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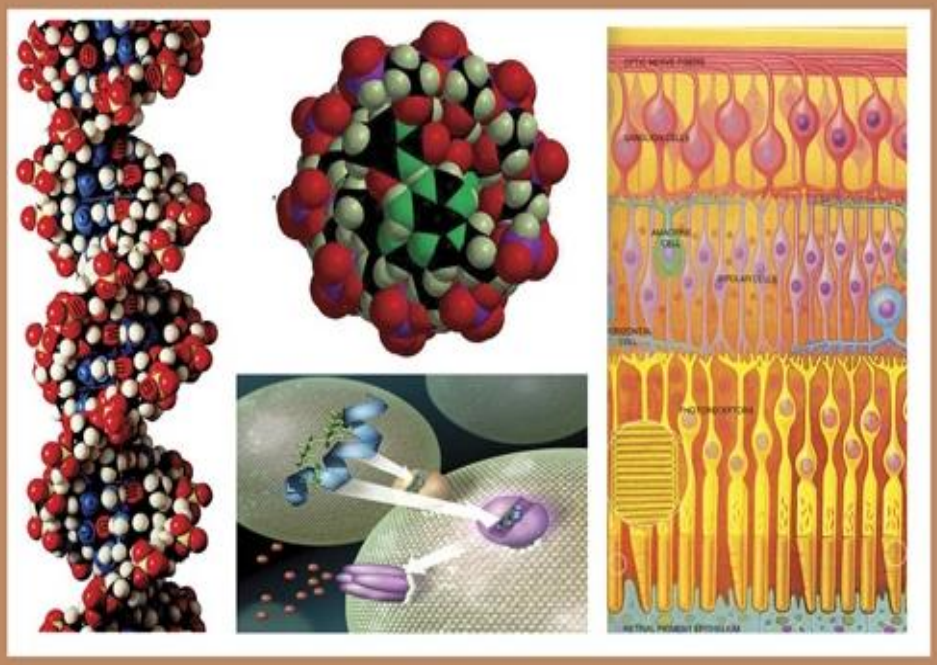

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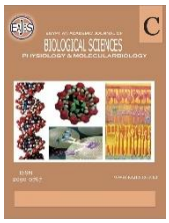

Analysis of Chosen Polymorphisms rs1982073 T/C, rs1800471 C/G TGFβ1 in Pathogenesis of Hashimoto's Diseases

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ABSTRACT

Hashimoto's thyroiditis is an autoimmune disorder that primarily affects the thyroid gland, leading to chronic inflammation and impaired thyroid function. On the other hand, transforming growth factor beta-1 (TGF-β1) is a protein that plays a crucial role in regulating various cellular processes, including immune responses and tissue remodelling. The objective of this study was to investigate the association of TGFβ-1 levels and its gene polymorphisms in Hashimoto's Thyroiditis (HT) patients and the immune system attack of the thyroid gland cells, by the presence of antibodies, including thyroglobulin antibody (TG-AB) and thyroid peroxidase antibody (TPO-AB), which serve as biochemical markers for Hashimoto's Thyroiditis. A case-control study encompassed a cohort of 100 female subjects within the age range of 20 to 53 years, the study consisted of a group of diagnosed Hashimoto's thyroiditis patients and an equivalent number of healthy volunteers who were carefully selected to match the age and gender characteristics of the patient group. The study employed allele-specific arm-PCR technique to examine the genotype of TGF-β1 gene polymorphisms. Furthermore, levels of T3, T4, TSH, fT3, fT4, Anti TPO, and Anti-TG were quantified using the ELISA method, utilizing specific commercial kits designed for analysis within the respective study groups. Current work showed a highly significant variation in TGFβ1 levels in HT patients group when compared with healthy group, in addition to these findings suggest that the presence of the CG, GG genotype of rs1800471 and the CT, TT genotypes in rs1982073 are associated with an increased susceptibility to HT disease. The present study concluded that genotypes distribution and associated risks highlight the potential role of this genetic variation in the development and manifestation of HT disease.

