Original Article

# Expression of immune checkpoint protein in oral squamous cell carcinoma and its clinicopathological correlation: A tertiary care center cross-sectional study

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## Abstract

**Background:** Recent evidence suggests that oral squamous cell carcinoma (OSCC) patients who exhibit the immunohistochemical expression of immune checkpoint protein programmed cell death ligand 1 (PD-L1) are more likely to have a poor clinical outcome and may serve as an independent prognostic marker.

**Aims and Objectives:** This study aimed to assess the immunohistochemical expression of immune checkpoint protein PD-L1 in OSCC and its clinicopathological correlation.

**Materials and Methods:** OSCC cases were included in the study. This was a tertiary care center cross-sectional one-year duration study. Histomorphological diagnosis and immunohistochemical expression of PD-L1 were performed after taking ethical clearance. The statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 21.0 statistical analysis software.

**Results:** A total of 106 cases of OSCC were included in the study. Histologically, the majority of cases (58.5%) were graded as well differentiated, followed by moderately differentiated (58.5%) and poorly differentiated (4.7%), respectively. In PD-L1 immunohistochemical expression, score 1+ was accorded to 37 (34.9%), 2+ was accorded to 31 (29.2%), and score 3+ was accorded to 33 (31.1%) cases. Tumor size, pattern, depth of invasion lymphovascular invasion (LVI), and perineural invasion (PNI) were found to be significantly associated with PD-L1 immunohistochemical scores.

**Conclusions:** We concluded that the immunohistochemical expression of immune checkpoint protein PD-L1 positivity in tumor cells was seen in the majority of the cases (60.37%) in our patient. This suggests that the PD-1 or PD-L1 pathway plays a significant role in tumor immune evasion in OSCC.

**Keywords:** Immune checkpoint protein programmed cell death ligand 1 (PD-L1), immunohistochemistry, oral squamous cell carcinoma

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## INTRODUCTION

Cancers of head and neck region are the ninth most common malignancy worldwide and the third most common malignancy in developing countries. More than ninety percent of head and neck cancers are of squamous epithelial origin.[1] In the head and neck region, the majority

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is oral squamous cell carcinoma (OSCC) that accounts for more than 550,000 cases annually worldwide and is currently one of the leading causes of cancer-related deaths.[2,3] The expression of programmed cell death ligand 1 (PD-L1) in tumor cells activates the PD-L1 or programmed death 1 (PD-1) pathway by binding to the PD-1 receptor on activated T-lymphocytes and downregulates the proliferation of antigen-stimulated lymphocytes, resulting in lymphocyte exhaustion and attenuation of the immune response.[4] Recent evidence suggests that OSCC patients who exhibit high levels of PD-L1 expression are more likely to have a poor clinical outcome and that PD-L1 expression, as determined by immunohistochemical staining, may serve as an independent prognostic marker.[5]

### Aim and objectives

This study aimed to assess the immunohistochemical expression of PD-L1 in OSCC and its clinicopathological correlation.

## MATERIALS AND METHODS

This was a tertiary care center cross-sectional one-year duration study. A total of 106 cases of oral squamous cell carcinoma diagnosed on histopathological examination (HPE) were included in the study. Patients were admitted and enrolled under the department of surgical oncology. Histomorphological evaluation and immunohistochemistry for PD-L1 were determined after taking ethical clearance from the committee and written consent from patients. Immunohistochemistry was performed with antibodies to PD-L1. PD-L1 was manufactured by Cell Signalling Technology (rabbit monoclonal clone E1L3N to PD-L1, diluted in tris-buffered saline (TBS), in a dilution of 1:100). Primary antibody (anti-PD-L1 antibody (E1L3N) CST, rabbit monoclonal antibody against PD-L1, clone E1L3N) and secondary antibody (Dako Envision™ FLEX Mini Kit, high pH (Link)) were used in the study. Normal human placenta was used as positive control, as recommended by the manufacturer. Immunohistochemical analysis of placental tissue shows strong expression of syncytiotrophoblast cells with absent PD-L1 immunoreactivity in cytotrophoblast cells. Negative control sections were processed by omitting primary antibodies. Immunohistochemistry (IHC) was evaluated using combined proportion score (CPS) at 200× magnification. CPS is the number of PD-L1 staining in tumor cells, macrophages, and lymphocytes divided by total viable tumor cells, multiplied by 100. CPS >1 was scored as positive. CPS <1 or no expression in tumor or immune cells was scored as negative. IHC expression for PD-L1 in tumor cells was evaluated by two observers separately. Interobserver agreement of 97.1% was present, which is in the acceptable range. Tumor cell population displays 1+ (<1%), 2+ (>1- 19%), or 3+ (>20%) membranous positivity for PD-L1, with intensity scoring as follows: 0: no staining, 1+: weak equivocal staining, 2+: moderate staining, and 3+: strong staining. Positive cases were further scored by a two-tiered system into low and high expressions. For the purpose of subsequent analysis, scores 0 and 1 have been considered "low scores" and scores 2 and 3 have been considered "high scores." tumor infiltrating lymphocytes (TIL) displaying membranous and cytoplasmic expression and tumor cell displaying membranous expression were considered positive finding. TIL grades were defined as mild, either a mild or moderate focal or multifocal TIL infiltrate, moderate as either a moderate focal or marked multifocal, and marked as marked diffuse TIL infiltrates.

The statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 21.0 statistical analysis software. The values were represented in number (%), mean ± SD, Chi-square test, and level of significance.

## RESULTS

The present study was conducted to evaluate immune checkpoint protein PD-L1 expression in OSCC and to carry out its clinicopathological correlation. For this purpose, a total of 106 samples were obtained from the surgical oncology department. The age of the patients ranged from 22 to 75 years. The mean age of the patients was 45.43 ± 12.97 years. The age group of 40 to 60 years was most affected. The majority of patients (83%) were males, and 17% were females. A total of 63 (59.4%) patients had received neoadjuvant chemotherapy (NACT). Tongue was the most commonly involved site (n = 37; 34.9%) followed by gingiva (n = 23, *P* = 21.7%), buccal mucosa (n = 12; 11.3%), and lip (n = 7; 6.6%). A total of 27 (25.5%) patients had involvement of more than one site and were placed in the mixed category. The left side was more commonly involved (n = 51; 48.1%) than the right side (n = 49; 46.2%). There were six (5.6%) cases showing bilateral involvement. A history of tobacco chewing with smoking was reported by 92 (86.8%) cases.

A total of 44 (41.5%) cases each had lesion size <2 cm, and in next 44 (41.5%) cases, lesion size was 2–4 cm, while the remaining 18 cases (17.0%) had lesion size of >4 cm. Histologically, cases (58.5%) were graded as well differentiated, followed by moderately differentiated (58.5%) and poorly differentiated (4.7%),

respectively [Figures 1-3]. Ulceroproliferative or nodular pattern (n = 51; 48.1%) was the most common followed by infiltrative (n = 48; 45.2%) and patch (n = 7; 6%) patterns, respectively. A total of 48 cases (45.3%) had depth of invasion >45.3% and 28 cases (26.4%) had evident lymphovascular invasion (LVI), whereas 21 cases (19.8%) had perineural invasion (PNI). Tumor infiltrative lymphocytes were graded as mild, moderate, and dense in 17 (16%), 40 (37.7%), and 49 (46.2%) cases, respectively [Table 1].

In the majority of cases (62.3%), tumor extended up to muscle followed by submucosa (25.5%), bone (9.4%), and skin or dermis (2.6), respectively. The advancing edge was infiltrative in 87 (82.1%) and pushing in 19 (17.9% cases). Nodal metastasis was present in 38 (36.9%) cases. Pathologically, tumor stage was diagnosed as T1, T2, T3, and T4a in 17 (16%), 46 (43.4%), 25 (23.6%), and 18 (17%) cases. Pathologically, majority of cases had nodal status: N0 (n = 65; 61.3%) followed by N1 (n = 20; 18.9%), N2b (n = 10; 9.4%), N3b (n = 8; 7.5%), Nx (n = 2; 1.9%), and N2c (n = 1; 0.9%), respectively.

