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High Expression of Immune Checkpoint Molecules in Different Types of Thyroid Cancer

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ABSTRACT

This study aimed to investigate the expression of programmed cell death protein-1 (PD-1) and its ligand (PD-L1) immune checkpoint molecules in thyroid carcinomas and determine their association with the clinicopathological characteristics of patients.

Thyroid tissue specimens from 100 patients diagnosed with primary thyroid carcinomas including papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), medullary thyroid carcinoma (MTC), and anaplastic thyroid carcinoma (ATC) were collected. Sections were prepared from formalin-fixed paraffin-embedded samples, and PD-1 and PD-L1 expressions were examined using immunohistochemistry.

PD-1 was detected in tumor-infiltrating lymphocytes (TILs) in 88% of the patients and tumor cells in 28% of the patients with 10% in PTC, 5% in FTC, 5% in MTC, and 8% in ATC). PD-L1 was found in tumor cells and TILs in 30% and 79% of the patients, respectively. Moreover, a significant difference was observed in PD-1 and PD-L1 expression between tumor cells and TILs across different tumor types. However, their expression in tumor cells and TILs was significantly higher in ATC compared to other tumor types. Additionally, the expression of PD-1 and PD-L1 was significantly associated with an advanced stage, higher tumor size, tumor necrosis, and mitosis. A significant positive correlation was also observed between the expression of PD-1 and PD-L1 in tumor cells and TILs.

The higher expression of PD-1 and PD-L1 may contribute to tumor progression. Therefore, combinational immunotherapy by these immune checkpoint inhibitors might be a promising strategy for clinical improvement in patients with thyroid cancer, especially those with ATC.

Keywords: Immune checkpoint; Immunohistochemistry; Programmed cell death 1 receptor; Programmed cell death ligand; Thyroid cancer

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INTRODUCTION

Thyroid cancer stands out as the most prevalent malignancy in the endocrine system and holds the ninth position in terms of cancer prevalence overall, with nearly 52,000 new cases and 2000 fatalities recorded annually in the United States alone.[1] Notably, the incidence of thyroid cancer in women surpasses that in men by threefold, and this gender disparity persists as an ongoing trend with each passing year. While the mortality rate associated with this disease generally remains low, certain cases exhibit heightened risks of metastasis and ultimately death.[2] Primary thyroid cancers encompass 4 main types: differentiated thyroid cancers, including papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), and medullary thyroid carcinoma (MTC), as well as poorly differentiated thyroid cancer, notably anaplastic thyroid carcinoma (ATC). Among these, PTC comprises approximately 90% of cases, while ATC represents a rare but highly aggressive variant, constituting around 2% of diagnoses. While differentiated thyroid cancers generally carry a favorable prognosis, ATC stands out for its aggressive nature and high fatality rate, approaching nearly 100%, making it one of the deadliest solid tumors in humans, not solely within thyroid cancers.[3] Thyroid cancer treatment typically entails a multifaceted approach involving surgical intervention and pharmacotherapy. Following procedures such as hemithyroidectomy or total thyroidectomy, adjunctive therapies including radioactive iodine therapy, thyroid stimulating hormone suppression therapy, and targeted therapy are commonly employed to eradicate residual malignant cells and mitigate the risk of metastasis.[4,5] An emerging strategy in thyroid cancer treatment involves immunotherapy, which targets immune checkpoint molecules to enhance the body's natural defenses against cancer cells.[6] These molecules play a crucial role in regulating immune responses, ensuring self-tolerance, and preventing autoimmune diseases, ultimately safeguarding tissues from potential damages caused by immune reactions.[7] In recent decades, extensive research has been conducted on immune checkpoints, leading to the identification of numerous molecules with clinical and diagnostic significance.[8]

