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Original Research Article

Expression of PD-L1 (Programmed Cell Death Ligand-1) in Oral Squamous Cell Carcinoma: A Potential Immunomodulator Tool for Management

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Abstract:

Background: Oral squamous cell carcinoma (OSCC) is a common malignancy with increasing incidence and mortality. Now it is important to take step to improve therapeutic strategies for the patients affected by OSCC. In the advent of immunotherapy, Anti PD-L1 seems to be effective in the treatment of many malignancies.

Objectives: To demonstrate the expression of Programmed Cell Death Ligand-1 (PD-L1) in Oral squamous cell Carcinoma (OSCC) by means of immunohistochemistry (IHC).

Material and Method: In this study, immunohistochemical staining in 25 Cases of OSCC was done by Ventana PD-L1(SP263) Rabbit monoclonal primary antibody and expression of PD-L1 was assessed as per FDA approved criteria. Only membranous staining on tumor cells was considered positive. For statistical analysis, SPSS software version 23 was used.

Result: In this study**,** PD-L1 was expressed in 23 Cases out of 25 Cases (92%)of OSCC. Expression was negative in only 2 cases of OSCC. We also found that expression was high in Cases of Alveolar ridge and Buccal Mucosa and low in the lesions of tongue.

Conclusion: Our study supports that PD-L1 which is an immunomodulator is significantly expressed and play role in Oral squamous cell carcinoma. Anti PD-L1 immunotherapy in patients of OSCC could be a new direction in the treatment of oral cancers.

Keywords: Immunomodulator, PD-L1, Oral squamous cell Carcinoma (OSCC).

Introduction

Oral squamous cell carcinoma (OSCC) is the most common tumor of the oral cavity with an increasing incidence worldwide, with up to 370,000 new cases per year [1]. Pathogenesis of OSCC is multifactorial [2]. Among men, it is the second most common site and in women it is the fourth in India[3]. Commonest sites are gingivobuccal sulcus of mandible followed by the tongue and floor of the mouth [4]. Despite of many treatment modalities, overall survival has not improved in last few decades. For this reasons it is important to find out relevant biomarker and develop effective targeted therapy for OSCC.

PD-1 is an immune checkpoint protein expressed on the surface of immune cells such as cytotoxic T cells. PD-L1 and PD-L2 are overexpressed on the surface of cancer cells, bind to the trans membrane protein PD-1 on T cells and inhibit cytotoxic immune response of T cells and protect themselves from immune surveillance [5]. By Blocking the interaction of PD-L1 on cancer cells with its receptor PD-1 on T cells by targeted therapy can regain cytotoxic nature of T cell and kill cancer cells.

PD-L1 expression is seen in many human cancers including pancreatic cancers, gastric cancers, lung cancers and Breast cancers [6]. But role of PD-L1 in oral cancer is not yet fully understood. So in such phase of on-going researches we aim to determine expression of PD-L1 in OSCC.

Material and Methods

We conducted a cross sectional study in 25 cases of OSCC in the time period of one year after approval from ethical committee. Written informed consent was taken and all parameters were obtained from medical records. Among 25 patients selected in this study, we examined patient's gender, age, locality, socioeconomic status, primary site of OSCC and habit of tobacco chewing.

Paraffin-embedded blocks containing OSCC tissue samples were prepared. Hematoxylin and Eosin staining was done and examined under light microscope.

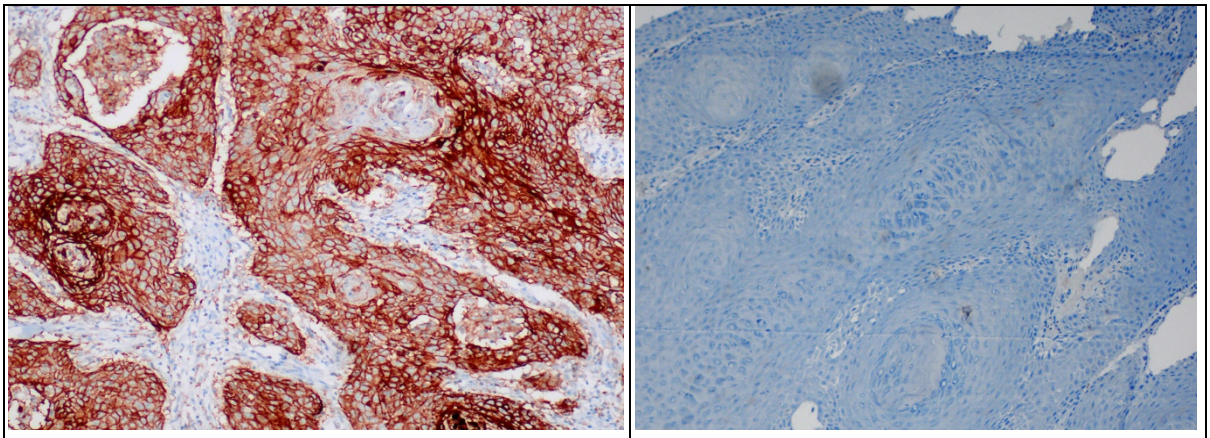
For immunohistochemistry, section of 3-4 µ was taken and stained with Ventana PD-L1 SP263 Rabbit monoclonal primary antibody. Evaluation of PD-L1 was done in tumor areas comprising of at least 100 viable cells and only membranous staining of tumor cells was considered positive. Scoring was done as follow- Score 0- Negative or <1%, Score 1- 1-10%, Score 2- 11-25%, Score 3- 26-50%, Score 4- More than 50%.[7]

