

ABSTRACT

Importance: Aggressive variant of papillary thyroid cancer (PTC) accounts for 5% of cases and sometimes become radioactive-iodine refractory, leaving limited treatment options. Additionally, lymph node metastases are common and associated with increased recurrence, decreased survival, and often requires revision surgery in an operated field that has also potentially received radioactive iodine, carrying higher complication rates.

Objectives: To assess PD-L1 levels in serum and tumor of patients with PTC and its association with clinicopathologic characteristics including aggressiveness and lymph node metastasis.

Design, setting and participants: A retrospective chart review was conducted to identify patients with PTC at Louisiana State University Health Sciences Center Shreveport (LSUHSC-S) and Overton Brooks Veterans Affairs Medical Center.

Main outcomes and measure: Enzyme-linked immunosorbent assay (ELISA) was used to determine serum levels of PD-L1. Tumor was immunostained for PD-L1 (E1L3N). Clinical data were obtained from chart review and clinical databases.

Results: Among 98 subjects included in the analysis, average PD-L1 serum levels of aggressive PTC were higher than that of non-aggressive PTC (53.97; 95% CI, 20.21 to 87.75). Extrathyroidal extension (63.93; 95% CI, 30.31 to 97.55) and TNM stage (132.28; 95% CI, 67.98 to 196.59) showed associations with higher PD-L1 serum levels. PD-L1 expression in tumor was also associated with malignancy, and metastatic lymph nodes.

Conclusion and relevance: Findings suggest patients with aggressive and advanced stage PTC express higher levels of PD-L1. Results support PD-L1's role as a potential prognostic marker for aggressive PTC. PD-L1 warrants further exploration as a potential target for immunotherapy.

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Serum PD-L1 As A Potential Prognostic Marker in Papillary Thyroid Carcinoma

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BACKGROUND

- Papillary thyroid cancer (PTC) accounts for 80% of thyroid cancers.

- Fortunately, PTC has the best prognosis, however, 5% of PTC patients are considered to have aggressive disease and can become radioactive-iodine (RAI) refractory.

- Patients with aggressive PTC have limited treatment options and a 10-year survival rate of <10%.^{1,2}

- 5–15% of patients become RAI refractory 10-13 and prognosis is poor.

- Survival for patients with RAI-refractory differentiated thyroid carcinoma and distant metastases is around 2.5–3.5 years.^{3, 4}

- The anti-tumor immune response is often suppressed as a result of the upregulation of Programmed Death Ligand-1 (PD-L1) in the tumor microenvironment.

- PD-L1 binds to PD-1 receptors on T cells, and the PD-1/PD-L1 interaction inhibits T cell proliferation and induces T cell apoptosis.

- PD-L1 as a biomarker could potentially determine patients that may need more extensive surgery to include neck node management and lay the foundation for possible immune checkpoint inhibitor therapy.

OBJECTIVES

The purpose of this study is to evaluate the prognostic value of serum PD-L1 in PTC.

- Determine whether serum PD-L1 is associated with clinicopathologic characteristics, including aggressiveness, risk of recurrence, disease-free survival and TNM stage.

- Determine the immunohistochemical patterns of PD-L1 in tumor tissue and compare it with serum samples.

METHODS

Patients and Sample Collections

- Chart reviews were conducted in 98 patients undergoing total thyroidectomy to identify demographic variables, comorbidities, aggressive PTC features (extrathyroidal extension, extra-nodal extension, aggressive histology, and distant metastases), metastatic lymph nodes, cancer staging and recurrence. Cancer staging was performed according to AJCC Cancer Staging Manual 8th edition.⁵

Immunohistochemistry

- PTC (n=40) samples were immunostained for PD-L1 (E1L3N; #13684 Cell Signaling Technology, 1:200 dilution).
- PD-L1 IHC expression was evaluated by two pathologists who were blinded to the clinical details.
- Positive PD-L1 staining is defined as complete and/or partial circumferential linear plasma membrane staining at any intensity that can be differentiated from background and diffuse cytoplasmic staining.⁶

