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Interleukin 9 +176 rs1799962 Polymorphism is Associated with Susceptibility to Thyroiditis in Iraqi Patients

Ashwak T. Humadi[1], Maha Falih Nazzal[1], Ibtesam Badday Hassan[1*], Alaudeen Sadeq Khalaf[1]

Department of biology, College of Education for pure science, University of Diyala, Iraq[1]

Corresponding author: 1*

ABSTRACT— The current study aimed to evaluate the association between SNP of IL-9 gene at position +176 and in patients 60 samples with thyroiditis, divided into 30 samples of hyperthyroidism, 5 male and 25 females, their age 23-60 years, and hypothyroidism patients 30 females their age 21-60 years, and controls 31 samples, 3 male and 28 females their age 18-45 years. This study showed a significant increase for both hyper and hypo groups compared with control (42.90±2.11), (41.47±2.11) and (28.0±1.55), under ($P<0.05$). Study showed, that homozygous CC genotype and C allele is EF for Hashimoto thyroiditis (2.20 and 1.30), with RR (23.6% and 11.9%) respectively, while the heterozygous TC genotype and allele T were considered PF from Hashimoto thyroiditis (0.32 and 0.77) respectively. The current study showed, homozygous TT genotype and T allele were considered EF for Graves' disease (4.20 and 3.56), with RR (50.8% and 52.8%) respectively. While the homozygous CC genotype and C allele recorded a significant decrease in patients CC genotype and C allele were considered PF from Graves' disease 0.30 and 0.28 respectively. Same result recorded TC genotype (13.33%), also considered PF from Graves' disease 22.58 %. This study suggest that SNP of IL-9 gene at position +176 may have a role in pathogenic mechanisms, and an association (positive and negative) with Graves' disease and Hashimoto thyroiditis in samples of Iraqi patients.

KEYWORDS: Graves' disease, Hashimoto thyroiditis and IL-9

1. INTRODUCTION

Thyroiditis is an inflammatory condition affecting the thyroid gland. It may be painful and tender when caused by an infection or trauma, or painless when caused by an autoimmune condition or medications [1]. Autoimmune thyroid diseases AITD, characterized by autoantibody production and lymphocyte infiltration that usually result in cell destruction and tissue inflammation [2]. The most common organ-specific autoimmune disorder is autoimmune thyroid disease AITD. Due to loss of immune tolerance and reactivity to thyroid auto antigens, AITD production occurs: thyroid peroxidase TPO, thyroid stimulating hormone receptor TSHR and thyroglobulin TG. This contributes to T cells and B cells infiltrating the gland, which generate antibodies unique to clinical manifestations, for chronic autoimmune thyroiditis cAIT and hyperthyroidism in Graves' disease GD [3]. As a result of losing the thyroid antigen tolerance due to many factors such as genetic predisposition, environmental chemicals, dietary iodine, interferon alpha INF-a, molecular mimics, medicines, and selenium [4]. cytokines are divided according to their location of secretion into lymphokines, which are those cytokines that are secreted by T-lymphocyte cells and B cells mediated pathways, including the synthesis of IL-12, IFN-γ and TNF-alpha, have been shown to play a significant role in the destruction of thyrocytes and thus in ATD pathogenesis in rat model studies [5]. It is thought that antibody production in Graves' disease is triggered by the activation of helper T cell (CD4 +), followed by recruitment of B cells into the thyroid gland as these cells produce thyroid antibody-specific

antibodies. In Hashimoto's thyroiditis, activated CD4 +T cells produce an antiviral, causing the thyroid cells to display MHC class II molecules and this broadens the repertoire of autonomous T cells and prolongs the inflammatory response [6]. While thyroid antibodies are used to track the presence of autoimmune thyroiditis, they are not generally considered to be a direct contribution to thyroid destruction [7], [8]. IL-9 is a pleiotropic cytokine that affects hematopoietic progenitor cells, lymphocytes, mast cells, airway smooth muscle cells, and epithelial cells in both direct and indirect ways. IL-9 can play a role in Th1/Th17-mediated inflammation and Treg response. However, its primary function in immunity is typically related to allergic inflammation and extracellular parasite immunity. Recently, several studies have suggested that IL-9 could play a role in the pathogenesis of neoplasia surprisingly; IL-9 can induce Th17 cell differentiation and mediate autoimmune and inflammatory diseases. IL-9 is released by Th17 cells, which primarily secrete IL-17A and IL-17F [9], [10]. IL-9 is produced by variety of cells including mast cells, NKT cells, Th2, Th17, Treg, ILC2, and Th9 cells. Th9 cells are regarded as the major CD4+ T cells that produce IL-9, additionally; it gives rise to the multiplication of hematologic neoplasias and also Hodgkin's lymphoma in humans, but IL-9 also has antitumor properties in solid tumors, for example melanoma [11]. One study revealed that Hashimoto thyroiditis (HT) patients with papillary thyroid cancer (PTC) develop significantly higher IL-9 concentrations than patients with PTC without HT. This is important because of the fact that HT has higher concentrations of cytokines that are inflammatory, and IL-9 has a common role in inflammation. PTC patients have a higher concentration of IL-9 than controls and single HT patients with normal IL-9 levels [12].

