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PDL1 Immunohistochemical Expression in Thyroid Neoplasms: A Possible Therapeutic Target

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Abstract

Background: PD-L1 expression is being considered a potential biomarker for the response of PD-L1 agents.

Aim of Study : We aimed to investigate the expression of PD-L1 in cases of thyroid neoplasms from Egyptian patients trying to correlate PD-L1 immunohistochemical expression and pertinent clincopathological features of thyroid neoplasms that will help to provide better treatment options that might be effective against aggressive malignancy.

Material and Methods: Forty cases of benign and malignant thyroid neoplasms were tested for PD-L1 positivity scores based on the percentage positivity and staining intensity. A total score was then obtained (ranging from 0 to 7) by adding the percentage positivity scores and intensity scores for each section (H-Score). Correlation with clinico-pathological parameters was done.

Results: PDL-1 expression positivity was seen in 82.5% of cases while 17.5% showed negative PDL-1 expression. H-score of studied cases was as follows; 0 (7 cases; one was bengin and 6 were malignant), 1(8 cases; three were benign and 5 were malignant), 2 (6 cases; four were benign and two were malignant), 3 (No case scored as 3), 4 (5 cases; all are malignant), 5 (4 cases; all are malignant), 6 (5 cases; 2 were benign and three were malignant) and 7 (5 cases; 2 were benign and three were malignant).

Conclusion: The presence of PD-L1 in thyroid cancers indicates that there is a sizable group of thyroid cancer patients who might potentially benefit from immunetherapy. Unfortunately, PDL-1 could not be used to differentiate between benign and malignant thyroid neoplasms.

Key Words: *Malignant thyroid neoplasms – Benign thyroid neoplasms – PDL-1 – Immunohistochemical.*

Introduction

AROUND 80% of adults will have at least one thyroid nodule by the time they reach 70 years of age, being benign in 90-95% of cases but thyroid carcinomas (TC) are rapidly increasing in both men and women **[1]**.

Thyroid cancers mostly well differentiated papillary and follicular TCs have excellent prognosis if detected early and treated appropriately **[2]**. While 50% of the deaths are due to anaplastic TC annually, the remainder is due to aggressive variants of metastatic papillary and follicular TCs which have higher risk of recurrence, shortened disease free survival, and death **[3]**. Programmed death ligand 1 (PD-L1, also known as CD274) show great promise for the treatment of various tumors, including melanoma and non-small-cell lung cancer **[4]**. In 2018, the Nobel Prize in Physiology was awarded to Tasuku Honjo and James P. Allison for discovering that immune regulation by PD-L1/PD-1 was a successful anti-cancer therapeutic approach **[5]**.

Based on the premise that anti-PD-1 therapy functions by blocking interactions between PD-1 and PD-L1, PD-L1 expression is being considered a potential biomarker for response of anti-PD-1 or PD-L1 agents **[6]**.

The frequency of PD-L1 positivity ranges from 23% to 87.5% of thyroid carcinoma, which varies according to studies and is higher than other cancer types **[6-9]**.

In the present study, we investigated the expression of PD-L1 in cases of thyroid neoplasms from Egyptian patients trying to correlate PD-L1 immunohistochemical expression and pertinent clincopathological features of thyroid neoplasms that will help to provide better treatment options that might be effective against aggressive malignancy.

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Material and Methods

Forty cases of both benign and malignant thyroid neoplasms were included. specimens were collected from the Pathology Department, Faculty of Medicine, Cairo University. In the period from April 2020 to May 2021. All procedures performed were in concordance with the ethical guidelines of the Kasr Alainy Research Ethics Committee (REC), which operates in accordance with ICH GCP guidelines and related local and institutional regulations and strategies directing REC operation with a ref no. (N-1022023). The clinical data were obtained from the pathology requisition sheets enclosed.

