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EXPRESSION OF ANTI-PROGRAMMED DEATH-LIGAND 1 AND ANTI-CLUSTER OF DIFFERENTIATION 8 ANTIBODIES IN ORAL SQUAMOUS CELL CARCINOMA: A CROSS-SECTIONAL STUDY

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ABSTRACT

Objective: To determine the immunohistochemical expression of anti-PD-L1 and anti-CD8 antibodies in oral squamous cell carcinoma **(**OSCC).

Methods: A six-month cross-sectional analysis of 150 histopathologically proven OSCC cases was carried out at Khyber Medical University (KMU) from 1st November 2025 to April 30th, 2026. Formalin-fixed paraffin-embedded (FFPE) tissue sections were stained with anti-PD-L1 (clone 22C3) and anti-CD8 antibodies (clones C8/144B). Biomarker expression was compared to clinicopathological factors using chi-square, independent t-test, one-way ANOVA, Spearman association test, and binary logistic regression.

Results: PD-L1 positivity was found in 56.0% of patients, with significant CD8+ lymphocyte infiltration in 54.0%. Significant correlations were seen between PD-L1 expression and advanced TNM stage (p0.001), poor histological differentiation (p=0.001), lymph node metastases (p=0.008), and strong CD8 infiltration (p<0.001). The number of CD8+ cells differed significantly between well-differentiated and early-stage tumors (p<0.001). There was a moderate positive correlation between PD-L1 expression and CD8 infiltration (r_s=0.421, p<0.001). Logistic analysis identified advanced stage, poor differentiation, lymph node positivity, significant CD8 infiltration, and older age as independent determinants of PD-L1 positivity.

Conclusions: In OSCC, PD-L1 expression was substantially related to aggressive clinicopathological characteristics and increased infiltration of immune cells positive for CD8. A panel of immunological biomarkers that includes both PD-L1 and CD8 could be relevant for patient stratification and treatment selection.

KEYWORDS: Oral squamous cell carcinoma; PD-L1; CD8-positive tumor-infiltrating lymphocytes

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common oral malignancy, accounting for over 90% of all cases.[1] It remains a severe health burden worldwide, particularly in South Asian countries, where tobacco, areca nut, and alcohol usage are common, leading to a disproportionately high illness burden.[2] Despite advances in surgery, radiation, and chemotherapy in recent decades, the 5-year survival rate for OSCC has only slightly increased due to delayed diagnosis, high rates of locoregional recurrence, and cervical lymph node metastases.[3] The biological variability of OSCC emphasizes the necessity of identifying and having access to biomarkers that can improve prognosis accuracy and aid in the creation of customized treatment strategies.[4]

The tumor immune microenvironment is critical to tumor growth because it is not fully dependent on malignant epithelial cells' inherent features.[5] The PD-1/PD-L1 pathway is a critical immune regulatory system that allows tumor cells to elude host immune monitoring.[6] In the tumor microenvironment, PD-L1 is expressed on the membranes of tumor cells and immune cells; it interacts with PD-1 receptors on activated T lymphocytes, inhibiting their proliferation, cytokine generation, and killing function.[7]

On the other hand, CD8+ cells (cytotoxic T lymphocytes) are the primary effectors that recognize and destroy malignant cells.[8] Elevated infiltration of CD8+ T cells is a general indicator of an active anti-tumor immune

response, although it can be dysfunctional in the presence of elevated PD-L1.[9] Thus, assessing PD-L1 expression and CD8-positive TILs would be useful in understanding the immunological profile of OSCC.[10]
The introduction of innovative immunotherapies, such as immune checkpoint inhibitors against the PD-1/PD-L1 pathway, has transformed the treatment of various solid malignancies, including recurrent/metastatic head and neck squamous cell carcinoma.[11] However, the expression of PD-L1 and the density of CD8+ tumor-infiltrating lymphocytes can range dramatically between OSCC patients, as well as across different differentiation, stage, and geographical groups.[12] Furthermore, the relationship between PD-L1 expression and the presence of CD8-positive immune cells varies between studies, with some revealing a positive link, indicating adaptive immunology, while others show an immunosuppressive tumor phenotype.[10, 13, 14]
There is little evidence available on the co-expression of PD-L1 and CD8 in the local OSCC community, and the presence of distinct environmental risk factors and demographic characteristics may influence the tumor's immunobiology. Identifying the expression profile of these immune biomarkers could improve our understanding of TME and provide evidence for the possible use of immunotherapy in this setting. The purpose of this study was to examine the immunohistochemical expression of anti-PD-L1 and anti-CD8 antibodies in OSCC tissue and assess their distribution in relation to the clinicopathological characteristics of the local population..