Immuno-expression of PD-L1 marker was seen in 101 (95.3%) cases, and in the rest of five cases, the tissue was exhausted for the IHC panel. Among cases with IHC expression, score 1+ was accorded to 37 (34.9%); score 2+, to 31 (29.2%); and score 3+, to 33 (31.1%) cases [Table 2; Figures 1-4]. Age and gender did not show a significant association with PD-L1 immuno-expression pattern ($P > 0.05$). No significant association of IHC scores was observed with tobacco smoking habit; however, site of involvement and NACT status were significantly associated with IHC scores. It was seen that the majority of cases being involved of tongue, gingiva, and lip had high

**Table 1: Macroscopic and microscopic histopathological findings**

| Characteristics | No. | % |
|---|---|---|
| Size | | |
| ≤2 cm | 44 | 41.5 |
| 2–4 cm | 44 | 41.5 |
| >4 cm | 18 | 17.0 |
| Histological grade | | |
| Well differentiated | 62 | 58.5 |
| Moderately differentiated | 39 | 58.5 |
| Poorly differentiated | 5` | 4.7 |
| Pattern | | |
| Ulceroproliferative/nodular | 51 | 48.1 |
| Infiltrative | 48 | 45.2 |
| Patch | 7 | 6.0 |
| DOI | | |
| ≤10 mm | 58 | 54.7 |
| >10 mm | 48 | 45.3 |
| Evident LVI | 28 | 26.4 |
| Evident PNI | 21 | 19.8 |
| TIL | | |
| Mild | 17 | 16.0 |
| Moderate | 40 | 37.7 |
| Dense | 49 | 46.2 |

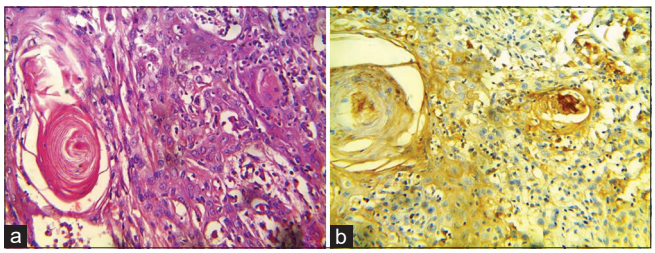


**Figure 1:** (a) Squamous cell carcinoma—well differentiated (40X, H&E stain) (b) PD-L1 immuno-expression in the above case with score 1+ (40X, IHC stain)

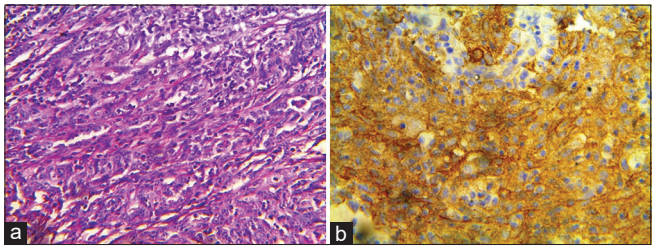


**Figure 3:** (a) Squamous cell carcinoma—poorly differentiated (20X, H&E stain) (b) PD-L1 immuno-expression in the above case with score 3+ (40X, IHC stain)

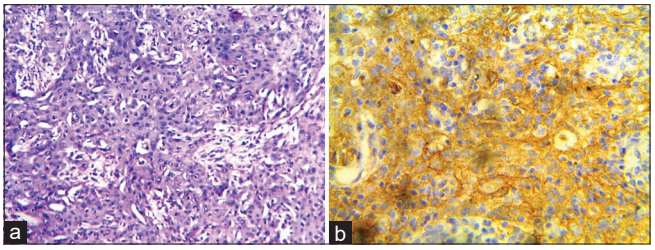


**Figure 2:** (a) Squamous cell carcinoma—moderately differentiated (20X, H&E stain) (b) PD-L1 immuno-expression in the above case with score 2+ (40X, IHC stain)

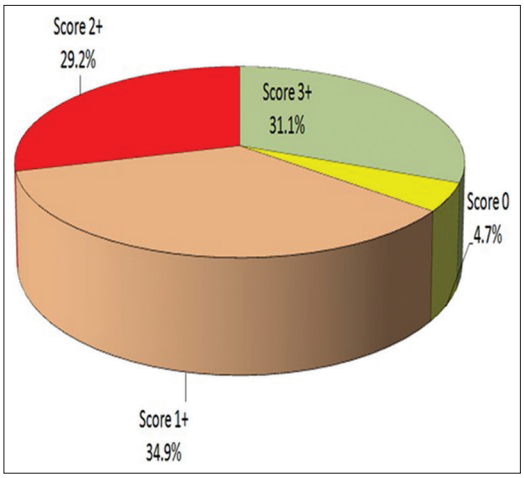


**Figure 4:** Distribution of cases according to PD-L1 immunohistochemical expression score

scores, whereas the majority of cases being involved of buccal mucosa and mixed sites had low scores. Statistically, this difference was significant ($P$ = 0.015). Similarly, those having history of NACT tended to have higher scores (69.8%) as compared to those not having a history of NACT (46.5%) ($P$ = 0.016) [Table 3].

