http://doi.org/10.33279/jkcd.v16i02.967

Original Article

EXPRESSION OF PROGRAMMED DEATH LIGAND-1 (PD-L1) IN ORAL POTENTIALLY MALIGNANT DISORDERS (OPMDS): A COMPARATIVE IMMUNOHISTOCHEMICAL STUDY

Ammara Khattak[1], Najma Baseer[2], Khurshid Ali[3], Sanna Safi[4]

[1]Department of Oral Biology, Khyber College of Dentistry, Peshawar, Pakistan
[2]Department of Anatomy, Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan
[3]Department of Oral Pathology, Khyber College of Dentistry, Peshawar, Pakistan
[4]Department of Oral Medicine, KMU Institute of Dental Sciences, Khyber Medical University, Kohat, Pakistan

ABSTRACT

Objectives: *This lab-based cross-sectional study aimed to assess PD-L1 expression in Oral Potentially Malignant Disorders (OPMDs) and compare it with Oral Squamous Cell Carcinoma (OSCC).*

Materials and Methods: *Nineteen OPMD samples (14 biopsies and 5 cytology) were included in the study. Histopathological reports confirmed the diagnosis of biopsy samples and grouped them into dysplasia (n=6), chronic inflammation (n=5), verrucous leukoplakia VL (n=1), oral lichen planus OLP (n=1), and oral submucous fibrosis OSMF (n=1). The cytology samples were grouped into white oral lesions (n=3), and chronic oral ulcerative lesions (n=2) based on their clinical features. Additionally, five OSCC cases and five normal oral mucosa (NOM) samples were taken as positive and negative controls, respectively. Immunohistochemical staining was done to determine the qualitative and quantitative expression of PD-L1 using Image J software.*

Results: *There was 74% expression of PDL-1 in the OPMDs biopsy samples, 80% in OPMDs cytology samples, and 88% in the OSCC samples. A statistically significant difference was noted for PD-L1 expression between the OPMDs biopsy and OSCC groups (p-value=.013). However, this difference was insignificant in terms of cell count (p=0.47). Among OPDMs, the highest expression was seen in the Chronic oral ulcerative lesion among cytology (96%) samples and dysplasia (78%) among the biopsies.*

Conclusion: *Increased expression of PD-L1 in OPMDs suggests its role in immune-modulation and progression to malignancy. Therefore, it could serve as a predictive diagnostic marker for the malignant transformation of OPMDs.*

Key words: *Dysplasia, Immunotherapy, Immune checkpoint inhibitors, Oral Potentially Malignant Disorders, Oral Squamous Cell Carcinoma, Programmed Death-Ligand 1.*

Cite as: Khattak A, Baseer N, Ali K, Safi S. Expression of programmed death ligand-1 (pd-l1) in oral potentially malignant disorders (opmdS): a comparative immunohistochemical study. Journal of Khyber College of Dentistry Jun 2026, Vol. 16, No. 2. http://doi.org/10.33279/jkcd.v16i02.967

INTRODUCTION

Oral Cancer is a devastating disease. It is the sixth most common cancer worldwide[1]. It has an annual incidence of 300,000 cases and a mortality rate of 145,000 deaths worldwide[2]. Among oral cancers, OSCC is the most prevalent cancer with a five-year survival rate of 50% to 60%[3].

OSCC is commonly preceded by a premalignant phase, with alterations at the cellular and tissue levels. The premalignant disorders are grouped into OPMDs. Histologically these changes are limited to epithelium and are called oral epithelial dysplasia[4].

Correspondence:
Najma Baseer
Professor
Department of Anatomy, Institute of Basic Medical Sciences, Khyber Medical University, Peshawar Pakistan
Email: drnajma.ibms@kmu.edu.pk

Date Submitted: December 2025
Date Revised: March 2026
Date Accepted: April 2026

There is a 3-36% chance of malignant transformation in dysplastic tissues[5]. Oral Potentially malignant disorders (OPMDs) like leukoplakia and erythroplakia, lichen planus, and submucous fibrosis tend to develop into malignancies with time. Between 11% and 40% of these cases are resolved without surgical intervention[6]. Oral leukoplakia is the most common OPMD with a 0.1-18% chance of malignant transformation[6]. Predicting malignant transformation in OPMD would help in earlier diagnosis and treatment thus reducing morbidity and mortality and improving quality of life[4].