INTRODUCTION

Hashimoto's thyroiditis (HT), is a prevalent autoimmune disease specifically targeting the thyroid gland. It affects approximately 5% of the population (Hiromatsu *et al.*, 2013). The development of HT is multifactorial, involving a complex interplay of various genetic and environmental factors that influence the occurrence of autoimmune reactions against the thyroid gland(Rayman & Hu, 2016).

In Hashimoto's thyroiditis (HT), the primary immune mechanism at involves the infiltration of cytotoxic lymphocytes into the thyroid gland. This infiltration leads to the destruction of thyroid cells; the immune system generates antibodies that specifically target the thyroid follicles.

These antibodies, namely thyroglobulin antibody (TG-AB) and thyroid peroxidase antibody (TPO-AB), are produced. The presence of TG-AB and TPO-AB serves as biochemical markers indicating the occurrence of HT (Babiker, 2018; Ragusa *et al.*, 2019). These antibodies play a significant role in the pathogenesis of HT, as they can activate complement components and cause toxicity to thyroid cells (Majid *et al.*, 2020). Studies indicate that Hashimoto's thyroiditis occurs more frequently in women between the ages of 30 and 50, with an approximately eight-fold higher incidence compared to males (Lokhande, 2012).

The Transforming Growth Factor beta (TGFβ) family consists of three isoforms, namely TGFβ1, TGFβ2, and TGFβ3, which are multifunctional cytokines involved in regulating various physiological processes, including cell proliferation, differentiation, migration, apoptosis, and immune responses (Massagué, 2012). TGFβ is produced and secreted by nearly all cells, including certain types of white blood cells (Morikawa *et al.*, 2016; Santibañez *et al.*, 2011). As well as TGFβ levels are typically tightly controlled to maintain tissue balance, they can be dysregulated in certain disease conditions such as cancer, fibrosis, and inflammation. Excessive production of TGFβ in these states contributes to disease progression by influencing cell growth and movement (Aventaggiato *et al.*, 2022).

The progress of thyroid autoimmunity in Hashimoto's thyroiditis (HT) is influenced by a combination of genetic predisposition and dysregulation of immune mechanisms. The autoimmune destruction of the thyroid gland is facilitated by immune cell reactivity, which is guided by regulatory and effector cytokines (Ouyang *et al.*, 2009; Syed, 2016).

Despite numerous studies, the role of cytokines in HT development remains unclear and inconclusive (Morikawa *et al.*, 2016). However, scientific evidence supports the significant role of TGFβ1 in the pathophysiology of autoimmune thyroid diseases. It has been found that this growth factor affects the severity, progression, or course of autoimmune thyroid disease in a condition-specific manner *(Kardalas et al*., 2021). The role of TGFβ1, whether it acts as an inhibitor or facilitator of thyroid autoimmunity, depends on factors such as the rate of TGFβ1 synthesis, the specific genotypic and phenotypic features of the autoimmune thyroid disease (Sacristán-Gómez *et al*., 2023).

The human TGFβ1 gene is located in the chromosomal region 19q13.2 and encodes a 25-kDa multifunctional homodimer protein consisting of 112 amino acids (Su *et al.,* 2022). Within this gene, five polymorphisms have been identified (Baran *et al.,* 2007). Polymorphisms in genes encoding transforming growth factor beta (TGFβ1) have been the subject of extensive research due to their documented association with a range of immune-mediated diseases(Gerenova & Stanilova, 2016). Specific single-nucleotide polymorphisms (SNPs) within the TGFβ1 gene have been delineated, including +869T/C and +915G/C. These SNPs lead to distinct amino acid substitutions at codons 10 (leucine to proline) and 25 (arginine to proline), respectively. Importantly, it has been observed that the presence of TGFβ1 +869 T and +915 G alleles is correlated with augmented expression levels of the TGFβ1 protein. This implies that these genetic variations might exert functional effects, potentially influencing TGFβ1 signalling and immune responses within individuals harbouring these alleles (Ahmed *et al*., 2020; Rice *et al*., 2021).