Two sections of 4-µm thickness were cut from formalin-fixed paraffin-embedded tumor (FFPE) blocks. One slide for Hematoxylin and Eosin (H&E) staining for histopathological reassessment and one positively charged slide for immunohistochemical staining by ready-to-use PDL-1 rabbit monoclonal antibody (AVI 3171 G, Biocare Medical, Pacheco, CA 94553, USA). The staining procedure was conducted using the Benchmark XT IHC/ISH staining module (Ventana, Medical Systems, Roche Group, California, USA).

Sections obtained from human tonsils, which exhibited strong intensity of PD-L1 immunostaining served as a positive control. Negative control was obtained by omitting the primary antibody.

Interpretation of immunostaining:

All sections were screened to disclose the areas with well-preserved tissue architecture and cell morphology for scoring of immunoreactivity. Areas with deterioration of tissue morphology due to processing were discarded in the analysis.

Brown membranous with or without cytoplasmic staining was defined as PD-L1 positivity.

Immunostaining scores were based on the percentage positivity and staining intensity as described by Chowdhury et al. **[10]**, Hsieh et al., **[11]**.

Percentage positive scores were assigned according to the following scale: 0 0%; 1 11–30%; 2 31-50%; 3 51- 70%; and 4 71% 71%. Staining intensity was scored semiquantitatively as follows: 0 (none); 1 (mild); 2 (moderate) and 3 (intense). A total score was then obtained (ranging from 0 to 7) by adding the percentage positivity scores and intensit yscores for each section (H-Score) **[10,11]**.

Statistical methods:

All analyses were done using SPSS (Statistical Package for Social Sciences) software, version 26, Chicago, IL, USA. Categorical variables were expressed as frequencies and percentages. Chi-square test was used for testing proportion independence to rule out any significant correlation between PDL-1 expression and other clinicopathological variables included in the study. One-way ANOVA was used to compare different groups with quantitive variables. *p*-value was set significant if 0.05 level.

Results

This retrospective study included 40 thyroid neoplasms with 12 cases (30%) of benign tumors (follicular adenoma) and 28 cases (70%) of malignant tumors (carcinomas). The majority of studied cases were females (67.50%) while males represented 32.50%. The female to male ratio was 2.07:1

The patients' ages ranged from 17 to 66 years with a mean age of 40±13.00 years and a median of 38.

We observed PDL-1 expression positivity in 33 of our cases (82.5%) while 7 cases (17.5%) showed negative PDL-1 expression (Figs. 1,2).

PDL-1 immunohistochemial expression profiles in relation to thyroid neoplasms patients' clinicopathologic characteristics are displayed in Table (1).

H-score of our studied cases was as follows; 0 (7 cases; one was bengin and 6 were malignant), 1(8 cases; three were benign and 5 were malignant), 2 (6 cases; four were benign and two were malignant), 3 (No case scored as 3), 4 (5 cases; all are malignant), 5 (4 cases; all are malignant), 6 (5 cases; 2 were benign and three were malignant) and 7 (5 cases; 2 were benign and three were malignant).

Correlations between clinico-pathologic parameters for studied cases and H-score among positive cases are demonstrated in Table (2).

Discussion

Thyroid cancer represents the fifth most common cancer in women in the United States **[12]**. Its annual incidence has tripled over the last twenty years **[13]**. Similarly, in Egypt, thyroid cancer ranks fifth among females accounting for 3.6% of all malignancies in women **[14]**. 2 Although originating from the same cell type, thyroid cancers display different morphological features, functional behavior, and grade of differentiation as a result of heterogeneous genetic alterations **[15]**.

Table (1): PDL-1 immunohistochemial expression in relation to thyroid neoplasms patients' clinicopathologic characteristics.