Staining intensity to be scored as follow: No, Weak, Moderate, Strong.

PD-L1 expression of at least in 1% of tumor cells was considered as positive and <1% as Negative [7]. In the previous studies, it is found that Patients with high expression of PD-L1 on tumor cells respond well to Anti PD-L1 therapy. So as per FDA approved criteria, tumor cell expressing PD-L1 of > 25% was considered as High expression of PD-L1 [8].

Statistical Analysis Used

Qualitative Data was expressed as mean and standard deviation, Whereas Quantitative data as Frequency and Percentages. Association of PD-L1 with all the Parameters was determined by applying the chi-squared test and P value of less than 0.05 was considered significant.



A **B**

Figure 1: Immunohistochemical expression of PD-L1 in Oral squamous cell carcinoma (A) Positive membranous staining of PD-L1 on tumor cells (B) Negative Staining

Results and Observations

Table 1-summarises the distribution of parameters included in our study. In this study, patient's age ranged from 35-80 years with mean age-50.5 ± 13.4 yrs. Among 25 Cases ,22 (88 %)were male and 3(12 %) were female. 13 patients belong to urban locality and 12 were belong to rural locality. Out of 25 cases, 20 cases were tobacco chewer and 5 cases had no history of tobacco chewing. In this study, 15(60%) cases were from buccal mucosa, 4 (16%)cases were from alveolar ridge, 3(12%) from Retromolar Trigone and 3(12%) from tongue.

Table 1: Parameters Included In Our Study

Parameters	**Number of patient's n (%)**
Age (in years, Mean Age)	50.5 ± 13.4
Gender	
Male	22 (88 %)
Female	3 (12 %)
Locality	
Rural	12 (48 %)
Urban	13 (52%)
Socioeconomic Status	
Upper and Upper Middle Class	8 (32%)
Lower Middle Class	8 (32%)
Upper Lower and Lower Class	9 (36%)
Tobacco Usage habits	
No	5 (20%)
Tobacco chewer	20(80%)

Site of Lesion	
Buccal Mucosa	15(60%)
Alveolar Ridge	4 (16%)
Retromolar Trigone	3 (12%)
Tongue	3 (12%)

Table 2 Expression of PD-L1 in tumor cells in 25 cases of oral squamous cell carcinoma. Immunohistochemical staining of PD-L1 in tumor cells was assessed as membranous staining in 100 viable cells.

Intensity of staining and percentage of positive tumor cells was scored as mentioned in methodology section. In the result we found that among 25 cases, 12 (48.0%) were of moderate staining, 7(28.0%) were of weak staining, 4(16.0%) were of strong intensity and 2(8.0%) showed negative staining.

Most cases in our study showed Score 2 positive expression of PD-L1 (18-32.0%). High expression of PD-L1(>25%) shown by 11 /25 cases (44%) and low expression by 12/25 cases (48%). Negative or no expression of PD-L1 was seen in 2/25 cases (8%).