The +869T/C polymorphism, located in the promoter region of the TGFβ1 gene, is of particular interest as it could influence gene expression and may be a candidate locus for genetic susceptibility(Stanilova *et al.*, 2018). Some studies have investigated the association between different TGFB1 SNPs and Hashimoto's thyroiditis (HT) susceptibility and severity, but the results have been contradictory(Shah *et al.*, 2006).

In addition, the role of TGFB1 SNPs has been studied in relation to other diseases, such as psoriasis, with some studies showing positive associations (Ahmed *et al.,* 2020; Huang *et al.*, 2015). SNPs within the TGF-β1 gene have undergone comprehensive cataloguing. It has been observed that the presence of TGF-β +869 T and +915 G alleles is associated with heightened expression levels of the TGF-β1 protein. In a study conducted in Argentina, the association between TGF-β1 +869 and +915 SNPs and Hashimoto's thyroiditis (HT) in both pediatric and adult populations was thoroughly investigated.(Yousefi *et al.,* 2019).

MATERIALS AND METHODS

Study Design:

A case-control study was conducted on 100 female participants aged 20-53 years, comprising diagnosed Hashimoto's thyroiditis patients and an equal number of healthy volunteers were matched for their age and gender The subjects included in the study were selected based on specific inclusion and exclusion criteria, by applying these inclusion and exclusion criteria, the study aimed to ensure a homogeneous group of patients with Hashimoto's thyroiditis, minimizing potential confounding factors and allowing for a more focused investigation of the genetic association of TGFB1 SNPs with the disease.

Methods:

Participant in this study, a venous whole blood sample of five millilitres was obtained. The collected blood samples were then left to clot at room temperature and subsequently subjected to centrifugation at a speed of 5000 times the force of gravity (5000xg) for a duration of 5 minutes. Following centrifugation, the resulting sera were carefully collected and stored at a temperature of -18ºC until they were utilized for measuring thyroid function tests, as well as thyroid peroxidase antibody (TPO-AB) and thyroglobulin antibody (TG-AB). These measurements were performed using a Sandwich-ELISA kit manufactured by Sunlong company, Chain. The genotyping of TGFβ1 polymorphism(rs1982073C/T) and (rs1800471C/G) were analysed by using Allele Specific Arm polymerase chain reaction.

Statistical Analysis:

The statistical analysis of the obtained results in the current study was conducted using the 26th edition of the Statistical Package for the Social Sciences (SPSS). The outcomes were presented in terms of Mean ± Standard Deviation (Mean±SD), which provides information about the average value of the measurements along with the dispersion or variability around the mean. The Student's t-test is a statistical test used to determine if there is a significant difference between the means of two independent groups. To determine the relationships among the measurable factors in the study, Pearson's correlation analysis was applied. A p-value less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

The focus of the study was to examine the TGF-β1levels and its potential genetic variations and explore its role as a susceptibility factor in Hashimoto's thyroiditis (HT), furthermore, the study aimed to investigate the relationship between genetic variations in the TGF-β1 gene and various biochemical parameters associated with HT. These parameters were divided into two groups: demographic factors (such as age, body mass index [BMI], systolic and diastolic blood pressure) and thyroid function tests (including T3, T4, TSH, free T3 [fT3], free T4 [fT4], anti-thyroid peroxidase [anti-TPO] antibodies, and anti-thyroglobulin [anti-TG] antibodies). Specifically, the study sought to determine whether the single nucleotide polymorphisms (SNPs) +869T/C (rs1982073) and +915C/G (rs1800471) in the promoter region of the TGFβ1 gene were associated with genetic susceptibility and clinical characteristics of Iraqi individuals with HT.

