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Association of immune response with overall and disease-free survival in laryngeal squamous cell carcinomas

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ABSTRACT

Objectives: This study aimed to examine the relationship between checkpoint receptors (PD-1, PD-L1, PD-L2, CTLA-4) and lymphoid infiltration level (TILs) with prognostic features of patients with laryngeal squamous cell carcinoma (LSCC).

Methods: A retrospective study was designed at a tertiary referential university hospital between April 2008 and December 2020. The surgical specimen of the patients who met the eligibility criteria were re-examined histopathological, sociodemographic, clinical, pathological, and follow-up findings of patients were determined. The impact of PD-1, PD-L1, PD-L2, CTLA4, and TILs levels for the presence of cancer recurrence, disease-specific mortality, overall survival (OS), disease-free survival (DFS) was investigated.

Results: Forty-five patients with LSCC were included in the study. The mean follow-up period was 48.3 ± 14.3 months (min: 36, max 84). TILs scores were detected significantly lower in patients with distant metastasis and recurrence ($p = 0.046$ and 0.010). Also, only TILs was a significant risk factor for recurrence and survival among the PD-1, PD-L1, PD-L2, CTLA-4, and TILs (HR = 0.217 CI: 0.070–0.679, $p = 0.009$ and HR = 0.566, CI: 0,321–980, $p = 0.048$). Similarly, for the TILs score: > 1 was significant for DFS. (Long-Rank = 0.009). The examined markers and TILs scores were not a significant predictive factor for OS.

Conclusion: An increase in TILs density in LSCCs is associated with a better prognosis. However, PD-1, PD-L1, PD-L2, CTLA-4 could not be associated with prognosis. Controlled studies combined with immunotherapy treatment results are needed to reveal their role as a marker and prognostic factor of the anti-tumor immune response.

1. Introduction

Squamous cell carcinoma of the larynx (LSCC) remains the most common cancer carcinoma of the upper airways following lip and oral cavity carcinoma. The majority of patients are diagnosed at advanced stages, and unfortunately, despite advances in diagnosis and treatment, 5-year survival rates are reported to be between 63 and 66% [1]. Therefore, oncological research for advanced LSCC and metastatic disease has focused on tumor biology and continues to develop prognostic markers and potential therapeutic targets.

Tumor-infiltrating lymphocytes (TILs) are considered to be the most important effectors of the host anti-tumor immune response and have been associated with a better prognosis [2,3]. Programmed death receptor 1 (PD-1) is a transmembrane receptor in protein structure and plays a role in hiding the cancer cell from immune response. PD-1 is expressed on the surface of T cells and regulates the immune response by inhibiting the activation of lymphocytes. It has two ligands, PD-L1 and PD-L2. The interaction of the PD-1 receptor with its ligands is an example of an escape mechanism from the control of the immune system by intensifying the negative signal transmitted to tumor-infiltrating lymphocytes, impairing the function of tumor cells. In recent studies, PD-1 and its ligand PD-L1 were found to be significantly higher in laryngeal cancer than in normal healthy tissue [4].

In recent years, immunotherapy with monoclonal antibodies that

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inhibit PD-1 has been an important development in the treatment of neoplastic diseases. Immunotherapy with the anti-PD1 antibody pembrolizumab and nivolumab has been approved by the US Food and Drug Administration and The European Commission for patients with platinum-resistant, recurrent or metastatic head and neck squamous cell carcinoma (HNSCC). PD-1 monoclonal antibodies (nivolumab and pembrolizumab) were initially approved in patients with recurrent and metastatic, unresponsive HNSCC to platinum and fluorouracil, and PD-L1 expression in tumor tissue with a tumor proportion score of ≥50%. After 2019, PD-1 inhibitors were approved as first-line treatment in patients with metastatic or unresectable, recurrent HNSCC, either alone or in combination with ≥1 combined positive score in immunohistochemical staining [5,6].

Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), a negative regulator of T cell activation, is expressed in response to the immune process and inhibits the uncontrolled immune activity. CTLA-4 prevents the formation of chronic autoimmune inflammation and becomes a preventative factor for the development of the endogenous immune response in the antitumor response. Tumor cells benefit from negative immune regulation of CTLA-4 and generate signals that inhibit T cell activation via CTLA-4. So, the most important examples of immune checkpoint receptors are CTLA-4 and PD-1 [7,8]. PD-1 and CTLA 4 levels were found significantly associated with the immune response to the tumor and the degree of infiltration of cancer tissue by TIL [9–11].

Therefore, this study, primarily, was aimed to examine the relationship between PD-1, PD-L1, PD-L2, CTLA-4, and TILs levels in tumor tissue with clinical, pathological, and prognostic features of the disease. Secondly, it aimed to examine the effects of the levels of these markers on disease-free survival (DFS) and overall survival (OS).

2. Materials and methods

This retrospective study was designed at a tertiary referential university hospital between April 2008 and December 2020. Patients with a diagnosis of laryngeal squamous cell carcinoma (LSCC) were identified from the archive records. The eligibility criteria were to have undergone a total laryngectomy with neck dissection, not to have received preoperative radiotherapy or systemic chemotherapy, not to have had a partial laryngectomy before, and to have a preoperative PET/CT scan. The surgical specimen of the patients who met the eligibility criteria were re-examined histopathologically. Patients who have not continued follow-up visits after surgery and lost due to intraoperative complications were not included in the final analysis.