	PDL-1 Immunohistochemical Expression		Total	*p*-value
	Negative	Positive		
Behavior:				
Malignant	6 (21.5%)	22 (78.5%)	28	0.946
Benign	1 (8.5%)	11 (91.5%)	12	
Age group:				
<55				
Malignant	5 (22.7%)	17 (77.3%)	22	
Benign	1 (8.5%)	11 (91.5%)	12	
55				
Malignant	1 (16.6%)	5 (83.4%)	6	
Benign	0 (0.0%)	0 (0.0%)	0	
Gender:				
Male				
Malignant	1 (12.5%)	7 (87.5%)	5	
Benign	0 (0.0%)	5 (100%)	7	
Female:				
Malignant	5 (25%)	15 (75%)	8	
Benign	1 (14.2%)	6 (85.8%)	20	
Age:*				
<55	5 (22.7%)	17 (77.3%)	22	
55	1 (16.6%)	5 (83.4%)	6	
Gender:*				
Male	1 (12.5%)	7 (87.5%)	8	0.365
Female	5 (25%)	15 (75%)	20	
pT:*				
T1	2 (28.5%)	5 (71.5%)	7	0.173
T2	3 (25%)	9 (75%)	12	
T3	1 (11%)	8 (89%)	9	
N:*				
N0	5 (23.8%)	16 (76.1%)	21	0.497
N1	1 (14.2%)	6 (85.7%)	7	
Stage:*				
Stage I	3 (21.5%)	11 (78.5%)	14	
Stage II	2 (25%)	6 (75%)	8	
Stage III	1 (33.3%)	2 (66.7%)	3	
Stage IV	0 (0.0%)	3 (100%)	3	
Multicentricity:*				
Absent	4 (26.6%)	11 (73.4%)	15	
Present	2 (15.3%)	11 (84.7%)	13	
Extrathyroidal extension:*				
Absent	5 (35.7%)	9 (64.3%)	14	0.094
Present	1 (7.1%)	13 (92.3%)	14	
Co-existing pathology:*				
Absent	3 (18.75%)	13 (81.25%)	16	
FA	1 (50%)	1 (50%)	2	
HT	1 (16.6%)	5 (83.4%)	4	
MNG	1 (25%)	3 (75%)	6	0.145

* : Parameter studied among malignant cases.
FA : Follicular adenoma.
HT : Hashimoto's thyroiditis.
MNG : Multi-nodular goitre.

Table (2): PDL-1 H-score expressions in relation to patients' clinicopathologic characteristics among positive cases.

