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Research Article

Association between IL-2 Levels and *IL-2-rs2069762 Gene* Polymorphism in Patients with Hashimoto's Disease

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Abstract:

Background: Hashimoto's thyroiditis (HT) is one of the most prevalent organ-specific autoimmune diseases worldwide at any age, and is induced by loss of immune tolerance of thyroid gland. Over the past few decades, its occurrence has increased. It is a classic example of an organ-specific autoimmune disease, wherein in genetically predisposed individuals, environmental and existential circumstances set off the immune system's attack against thyroid antigens.

Aim: The present study aims to evaluate the role of IL-2 genes polymorphisms and their serum levels in the predisposition to HT.

Methods: The research project included 60 patients with Hashimoto's thyroiditis disease, ages ranging from 21 to 65, who hospital admission between March and August of 2023. The other group consisted of 60 people who appeared to be health individuals. Five milliliters of blood were taken, two milliliters of every specimen being used for the PCR amplification and *IL-2 (rs2069762)* gene detection (RFLP-PCR) method. The remaining (3ml) for IL-2 ELISA (Mabtech USA) Kit test.

Results: The IL-2 genotype did not statistically correlate with a higher risk of HT (P= 0.352). On the other hand, those with the TG genotype had greater serum levels of IL-2 than those with the TT or GG genotype (P= 0.172). HT patients' serum IL-2 levels were statistically greater than those of the healthy control group. **Conclusions:** a insignificant correlation between *IL-2* genotype and serum IL-2 concentration in patients with HT.

Keywords: Hashimoto's thyroiditis, *IL-2*, polymorphism

Introduction

Hashimoto's thyroiditis (HT) is one of the utmost prevalent organ-specific autoimmune diseases worldwide at any age, and is induced by loss of immune tolerance of thyroid gland. Over the past few decades, its occurrence has increased. It is a classic example of an organ-specific autoimmune disease, wherein in genetically predisposed individuals, environmental and existential circumstances set off the immune system's attack on thyroid antigens [1]. Because of the disturbance of thyroid architecture and decreasing functionality, increasing hypothyroidism (HT) complicates the course of HT

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[2]. Although Hashimoto's thyroiditis can occur at any age, women from 30 to 60 years old are most likely to get it. With a roughly 8:1 incidence ratio, HT is very common in females. Furthermore, whites and Asians are more likely to have it than African-Americans. However, research showed that roughly 10% of the population suffered with HT based on laboratory results of thyroid autoantibodies in females. Although the condition's precise incidence is unknown, Grave's disease is likely to be comparable. Studies show that it is more common in areas where the consumption of iodine is high and among those with genetic predispositions [3]. Other studies estimate the prevalence among the general population to be approximately 2% [4]. According to the United States epidemiological study, also known as the Wickham survey, the prevalence of hypothyroidism is less than 0.1% in males and 1.5% in females. The number of cases of Hashimoto's thyroiditis has reportedly grown over the past few decades. This increase may be related to the sensitivity of modern diagnostic modalities such as needle biopsies and serological testing, which are more sensitive than older techniques [5].

In the investigation conducted by Aouda *et al* [6] in Al-Hilla teaching hospitals, 175 patients had thyroid surgery in order to determine the incidence of Hashimoto's thyroiditis (13.4%) and the percentage of individuals with thyroiditis relative to all cases taken. Additionally, the study aimed to determine the relationship between thyroid function and histopathological status in patients with thyroiditis diagnoses. Of these patients, 18 had thyroiditis. When compared to groups with an adequate iodine status, the frequency of hypothyroidism is greater in those with high iodine intake or a severe iodine deficit. When iodine insufficiency becomes less severe, the incidence falls, and when iodine intake changes from mild deficiency to optimal or excessive intake, the prevalence rises [7].

Genetic predisposition to HT has been suggested by twin studies that report disease concurrence of 33% in sibling, and 55% monozygotic twins compared with 0% in dizygotic twins [8]. A number of genes have been linked to AITDs: some are unique to hypothyroidism (HT), while others are shared by both conditions and non-thyroidal autoimmune illnesses. Most genes associated with susceptibility are involved in immune-regulatory functions, including lymphocyte activation, antigen presentation, peripheral and central tolerance, and tolerance. Changes in these genes' activities brought on by polymorphisms may cause the emergence of asthma [9].