Age, gender, smoking, T stage of LSCC, and follow-up period were determined. The presence of cancer recurrence, overall survival rate, time of recurrence or loss was also determined. Perineural and vascular invasion, lymph node metastasis, lymphoid response to the tumor (absent, mild, moderate, prominent) were examined in the pathology specimen. The presence of distant metastases was determined. Immunohistochemical (IHC) staining was repeated for PD-1, PD-L1, PD-L2, and CTLA4, and the percentage of staining was determined by two different pathologists. Inflammatory response to tumor (TILs) was scored 0: absent, 1: mild, 2: moderate, and 3: prominent and evaluated separately. While determining the percentages of IHC staining, the average of two different measurements was taken. Inter-class correlation coefficient (Cronbach's Alpha) and *p*-value were calculated for two different examination.

2.1. Tissue microarray (TMA)

H&E stained preparations of the patients included in the study which contained primary tumor tissue, and representative tumoral areas were chosen for sampling. From tumoral areas detected in archived paraffin blocks of these selected preparations, 1.5 mm-diameter tissue cores per donor block were punched out and transferred to a recipient block with a maximum of 24 cores using a tissue microarrayer [12]. Thin sections (4 μm) were consecutively cut from the tissue microarrays and transferred to polylysine-coated glass slides. H&E staining was performed for confirmation of tumor tissue.

Table 1
Summary of clinical and pathological features.

		n	%
Gender	Female	2	4.4
	Make	43	95.6
Smoking	−	3	6.7
	+	42	93.3
T stage	pT3	14	31.
	pT4	31	68.
Neural invasion	−	37	84.1
	+	7	15.9
Vascular invasion	−	33	75.0
	+	11	25.0
Lymphoid response	No	9	20.0
	Mild	17	37.8
	Moderate	9	20.0
	Severe	10	22.2
pN	−	21	48.8
	+	22	51.2
Distant metastasis	−	34	75.6
	+	11	24.4
Recurrence	−	33	73.3
	+	12	26.7
Survival	Ex	4	8.9
	Yes	41	91.1
Adjuvant RT	−	5	11.4
	+	39	88.6
Adjuvant CT	−	24	54.5
	+	20	45.5

2.2. Immunohistochemistry (IHC)

Serial sections of TMA were baked over-night at 60 °C, dewaxed in xylene and exposed to graded alcohol for gradual hydration. The sections were treated with heat-induced epitope retrieval in microwave (in 10 mM/L citrate buffer, pH 6.0, for 20 min, followed by cooling at room temperature for 20 min). Blocking of endogenous peroxidase and biotin were performed by 3%H_2O_2. Samples were incubated with primary anti-PDL-1 (Abcam, ab205921), anti PD-1 (CD279, Bioss, bs1867-R); anti PDL-2 (B7-DC, Bioss, bs1868-R) anti-CTLA4 (Santacruz, sc376016) antibodies at dilution of 1:200 for 60 min for all four. Negative controls were analyzed on sections incubated using non-immune mouse serum devoid of primary antibody. Secondary antibody was performed and visualization was done by diaminobenzidine with hematoxylin counterstain.

PD-1, PD-L1, PD-L2, CTLA-4, and TILs values were compared between study groups by *t*-test or Mann-Whitney *U* test. Cut-off values were determined for these parameters by ROC analysis and the area under the curve (AUC) was determined. According to this determined cut-off value, patients were divided into groups as < cut-off or ≥ cut-off for each IHC marker. PD-1, PD-L1, PD-L2, CTLA-4, and TILs were examined by Cox regression analysis as an independent risk factor for cancer recurrence and survival, and Hazard Ratio was calculated. Long-Rank comparisons and Kaplan Meier analysis were made to determine possible risk factors associated with recurrence and survival among the groups made according to obtained cut-off values.

SPSS 22.0 program (IBM Corp., Armonk, NY, USA) was used for statistical analysis. The study was carried out in accordance with the 1964 Helsinki Declaration and subsequent amendments. The institutional review board was approved by the ethic committee of affiliated university for the study.

3. Results

Forty-five patients diagnosed with laryngeal squamous cell

Table 2
Summary of scale variables.

	m	SD	m	Min.	Max.
Age	61.7	9.4	60	38.0	89
Follow-up (months)	48.3	24.3	48	9.0	84
Disease-free survival	43.8	26.7	48	6.0	84
PD-1	19.0	13.8	15	0.0	60.00
PD-L1	24.8	22.3	20	0.0	80.00
PD-L2	21.2	21.9	15	0.0	80.00
CTLA-4	16.2	16.0	13	0.0	70.00
TILs score	0.9	0.7	1	0.0	3.0

carcinoma (LSCC) were included in the study. Forty-three (% 95.6) of the patients were male and the mean age of the patients was 61.7 ± 9.4 (min: 38, max: 89). The clinical and pathological features of the patients and findings for IHC staining of the specimens are given in Tables 1 and 2. Among the inflammatory markers, the most intense staining was detected for PD-L1 (24.8 ± 22.3%).

The mean follow-up period after surgery was 48.3 ± 14.3 months (min > 36). The inflammation score detected in the tumor tissue was found to be significantly lower in patients with distant metastasis and recurrence ($p = 0.046$ and 0.010, Mann Whitney U test). Univariate analysis of PD-1, PD-L1, PD-L2, CTLA-4 inflammatory markers according to clinical and pathological variables is given in Table 3. According to Cox regression analysis, only TILs were found to be a significant risk factor for recurrence and survival among the PD-1, PD-L1, PD-L2, CTLA-4, and TILs variables (HR = 0.217 CI: 0.070–0.679, $p = 0.009$ and HR = 0.566, CI: 0.321–0.980 $p = 0.048$).