2. Materials and Methods

2.1 Subjects

This study was conducted for a period from the beginning of October 2020 to the end of January 2021, as blood samples were collected from patients with thyroiditis, as well as healthy people from Baquba Teaching Hospital and Al-Batoul Hospital Education Feminine and Pediatric in Diyala Governorate. After the diagnosis by taking laboratory analyzes TSH, T3 and T4 and specialist physician, the samples were divided into three groups have been investigated. An informed consent was obtained from all patients.

2.1.1 Control Group

28 female and 3 males healthy participants included in this study as a control group. Their ages ranged between (18-45) years.

2.1.2 Hyperthyroidism Group

25 female and 5 male patients, their ages ranged between 23-60 years.

2.1.3 Hypothyroidism Group

30 female patients, their ages ranged between 21-60 years.

2.2 Detection of IL-9 Polymorphism

Genomic DNA was extracted from EDTA blood using Wizard Genomic DNA Purification Kit (G- Spin TM Total Korea), followed by electrophoresis on 2% agarose-gel by CTSPCRSSP Tray Kit (G- Spin TM Total Korea). Amplification of the IL-9 gene Polymerase chain reaction (PCR) augmentation of the polymerase chain reaction to amplify the promoter region of the IL-9 gene, using a nucleotide primer designed by Dr. Ibtesam Badday Hassan and sent to the company (Macrogen).

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Primer name	(5′-3′) Seq	Product size	Annealing temp., (°C)
rs1799962- F	CCTTCGTTAGAACACCCATGA	+176 bp	57
rs1799962-R	AGACAGGGATTCTGGTGTGA		

2.3 Statistical Analysis

Used Mean ± SE and Tukey test referred to significant differences. Genotypes of IL-9 +176 SNP were presented as percentage frequencies, these estimations were calculated by using the WINPEPI computer programs for epidemiologists. The latest version of the WINPEPI package is available free online at http://www.brixtonhealth.com.

3. Results and Discussion

3.1 Thyroiditis disease and age groups

This study showed that patients with an age group between (21-65) year that the incidence rate of Hypothyroidism patients in females (41.47±2.11) compared with healthy subjects with an age group between 18-45 (28.0±1.55) and upon statistical decomposition there is a significant difference, as for the infection rate of patients with Hyperthyroidism with an age group between 23-75 (42.90±2.11) Comparison with healthy controls there was a significant difference. As shown in Table 1.

Table 1 Thyroiditis disease and age groups

Groups	Gender	Mean ± Std. Error of Mean	Probability
Control	Male	36.67±6.01	0.570
	Female	27.04±1.53	
	Total	28.00±1.55	B
Hypothyroidism	Female	41.47±2.11	
	Total	41.47±2.11	A
Hyperthyroidism	Male	44.80±6.95	0.992
	Female	42.52±2.188	
	Total	42.90±2.11	A

- Values are Mean ± SE.
- Tukey test: similar letters referred to non- significant differences (P> 0.05) while the different letters referred to a significant differences (P <0.05).

The current study agreed with a study [13]. The incidence rate in Graves' disease was 41 and the incidence rate in Hashimoto thyroiditis was 40. A study in Japan had an incidence rate of 49 in Graves' disease [14] a study [15]. The incidence rate for Hashemoto patients was 45 a study of the incidence rate of 41 in Graves' patients and the incidence rate of 46 in Hashmioto thyroiditis [16]. This study is agreed with study [17]. The incidence rate for Graves' patients was 41.A study [18]. The reason may be due to the spread of Hashimoto's disease in females had low levels of vitamin D. A link exists between vitamin D deficiency and the development of HT. The severity of vitamin D deficiency may also be related to the development of thyroid damage. The reason for the differences is due to hormonal factors specific to sex [19]. The results of the current study showed, as shown in Table 2, that the homozygous genotype CC and allele C were recorded. A significant increase in the group of patients with, Hashimoto thyroiditis according to the