Multifunctional cytokine interleukin-2 (IL-2) has the ability to stimulate T cell proliferation. Recombinant IL-2 has been applied to the management of cancer and autoimmune diseases [10]. The pathophysiology of various autoimmune diseases demonstrates the important function that IL-2 plays in both activating and controlling the immune system. The IL-2 gene comprises five exons, is situated in the 4q27 chromosomal region, and has a restriction site at 2069762 (rs2069762) [11]. The generation of antibodies in autoimmune disorders has been connected to certain SNPs in the IL gene [12]. A study suggested that IL-2 (rs2069762) were linked to autoimmune diseases such as hematological-bleeding conditions and Addison's syndrome [13].

Materials and Methods

From March to August 2023, 60 patients with Hashimoto's disease, ranging in age from 21 to 65, participated in the present study. As a control group, another group of sixty

clinically deemed healthy people without a history of systemic disease was also included in this study. Patients with malignant tumors, hypertension, diabetes mellitus, chronic kidney disease, infectious disorders, or other autoimmune diseases were not included in our study. Five milliliters of blood were drawn via vein puncture with aseptic technique and disposable syringes. For every specimen, two milliliters were put into an EDTA tube, and these were instantly frozen at -20 degrees Celsius until they were needed again. This prevented repeated thawing and freezing during the polymerase chain reaction amplification and identification of the IL-2 (rs2069762) gene (RFLP-PCR) method. To prevent repeated thawing and freezing for the IL-2 ELISA Kit (Mabtech USA) test, the remaining (3 ml) were transferred to sterile Gel tubes, allowed to clot at room temperature, and centrifuged at 2500 rpm for 10 minutes. The separated serum was then saved in Eppendorf tubes and frozen at -20 C until further use. The ethics of Al-Diwaniyah Teaching Hospital were followed in this study, and each participant gave their verbal informed consent.

Results

The characteristics of our patients and control subjects, who were statistically comparable for age and sex, are summarized in Table 1. The mean age of patients was 38.17±1.5 and that of control subjects was 39.6 ±1.5 years, however, this difference was not statistically significant between patients with Hashimoto's disease and control (P = 0.501). Patients' group included 6 (10.0 %) males and 54 (90.0%) females, whereas, control group included 13 (21.7%) males and 47 (78.3%) females and there was no significant difference in the frequency distribution of patients and control subjects according to gender (P = 0.080). To prevent age and gender bias in case control studies like this one, the current finding that is, the absence of a substantial difference in the distribution of individuals in both groups is a necessary condition.

The present results show the mean levels of Thyroid-stimulating hormone (TSH) were 8.31±2.06 and 1.9±0.160, in patients with Hashimoto's disease and control subjects respectively; the level was greater in patient's group in compared with normal control group and the difference was highly significant (P= 0.001). Also the mean levels of Triiodothyronine (T3) were 96.4±11.40 ng/dl and 132.60 ± 5.78 ng/dl, in patients with Hashimoto's disease and healthy subjects subjects respectively; the level was lower in patients group in compared with normal control group and the difference was highly significant (P= 0.001). While the mean levels of Thyroxine (T4) were 6.82 ±0.380 µg/dL and 8.44±0.211 µg/dL, in patients with Hashimoto's disease and healthy control subject respectively; the level was lower in patients group in comparison with normal control group and the difference was significant (P = 0.001).

Regarding the mean levels of Anti-TPO, the current findings indicate the mean concentration of Anti-TPO in Hashimoto's disease patients was highly significantly greater than normal control group, 444.6±72.86 IU/mL versus 7.09 ±0.73 IU/mL respectively, (p= 0.001).

Distribution of *IL-2 (rs2069762) gene* polymorphism was detected by RFLP-PCR technique, at this locus there are three genotypes, TT, TG and GG. Different genotypes resulted to different digested products of non-digested fragment for TT (413bp) and completely digested for GG (388+25bp) and three bands for TG heterozygote genotype