METHODS

An analytical cross-sectional study was designed at the Department of Oral Pathology, Khyber Medical University, Peshawar. The study lasted for six months, beginning on 1st November, 2025, and ending on April 30, 2026. The sample size was calculated using OpenEpi version 3.01, which is software designed to estimate a single population proportion. Based on Greeshma et al.'s (2023) study, which found PD-L1 positivity in roughly 56% of oral squamous cell carcinoma (OSCC) cases, the population percentage (P) was set at 56%.[15] Using a 95% confidence level (Z = 1.96) and an 8% margin of error, the minimum required sample size was determined to be 150 cases. A non-probability consecutive sampling technique was used. The archived blocks of all patients who had histopathologically confirmed OSCC and met the inclusion criteria during the study period were recruited sequentially until the requisite sample size of 150 was met.
The study comprised patients with histopathologically confirmed primary OSCC who were 18 years old or older. The cases were chosen only if they had enough FFPE tissue blocks for IHC staining and complete clinicopathological data such as age, gender, tumor location, histological grade, and TNM stage. Tissue blocks were excluded from the study if they were insufficient or poorly preserved, if OSCC was recurrent or metastatic in the oral cavity, if the tissue was obtained after neoadjuvant chemotherapy, radiotherapy, or immunotherapy, or if the patient had incomplete clinical or histopathological records.
Demographic and clinicopathological data, including age, gender, tumor site, TNM stage, histological grade, lymph node status, and pertinent clinical history, were extracted from hospital records and entered into a structured data collection form. Block selection was carried out after analyzing hematoxylin and eosin-stained slides to confirm the diagnosis and the existence of live tumor tissue suitable for use as representative tumor blocks. The fresh 4-µm thick slices were placed on slides coated with poly-L-lysine. Monoclonal antibodies (mAbs) against CD8 (clone C8/144B) and PD-L1 (clone 22C3) were utilized for immunohistochemistry in accordance with the manufacturer's standard protocols. This was followed by epitope retrieval using citrate buffer (pH 6.0) and primary antibodies. A horseradish peroxidase polymer detection method with chromogen '3,3′-diaminobenzidine' (DAB) was utilized, and the slides were counterstained with hematoxylin. Each staining was accompanied by positive and negative controls to ensure quality. These antibody clones are among the most commonly used and validated for PD-L1 and CD8 immunohistochemistry in OSCCs.
The slides were reviewed separately by two expert oral pathologists without clinical information, and the results were compared. The percentage of cells with partial or complete tumor membrane staining was utilized to assess PD-L1 expression. Tumors with ≥1% membrane staining were classified as PD-L1 positive. CD8 expression was measured by counting positive tumor-infiltrating cells in five high-power fields (400×) and averaging the results for each case. After splitting the cases into two groups based on the median CD8 count, they were classified as having low or high CD8 infiltration.

The data obtained were entered into Microsoft Excel and analyzed with IBM SPSS Statistics version 26.0. Continuous data, including age and CD8+ lymphocyte counts, were provided as mean ± SD. Categorical variables such as sex, tumor site, histological grade, TNM stage, PD-L1 expression status, and CD8 infiltration category were represented as frequencies and percentages. The Shapiro-Wilk test was used to determine if the continuous variables were normally distributed. The chi-square test was performed to examine the relationship between PD-L1 expression and clinicopathological factors.

The independent-samples t-test was performed to compare the number of CD8+ cells in two groups. A one-way ANOVA was performed to compare more than two groups. The relationship between PD-L1 expression and the presence of CD8+ tumor-infiltrating lymphocytes was determined using Spearman's rank association coefficient. Variables having a p-value <0.20 in univariate analysis were included in a multivariable binary logistic regression model to assess the independent variables for PD-L1 positivity. Adjusted odds ratios (AORs) with 95% confidence intervals (CIs) were calculated, with a p-value <0.05 indicating statistical significance.

