# Genotyping polymorphism of PD-1 and PDL-1 in papillary thyroid carcinoma of Iraqi patients

Ali Shafeeq Neamah[1], Asaad AL Janabi[2]
[1]Al-Mustaqbal University College, Department of Dentistry/Iraq
[2]Faculty of Medicine, University of Kufa, Iraq
Email: ali.shafek.nama@uomus.edu.iq

**Abstract**
Papillary thyroid carcinoma (PTC) is the most common form of well-differentiated thyroid cancer, and the most common form of thyroid cancer to result from radiation exposure. Papillary carcinoma appears as an irregular solid or cystic mass or nodule in normal thyroid parenchyma. As the understanding of thyroid tumor formation advanced, the focus of genetic alterations shifted from single genetic mutations to molecular polymorphism and panels of multiple mutations. Aims of the study: To determine if pdl-1 and PD-1 polymorphism are frequent events and play a crucial role in papillary thyroid tumorigenesis and to correlate PDL-1 and PD-1 with other prognostic parameters such as age, sex, stage, and grade of the tumor.Results: The current study of PDL1 and PD-1 polymorphism n papillary thyroid carcinoma. regarding PD1 genotyping, in the assessment of rs11568821 SNPs, GG dominant genotype is present in about 52 % of cases, while the CC genotype is present in about 40.40 % in the assessment of rs4143815G>C SNP in the PDL-1 gene. Conclusion: We can conclude from the current study that PDL1 polymorphism that the molecular mutant genotyping CC is most dominant than other GG, GC, or deletion mutations. On the other hand, Regarding the PD-1 gene, Genotyping GG is more predominant than other GA, AA, Or deletion mutation.
Keywords: Papillary Thyroid Carcinoma, PDL-1, CD274, PD-1, Polymorphism.

## 1. Introduction

Approximately 80% of thyroid cancer cases are papillary thyroid carcinomas [1]. Additionally, it is the most common subtype of thyroid cancer in nations with diets high in iodine or sufficient in iodine [2]. PTC is becoming more prevalent [3]. Uncertainty over the causes could be due to advancements in cancer detection technology [4]. PTC has infrequently been identified as a congenital tumor and can happen at any age [5]. With a mean age of 40 years, it is typically discovered in individuals between their third and fifth decades of life. Women are affected by PTC more frequently than men in ratios of 2:1 to 4:1, and the incidence of the condition rises with age [6]. There is a lot of curiosity in PTC etiology. A prior history of radiation exposure is the only prominent or well-established environmental risk associated with the advancement of PTC [7]. The atomic bombings of Hiroshima and Nagasaki at the close of World War II in 1945 and the Chornobyl nuclear power plant explosion in 1986 both confirmed the radiation's ability to cause cancer [8]. Other potential risk factors are having a family history of PTC or having an underlying benign thyroid condition [9].

Significant molecular genetic modification investigations carried out over the past two decades have improved our understanding of PTC progression. PTC is frequently characterized by point mutations in the RAS or BRAF proto-oncogenes, RET chromosomal rearrangements, or both, all of which can cause the activation of the mitogen-activated protein kinase (MAPK) cascade. Nearly 70% of PTC cases had mutations in the BRAF, RAS, or RET genes [10]. Following the alterations, additional genetic occurrences could produce a wide variety of PTC variations [11]. The various histopathologic characteristics can be used to distinguish these variants. The classical, follicular, and tall cell varieties are the most prevalent. Tall cell and columnar cell forms of PTC are physiologically the most aggressive. [12].

As the understanding of thyroid tumor formation advanced, the focus of genetic alterations shifted from single genetic mutations to molecular signatures and panels of multiple mutations. This shift in approach happened because investigators realized that the progression from a normal cell to a tumor is a complex process that likely involves multiple genetic events, making identification of a single mutation responsible for sporadic thyroid cancer unlikely. Instead, a spectrum of many different mutations might be responsible for sporadic thyroid cancer (13).

## 2. Methodology

This study was designed as a cross-sectional one; cases were collected from October 2020 to October 2021 at AL-Sadder teaching hospital and some private laboratories.

It involved 52 cases of papillary thyroid carcinoma that underwent total surgical thyroidectomy and six paraffin blocks of normal placental tissues as controls. Their clinical data regarding the tumor's age, sex, site, and other pathological parameters for each patient were obtained from pathological reports. Their ages ranged between 18 and 60 years.

All cases were submitted for resectioning and staining with H&E for reexamination and assurance

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of histopathological diagnosis. Then a molecular study was applied to all cases.