According to ROC analysis, the highest sensitivity (0.500) and specificity (0.724) for tumor recurrence among PD-1, PD-L1, PD-L2, CTLA-4, and TILs markers were determined for the 25.0% cut-off value of PD-L1 (AUC = 0.576, $p = 0.448$). However, for the 1.0 cut-off value of TILs, the sensitivity was 0.333 and the specificity was 0.310, for tumor recurrence (AUC = 264, $p = 0.019$, Table 4). The calculated cut-off values of the examined markers for mortality, and the sensitivity and specificity values according to these cut-off values are given in Table 5.

Examined IHC markers were divided into two groups according to the cut-off values obtained from the ROC analysis. These groups were compared for survival and recurrence with Kaplan Meier plots and Long-Rank tests. Accordingly, only TILs >1 values were found to be significant

Table 3
The mean and standard deviation of PD-1, PD-L1, PD-L2, CTLA4, and TILs score according to subgroups.

		PD-1			PDL-1			PD-L2			CTLA-4			TILs		
		m	SD	p	m	SD	p	m	SD	p	m	SD	p	m	SD	p
Gender	Female	22.50	10.61	0.717	50.00	21.21	0.102	10.00	14.14	0.464	30.00	7.07	0.216	1.50	0.00	0.178
	Male	18.82	14.01		23.52	21.87		21.77	22.18		15.55	16.06		0.83	0.69	
Smoking	−	6.67	5.77	0.109	18.33	14.43	0.611	12.50	6.61	0.481	0.83	1.44	0.020	0.50	0.50	0.360
	+	19.91	13.80		25.24	22.86		21.88	22.52		17.37	16.01		0.88	0.70	
Neural invasion	−	18.90	13.63	0.570	25.84	23.51	0.335	19.57	19.91	0.144	16.79	16.78	0.364	0.95	0.70	0.114
	+	15.71	12.39		16.79	14.91		32.86	29.60		10.71	10.07		0.50	0.50	
Vascular invasion	−	20.53	14.36	0.244	24.16	22.98	0.738	21.86	22.21	0.813	14.94	15.61	0.604	0.88	0.64	0.407
	+	14.77	12.37		26.86	22.35		20.00	22.94		17.95	16.88		0.68	0.78	
Lymphoid response	No	18.33	8.75	0.835	24.22	24.65	0.842	24.17	22.29	0.319	16.61	8.67	0.726	0.28	0.57	0.026
	Mild	19.71	17.39		26.32	19.61		18.65	23.31		18.88	21.69		0.88	0.67	
	Moderate	21.44	13.43		19.69	21.23		14.72	19.06		11.94	13.22		1.06	0.53	
	Severe	16.11	11.93		26.83	28.43		29.72	21.88		14.94	11.56		1.15	0.71	
pN	−	17.21	13.07	0.305	19.48	18.87	0.084	19.78	20.22	0.632	12.48	10.63	0.079	0.67	0.68	0.056
	+	21.75	14.85		31.78	24.91		23.14	24.50		21.21	19.18		1.07	0.66	
Distant metastasis	−	17.17	11.13	0.259	27.14	22.40	0.208	21.73	21.34	0.801	17.59	17.78	0.344	0.96	0.63	0.046
	+	25.00	19.86		16.90	21.24		19.77	24.53		12.23	8.52		0.55	0.79	
Recurrence	−	18.52	12.32	0.723	23.61	23.23	0.595	19.55	18.44	0.409	15.16	14.91	0.492	1.02	0.67	0.010
	+	20.21	17.60		27.71	20.43		25.75	29.77		18.96	18.99		0.42	0.56	
Survival	Ex	22.50	16.58	0.599	12.25	8.23	0.244	5.63	9.66	0.137	13.63	3.04	0.738	0.38	0.48	0.145
	Yes	18.63	13.68		26.04	22.96		22.80	22.23		16.49	16.78		0.90	0.69	

Table 4
ROC analysis of variables for recurrence.

Variables	Area	Std. error	p	95% CI		Cut off	Sensitivity	Specificity
				Lower bound	Upper bound			
PD-1	0.489	0.105	0.909	0.283	0.694	18.75	0.417	0.552
PD-L1	0.576	0.098	0.448	0.383	0.769	25.00	0.500	0.724
PD-L2	0.539	0.113	0.699	0.318	0.760	13.75	0.500	0.517
CTLA-4	0.542	0.096	0.678	0.354	0.729	13.75	0.500	0.517
TILs	0.264	0.086	0.019	0.095	0.434	1	0.333	0.310

Table 5
ROC analysis of variables for mortality.