RESULTS

The study comprised 150 cases of histopathologically diagnosed OSCC. The average age of participants was 55.8 ± 11.9 years, with 69.3% being above 50. The study population consisted of 68.0% males. The most common sites of involvement were the buccal mucosa (40.7%) and the tongue (26.0%). The most common stage of most cancers was III-IV (64.0%), with cervical lymph node involvement in 58.0% of patients. The tumors were classified histologically as well differentiated (45.3%), moderately differentiated (38.0%), and poorly differentiated (16.7%). Immunohistochemical staining revealed that 56.0% of the patients were PD-L1 positive, with 54.0% having a high CD8-positive tumor-infiltrating cell count. The average number of CD8-positive cells was 46.5 ± 17.3 per HPF. (Table 1; Figure 1)

Table 1. Demographic, Clinicopathological and Immunohistochemical Characteristics of Patients with Oral Squamous Cell Carcinoma (n=150)

Variable	Category	n (%) / Mean ± SD
Age (years)	Mean ± SD	**55.8 ± 11.9**
	<50 years	46 (30.7)
	≥50 years	104 (69.3)
Gender	Male	102 (68.0)
	Female	48 (32.0)
Tumor Site	Buccal mucosa	61 (40.7)
	Tongue	39 (26.0)
	Gingiva	20 (13.3)
	Floor of mouth	15 (10.0)
	Palate	15 (10.0)
Histological Grade	Well differentiated	68 (45.3)
	Moderately differentiated	57 (38.0)
	Poorly differentiated	25 (16.7)
TNM Stage	I–II	54 (36.0)
	III–IV	96 (64.0)
Lymph Node Status	Negative	63 (42.0)
	Positive	87 (58.0)
PD-L1 Expression	Positive	84 (56.0)
	Negative	66 (44.0)
CD8 Infiltration	High	81 (54.0)
	Low	69 (46.0)
CD8 Cell Count (cells/HPF)	Mean ± SD	**46.5 ± 17.3**

Significantly higher CD8 cell counts were observed among patients aged <50 years (p=0.046), those with well-differentiated tumors (p<0.001), and those with early-stage disease (p<0.001). No statistically significant difference in CD8 cell counts was found between male and female patients (p=0.328). (Table 2)

Table 2. Normality Assessment and Comparison of CD8 Cell Counts According to Clinicopathological Characteristics

Variable	Category	Mean CD8 cells/HPF Mean± SD	Test Statistic	p-value
Age	<50 years	49.8 ± 15.4	t=2.01	0.046*
	≥50 years	45.0 ± 17.9		
Gender	Male	45.2 ± 17.5	t=0.98	0.328
	Female	49.1 ± 16.8		
Histological Grade	Well differentiated	54.3 ±15.1	F=14.87	<0.001*
	Moderately differentiated	44.8 ±15.6		
	Poorly differentiated	32.5 ±12.7		
TNM Stage	I–II	53.6 ±14.2	t=4.11	<0.001*
	III–IV	42.5 ±17.0		

Advanced TNM stage (p=0.001), poor histological differentiation (p=0.001), lymph node metastasis (p=0.008), high CD8+ lymphocyte infiltration (p<0.001), and older age (p=0.043) were all significantly associated with positive PD-L1 expression. However, no significant association was observed with gender (p=0.389) or tumor site (p=0.565). (Table 3)

Table 3. Association of PD-L1 Expression with Clinicopathological Characteristics and CD8 Infiltration

