

Immunohistochemical expression of programmed death ligand 1 in squamous cell carcinoma of head and neck

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ABSTRACT

Objective: To assess the immunohistochemical expression and clinicopathological features of Programmed Death Ligand 1 in the head and neck squamous cell carcinoma (HNSCC).

Material and Methods: This Cross-sectional study was conducted at the Histopathology department, Rehman Medical Institute, Peshawar, Sep 2023 to Dec 2024. Patients with histologically confirmed head and neck squamous cell carcinoma were included in the study. Sections from Formalin fixed tissue blocks underwent immunohistochemical staining by PD-L1 antibody followed by analysis. Demographic characteristics and histopathological parameters were recorded.

Results: Among 92 participants we observed a male predominance with male to female ratio of 3.8:1 with a median (IQR) age of 59.62 (56.85-62) years. The frequency of PD-L1 positivity was observed in 84 (91.3%) patients. The most common site of the tumor was the oral cavity in 36 (60.9%). We observed that lymphovascular and perineural invasion was not significantly associated with PD-L1 positivity. T-stage of the tumors revealed that most tumors had T1 followed by T2 stage, while N-staging revealed most specimens 39 (46.4%) had N1 stage, followed by N0, N2, and N3 stages with a significant correlation to PD-L1 positivity. Tumors with extranodal extension and moderate tumor-infiltrating lymphocytes histologically revealed PD-L1 expression in 36 (77%) and 59 (70.2%) with p=0.920 and p=0.239 respectively.

Conclusion: We conclude that there is a predominance of PD-L1 positivity in HNSCC with a significant correlation between PD-L1 positivity and nodal metastasis. These findings may lead to the development of immunotherapeutic strategies and identification of patients who may benefit from PD-L1 targeted therapies.

Keywords: Immunohistochemistry, PD-L1, Squamous cell carcinoma of head and neck, Immunotherapy

This article can be cited as: Khan M, Safdar A, Mahmood R, Jamil A, Javaid F, Muhammad I. Immunohistochemical expression of Programmed Death Ligand 1 in squamous cell carcinoma of head and neck. Pak J Pathol. 2025; 36(1): 12-18.

DOI: <https://doi.org/10.55629/pakipathol.v36i1.890>

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is one of the most common malignancies arising from the squamous cells of the nasopharynx, paranasal sinuses, oral cavity, pharynx, larynx, and salivary glands [1]. It is a major health concern due to its increasing incidence with approximately 890,000 new cases and a mortality of 450,000 per year worldwide as per the data of GLOBOCAN [2].

An estimate of 90% of HNSCCs are squamous cell carcinoma, and due to limited treatment options, warrants early diagnosis with prompt intervention [2].

Several risk factors, including the use of tobacco, human papillomavirus, and low socioeconomic status, are the known risk factors of HNSCC [3]. Increase in the number of cases and higher rates of mortality are alarming for healthcare workers. Multimodal treatment options including radiotherapy, chemotherapy, and surgical options are adopted in patients with HNSCC. However, regardless of the treatment, there is a higher incidence of recurrence and metastasis [4]. Advances in the field of histopathological diagnostics have enabled healthcare workers to identify biomarkers in cancerous cells which may be used for initiating

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Receiving Date: 03 Jan 2025

Revision Date: 18 Feb 2025

Acceptance Date: 04 Mar 2025



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targeted therapies against cancerous cells for better outcomes.

Novel therapies including immune-therapies are emerging techniques for modifying and harnessing the immune system of the human body. Programmed death ligand 1 (PD-L1) is a protein that binds to the protein Programmed death 1 (PD1) receptors on T cells, and binding of PD-L1 to its receptors prevents the T cells from killing the cancerous cells. Immune checkpoint inhibitors (anticancer drugs) avoid the binding of PD-L1 to PD1 receptors and resume the defensive function of T cells against the cancerous cells. Targeting PD-L1 in advanced melanoma, Hodgkin lymphoma, and other solid tumors has revealed a high success rate, and this signifies the importance of targeted immunotherapy in treating cancer [5]. However, the limited number of trials on PD-L1 expression in HNSCC leads to an existing gap in research.

This paper aims to assess the expression of PD-L1 expression and its clinicopathological relevance in HNSCC. This will help in exploring the potential of PD-L1 as a predictive targeted biomarker for treatment in patients diagnosed with HNSCC for better patient outcomes. In the future, this will pave the way for further research to initiate immunotherapies against PD-L1 or combination immunotherapies targeting other checkpoints as well.

MATERIAL AND METHODS

This cross-sectional study was conducted at Baitman Medical Institute, Peshawar from September 2023 to December 2024 after obtaining approval of the ethical review committee under ERC # 42 dated 6th September 2023.

Sample size $n=81$ was calculated using the WHO sample size calculator with an 8% level of significance and 95% power of test when the reported prevalence of head and neck squamous cell carcinoma was 16% [8]. Sampling was done using a non-probability consecutive technique.