Variables	Area	Std. error	p	95% CI		Cut off	Sensitivity	Specificity
				Lower bound	Upper bound			
PD-1	0.557	0.156	0.709	0.253	0.862	22.50	0.500	0.703
PD-L1	0.301	0.092	0.195	0.119	0.482	12.50	0.750	0.351
PD-L2	0.260	0.124	0.119	0.017	0503	2.0	0.500	0.216
CTLA-4	0.514	0.084	0.930	0.348	0.679	11.25	0.750	0.459
TILs	0.291	0.112	0.173	0.071	0.510	1	0.250	0.378

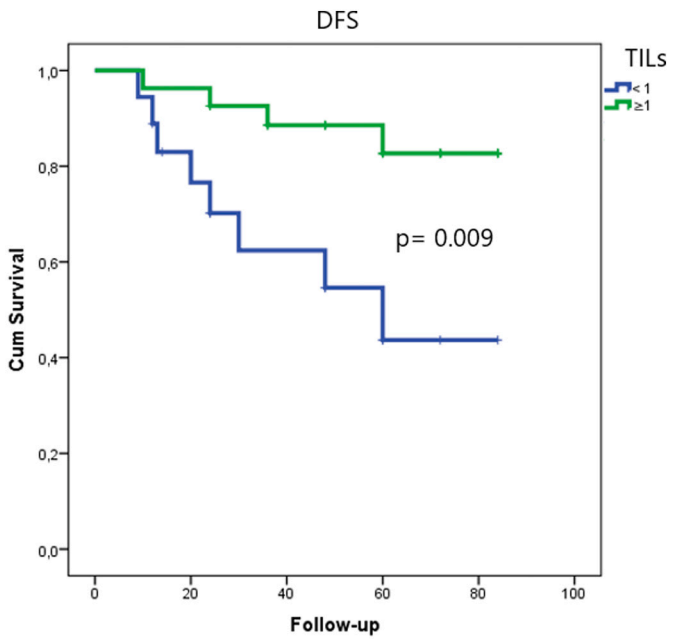


Fig. 1. Kaplan-Meier plot of disease-free survival for '1' cut-off value of TILs.

for DFS (Long-Rank = 0.009, Fig. 1).

Cronbach's Alpha and *p* values for the percentages obtained by 2 different pathologists evaluating IHC staining are as follows; PD-1: 0.049; 0.446, PD-L1: 0.523; 0.025, PD-L2: 0.556; 0.016, CTLA-4: 0.291; 0.752, TILs: 0.460; 0.034 (Figs. 2, 3, 4, 5, 6).

4. Discussion

4.1. Main findings

In this study, biomarkers were expressed as percentages, and TILs were graded as 0–4. Consistent with the general literature, the score of TILs was found to be significantly lower in patients with distant metastases and in patients with recurrence, compared to the univariate analysis. In addition, CTLA-4 was found to be significantly higher in smokers. In addition, among the PD-1, PD-L1, PD-L2, CTLA-4, and the score of TILs variables, only the score of TILs was found to be an independent risk factor for both cancer recurrence and disease-related mortality. Among these variables, only the presence of TILs (score ≥ 1) made a significant positive contribution to only DFS.

The inclusion of these prognosis-related immune checkpoint receptors in the routine pathological examination will provide clinicians with an important insight into the immunotherapy option. Although initiating treatment with immunotherapy alone is an option approved by the literature, it will be a difficult decision for many clinics. However, at least the addition of immunotherapy to conventional chemotherapy regimens may provide favorable prognostic improvement.

4.2. Interpretation

The importance of TILs is increasing as evidence emerges of the prognostic and potentially predictive importance for many different tumor types. For the evaluation of TILs, morphological evaluation of lymphocytic infiltrates on formalin-fixed paraffin-embedded sections, as well as detection of T-cell subpopulations ($CD3^+$, $CD8^+$) or immunophenotyping methods can be used.

Investigation of the immune system and tumor microenvironment in larynx squamous cell carcinomas (LSSC) is important for prognosis and treatment planning. PD-1 and CTLA-4 are the two immune checkpoint receptors most detailed studied as immunotherapy targets. Both receptors regulate their responses at different levels and by different mechanisms and are used by tumor cells to inhibit the antitumoral immune response [13]. Furthermore, clinical responses to immune checkpoint inhibitors are thought to be associated with increased TILs or TIL subtypes [14].

Although most analyzes of PD-1 ligand expression have focused on PD-L1, PD-L2 is another ligand for PD-1. Expression of PD-L2 was found in some HNSCC with the absence of PD-L1 [14,15]. So, in this study, both PD-L1 and PD-L2 ligands were examined.

In studies conducted with different organ cancers, it has been suggested that PD-L1 expression may be correlated with poor or better prognosis, or it does not show any association with prognosis. Obtaining these different results are thought to be related to differences in IHC techniques, the type of cancer, the stage, and the treatment history of cancer [14,16–20].

It is also controversial whether there is a significant relation between PD-L1 expression and prognosis in head and neck cancers. In a meta-analysis for oral SCC, PD-L1 was reported to be associated with poor prognosis. The results of studies investigating the relationship between PD-L1 expression and prognosis in laryngeal cancers similarly had variable results [10,12,21–25].