In recent years, certain immune checkpoint molecules, notably programmed cell death 1 (PD-1) and programmed cell death 1 ligand (PD-L1), have attracted significant attention, leading to the development of numerous checkpoint inhibitors targeting them.[9] PD-1 (CD279), as a cell surface receptor and a homolog of the CD28 molecule, initiates the immune system's inhibitory responses. PD-1 is a regulator of acquired immune responses, the presence of which is vital for inducing immunological tolerance and preventing immunopathological effects during many immune responses PD-1. The molecule functions as a cell surface receptor sharing homology with CD28. It plays a pivotal role in initiating inhibitory responses within the immune system and acts as a critical regulator of acquired immune responses.[10] The interplay between PD-1 and PD-L1 within the tumor microenvironment acts to suppress immune responses against cancer, representing a pivotal mechanism for tumor cells to evade immune detection.[11,12] Overexpression of PD-1 and PD-L1 has been documented in numerous human malignancies, including seminoma dysgerminoma as well as cancers of the gastric, colorectal, breast, and bladder.[13-17]

Furthermore, the expression of these molecules has been reported in some types of thyroid cancer, including PTC, FTC, and ATC.[18,19] Interestingly, our previous study introduced PD-1.5 C/T polymorphism and GT haplotype, resulting from PD-1.3 G and PD-1.5 T, as the genetic markers associated with developing thyroid cancer among the Iranian population.[20] Numerous immune checkpoint inhibitors, including pembrolizumab (anti-PD-1) and durvalumab (anti-PD-L1), have emerged, showing promising potential in enhancing immune responses against tumors and thereby impacting the treatment of various cancers.[21] In this study, our objective was to explore and compare the expression of PD-1 and PD-L1 in 4 subtypes of thyroid cancer within both tumor cells and tumor-infiltrating lymphocytes (TILs), aiming to ascertain their correlation with the clinicopathological characteristics of patients.

MATERIALS AND METHODS

Study Population

Thyroid tissue specimens from 100 patients diagnosed with primary thyroid cancers, including PTC (n=48), FTC (n=21), MTC (n=21), and ATC (n=10), who underwent total thyroidectomy between 2012 and 2019 at Chamran Hospital, Shiraz, Iran, were selected for this study. The tissue sections were stained with hematoxylin and eosin and evaluated by a pathologist to confirm the histologic type of thyroid cancer. Formalin-fixed paraffin-embedded (FFPE) samples from eligible

patients were retrieved from the archive, and clinicopathological information was obtained from the patients' medical records. The study protocol was approved by the Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.REC.1398.1211).

PD-1 and PD-L1 Immunohistochemistry

Two consecutive sections of FFPE tissue from the patients were cut at a thickness of 5 µm and mounted on glass slides coated with poly-L-lysine. The sections underwent deparaffinization in a hot air oven at 61°C, followed by rehydration using fresh xylene and a series of decreasing concentrations of ethanol. Antigen retrieval for PD-1 and PD-L1 was performed using the heat-induced epitope retrieval (HIER) method with Tris-EDTA retrieval buffer (pH 9). Endogenous peroxidase activity and nonspecific hydrophobic interactions were blocked with 10% H_2O_2 and 10% goat serum, respectively. Subsequently, a mouse anti-PD-1 monoclonal antibody (SB-019261; Sinabiotech, Tehran, Iran) and a mouse anti-PD-L1 monoclonal antibody (Sinabiotech, Tehran, Iran), both diluted at 1:200, were applied at room temperature for 1 hour. Visualization was carried out using an immunoperoxidase diaminobenzidine (DAB) Kit (MAD-001811QK, Master Diagnostica, Spain) according to the manufacturer's instructions. Finally, all sections were counterstained with hematoxylin. Normal tonsil tissues served as a positive control for both markers, while ATC tissue was immunostained without the application of primary antibodies for use as a negative control.

Evaluation of Immunohistochemistry

PD-1 and PD-L1 expression levels were evaluated by an expert pathologist who was blinded to the patients' clinicopathological information. Only cells showing membranous staining were considered positive for PD-1 and PD-L1. The immunostaining scores for tumor cells were categorized as negative (0), weak (1+), moderate (2+), and strong (3+) (Figure 1). For TILs, the assessment was based on the percentage of positive cells and grouped as negative (0), weak (<10%), moderate (10–40%), and strong (>40%) (Figure 2). The evaluation of TILs followed the guidelines for breast cancer recommended by the International TILs Working Group in 2014.[22]

Statistical Analyses

All statistical analyses were conducted using IBM SPSS software version 22. The expression of molecules across multiple groups was assessed using the Kruskal-Wallis nonparametric test, followed by pairwise comparisons using the Mann-Whitney U test in case of significance. Statistical significance was defined as a *p* values<0.05. Additionally, Spearman correlation analysis was employed to explore the relationship between the expressions of different molecules.

