



IMMUNOHISTOCHEMICAL ANALYSIS OF PD-L1 IN ORAL SQUAMOUS CELL CARCINOMA AND ITS ASSOCIATION WITH CLINICOPATHOLOGICAL FEATURES .

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INTRODUCTION

With an increasing incidence worldwide, with up to 370,000 new cases per year. Although recent therapeutic opportunities have improved, OSCC is still responsible for up to 177,000 deaths annually, with a 5-year overall survival (OS) rate of around 60%.

Despite rapid advances in treatment modalities, including chemotherapy, radiotherapy, and target therapy, surgery is still a mainstream treatment for OSCC.

Emerging cancer immunotherapy using immune checkpoint blockade (ICB) has brought about drastic changes in the field of clinical oncology. This immunotherapy is based on the different survival strategies of tumor cells , One of these potential routes of tumor escape from the action of the immune system is the expression of the PD1 ligand protein (PD-L1) by tumor cells, which induces anergy in cytotoxic CD8 lymphocytes and prevents them from attacking the tumor cells by interacting with their PD1 protein.

Blocking the binding of PD-L1 to PD-1 via an immune checkpoint inhibitor allows T cells to destroy tumor cells .Such targeted immunotherapy can be successfully achieved through the use of monoclonal antibodies directed either against PD-L1 or PD-1.

The expression of PD-L1 can be evaluated on both tumor cells and immune cells with immunohistochemistry ,positive staining and the patterns of PD-L1 immunoexpression represent one of the most important indicators for correctly selecting patients eligibility for immunotherapy.

Solid cancers are also infiltrated to varying degrees by immune cells [tumor-infiltrating lymphocytes (TILs)], and this phenomenon was suggested to be a manifestation of the host immune response against cancer cells .

Studies demonstrated a positive association of high PD-L1 expression and increased TIL frequency with longer survival, also a significant positive association between PD-L1 expression and the frequency of TILs was found.

Consistent with this possibility, PD-L1 expression has been reported to be the most important predictor of the responsiveness of various cancer types to PD-L1 or PD-1 blocking antibodies .

AIMS AND OBJECTIVES

- To Study Clinicopathological characteristics and Tumour infiltrating lymphocytes in Oral squamous cell carcinoma .
- To Study PD-L1 expression in Oral squamous cell carcinoma.
- To correlate PD-L1 expression with Tumour infiltrating lymphocytes (TIL's)

INCLUSION CRITERIA

- All cases of Oral squamous cell carcinoma involving the Buccal mucosa.
- All patients above 18 years of age and all genders.

EXCLUSION CRITERIA

- Inadequate biopsies .
- Patients with oral squamous cell carcinoma involving anatomical sites other than the buccal mucosa (e.g., tongue, floor of mouth, gingiva, palate, oropharynx, and lips).
- Resection specimens were excluded; only biopsy samples were included in the study.

MATERIALS AND METHODS

The present retrospective study was conducted in the Department of Pathology, Mahadevappa Rampure Medical College, Kalaburagi, over a period of one year, from January 2024 to January 2025, and included a total of 42 cases.

Immunohistochemical Staining :

A total of 42 paraffin-embedded tissue sections from histopathologically confirmed cases of squamous cell carcinoma of the buccal mucosa were subjected to immunohistochemical staining using the PD-L1 antibody clone CAL10 (rabbit monoclonal, Biocare Medicals, USA), and membranous expression of PD-L1 was evaluated.

Slides were evaluated by at least two of the Authors in a blinded manner, using a light microscope. The evaluation of immunohistochemical staining was done by Tumour proportion score (TPS). A minimum of 100 viable tumor cells were required for evaluation. Necrotic areas were excluded from scoring.

Any degree of staining intensity was considered positive. only partial or complete membrane staining was evaluated, cytoplasmic staining was excluded.

PD-L1 expression in OSCC was quantified using the Tumor Proportion Score (TPS), defined as the percentage of viable tumor cells showing membranous PD-L1 staining ($\geq 1+$).

