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The Role of Il-4 −590 (C>T) Gene As A Diagnostic Biomarker Of Hashimoto Thyroiditis Disease Patients In Al-Najaf Provenance /Iraq

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Abstract: *The study of interleukin 4 (IL-4) genotyping in Hashimoto patients is considered one of the challenges of sustainable development. Hashimoto disease is an autoimmune disease that causes the immune system to attack the cells of the thyroid gland, causing the death of the cells that produce thyroid hormones, and thus leading to chronic hypothyroidism. the current study aims to determine the genotyping of IL-4 -590C>T in the DNA of Hashimoto patients and compared it with healthy subjects. To achieve this goal, blood samples were collected from 54 Hashimoto's disease patients and 25 healthy as control groups, during the period from March 2022 to September 2022. Genomic DNA was obtained from blood samples for molecular analysis to investigate the IL-4 -590 C/T gene polymorphism in patients and the control groups. Genotyping is done by using Amplification Refractory Mutation System – Polymerase Chain Reaction (ARMS-PCR) technique. The current study, included 50/54 females and 4/54 males with Hashimoto thyroiditis with ages ranging from 20-59 years old. The results showed that the age group 40-49 years were the most affected, Also, the body mass index was had increased among patients. The CT genotype is present in 74% of Hashimoto patients while 16% of controls. The C allele was found at a high rate in both patients and control with percentages of 100% and 96% respectively. The Hashimoto patients have more frequency of CT genotyping of IL-4 -590 C>T than control, while C Allele was high in both Hashimoto patients and controls. The -590 CC genotype in the IL4 gene may consider to be a strong predictive factor for the development of hypothyroidism in Hashimoto disease (HD).*

Keywords: *Genotyping, IL-4-590C/T, Hashimoto, BMI, C Allele,T Allele.*

1.Introduction

Hashimoto Thyroiditis (HT) an autoimmune disorder involving chronic inflammation of the thyroid is the most typical case of organ-specific autoimmune disease and the leading cause of hypothyroidism in regions of the world where iodine is abundant [1] . The earliest description this disease in 1912 by **Hakaru Hashimoto** who described four ladies who had a disorder he initially called Struma lymphomatosa [2].

HT is defined by a progressive loss of thyroid function, goitre, or both as a result of the thyroid gland being destroyed by the immune system through thyrocyte apoptosis[3]. The pathophysiology of HT is thought to be explained by several factors: The first of these factors is molecular mimicry. Here, it is believed that an immune response against an antigen that resembles endogenous proteins structurally is what causes HT [2]. Other factors are bystander activation. In this situation, a virus that enters the thyroid gland or activates non-specific lymphocytes there may produce cytokines that in turn activate local thyroid-specific T cells and favor an inflammatory response (i.e. thyroiditis) [3]. Antigen presentation cells (APC) Nonlinear cytokine production causes self-apoptosis in thyrocytes by inducing the expression of Fas and Fas ligands in autoimmune thyroiditis and Th1 cells [4].

IL-4 was initially identified as a 129-amino acid low molecular weight polypeptide produced by T cells and encoded by the IL-4 gene on chromosome 5q23.31. Gene polymorphisms play important roles in controlling the expression of cytokines; for instance, it has been reported that the substitution of C by T at position 590 of the IL4 gene is associated with reduced IL4 expression, whereas the TT genotype upregulates the production of this cytokine[5] . The study aim to detect the genotyping of IL-4 -590C>T among Hashimoto patients and controls.

2. Methodology

The study included 54 pathological samples of people with Hashimoto disease and 25 samples from Healthy People. The majority of the studied Hashimoto samples was (45/54) female, compared to (4/54) male, and the age category ranged between 20-59 years old. One ml of blood was collected from patients with Hashimoto's disease and control, DNA was extracted from blood using the Favorprep kit from (FAVORGEN BIOTECH CORP). The amplification refractory mutation system (Polymerase Chain Reaction-PCR) method was used to genotype the IL-4 gene at position 590 (C>T). According to [6], primers were manufactured at Microgen Company (Korea AZ) and used at this location, Table (1).