(413+388+25 bp), figure 1. In every research group, the genotype distribution did not deviate from the Hardy-Weinberg equilibrium. The frequencies of *IL-2 T/G* and GG genotypes were higher in Hashimoto's disease than in their controls, but without reaching to a statistically significant level (P=0.352). Also, *the G allele of IL-2* polymorphism was more frequently observed with SLE compared to healthy subjects (P=0.085), table 3. Mean serum concentration of IL-12 level in hashimoto's disease was significantly higher than that of normal control group, 473.21 ±27.3 versus 383.43 ±43.2 and P-value was (P < 0.001), table 4 and figure 2. Specifically, the presently available results indicate that patients with the IL-2 (rs2069762) T/G and TT genotypes present with higher serum IL-2 levels compared to those with the IL-2 (rs2069762) GG genotype (both P < 0.05), following a systematic comparison between IL-2 (rs2069762) genotypes and serum IL-2 levels. Nonetheless, no statistically significant variation was seen in serum IL-2 levels between subjects bearing the IL-2 (rs2069762) T/G and IL-2 (rs2069762) TT genotypes (P > 0.05) table 5.

Table 1. Demographic characteristics of patients with Hashimoto's disease and control subjects

Characteristic	Patients *n* = 60	Control *n* = 60	*P*
Age (years)			
Mean ±SE	38.17±1.5	39.6 ±1.5	0.501 † NS
Range	21 - 65 years	21- 63 years	
Gender			
Male, *n* (%)	6 (10.0%)	13 (21.7%)	0.08 ¥ NS
Female, *n* (%)	54 (90.0%)	47 (78.3%)	

n: number of cases; **SE**: standard Error of mean; †: independent samples t-test; ¥: Chi-square test; **NS**: not significant at *P* > 0.05.

Table 2. Mean levels of thyroid hormone levels in patients with Hashimoto's disease and healthy control subject.

	Cases –control comparison		***P***
	Patients ***n*** **= 60**	**Control** ***n*** **= 60**	
TSH (uIU/mL)			
Mean± SE	**8.31±2.06**	**1.9±0.160**	**< 0.001** † **HS**
Range	**1.00- 99.00**	**0.38-5.40**	
T3 (ng/dl)			
Mean± SE	**96.4±11.40**	**132.60 ± 5.78**	**< 0.001** † **HS**
Range	**1.45- 310.00**	**80.00-197.00**	
T4 (µg/dL)			
Mean± SE	**6.82 ±0.380**	**8.44±0.211**	**< 0.001** † **HS**
Range	**0.90-15.50**	**5.10-12.64**	
Anti-TPO (IU/mL)			
Mean± SD	**444.6±72.86**	**7.09 ±0.73**	**< 0.001** † **HS**
Range	**17.00- 2468.00**	**1.5-33.00**	

n: number of cases; **SD**: standard deviation; †: Independent T test; HS: Highly significant at P ≤ 0.001; NS: not significant at P > 0.05.

Table 3. *IL-2 (rs2069762)* POLY genotype frequency in Patients and healthy control.

Mode	*IL-2 (rs2069762)*	Patients *n* = 60	Control *n* = 60	*P*	OR	95% CI
Co-dominant	GG	18 (30.0%)	12 (20.0%)	0.352 ¥ NS	1.92	0.78-4.71
	T/G	17 (28.3%)	16 (26.7%)		1.36	0.57 -3.21
	TT	25 (41.7%)	32 (53.3%)		Reference	
Dominant	GG+T/G	35 (58.3 %)	28 (46.7%)	0.201 ¥ NS	Reference	
	TT	25 (41.7%)	32 (53.3%)		0.625	0.30-1.28
Recessive	GG	18 (30.0%)	12 (20.0%)	0.206 ¥ NS	1.71	0.74-3.96
	T/G+TT	42 (70.0%)	48 (80.0 %)		Reference	
Alleles	G	53 (44.2%)	40 (33.3 %)	0.085 ¥ NS	1.58	0.93-2.67
	T	67 (55.8%)	80 (66.7 %)		Reference	

