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GENOTYPING OF *IL13*-1024 (C/T) GENE AMONG IRAQI THYROID GOITER PATIENTS

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ABSTRACT : This study dealt with *IL-13* 1024 (C/T) gene genotyping among patients with Thyroid goiter in Iraq. Forty blood samples from patients with Thyroid goiter were collected and compared with 30 healthy persons as controls. The genotyping results of *IL-13* 1024 (C/T) gene using ARMS-PCR revealed presence TT, CC and CT genotypes beside T and C alleles. The T allele and TT genotype frequency were higher in Thyroid goiter patients compared to the same genotype and allele in healthy persons (P = 0.060). These increasing results were related with increasing risk factor of Thyroid goiter (odds ratio [OR] 2.15; 95% confidence interval [CI] 0.99–71.4). No significant differences between genotypes for Thyroid goiter patients and controls were revealed by using Hardy-Weinberg distribution. In conclusion, Thyroid goiter increasing risk was related with the TT and TC genotypes and T allele and these are showed as etiological fraction (EF) with risk having Thyroid goiter, while the CC genotype ratio percentage in healthy persons was higher in comparisons with Thyroid goiter patients suggesting the CC genotype have preventive fraction (PF).

Key words : *IL13* -1024 (C/T) gene, thyroid goiter, ARMS-PCR, autoimmune disease.

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INTRODUCTION

Thyroid goiter is one of the autoimmune and endocrine system diseases, which includes a number of pathological conditions that leads to a functional impairment in it and thus an increase in its size (Weetman, 1996; Davies, 2000; Huber *et al*, 2008). Goiter disease arises from an interaction between environmental factors (iodine, smoking, viral and bacterial infection, pregnancy) and genetic factors (genetic predisposition to developing the disease) (Pearce and Leech, 2004). Thyroid autoimmune disease is the most common organ-specific autoimmune disease, affecting 2-5% of the world's population (Simmonds and Gough, 2004), with a large difference in their incidence between the sexes (5-15% in women and 1-5% in men) (Dayan and Daniels, 2004). Cytokines play an important role in AITDs, through its role in degrading autoimmune tolerance, presentation of autoantigens, activation of B and T lymphocytes, production of autoantibodies, and autoimmune inhibition. Given the important role of cytokines produced by inflammatory cells within the thyroid and follicular cells in the immune and inflammatory responses to disease, the genes encoding these cytokines are disease candidates (Ajjan *et al*, 1997 and Xiaoheng *et al*, 2017). Interleukin 13 (IL-13) is known to be a pluripotent cytokine produced by Th2 cells, as well as by many cells including Th1 cells, Th17 cells (Gallo *et al*, 2012), and mast cells in atopic dermatitis (Obara *et al*, 2002), eosinophils in granulomas (Reiman *et al*, 2006), basal cells (Akdis *et al*, 2011), natural killer T cells (NK) (Fuss *et al*, 2004), phagocytes in pulmonary fibrosis (Aoki *et al*, 2015). The complementary deoxyribonucleic acid-cDNA of interleukin-13 was cloned in 1993 and was shown to have single open-reading frame (ORF) with 132 amino acids (McKenzie *et al*, 1993 and Minty *et al*, 1993) and it has a 25% similarity with interleukin 4 (IL-4) at the amino acid level (Chomarat and Banchereau, 1998). *IL-13*-1024 (C/T) gene encoding IL-13 was located 12 kilobases from the gene encoding IL-4 and consists of 4 exons and 3 introns (Smirnov *et al*, 1995) at location (5q23-31) (Minty *et al*, 1993). IL-13 plays a major role in asthma and allergy diseases (Vladich *et al*, 2005; Lee *et al*, 2016; Doran *et al*, 2017) and several autoimmune diseases (Spadaro *et al*, 2002; Wang *et al*, 2018). This work was dealt with

IL-13-1024 (C/T) genotyping among patients with Thyroid goiter in Iraq for determining its role susceptibility.

MATERIALS AND METHODS

Subjects : Forty Benign Multinodular goiter patients (10 patients having family history of Grave's disease, 7 patients having family history of Hashimoto's thyroiditis and 23 patients with no family history of AITDs) and 30 controls (healthy) were enrolled in this study. Peripheral blood samples (1.5 ml) for DNA extraction were collected from controls and patients form the period 1/1/2019 to 1/4/2019. Patients were newly diagnosed by Endocrinologists at Baghdad Teaching Hospital, Medical city, Iraq, as clinical and laboratory examination was adopted in determining the disease. All subjects ranged age from 28 to 60 years.

