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Genetic Association Between Single Nucleotide Polymorphisms of the *HLA-G* Gene and Papillary Thyroid Carcinoma in Iraqi Patients

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Abstract

This study was designed to investigate the relationship between HLA-G gene SNPs in exons 2 and 8 and papillary thyroid carcinoma (PTC). Blood samples from 100 patients, categorized into pre- and post-radioactive therapy groups, and 50 healthy subjects were analyzed via PCR and direct sequencing for allele and genotype frequencies. PTC was eight times more prevalent in women. Thyroid assessment hormones exhibited reduced T3 levels pre- and post-radioactive iodine therapy and increased T4 levels post-therapy (49.80 ng/ml vs. control 16.84 ng/ml). Notably, TSH levels significantly decreased before and after therapy in PTC patients versus controls (P = 0.005). HLA-G gene SNPs were linked to PTC risk: in exon 2, genotype AA posed risk (OR = 16.10 and 10.58; P = 0.001) pre- and post-therapy, while homozygous TT in exon 8 increased risk (OR = 7.30; P = 0.001; OR = 4.41; P = 0.018), and heterozygous CT was protective (OR = 0.27; P = 0.016) before therapy. These findings suggest homozygous SNPs contribute to the genetic risk factors for PTC in the Iraqi population.

Keywords: DNA Sequencing, Genotyping, HLA-G, Papillary thyroid carcinoma, Single Nucleotide Polymorphisms.

الارتباط الوراثي بين تعدد أشكال النيوكليوتيدات المفردة لجين *HLA-G* ومرضى سرطان الغدة الدرقية الحليمي في المرضى العراقيين

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الخلاصة

صممت هذه الدراسة للتحقيق في العلاقة بين SNPs الجين HLA-G في exons 2 و 8 وسرطان الغدة الدرقية الحليمي .تم تحليل عينات الدم من 100 مريض، مصنفة في مجموعات العلاج قبل وبعد الإشعاع ، و 50 شخصًا صحيًا عبر PCR والتسلسل المباشر لترددات الأليل والنمط الجيني. كانت PTC أكثر انتشارًا في النساء من الرجال بمعدل ثمان مرات. أظهر تقييم هرمونات الغدة الدرقية انخفاض مستويات T3 قبل وبعد العلاج باليود المشع، وزيادة مستويات T4 بعد العلاج (49.80 نانوغرام / مل مقابل التحكم 16.84 نانوغرام / مل). والجدير بالذكر أن مستويات TSH قلت بشكل ملحوظ قبل وبعد العلاج في مرضى سرطان الغدة الدرقية الحليمي مقابل عوامل السيطرة (P = 0.005). تم ربط تغايرات الاقاعدة الواحدة في الجين HLA-G بمخاطر PTC في exon 2 ، حيث اعتبر النمط الجيني AA عامل خطورة (OR = 16.10 and 10.58; P = 0.001) قبل وبعد العلاج, بينما تزيد TT المتماثلة الزيجوت في exon 8 من

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المخاطر (OR = 7.30; P = 0.001; OR = 4.41; P = 0.018). اما الطراز الوراثي CT غير المتماثل فقد اعتبر كعامل وقائي (OR = 0.27; P = 0.016) قبل العلاج. تشير هذه النتائج إلى أن SNPs المتماثلة الزيجوت تساهم كعوامل خطر وراثية لـ PTC في السكان العراقيين. باختصار، افترض هذا البحث أن هذه النيوكلوتايد في شكل متماثل الزيجوت الطافرة هي عوامل خطر وراثية محتملة لتطور سرطان الغدة الدرقية الحليمي في السكان العراقيين.