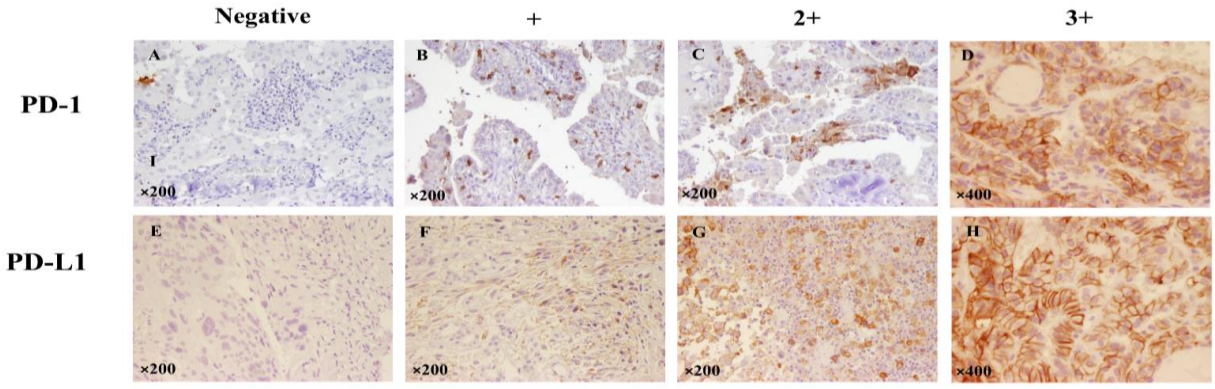

Figure 1. The scoring system for programmed cell death protein-1 (PD-1) and its ligand (PD-L1) expression in tumor cells ranged from 0 to 1+, 2+, and 3+, respectively, reflecting increased expression levels. The brown dot indicates positive expression for PD-1 and PD-L1.

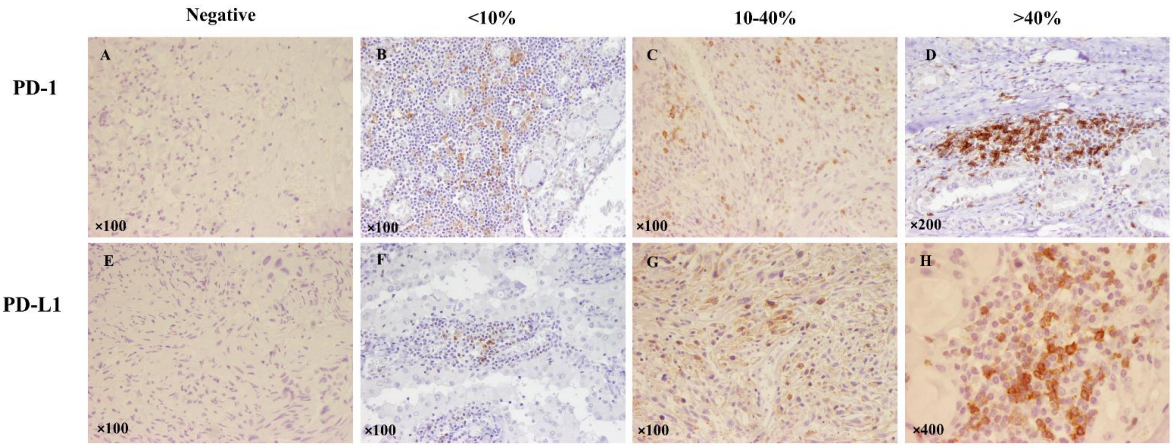

Figure 2. The scoring system of programmed cell death protein-1 (PD-1) and its ligand (PD-L1) expression in tumor-infiltrating lymphocytes (TILs) scored from 0 to<10%, 10–40%, and>40%, respectively, based on the increased expression. (A–D) Scoring the expression of PD-1 molecule in TILs. (E–H) Scoring of PD-L1 expression in TILs.