Variable	Category	PD-L1 Positive n (%)	PD-L1 Negative n (%)	Statistic	p-value
Age	<50 years	21 (45.7)	25 (54.3)	4.08	0.043*
	≥50 years	63 (60.6)	41 (39.4)		
Gender	Male	60 (58.8)	42 (41.2)	0.74	0.389
	Female	24 (50.0)	24 (50.0)		
Tumor Site	Buccal mucosa	38 (62.3)	23 (37.7)	2.96	0.565
	Other sites	46 (51.7)	43 (48.3)		
Histological Grade	Well differentiated	29 (42.6)	39 (57.4)	15.12	0.001*
	Moderately differentiated	37 (64.9)	20 (35.1)		
	Poorly differentiated	18 (72.0)	7 (28.0)		
TNM Stage	I–II	22 (40.7)	32 (59.3)	11.37	0.001*
	III–IV	62 (64.6)	34 (35.4)		
Lymph Node Status	Negative	28 (44.4)	35 (55.6)	7.12	0.008*
	Positive	56 (64.4)	31 (35.6)		
CD8 Infiltration	High	57 (70.4)	24 (29.6)	17.84	<0.001*
	Low	27 (39.1)	42 (60.9)		

Spearman's rank association analysis revealed a moderate positive association between PD-L1 expression and the density of CD8+ TILs (p<0.001), implying that there was also a positive association between PD-L1 expression and the infiltration of CD8 lymphocytes. (Table 4)

Table 4. Association Between PD-L1 Expression and CD8 Cell Count

Variables	Spearman's association coefficient (r_s)	p-value
PD-L1 expression vs. CD8-positive lymphocyte count	0.421	<0.001

Poorly differentiated OSCC (p=0.005), advanced TNM stage (p=0.004), lymph node positivity (p=0.022), high infiltration of CD8 cells (p<0.001), and age ≥50 years (p=0.045) were independent predictors of PD-L1 positivity, while gender was not (p=0.481). The regression model was found to be well calibrated, had reasonable explanatory power, and did not suffer from multicollinearity. (Table 5)

Table 5. Multivariable Binary Logistic Regression Analysis for Predictors of PD-L1 Positivity

Variable	Adjusted OR	95% CI	p-value
Age ≥50 years	1.78	1.01–3.15	0.045*
Male gender	1.26	0.66–2.39	0.481
Poorly differentiated tumor	3.12	1.41–6.89	0.005*
Stage III–IV	2.69	1.35–5.36	0.004*
Lymph node positivity	2.15	1.12–4.14	0.022*
High CD8 infiltration	3.81	1.95–7.44	<0.001*
Model diagnostics: *Hosmer–Lemeshow $\chi^2 = 5.82$ ($p = 0.668$); Nagelkerke $R^2 = 0.39$; VIF range = 1.12–1.86.*			