No significant association of histological grade, LVI, and tumor infiltrative lymphocyte status could be seen with PD-L1 immuno-expression scores; however, tumor size, pattern, depth of invasion, and PVI were found to be significantly associated with immunohistochemical scores. The majority of cases with tumor size 2 cm or more had high scores as compared to majority of <2 cm size tumors who had low scores, thereby showing a significant difference ($P$ = 0.030). The majority of cases with ulceroproliferative (64.7%) and infiltrative (62.5%) lesions had high scores as compared to the majority of patch lesions who had low scores (85.7%), thereby showing a significant difference ($P$ = 0.035). The majority of patients having >10 mm deep invasion had high scores (75%), whereas the majority of patients having <10 mm deep lesion (51.7%) had low scores ($P$ = 0.005). The proportion of patients having evidence of perineural neural invasion had high scores in a significantly higher proportion of patients (85.7%) as compared to those having no evidence of PVI (54.1%) ($P$ = 0.008) [Table 4]. The proportion of cases having tumor extension up to submucous was significantly lower (33.3%) as compared to those having extension up to other sites (66.7% to 70%) ($P$ = 0.011). The proportion of cases with high scores was higher among those having infiltrative advancing edge (65.5%) as compared to those having pushing edge (36.8%) ($P$ = 0.021). The proportion of cases with high scores was higher among those having lymph node metastasis (76.3%) as compared to those not having lymph node metastasis (52.3%) ($P$ = 0.016). With increasing tumor stage, a significant increase in proportion of patients with high scores was seen (35.3% and 56.4% in cases with T1 and T2 stages as compared to 68% and 83.3% in T3 and T4a stages) ($P$ = 0.025) [Table 5].

Of the 37 patients who were alive, 16 (43.2%) had low scores and 21 (56.8%) had high scores, whereas all five (100%) of those who died had high IHC scores. Though the proportion of patients with high score was higher among those who died (100%) as compared to those who survived (56.8%), this difference was not significant statistically ($P$ = 0.062).

**Table 2: Distribution of cases according to PD-L1 immunohistochemical expression scores**

| Score | No. of samples | Percentage |
|---|---|---|
| 0 | 5 | 4.7 |
| 1+ | 37 | 34.9 |
| 2+ | 31 | 29.2 |
| 3+ | 33 | 31.1 |

**Table 3: Association of PD-L1 immuno-expression with clinical profile and treatment/personal history**

| Characteristics | Total | Low scores ($n$=42) | | High scores ($n$=64) | | $P$ | |
|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | $\chi^2$ | $P$ |
| Site | | | | | | | |
| Tongue | 37 | 9 | 24.3 | 28 | 75.7 | 12.27 | 0.015 |
| Gingiva | 24 | 10 | 41.7 | 14 | 58.3 | | |
| Buccal mucosa | 11 | 8 | 72.7 | 3 | 27.3 | | |
| Lip | 7 | 1 | 14.3 | 6 | 85.7 | | |
| Mixed | 27 | 14 | 51.9 | 13 | 48.1 | | |
| Laterality | | | | | | | |
| Left | 51 | 17 | 33.3 | 34 | 66.7 | 1.68 | 0.431 |
| Right | 49 | 22 | 44.9 | 27 | 55.1 | | |
| Bilateral/center | 6 | 3 | 50.0 | 3 | 50.0 | | |
| Duration | | | | | | | |
| ≤6 months | 45 | 22 | 48.9 | 23 | 51.1 | 5.12 | 0.078 |
| 6–12 months | 39 | 10 | 25.6 | 12 | 74.5 | | |
| >12 months | 22 | 10 | 45.5 | 12 | 54.5 | | |
| Tobacco/smoking | | | | | | | |
| No | 14 | 4 | 28.6 | 10 | 71.4 | 0.823 | 0.364 |
| Yes | 92 | 38 | 41.3 | 54 | 58.7 | | |
| NACT | | | | | | | |
| No | 43 | 23 | 53.5 | 20 | 46.5 | 5.81 | 0.016 |
| Yes | 63 | 19 | 30.2 | 44 | 69.8 | | |