Expression levels were categorized as follows:

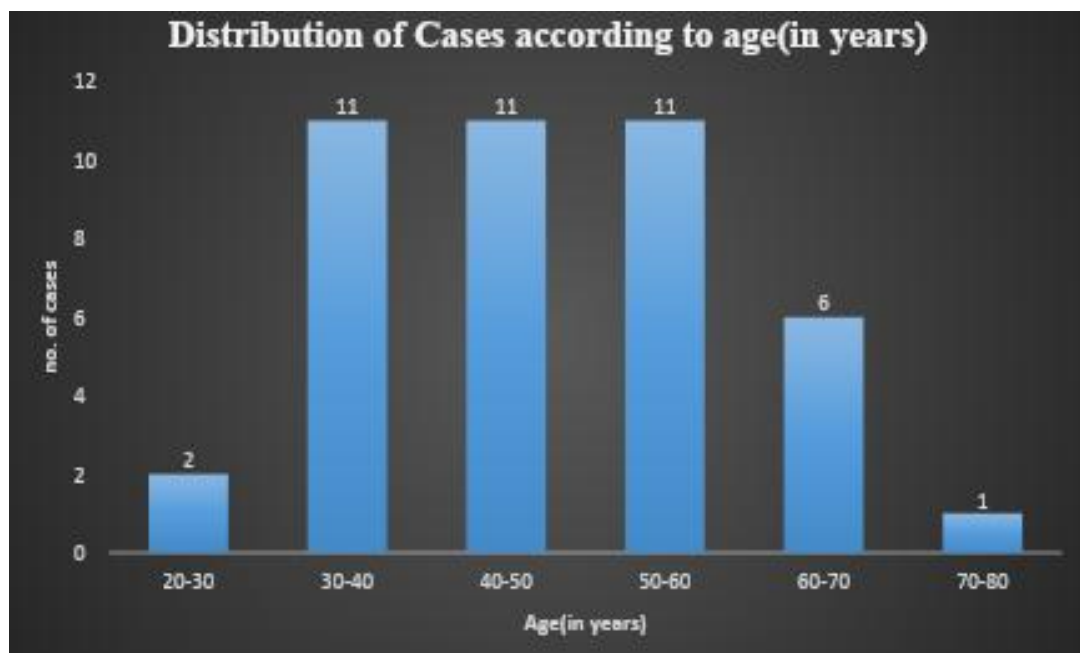
- TPS $< 1\%$ (negative)
- TPS 1–49% (positive),
- TPS $\geq 50\%$ (high expression).
- Evaluation of TIL's : The number of TILs was counted in at least four different high-power fields (hpf; $\times 40$ objective, $\times 10$ eyepiece) for each specimen.
- Fields with the most abundant TILs in a specimen were selected for counting.
- Only stromal TILs were evaluated , TILs inside tumour nests were excluded.
- Percentage of stroma occupied by mononuclear inflammatory cells was estimated , excluding granulocytes.
- A semi quantitative percentage scoring was applied :
- $< 10\%$ - Low TIL's : Immune desert or exclusion
- 10-40% - Moderate TIL's : Partial immune response
- $> 40\%$ - High TIL's : Active immune microenvironment

RESULTS

A total of 42 cases of oral squamous cell carcinoma (OSCC) involving the buccal mucosa were analyzed.

Table 1: Distribution of Cases according to age(n=42)

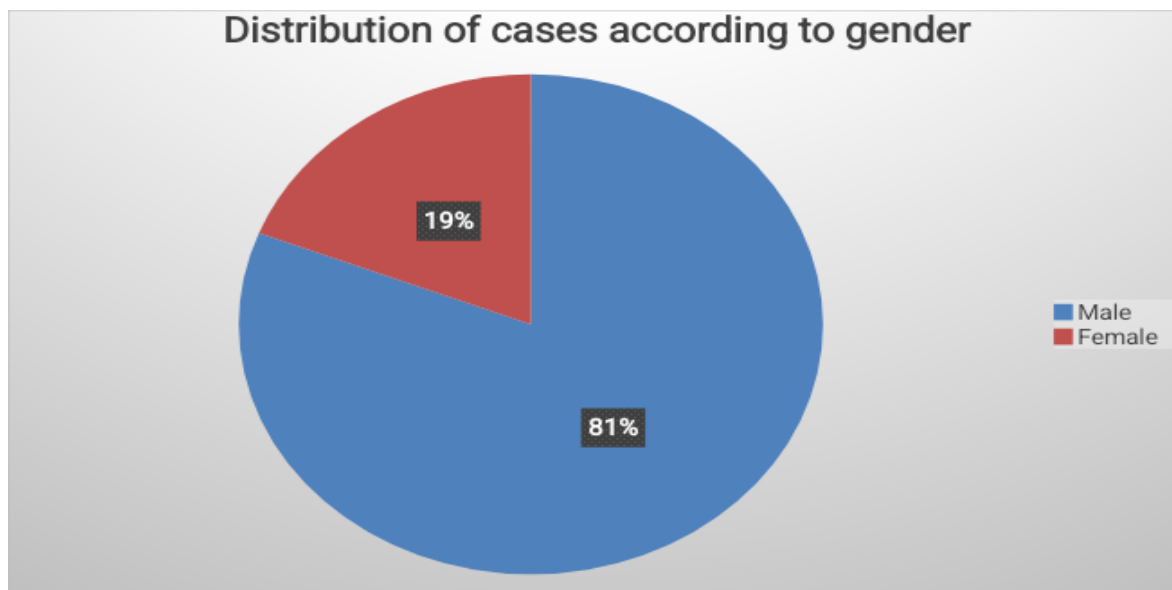
Age(in years)	Number of Cases	Percentage
20-30	02	4.76
30-40	11	26.19
40-50	11	26.19
50-60	11	26.19
60-70	06	14.29
70-80	01	2.38
Total	42	100
Mean $\pm$ SD	47.62 $\pm$ 11.96	