Tumour cells undergo genetic and epigenetic changes, resulting in products that can be targeted by immune system and is used in Immunotherapy. Checkpoint blockade blocks inhibitory signals from cancer cells and enhances the immune system to fight cancer[7,8]. Different Checkpoint inhibitors have been identified and are under clinical trial; among them CTLA-4 and PDL-1/PD-L1 are the most important ones. PD-1/PD-L1 therapies have been approved for treating melanoma, non-small cell lung cancer (NSCLC)[8,9] renal cell carcinoma, and triple-negative breast cancers[10].

Numerous researchers have investigated the expression of PDL-1 in OSCC, and as the stage advances, the PD-L1 expression is increased[11]. It shows that grade of malignancy also increases with increased expression. Immunological methods of blocking the PD-1/PD-L1 pathway for treating OSCC have been used and shown positive results.

Demonstrating detectable PD-L1 expression in early lesions could establish it as a viable screening biomarker. Cytological evaluation may provide a minimally invasive approach for early-stage lesions where a biopsy is not yet indicated, potentially paving the way for targeted immunotherapy interventions to intercept and arrest the progression of OPMDs into advanced malignancies.

We hypothesized that PD-L1 expression increases progressively across the clinical spectrum of OPMDs, and that its upregulation can be reliably detected via both cytological and histopathological methods. Thus, our study aimed to determine the expression of PD-L1 in OPMDs, especially those at a very early stage. If PD-L1 detection is possible with the help of cytology or biopsy techniques, then such suspected lesions can be treated with immunotherapy, and their progression into malignancy can be stopped at an earlier stage.

MATERIALS AND METHODS

This lab-base cross sectional study was conducted for a period of 6 months, after obtaining ethical approval from Khyber Medical Univeristy (KMU) Peshawar, Khyber College of Dentistry (KCD) Peshawar and Rehman Medical Instutue (RMI) Peshawar. Samples were collected from KCD and RMI. The lab work was conducted at the Histopathology Lab of the Institute of Basic Medical Sciences (IBMS), KMU. The study was conducted following the Declaration of Helsinki, and informed consent was obtained from all participants. The sample size was calculated using Slovin's formula for an unknown population ($n = N / [1 + Ne^2]$)[12]. Based on a clinical flow of three patients per month, the total estimated population over six months was ($N = 18$), with a 5% margin of error ($e = 0.05$), the calculated sample size was ($n = 18.5$), rounded to 19. A total of 19 samples (14 biopsies and 5 cytology samples) of OPMD were collected using a non-probability, purposive sampling technique. Histopathological reports confirmed the diagnosis of biopsy samples. Based on the histopathology reports, the OPMDs were grouped into dysplasia (n=6), chronic inflammation (n=5), VL (n=1), OLP (n=1), and OSMF (n=1). For cases where biopsy was not feasible, cytology was obtained and grouped into white oral lesions (n=3) and chronic oral ulcerative lesions (n=2) based on their clinical features. Additionally, five OSCC cases were included as positive controls and five NOM cases as negative controls. Immunohistochemical staining was performed to study the expression of PD-L1. Image J software was used for image analysis.

Inclusion criteria: Patients presenting with persistent oral mucosal lesions—classified as white, red, or mixed in appearance—suspected of malignancy were included in the study. Additionally, cytology samples were obtained from lesions of similar appearance when a biopsy was not feasible due to clinical limitations.

Exclusion criteria: While patients with a history of prior treatment for oral lesions—including corticosteroid therapy, chemotherapy, radiotherapy, or surgical intervention—were excluded. Additionally, cases presenting with oral reactive lesions such as

pyogenic granuloma, peripheral giant cell granuloma, or denture-induced epulis (euploid lesions) were not included. Other exclusions comprised granulomatous lesions of the oral cavity and benign odontogenic tumors such as peripheral ameloblastoma.