## DISCUSSION

The findings of the present study are recapitulated and compared with results of other authors, indicating agreement or contrast with previously published work. The patients in our study were aged between 25 and 72 years with a mean age of 45.43+–12.97 years. There was no significant correlation between increasing age and PD-L1 expression in our study. Similar observation was made in previous studies conducted on OSCC and PD-L1 expression correlations by Maruse Y *et al*., Chen XJ *et al*., Kogashiwa Y *et al*., and Straub M *et al*.[4,6-8]

The male-to-female ratio was 4.9:1 (M = 88, F = 18) in our study. The most likely reason can be that the consumption of tobacco in males is more as compared to females, which is a leading risk factor for oral cancer development and also that the number of females presenting to a tertiary care center are less in a developing country like India. PD-L1 expression did not correlate significantly with sex; however, it was observed that a higher percentage of female patients (77.8%) expressed high PD-L1 as compared to male patients (56.8%). In the study conducted by Kogashiwa Y *et al*.[7] and Lin YM *et al*.,[5] PD-L1 expression was significantly correlated with sex.

Female patients expressed high PD-L1. Kim HS *et al*.[9] and Chen TC *et al*.[10] in their study also found a correlation

**Table 4: Association of PD-L1 immuno-expression with histopathological profile**

| Characteristics | Total | Low (0, 1+) (*n*=42) | | High (2+, 3+) (*n*=64) | | *P* | |
|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | $\chi^2$ | *P* |
| Size | | | | | | | |
| ≤2 cm | 44 | 24 | 54.5 | 20 | 45.5 | 7.02 | 0.030 |
| 2–4 cm | 44 | 13 | 29.5 | 31 | 70.5 | | |
| >4 cm | 18 | 5 | 27.8 | 13 | 72.2 | | |
| Histological grade | | | | | | | |
| WD | 62 | 28 | 45.2 | 34 | 54.8 | 2.08 | 0.355 |
| MD | 39 | 12 | 30.8 | 27 | 60.0 | | |
| PD | 5 | 3 | 60.0 | 2 | 40.0 | | |
| Pattern | | | | | | | |
| Ulceroproliferative | 51 | 18 | 35.3 | 33 | 64.7 | 6.71 | 0.035 |
| Infiltrative | 48 | 18 | 37.5 | 30 | 62.5 | | |
| Patch lesion | 7 | 6 | 85.7 | 1 | 14.3 | | |
| DOI | | | | | | | |
| <10 mm | 58 | 30 | 51.7 | 28 | 48.3 | 7.84 | 0.005 |
| >10 mm | 48 | 12 | 25.0 | 36 | 75.0 | | |
| LVI | | | | | | | |
| Evident | 28 | 8 | 28.6 | 20 | 71.4 | 1.94 | 0.163 |
| Not evident | 78 | 34 | 43.6 | 44 | 56.4 | | |
| PNI | | | | | | | |
| Evident | 21 | 3 | 14.3 | 18 | 85.7 | 7.03 | 0.008 |
| Not evident | 85 | 39 | 45.9 | 46 | 54.1 | | |
| TIL | | | | | | | |
| Mild | 17 | 9 | 52.9 | 8 | 47.1 | 1.51 | 0.471 |
| Moderate | 40 | 15 | 37.5 | 25 | 62.5 | | |
| Dense | 49 | 18 | 36.7 | 31 | 63.3 | | |