¥: Chi-square test; NS: not significant at $P > 0.05$

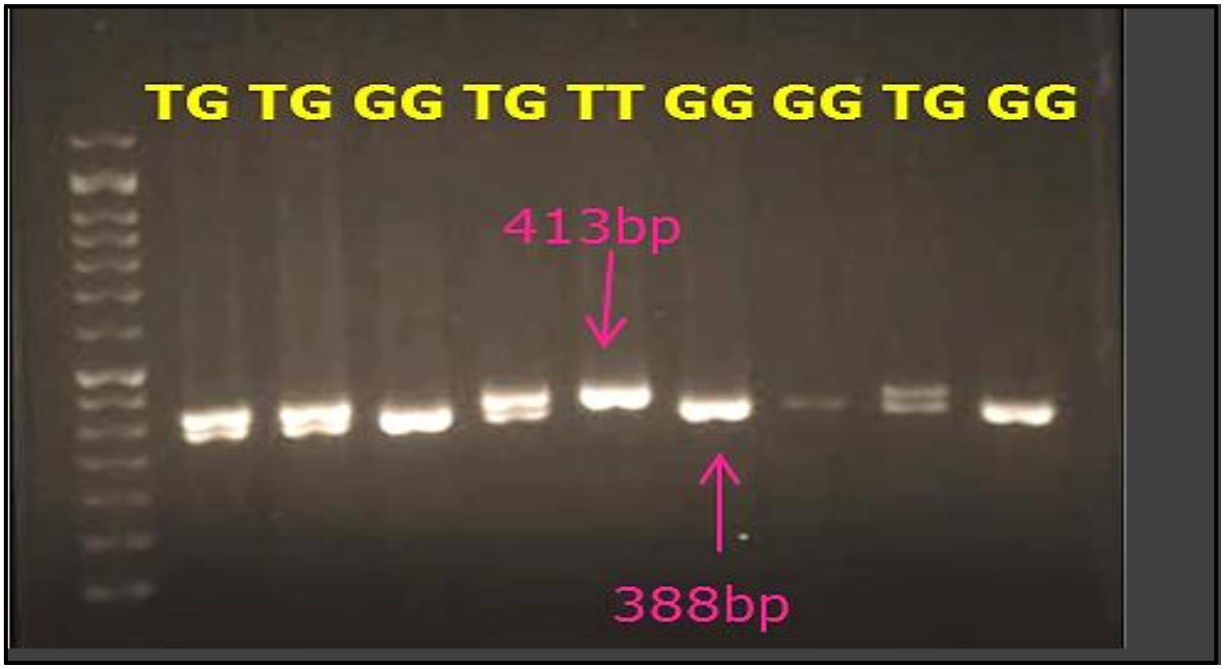

Figure 1. Agarose gel electrophoresis image that show the RFLP-PCR product analysis of *IL-2 (rs2069762)* gene polymorphism. Different genotypes resulted to different digested products of non-digested fragment for TT (413bp) and completely digested for GG (388+25bp) and three bands for TG heterozygote genotype (413+388+25 bp).

Table 4. The compared between the study groups regarding IL-2 levels.

IL-2 levels	Patients	Healthy control	P value
Mean± SD	473.21 ±27.3	383.43 ±43.2	<0.001 ¥ (S)
Range	197.42- 660.31	101.21- 531.21	