	H-score						Total	p-value
	1	2	4	5	6	7		
Behviour:								
Malignant	5 (15.2%)	2 (6.1%)	5 (15.2%)	4 (12.1%)	3 (9.1%)	3 (9.1%)	22 (66.7%)	0.170
Benign	3 (9.1%)	4 (12.1%)	0 (0%)	0 (0%)	2 (6.1%)	2 (6.1%)	11 (33.3%)	
Gender:								
Male	3 (9.1%)	2 (6.1%)	0 (0%)	2 (6.1%)	1 (3%)	4 (12.1%)	12 (36.4%)	0.162
Female	5 (15.2%)	4 (12.1%)	5 (15.2%)	2 (6.1%)	4 (12.1%)	1 (3%)	21 (63.6%)	
Diagnosis:								
Hurthle cell carcinoma	1 (4.5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4.5%)	0.52
Poorly differetiated carcinoma	1 (4.5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4.5%)	2 (9.1%)	
Medullary carcinoma	0 (0%)	0 (0%)	0 (0%)	1 (4.5%)	0 (0%)	0 (0%)	1 (4.5%)	
Anaplastic carcinoma	0 (0%)	1 (4.5%)	2 (9.1%)	0 (0%)	0 (0%)	0 (0%)	3 (13.6%)	
Papillary thyroid carcinoma	3 (13.6%)	1 (4.5%)	3 (13.6%)	3 (13.6%)	3 (13.6%)	2 (9.1%)	15 (68.2%)	
T stage:*								
T1	2 (9.1%)	0 (0%)	0 (0%)	1 (4.5%)	1 (4.5%)	1 (4.5%)	5 (22.7%)	0.149
T2	2 (9.1%)	0 (0%)	3 (13.6%)	1 (4.5%)	2 (9.1%)	1 (4.5%)	9 (40.9%)	
T3	1 (4.5%)	2 (9.1%)	2 (9.1%)	2 (9.1%)	0 (0%)	1 (4.5%)	8 (36.3%)	
LN:*								
N0	3 (13.6%)	2 (9.1%)	4 (18.2%)	2 (9.1%)	3 (13.6%)	2 (9.1%)	16 (72.7%)	0.621
N1	2 (9.1%)	0 (0%)	1 (4.5%)	2 (9.1%)	0 (0%)	1 (4.5%)	6 (27.3%)	
Stage:*								
Stage I	1 (4.5%)	0 (0%)	3 (13.6%)	2 (9.1%)	1 (4.5%)	2 (9.1%)	9 (40.9%)	0.259
Stage II	1 (4.5%)	1 (4.5%)	2 (9.1%)	1 (4.5%)	2 (9.1%)	0 (0%)	7 (31.8%)	
Stage III	0 (0%)	1 (4.5%)	0 (0%)	1 (4.5%)	0 (0%)	1 (4.5%)	3 (13.6%)	
Stage IV	3 (13.6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (13.6%)	
Multicentericiry:*								
Absent	2 (9.1%)	1 (4.5%)	3 (13.6%)	3 (13.6%)	1 (4.5%)	1 (4.5%)	11 (50%)	0.840
Present	3 (13.6%)	1 (4.5%)	2 (9.1%)	1 (4.5%)	2 (9.1%)	2 (9.1%)	11 (50%)	
Extrathyroidal extension:*								
Absent	2 (9.1%)	1 (4.5%)	2 (9.1%)	2 (9.1%)	1 (4.5%)	1 (4.5%)	9 (40.9%)	0.848
Present	3 (13.6%)	1 (4.5%)	4 (18.2%)	2 (9.1%)	1 (4.5%)	2 (9.1%)	13 (59.1%)	
Co-existing pathology:*								
Absent	3 (13.6%%)	1 (4.5%)	5 (22.7%)	2 (9.1%)	1 (4.5%)	1 (4.5%)	13 (59.1%)	0.087
FA	0 (0%)	1 (4.5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4.5%)	
HT	1 (4.5%)	0 (0%)	0 (0%)	0 (0%)	2 (9.1%)	1 (4.5%)	4 (18.2%)	
MNG	1 (4.5%)	0 (0%)	0 (0%)	2 (9.1%)	0 (0%)	1 (4.5%)	4 (18.2%)	

* : Parameter studied among malignant cases.
FA : Follicular adenoma.
HT : Hashimoto's thyroiditis.
MNG : Multi-nodulaar goitre.

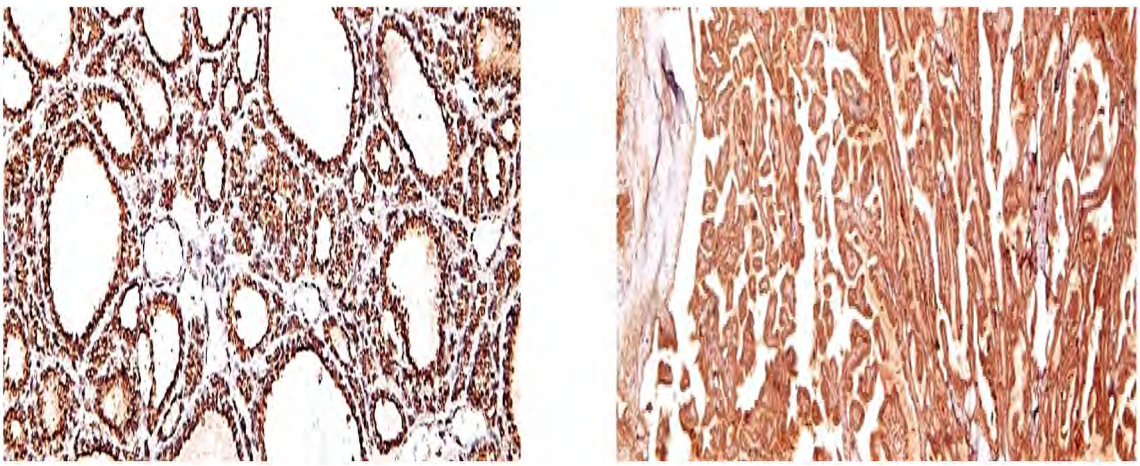

Fig. (1): Positive PDL-1 staining (H-score 7) in a case of; Left: Follicular adenoma, Right: Papillary thyroid carcinoma (IHC x 400 original magnifications).