**Table 5: Association of PD-L1 immuno-expression with histopathological findings and pathological TNM staging**

| Characteristics | Total | Low (0, 1+) (*n*=42) | | High (2+, 3+) (*n*=64) | | *P* | |
|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | $\chi^2$ | *P* |
| Tumor extension | | | | | | | |
| Submucous | 27 | 18 | 66.7 | 9 | 33.3 | 11.09 | 0.011 |
| Muscle | 66 | 20 | 30.3 | 46 | 69.7 | | |
| Bone | 10 | 3 | 30.0 | 7 | 70.0 | | |
| Skin/dermis | 3 | 1 | 33.3 | 2 | 66.7 | | |
| Advancing edge | | | | | | | |
| Infiltrative | 87 | 30 | 34.5 | 57 | 65.5 | 5.36 | 0.021 |
| Pushing | 19 | 12 | 63.2 | 7 | 36.8 | | |
| Nodal metastasis (*n*=103) | | | | | | | |
| Absent | 65 | 31 | 47.7 | 34 | 52.3 | 5.82 | 0.016 |
| Present | 38 | 9 | 23.7 | 29 | 76.3 | | |
| Tumor stage (pT) | | | | | | | |
| T1 | 17 | 11 | 64.7 | 6 | 35.3 | 9.33 | 0.025 |
| T2 | 46 | 20 | 43.5 | 26 | 56.5 | | |
| T3 | 25 | 8 | 32.0 | 17 | 68.0 | | |
| T4a | 18 | 3 | 16.7 | 15 | 83.3 | | |
| T4b | 0 | 0 | 0 | 0 | 0 | | |
| Nodal stage (pN) | | | | | | | |
| Nx | 2 | 1 | 50.0 | 1 | 50.0 | 6.14 | 0.293 |
| N0 | 65 | 31 | 47.7 | 34 | 52.3 | | |
| N1 | 20 | 6 | 30.0 | 14 | 70.0 | | |
| N2a | 0 | 0 | 0 | 0 | 0 | | |
| N2b | 10 | 3 | 30.0 | 7 | 70.0 | | |
| N2c | 1 | 0 | 0.0 | 1 | 100.0 | | |
| N3a | 0 | 0 | 0 | 0 | 0 | | |
| N3b | 8 | 1 | 12.5 | 7 | 87.5 | | |

of PD-L1 high expression with female sex; however, this was not statistically significant. SCC was studied at multiple sites in oral cavity in our study. The maximum percentage of cases that expressed high PD-L1 was observed in the lip (85.7%) followed by the tongue (75.7%) and buccal mucosa (58.3%). There was a statistically significant correlation between these sites and PD-L1 expression ($P = 0.015$). A similar trend was projected in the study conducted by Maruse Y *et al*.,[4] in which the maximum percentage of high PD-L1 expressing cancers were from the tongue and buccal mucosa; however, this was not statistically significant.

In our study, PD-L1 expression in tobacco consumers did not show much difference in intensity. However, the percentage of nonconsumers of tobacco showing high PD-L1 expression was more (71.4%). Similar observation was made in the study conducted by de Vicente JC *et al*. and Chen XJ *et al*.[6,11] There was statistically significant correlation of PD-L1 expression observed in our study between the cases that have history of NACT and those that did not have it. A large percentage of cases that took NACT showed high PD-L1 expression (69.8%). Kim HS *et al*.[9] included some cases of post-NACT OSCC in their study and observed a similar trend that higher percentage of cases with NACT intake showed high PD-L1 expression (77.14%). This could be explained by taking into consideration the studies conducted by Leduc C *et al*.[12] and Ock CY *et al*.[13] They studied PD-L1 expression in cases of OSCC before and after chemotherapy and evaluated the effects of NACT on tumor expression of PD-L1 and found that chemotherapy increases PD-L1 expression in tumor cells.

In our study, tumor size was divided into three categories as mentioned before and PD-L1 expression was significantly correlated with size greater than 2 cm in comparison with size <2 cm. Yoshida S *et al*.[14] in their study correlated PD-L1 expression with tumor volume. They found that PD-L1-positive case percentage increased from 23% in tumors less than 2000 $mm^3$ to 79% in tumors 2,000–5,000 $mm^3$. The depth of tumor invasion also showed statistically significant correlation with PD-L1 expression, suggesting that as the invasion of tumor increases, the tumor cells show high PD-L1 expression concluding that evasion from tumor immunity is more in those tumors that have a tendency to invade more. In our study, the immunohistochemical expression of immune checkpoint protein PD-L1 expression showed statistically significant correlation with LVI and PNI. However, cases that had LVI showed larger percentage of high PD-L1 expressing cases. In study conducted by Chen SC *et al*.,[15] PD-L1 expression also did not statistically correlate with LVI or with PNI; however, cases that had