1. Introduction

Papillary thyroid carcinoma (PTC) is the most prevalent kind of well-differentiated thyroid cancer, manifesting as an irregular solid or cystic mass or nodule in the normal thyroid parenchyma. Several risk factors enhance the likelihood of getting PTC, including radiation exposure and certain hereditary disorders [1]. PTC pathogenesis is a multi-step process that includes mutagenesis, epigenetics, and genetic variation [2]. In the Iraqi population, thyroid cancer is one of the most common cancer types after breast cancer, lung cancer, colorectal cancer, and leukemia. The annual incidence of thyroid cancer is 11.93 per 100,000 people, with 1660 new cases (4.9%) for both sexes (Ministry of Health, Iraqi Cancer Board, 2020). The HLA-G gene is found on chromosome 6 in region 6p21.3, in the major histocompatibility complex (MHC) class I gene cluster. Alternative RNA splicing can generate seven isoforms, including four membrane-bound isoforms (HLA-G1, G2, G3, and G4) and three secreted and soluble isoforms (HLA-G5, G6, and G7) [3–5]. Natural killer (NK) cells, T lymphocytes, and antigen-presenting cells (APCs) are inhibited by HLA-G. Therefore, it is tempting to believe that HLA-G expression promotes tumor growth by allowing tumor cells to evade detection by the host immune system. It has been reported that HLA-G gene polymorphisms affect expression, mRNA stability, and the concentration of soluble HLA-G [6]. Radioactive iodine (RAI) is utilized in the treatment of hyperthyroidism and certain types of thyroid cancer. The thyroid gland absorbs almost all of the iodine in the body. Consequently, radioactive iodine (RAI, also known as I-131) can be used to treat thyroid cancer. The RAI primarily accumulates in thyroid cells, where it can harm the thyroid gland and any other thyroid cells (including cancer cells) that absorb iodine while having minimal effect on the rest of the body. In this investigation, the effect of RAI on Iraqi patients with papillary thyroid carcinoma is investigated [7].

2. Material and methods

2.1 Samples collection

This study included 100 patients with papillary thyroid cancer (PTC) who consulted the Nuclear Medicine Hospital in Baghdad, Iraq, between February 2021 and August 2021. The patients were divided into two groups: 50 patients with pre-radioactive iodine therapy and 50 patients with post-radioactive iodine therapy (RAI). The investigation excluded patients with anatomical, endocrine, or metabolic disorders, as well as immunodeficiency and autoimmune diseases. The medical ethics committee of the University of Baghdad, College of Science, approved the investigation under the reference number CSEC/1022/0132. The control group consisted of 50 unrelated Iraqis without any diseases. All participants gave informed consent and agreed to donate blood samples for this case-control study.

2.2 Genotyping of HLA-G SNPs

Using the ReliaPrep^{TM} Blood gDNA Miniprep System (Promega, USA), genomic DNA was extracted from whole blood, added to EDTA containers, and then the purity and concentration were determined. The exons 2 and 8 of *the HLA-G* gene were amplified by polymerase chain reaction (PCR). The primers for exon 2 were the forward sequence (5'-TCCATGAGGTATTTCAGCGC-3') and reverse sequence (5'-CTGGGCCGGAGTTACTCACT-3'), while the primers for exon 8 were the forward sequence (5'-GTGATGGGCTGTTTAAAGTGTCACC-3) and the reverse sequence (5'-

GGAAGGAATGCAGTTCAGCATGA-3); these primers were designed in this study. The total PCR reaction was 30µl, which consisted of 15 µl Go Taq® Green Master Mix, 0.5 µl each forward and reverse primer (10 mM), 2 µl DNA (100 ng), and 12 µl nuclease-free distilled water. PCR conditions were programmed for exon 2 as follows: one cycle of initial denaturation at 95 °C for 5 minutes, 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 60 °C for 30 seconds, and extension at 72 °C for 30 seconds, followed by one cycle of a final extension step at 72 °C for 10 minutes. While PCR was programmed for exon 8 as follows: an initial denaturation step at 94 °C for 5 minutes, followed by a final expansion of 35 cycles of denaturation at 94 °C for 20 s, annealing at 64 °C for 30 s, and extension at 72 °C for 60 s, followed by one cycle of a final extension step at 72 °C for 10 minutes. The amplified PCR products (281 bp for exon 2 and 210/224 bp for exon 8) were electrophoresed on 2% and 2.5%, respectively, of agarose gels to confirm amplification. The PCR results were then submitted for Sanger sequencing on a Macrogen Corporation (South Korea) ABI3730XL digital DNA sequencer. Geneious software was used to identify genotypes and alleles following alignment with an NCBI reference sequence.