The statistical analysis of age in the study groups revealed no significant differences between the HT patients and the corresponding control group (p=0.435). However, there was a highly significant

increase in BMI among the HT patients compared to the healthy control subjects (p=0.000). Furthermore, both systolic and diastolic blood pressure measurements showed a significant increase in HT patients compared to the healthy control subjects (p=0.000). These findings indicate that HT patients had higher BMI and blood pressure levels compared to the control group, suggesting potential associations between HT and these parameters, as shown in Table 1.

Table 1: Demographic Characteristics of Study Group.

Parameters	Healthy women Mean ± SD	Patient women Mean ± SD	P Value
Age (year)	37.571 ± 11.496	42.340 ± 10.231	0.435
BMI(Kg/m²)	23.602 ± 2.859	32.303 ± 5.604	0.000
WHR	0.776 ± 0.059	0.960 ± 0.105	0.005
Systolic blood pressure (mmHg)	115.500 ± 8.149	146.000 ± 18.516	0.000
Diastolic blood pressure (mmHg)	73.625 ± 7.925	87.700 ± 11.438	0.000

P value < 0.05 was significant, and P value >0.05 was insignificant.

The study observed a significant rise in the levels of thyroid-stimulating hormone (TSH), as well as thyroid peroxidase antibody (TPO-AB) and thyroglobulin antibody (TG-AB), among patients diagnosed with Hashimoto's thyroiditis (HT) in comparison to the healthy control group. Conversely, a significant decrease was noted in the levels of triiodothyronine (T3), thyroxine (T4), free triiodothyronine (fT3), and free thyroxine (fT4) in the sera of HT patients when compared to the healthy control group. Also, a significantly higher mean serum level of TGFβ1 was observed in HT patients compared to the controls in this study.

Table 2: Levels of (Mean ± SD) of Thyroid Function Tests and TGFβ1 in Sera of Study Group.

Markers	Control No. Mean ± SD	Patients No. Mean ±SD	P Value
T3 nmol/L	2.243 ± 0.955	0.893 ± 0.193	0.004
T4 nmol/L	121.679 ± 16.910	51.593 ± 9.941	0.000
TSH µmol/ml	1.714 ± 0.860	10.151 ± 11.213	0.009
fT3 pmol/L	3.132 ± 0.444	1.684 ± 0.259	0.030
fT4 pmol/L	1.006 ± 0.230	0.445 ± 0.139	0.000
Anti-TG IU/ml	138.603 ± 58.246	422.444 ± 115.061	0.000
Anti-TPO IU/ml	225.647 ± 67.019	593.234 ± 192.442	0.001
TGFβ1	13.027 ± 3.812	21.459 ± 5.975	0.02

P value < 0.05 was significant, and P value >0.05 was insignificant.

According to the study findings, there was a notable decrease in the levels of triiodothyronine (T3), thyroxine (T4), free triiodothyronine (fT3), and free thyroxine (fT4) in the sera of patients with Hashimoto's thyroiditis (HT) compared to the healthy control group. Conversely, the study reported a significant increase in the levels of (TSH), (TPO-AB), (TG-AB), and (TGFβ1) in HT patients compared to the healthy control subjects, as shown in Table 2. The genotype frequencies of TGF-β1 SNP rs1800471 (C/G) were found to be consistent with Hardy-Weinberg equilibrium in both control subjects and HT patients, this indicates that the distribution of genotypes for this SNP in the population is in line with the expectations under the equilibrium. On the other hand, the

genotype frequencies of TGF-β1 SNP rs1982073 (T/C) were found to be inconsistent with Hardy-Weinberg equilibrium in both control subjects and HT patients. This suggests that there may be factors influencing the genotypic distribution of this SNP in the studied population that deviate from the equilibrium expectations. The allelic and genotypic distributions of TGF-β1 SNP rs1800471 (915C/G) showed significant differences between the control subjects and HT patients. In Tables 3 and 4, the frequency distribution of the TGFβ1 SNP rs1982073 (T/C) alleles exhibited significant differences between the patient and control groups. The C allele was found to be less frequent in the patient group, accounting for 38% of the alleles, while it was more prevalent in the control group, comprising 69.5% of the alleles. In contrast, the T allele showed a higher frequency in the patient group, constituting 62% of the alleles, whereas it was less common in the control group, making up only 30.5% of the alleles.