Genotyping : Peripheral blood samples was used for isolating DNA by using ReliaPrep™ Blood gDNA Miniprep System Kit (Promega-USA). The genotyping of *IL-13*-1024 (C/T) gene was done by using ARMS-PCR technique. Gene primer was designed according to Hummelshoj *et al* (2003). Table 1 showed the primers sequences which synthesized in Alpha Company, Canada.

ARMS-PCR : This method was applied for *IL-13* -1024 (C/T) gene genotyping. The DNA template and primers were mixed with PCR PreMix (Bioneer, Korea) tubes. The volume of reaction was completed to 20 µl with nuclease-free water (Table 2). All mixer reactions were described in Table 3 and placed into thermal cycler (Esco, Singapore).

The PCR products were resolved using agarose gels (2%). The ladder (Bioneer, Korea) was loaded on the agarose gel. Bromophenol blue dye (2 µl) was also loaded with all mixed reaction samples. Agarose gel electrophoresis was done using 80 Volts for 2 hrs. Finally, the agarose gel was stained with ethidium bromide (Promega, USA) for 20 min. Gel documentation system (Biocom, USA) was used for documenting the agarose gel.

Table 1 : The Primers sequences for *IL-13*-1024 (C/T) gene.

Primer	Sequence (5´ → 3´)	Product size (bp)
T allele (F)	5'-TTCTGGAGGACTTCTAGGAAAAT-3'	319
C allele (F)	5'-TTCTGGAGGACTTCTAGGAAAAC-3'	319
Reverse	5'-GGAGATGGGGTCTCACTATG-3'	-

Table 2 : The PCR mix reaction for genotyping of *IL-13*-1024 (C/T) gene.

Component		Volume (µl)	Final concentration
AccuPower PCR PreMix		Stock	1X
Primers	T allel (F)	1	1.0 µM
	C allel (F)	1	1.0 µM
	Reverse	1	1.0 µM
DNA template		2	100 ng
Nuclease-Free water		16	-

Table 3 : PCR conditions for genotyping of *IL-13*–1024 (C/T) gene.

Steps	Temperature (^{0}C) and cycles		Time
Denature template	94		3 min
First Initial denaturation	94	15 cycles	30 sec
First Annealing	63		60 sec
First Extension	72		60 sec
Second Initial denaturation	94	20 cycles	30 sec
Second Annealing	60		30 sec
Second Extension	72		60 sec
Final Extension	72		5min
Incubation	4		5min

Statistical analysis : Significant differences and frequencies data for patients and healthy persons were analyzed using Fisher's test. The odds ratio (OR) and confidence intervals (CI) were analyzed using Compare 2 Ver.3.04 program designed by J. H. Abramson/2003–2013. Deviations were analyzed using Hardy-Weinberg equation available at website (offline) www.had2know.com/academics.html.

RESULTS AND DISCUSSION

The genotyping of *IL13*-1024 (C/T) by using ARMS-PCR showed presence three genotypes TT, TC and CC. T and C alleles also found using the T, C and reverse primers (Fig. 1). *IL13*-1024 (C/T) genotype frequencies were agreed with Hardy-Weinberg equilibrium in Thyroid goiter patients and healthy persons. The percentage of recurrence of the TT genotype was higher in the sample of patients in comparisons with healthy persons, and the percentages were 15% and 3.33%, respectively, with no significant difference, as the probability value was 0.226 at ($P<0.05$) using the Fisher Exact test, and the OR value was 5.12 and the CI value ranged from 0.60-43.46. The TT genotype also appeared as Etiological Fraction (EF) genotype for goiter, with a value of 0.21(Table 4). The T allele increased significantly frequency in patients in