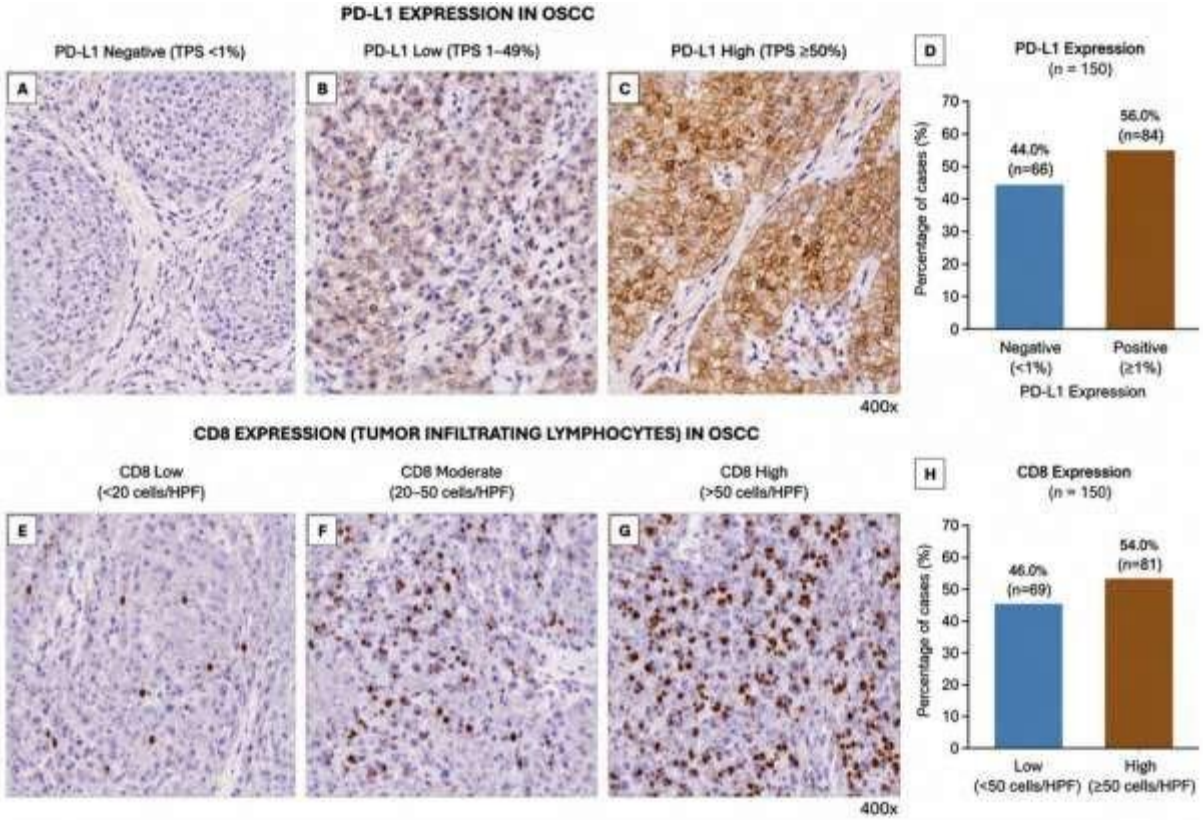

Figure 1: IHC expression of PD-L1 and CD8 in oral squamous cell carcinoma (OSCC).

A–C: PD-L1 (clone 22C3) expression showing negative (<1% TPS), low (1–49% TPS), and high (≥50% TPS) membranous staining. **D:** Distribution of PD-L1 expression (n=150). **E–G:** CD8-positive tumor-infiltrating lymphocytes showing low (<20 cells/HPF), moderate (20–50 cells/HPF), and high (>50 cells/HPF) infiltration. **H:** Distribution of CD8 infiltration (n=150). Brown staining indicates positive immunoreactivity; hematoxylin counterstain. Original magnification: ×400.

DISCUSSION

In the current investigation, the immunohistochemical expression of PD-L1 and CD8 was examined in OSCC, and 56.0% of OSCC were found to be PD-L1 positive, while 54.0% of OSCC had significant CD8-positive tumor-infiltrating lymphocyte (TIL) density. Furthermore, PD-L1 expression was strongly linked to advanced tumor stage, low histologic grade, lymph node metastases, and high CD8 infiltration. The results show that the activation of the

PD-1/PD-L1 immune checkpoint pathway has a major impact on the tumor immune microenvironment in OSCC and could help identify patients who may be suitable subjects for immune checkpoint inhibitor therapy.

The current study's PD-L1 positivity rate (56.0%) was similar to that reported by Greeshma et al. (2023), who employed immunohistochemistry to detect PD-L1 expression in OSCCs, which increased as the disease progressed, with an expression rate of roughly 56%.[15] Similarly, Kujan et al. (2022) found that PD-L1 expression increased considerably from oral potentially malignant diseases to OSCC, implying that PD-L1 expression becomes increasingly dysregulated during oral carcinogenesis.[16] These similarities support the conclusion that PD-L1 is frequently elevated in OSCC and could be employed as an effective biomarker for immunotherapeutic decision-making.

In the current study, PD-L1 expression was substantially associated with poor histological differentiation. The findings are consistent with those of Lequerica-Fernández et al. (2021), who discovered that aggressive tumors exhibited greater levels of PD-L1 expression and altered immune cell infiltration, indicating a shift toward an immunosuppressive tumor environment.[17] Similarly, Greeshma et al. (2023) found that PD-L1 was more highly marked in lesions with advanced histological alterations, emphasizing the role of PD-L1 in tumor progression.[15] Some systematic reviews have found that PD-L1 is associated with poor clinicopathological features, although its independent prognostic function for survival remains equivocal due to variations in antibodies employed, scoring systems, and positivity cut-off points between studies.[18, 19]

The current investigation discovered that advanced TNM stage and cervical lymph node metastases were independently linked with PD-L1 positivity. Jha et al. (2023) and Geum et al. (2022) published similar results, demonstrating that PD-L1 expression was higher in advanced tumors and metastatic disease.[20, 21] Similarly, Anconelli et al. (2026) presented information indicating that PD-L1 expression was frequently associated with lymph node metastases and advanced clinicopathological stage, albeit most of this evidence was debatable regarding its relationship with long-term survival.[22] Together, these findings suggest that PD-L1 may be a predictor of tumor aggressiveness, but not necessarily of prognosis.