2.3 Statistical analysis

The statistical analysis SPSS Statistics 26 (from IBM) was used to detect the effect of different factors on study parameters. A one-way ANOVA and t-test were used to significantly compare the means. The Chi-square test was performed to compare percentages with 0.05 and 0.01 probability. In this study, the odds ratio and confidence interval are estimated. The WINPEPI program was used to detect the odd ratio.

3. Results and Discussion

In this study, there were no significant differences ($P > 0.05$) between the patient and control groups in terms of gender distribution or mean age. The results showed that the mean ± SD age for males in pre-, post-therapy, and control groups was 39.67±8.165, 36.75±16.317, and 38.18±14.806, respectively, while the mean ±SD age for females in pre-, post-therapy, and control groups was 36.79±8.115, 36.80±9.870, and 42.85±14.260, respectively (Table.1). The results in Table 1 revealed that the number of women with PTC was almost eight times that of men. Women had a nine-fold increased incidence of papillary thyroid cancer (PTC) compared to men. This is due to the interaction of female hormone effects, genetic factors, and chromosomal X abnormalities [8, 9]. Thyroid diseases were found to be more common in women than men, according to several studies. Skewed X chromosome inactivation (XCI), in which a female's X chromosome is inactivated for unknown reasons, has been proposed as a possible explanation [10].

Table 1: Prevalence of PTC according to gender and age

Age groups	Pre therapy group (No. =50)		Post-therapy group (No.=50)		Control group (No.=50)	
	Male	Female	Male	Female	Male	Female
20>-29	1 (2.2%)	8 (17.7%)	1 (2.2%)	9 (20%)	6 (12.5%)	6 (12.5%)
30-39	2 (4.4%)	13 (28.88%)	2 (4.4%)	16 (35.55%)	5 (26%)	7 (14.5%)
40-49	3 (6.66%)	17 (37.77%)	0	13 (28.88%)	4 (8.3%)	3 (6.25%)
50-59	0	1 (2.2%)	1 (2.2%)	2 (4.4%)	7 (14.5%)	6 (12.5%)
60-69	0	0	0	1 (2.2%)	0	4 (8.3%)
mean± SD	39.67±8.165	36.79±8.115	36.75±16.317	36.80±9.870	38.18±14.806	42.85±14.260
Std. Error	3.333	1.299	8.159	1.541	3.157	2.797
Maximum	49	52	59	61	57	75
Minimum	27	22	20	21	16	26
p-value	0.4 N. S		0.9 N. S		0.2 N. S	
*** (P≤0.05), ** (P≤0.01), NS: Non-Significant**						

For the ABO system, the PTC group had a higher prevalence of blood type O, which was statistically significant with a *P*-value of 0.0001, as shown in Table 2. There were no publications that linked the blood group to PTC or any other histological kind of thyroid cancer. On the other hand, some researchers found a correlation between blood group type and several types of malignancy. Su *et al*. (2001) [11] discovered that males with blood type B are more likely to develop esophageal cancer. Blood type A is associated with an increased risk of cancer in multiple sites, including gastric cancer (particularly in individuals with a documented family history), laryngeal and hypopharyngeal malignancy, pancreatic cancer, breast cancer, colorectal cancer, and rectal carcinoma [12–17]. According to [18], the type A blood group also increases the risk of other malignancies, in addition to cervical and ovarian cancer. More research is needed to prove that there is a link between PTC and blood groups and to figure out how ABO blood type or closely related genetic variations may affect PTC cancer risk.

Table 2: Relationship between the ABO blood groups and papillary thyroid cancer (PTC)

Group	Total No	A No. (%)	B No. (%)	AB No. (%)	O No. (%)	P-value
Patients	100	12 (8.89%)	14 (13.33%)	8 (6.67%)	66 (71.11%)	0.0001 **
Control	50	13 (25.00%)	10 (18.75%)	7 (14.58%)	20 (41.68%)	0.0427 *
P-value	---	0.371 NS	0.512 NS	0.781 NS	0.0001**	---
*** (P≤0.05), ** (P≤0.01) , NS: Non-Significant.**						

