Immunohistochemical expression of PDL1 in papillary thyroid carcinoma and its correlation with clinicopathological parameters

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Abstract
Background: Programmed death-ligand 1 (PD-L1) expression has been reported in several malignancies, but the expression of PD-L1 in papillary thyroid cancer (PTC) has been characterized rarely. The aim of this study was to assess PD-L1 expression and its associations with clinicopathologic factors in PTC. Methods: Immunohistochemistry staining was conducted retrospectively to evaluate the expression of PD-L1 in a total of 52 PTC tumors. The correlations between PD-L1 expressions with clinicopathologic features were analyzed. Results: PD-L1 expression was positive in 59% (28/52) of PTC tumor tissues. In clinicopathologic analyses, this positive staining of PD-L1 was highly significantly linked to Pn Stage (p = 0.001), initial metastasis (p = 0.001), Tumour size (p = 0.003) and TNM staging (p = 0.001), significantly correlated with age (p = 0.03), and gender (p = 0.05), and non-significant correlation(p = 0.269) regarding presence or absence of other related background diseases. Conclusions: PD-L1 is important in determining the aggressiveness of PTC and could predict the prognosis of patients. Therefore, inhibition of PD-L1 is suggested as a potential strategy for the treatment of advanced PTC with high expression of PD-L1.
Keywords: biological marker; papillary thyroid cancer; programmed death-ligand 1; survival analysis.

1. Introduction

The yearly incidence of thyroid cancer in the United States is 14.42 per 100,000 persons, according to statistics from 2013. There has been an increase in both the incidence of thyroid cancer and the mortality rate associated with thyroid cancer over time. Thyroid cancer is the fourth most prevalent type of cancer in American women (1). Papillary, follicular, and anaplastic malignancies are formed from thyroid follicular epithelial cells. Other thyroid cancers spread to the thyroid gland from different kinds of tumors. Papillary TC (PTC), which makes up around 80% of TC, is the most prevalent (2). Patients with PTC or follicular TC (FTC), two different forms of TC, can frequently be successfully treated. The tumor is more aggressive and likely to spread earlier in people with medullary or anaplastic TC than in those with other kinds of TC (3). Immune control point regulators are essential for reducing collateral tissue damage in chronic infections and preventing autoimmunity. They also aid in controlling how the immune system reacts. Antibodies can easily block immunological control points since most of them are started by ligand-receptor interactions. You can buy some recombinant receptor and ligand forms (4).
The CTLA-4 co-inhibitory receptor was the first immune checkpoint regulator to be identified (5,6). The investigation into the relationship between the PD-1 receptor and PD-L1 received the greatest interest. A cell-surface glycoprotein called PD-L1 sometimes called CD274 is expressed in various organs, including tumors. Cytotoxic T-lymphocytes (CTLs) that express PD-L1 can become inactive through the inhibitory receptor and PD-1 (7).
The expression of the PD-L1 immune escape system increased in response to cancer cells. The discovery of the interaction between PD-1 and PD-L1 led to the development of immunotherapy as a novel, inspirational treatment for cancer resistance. Depending on the individual, it may be promising for aggressive TC and ATC patients to use immunotherapy (8).
Recent research has examined the use of immune checkpoint inhibitors in severe tumor cell (TC) disease (9,10). PTC patients have lower levels of programmed death-ligand 1 (PD-L1) expression than patients with FTC and ATC. It has been noted that patients with lymph node metastases expressed PD-L1 more frequently (11, 12).

2. Materials and Methods

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 immunohistochemical study with PDL1 and a molecular study was applied to all cases. The PD-L1 (clone 28-8) protein expression is measured using the Combined Positive Score (CPS), which is positive if the CPS is ≥1%. Immunohistochemistry was assigned percentage ratings based on the amount of positive staining and the number of stained cells. Negative signified no measurable IHC reaction, while 1, 2, and 3 denote low, moderate, and high relative intensities on the scale. Each sample had ten fields where positive cells were enumerated, and the average of the ten fields was assigned to one of the following four groups (13):

1. Negative score: Less than 1 %.
2. Score 1: More than 1- less than 5%.
3. Score 2: More than 5 to >50%.
4. Score 3: More than 50%.

3. Statistical Analysis

Data were statistically computerized using SPSS version 20 for windows. Expressive statistics are presented as frequencies as percentages. Chi-square and Fisher's exact tests were used for categorical variables when appropriate. T-table's test was used for continuous variables. The differences were considered significant when the probability (*P*-value) was less than 0.