Biopsy samples were stored in 10% formalin. Cytology samples were collected using a cytobrush with rolling strokes on lesions, applied on charged IHC slides and glass slides for H&E staining after fixing in 95% Ethyl alcohol for 6-12 hours.

Tissue processing of biopsy samples used an automated tissue processor (LUPTEC PT09 TS) and made into paraffin blocks. Thin 5µm sections were mounted on charged IHC slides and plain slides for H&E staining.

Programmed Death Ligand-1 Immunohistochemistry involved deparaffinizing tissue slides, performing heat-induced epitope retrieval (HIER) using EDTA buffer, applying peroxidase-blocking solution, and treating with AntiPD-L1 (Abcam; ab205921). Staining included 3,3'-Diamino Benzidine (DAB) application and counterstaining with hematoxylin. Slides were dehydrated, cleared in xylene, and mounted with DPX. PD-L1 expression analysis used a Nikon microscope and Image J software to calculate optical density and positive cell count.

The data was analysed using SPSS (version 22). Normality was assessed using the Shapiro-Wilk test, the data showed a normal distribution. An independent sample t-test was used, with $p \leq 0.05$ as significant.

RESULT

Table 1 summarizes patient demographics. The mean age was 61.4±11.6 years for OPMD biopsy

Table 1: Demographic data of groups and site distribution

Parameters		OPMD group	OSCC group	NOM group
Age (mean±SD)		59.11±11.8	73±8.9	48±10.5
Gender	Male	14(73.6%)	4(80%)	3(60%)
	Female	5(26.3%)	1(20%)	2(40%)
Tobacco status	Users	12(63.2%)	5(100%)	0
	Non-users	7(36.8%)	0	5(100%)
Location	Tongue	7(36.8%)	3(60%)	0
	Buccal vestibule	3(15.8%)	0	0
	Labial vestibule	5(26.3%)	2(40%)	0
	Palate	2(10.5%)	0	0
	Gingiva and retromolar pad	2(10.5%)	0	5(100%)

OPDM= Oral potentially malignant disorders, OSCC= Oral squamous cells carcinoma, NOM= Normal Mucosa

Table 2: Optical Density and Percentage Expression of OPMD Groups

OPMDs groups	Diagnostic Groups	Mean OD/ OD±SD	Percentage Expression
Biopsy	Dysplasia	0.39±.038	78%
	Chronic Inflammation	0.38±.04	76%
	Verrucous leukoplakia	0.32*	64%
	Submucous fibrosis	0.30*	60%
	Lichen planus	0.28*	56%
	Clinical presentation	**Optical Density**	**Percentage Expression**
Cytology	White patch with a rough surface and irregular margins on the palate	0.44	88%
	Elevated grey-white patch in labial sulcus	0.31	62%
	Chronic oral ulcerative lesion	0.46	92%
	Chronic oral ulcerative lesion	0.48	96%
	White elevated area on the tongue	0.33	66%

*Only one sample was present, so Standard Deviation was not possible

samples and 53.4±11.5 years for cytology samples. Biopsy samples included nine males and five females, while cytology samples had five males. Tobacco use was observed in eight (57.1%) biopsy cases and four (80%) cytology cases.

PD-L1 showed membranous and cytoplasmic expression. The mean optical density was 0.37 ± 0.05 for the biopsy group and 0.40 ± 0.70 for the cytology group. Mean percentage expression was 74% for biopsies, 80% for cytology, and 88% for OSCC. Optical density and percentage expression data are summarized in Table 2.