Table 2: Distribution of Cases based on PDL-1 Expression

PD-L1 Expression	Number of Participants (N) Percentage (%)
Staining Intensity	
No	2(8. 0%)
Weak	7(28. 0%)
Moderate	12(48. 0%)
Strong	4 (16. 0%)
Percentage of Tumor Cells expressing PD-L1	
Score-0 -<1%	2(8. 0%)
Score-1 -1-10% Cells	4(16. 0%)
Score-2-11 to 25% Cells	8(32. 0%)
Score-3-26% to 50% Cells	6(24. 0%)
Score-4- More than 50% Cells	5(20. 0%)

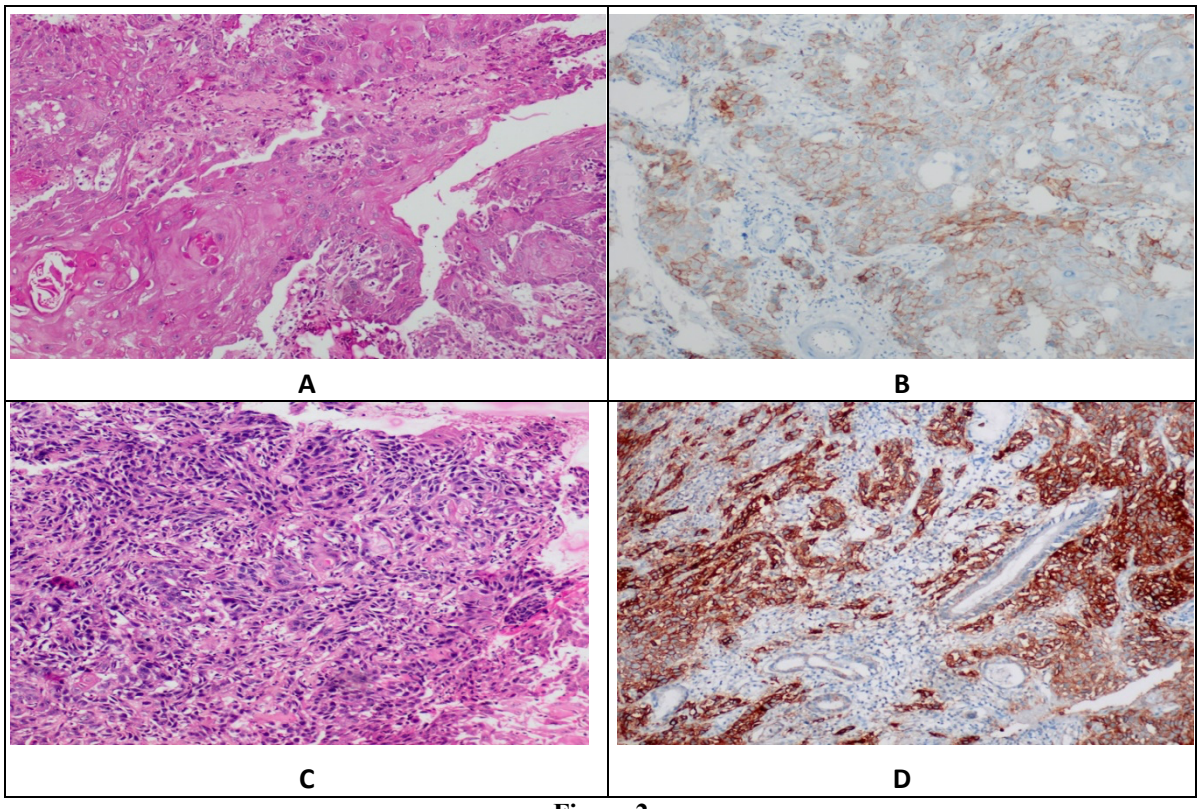


Figure 2

Figure 2 A & B- H and E stained section of oral squamous cell carcinoma show tumor cells with its corresponding IHC expression of PD-L1 showing weak intensity expression. C & D-H and E stained section show sheets of malignant squamous cells with its corresponding IHC expression of PD-L1 showing Strong intensity expression.

Table 3: Association Between PD-L1 expression and Different Parameters

Parameters		Percentage of Tumor Cell Expressing PD-L1			P-Value
		No or < 1% (Negative expression)	Up to 25% (Low Expression)	More than 25% (High Expression)	
Age (in completed years)	40 and younger	0	4 (44. 4%)	5 (55. 6%)	0. 118
	41 to 55	2 (28. 5%)	3 (43.0%)	2 (28.5. 0%)	
	56 to 70	0	4(66. 7%)	2 (33. 3%)	
	>70	0	1 (33. 3%)	2 (66.7%)	
Sex	Male	2 (9.2%)	10(45.4%)	10 (45. 4%)	0.174
	Female	0	2 (66. 6%)	1 (33.4%)	
Locality	Rural	2(16. 6%)	6 (50.0%)	4 (33. 4%)	0. 162
	Urban	0	6(46.1%)	7 (53.9%)	
Socio Economic Status	Upper and Upper Middle Class	0	5(62. 5%)	3 (37. 5%)	0. 095
	Lower Middle Class	0	5(62.5%)	3 (37.5%)	
	Upper Lower and Lower Class	2 (22.2%)	2(22.2%)	5 (55.6%)	
Habit	Tobacco use				0.145
	None	0 (0%)	4 (80.0%)	1 (20.0%)	
	Tobacco Chewing	2 (10%)	8 (40%)	10 (50%)	
Site of Lesion	Buccal Mucosa	2 (13. 3%)	6 (24.0%)	7 (46. 7%)	**0. 013**
	Alveolar Ridge	0	1 (25.0%)	3(75.0%)	
	Retromolar Trigone	0	2 (66.6%)	1 (33.4%)	
	Tongue	0	3 (100%)	0	