ELISA

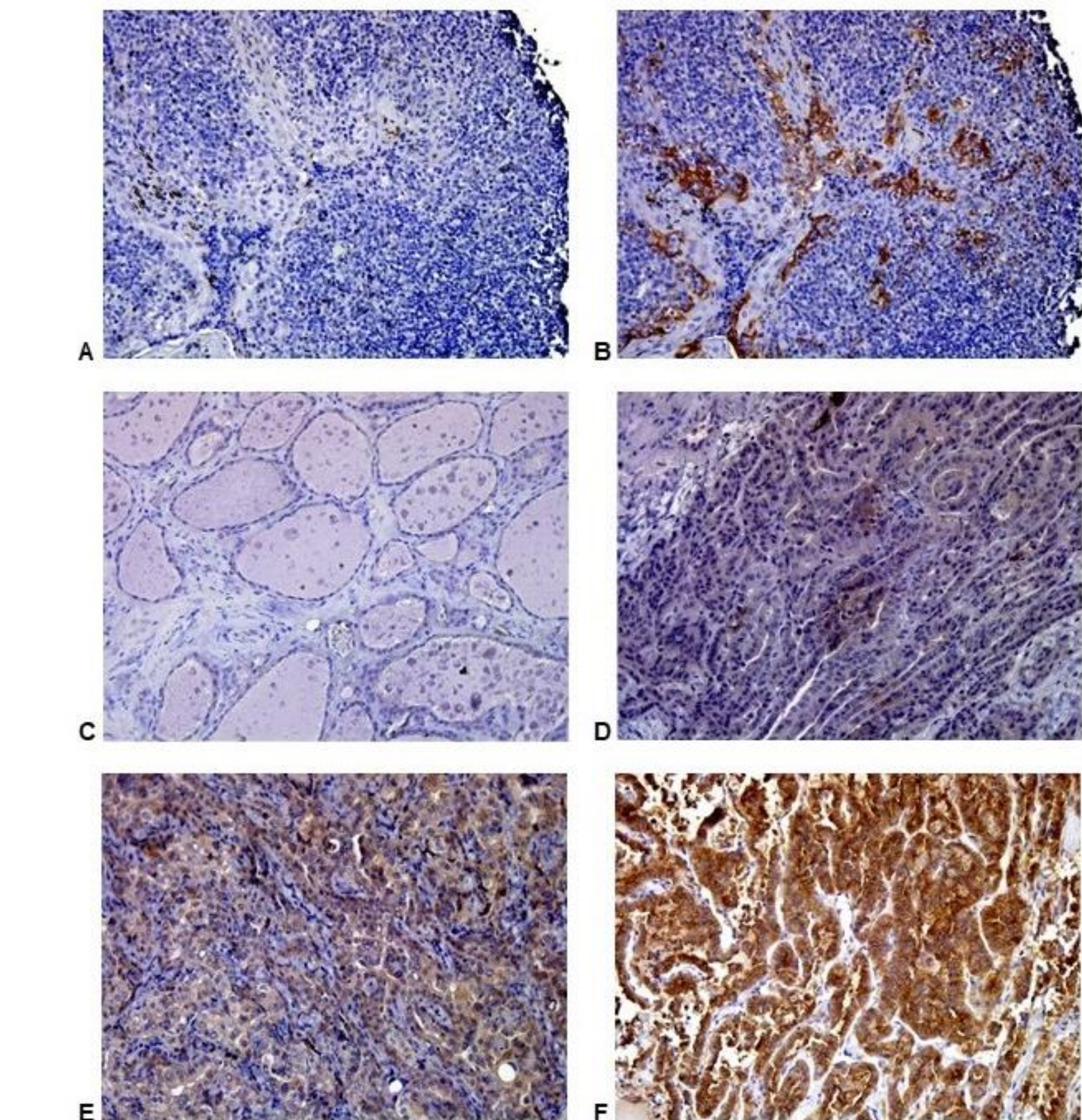
- Serum PD-L1 levels of 98 patients were measured with ELISA R&D system kit by obtaining frozen serum of:
 - 31 healthy volunteers (no known thyroid disease).
 - 48 patients with benign thyroid disease (multinodular goiter, hyperplasia, follicular adenoma, and Hashimoto's disease).
 - 30 PTC patients.

Statistical Analysis

- Differences in serum ELISA PD-L1 levels were estimated using ANOVA.
- Odds ratios (ORs) and Cohen's f effect sizes were also calculated. Power analysis was performed to evaluate the differences of PD-L1 serum levels between the aggressive and non-aggressive group, at an alpha of 0.05.
- Data management and statistical analysis was performed using Stata/IC, version 16 (StataCorp LLC).

RESULTS

Figure 1. Immunohistochemical analysis of PD-L1 in thyroid tissue.