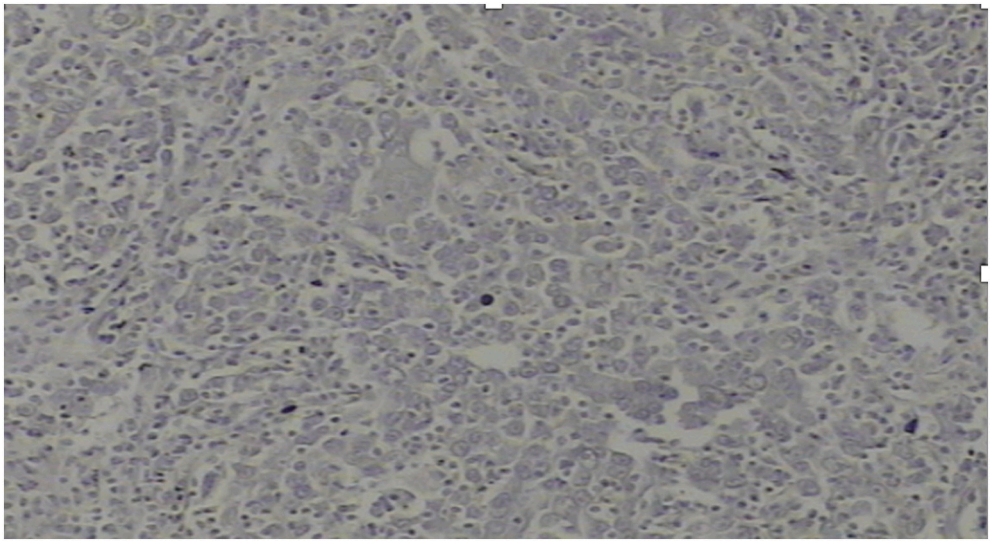


Fig. 2. Example of stromal prominent infiltration of TILs.

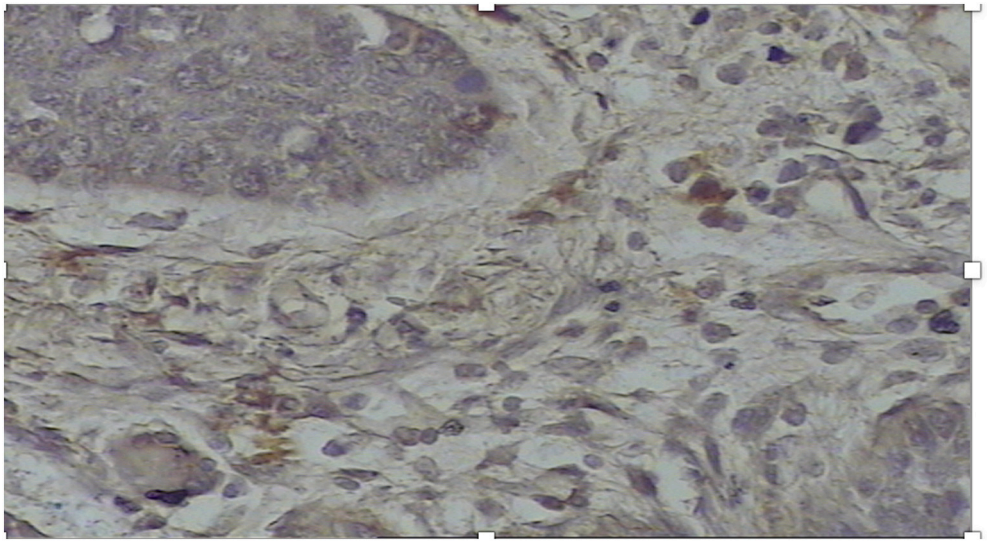


Fig. 3. Immunohistochemical staining for laryngeal cancer tissue with PD-1.

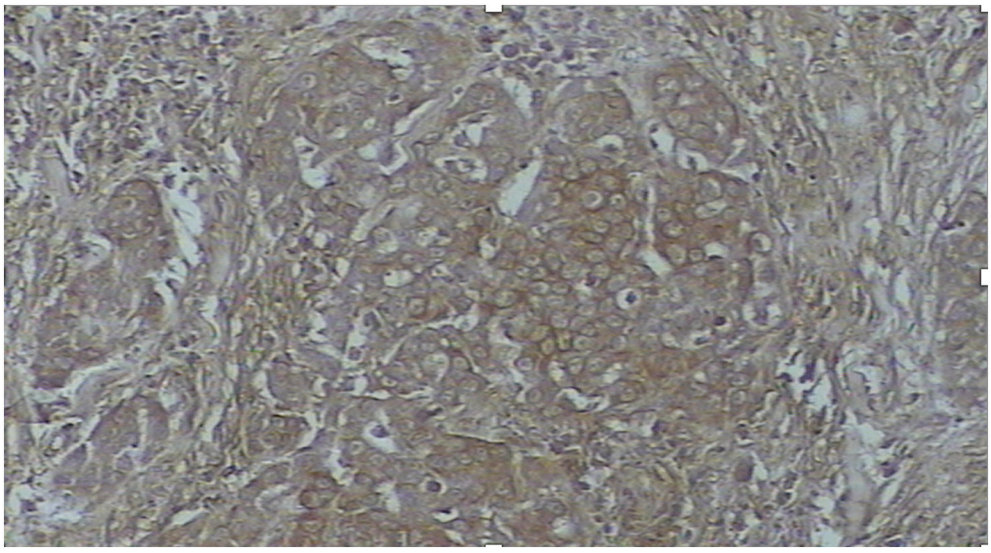


Fig. 4. Immunohistochemical staining for laryngeal cancer tissue with PD-L1.

Birtalan et al. detected an improved disease-specific survival (DSS) in PD-L1 positive (for TILs) laryngeal tumors compared with the PD-L1 negative group. Although, PD-L1 expression on tumor cells had not a significant correlation with DSS. They reported that PD-L1 did not been proven an independent prognostic factor among sex, tumor localization, and stage, in the multivariate analysis. Also, in this study, PD-L1 positivity correlated with CTLA-4 positivity. However, the relevance of PD-L2, CTLA-4, or TILs density to prognosis was not mentioned in this study. In addition, this study also includes other HNSCCs, and the correlation of these receptors and ligands with human papillomavirus (HPV) status was examined [23]. Yu et al. [24] found increased PD-L1 rates in patients with laryngeal carcinoma compared to patients with leukoplakia and stated that PD-L1 is an important factor of tumor microenvironment by immunosuppression. Additionally, they reported that the patients with a high expression of PD-L1 also had a larger size, higher differentiation, and lower N stage cancer. But they did not detect a significant association between PD-L1 expressions with TNM stage or mortality rate [24].