The age of patients ranged from 18 to 80 years, with a mean age of 48.05 years. The majority of patients (78.57%) were in the 3rd to 5th decades of life.

Table 2 : Distribution of cases accoring to Gender (n =42)

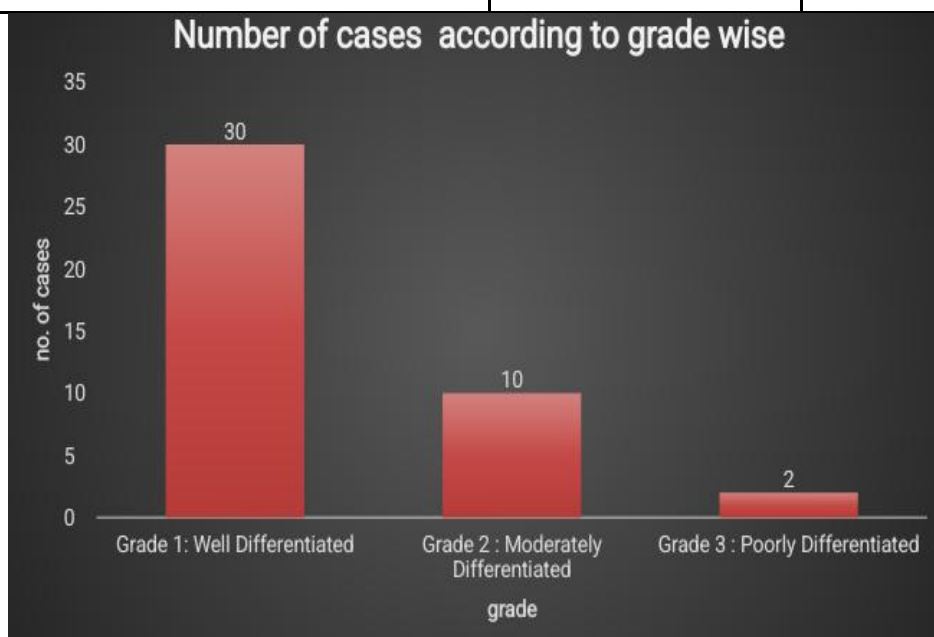
Gender	Number	Percentage
Male	34	81
Female	8	19
Total	42	100



Of the 42 cases 34 were males (81 %) and 8 were female (19%) , The ratio of male to female is 4.3 : 1 , showing male predominance.

Table 3 : Distribution based on histomorphological grading of Oral squamous cell carcinoma (n=42)

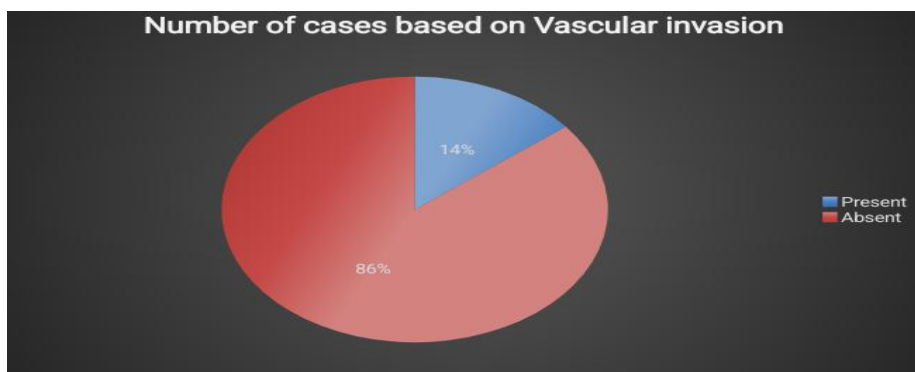
Grade	Number of cases	Percentage
Grade 1 Well Differentiated	30	71.4
Grade 2 Moderately Differentiated	10	23.9
Grade 3 Poorly Differentiated	2	4.7
Total	42	100



Out of 42 cases of squamous cell carcinoma involving the buccal mucosa, the majority , 71.4 % were classified as Grade 1 (well-differentiated), followed by Grade 2 (moderately differentiated). The least cases were Grade 3 (poorly differentiated) .

