Sci 2017;9:7-12.
22. Saptarini NM, Wibowo MS, Gusdinar T. Correlation study of age, disease duration, and erythrocyte sedimentation rate among the Indonesian rheumatoid arthritis patients. Int J Pharm Pharm Sci 2015;7:274-7.
23. Santoso DI, Yunita S, Paramita N, Andraini T, Kartinah NT, Bayani GF, *et al*. Effect of *Hibiscus sabdariffa* Linn on IL-6 and TNF-α levels in overtrained rat heart. Int J Appl Pharm 2019;11:42-5.