RESULTS

Clinicopathological Characteristics of the Patients

In this study, we used 100 samples of thyroid cancer patients who underwent total thyroidectomy as primary treatment. Among the 100 patients included in the study, 61 were female and 39 were male, with a mean age of 51.95±17.5 years (range: 14-89). Furthermore, 34 patients were classified as having stage I disease, 29 patients as stage II, 24 patients as stage III, and 13 patients as stage IV disease. Table 1 provides an overview of the clinicopathological characteristics of the patients.

Clinicopathological Significance of PD-1 and PD-L1 Expression

We investigated the expression of PD-1 and PD-L1 in tumoral, peritumoral, and normal thyroid tissues using the immunohistochemistry method. No PD-1 or PD-L1 expression was detected in peritumoral and normal thyroid tissues. PD-1 expression was observed in TILs in 88% of the patients, while PD-L1 expression was found in tumor cells and TILs in 30% and 79% of the patients, respectively. Significant disparities were noted in the expression levels of PD-1 and PD-L1 among tumor cells across various types of thyroid tumors (PD-1: p=0.001, 95% CI [0.11–0.56] for PTC, 95% CI [0.04–0.44] for FTC, 95% CI [0.04–0.44] for MTC, and 95% CI [0.62–1.98] for ATC; PD-L1: p<0.001, 95% CI [0.2–0.63] for PTC, 95% CI [−0.30–0.7] for FTC, 95% CI [−0.02–0.31] for MTC, and 95% CI [0.71–2.09] for ATC.

Particularly noteworthy was the considerably elevated expression of PD-1 and PD-L1 in ATC compared to other tumor types. Furthermore, ATC demonstrated the highest PD-1 expression among TILs compared to other tumor types. Similar findings were noted for the expression of PD-1 and PD-L1 in TILs across different types of thyroid tumors (PD-1: p=0.001, 95% CI [0.76–1.24] for PTC, 95% CI [0.93–1.36] for FTC, 95% CI [0.62–1.29] for MTC, and 95% CI [1.69–2.51] for ATC; PD-L1: p<0.001, 95% CI [0.65–1.10] for PTC, 95% CI [1.08–1.5] for FTC, 95% CI [0.53–1.19] for MTC, and 95% CI [1.49–2.31] for ATC). PD-1 expression in tumor cells was detected in 28% of the patients. Additionally, a significant association was found between PD-1/PD-L1 expression and the TNM stage, T stage, tumor mitosis, necrosis, and tumor cell atypia. However, no significant association was observed between the PD-1/PD-L1 status and other clinicopathological parameters such as age, sex, tumor encapsulation, tumor size, lymphoid invasion, vascular invasion, capsular invasion, extrathyroidal extension, and parathyroid gland involvement. Table 2 summarizes the association between the PD-1/PD-L1 status and clinicopathological parameters.

Table 1. Clinicopathological characteristics of the patients with thyroid cancer

Characteristics	N (%)	Characteristics	N (%)
Age (year)		**Tumor cell atypia**	
<45	28	**Absent**	**70**
≥45	72	**Present**	**30**
Sex		**Encapsulation**	
Female	61	**Absent**	**25**
Male	39	**Complete**	**36**
		Partial	**35**
		Unknown	**4**
Tumor type		**Capsular invasion**	
PTC	48	**Positive**	**28**
FTC	21	**Negative**	**34**
MTC	21	**Unknown**	**38 (38%)**
ATC	10		
TNM stage		**Vascular invasion**	
I	34	**Positive**	**74**
II	29	**Negative**	**26**
III	24 13		
IV			
T stage		**Lymphoid invasion**	
T1	27	**Positive**	**82**
T2	37	**Negative**	**18**
T3	28		
T4	8		
Lymph node		**Extrathyroidal**	
involvement	13	**extension**	**78**
Absent	23	**Absent**	**21**
Present	64	**Present**	**1**
Unknown		**Unknown**	
Tumor size		**Parathyroid gland**	
≤2cm	27	**Positive**	**83**
2–4cm	39	**Negative**	**8**
>4cm	34	**Unknown**	**9**
Tumor location		**Inflammatory disease**	
Left	30	**Absent**	**19**
Right	48	**Present**	**81**
Right and left	22		
Tumor necrosis		**Tumor mitosis**	
Absent	85	**Absent**	**79**
Present	15	**Present**	**21**

ATC: anaplastic thyroid carcinoma; FTC: follicular thyroid carcinoma; MTC: medullary thyroid carcinoma; PTC: papillary thyroid carcinoma; TILs: tumor-infiltrating lymphocytes.