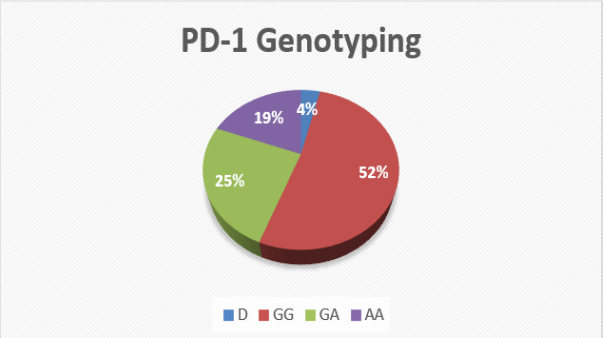
The sequences of primers sets used in this study are mentioned in table (1)

| Table (1): the primers Sequences for target gene amplification | |
|---|---|
| Primer and rs | sequences |
| PD-L1 rs4143815 | FO: CTGTGACAGGGAGAAAGGATACTTCTG RO: AGCAAGTTTAGTTTGGCGACAAAATTGT FI: TTTGCCTCCACTCAATGCCTCAATATC RI: AACACTGAGACTCTCAGTCATGCAGAATAC |
| PD-1rs11568821 | F: CTCACATTCTATTATAGCCAGGACC R: TAAGATAAGAAATGACCAAGCCCAC |

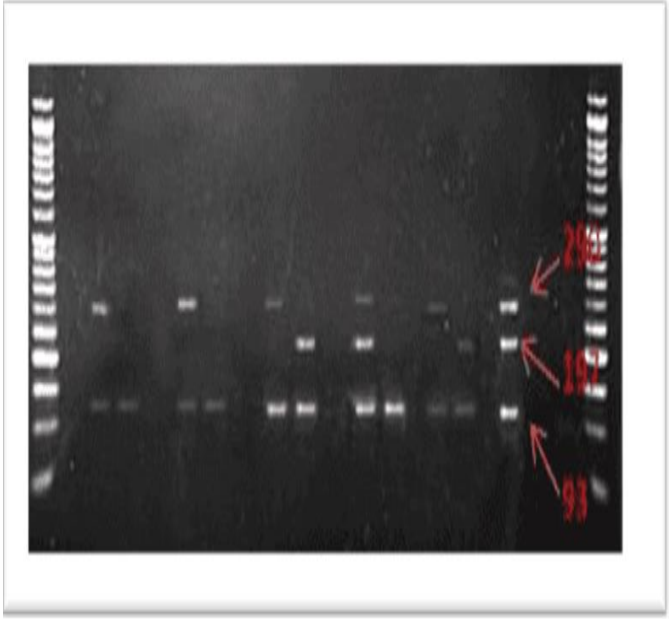
## 3. Results

### PD-1 and PDL-1 rs4143815 Genotyping

PCR-RFLP implemented the genotyping of PD-1 rs11568821 to generate 290 bp, 197, and 93 bp. The results show that the GG was more frequent in (27) cases 51.9%, GA was observed in (13) cases 25.0 %, AA was observed in (10) cases 19.2%and deletion mutation (did not amplification target sequence) was observed in (2) study cases 3.8%. (Figure 1) (Figure 2)




*(Figure 1): The PD-1 genotyping distribution in study cases.*




*(Figure 2): The PD-1 genotyping in study cases, homozygote GG (290bp), heterozygote GA (197+93bp), homozygote AA (93bp) genotypes and deletion mutation, 1% agarose, 70V, 20mA, 0.5 X TBE buffer for 45min with ethidium bromide staining. DNA ladder (50-2000 bp).*