4. Results

Negative score I expression was reported in 24 (46.16%) out of 52 cases of papillary thyroid carcinoma. In contrast, PDL1 positive expression was reported in 28 (59.84. %) cases, score II positive expression was reported in 12 (23.07%), score III positive cases were reported in 12 (23.07) cases, score IV positive cases were reported in 4 (7.70%) cases.

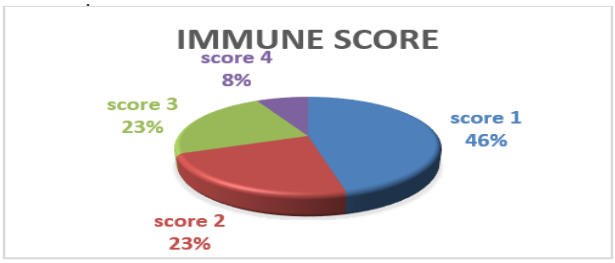

Fig 1: Immunohistochemical expression of PDL1 in neoplastic papillarythyroid tissues.

Table.1: PDL1 Immunohistochemical expression vs. clinicopathological parameters:

Characteristic of patients		immuno_scoring				P value
		I n(%)	II n(%)	III n(%)	IV n(%)	
age	<30	6 (35.3)	7(41.2)	4(23.5)	0 (0.0)	0.03
	30-45	16(64.0)	3(12.0)	3(12.0)	3 (12.0)	
	>45	2 (20.0)	2 (20.0)	5 (50.0)	1 (10.0)	
Gender	Female	23 (53.5)	9(20.9)	9(20.9)	2 (4.7)	0.05
	Male	1 (11.1)	3 (33.3)	3 (33.3)	2 (22.2)	
pN_stage	N0	11 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.0001
	N1a	13 (81.2)	3 (18.8)	0 (0.0)	0 (0.0)	
	N2b	0 (0.0)	9 (42.9)	12 (57.1)	0 (0.0)	
	Nx	0 (0.0)	0 (0.0)	0 (0.0)	4 (100.0)	
Initial metastasis	no Initial metastasis	24 (80.0)	6 (20.0)	0 (0.0)	0 (0.0)	0.0001
	Initial metastasis	0 (0.0)	6 (27.3)	12 (54.5)	4 (18.2)	
Tumor size	less than 2 cm	0 (0.0)	4 (20.0)	12 (60.0)	4 (20.0)	0.003
	more than 2 cm	24 (75.0)	8 (25.0)	0 (0.0)	0 (0.0)	
Other diseases	Hashimoto thyroiditis	0 (0.0)	1 (11.1)	8 (88.9)	0 (0.0)	0.269
	nodular follicular hyperplasia	0 (0.0)	0 (0.0)	4 (50.0)	4 (50.0)	
	No disease	24 (68.6)	11 (31.4)	0 (0.0)	0 (0.0)	
Stage	1	6 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.001
	2	18 (58.1)	12 (37.7)	1 (3.2)	0 (0.0)	
	3	0 (0.0)	0 (0.0)	10(100.0)	0 (0.0)	
	4	0 (0.0)	0 (0.0)	1 (20.0)	4 (80.0)	

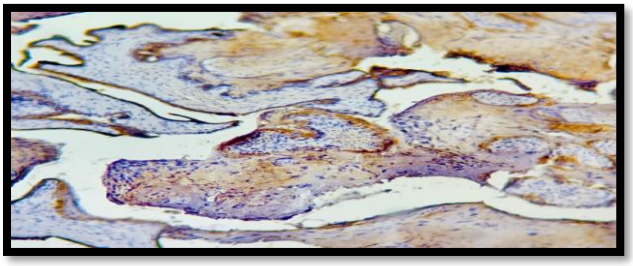

Figure (2): placental tissue positive for PDL1 strong membraneous stain. Positive Syncytiotropoblast cells 400x.

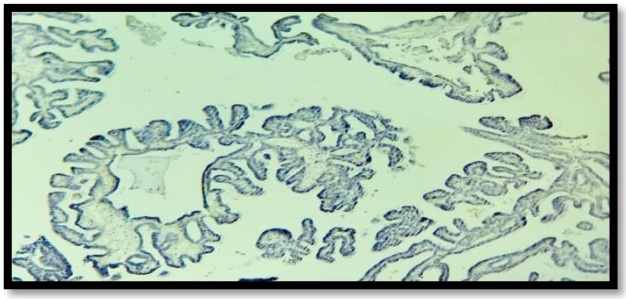

Figure (3): papillary thyroid carcinoma negative for PDL1. IHC. 100x

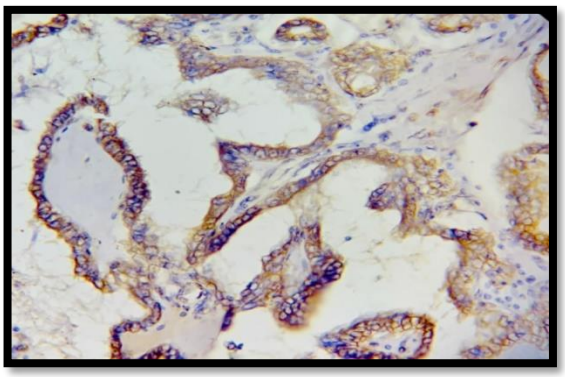

Figure (4): papillary thyroid carcinoma positive for PDL1 strong membranous stain. IHC. 400x.