Table 2. Patients characteristics by programmed cell death protein-1/ programmed cell death protein-ligand1 (PD-1/PD-L1) status

Characteristics	PD-1 TUM (N)					PD-L1 TUM (N)					PD-1 TILS (N)					PD-L1 TILS (N)				
	0	1+	2+	3+	*p*	0	1+	2+	3+	*p*	0	<10%	10-40%	>40%	*p*	0	<10%	10-40%	>40%	*p*
Age																				
<45	18	9	0	1	0.387	19	7	0	2	0.841	5	15	5	3	0.593	8	13	6	1	0.597
≥45	54	12	3	3		51	13	6	2		13	43	12	4		13	43	13	3	
Sex																				
Female	42	14	2	3	0.368	41	12	6	2	0.385	14	31	10	6	0.733	12	29	16	4	**0.032**
Male	30	7	1	1		29	8	0	2		4	27	7	1		9	27	3	0	
Tumor type																				
PTC	38	7	0	3	**0.001**	33	12	1	2	**<0.001**	12	28	4	4	**<0.001**	15	26	5	2	**<0.001**
FTC	16	5	0	0		17	2	1	1		1	16	4	0		0	15	6	0	
MTC	16	5	0	0		18	3	0	0		5	13	2	1		6	13	1	1	
ATC	2	4	3	1		2	3	4	1		0	1	7	2		0	2	7	1	
TNM stage																				
I	22	10	0	2	**<0.001**	24	7	1	2	**0.002**	5	18	6	5	**<0.001**	9	17	5	3	**0.008**
II	25	3	0	1		25	4	0	0		8	18	3	0		8	16	5	0	
III	22	2	0	0		17	5	1	1		5	4	7	2		4	18	2	0	
IV	3	6	3	1		4	4	4	1		0	4	7	2		0	5	7	1	
T stage																				
T1	17	8	0	2	**0.002**	19	6	0	2	**<0.001**	5	14	4	4	**0.002**	9	14	2	2	**0.001**
T2	31	6	0	0		31	5	1	0		7	25	3	2		6	22	7	2	
T3	22	4	1	1		19	7	1	1		6	18	4	0		6	19	3	0	
T4	2	3	2	1		1	2	4	1		0	1	6	1		0	1	7	0	
Lymph node involvement																				
Absent	9	1	2	1	0.906	9	2	1	1	0.456	0	9	3	1	0.201	1	8	4	0	0.281
Present	15	7	0	1		12	8	2	1		6	11	5	1		6	12	4	1	

Table 2. Continued...