Karpathiou et al. [13] found while highly T lymphocyte and dendritic cell response in HNSCC was associated with good prognosis. They showed that While PD-L1 expression had a significant correlation with rich lymphocytes infiltration and lower aggressive cancer, it did not affect prognosis. CTLA4 expression by tumor cells was associated with worse DSS and OS but the lymphocytic expression of CTLA-4 was not associated with prognosis. Additionally, CTLA-4 expression by lymphocytes was significantly associated with rich lymphocytic response, less aggressive cancer, and higher N stage. Also, no association was shown between PD-L1 and CTLA-4 expression [13].

The use of PD-L1 as a biomarker in head and neck cancers is confusing due to heterogeneous expression in the tumor and lymphocytes, the absence of a cut-off value for positivity, different antibody clones, and variable scoring systems for measurement. PD-L1 expression was evaluated as 0 (<6%), 1 (6%–25%), 2 (6%–50%), 3 (51%–75%) and 4 (positive rate > 75%), or with a semi-quantitative scale for 4 grades: grade 0 (negative), grade 1 (weak positive), 2 (moderately positive), and 3 (strongly positive) by Yu et al. [24]. Vassilakopoulou et al. [12] determine a scale for density of TILs as <30%, 30–60% and >60% and survival analysis performed according to this groups. Additonally, they found a cut-off value 59% of PD-L1 for analysis. They observed no statistically significant correlation between TILs and T stage, grade, nodal

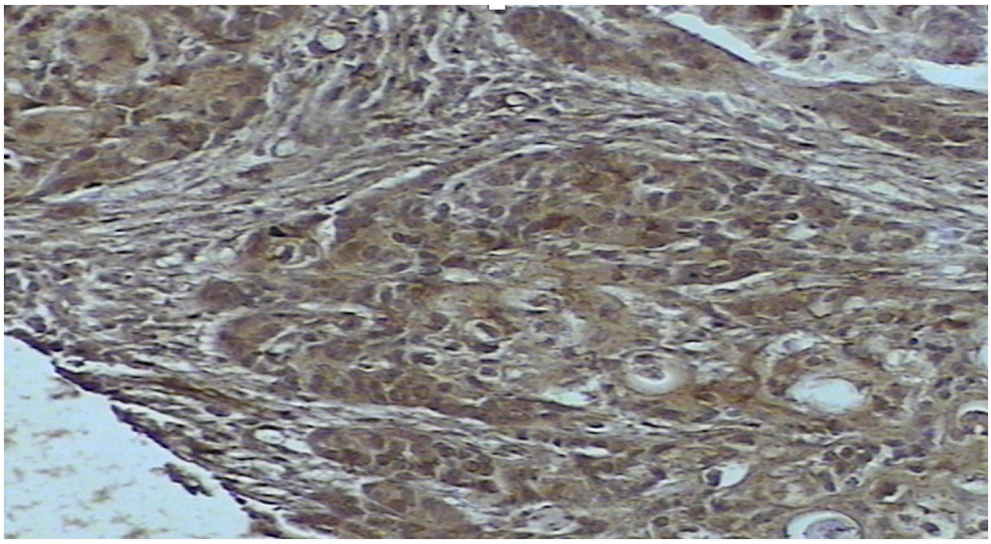


Fig. 5. Immunohistochemical staining for laryngeal cancer tissue with PD-L2.

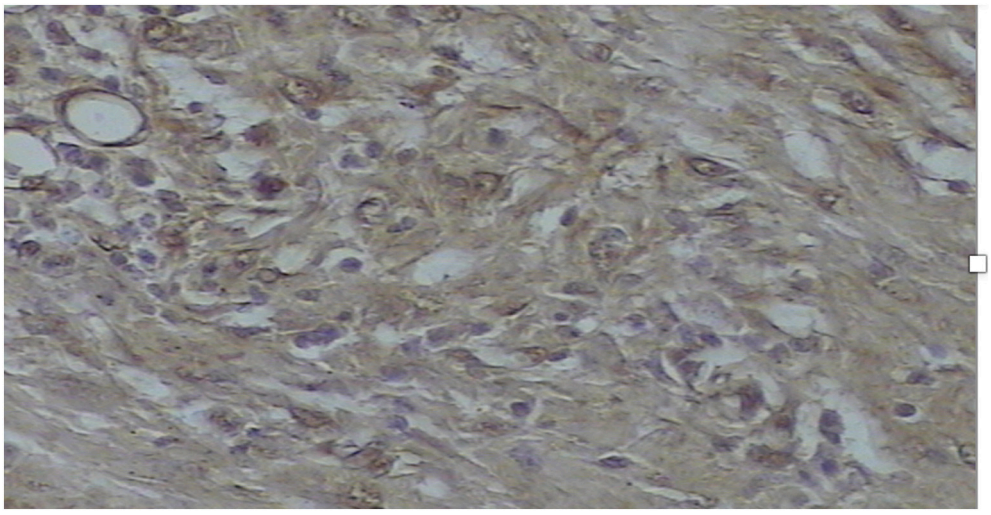


Fig. 6. Immunohistochemical staining for laryngeal cancer tissue with CTLA-4.

stage, or smoking behavior. They noted lower expression of PD-L1 in smokers, but stronger staining in node-positive patients. They found that the density of stromal TILs and overexpressing PD-L1 was associated with superior survival outcome [12]. In addition, it is important to evaluate other processes such as angiogenesis together in order to create a standard profile for the selection of suitable candidates for immunotherapy, as the presence of other immunoreceptors is also effective in prognosis [26].