Table 4 : Distribution based on Vascular invasion (n=42)

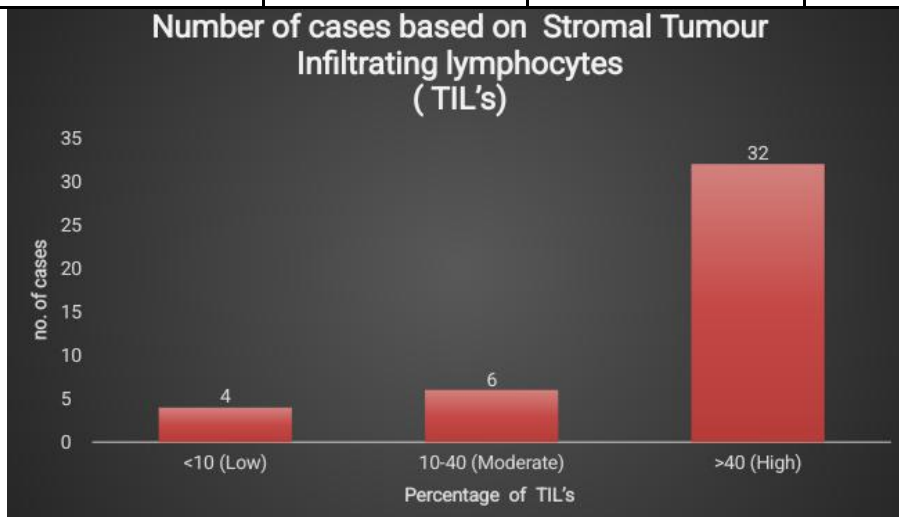
Vascular invasion	Number of cases	Percentage
Present	6	14.28
Absent	36	85.72
Total	42	100



Out of 42 cases , only 6 cases showed Vascular invasion while in majority of cases , i.e 85.72 % , there was no vascular invasion noted.

Table 5 : Distribution Based on Stromal Tumour Infiltrating lymphocytes (TIL's) (n =42)

	Score	number of cases	Percentage	Immune response
<10%	Low TIL's	4	9.52	Immune exclusion
10-40%	Moderate TIL's	6	14.28	Partial immune response
>40%	High TIL's	32	76.19	Active immune microenvironment
	Total	42	100	

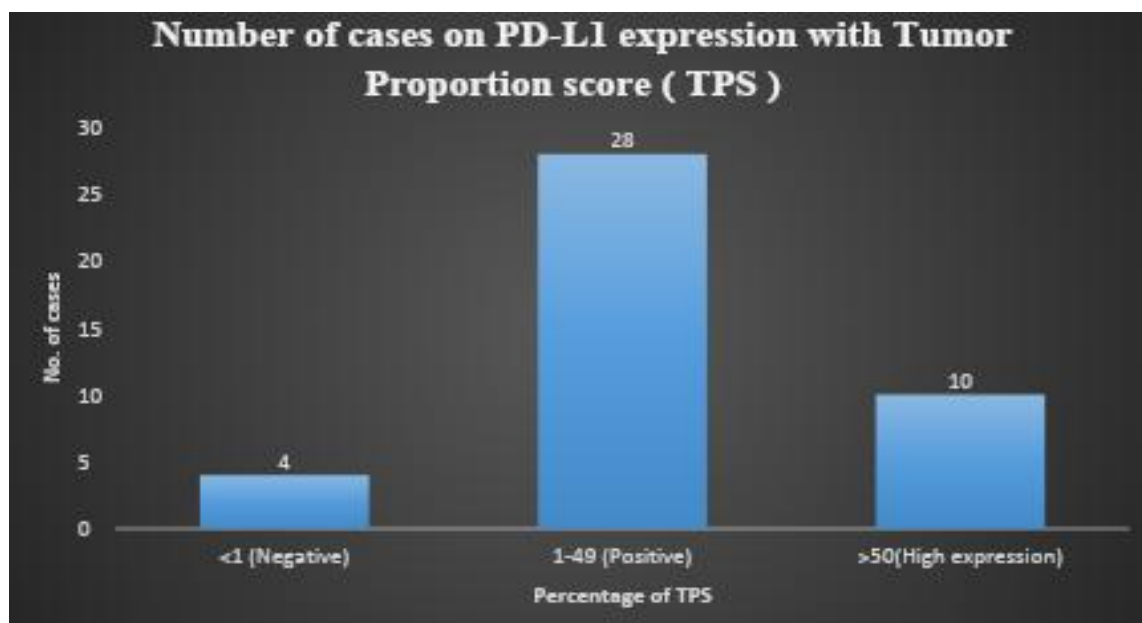


Out of 42 cases, 32 cases (76.19%) showed TIL's more than 40% indicating active

immunEmicroenvironment whereas only 4 cases (9.52%) showed less than 10 % TIL's , indicating possibility of immune exclusion.