Tumor size																		
≤2cm	17	8	0	2	0.056	19	6	2	2	**0.031**	5	14	4	4	0.396	9	14	2	2	0.219
2-4cm	33	6	0	0		32	6	0	2		7	27	3	2		6	23	8	2	
>4cm	22	7	3	2		19	8	5	2		6	17	10	1		6	19	9	0	
Tumor location																				
Left	16	10	1	3	**0.005**	18	8	4	0	0.105	6	16	7	1	0.364	7	15	7	1	0.376
Right	41	7	0	0		39	5	1	3		10	26	5	4		12	27	7	2	
Right and Left	15	4	2	1		13	7	1	1		2	13	5	2		2	14	5	1	
Tumor necrosis																				
Absent	66	16	1	2	**0.001**	64	16	2	3	**0.003**	18	52	10	5	**0.001**	20	51	11	3	**0.002**
Present	6	5	2	2		6	4	4	1		0	6	7	2		1	5	8	1	
Tumor mitosis																				
Absent	61	15	0	3	**0.017**	60	14	2	3	**0.009**	17	49	8	5	**0.001**	20	46	10	3	**0.002**
Present	11	6	3	1		10	6	4	1		1	9	9	2		1	10	9	1	
Tumor cell atypia																				
Absent	54	13	0	3	0.070	54	12	1	3	**0.014**	16	42	8	4	**0.007**	19	40	9	2	**0.002**
Present	18	8	3	1		16	8	5	1		2	16	9	3		2	16	10	2	
Encapsulation																				
Absent	17	4	1	3	0.192	17	7	0	1	0.421	8	10	4	3	0.766	9	9	5	2	0.615
Complete	30	5	0	1		28	5	1	2		4	24	7	1		5	22	8	1	
Partial	23	10	2	0		22	8	5	0		5	22	6	2		5	23	6	1	
Extrathyroidal extension																				
Absent	58	16	1	3	0.218	59	15	2	2	**0.021**	16	46	11	5	0.161	18	44	12	4	0.139
Present	13	5	2	1		11	5	4	1		2	12	6	1		2	12	7	0	
Inflammatory disease																				
Absent	12	6	1	0	0.165	10	7	2	0	0.222	2	10	7	0	**0.038**	2	11	6	0	0.975
Present	60	15	2	4		60	13	4	4		16	48	10	7		19	45	13	4	

TUM: tumor cells; TILs: Tumor-infiltrating lymphocytes; PTC: papillary thyroid carcinoma; FTC: follicular thyroid carcinoma; MTC medullary thyroid carcinoma; ATC anaplastic thyroid carcinoma

Correlation between PD-1 and PD-L1 Expression

According to the analysis performed by the Spearman test, it was found that PD-1 expression in tumor cells had a significant positive correlation with PD-1 in TILs (p<0.001, r=0.393) and PD-L1 expression in tumor cells (P <0.001, r=0.499) and TILs (p=0.001, r=0.316). Moreover, there was a significant positive correlation between PD-L1 expression in tumor cells and PD-1 and PD-L1 in TILs (p<0.001, r=0.378 and p=0.001, r=0.298; respectively). Furthermore, PD-1 expression in TILs had a significant positive correlation with PD-L1 in TILs (p<0.001, r=0.680). Table 3 shows the correlations between PD-1/PD-L1 statuses.

According to Spearman's test, PD-1 expression in tumor cells showed a weak positive correlation with PD-1 in TILs (p<0.001, r=0.393), a moderate positive correlation with PD-L1 expression in tumor cells (p<0.001, r=0.499), and a weak positive correlation with PD-L1 in TILs (p=0.001, r=0.316). Additionally, PD-L1 expression in tumor cells correlated positively with PD-1 and PD-L1 in TILs (p<0.001, r=0.378, which is considered weak, and p=0.001, r=0.298, also considered weak, respectively). Furthermore, PD-1 expression in TILs was strongly positively correlated with PD-L1 in TILs (p<0.001, r=0.680). Table 3 summarizes these correlations between PD-1/PD-L1 statuses.

Table 3. **Spearman correlation between PD-1/PD-L1 status across all cancer types**

Variables		PD-1 Tumor	PD-L1 Tumor	PD-1 TILs	PD-L1 TILs
PD-1 expression in Tumor tissue	Correlation Coefficient	1.000	0.499**	0.393**	0.316**
	Sig. (2-tailed)	.	**0.000**	**0.000**	**0.001**
	N	100	100	100	100
PD-L1 expression in Tumor tissue	Correlation Coefficient	0.499**	1.000	0.378**	0.298**
	Sig. (2-tailed)	**0.000**	.	**0.000**	**0.003**
	N	100	100	100	100
PD-1 expression by TILs	Correlation Coefficient	0.393**	0.378**	1.000	0.680**
	Sig. (2-tailed)	**0.000**	**0.000**	.	**0.000**
	N	100	100	100	100
PD-L1 expression by TILs	Correlation Coefficient	0.316**	0.298**	0.680**	1.000
	Sig. (2-tailed)	**0.001**	**0.003**	**0.000**	.
	N	100	100	100	100

PD-1: programmed cell death protein-1; PD-L1: programmed cell death protein-ligand1; TILs: Tumor-infiltrating lymphocytes; Statistical significance levels are indicated as follows: (* p<0.05, ** p<0.01, *** p<0.001, ****p<0.0001).