4.3. Strengths and limitations

The International Immuno-Oncology Biomarkers Working Group has recommended a standardized methodology for the evaluation of TIL for head and neck tumors [27]. In our study, morphological evaluation of TILs in accordance with this methodology was performed. Recent studies applied the TILs scoring guidelines recommended by this consensus [27] for LSSC, and both studies found that the TILs score was an independent prognostic factor for disease-free and overall survival [12,28,29]. Morphological evaluation of TILs with the suggested method provides quick identification of patients with a high risk of cancer recurrence and short survival without extra cost in routine clinical practice. Tissue microarrays (TMA) allow simultaneous analysis of multiple case samples on a single slide, saving time, and this method was used and suggested in similar studies previously [10,12,23,30]. However, there is a risk of misrepresenting the entire tumor due to intratumoral expression heterogeneity. For this reason, 2 samples were prepared per patient specimen in our study. The retrospective design of our study and the small number of cases are main limitations of this study. In addition, the inability to compare with healthy tissue is another limitation. None of these patients received immunotherapy, so it is a deficiency that these markers, an oncological treatment target, cannot be correlated with treatment outcomes.

5. Conclusion

An increase in TILs density in LSCCs is associated with a better prognosis. However, PD-1, PD-L1, PD-L2, CTLA-4 could not be associated with prognosis. In addition, standard techniques are needed for the objective measurement of the expression of these molecules. However, prospective controlled studies with the expression of immune checkpoint receptors combined with immunotherapy treatment results are needed to reveal their role as a marker and prognostic factor of the anti-

tumor immune response.

CRediT authorship contribution statement

Conceptualization, methodology: AFB, SA.
Formal analysis: Aİ, AFB.
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Declaration of competing interest

The authors declare no conflict of interest.