Table 6 : Distribution based on PD-L1 expression with Tumour Proportion score (TPS) (n=42)

TPS	PD-L1 Expression	number of cases	Percentage
<1%	Negative	4	9.52
1-49%	Positive	28	66.67
>50%	High expression	10	23.80
	Total	42	100

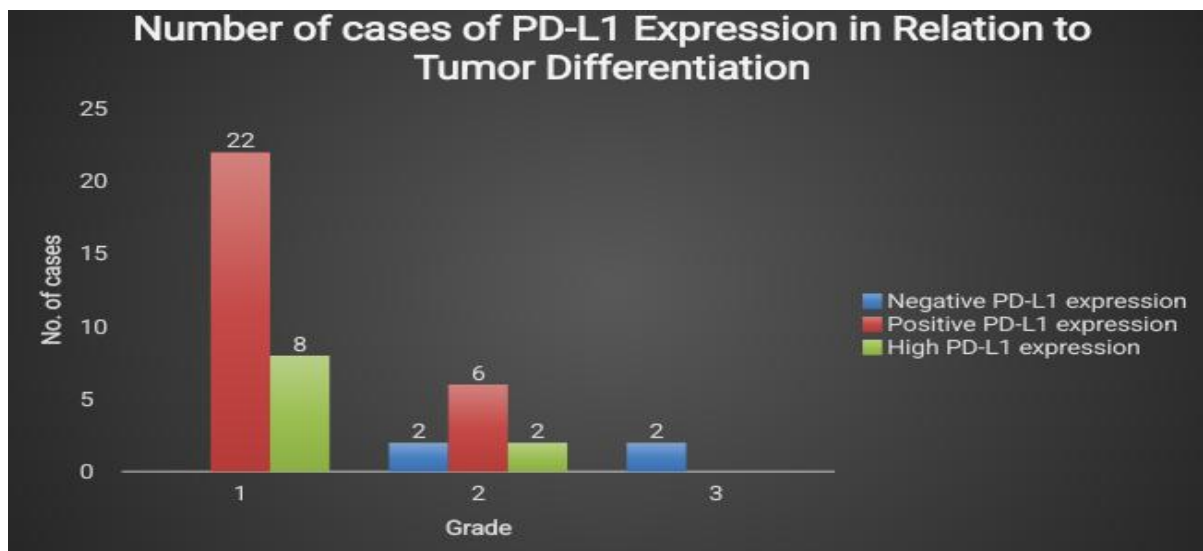


out of 42 cases , majority of them (66.67%) , showed positive PD-L1 expression followed by 23.8% cases showing high expression with a TPS of more than 50% , 9.52% cases showed negative PD-L1 expression with TPS less than 1 % .

Table 7 : Distribution of PD-L1 Expression in Relation to Tumor Differentiation and p-value by Chi-square.

GRAD E	Negative PD-L1 expression	Positive PD-L1 expression	High PD-L1 expression	Total cases	p-value
1	0(0.0%)	22(73.33%)	8(26.67%)	30(100%)	0.0001(S)
2	2(20%)	6(60%)	2(20%)	10(100%)	
3	2(100%)	0(0.0%)	0(0.0%)	2(100%)	
Total	4	28	10	42	