The highest expression among the biopsy samples was observed in the Dysplasia group (78%) whereas in cytology group the highest expression was observed in a case of chronic oral ulcerative lesion (96%). Figure 1

PD-L1 expression was higher in OSCC (Fig 2d) as compared to other samples of OPMD (Figure 2)

An Independent sample t-test was applied to find the mean difference between the biopsy group (0.037±.05) and the OSCC (OD= 0.44±0.03). There was a statistical difference between them (p-value= 0.013). There was no statistically significant difference between the cytology group and OSCC group (p-value= 0.37). The NOM showed negative PD-L1 expression.

The mean cell count of the premalignant group was 44.04±22.97 and that of the OSCC group was 52.14±11.88. Independent sample t-tests were carried out to determine the mean difference between the cell count values of OPMDs and OSCC groups. The p-value was 0.47, which showed that there was no statistically significant difference between the cell count values of the two test groups. However, the mean cell count in the OSCC group was more than that of the pre-malignant group.

DISCUSSION

Our findings showed 74% of PD-L1 expression in OPMD biopsy samples and 80% in OPMD cy-

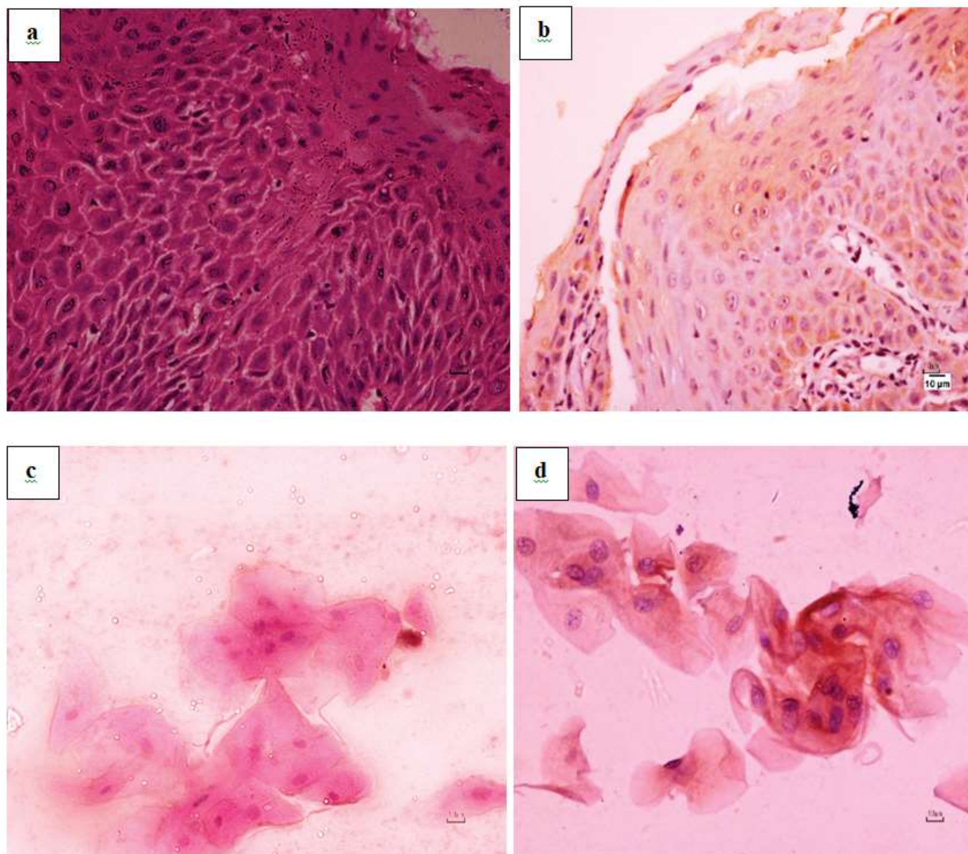


Fig 1: Photomicrograph of a case of Dysplasia H&E staining (a) and IHC staining (b) at 400X magnification showing 78% expression of PD-L1 (white arrowhead). Cytology image of a case of chronic oral ulcerative H&E staining(c) and IHC staining (d) showing 96% expression of PD-L1 at 400X magnification. (Scale bar 40X=10µm)