†: Mann-Whitney U test; **S**: significant at $P \leq 0.05$

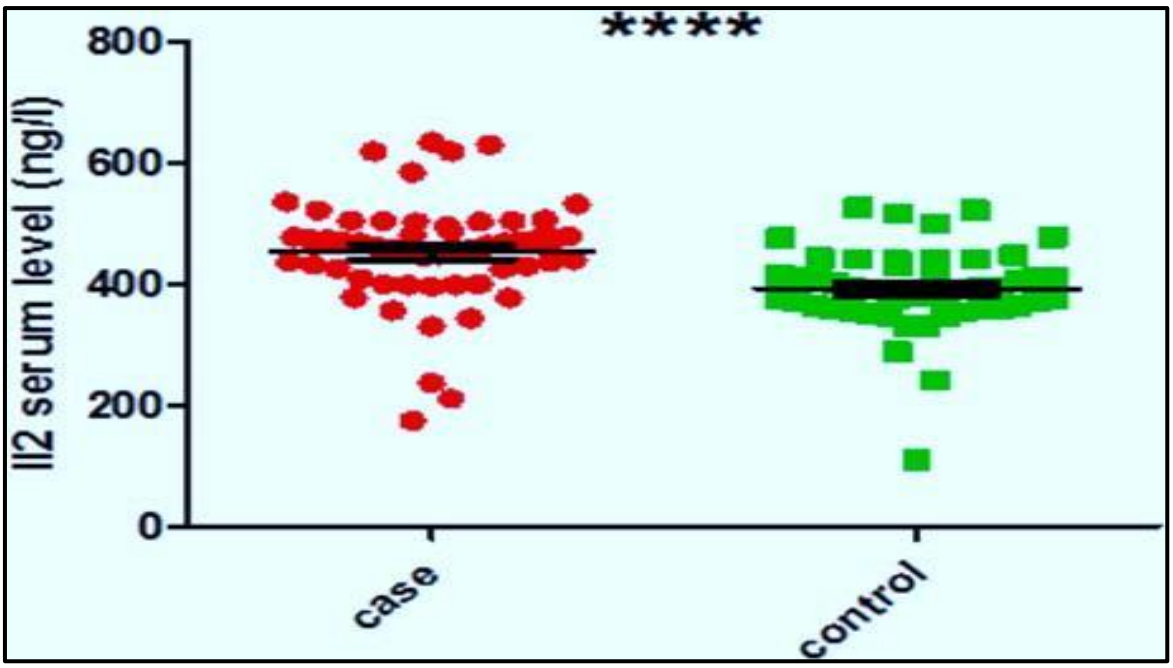

Figure 2. Comparison of the IL-2 concentration among hashimoto's disease patients and control group.

Table 5. Association between *IL-2 (rs2069762) genotype and serum IL-2* levels in patient with Hashimoto's disease.

Serum Levels	***IL-2 (rs2069762) genotype***			
	TT genotype	***TG genotype***	***GG genotype***	***P***
IL-2 levels				
Mean± SE	**424.8** ± 19.33 [A]	**446.3**± 15.03 [A]	**411.8**± 19.19 [A]	**P=0.172 † NS**
Range	110.1 – 610.1	174.7 – 635.5	211.0- 585.8	
Different latters denote to the significant differences at p< 0.05				

n: number of cases; **SD**: standard deviation; †: one way ANOVA; **S**: significant at $P < 0.05$

Discussion

Hashimoto's thyroiditis, one of the most common autoimmune diseases and the most common endocrine sickness, is described as chronic inflammation of the thyroid gland [14]. While Hashimoto's thyroiditis can occur at any age, women between the ages of 30 and 60 are most likely to acquire it. Although the condition's precise incidence is unknown, Grave's disease is likely to be comparable [3]. The present finding show mean

age of patients was 38.17±1.5 years and that of control subjects was 39.6 ±1.5 years and there was no significant difference between patients and control subjects in mean age (P = 0.501). This results similar to the results of Maitham Al-Khateeb *et al*. [15], who showed the mean age of HT was 37.4 ±13.3 years. Closely related findings have been observed in other Iraqi studies: Al-Mofarji [16] discovered that patients between the ages of 30 and 49 had the highest prevalence of HT disorder; Al-Wayly [17] reported that patients in this age group had the highest prevalence of HT present; and Amin et al. [18] found that patients in this age range had the highest frequency of autoimmune thyroiditis. According to Taha *et al.*[19], thyroid disorders are frequently discovered in middle age. Numerous epidemiological studies conducted worldwide suggest that while HT can strike anyone at any age, its prevalence is highest in people between the ages of 30 and 60 [20].

The incidence ratio of Hashimoto's thyroiditis is approximately 8:1, making it quite common in females [3]. Based on the current data, the patient group consisted of 6 (10.0%) males and 54 (90.0%) females, while the control group had 13 (20.6%) males and 50 (79.4%) females. The frequency distribution of patients and control subjects did not differ significantly based on gender (P = 0.102). These findings suggest that women are more likely than men to develop HT illness; in other words, women's gender increases the likelihood of getting autoimmune hypothyroidism. The current observation is extremely significant (P = 0.001) and is consistent with the findings of Abdullah et al. [21] that the majority of HT patients were female (82%). Results from several epidemiological studies around the world showed that, for example, autoimmune thyroid illnesses strongly impact women. According to Pirahanchi *et al.* [22], ladies have HT at least ten times more than males do. The identical results were discovered by [23]. The loss of self-tolerance to X-linked antigens could explain the female domination of Hashimoto's disease. New research indicates that imbalanced XCI, or the deactivation of chromosomal X in roughly 80% of cells, may put females at risk for developing AITD [24]. Additionally, because estrogen increases a woman's susceptibility to autoimmune diseases by inducing a pro-inflammatory response, the function of estrogen-induced immune response has also been proposed [25]. Through a receptor on or inside immune cells, the sex hormone directly interacts with immune system cells. The effects of steroid hormones on the generation of antibodies and the proliferation of immune cells are well-known [26]. There were sixty HT patients and sixty healthy controls in this case-control study. Serum levels of T3, T4, TSH, and anti-TPO were measured for each of the individuals under investigation. According to the current research, HT patients' serum TSH values were significantly higher (P <0.001) than those of the control group. The mean TSH levels in the patient group were greater than those in the control group, coming in at 8.31±2.06 versus 1.9±0.160 uIU/mL, respectively. Patients' serum T3 concentrations were substantially lower than those of the healthy subjects. However, the sufferers' serum T4 concentration was noticeably lower than that of the healthy individuals. Compared to the control group, Hashimoto's patients had significantly higher levels of thyroid auto-antibodies, or anti-TPO; see table [2]. According to the current findings, the mean levels of anti-TPO in patients with Hashimoto's disease were found to be substantially greater than those in healthy control subjects, ranging from 7.09 ±0.73 IU/mL to 444.6±72.86 IU/mL, respectively (P <0.001).