Table (1): Primers for IL-4 −590 (C>T) genotyping

Primer	5-Sequence-3	size(bp)
T allele	ACACTAAACTTGG GAGAACATTGTT	216
C allele	ACACTAAACTTGG GAGAACATTGTC	248
Reverse	GAATTTGTTAGTA ATGCAGTCCTCC	

Molecular analysis: To genotype IL4 590 (C>T), ARMS-PCR was utilized. With minor adjustments, Thermal Cycler PCR Analytic Jena (Jena, German) was created by [6] . Primer and DNA template were added to PCR PreMix tubes, and 20 L of nuclease-free water was used to create the final volume for the PCR reaction Table (2). Table (3) lists the PCR conditions for each reaction mixer. 1.5% agarose gels were used to resolve the PCR products. On an agarose gel containing ethidium bromide stain (Promega, USA), DNA from patients and healthy samples were added along with a 1000bp ladder (Pioneer, Korea). Using gel electrophoresis, the bands were separated according to their base pair and detected by using a gel documentation system (Biocom, USA).

Table (2): PCR mix reaction for genotyping of IL-4 gene position −590 (C>T)

Component	Volume (μl)	Final concentration
Each primer (T or C allele + reverse)	2	1 μM
DNA template	5	100 ng
Nuclease-free water	11	-
Final volume	20	-

***T and reverse primers were used for detecting C allele, and T and reverse primers were used for detecting C allele.

Table (3): PCR conditions for genotyping of IL-4 gene position −590 (C>T)

Steps	Tem. (∘ C)	Cycles	Time (sec)
Denature template	96		50
First initial denaturation	95		15
First annealing	65	10 cycles	50
First extension	72		40
Second initial denaturation	95		50
Second annealing	59	20 cycles	50
Second extension	72		50
Final extension	72		420
Incubation	4		300

Statistical Analysis: Percentage frequencies between patients and controls were given by Penetrate [24].

3.Results and Discussion

The study included 54 pathological samples of people with Hashimoto disease and 25 samples from Healthy People. The majority of the studied Hashimoto samples was (50/54) female, compared to (4/54) male , and the age category ranged between 20-59 years old, and their BMI as Table(4) .

Table (4): Demographic study of Hashimoto patients

Parameters	Values	Percentage
Gender		
Male	4	7.4%
Female	50	92.59%
Average Age	40	
Age Category		
20-29	9	16.66%
30-39	12	22.22%
40-49	19	35.18%
50-59	14	25.92%
Increase in BMI	50	92.59%
Normal BMI	4	7.40%

The demographic of the current study of Hashimoto patients showed that most patients were women (92.59%) compared to men (7.4%). Women are five to eight times more likely than men to develop thyroid disease, according to the American Thyroid Association that occurs because of a relationship between the fluctuation of hormones during the menstrual cycle and thyroid hormones. Thyroid problems can happen at any time but they are especially common in women during and after menopause when hormone levels are changing [7]. These results agree with the results of [8,9,10,11] that expected that when a woman undergoes menopause, more of hormonal levels change because menstruation has a role in the regulation of the female hormone especially the estrogen hormone is responsible for many mechanisms in the female body. Simmonds *et al* 2014 and Effraimidis and Wiersinga found that the women infected by

Hashimoto's disease may occur in high percentage because of the loss of self-tolerance to the X-linked antigens which lead to autoimmune thyroid disease[12,13].

Also, Current results showed about (35.13%) of patients were at age (40-49) years,, and these results are closely related to another study performed on Hashimoto patients [14,15] that explained Hashimoto may occur at any age during life but it is largely increased in patients aged (30 - 60) years [16,17].

There was an increase in the body mass index (BMI) of Hashimoto's patients by 92.59% with a high BMI >25 , while only 7.4% of the patients had a normal BMI <25. These results agree with the finding of Popławska *et al.*, that he observed 72% of Hashimoto patients have an increase in BMI [18]. Rong *et al*., in their study, show that the risks of HT in the obese population increased by 91% [19].