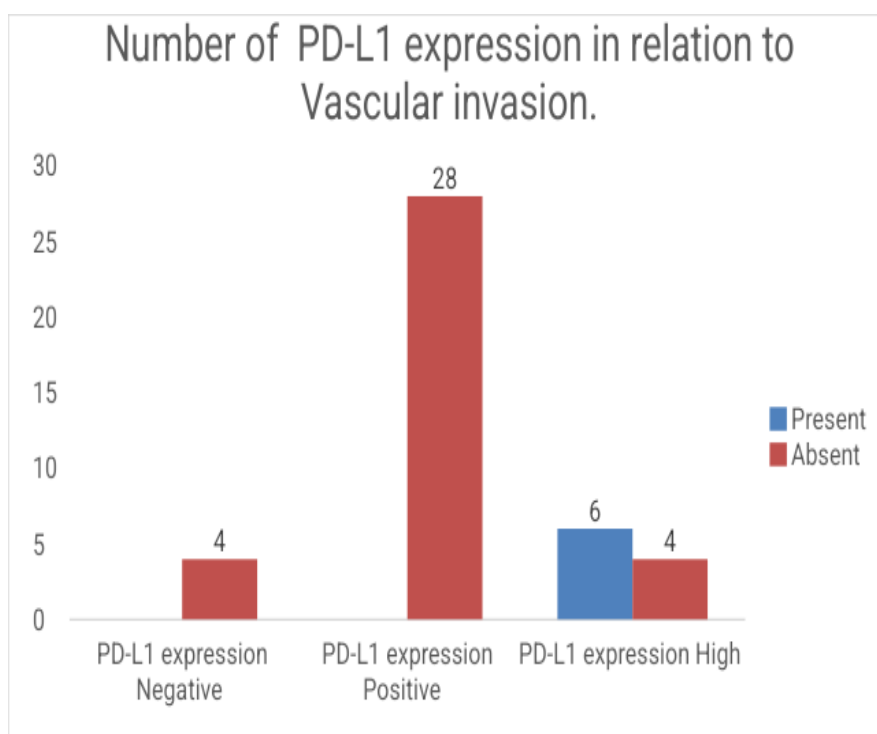
S: significant



PD-L1 expression was inversely related to tumor grade ($p = 0.0001$) and absent in all poorly differentiated cases.

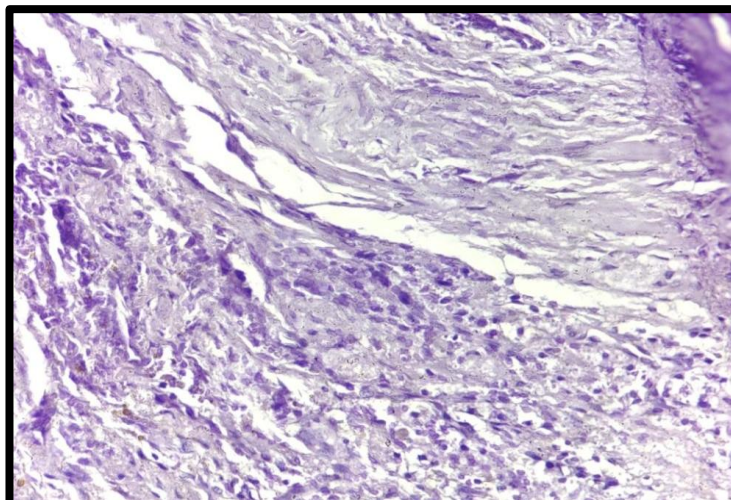
Table 8 : Distribution of PD-L1 expression in relation to Vascular invasion and p-value by Chi-square.

Vascular invasion	PD-L1 expression Negative	PD-L1 expression Positive	PD-L1 expression High	p-value
Present	0	0	6	<0.0001(S)
Absent	4	28	4	
Total	4	28	10	

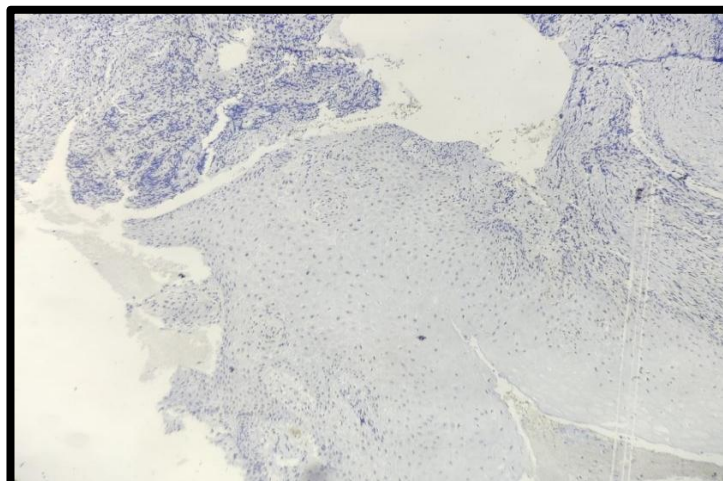


High PD-L1 expression was significantly associated with vascular invasion ($p < 0.0001$).