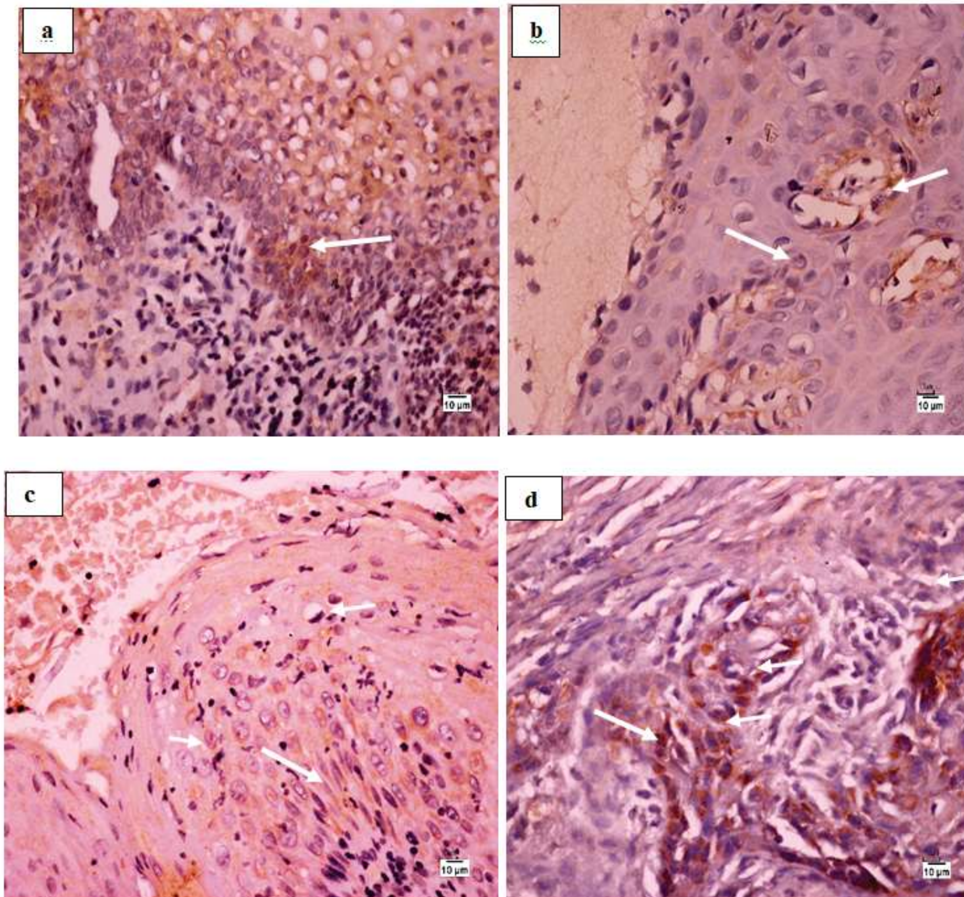


Fig 2: Photomicrograph of IHC showing PD-L1 expression in case of (a) chronic Inflammation (76%), (b) VL (64%), (c) OSMF (60%), (d)OSCC (88%) at 400X magnification, showing PD-L1 expression (white arrowhead)

tology samples, the OSCC group exhibited an 88% expression of PD-L1.

There are no studies reported regarding the evaluation of PD-L1 expression in persistent oral lesions through cytology. Both Gosney et al[13] and Lozano et al[14] support the reliability of cytology specimens for PD-L1 assessment in NSCLC, showing them to be comparable to histology and feasible for accurate evaluation. High PD-L1 expression in our studies indicated that the lesions were progressing to carcinoma. If they are subjected to immunotherapy, then positive results can be observed.

There was decreased expression of PD-L1 in the OPMD in comparison to the OSCC group. Pachpande et al. similarly, reported higher PD-L1 expression in OSCC patients compared to OPMD patients[15]. Similarly, Kouketsu et al. reported 67.9% PD-L1 expression in OSCCs and 26.6% in oral epithelial precursor lesions[16]. Chen and Mellman., found high expression of PD-L1 in 97.6% of OSCC samples, compared to 61.9% in oral leukoplakia cases, while the normal mucosa cases showed no PD-L1 expression[9]. The increased PD-L1 expression in OSCC suggests its role in creating an immunosuppressive environment that supports tumour progression from premalignant to malignant stages.