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References

[1] Steuer CE, El-Deiry M, Parks JR, Higgins KA, Saba NF. An update on larynx cancer. CA Cancer J Clin 2017;67(1):31–50.
[2] Wondergem NE, Nauta IH, Muijlwijk T, Leemans CR, van de Ven R. The immune microenvironment in head and neck squamous cell carcinoma: on subsets and subsites. Curr Oncol Rep 2020 Jun 29;22(8):81. https://doi.org/10.1007/s11912-020-00938-3.
[3] Qin Y, Zheng X, Gao W, Wang B, Wu Y. Tumor microenvironment and immune-related therapies of head and neck squamous cell carcinoma. Mol Ther - Oncolytics [Internet] 2021;20(March):342–51. https://doi.org/10.1016/j.omto.2021.01.011. Available from.
[4] Kowalski A, Malinowska K, Olszewski J, Zielińska-Bliźniewska H. Expression of programmed death receptor 1 (Pd-1) gene and its ligand (pd-l1) in patients with laryngeal cancer. Biomolecules 2021;11(7):1–9.
[5] Seiwert TY, Burtness B, Mehra R, Weiss J, Berger R, Eder JP, et al. Safety and clinical activity of pembrolizumab for treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-012): an open-label, multicentre, phase 1b trial. Lancet Oncol [Internet] 2016;17(7):956–65. https://doi.org/10.1016/S1470-2045(16)30066-3. Available from:.
[6] Rini BI, Battle D, Figlin RA, George DJ, Hammers H, Hutson T, et al. The society for immunotherapy of cancer consensus statement on immunotherapy for the treatment of advanced renal cell carcinoma (RCC). J Immunother Cancer 2019;7(1):1–31.
[7] Rieckmann T, Münscher A, Busch CJ, Dogan VD. Current studies of immunotherapy in head and neck cancer. Clin Otolaryngol 2018 Feb;43(1):13–21. https://doi.org/10.1111/coa.12895. Epub 2017 May 29.
[8] Hussaini S, Chehade R, Boldt RG, Raphael J, Blanchette P, Maleki Vareki S, et al. Association between immune-related side effects and efficacy and benefit of immune checkpoint inhibitors – A systematic review and meta-analysis. Cancer Treat Rev [Internet] 2021;92(October 2020):102134. https://doi.org/10.1016/j.ctrv.2020.102134. Available from.
[9] Liu J, Kong X, Huang T, Wang R, Li W. Clinical Implications of Aberrant PD-1 and CTLA4 Expression for Cancer Immunity and Prognosis: A Pan-cancer Study 2020;11(September).
[10] Alessandrini L, Franz L, Ottaviano G, Ghi MG, Lanza C, Blandamura S, et al. Prognostic role of programmed death ligand 1 (PD-L1) and the immune microenvironment in laryngeal carcinoma. Oral Oncol [Internet] 2020;108(April):104836. https://doi.org/10.1016/j.oraloncology.2020.104836. Available from:.
[11] Sautès-Fridman C, Petitprez F, Calderaro J, Fridman WH. Tertiary lymphoid structures in the era of cancer immunotherapy. Nat Rev Cancer [Internet] 2019;19(6):307–25. https://doi.org/10.1038/s41568-019-0144-6. Available from:.
[12] Vassilakopoulou M, Avgeris M, Velcheti V, Kotoula V, Rampias T, Chatzopoulos K, et al. Evaluation of PD-L1 expression and associated tumor-infiltrating lymphocytes in laryngeal squamous cell carcinoma. Clin Cancer Res 2016;22(3):704–13.
[13] Karpathiou G, Casteillo F, Giroult JB, Forest F, Fournel P, Monaya A, et al. Prognostic impact of immune microenvironment in laryngeal and pharyngeal squamous cell carcinoma: immune cell subtypes, immuno-suppressive pathways and clinicopathologic characteristics. Oncotarget 2017;8(12):19310–22.
[14] Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer [Internet] 2012;12(4):252–64. https://doi.org/10.1038/nrc3239. Available from:.
[15] Yearley JH, Gibson C, Yu N, Moon C, Murphy E, Juco J, et al. PD-L2 expression in human tumors: relevance to anti-PD-1 therapy in cancer. Clin Cancer Res 2017;23(12):3158–67.
[16] Hamanishi J, Mandai M, Iwasaki M, Okazaki T, Tanaka Y, Yamaguchi K, et al. Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. Proc Natl Acad Sci U S A 2007;104(9):3360–5.
[17] Ohigashi Y, Sho M, Yamada Y, Tsurui Y, Hamada K, Ikeda N, et al. Clinical significance of programmed death-1 ligand-1 and programmed death-1 ligand-2 expression in human esophageal cancer. Clin Cancer Res 2005;11(8):2947–53.
[18] Wu C, Zhu Y, Jiang J, Zhao J, Zhang XG, Xu N. Immunohistochemical localization of programmed death-1 ligand-1 (PD-L1) in gastric carcinoma and its clinical significance. Acta Histochem 2006;108(1):19–24.
[19] Ghebeh H, Mohammed S, Al-Omair A, Qattan A, Lehe C, Al-Qudaihi G, et al. The B7–H1 (PD-L1) T lymphocyte-inhibitory molecule is expressed in breast cancer patients with infiltrating ductal carcinoma: correlation with important high-risk prognostic factors. Neoplasia 2006;8(3):190–8.
[20] Hino R, Kabashima K, Kato Y, Yagi H, Nakamura M, Honjo T, et al. Tumor cell expression of programmed cell death-1 ligand 1 is a prognostic factor for malignant melanoma. Cancer 2010;116(7):1757–66.
[21] Lin YM, Sung WW, Hsieh MJ, Tsai SC, Lai HW, Yang SM, et al. High PD-L1 expression correlates with metastasis and poor prognosis in oral squamous cell carcinoma. PLoS One 2015;10(11):1–11.
[22] Chen L, Jin M, Li C, Shang Y, Zhang Q. The tissue distribution and significance of B7–H4 in laryngeal carcinoma. Oncotarget 2017;8(54):92227–39.
[23] Birtalan E, Danos K, Gurbi B, Brauswetter D, Halasz J, Piurko VK, et al. Expression of PD-L1 on immune cells shows better prognosis in laryngeal, oropharyngeal, and hypopharyngeal cancer. Appl Immunohistochem Mol Morphol 2018;26(7):e79–85.
[24] Yu D, Cheng J, Xue K, Zhao X, Wen L, Xu C. Expression of programmed death-ligand 1 in laryngeal carcinoma and its effects on immune cell subgroup infiltration. Pathol Oncol Res 2019;25(4):1437–43.
[25] Yang S-M, Wu M, Han F-Y, Sun Y-M, Yang J-Q, Liu H-X. Role of HPV status and PD-L1 expression in prognosis of laryngeal squamous cell carcinoma. Int J Clin Exp Pathol [Internet] 2021;14(1):107–15.
[26] Franz L, Alessandrini L, Calvanese L, Crosetta G, Frigo AC, Marioni G. Angiogenesis, programmed death ligand 1 (PD-L1) and immune microenvironment association in laryngeal carcinoma. Pathology 2021 Dec;53(7):844–51. https://doi.org/10.1016/j.pathol.2021.02.007.
[27] Hendry S, Salgado R, Gevaert T, Russell PA, John T, Thapa B, et al. Assessing tumor-infiltrating lymphocytes in solid tumors: a practical review for pathologists and proposal for a standardized method from the international immuno-oncology biomarkers working group: part 2: TILs in melanoma. Gastrointestinal Tract Carcinom Adv Anat Pathol 2017;24(6):311–35.
[28] Wang J, Wang S, Song X, Zeng W, Wang S, Chen F, et al. The prognostic value of systemic and local inflammation in patients with laryngeal squamous cell carcinoma. Onco Targets Ther 2016;9:7177–85.
[29] Wang L, Heckmann BL, Yang X, Long H. Osteoblast autophagy in glucocorticoid-induced osteoporosis. J Cell Physiol 2019;234(4):3207–15.
[30] Malm IJ, Rooper LM, Bishop JA, Ozgursoy SK, Hillel AT, Akst LM, et al. Molecular and immunologic analysis of laryngeal squamous cell carcinoma in smokers and non-smokers. Am J Otolaryngol - Head Neck Med Surg [Internet] 2019;40(2):213–7. https://doi.org/10.1016/j.amjoto.2018.11.009. Available from:.