In this research, thyroid assessment hormones (T3, T4, and TSH) demonstrated a substantial difference between patients before and after RAI therapy for PTC patients and controls. Table 3 shows that patients before and after treatment with RAI had a significant decrease ($p = 0.030$) in the mean ± SE of T3 (1.829±0.34 and 1.318 ± 0.12 ng/ml, respectively) in comparison with control (2.184±0.16 ng/ml) and a decrease in the mean ± SE of T4 before therapy (13.32 ± 2.58) ng/ml, while there was a significant increase ($p = 0.0001$) in the mean ± SE after therapy with RAI (49.80 ± 9.19) ng/ml when compared with control (16.84 ± 0.40) ng/ml. Regarding TSH levels, the results found that there was a significant decrease ($p = 0.005$) of TSH before and after therapy (14.72 ± 3.36 and 9.72 ± 3.21) ulU/ml, respectively, in comparison with controls (2.50 ± 0.15) ulU/ml .On the outer layer of thyroid follicular cells, radioiodine is transferred and concentrated via sodium-iodide transport. The sodium-iodide symporter (NIS) is present in numerous iodine-collecting tissues, including the stomach, salivary glands, lactating breasts, thymus, nasal mucous membrane, lacrimal glands, and placenta [19]. Poorly differentiated or undifferentiated thyroid carcinomas are unable to concentrate iodine, exhibit thyroid stimulating hormone (TSH) receptors, or produce thyroglobulin (Tg). Due to the absence of iodine in medullary cancer, lymphoma, and anaplastic cancer, RAI is contraindicated for these malignancies [20].

Table 3: A comparison study between patients and controls in thyroid function hormones

Groups	T3 ng/ml (mean ±SE) NO.=50	T4 ng/ml (mean ±SE) NO.=50	TSH ulU/ml (mean ±SE) NO.=50
Pre-therapy	1.829±0.34	13.32±2.58	14.72±3.36
Post- therapy	1.318 ± 0.12	49.80±9.19	9.72±3.21
Control	2.184±0.16	16.84±0.40	2.50±0.15
p-value	0.030*	0.0001**	0.005**

* (P≤0.05), ** (P≤0.01), NS: Non-Significant. T3= thyroxin 3, T4== thyroxin 4, TSH= Thyroid Stimulating Hormone

This research studied the alleles and genotype frequencies of HLA-G gene SNPs in exons 2 and 8 (rs1130355 and rs567747015, respectively) among Iraqi patients with PTC and healthy controls. Before sequencing, agarose gel electrophoresis was used to identify the amplified HLA-G gene regions from the PCR results. PCR products for the specific amplified regions were sequenced to determine genotype allele frequencies for the expected SNPs pre- and post-therapy to illustrate the nucleotide sequence of the specific regions in patients with papillary thyroid carcinoma (PTC) and healthy controls (Figures 1 and 2).

Figure 1: The PCR products for exon 2 have a molecular size of 281 bp. M stands for marker; lanes 1–5 represent control samples, while lanes 6–18 represent patient samples