We observed a spectrum of 78% to 56% expression of PD-L1 in OPMDs, with the highest in dysplasia (78%), indicating a higher malignant transformation risk as compared to OPMD without dysplasia. Dave et al. reported significantly increased PD-L1 expression in progressing dysplasia versus non-progressing dysplasia ($p<0.01$)[4]. Whereas, Saeed et al. found 8% PD-L1 positivity in OPMD with and without dysplasia, showing no significant difference ($p=0.95$), suggesting dysplasia does not affect PD-L1 expression[17]. Similarly Hanroongsri et al. reported conflicting results, of higher PD-L1 expression in mild OED (87%) compared to OSCC (53%)[18]. They observed increased positive cell count ($p<0.001$) and intensity score ($p=0.002$) in OED

versus OSCC. They suggested increased expression in OED may help to evade immune attack. Heterogeneity in the expression of PD-L1 suggests the need for the development of a standardized method for evaluating PD-L1 expression.

Our study showed 76% PD-L1 expression in chronic inflammation, indicating molecular changes linked to dysplasia development. Dave et al. reported increased inflammatory cells in OPMD and tumors, suggesting a positive association between chronic inflammation and tumor progression[4].

In our study, VL showed decreased PD-L1 expression of 64%, likely due to the absence of dysplastic changes in the epithelium, with only hyperkeratosis observed. Contradicting Pachpande et al[15] who reported high PD-L1 expression in oral leukoplakia due to its association with dysplasia. Reduced expression was also found in OSMF, consistent with Pachpande et al., findings of low PD-L1 expression in OSMF in 86% of cases[15]. However, Quan et al,. reported higher PD-L1/PD-1 expression in OSCC linked to OSMF compared to OSCC without OSMF ($p = 0.006$), suggesting PD-L1/PD-1 plays a role in OSMF's malignant transformation[19]. In our samples, the lowest expression of PD-L1 was shown by OLP (56%). Minimum expression in OLP was also reported by Pachpande et al[15]. This decreased expression indicates reduced risk of malignant transformation of OLP. Costa et al. results are also parallel with our study. They reported negative PD-L1 expression in 66.6% of cases of OLP[20].

These discrepancies of concordant and discordant results underline the complexity of PD-L1's role in malignant transformation.

One of the major limitations of the study was its smaller sample size and inadequate representation of certain types of OPMD, thus limiting the generalizability of our findings. Multivariable statistical modelling could not be performed due to the limited sample size and incomplete availability of data on potential confounding variables. Therefore, the findings should be interpreted cautiously, as unmeasured or residual confounding may have influenced the observed associations. Future studies with larger, multicenter, prospectively designed datasets are recommended to adjust for important confounders such as age, gender, tobacco use, betel/areca nut use, lesion site, OPMD diagnosis, and dysplasia grade.

CONCLUSION

A notable proportion of OPMD show higher PD-L1, displaying its role in malignant transformation. Therefore, Immunotherapy blocking PD-L1 checkpoints could be considered for premalignant cases with PD-L1 positivity.