DISCUSSION

This study aimed to investigate the expression of PD-1 and PD-L1 in tumor cells and TILs of thyroid cancer and determine their association with the patients' clinicopathological characteristics. Unlike previous studies that investigated PD-1/PD-L1 expression in tumor cells or TILs in 1 or 2 types of thyroid cancer, we studied and compared the expression of these molecules in 4 types, including PTC, FTC, MTC, and ATC, for the first time.

Our study showed that PD-1 expression in TILs in different types of thyroid cancer was significantly different from each other. It seems that this significant difference is primarily due to the high expression of PD-1 in the TILs of the ATC type. ATC is the most aggressive type of thyroid cancer, and the infiltration of TILs in this type is much more than in other types. As a result, it can be inferred that a significant enhancement of PD-1 in TILs in ATC is due to the increased aggressiveness of this type and higher infiltration of TILs into the ATC tumors. Similar to our results, several previous studies proved a significant enhancement expression of this molecule in TILs of PTC, ATC, and MTC.[19,23,24] Additionally, we demonstrated the presence of PD-1 on the surface of thyroid tumor cells.

In 28% of the patients (10% PTC, 5% FTC, 5% MTC, and 8% ATC), PD-1 expression in tumor cells

was observed for the first time. The expression of PD-1 in tumor cells across different types of thyroid cancer exhibited significant variation. Notably, prior research on PD-1 expression in thyroid cancer has not reported its presence in tumor cells. However, PD-1 expression has been documented exclusively in tumor cells of certain cancers, including melanoma, lung, and liver cancers.[25,26] There are conflicting findings regarding the implications of PD-1 expression in tumor cells. Wang et al conducted a study on lung cancer cells and found that the interaction of PD-1 with its ligands can inhibit the extracellular signal-regulated kinase (ERK) and Ak strain transforming (AKT) pathways, leading to tumor growth inhibition.[27] Conversely, Li et al, in their study on hepatocellular carcinoma cells, concluded that PD-1 interaction with its ligand activates the mammalian target of the rapamycin (Mtor) pathway, resulting in increased tumor cell proliferation.[25,28] In our study, we primarily observed PD-1 expression in thyroid tumor cells of patients with advanced stages (Table 2). These findings suggest that PD-1 expression in thyroid tumor cells may potentially promote tumor proliferation and aggressiveness in these patients. However, further studies are necessary to precisely determine the consequences of PD-1 expression in thyroid tumor cells.

In addition, we investigated the expression of PD-L1 in tumor cells and TILs of thyroid cancer. Our study revealed that 30% of the patients (15% PTC, 4% FTC, 3% MTC, and 8% ATC) exhibited PD-L1 expression in tumor cells, while 79% of the patients (33% PTC, 21% FTC, 15% MTC, and 10% ATC) showed PD-L1 expression in their TILs. Furthermore, we observed significant differences in PD-L1 expression in tumor cells and TILs among the various types of thyroid cancer. Bi et al, reported a PD-L1 expression rate of 21% in both tumor cells and TILs among MTC patients.[24] Rosenbaum et al, reported a positive PD-L1 expression rate of 25% in ATC patients.[29] A comparative study conducted by Ahn et al, showed that PD-L1 was expressed in 6% of PTC patients, 7.6% of FTC patients, and 22.2% of ATC patients.[18,30] Additionally, a meta-analysis study by Wan et al, demonstrated a significant increase in PD-L1 expression across the 4 types of thyroid cancer.[31] PD-L1 has emerged as a potential biomarker for predicting prognosis, disease recurrence, and guiding treatment planning in thyroid cancer patients.[12,31,32]