| Table (2 ) PD-1 genotyping vs clinicopathological parameters : | | | | | | |
|---|---|---|---|---|---|---|
| Characteristic of patients | | PD1_Genotyping | | | | P value |
| | | D n (%) | GA n (%) | GG n (%) | AA n(%) | |
| Age | <30 | 0 ( 0.0 ) | 4 (23.5) | 11 (64.7) | 2 ( 11.8 ) | 0.21 |
| | 30-45 | 2 (8.0) | 8 (32.0) | 11 ( 40.0 ) | 4 (16.0) | |
| | >45 | 0 ( 0.0 ) | 1 (10.0) | 5 ( 50.0 ) | 4 ( 40.0 ) | |
| Gender | Female | 2 (4.7) | 12 (27.9) | 22 (51.2) | 7 (16.3) | 0.49 |
| | Male | 0 (0.0) | 1(11.1) | 5 (55.6) | 3 ( 33.3 ) | |
| pN_stage | N0 | 2 ( 18.2 ) | 9 ( 81.8 ) | 0 ( 0.0 ) | 0 ( 0.0 ) | 0.0001 |
| | N1a | 0 ( 0.0 ) | 4 ( 25.0 ) | 12 ( 75.0 ) | 0 ( 0.0 ) | |
| | N2b | 0 ( 0.0 ) | 0 ( 0.0 ) | 15 ( 71.4 ) | 6 ( 28.6 ) | |
| | Nx | 0 ( 0.0 ) | 0 ( 0.0 ) | 0 ( 0.0 ) | 4 ( 100.0 ) | |
| Initial metastasis | no Initial metastasis | 2 ( 6.7 ) | 13 ( 43.3 ) | 15 ( 50.0 ) | 0 ( 0.0 ) | 0.0001 |
| | Initial metastasis | 0 ( 0.0 ) | 0 ( 0.0 ) | 12 ( 54.5 ) | 10 ( 45.5) | |
| Tumors size | less than 2 cm | 0 ( 0.0 ) | 0 ( 0.0 ) | 10 (50.0 ) | 10 (50.0 ) | 0.003 |
| | more than 2 cm | 2 (6.2) | 13 ( 40.6 ) | 17 ( 53.1 ) | 0 (0.0 ) | |
| Other diseases | Hashimoto thyroiditis | 0 ( 0.0 ) | 0 ( 0.0 ) | 7 ( 77.8 ) | 2 ( 22.2 ) | 0.37 |
| | nodular follicular hyperplasia | 2 ( 5.7 ) | 13 (37.1 ) | 20 (57.1 ) | 0 ( 0.0 ) | |
| | No disease | 0 ( 0.0 ) | 0 ( 0.0 ) | 0 ( 0.0 ) | 8 ( 100.0 ) | |
| Stage | 1 | 2 ( 33.3 ) | 4 ( 66.7 ) | 0 ( 0.0 ) | 0 ( 0.0 ) | 0.001 |
| | 2 | 0 ( 0.0 ) | 9 ( 29.0 ) | 22 ( 71.0 ) | 0 ( 0.0 ) | |
| | 3 | 0 ( 0.0 ) | 0 ( 0.0 ) | 5 ( 50.0 ) | 5 ( 50.0 ) | |
| | 4 | 0 ( 0.0 ) | 0 ( 0.0 ) | 0 ( 0.0 ) | 5 ( 100.0) | |
| immuno_scoring | Negative | 2 ( 8.3 ) | 13 ( 54.2 ) | 9 (37.5) | 0 ( 0.0 ) | 0.20 |
| | 1 | 0 ( 0.0 ) | 0 ( 0.0 ) | 12 ( 100.0 ) | 0 ( 0.0 ) | |
| | 2 | 0 ( 0.0 ) | 0 ( 0.0 ) | 6 ( 50.0 ) | 6 ( 50.0 ) | |
| | 3 | 0 ( 0.0 ) | 0 ( 0.0 ) | 0 ( 0.0 ) | 4 ( 100.0) | |

The PD-L1 rs4143815 polymorphism implemented by PCR-ARMS shows two alleles (G, C) and three genotyping, including homozygote GG, heterozygote GC, homozygote CC genotypes Allele G=176 bp, Allele C=203 bp, and Control bands = 322 bp and deletion mutation (D). (Figure 3)
The results show that the CC was more frequent in (21) cases at 40.4%, GG observed in (16) cases at 30.8%, GC observed in (12) at 23.1%, and deletion mutation (did not amplification target sequence) was (3) cases at 5.8% in study cases. (Fig 4).

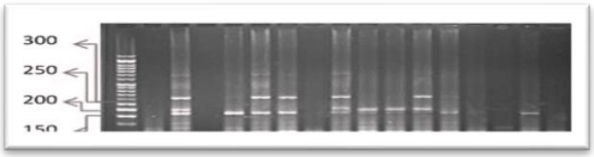



*Figure (3): the PD-L1 is genotyping in study cases, homozygote GG, heterozygote GC, homozygote CC genotypes and deletion mutation, 1% agarose, 70V, 20mA, 0.5TBE buffer for 45min with ethidium bromide staining. DNA ladder (50-2000 bp).*