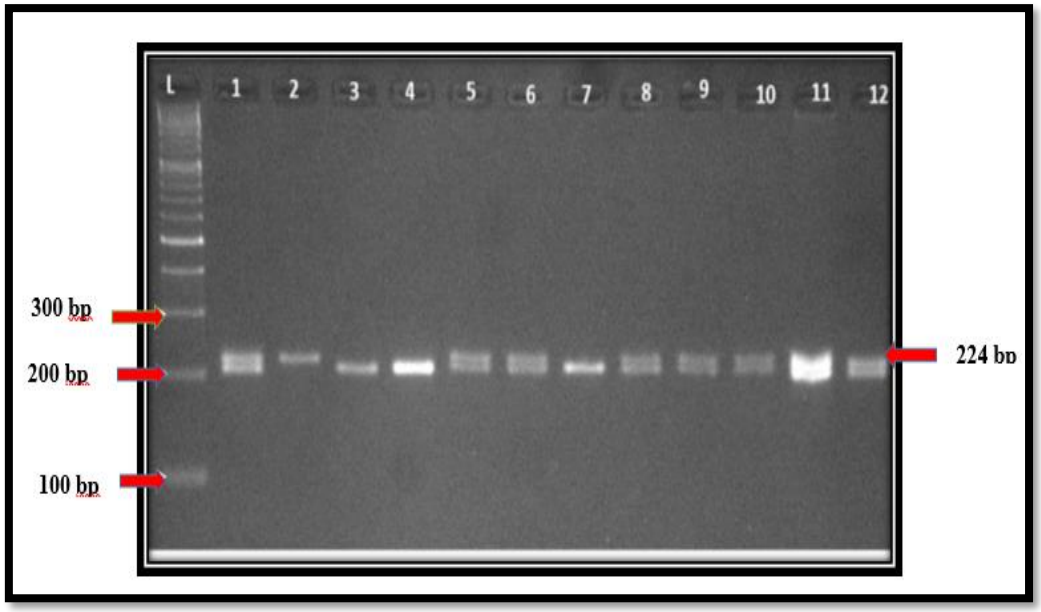

Figure 2: The PCR products for exon 8 have a molecular size of 224-bp band. M: marker; lanes 1–5: control samples; lanes 6–12: patient samples

SNPs (rs1130355, G>A, in exon 2) have been detected in three genotypes, GG, GA, and AA, and (rs567747015, C>T, in exon 8) with three genotypes, CC, CT, and TT, have been identified in both patients and controls. For exon 2, more mutant homozygotes have been

discovered in pre- and post-therapy patients than expected in the rs1130355 SNP. This shows that the (rs1130355) SNP in exon 2 of the HLA-G gene may have some importance for the development of PTC. There are many increased homozygotes of mutant type (AA) of the (rs1130355) SNP in PTC compared with healthy subjects, which showed low homozygotes of mutant type (AA) of this SNP. The distributions of genotype frequencies and alleles of the (rs1130355) SNP are reported in Table 4. The results revealed that there were greatly significant differences between genotypes AA in PTC of pre- and post-therapy patients in comparison with control groups (P = 0.001 for each), while there were no significant differences among patients in pre- and post-therapy and controls in terms of heterozygotes mutant (P = 0.372 and 0.330, respectively). The frequency of AA genotype in PTC of pre-, post-, and control patients was 70%, 46%, and 10%, respectively, and the odd ratio was 16.10; 95% CI = 4.33-65.69 and 10.58; 95% CI = 3.09-37.68, respectively. The significance of such a relationship was assessed by Fisher's exact possibility. Such an assessment is preferred because it allows for the correction of possibilities and is not affected by small numbers (less than 5). It seems that the disease can occur when the patients have homozygote mutant AA in pre- and post-therapy (the odd ratio of AA was 16.10 and 10.58, respectively). The SNP's A allele was detected in pre- and post-therapy (150% and 126%, respectively) of PTC patients and (64% of healthy controls), with significant differences in frequency among groups under study (OR = 6.38; 95% CI = 3.29–12.41, p = 0.001 and 3.44; 95% CI = 1.98–5.99, p = 0.001). The distributions of the (rs1130355) SNP of exon 2 for the HLA-G gene did not depart substantially from HWE (P > 0.05), as shown in Table 4. In exon 8, the heterozygote mutant (CT) of SNP (rs567747015) showed significant differences in pre-therapy patients with PTC (P = 0.016), while there was no significant difference for this genotype after therapy with RIT (P = 0.052). More mutant homozygotes (TT) have been discovered in pre- and post-therapy patients than expected in the rs567747015 SNP, while more mutant heterozygotes (CT) were found in control groups. This shows that the (rs567747015) SNP in exon 8 of the HLA-G gene may have some importance for the development of PTC. There are many increased homozygotes of mutant type (TT) of the (rs567747015) SNP in PTC compared with healthy subjects, which showed low homozygotes of mutant type (TT) of this SNP. The distributions of genotype and allele frequencies of the (rs567747015) SNP are demonstrated in Table 4. The results showed that there were highly significant differences between genotype TT in PTC in pre- and post-therapy patients in comparison with control groups (p = 0.001 and p = 0.018, respectively). The frequency of TT genotype in PTC in pre-, post-, and control patients was 48%, 34%, and 8%, respectively, and the odd ratio was 7.30 (95% CI = 2.04–32.34) and 4.41 (95% CI = 1.20–19.97), respectively. Fisher's exact possibility was used to determine the significance of the relationship. This method is favored because it allows for the correction of possibilities and is unaffected by small numbers (less than five). It appears that the disease can occur in patients with homozygous mutant TT before and after therapy (the odds ratio of TT was 7.30 and 4.41, respectively). The SNP's T allele was detected in pre- and post-therapy (102% and 80%, respectively) of PTC patients and (52% of healthy controls), with significant differences in frequency among groups under study (OR = 2.96; 95% CI = 1.57–5.62, p = 0.001 and 1.90; 95% CI = 1.00–3.62, p = 0.050), respectively. As shown in Table 4, the distributions of the (rs567747015) SNP in exon 8 of the HLA-G gene did not deviate significantly from HWE (P > 0.05).