Table 3 In all the parameters included in our study, no association found with age of patient, gender, locality, socioeconomic status and habit of tobacco. We found high expression of PD-L1 in alveolar ridge and buccal mucosa OSCC with statistically significant P value-0.013. Expression was low in cases of tongue OSCC.

Discussion

Immune tolerance is a major barrier in cancer immunotherapy and exists in a variety of human tumors. Within the tumor microenvironment, tumor cells rely on different mechanisms to protect themselves and escape the body's immune responses. PD-L1 also known as B7-H1 or CD274 is an important molecule in the tumor microenvironment and its upregulation is one of the key mechanisms of tumor immune evasion. Recent studies have found that PD-L1 is widely expressed in many types of human tumor tissue.

However, a clear consensus has not been reached on expression of PD-L1 in oral squamous cell carcinoma. Our study revealed that PD-L1 is highly expressed in oral squamous cell carcinoma. Its expression was not significantly correlated with age of patient, gender, locality, socioeconomic status and habit of tobacco, but was associated with site of lesion. In previous studies, Tim Muller et al (2017)[9] and Yu and colleague[10] revealed significant subset of head and neck cancers showing high expression of PD-L1. Similarly study of Wu and tang (2021)[11] revealed that PD-L1 was highly expressed in OSCC and higher PD-L1 expression associated with worst prognosis. The result of our study is consistent with these previous studies.

Despite of many studies performed, the clinicopathological effects of PD-L1 expression in patient with oral squamous cell carcinoma in previous studies remain unclear. In previous studies, Yong and Xin (2020)[12] and Nattinee (2020)[13] demonstrated high PD-L1 expression in women but there was no significant correlation seen between PD-L1 expression and sex of the patient in oral squamous cell carcinoma in other studies similarly to our study. Muller et al (2017)[9] observed no correlation between PD-L1 expression and causative agents like tobacco use and alcohol consumption. As Tobacco and smoke abuse is an important risk factor in oral squamous cell carcinoma accounts for 50% of all cancers of men and 25% of all cancers in women. Previous studies demonstrated different expression in oral squamous cell carcinoma depending on its location. Lukasz Jan Adamaski (2021)[14] also show lower expression in carcinoma of tongue and floor of the

mouth similar to our study. The site dependent differences reported by Green et al[15]and observed high tumor infiltrating lymphocytes in oropharyngeal cancers compared to other location may be contributing to difference in expression including many other factors. In our study most cases of oral squamous cell carcinoma were of buccal mucosa (15/25 cases). We demonstrated high expression of PD-L1 in Alveolar ridge (3/4 cases) and buccal mucosa (7/15 cases) with statistically significant P value-0.013. Low expression of PD-L1 seen in carcinoma of tongue (3/3).

Studies have shown that PD-1/PD-L1 not only participates in the regulation of tumor immunity, but also plays a role in tumorigenesis, growth and metastasis. At present many anti-PD-1 or anti-PD-L1 drugs have been used in clinical trials. A key feature of these treatments is good patient tolerance. PD-1/PD-L1 signalling pathway has been widely acknowledged and emphasized in research on cancer immunotherapy and has provided us with new molecular targets.

In a recent meta-analysis, Gandhi et al (2016)[16] found that IHC evaluation of tumor with high PD-L1 expression correlated with clinical response to PD-L1/PD-1 immunotherapy in various cancers and Topalian et al (2012)[17] reported that response to anti PD-1/PD-L1 targeted therapy was only observed in PD-L1 positive tumors. With our study, we can suggest that patient with high PD-L1 expression in oral squamous cell carcinoma should be targeted for immunotherapy to improve prognosis and clinical outcome.

Conclusion

In summary, PD-L1 is significantly expressed in oral squamous cell carcinoma. It is also highly expressed in alveolar ridge and buccal mucosa OSCC. This expression helps us in targeting oral cancer cases for immunotherapy.

Limitation-Larger sample will be required for more clear results.

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