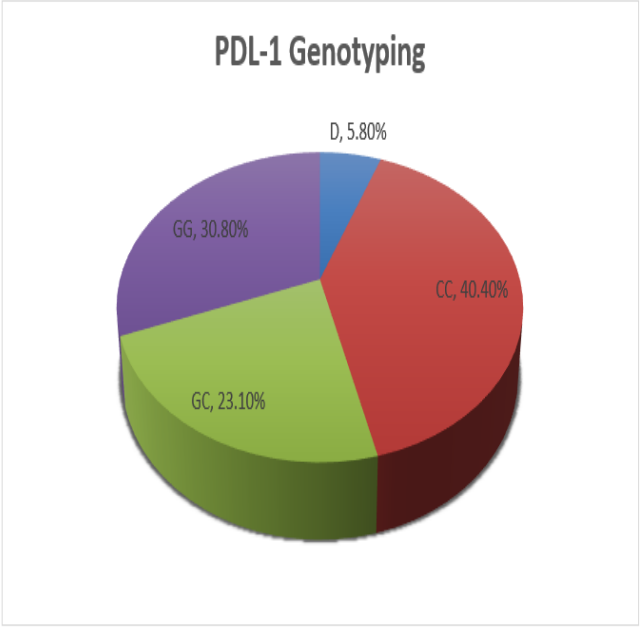



*Figure (4) The PD-L1 genotyping frequency in the study group.*

Table (3) PDL1 genotyping vs clinicopathological parameters:

| Characteristic of patients | | PDL1_Genotyping | | | | P value |
|---|---|---|---|---|---|---|
| | | D n(%) | CC n(%) | GC n(%) | GG n(%) | |
| Age | <30 | 1(5.9) | 7(41.2) | 5(29.4) | 4( 23.5 ) | 0.29 |
| | 30-45 | 2(8.0) | 10(40.0) | 7( 28.0 ) | 6(24.0) | |
| | >45 | 0 ( 0.0 ) | 4 (40.0) | 0 ( 0.0 ) | 6(60.0 ) | |
| Gender | Female | 2 (4.7) | 20(46.5) | 10( 23.3 ) | 11(25.6) | 0.05 |
| | Male | 1 (11.1) | 1(11.1) | 2 ( 22.2 ) | 5( 55.6) | |
| pN_stage | N0 | 3 ( 27.3 ) | 1(9.1 ) | 7( 63.6 ) | 0( 0.0 ) | 0.0001 |
| | N1a | 0 ( 0.0 ) | 16 ( 100.0 ) | 0 ( 0.0 ) | 0 ( 0.0 ) | |
| | N2b | 0 ( 0.0 ) | 4 ( 19.0 ) | 5 ( 23.8) | 12 ( 57.1 ) | |
| | Nx | 0 ( 0.0 ) | 0 ( 0.0 ) | 0 ( 0.0 ) | 4 ( 100.0 ) | |
| Initial metastasis | no Initial metastasis | 3 ( 10.0 ) | 20 ( 66.7 ) | 7 ( 23.3 ) | 0 ( 0.0 ) | 0.0001 |
| | Initial metastasis | 0 ( 0.0 ) | 1 ( 4.5 ) | 5 ( 22.7 ) | 16 ( 72.7) | |
| Tumors size | less than 2 cm | 0 ( 0.0 ) | 0 ( 0.0 ) | 4 (20.0 ) | 16 (80.0 ) | 0.003 |
| | more than 2 cm | 3 (9.4) | 21 ( 65.6 ) | 8 ( 25.0 ) | 0 (0.0 ) | |
| Other diseases | Hashimoto thyroiditis | 0 ( 0.0 ) | 0 ( 0.0 ) | 1 ( 11.1 ) | 8 ( 88.9 ) | 0.001 |
| | nodular follicular hyperplasia | 0 ( 0.0 ) | 0 ( 0.0 ) | 0 ( 0.0 ) | 8 ( 100.0 ) | |
| | No disease | 3 ( 8.6) | 21 ( 60.0 ) | 11( 31.4 ) | 0 ( 0.0 ) | |
| Stage | 1 | 3 ( 50.0 ) | 0 ( 0.0 ) | 3 ( 50.0 ) | 0 ( 0.0 ) | 0.001 |
| | 2 | 0 ( 0.0 ) | 21 ( 71.0 ) | 9 ( 29.0 ) | 1 ( 3.2 ) | |
| | 3 | 0 ( 0.0 ) | 0 ( 0.0 ) | 10 ( 100.0 ) | 0 ( 0.0 ) | |
| | 4 | 0 ( 0.0 ) | 0 ( 0.0 ) | 0 ( 0.0 ) | 5 ( 100.0) | |
| immuno_scoring | Negative | 3 ( 12.5 ) | 14 ( 58.3 ) | 7 ( 29.2 ) | 0 ( 0.0 ) | 0.001 |
| | 1 | 0 ( 0.0 ) | 7 ( 58.3 ) | 5 ( 41.7 ) | 0 ( 0.0 ) | |
| | 2 | 0 ( 0.0 ) | 0 ( 0.0 ) | 0 ( 0.0 ) | 12 ( 100.0 ) | |
| | 3 | 0 ( 0.0 ) | 0 ( 0.0 ) | 0 ( 0.0 ) | 4 ( 100.0 ) | |