Table 3: Hardy– Weinberg Equilibrium (HWE) for 869 SNP rs1982073 C > T gene for Control.

Genotype of control	Observed	Expected	X^2	P value
TT Reference	15	9.3	7.22	0.007
CT Heterozygote	31	42.4		
CC Recessive	54	48.3		
C	69.5%			
T	30.5%			

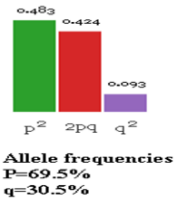
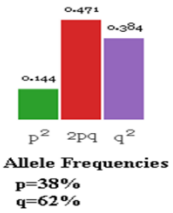


Table 4: Hardy – Weinberg Equilibrium (HWE) for 869 SNP rs1982073 C > T gene for Patients.

Genotype of Patient	Observed	Expected	X^2	P value
CC Recessive	18	14.44	2.3	0.1
CT Heterozygote	40	47.12		
TT Reference	42	38.44		
C	38%			
T	62%			



In Tables 5 and 6, the frequency distribution of the TGF-β1 SNP rs1800471 (C/G) alleles displayed marked differences between the patient and control groups. The C allele was found to be less frequent in the patient group, accounting for 25% of the alleles, while it was more prevalent in the control group, comprising 51.5% of the alleles. Conversely, the G allele exhibited a higher frequency in the patient group, constituting 75% of the alleles, whereas it was less common in the control group, making up only 48.5% of the alleles. These results indicate a notable disparity in allele frequencies between patients and controls, suggesting a potential association between the TGF-β1 rs1800471 SNP and the risk of developing the condition under investigation.

Table 5: Hardy– Weinberg Equilibrium (HWE) for 915 SNP rs1800471 C > G gene for Control.

Genotype of Control	Observed	Expected	X^2	P value
CC Recessive	50	26.52	88.34	0.01
CG Heterozygote	3	49.95		
GG Reference	47	23.53		
C	51.5%			
G	48.5%			

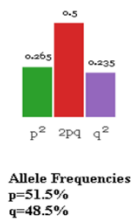


Table 6: Hardy – Weinberg Equilibrium (HWE) for 915 SNP rs1800471 C > G gene for Patients consistent.

Genotype of patients	Observed	Expected	X^2	P value
CC Recessive	16	6.25%	27.04	0.001
CG Heterozygote	18	37.5%		
GG Reference	66	56.25%		
C	25%			
G	75%			

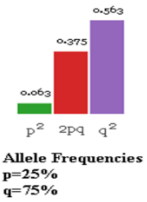


Table 7 demonstrated that the CG genotype of rs915 C/G of TGF-β1 gene polymorphisms was associated with an increased risk of HT susceptibility, with an odds ratio (OR) of 18.75 (CI 95% 4.8817 to 72.0158). Similarly, the GG genotype in the codominant model was associated with an increased danger of HT, with an OR of 4.4 (CI 95% 2.2323 to 8.6267) compared to the wild-type CC genotype. In the dominant model (CG + GG), the OR was 5.3 (CI 95% 2.7055 to 10.1876) indicating a five-fold increased risk of HT compared to the wild type. These findings suggest that the presence of the CG and GG genotype of rs1800471 are associated with infected to HT. The genotype distributions and associated risks highlight the potential role of this genetic variation in the development and manifestation of HT.

In the recessive model, individuals carrying the GG genotype of TGF-β1 SNP rs1800471 (915C/G) exhibit a significantly higher danger of developing (HT) compared to those with the CC + CG genotypes, with an odds ratio (OR) of 2.21 (CI 95% 2374 to 3.8725), this indicates a two-fold increased susceptibility to HT for individuals with the GG genotype in comparison to those with the CC + CG genotypes.