mentioned percentages (3.9% and 51.67%) respectively compared to the healthy group (control) and as follows (2.48% and 45.16%) according to Fisher's probability (P=0.184 and P= 0.587) among patients compared to control. The same result was recorded by homozygous genotype TT (3.6, 3.41) as shown in Table (3). The homozygous genotype CC and C allele is considered an etiological factor for the Hashimoto thyroiditis disease (2.20 and 1.30), with a risk ratio of (23.6% and 11.9%) respectively, as shown in Table (4). While the heterozygous genotype TC and the T allele recorded a significant decrease in patients according to the value (1.5% and 48.33%), respectively, compared to the healthy group, which was recorded (3.72% and 54.84%), respectively, and according to the Fisher probability (P=0.086 and P=0.587). Therefore, the TC genotype and the T allele are considered a protective factor or protection from the Hashimoto thyroiditis disease, as they reached (0.32 and 0.77), respectively. The results of the current study showed, as shown in Table 4, that the homozygous genotype TT and allele T were recorded.

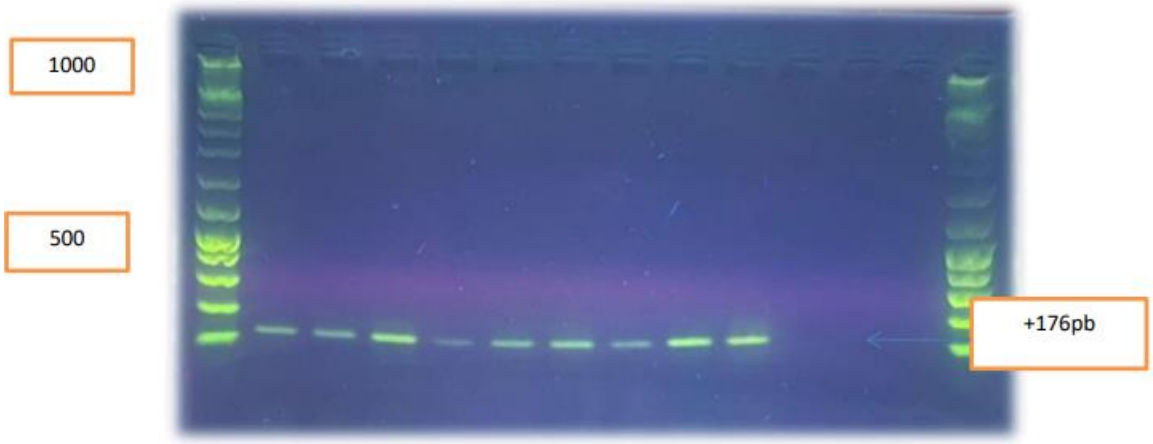

Figure 1: The product of amplification of the IL-9-rs gene 1799962 that causes thyroid-disease and carried on agarose gel at a concentration of 1.5% and an electrical potential difference of 90 volts for an hour and a half after staining with ethidium bromide dye and photographed under ultraviolet light.

Table 2 distributions of genotypes and allelic repeats of IL-9+ 176 gene at rs1799962 according to the Hardy-Weinberg equilibrium in Hashimoto thyroiditis patients in comparison with the healthy subjects in the study groups

Groups		IL - 9+ 176 gene at rs1799962 Genotypes or alleles						H-W X ^{2} P ≤
			TT	TC	CC	T	C	
Hashimoto thyroiditis (No. = 30)	Observed	No.	12	5	13	29	31	0.0003
		%	3.6	1.5	3.9	48.33	51.67	
	Expected	No.	7.01	14.98	8.01	Not Estimated		
		%	23.36	49.94	26.69			
Controls Observed (No. = 31)	Observed	No.	11	12	8	34	28	0.2238
		%	3.41	3.72	2.48	54.84	45.16	
	Expected	No.	9.32	15.35	6.32	Not Estimated		
		%	30.07	49.53	20.4			

Table 3 Statistical analysis of associations between IL-9 +176 genotypes or alleles in Hashimoto thyroiditis patients and controls

Type of Comparison	Statistical Evaluation Statistical Evaluation			Fisher's Exact Probability	95% Confidence Intervals
	IL-9+ 176	Relative	Preventive		

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	Genotype	Risk	or Fraction Etiological		
Hashimoto thyroiditis Versus Controls	TT	7.0%	1.21	0.795	0.44 - 3.36
	TC	26.5%	0.32	0.086	0.10 - 1.03
	CC	23.6%	2.20	0.184	0.76 to 6.37
	T	12.6%	0.77	0.587	0.38 to 1.56
	C	11.9%	1.30	0.587	0.64 to 2.63