LVI and PNI expressed high PD-L1 in large proportion. Roper E *et al*.[16] also did not find any statistically significant correlation between PD-L1 expression and LVI and PNI in cases of head and neck cutaneous SCC. Prabha S Mishra *et al*.[17] concluded that PD-L1 expression in head and neck squamous cell carcinoma (HNSCC) cases was independent of age, gender, tumor site, and tumor grade or stage. In Xiao Jie Chen *et al*.'s[6] study, the expression level of PD-L1 in OSCC was positively correlated with the pathological grade ($P < 0.0001$), but it was independent of age, gender, smoking, drinking, tumor size, lymph node status, or recurrence ($P > 0.05$). In this study, no statistically significant correlation was found between high PD-L1 expression in tumor cells and some clinical and histological parameters such as age and sex of patients, laterality of tumor, duration of symptoms, tobacco consumption, histological tumor grade, lymphoplasmacytic infiltration, and nodal status, whereas a correlation was found statistically significant between high PD-L1 expression and some other clinical and histological parameters such as site of tumor, size of tumor, pattern of growth, depth of tumor invasion, tumor extension, LVI, PVI, advancing edge of tumor, nodal metastasis, and T stage.

In cases that had high PD-L1 expression, an incremental trend in the number of tumor-infiltrating lymphocytes was observed in our study; however, this was not statistically significant. Roper E *et al*.[16] in their study conducted on head and neck cutaneous SCC observed that TIL was more in high PD-L1 expressing cases, but this was not statistically significant. Kogashiwa Y *et al*.[7] conducted the study on locally advanced oral SCC cases and PD-L1 expression was significantly correlated with cluster of differentiation (CD)8 + TILs. This difference with our study could be explained by the fact that we did not separate CD8 + and CD4 + TILs and as studied before.

CD8+ TILs are the main type of lymphocytes that have a key role in tumor immune escape mechanisms.

In our study, tumors that were locally advanced and reached up to muscle, bone, or skin expressed high PD-L1 as compared to those that were limited to the submucosa. This suggests that the PD-L1 expression by tumor cells increases as the tumor becomes locally advanced. It was also observed in this study that tumors having infiltrating edges expressed high PD-L1, again showing a change in PD-L1 expressivity of tumors as they become more infiltrative.

In this study, there was significant correlation seen between nodal metastasis and PD-L1 expression. The high percentage of cases that had PD-L1 expression was positive for nodal metastasis. Similar results were observed in the study conducted by Maruse Y *et al*. and Yoshida S *et al*. on cases of oral SCC.[4,14] This suggests that the PD-L1 expression increases as the tendency of nodal metastasis increases. When the correlation between PD-L1 expression and AJCC T stage and nodal stage was determined, it was observed that only T stage correlated significantly with the expression of PD-L1. A similar trend was observed in studies conducted by Kim HS *et al*.[9] and Maruse Y *et al*. that as the T stage increased, the PD-L1 expressing cases increased. However, their correlation was not statistically significant.[4] Taking N stage into consideration, harmony with our results was seen in many other studies conducted on oral SCC cases like Lin YM *et al*.[5] and Kogashiwa Y *et al*.[7]

Of 106 cases included in our study, only 42 patients were available for evaluation of prognosis, and the rest were lost to follow-up. Of these available patients, majority were alive, but those who died belonged to the category of high PD-L1 expression. This correlation, however, was not statistically significant. This may be attributed to short time of follow-up ranging from 3 months to 12 months. The cases were followed for an average time period of only 8.4 months after surgery. Also, around 60% patients were lost to follow-up. Maruse Y *et al*.[4] and Lin YM *et al*.,[5] in their studies on oral SCC, found that those tumor with high PD-L1 had poor survival rate as compared to those with low expression of PD-L1. However, the study conducted by Yoshida S *et al*.[14] on tongue SCC showed that there was no significant correlation between PD-L1 expression and patient outcome. Similarly, Kim HS *et al*.[9] found that there is no significant correlation between PD-L1 expression and overall survival of patients of oropharyngeal SCC. Kogashiwa Y *et al*.[7] found that in advanced OSCC, PD-L1 expression was associated with better patient survival.

The immunohistochemical expression of immune checkpoint protein PD-L1 was significantly correlated with various parameters of tumor and clinical profile of patients. These subsets of patients may benefit from immunotherapy, and survival rate may get better. Targeted drugs that are available against PD-L1 have shown in trials better tolerance and less complications as compared to conventional chemotherapy. Thus, on the basis of previous studies it can be suggested that in those cases in which PD-L1 expression is high, the role of immunotherapy can be significant.

## CONCLUSION

We conclude that the role of targeted therapy can be predicted by evaluating the immunohistochemical

expression of immune checkpoint protein PD-L1 expression in the cases of OSCC cases that may be benefited by immunotherapy.

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## Conflicts of interest

There are no conflicts of interest.

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