Table 4: Observed and expected numbers and percentage frequencies of *HLA-g* gene polymorphisms (rs1130355) and (rs567747015) genotypes and their HWE in patients and controls

***HLA-G* SNPs Genotype and allele frequency**	**Pre-RIT patients N=50 (%)**	**p-value OR(95%CI)**	**Post-RIT patients N=50 (%)**	**p-value OR(95%CI)**	**Controls N=50(%)**
***Exon 2* rs1130355 genotypes frequency**					
GG	10(20)	Reference	10(20)	Reference	23(46)
GA	5(10)	0.372 0.52(0.12-2.03)	17(34)	0.330 1.75(0.68 - 4.53)	22(44)
AA	35(70)	0.001** 16.10(4.33-65.69)	23(46)	0.001** 10.58(3.09 - 37.68)	5(10)
HWE *p*-value	0	-	0.055	-	0.937
***Exon 2* rs1130355 allele frequency**					
G	25(50)	Reference	37(74)	Reference	68(136)
A	75(150)	0.001** 6.38(3.29 - 12.41)	63(126)	0.001** 3.44(1.98 -5.99)	32(64)
***Exon 8* rs567747015 genotype frequency**					
CC	23(46)	Reference	27(54)	Reference	28(56)
CT	3(6)	0.016** 0.27(0.09 - 0.77)	6(12)	0.052 0.35(0.10 - 1.10)	18(36)
TT	24(48)	0.001** 7.30(2.04 - 32.34)	17(34)	0.018** 4.41(1.20- 19.97)	4(8)
HWE *p*-value	0	-	0	-	0.937
***Exon 8* rs567747015 allele frequency**					
C	49(98)	Reference	60(120)	Reference	74(148)
T	51(102)	0.001** 2.96(1.57 - 5.62)	40(80)	0.050* 1.90(1.00 - 3.62)	26(52)

*** (P≤0.05), ** (P≤0.01), NS: Non-Significant.**

No similar studies were found about these SNPs under study and their relationship with papillary thyroid carcinoma. These findings are consistent with those of [21], who discovered a similar correlation and reported that this SNP plays a significant role in the development of rheumatoid arthritis (RA). The researchers [22, 23] found that allelic variants of this SNP were responsible for recurrent pregnancy loss. Allele T in the SNP (rs567747015) was significantly associated with the illness (P = 0.02) and may be reported as a risk factor with an OR of 2.91 (Table 4). This SNP was discussed by [24], who found a link between *HLA-G* 3'UTR polymorphism and cervical cancer susceptibility. Also, [25] discovered a similar correlation and concluded that this SNP plays an essential role in chronic renal disease and allograft acceptance. Some researchers found that allelic variants of this SNP, namely allele G, were the cause of AIDS and CMV retinochoroiditis [26]. In 2015, researchers discovered that

HLA-G allele G has a substantial connection with a higher risk of breast cancer [27]. Due to the impact of this gene on immune system suppression and self-tolerance, these SNPs can be considered active immune regulation factors. Autoimmunity can result from a tolerance breakdown [28]. Our findings correspond with those of [29], who established the relevance of *HLA-G* polymorphisms in the development of thyroid cancer in Brazilian populations. Logistic regression analysis of the SNP (rs1130355) of exon 2 showed that the AA genotype was significantly linked to the risk of PTC in patients (both pre- and post-RIT) when compared to controls (Table 5). The genotype (GA+AA) under dominant models exhibited the same significant differences in pre-RIT and post-RIT patients in comparison to controls ($p \leq 0.01$). While the genotype GA in the codominant model demonstrated no significant differences in pre-RIT and post-RIT patients when compared to controls, p values were equal (0.372 and 0.330, respectively). Regarding the SNP (rs567747015) in exon 8, the genotype (TT) showed a significant association with PTC incidence before and after therapy (pre-RIT and post-RIT) in codominant and recessive models when compared with control groups. The genotype (CT+TT) under dominant models exhibited no significant differences in pre-RIT and post-RIT patients in comparison to controls ($p > 0.05$). While the genotype CT in the codominant model demonstrated a significant difference in pre-RIT but not post-RIT patients when compared to controls, the p-values were equal (0.016 and 0.052), respectively (Table 5). No previous study demonstrated any correlation between these SNPs in exons 2 and 8 of the *HLA-G* gene and PTC in patients before and after therapy.