LOW TIL DENSITY CORRESPONDING TO NEGATIVE PD-L1 EXPRESSION

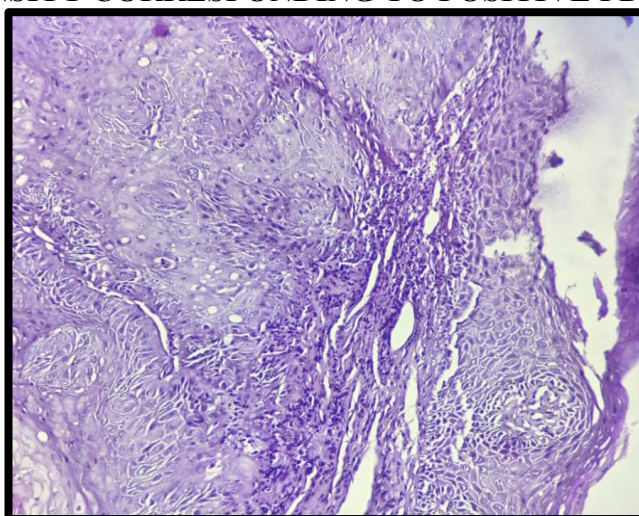


(H&E) stained section demonstrated **low tumor-infiltrating lymphocyte (TIL)** density, occupying less than 10% of the stromal area.

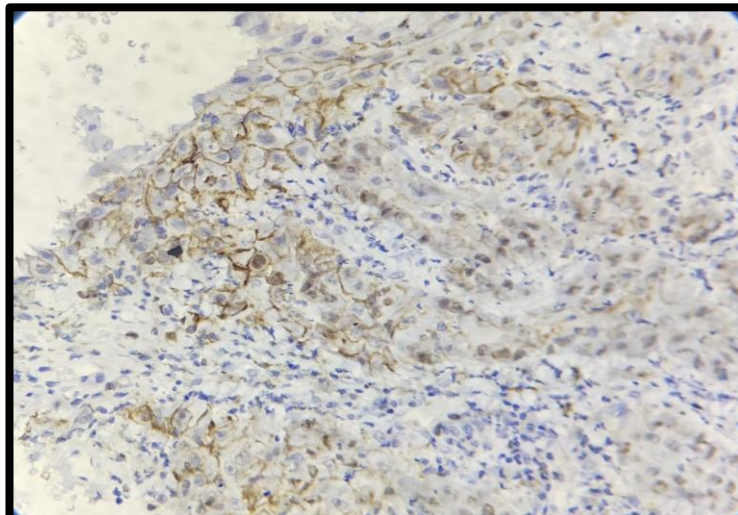


Immunohistochemistry (IHC) revealed **negative PD-L1 expression**, with a Tumor Proportion Score (TPS) of less than 1%.

MODERATE TIL DENSITY CORRESPONDING TO POSITIVE PD-L1 EXPRESSION

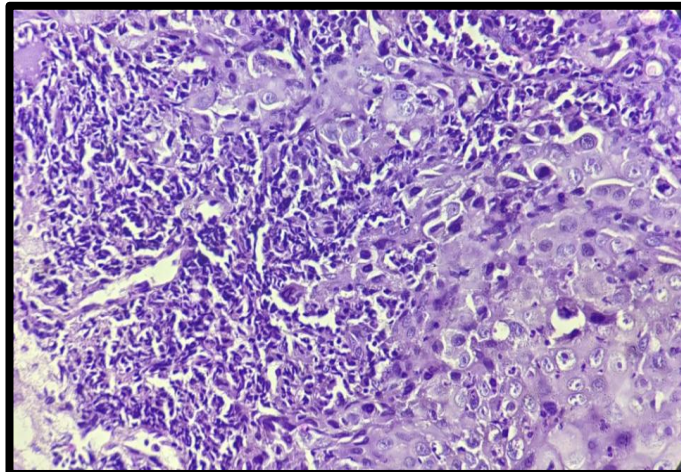


(H&E) stained sections demonstrated moderate tumor-infiltrating lymphocyte (TIL) density, occupying 10-40% of the stromal area

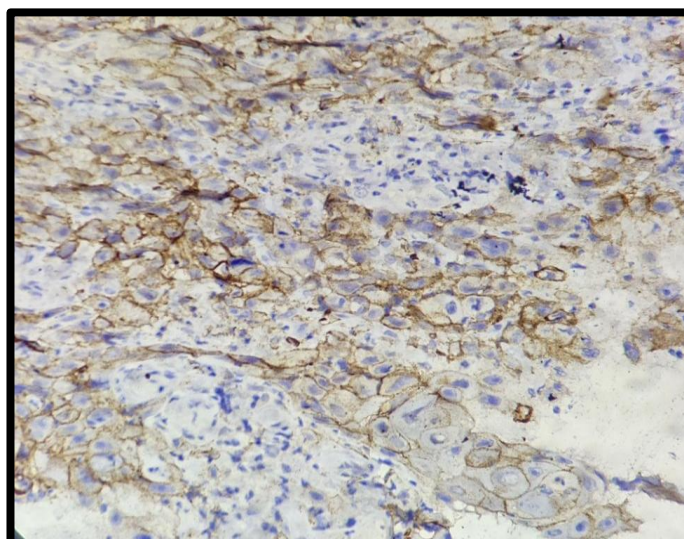


Immunohistochemistry (IHC) revealed positive PD-L1 expression, with a Tumor Proportion Score (TPS) of 20%