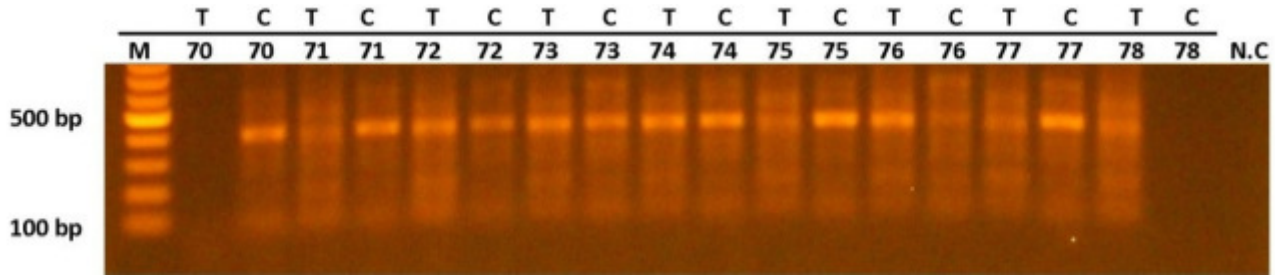

Fig. 1 : Agarose gel electrophoresis for T and C alleles of *IL-13* –1024 (C/T) gene among Thyroid goiter patients.

Table 4 : Frequency distribution of *IL-13*–1024 (C/T) gene genotypes in Thyroid goiter patients and healthy persons.

Gene position	Genotype	Patients No. (%)	Control No. (%)	OR (95% CI)	P-value
IL-13 ×1024 (C/T)	TT	6(15%)	1 (3.3%)	5.12(CI=0.60-43.46)	0.226
	E.F	0.21			
	TC	16(40%)	10(33.3%)	1.33(CI=0.50-3.53	0.624
	E.F	0.10			
	CC	18(45%)	19(63%)	0.47(CI=0.18-1.23	0.152
	P.F	0.33			

EF = etiological fraction; PF = preventive fraction; OR = odds ratio; CI = confidence intervals.

Table 5 : Allele frequencies of *IL-13*–1024 (C/T) in Thyroid patients and controls.

Gene position	Allele	Patients No. (%)	Control No. (%)	OR (95% CI)	P value
IL-13 ×1024 (C/T)	T	28(35%)	12(20%)	2.15(CI=0.99-4.71)	0.060*
	E.F	0.18			
	C	52(65%)	48(80%)	0.46(CI=0.21-1.02	
	P.F	0.42			

OR = odds ratio; CI = confidence intervals; *=Significant differences at $P < 0.05$ level by using Fisher's test; EF = etiological fraction; PF = preventive fraction.

comparison with healthy persons (35 versus 20%). In contrast, C allele frequency was higher significantly in healthy persons (80%) as compared with patients (65%). The OR for T and C alleles were 2.15 and 0.46, respectively. The results suggest that T allele have an etiological effect, while C allele have protective effect (Table 5). The CC genotype frequencies was observed to have an increased frequency in healthy persons in comparisons with patients (63.4 versus 45%) with no significant difference, while TT and TC genotype frequencies were increased in patients 40% and 15%, respectively in comparison with controls (3.3% and 33.3%, respectively) with no significant difference. The OR for TT, TC and CC genotypes was 5.12, 1.33, and 0.47, respectively (Table 4). The increasing risk of Thyroid goiter might be associated with TT and TC genotypes, while the genotype CC is suggesting to have a protective effect against Thyroid goiter. These results are agreed with those of the study by Hiromatsu *et al* (2005), who showed that the occurrence of polymorphisms in the IL-13 gene at the sites -1112 (C->T) and 2044 (G->A) confer genetic susceptibility to developing Graves' disease in Japanese population. Whereas the results of Bednarczuk *et al* (2003) indicated that the occurrence of polymorphisms in the IL-13 gene at sites -1112 (C->T) and 2044 (G->A) did not confer genetic susceptibility to developing Graves' disease. While, the study of Zhu *et al* (2010) indicated that rs40401 mutation in the IL-13 gene confer genetic susceptibility to developing Graves' disease in Chinese population. The study of Mestiri *et al* (2020) also indicated that the occurrence of the mutation rs1800925 in the *IL-13* gene confer genetic susceptibility to developing Graves' disease and Hashimoto's disease in the Tunisian population.

Agarose gel electrophoresis was performed using 2% agarose gel, 80 volts for 2 hrs. One band presence in T lane and absence of the same band in C lane refers to the TT genotype. In contrast, one band presence in C lane and absence of same band in T lane refers to the

CC genotype. Presence of two bands in both lanes refers to the TC genotype.

CONCLUSION

The Thyroid goiter increasing risk was related with the TT and TC genotype and T allele and these were showed as EF with having a risk with Thyroid goiter, while the CC genotype and ratio percentage of C allele in healthy persons was higher as compared to patients with Thyroid goiter suggesting that this genotype have a PF.

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