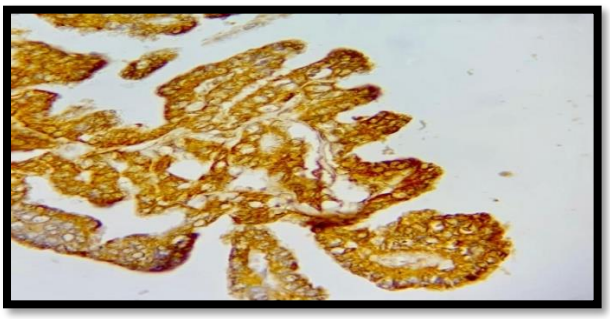

Figure (5): papillary thyroid carcinoma positive for PDL1 strong membraneous stain. IHC 400x.

5. Discussion

Clinicopathological parameters

In this study, female patients are more than males, about 82% are female in comparison to only about 17% are male; this agrees with many studies that show a female predominance regarding PTC. Regarding age parameters, age is categorized into three groups, less than 30 years, from 30 to 45 years, and above 45 years, the second group showed predominance. The prognosis of PTC decreases with age.

Regarding the TNM stage, there was a predominance of stage II in about 59%, which disagreed with many studies that showed that stage I is more predominant because of advances in early-stage radiographic screening. The predominance of stage II in this study may be due to factors of sample collection, and any samples should be taken from the patient subjected to radioactive iodine in a private hospital.

5.2 Discussion of Immunohistochemical results

In this investigation, along with the clinicopathological features of the tumors, we looked at the expression of PD-L1 in 52 cases of PTC with and without Hashimoto thyroiditis and nodular follicular hyperplasia.

In our study, patients with papillary thyroid carcinoma had lower levels of PD-L1 positive expression than in previous thyroid cancer studies (Table 1). Cunha et al. 2013 and Chowdhury et al. 2016 reported that up to 66.5 percent and 82.5 %% of papillary thyroid cancer cases were PD-L1 positive immunohistochemically. (14)(15).

It is understood that only membranous PD-L1 interacts with PD1+ T cells to be functionally significant (16). Therefore, membrane positive without cytoplasmic positivity was seen as a bad outcome. Future research must compare PD-L1 positive in thyroid cancer using various assays with validation and suggest a standardized PD-L1 evaluation process. (17) (Figure 1,2,,4)

Clinically, there was no significant association of PD-L1 expression with the presence or absence of other diseases like nodular follicular hyperplasia or Hashimoto thyroiditis. Moreover, there was a significant correlation between age, gender, and tumor size. This study showed a significant correlation between PD-L1 expression and clinicopathologic factors such as pN stage, first metastasis, and PTC stage.

Our results are consistent with earlier studies that have shown PTCs to form when there is Hashimoto thyroiditis. Although there was no correlation with the tumors' prognostic features, the presence of Hashimoto's thyroiditis was substantially correlated with PD-L1 expression. Expression of PD-L1 did differ with the PTC stage even when subcategorizes were made based on whether or not HT was present. (18).

All cases of PTC with Hashimoto s thyroiditis showed positive pdl-1 expression. On the other hand, only 3 cases of positive PTC showed histological features of nodular follicular hyperplasia.

Our study's sample size is a significant constraint, reflecting the disease's prevalence and the necessity for collaborative research. Small sample sizes can result in Type 1 errors, particularly for p-values between 0.01 and 0.05. Small sample size studies with a significance level of 0.05 should be treated with caution. The uneven expression of PD-L1, which may result in missing expression, is another drawback of our investigation. (19).

6. Conclusions

PDL1 could be considered as a prognostic marker in addition to its proposed target of the treatment of cancers.

there was a significant correlation between age, gender, and tumor size. This study showed a significant correlation between PD-L1 expression and clinicopathologic factors such as pN stage, first metastasis, and PTC stage.

The presence of Hashimoto thyroiditis and nodular follicular hyperplasia were non significantly correlated with Immunohistochemical expression

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