## 4. Discussion

### 5.3 The PD-L1 rs4143815 and PD-1 rs11568821 polymorphism

The current study investigated the rs4143815 polymorphism in the PD-L1 gene by ARMS and PD-1 rs11568821 implemented by PCR-RFLP.
The goal of the present study is to evaluate the genetic variation of PD-1 and PD-L1 that may be a benefit in the therapeutic strategies; some studies showed that The copy number gains of PD-L1 are linked with substantial therapeutic activity in several cancer types according to the tumor PD-L1 high levels and elevation of immune infiltrates for enhancement (14) (15).
Regarding PD-1 rs11568821 polymorphism in PTC showed that the GG was more frequent in 27 cases (51.9%), AA observed in 10 cases (19.02%), GA in 13 cases (25%), and deletion mutation (did not amplification target sequence) was 2 cases (3.8%) in study cases. Figure (4.19)
There was a non-significant correlation regarding age, gender, immunohistochemical scoring, and presence or absence of other related diseases such as nodular follicular hyperplasia and Hashimoto thyroiditis.and there was a significant correlation regarding pN stage, tumor size, initial metastasis,

and staging of diseases as a general
On the other hand, for rs4143815 polymorphism in the PD-L1 gene, results show two alleles (G, C) and three genotyping, including homozygote GG and heterozygote GC. Homozygote CC genotypes, in addition to deletion mutation, their distribution in the patients was 21 cases (40.4) % for CC, 12 cases (2.1)% for GC, and 16 cases (30.8)% for GG. Deletion mutation was 3 cases (3.85) % as shown in figure (4). This result indicated that the CC was associated with papillary thyroid carcinoma (PTC). There was a non-significant correlation of pdl-1 genotyping with age and the presence or absence of other histological background diseases and a significant correlation with gender, and there was a highly significant correlation with pN stage, size of the tumor, metastasis, Immunohistochemical expression, and other related background diseases.

### 5.3.1 Association of PD-1 and PD-L1 with gender

In the current study, gender did not affect the PD-1 genotyping; all genotypes had non-significant differences between males and females, as shown in tables (2), But there was a significant correlation regarding pdl-1 genotyping (table 3).
The associations of gene polymorphism with gender have been described in several studies, Brcic et al. (2021) did not find a significant effect of gender in the PD-1and PD-L1 expression in the malignant pleural mesothelioma (16); the sites of both genes did not relate with sex or located on the sex chromosomes.

### 5.3.2 The Hashimoto thyroiditis and nodular follicular hyperplasia effect in the PD-1 and PDL1 genotyping:

The genotyping of PD-L1 was non-significant differences with Hashimoto thyroiditis and nodular follicular hyperplasia (Table 3); also, non-significant variation was observed in PD-1 genotyping (table 2), and the association of PD-L1 and PD-1 with Hashimoto thyroiditis and nodular follicular hyperplasia have been studied utilized gene expression and immune histochemistry, Álvarez-Sierra et al., (2019) found that the PD-L1, was expressed by thyroid follicular cells in areas that also contain abundant PD-1 positive T cells but co-localization in thyroid follicular cells indicated only partial overlap between the smaller areas of the PD-L1+ and the larger areas of HLA class II+ expression). (17)

### 5.3.3 The grade and score of disease effect in the PD-1 and PD-L1 genotyping

Significant differences were observed in CC and GC of PD-L1that were more frequent in grades I than II; deletion mutation was also observed in all grades I and II. And its highly significant effect on the PD-1 genotypes (table 2, 3 ). To our knowledge, there is no available published paper concerned with the correlation of PD-1 and PDL-1 genotyping to the staging of PTC to be compared with our results.
As a result of the immune checkpoints' importance in cancer cells by causing T-cell exhaustion and immune evasion facilitate, the examination of these checkpoint's role in aggressive thyroid cancers like Poorly differentiated thyroid carcinoma and harnessing the power of new immune regulatory drugs are necessary and essential steps forward in thyroid cancer management, the association of genotyping with disease grade was detected in the present study, the previous study suggested that the presence of PD-L1 expression in tumor cells may be associated with more aggressive tumor behavior, and its agreement with the study conducted by Chowdhury et al., (2016)(18). in papillary thyroid carcinoma, albeit using a different methodology and lower thresholds for calculating PD-L1 expression.

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