Table 4 distribution of genotypes and allelic repeats of IL-9 gene at rs1799962 locus according to the Hardy-Weinberg equilibrium in Graves' disease patients in comparison with the healthy subjects in the study groups

Groups		IL - 9 gene at rs1799962 Genotypes or alleles						H-W X ^{2} P ≤
			TT	TC	CC	T	C	
Graves' Disease (No. = 30)	Observed	No.	20	4	6	44	16	0.0003
		%	66.67	13.33	1.8	73.33	26.67	
	Expected	No.	16.13	11.73	2.13	Not Estimated		
		%	53.78	39.11	7.11			
Controls Observed (No. = 31)	Observed	No.	10	7	14	27	35	0.0026
		%	32.26	22.58	45.16	43.55	56.45	
	Expected	No.	5.88	15.24	9.88	Not Estimated		
		%	18.96	49.17	31.87			

A significant increase in patients with according to the mentioned percentages (66.67% and 73.33%) respectively compared to the healthy group and as follows (32.26% and 43.55%) according to Fisher's probability (P=0.010 and P= 0.001) among patients compared to control. As shown in Table 5. The homozygous genotype TT and T allele is considered a etiological factor for Graves' disease (4.20 and 3.56), with a risk ratio of (50.8% and 52.8%) respectively, as shown in Table 5. While the homozygous genotype CC and the C allele recorded a significant decrease in patients according to the value (1.8 % and 26.67%), respectively, compared to the healthy group, which was recorded (45.16% and 56.45%), respectively. According to the Fisher probability (P=0.056 and P=0.001). Therefore, the CC genotype and the C allele are considered a protective factor or protection from the Graves' disease, as they reached (0.30 and 0.28), respectively.

Table 5 Statistical analysis of associations between IL-9+ 176 genotypes or alleles in Graves' disease patients and controls

Type of Comparison	Statistical Evaluation Statistical Evaluation			Fisher's Exact Probability	95% Confidence Intervals
	IL-9 +176 Genotype	Relative Risk	Preventive or Fraction Etiological		
Graves' disease Versus Controls	TT	50.8%	4.20	0.010	1.47 - 12.02
	TC	10.7%	0.53	0.508	0.14 - 1.99
	CC	31.5%	0.30	0.056	0.10 - 0.93
	T	52.8%	3.56	0.001	1.68 - 7.58
	C	40.6%	0.28	0.001	0.13 - 0.60

The same result was recorded by the TC genotype (13.33%), which is also considered a factor, protection and prevention from Graves' disease, as it reached (22.58 %). These results suggest that the genetic SNP of

the IL-9 may have a role in the pathogenic mechanism, and it showed an association (positive and negative) with Graves' disease in samples of Iraqi patients. These results suggest that genetic single nucleotide polymorphisms (IL-9) may have a role in the disease-causing mechanism, and they showed an association (positive and negative) with the Hashimoto thyroiditis in samples of Iraqi patients. Interleukin-9 has been shown to be an important growth factor and stimulator of cell types important in the pathogenesis of asthma [17]. This was shown by a study conducted on children with asthma; the serum levels of IL-9 were significantly higher in the patients group. Patients with TT and TC genotypes at rs2069882 had significantly higher levels of IL-9. In addition, patients with severe asthma had significantly higher levels of IL-9 in their blood. Those genotypes in single nucleotide polymorphisms can influence serum levels of IL-9 in asthmatics, which in turn can influence its severity [20]. Another Iranian study conducted on local women suffering from allergic rhinitis found that IL-9 single nucleotide polymorphisms showed different genetic and allelic distributions among patients compared to healthy controls, which confirms the existence of a significant association between mechanisms and genes in disease development [21]. The other study concluded the role of IL-9 SNPs in causing asthma is a result of international studies between IL-9 SNP, which indicated its role in the exacerbation and severity of asthma. Thyroiditis disease is a complex disease with a significant genetic predisposition IL-9 is a pleiotropic cytokine that affects different and distinct functions of different target cells such as T cells, B cells, mast cells and epithelial cells by activating the transcription factors STAT1, STAT3 and STAT5. Due to its multidirectional functions, IL-9 has been shown to be involved in many diseases, such as cancer, autoimmune diseases, thyroid diseases and other pathogen-mediated immune system-mediated diseases and the role of Th9- and IL-9- producing immune cells in the events Allergic asthma and its severity and severity in patients [13], [20], [22- 24].

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