In the present study, differentiated and early-stage tumors had significantly more infiltration of CD8+ T cells. Lequerica-Fernández et al. (2021) conducted a similar study, demonstrating that increased CD8+ TILs were associated with a better immune response and prognosis in OSCC.[23] Similarly, Ali et al. (2025) showed that as dysplasia progresses to carcinoma, there is a corresponding infiltration by cytotoxic T lymphocytes (CTL), suggesting that although tumor cells are actively suppressing the host immune system, they are simultaneously active in other immune pathways that promote tumor progression.[24]

The study found a strong correlation (r_s=0.421, $p<0.001$) between PD-L1 expression and CD8+ T cell density in TILs. This discovery aligns with the concept of adaptive immunological resistance, where activated CD8-positive T cells generate interferon-γ and induce the expression of PD-L1 on tumor cells, allowing them to avoid immune-mediated death. Similar positive associations have been described by Lequerica-Fernández et al. (2021) and Greeshma et al. (2023), both of whom concluded that PD-L1 upregulation frequently occurs in tumors with abundant CD8-positive immune infiltrates.[15, 23] The results suggest that the combined evaluation of PD-L1 and CD8 reveals a more complex understanding of the immune microenvironment of OSCC than either immune checkpoint or T cell alone.

Multivariable logistic regression analysis showed that advanced stage, poor differentiation, presence of lymph node metastasis, age, and CD8 infiltration were independent factors associated with PD-L1 positivity. Lu et al. (2024), Lenouvel et al. (2021), and Greeshma et al. (2023) have also suggested tumor stage and differentiation as major determinants of PD-L1 expression, similar to the clinicopathological association indicated in the present study.[15, 25, 26] However, patient population, ethnicity, antibody clones, tissue processing methods, and PD-L1 scoring systems may account for the variation reported in different studies, and therefore future studies should be based on a standardised immunohistochemical assessment.

The results of this study have significant clinical implications. The findings of this study suggest that combined IHC assessment of PD-L1 and CD8 may provide valuable information regarding the immune status and biological behavior of oral squamous cell carcinoma. Tumors that are highly positive for PD-L1 and have more CD8+ tumor-infiltrating lymphocytes could be used to classify patients who are likely to benefit from immune checkpoint inhibitor treatment. The inclusion of these biomarkers in routine histopathological assessment may assist in personalized treatment planning, aid in prognosis, and patient selection for immunotherapy and future clinical studies.

There were several limitations of this study. Its cross-sectional design hampered the evaluation of the prognostic value of PD-L1 and CD8 expression in overall or disease-free survival. Secondly, the study was performed in a single center and on a small sample size, potentially limiting the generalisability of the results to other populations. Third, the expression of PD-L1 and CD8 was only assayed by IHC, and other immune checkpoints or molecular biomarkers were not assessed to further characterize the tumor immune microenvironment. Fourth, because historical tissue specimens were used, there was insufficient data on prolonged clinical follow-up and treatment outcomes. Finally,

even when employing standardized laboratory techniques, inter-laboratory variances in IHC scoring systems and antibody result interpretation will complicate any comparison with other published findings.

CONCLUSION

The current investigation found that PD-L1 was substantially linked with advanced tumor stage, poor histological differentiation, lymph node metastasis, and a high density of CD8+ TILs, and that PD-L1 was positive in more than half of OSCC cases. The moderate positive relationship between PD-L1 expression and CD8 infiltration suggests that an adaptive immune resistance mechanism may exist within the tumor microenvironment. The findings indicate that a combination of PD-L1 and CD8 IHC labeling could be used to better describe the immune profile of OSCC patients, and they emphasize the potential of combined immunohistochemistry for predicting response to ICI therapy. Further multicenter prospective studies with long-term follow-up are required to substantiate the use of these biomarkers as prognostic and predictive markers, as well as to evaluate their potential utility in designing personalized treatment methods.

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