Our study revealed that, in comparison to a normal, healthy control group, the serum concentrations of TSH and anti-TPO were significantly elevated in Hashimoto's patients. But when compared to a typical, healthy control group, the patients' levels of T3 and T4 were lower. These findings may point to the emergence of sub-clinical hypothyroidism, which is characterized by naturally occurring T4 concentrations, a reduction in T3, and a higher level of increased TSH [3]. Elevated anti-TPO and TSH concentrations, along with normal T4 levels, in the presence of a subclinical HT problem. Anti-TPO antibodies are extensively accessible and often utilized in HT illness clinical diagnostic laboratories [27]. The primary anti-thyroid antibodies in Hashimoto's disease are these ones, and an increase in anti-TPO antibodies has been connected to future clinical signs of the illness's progression [28]. Moreover, Siriwardhane et al. [29] came to the conclusion that blood anti-TPO concentrations can be utilized as markers for early thyroid autoimmune development prediction. Additionally, they advise including these tests with the T3, T4, and TSH tests in the list of thyroid function tests. Current study examined the blood concentration of IL-2 in HT patient and the control group, and the results indicated that the patients' serum levels of IL-2 were greater than those of the control group. Numerous investigations have established a correlation between the serum level of IL-2 and other autoimmune disorders, such as multiple sclerosis, Grave's disease, autoimmune thyroid illnesses, and type 1 diabetes. It is believed that the pathophysiology of autoimmune thyroid disorders is significantly influenced by these alterations in pro-inflammatory cytokines. Since aberrant thyroid-immune interactions result from disturbances of thyroid self-tolerance, a variety of cytokines and their receptors are implicated. In the presence of impaired immunomodulatory T regulatory cells, thyrocytes acquire antigen-presenting cell properties that activate Th1 and Th2 effector immune cells. This leads to thyroid lymphocytic infiltration and B cell activation, which produces antibodies against thyroid antigens and, depending on the Th1-Th2 balance, either thyroid stimulation or destruction [(30)]. Disrupted immune systems are a major development of autoimmune disorders. Organs and tissues that are owned are protected by the elimination of the "foreign" antigen and physiologically sound examination. In the thymus, autoreactive cells are cloned, whereas Treg regulatory (suppressor) lymphocytes or energy are used in peripheral immune system organs to develop tolerance. A cytokine signaling molecule called interleukin (IL) -2 is the primary growth factor for T cells that regulates immunity. Human T cell proliferation and effector cell differentiation are dependent on IL-2 [31]. The thyroid's secretion of cytokines is responsible for the lymphocyte pool's growth and concentration within the thyroid. The thyroid cells themselves also play a part in this secretion. Additionally, the thyroid cells generate several pro-inflammatory chemicals, such as IL-2 and IFN-g, which have the tendency to worsen the autoimmune process [32].