REFERENCES

1. Abati, S., Bramati, C., Bondi, S., Lissoni, A. & Trimarchi, M. Oral Cancer and Precancer: A Narrative Review on the Relevance of Early Diagnosis. Int J Environ Res Public Health 17, 9160 (2020).
2. Rivera, C. Essentials of oral cancer. Int J Clin Exp Pathol 8, 11884–11894 (2015).
3. Anwar, N. et al. Oral cancer: Clinicopathological features and associated risk factors in a high risk population presenting to a major tertiary care center in Pakistan. PLoS One 15, e0236359 (2020).
4. Dave, K., Ali, A. & Magalhaes, M. Increased expression of PD-1 and PD-L1 in oral lesions progressing to oral squamous cell carcinoma: a pilot study. Scientific Reports 2020 10:1 10, 1–11 (2020).
5. Patel, N. R. et al. An Immunohistochemical Study of HIF-1 Alpha in Oral Epithelial Dysplasia and Oral Squamous Cell Carcinoma. Indian J Otolaryngol Head Neck Surg 71, 435–441 (2019).
6. Yagyuu, T. et al. Programmed death ligand 1 (PD-L1) expression and tumor microenvironment: Implications for patients with oral precancerous lesions. Oral Oncology 68, 36–43 (2017).
7. Liu, H. et al. Discovery of low-molecular weight anti-PD-L1 peptides for cancer immunotherapy. Journal for ImmunoTherapy of Cancer 7, (2019).
8. Farkona, S., Diamandis, E. P. & Blasutig, I. M. Cancer immunotherapy: The beginning of the end of cancer? BMC Medicine 14, (2016).
9. Chen, D. S. & Mellman, I. Oncology meets immunology: The cancer-immunity cycle. Immunity 39, 1–10 (2013).
10. Wang, F. et al. Discovery of a new inhibitor targeting PD-L1 for cancer immunotherapy. Neoplasia (United States) 23, 281–293 (2021).
11. Furman, D. et al. Chronic inflammation in the etiology of disease across the life span. Nature Medicine 25, 1822–1832 (2019).
12. Mukti, B. H. Sample size determination: Principles and applications for health research. Heal. Sci. Int. J. 3, 127–143 (2025).
13. Gosney, J. R., Boothman, A. M., Ratcliffe, M. & Kerr, K. M. Cytology for PD-L1 testing: A systematic review.

Lung Cancer 141, 101–106 (2020).

14. Lozano, M. D. et al. Programmed death-ligand 1 expression on direct Pap-stained cytology smears from non-small cell lung cancer: Comparison with cell blocks and surgical resection specimens. Cancer Cytopathol 127, 470–480 (2019).
15. Pachpande, P. S., Mandale, M. S., Bhavthankar, J. D., Humbe, J. G. & Zanwar, P. Assessment of programmed cell death ligand- 1 (PD-L1) expression in oral potentially malignant disorders and oral squamous cell carcinoma - An immunohistochemical study. IP Archives of Cytology and Histopathology Research 8, 180–188 (2023).
16. Kouketsu, A. et al. Expression of immunoregulatory molecules PD-L1 and PD-1 in oral cancer and precancerous lesions: A cohort study of Japanese patients. Journal of Cranio-Maxillofacial Surgery 47, 33–40 (2019).
17. Saeed, S. et al. Comparison of PD-L1 Expression in Oral Squamous Cell Carcinoma and Premalignant Lesions of Oral Cavity. Asian Pacific Journal of Cancer Prevention 23, 4039–4045 (2022).
18. Hanroongsri, J., Amornphimoltham, P., Younis, R. H. & Chaisuparat, R. Expression of PD-L1 and p-RPS6 in epithelial dysplasia and squamous cell carcinoma of the oral cavity. Frontiers in Oral Health 5, (2024).
19. Quan, H. et al. Differential expression of programmed death-1 and its ligand, programmed death ligand-1 in oral squamous cell carcinoma with and without oral submucous fibrosis. Arch Oral Biol 119, 104916 (2020).
20. Costa, N. L. et al. Evaluation of PD-L1, PD-L2, PD-1 and cytotoxic immune response in oral lichen planus.

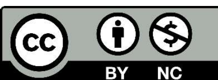
CONFLICT OF INTEREST
Authors declare no conflict of interest.
GRANT SUPPORT AND FINANCIAL DISCLOSURE
None declared.

AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	AK, NB
Acquisition, Analysis or Interpretation of Data:	AK, NB, KA, SS
Manuscript Writing & Approval:	AK, NB, KA, SS

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.



Ammara Khattak, et al. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License, which permits unrestricted use, distribution & reproduction in any medium provided that original work is cited properly.