In 2022, [30] will describe how the polymorphisms of HLA-G (such as rs567747015) can affect the plasma level of peripheral sHLA-G and play a key role in cervical carcinogenesis. The results of [31] found that the HLA-G genotype (rs1130355) was associated with an increased risk of miscarriage in a South Indian population. As these SNPs are close to the exon-intron boundary, they may play a role in splicing regulation. Also, [32] proved that rs1130355 and other genetic variants of HLA-G play important roles in reducing the dangers of COVID-19 disease through public health recommendations and individualized treatment. Numerous studies have proven the role of variability at the 3′ untranslated region of the HLA-G gene in many diseases such as recurrent pregnancy loss, AID, cytomegalovirus retinochoroiditis, and asthma [33–35].

Table 5: Logistic regression of *HLA-G* gene SNPs (rs1130355) and (rs567747015) of exon2 and exon8 in PTC patients compared to controls

SNPs of *HLA-G* gene	Models	Genotypes	Pre-RIT patients N= 50 (%)	*p*-value OR (95%CI)	Post-RIT patients N= 50 (%)	*p*-value OR (95%CI)	Control N=50(%)
Exon2 rs1130355	Codominant	GG	10(20)	Reference	10(20)	Reference	23(46)
		GA	5(10)	0.372 0.52(0.12-2.03)	17(34)	0.330 1.75(0.68 - 4.53)	22(44)
		AA	35(70)	0.001 16.10(4.33-65.69)	23(46)	0.001 10.58(3.09 - 37.68)	5(10)
	Dominant	GG	10(20)	Reference	10(20)	Reference	23(46)
		GA+AA	40(80)	0.010 3.41(1.30 - 9.28)	40(80)	0.010 3.41(1.30 - 9.28)	27(54)
	Recessive	GG+GA	15(30)	Reference	27(54)	Reference	45(90)
			35(70)	0.001	23(46)	0.001	

		AA		21.00(6.38 - 78.37)		7.67(2.42- 28.31)	5(10)
Exon8 rs567747015	**Codominant**	**CC**	23(46)	Reference	27(54)	Reference	28(56)
		CT	3(6)	0.016 0.27(0.09 - 0.77)	6(12)	0.052 0.35(0.10 - 1.10)	18(36)
		TT	24(48)	0.001 7.30(2.04 - 32.34)	17(34)	0.018 4.41(1.20- 19.97)	4(8)
	Dominant	**CC**	23(46)	Reference	27(54)	Reference	28(56)
		CT+TT	27(54)	0.424 1.49(0.63- 3.54)	23(46)	1.000 1.08(0.49 -2.37)	22(44)
	Recessive	**CC+CT**	26(52)	Reference	33(66)	Reference	46(92)
		TT	24(48)	0.001 10.62(3.10 - 45.58)	17(34)	0.003 4.72(1.81- 12.29)	4(8)

4. Conclusion

In conclusion, the single nucleotide variant rs1130355 of exon 2 is linked to the development of papillary thyroid carcinoma (PTC), and genotype AA is a risk factor. On the other hand, SNP rs567747015 of exon 8 was strongly linked to the disease when homozygous genotype TT was considered a risk factor for PTC and heterozygous genotype CT was thought to protect against the disease. In short, the idea behind this research was that these SNPs in mutant homozygotes could be genetic risk factors for papillary thyroid cancer in Iraqi people.

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Author's declaration

The local ethics council at the University of Baghdad approved this study.

Conflict of Interest

The authors report having no conflicts of interest.

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