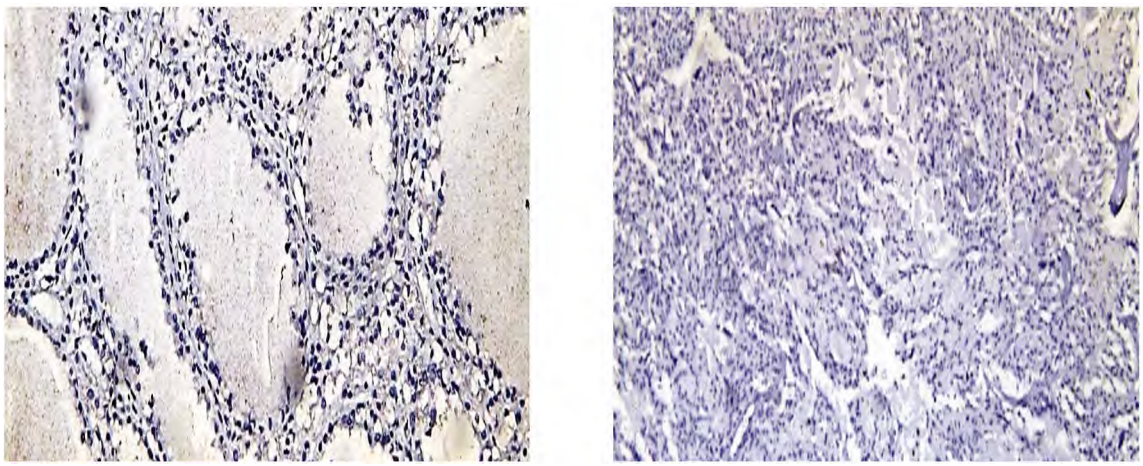

Fig. (2): Negative PDL-1 expression (H-score 0) in a case of; Left: Follicular adenoma, Right: Medullary thyroid carcinoma (IHC x 400 original magnifications).

We investigated the immunohistochemical expression of PDL-1 in forty thyroidectomy specimens diagnosed as thyroid neoplasms; benign (follicular thyroid adenoma) & malignant (papillary, follicular, anaplastic, medullary, poorly differentiatd thyroid carcinomas, & hurthle cell carcinoma) and its possible prognostic values.

Thirty-three out of our 40 cases (82.5%) showed positive PDL-1 expression while 7 cases (17.5%) showed negative PDL-1 expression. 78.5% of studied malignant cases showed positive PDL-1 expression while 91.5% of benign cases showed positive PDL-1 expression but with insignificant correlation (p=0.946).

Per our study, all papillary carcinoma cases showed positive PDL-1 expression. Chowdhury et al. **[10]** showed 123/185 (66.5%) of cases with positive PDL-1 expression, 53.25% of cases were positive for PDL-1 expression in the study done by Bai et al. **[16]**, and 50/75 (67%) of cases were positive for PDL-1 in the study done by Aghajani, et al. **[17]**. This difference may be due to different sample sizes, geographical locations, genetic differences, and different scoring systems used.

Regarding our anaplastic carcinoma cases; all showed positive PDL-1 expression, Chintakuntlawar et al. **[18]** showed 81.3% of cases positive for PDL-1 expression and Bastman et al. **[7]** showed 75% positive PDl-1 expression. Concerning poorly differentiated carcinoma cases; 66.5% showed positive PDL-1 expression. Bastman et al. **[7]** showed 100% positive expression. In contrast to medullary thyroid carcinoma (MTC); as we detected PDL-1 positivity in one out of four cases. In the study done by Bogiovanni et al. **[19]**, all of their 16 medullary thyroid carcinomas but one scored negatively in the tumor cells. This was explained by the fact that MTC has one of the lowest mutational loads and neo-antigen repertoires among all

solid tumors. Moreover, on histological examination, MTC usually has very few accompanying inflammatory cells.