About ARMS-PCR, the polymorphism of IL4 −590 (C>T) showed three genotypes (CC, CT, and TT) (Table 5 and Figure (1, 2) by using the specific C, specific T, and reverse primers the presence of C and T alleles Table (5).

Table (5): Distribution of IL4 −590 (C>T) genotypes in Hashimoto Patients and Control

Gene	Genotype	Hashimoto patients (n.54)	Control (n.25
IL-4 -590 (C>T)	CC CT TT	14 (25.92%) 40 (74.07%) 0 (0 %)	20 (80%) 4 (16%) 1 (4%)

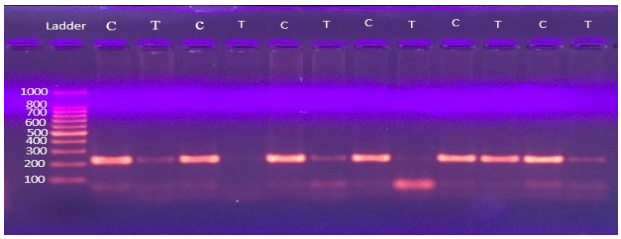

Figure (1): ARMS-PCR results of Hashimoto patients

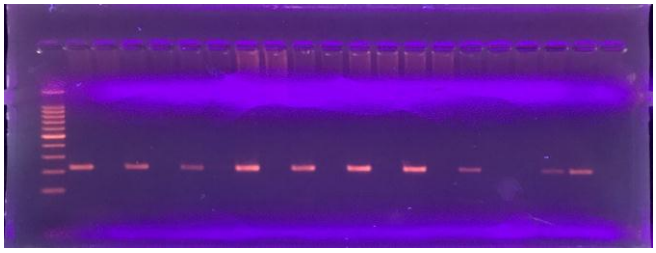

Figure (2) : ARMS –PCR results of Healthy people (Control)

Table (6): Allele frequencies of IL4 −590 (C>T) in Hashimoto Patients and Control

Gene position	Allele	Hashimoto patients (n.54)	Control(n.25)
IL-4-590 (C>T)	C T	54 (100%) 40 (74 %)	24 (96%) 5 (20%)

The severity of Hashimoto disease (HD) varies among patients and is difficult to predict when the disease is in the subclinical state and diagnosed by the presence of thyroid-specific autoantibody. IL-4, one of the key Th2 cytokines, stimulates humoral immunity and suppresses the production of inflammatory Th1 cytokines, including IFN-γ [20].

Genotyping of the current study by ARMS-PCR results of IL-4-590 C>T shows that most Hashimoto patients have CT genotyping in comparison with Control that having in most CC genotypes of IL-4 -590C>T. These results agree with the results of[21].

C Allele was found with a high percentage in patients and controls that were (100% and 96% respectively, While T Allele was found in patients with a high percentage compared with the control that was (74% and 20% respectively). Such results suggest that the C allele has a predisposing effect, while the T allele has a protective effect. These findings were resembles the results of Nakashima *et al*., who said that the individuals who carry the T allele in −590C>T polymorphism of the IL4 gene have a higher proportion of IL-4–producing T-helper cells [21].

Studies by Takashi *et al*., show when Hashimoto patients have the IL-4 −590 CC

genotype they would be expected to develop the severe disease (hypothyroidism) before 50 years of age. A mechanism to explain this phenomenon is that in HD patients with the −590 CC genotype, the proportion of IL-4–producing T-helper cells is lower than that in HD patients with the −590TT or −590CT genotype, which results in higher activity of inflammatory Th1 cytokines and more rapid progression of thyroid destruction, followed by the early development of hypothyroidism[22,24,25]

Conclusion

The -590 CC genotype in the *IL4* gene may consider to be a strong predictive factor for the development of hypothyroidism in HD. The Hashimoto patients have more frequency of CT genotyping of IL-4 -590 C>T than control, while C Allele was high in both Hashimoto patients and controls.

Ethics

This study was conducted under approval by the medical ethics committee at the University of Kufa (2017). Verbal and written consent was provided by parents and agreement for publication was obtained from both participants and researchers.

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