Table 7: The Allele Frequencies and Genotype of rs915 C/G of TGF-β1 Gene in Ht Patients and Control.

915C>G	Control No. = 100	Patient No. = 100	OR (CI 95%)	P Value
Codominant				
CC	50	16	1.0	
CG	3	18	18.75 4.8817 to 72.0158	0.0001
GG	47	66	4.4 2.2323 to 8.6267	0.0001
Dominant				
CG+GG	50	84	5.3 2.7055 to 10.1876	0.0001
Recessive				
CC+CG	53	34	1.0	
GG	47	66	2.2 1.2374 to 3.8725	0.0001
Additive				
C	103	50	1.0	
G	97	150	3.2 2.0858 to 4.8653	0.0001

P value < 0.05 was significant, and P value >0.05 was insignificant.

Similarly, for TGF-β1 SNP rs1982073 (869C/T), the allelic and genotypic distributions reveal notable distinctions between control subjects and HT patients. The CT genotype is associated with a four-fold increased risk of HT susceptibility relative to the wild-type CC genotype. In the codominant model, the TT genotype demonstrates an eight-fold higher risk of HT compared to CC. Furthermore, in the dominant model (CT + TT), there is a five-fold increased risk of HT infection in comparison to the wild type. Additionally, the recessive model for rs1982073 indicates that individuals with the TT genotype possess a four-fold heightened susceptibility to HT when compared to those with the CC + CT genotypes. These findings highlight the potential contribution of the GG genotype in rs1800471 and the CT or TT genotypes in rs1982073 to an elevated risk of HT development.

It is crucial to emphasize that further research, including replication studies, is warranted to validate these observations and gain a more comprehensive understanding of the association between these TGF-β1 SNPs and HT susceptibility, as shown in Table 8.

Table 8: The Allele Frequencies and Genotype of rs869 C/T of TGF-β1 Gene in Ht Patients and Control.

869C/T	Control No. = 100	Patients No. = 100	OR (CI 95%)	P Value
Codominant				
CC	54	18	1.0	
CT	31	40	3.87 1.9026 to 7.8759	0.0002
TT	15	42	8.4 3.7930 to 18.6026	0.0001
Dominant				
CT+TT	46	82	5.4 2.8082 to 10.1842	0.0001
Recessive				
CC+CT	85	58	1.0	
TT	15	42	4.1 2.0841 to 8.0795	0.0001
Additive				
C	139	76	1.0	
T	61	124	2.7 2.4553 to 5.6297	0.0001

P value < 0.05 was significant, and P value >0.05 was insignificant.

Hashimoto's thyroiditis (HT) is an autoimmune thyroid illness characterized by chronic inflammation of the thyroid gland, leading to hypothyroidism. TGF-beta1 (transforming growth factor-beta-1) is a cytokine involved in various cellular processes, including immune regulation and inflammation.

Research studies have suggested that TGF-beta-1 plays a significant role in the pathogenesis of Hashimoto's thyroiditis. TGF-beta-1 has both pro-inflammatory and anti-inflammatory effects, depending on the context. In HT, TGF-beta1 may contribute to the chronic inflammation seen in the thyroid gland, leading to the destruction of thyroid tissue and subsequent hypothyroidism (Consolandi *et al.*, 2015; Sun *et al.*, 2015). Additionally, TGF-beta-1 plays a crucial role in the differentiation and function of regulatory T cells (Tregs). Tregs help control the immune response and maintain self-tolerance, preventing the immune system from attacking its tissues. Dysregulation of

Tregs may contribute to the development of autoimmune diseases like Hashimoto's thyroiditis (Choileain & Redmond, 2006; Kong *et al.*, 2010). TGF-beta-1 is also involved in tissue fibrosis, which is a hallmark of HT. Fibroid in the thyroid can lead to the enlargement of the gland (goitre) (Gill *et al.*, 2002; Hsieh *et al.*, 2010). Given the role of TGF-beta-1 in inflammation and fibrosis, it has been suggested as a potential therapeutic target for Hashimoto's thyroiditis, but further research is wanted in this area (Yang *et al.*, 2020).