Furthermore, this cytokine stimulates T lymphocytes and causes molecules to emerge on their surface that allow T cells to undergo apoptosis, which has a beneficial influence on the immune response. Numerous investigations have verified the correlation between the level of IL-2 in serum and numerous autoimmune disorders, such as type 1 diabetes [33], juvenile atopic dermatitis [34], and autoimmune thyroid illnesses. The pro-inflammatory cytokine IL-2 mean serum concentrations of patients are significantly higher than those of controls, as demonstrated by the current data. These findings are

consistent with those of Liang *et al.* [35], who found that patient serum levels of IL-2 were 5.2 times higher than those of healthy controls. When comparing patients to controls, higher serum concentrations of the pro-inflammatory cytokine IL-2 were found, which suggests a high Th2 cytokine profile in the pathophysiology of HT. In an observation performed by Tahir *et al.* [36], on Acromegaly sufferers, IL-2 ranges have been higher in patients with Acromegaly than in the Acromegaly group. In 2020, Al-Asadi *et al.*,[37] showed that the presence of detectable amounts of IL-2 was statistically non-significant between diabetic patients and non-diabetic patients. One important functional component involved in immune regulation and function is the IL-2 gene [35]. Analysis was done on polymorphisms at the IL-2 promoter's -330 locus in both HT patients and healthy controls. Hardy-Weinberg equilibrium was seen in the IL-2 allele frequency distribution at the -330 gene ($P > 0.05$; data not shown). Next, potential associations between the incidence rates of HT and the genotype distribution of the -330 IL-2 promoter sites were examined. The HT group had a higher frequency of T/G genotype single nucleotide polymorphisms (SNPs) in both loci, as indicated by Table 3. These findings suggested that the HT susceptibility may be linked to the comparatively high prevalence of heterozygous T/G SNP on the alleles. Also the present investigation shows that *rs2069762 G* alleles were non-significantly more frequent in patients with HT (44.2% in HT, p= 0.085) compared with healthy control (33.3%) (Table 3). The GG genotype in Co-dominant model was non-significantly associated with HT (30% in case vs 20.0% in control, P value=0.352, OR=1.92) which indicates non-significant association of -330 IL-2 SNP with HT.

The 330 region of the IL 2 promoter contained a higher frequency of GG SNPs in the thyroid cancer group, according to the RFLP data of Martins et al. [38]. These findings suggested a possible link between the occurrence of thyroid cancer and the alleles' comparatively high homozygous GG SNP rates. Liang et al. [35], in agreement with our results, reported that the -330 region of the IL-2 promoter included a greater frequency of GG SNPs in the GD group. suggested that the occurrence of GD may be linked to the alleles' comparatively high prevalence of homozygous GG SNPs. Therefore, at least in the Asian population, SNPs may function [39-42] as bio marker in GD patient.

In Ulm and the surrounding area in southwest Germany, a study by Howson et al. [39] found a higher incidence of the IL2 genetic variant (rs2096762) in type 2 diabetes mellitus. According to Matesanz *et al.* [40], there is a correlation between the IL2 gene's T/T and −330 T/G genotypes and the likelihood of developing a secondary progressive (SP) course of multiple sclerosis. The −330 G allele exhibited significantly greater levels of gene expression in IL2 promoter luciferase constructs transected in the Jurkat cell line, according to their findings [43-46]. The present results show non-significant increased the serum IL-2 levels in TG genotype 446.3± 15.03, in compared to TT genotype and GG genotype respectively, 424.8 ± 19.33 and 411.8± 19.19, (P=0.172). When it is shown that changes in the IL-2 gene promoter activity are often responsible for changes in the *IL-2 (rs2096762)*. The expression of interleukin-2 and its receptor subunits can be affected by polymorphisms, which can also have an impact on the activity of the IL2 signaling pathway. Consequently, this may impact the performance of CD4+CD25+ Tregs and ultimately encourage the onset of autoimmune diseases [41]. The current findings are consistent with those of Wiśniewski and Sworczak [42] who

shown that changes in gene promoter activity frequently lead to changes in gene expression levels. Sayad and Movafagh [43] found that among Iranian MS patients, those with the −330 T/T and T/G genotypes had greater levels of IL2 than those with the −330 G/G genotype. The current findings did not align with the findings of Hoffman et al. [44] who reported a rise in IL2 production in individuals with a genotype of -330 G/G. Previous research in neuroendocrine tumors showed a correlation between serum cytokine levels [47-50] and a polymorphism mutation upstream to the IL2 gene promoter (-330 T/G, rs2060762).

Conclusions

Serum IL-2 levels were more elevated in Hashimoto's disease cases than in controls, contributing to SLE pathogenesis. The polymorphisms of *IL-2-330T>G genes* had influence on the susceptibility to Hashimoto's disease. Also indicate significant association between of *IL-2-330T>G genes* polymorphisms and serum IL-2 levels.

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