HIGH TIL DENSITY CORRESPONDING TO HIGH PD-L1 EXPRESSION



(H&E) stained section revealed a **high density of tumor-infiltrating lymphocytes (TILs)**, occupying more than 40% of the stromal area.



Immunohistochemistry (IHC) demonstrated **high PD-L1 expression**, with a Tumor Proportion Score (TPS) of 63.75%.

DISCUSSION

- Programmed cell death-ligand 1 (PD-L1) plays a crucial role in tumor immune evasion and has emerged as a significant biomarker in oral squamous cell carcinoma (OSCC).
- All poorly differentiated (Grade 3) tumors in the present study were negative for PD-L1, while the majority of well-differentiated (Grade 1) and moderately differentiated (Grade 2) tumors showed positive or high PD-L1 expression. These findings align with existing literature, suggesting that PD-L1 expression is typically higher in early-stage or lower-grade tumors and tends to diminish as tumors become more aggressive and dedifferentiated.
- **Nocini et al. (2022)** similarly reported increased PD-L1 expression in early-stage tumors and precursor lesions, indicating that PD-L1 upregulation may serve as an early immune escape mechanism. **Kogashiwa et al. (2017)** further supported this by demonstrating better prognosis in PD-L1-positive OSCC cases, particularly in locally advanced disease. Likewise, **Leporace-Jiménez et al. (2024)** noted a correlation between PD-L1 expression and favorable clinicopathological features, though heterogeneity in study designs and PD-L1 scoring systems limits direct comparison.
- In the study, no significant association was found between PD-L1 expression and demographic factors such as age and gender. However, a strong positive correlation was observed between stromal tumor-infiltrating lymphocytes (sTILs) and PD-L1 expression.
- All PD-L1 positive cases demonstrated moderate to high TIL density, and high PD-L1 expression was seen exclusively in tumors with high TILs. This supports the concept of adaptive immune resistance, wherein tumor cells upregulate PD-L1 in response to immune cell infiltration. This mechanism has also been emphasized by **Takahashi et al. (2019)**, who underlined the pivotal role of the immune microenvironment in modulating PD-L1 expression in OSCC.
- The present study found that all OSCC cases exhibiting vascular invasion demonstrated high PD-L1 expression. This suggests a potential link between vascular invasion and PD-L1 upregulation, reflecting a more aggressive tumor phenotype with enhanced immune evasion capabilities. **Pachpande et al. (2023)** also identified PD-L1 expression as being associated with histological indicators of progression in both potentially malignant and malignant oral lesions. However more number of cases need to be studied to arrive at a conclusion.
- From a therapeutic perspective, PD-L1 expression has emerged as a predictive biomarker for response to immune checkpoint inhibitors, particularly anti-PD-1 therapies such as pembrolizumab. **KEYNOTE-012 (Seiwert et al., 2016)** and **KEYNOTE-048 (Burtneess et al., 2019)** demonstrated improved clinical outcomes in PD-L1-positive head and neck squamous cell carcinoma patients, especially when the Combined Positive Score (CPS) was ≥ 1 . These trials reinforce the value of PD-L1 testing in guiding patient selection for immunotherapy.

CONCLUSION

- Taken together, the findings suggest that PD-L1 expression in OSCC is closely linked to tumor differentiation, immune infiltration, and invasive behavior. The absence of PD-L1 in poorly differentiated tumors raises questions about the efficacy of immunotherapy in such cases, whereas the presence of PD-L1 in tumors with vascular invasion and high TIL density suggests a subset of aggressive but immunologically active tumors that may benefit from targeted immune-based treatments.
- Personalized immunotherapeutic approaches, tailored based on PD-L1 status, TIL density, and histological differentiation, may improve treatment outcomes and patient selection.
- However, the absence of PD-L1 expression in poorly differentiated OSCC may limit the effectiveness of immune checkpoint inhibitors in such cases, highlighting the need for broader biomarker profiling and standardized evaluation protocols to optimize treatment strategies.

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