Some studies have identified genetic variants in the TGF-beta-1 gene that may be associated with an increased danger of developing HT (Munoz, 2004; Ying *et al.*, 2018).

Genetic Associations Have Been Investigated in the Context of Hashimoto's Thyroiditis and TGF-beta-1:

Several studies have explored the potential role of genetic variants within the TGF-beta1 gene in the development and progression of Hashimoto's thyroiditis. One study conducted in 2004 by Kula et al. examined the association between TGF-beta1 gene polymorphisms and the course of autoimmune thyroid disease in children. The researchers found that a specific genetic variant, known as the T869C polymorphism, was relating to an increased danger of developing HT. This variant was more frequently observed in children with HT compared to healthy controls (Munoz, 2004).

Another study published in 2014 by Mao *et al.* investigated TGF-beta-1 gene polymorphisms in Chinese patients with autoimmune thyroid diseases, including Hashimoto's thyroiditis. The researchers found that a different genetic variant, the G915C polymorphism, was relating to an increased susceptibility to autoimmune thyroid diseases. Specifically, the G915C variant was more prevalent in patients with HT compared to control groups.

These studies suggest that certain genetic variants within the TGF-beta1 gene may influence the risk of developing Hashimoto's thyroiditis. However, it's important to note that the exact mechanisms by which these genetic variants affect disease susceptibility or progression are still not fully understood. Further research is needed to elucidate the functional implications of these genetic variations and their specific contributions to the development of HT. A study by Ban *et al.* published in 2005 examined the association between TGF-beta-1 gene polymorphisms and the susceptibility to autoimmune thyroid diseases, including Hashimoto's thyroiditis, in Korean population. The researchers identified that the T869C polymorphism in the TGF-beta-1 gene was associated with an increased risk of certain autoimmune thyroid diseases, including HT (Ban & Tomer, 2005). In 2014, a study conducted by Wang et al. explored the association between gene of TGF-beta-1 polymorphisms and the danger of developing HT in a Chinese Han population. Researchers found that the T869C polymorphism was notably associated with increasing susceptibility to Hashimoto's thyroiditis, particularly in females (Du *et al.*, 2014). As well as a study by Ding, Duan et al. published in 2017 investigated the association between TGF-beta-1 gene polymorphisms and the development of autoimmune thyroid diseases, including Hashimoto's thyroiditis, in a Turkish population. The researchers identified that the C-509T polymorphism in the TGF-beta-1 gene was significantly associated with an increased risk of developing autoimmune thyroid diseases (Ding *et al.*, 2017).

These findings align with the previous study discussed, collectively providing additional evidence supporting the potential genetic associations between gene of TGF-beta-1 polymorphisms and the danger of developing Hashimoto's thyroiditis. However, it is crucial to acknowledge that further research is necessary to validate these findings and to gain a deeper understanding of the underlying mechanisms by which these genetic variants contribute to the development and progression of the disease.

Conclusion:

In conclusion, the present study

examined the association between TGF-beta-1 and Hashimoto's thyroiditis, focusing on the role of genetic variations within the TGF-beta-1 gene. The study identified specific genetic polymorphisms, namely rs1982073 and rs1800471, that were found to be more prevalent in individuals with HT compared to the control groups. These genetic variants may contribute to the development and progression of Hashimoto's thyroiditis by influencing immune responses, inflammation, or fibrotic processes associated with the disease. Moreover, the rs1982073 and rs1800471 SNPs had notable effects on (TPO-AB) and (TG-AB) antibodies, indicating their potential impact on the immune system. However, the precise mechanisms through which these genetic variations influence susceptibility and progression of Hashimoto's thyroiditis are not yet fully understood. Further research is necessary to elucidate the functional implications of these genetic associations and to better comprehend the specific roles of TGFβ1 gene polymorphisms in the pathogenesis of Hashimoto's thyroiditis.

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