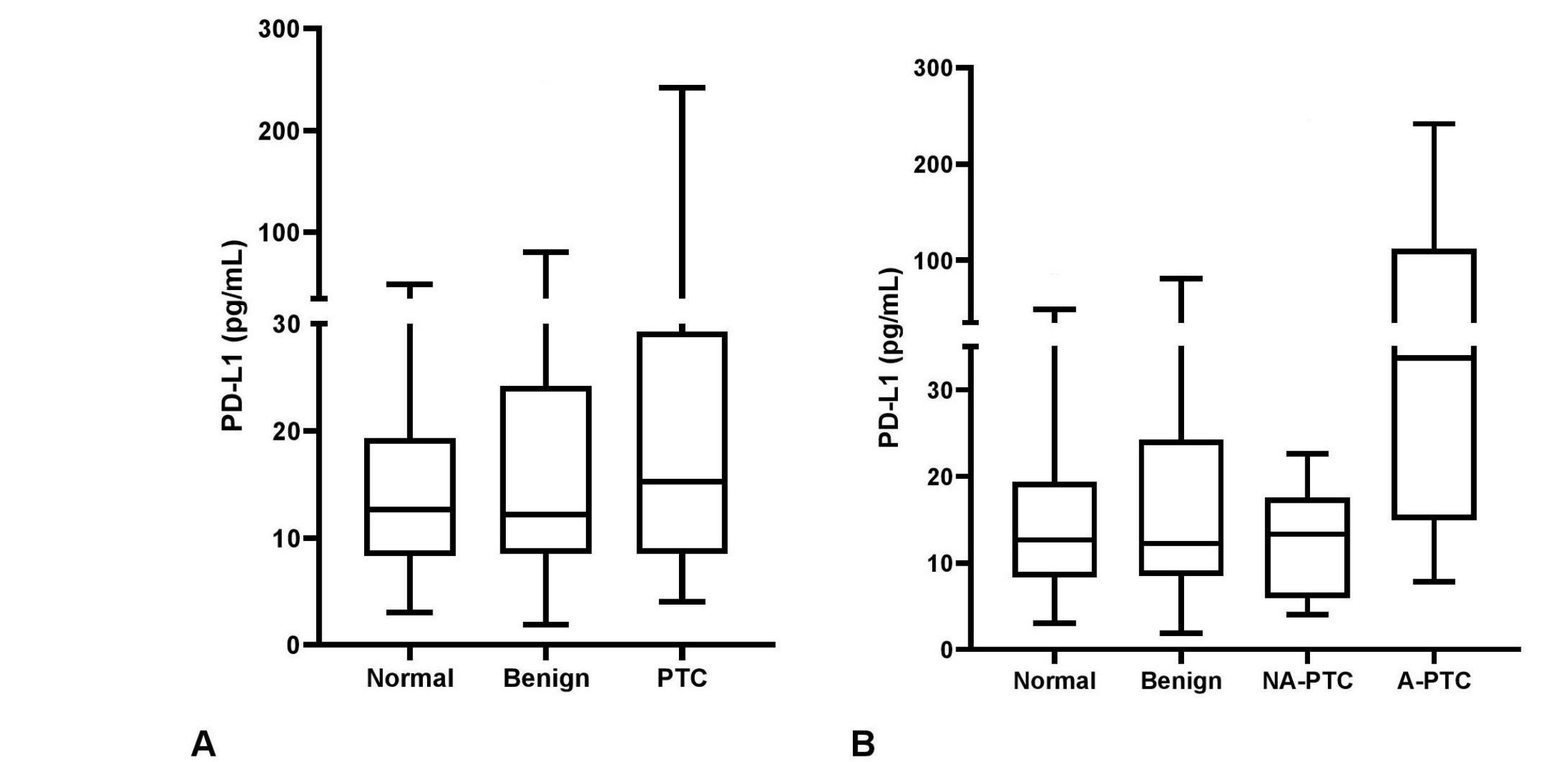


The representative photomicrographs show the different staining intensity (1+, low; 2+, moderate and 3+, strong) of PD-L1 in tonsil (control tissue) and thyroid tissue.

- A) Human tonsil (20X), negative control showing no detectable immunostaining for PD-L1.
- B) Human tonsil (20X), positive control showing strong (3+) membrane and cytoplasmic staining of cells at the tonsil crypts.
- C) Benign thyroid tissue (20X), showing no detectable immunostaining for PD-L1.
- D) PTC (20X), showing low (1+) and moderate (2+) cytoplasmic staining.
- E) PTC (20X) showing strong (3+) membrane staining and moderate (2+) and strong (3+) cytoplasmic staining.
- F) PTC (20X) showing strong (3+) membrane staining and strong (3+) cytoplasmic staining.

No association was found between the expression of PD-L1 in non-aggressive and aggressive PTC in tumor (1.58; 95% CI, 0.34 to 7.22).

Figure 2. Serum and tumor PDL1 levels in normal, benign and papillary thyroid cancer.



- A) Average PD-L1 serum in PTC is higher compared to normal (20.40; 95% CI, 1.17 to 39.62).
- B) Average serum PD-L1 of aggressive PTC (A-PTC) is higher than that of normal (50.99; 95% CI, 33.17 to 68.81), benign (47.80; 95% CI, 30.55 to 65.04) and non-aggressive PTC (NA-PTC) (53.98; 95% CI, 34.21 to 73.75). Average serum PD-L1 of non-aggressive PTC is not different from normal (2.99 pg/mL; 95% CI, -4.67 to 10.65) or benign (6.18; 95% CI, -1.18 to 13.54).

CONCLUSIONS

- PTC patients express high levels of PD-L1 at the primary site compared to healthy subjects and patients with benign thyroid disease.
- Aggressive PTC correlated with higher PD-L1 levels in plasma, especially those with extrathyroidal extension.
- Patients with metastatic lymph nodes showed increased expression of PD-L1 at the primary site.

These results demonstrate an association between PD-L1 and aggressive and advanced stage PTC, suggesting that PD-L1 may play a role as a prognostic marker. PD-L1 warrants further exploration as a potential target for immunotherapy in such patients.

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