The present study showed that PD-1 and PD-L1 expression in tumor cells and TILs was significantly associated with the TNM stage of the patients. In this regard, the PD-1 /PD-L1 expression in stage IV was found to be significantly higher than in the other stages. Higher expression of the PD-1 and PD-L1 expression in the advanced stage may suggest a critical role for these molecules in thyroid cancer progression. Similarly, Uhercik et al showed a significant association between PD-1 expression and the TNM stage in breast cancer patients. The current study revealed a significant association between PD-1 and PD-L1 expression in both tumor cells and TILs and the TNM stage of the patients. Specifically, PD-1 and PD-L1 expression in stage IV was notably higher compared to other stages. This heightened expression in advanced stages may indicate a crucial role for these molecules in thyroid cancer progression.[33] Ma et al, found a significant relationship between the expression of PD-L1 in cholangiocarcinoma cells and TILs with the TNM stage.[33,34] In contrast, several previous studies have demonstrated no significant association between PD-L1 expression and the TNM stage in different types of thyroid cancer.[18,19,23,24] In our study, we explored the relationship between 3 crucial parameters—tumor cell mitosis, tumor cell necrosis, and tumor cell atypia—and the expression of PD-1 and PD-L1 molecules in thyroid tumors for the first time. Our analysis revealed a significant association between the expression of both PD-1 and PD-L1 in tumor cells and TILs at the tumor site and tumor cell mitosis and necrosis. Additionally, we found a significant association between the expression of PD-1 and PD-L1 in TILs and the expression of PD-L1 in tumor cells with tumor cell atypia. Moreover, we observed that tumor cell mitosis, necrosis, and atypia were more prevalent in patients with aggressive tumor types and advanced stages. Interestingly, the expression of PD-1 and PD-L1 was also higher in these patients. Therefore, we propose that these significant relationships may be linked to aggressive behaviors and tumor progression in thyroid cancer. The tumor microenvironment of thyroid tumors is full of immune inhibitory elements. One of the most important of them is immune checkpoint molecules. Blocking of these molecules and improving immune responses against tumors are recently considered a promising approach in the treatment of thyroid cancer. In this regard, it has been reported that spartalizumab and pembrolizumab, both as anti-PD-1 monoclonal antibodies, improved the survival rate of patients with ATC. The tumor microenvironment of thyroid tumors is

rich in immune inhibitory elements, with immune checkpoint molecules playing a crucial role among them. Blocking these molecules and enhancing immune responses against tumors have emerged as promising approaches in the treatment of thyroid cancer. In this context, it has been reported that spartalizumab and pembrolizumab, both anti-PD-1 monoclonal antibodies, have improved the survival rate of patients with ATC.[35,36] However, recent investigations suggest that a combination of immune checkpoint inhibitors will be much more effective than single immune checkpoint blockade therapies. In this respect, numerous studies have shown that the expression of these molecules is related to each other.[36,37]

Before drawing conclusions, it is crucial to acknowledge the limitations of our study. Firstly, the relatively small sample size of our study cohort may limit the generalizability of our findings to a broader population of thyroid cancer patients. Additionally, the retrospective nature of our study design may introduce biases and limitations inherent to retrospective data analysis, such as incomplete or missing data. Furthermore, while we investigated the relationship between PD-1 and PD-L1 expression and various clinicopathological parameters, the observational nature of our study prevents us from establishing causality between these variables. Moreover, the lack of functional studies limits our understanding of the mechanistic implications of PD-1 and PD-L1 expression in thyroid cancer progression. Additionally, our study primarily focused on PD-1 and PD-L1 expression, potentially overlooking other immune checkpoints and molecular pathways that could contribute to thyroid cancer progression. These limitations should be taken into account when interpreting the findings of our study and designing future research endeavors in this field.

In conclusion, our study demonstrated a significant positive correlation between the expression of PD-1 and PD-L1 in tumor cells and TILs. This finding is consistent with the results of Bai et al, who also reported a significant correlation between PD-1 and PD-L1 expression in PTC.[23] Additionally, Shi et al, have reported a significant correlation among PD-1, PD-L1, Tim-3, and cytotoxic T-lymphocyte associated protein 4 (CTLA-4) in medullary thyroid cancer, which were associated with tumor recurrence and advanced disease stages.[38] In summary, our study introduces novel insights into the expression patterns of PD-1 and PD-L1 molecules and elucidates their critical correlations with major disease features. Furthermore, these findings have potential implications for the design of combinational immunotherapy strategies, particularly involving immune checkpoint inhibitors.

STATEMENT OF ETHICS

The study was approved by the Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.REC.1398.1211).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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