Given the fact that immune regulation by PD-L1/PD-1 was a successful anti-cancer therapeutic approach **[20]** and this high prevalence of positive PDL-1 expression in thyroid malignancy we found so thyroid cancer patients (whose tumors expressing this protein) may benefit from that immunotherapy especially those that cannot be treated surgically or that do not respond to traditional treatment options.

We observed an increased expression of PD-L1 in papillary thyroid carcinoma arising in a background of Hashimoto's thyroiditis, which agreed with Chowdhury et al. **[3]** who suggested that the chronic inflammation might provide a microenvironment enriched with different cytokines such as IFNγ, IL-1, IL-10, IL-6 that could trigger upregulation of PDL1 expression. While we found no statistically significant correlation between H-score and behavior among positive cases. This was in disconcordance to results reported by Tuccilli et al. **[21]** who found that the presence of PD-L1 expression in tumor cells may be associated with more aggressive tumor behavior, the statistical significance of this finding is limited by the size of the study, and larger series would be useful to further assess these observations.

Conclusions:

In conclusion, taking together, our findings and published data, we can assume that the presence of PD-L1 in thyroid cancers indicates that there is a sizable group of thyroid cancer patients who might potentially benefit from immunetherapy and underlines the need for further clinical trials supported by additional molecular techniques to offer individualized therapeutic options other than and together with limited traditional surgery, radioiodine therapy, chemotherapy, radiotherapy, or molecular targeted therapy. However, unfortunately, PDL-1 could not be used to differentiate between benign and malignant thyroid neoplasms.

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التعبير النسيجى المناعى عن PDL1 فى أورام الغدة الدرقية : هدف علاجى محتمل

يعتبر تعبير PD-L1 علامة بيولوجية محتملة لاستجابة عوامل PD-L1.

لقد هدفنا إلى التحقيق فى التعبير عن PD-L1 فى حالات أورام الغدة الدرقية من المرضى المصريين كمحاولة لربط التعبير الكيميائى المناعى PD-L1 والسمات الباثولوجية ذات الصلة لأورام الغدة الدرقية والتى ستساعد على توفير خيارات علاج أفضل قد تكون فعالة ضد الأورام الخبيثة العدوانية.

تم اختبار أربعين حالة من أورام الغدة الدرقية الحميدة والخبيثة للحصول على درجات إيجابية PD-L1 بناءً على النسبة المئوية الإيجابية وشدة الصبغة. ثم تم الحصول على مجموع نقاط (تتراوح من ٠ إلى ٧) عن طريق إضافة النسبة المئوية لدرجات الإيجابية ودرجات الشدة لكل قسم (H-Score). تم عمل الارتباط مع المعلمات السريرية المرضية.

شوهدت إيجابية تعبير PD-L1 فى ٨٢.٥٪ من الحالات بينما أظهر ١٧.٥٪ تعبير PD-L1 سلبى. كانت درجة H للحالات المدروسة على النحو التالى، ٠ (٧ حالات، واحدة كانت حميدة و ٦ حالات خبيثة)، ١ (٨ حالات ، ثلاث حالات حميدة و ٥ أورام خبيثة)، ٢ (٦ حالات، أربع حالات حميدة واثنتان خبيثتان)، ٣ (لم يتم تسجيل أى حالة على أنها ٣)، ٤ (٥ حالات، جميعها خبيثة)، ٥ (٤ حالات، جميعها خبيثة)، ٦ (٥ حالات، ٢ كانت حميدة وثلاث حالات خبيثة) و ٧ (٥ حالات، ٢ كانت حميدة وثلاث حالات خبيثة).

فى الختام، يشير وجود PD-L1 فى سرطانات الغدة الدرقية إلى وجود مجموعة كبيرة من مرضى سرطان الغدة الدرقية الذين قد يستفيدون من العلاج المناعى. لسوء الحظ، لا يمكن استخدام PD-L1 للتمييز بين أورام الغدة الدرقية الحميدة والخبيثة.