Patients of either gender with confirmed biopsy-proven diagnosis of head and neck

squamous cell carcinoma were included in the study. Patients with suspicion of HNSCC with inadequate biopsy specimens were excluded from the study.

A total of 92 samples were included in the research and demographic characteristics of all the patients were recorded. Tissue blocks, formalin fixed and embedded in paraffin (FFPE blocks) of histopathologically confirmed squamous cell carcinoma of head and neck were collected. Tissue sections of 5 micrometer thickness were obtained, which were mounted onto glass slides. These slides were then heated at 60 °C to obtain optimum adhesion of the section. The staining procedure consisted of deparafinization by xylene followed by rehydration via series of alcohol solutions of decreasing strength. This was followed by Antigen retrieval which was achieved via Heat Induced Antigen Retrieval method. Background staining was minimized by blocking endogenous peroxidase activity. This was followed by application of primary antibody i.e. Monoclonal Rabbit anti PD-L1 Antibody Clone 28-8 by Cell Marque which was incubated for a period of 1 hour. After rinsing with Phosphate Buffer Saline, the Secondary conjugated antibody was applied. To demonstrate the colour change, Diaminobenzidine substrate was utilized. Final steps included counterstaining with Hematoxylin, dehydration and mounting with coverslip. Tonsillar tissue was taken as control. PD-L1 identification was done by visualizing the stained cell membrane of tumor cells and a Combined Positive Score (CPS) was used for evaluation. Determination of the CPS score was done by the total number of PD-L1 staining cells divided by the total number of viable tumor cells multiplied by 100. A CPS of <1 was recorded as PD-L1 negative while a CPS of >1 was taken as PD-L1 positive. Specimen with CPS >1 is shown in Figure-II. The results were analyzed independently by two Histopathologists. Clinicopathological parameters of each specimen and PD-L1 expression were recorded on a predesigned Performa.

Data entry and analysis were done using the Statistical Package for the Social Sciences software version 23. For qualitative variables,

frequencies and percentages were calculated. We used Pearson chi-square test for analyzing the association between PD-L1 and qualitative variables like tumor site, lymphovascular invasion, perineural invasion, N-stage, T-stage, grade of tumor, extra nodal extension and tumor infiltrating lymphocytes. For scale variables like age, the distribution of the data was analyzed using the Shapiro-Wilk and Kolmogorov-Smirnov tests. For scale data with non-normal distribution, median and interquartile range were computed. The p-value of <0.05 was considered as significant.

RESULTS

Among 92 participants we observed a male predominance with a male-to-female ratio of 3.8:1 and a median (IQR) age of 59.62 (56.85-62) years. The majority of the participants diagnosed with squamous cell carcinoma had a history of smoking observed in 65 (70.7%). We concluded that the frequency of PD-L1 positivity was observed in 84 (91.3%) patients. Demographic characteristics of patients are shown in Table-I. The most common site of the tumor was the oral cavity in 56 (60.9%) followed by the larynx in 28 (30.4%) and the hypopharynx

in 8 (8.7%) patients ($p = 0.084$). Oral squamous cell carcinoma is shown in Figure-1. When analyzing lymphovascular invasion we observed that 84/92 (91.3%) were PD-L1 positive out of which 13 (15.5%) had lymphovascular invasion while 71 (84.5%) had no lymphovascular invasion with a p-value of 0.230. Similarly, perineural invasion was present in 23(25%) of the cases and absent in 69 (75%) of the cases, with PD-L1 positivity observed in 23 (27.4%) of the cases ($p = 0.087$). T-stage of the tumors revealed that most tumors were T1 followed by T2 stage, and correlation with PD-L1 positivity was not significant with a p-value of 0.213. N-staging of tumors revealed most specimens 39 (46.4%) had N1 stage followed by N0, N3 and N2 stage revealing a significant correlation with PD-L1 expression ($p < 0.001$). Tumors with extra nodal extension and moderate tumor-infiltrating lymphocytes histologically revealed higher PD-L1 expression observed in 30 (35.7%) and 59 (64.2%) with $p=0.920$ and $p=0.239$ respectively. The correlation of PD-L1 expression and clinicopathological parameters is shown in Table-II.

Table-I: Demographic Characteristics and PD-L1 status (n=92).

Variable	Results
Age in years - Median (IQR)	59.62 (56.85-62)
Gender n (%)	Male 73 (79.3%) Females 19 (20.7%)
Smoking n (%)	Yes 65 (70.7%) No 27 (29.3%)
PD-L1 positivity n (%)	Yes 84 (91.3%) No 8 (8.7%)

Table-II: Correlation of PD-L1 expression and clinicopathological parameters (n=92).

Parameters		PD-L1 positive (n=84)	PD-L1 Negative (n=08)	p-value
Tumor site n (%)	Oral cavity	54 (64.3%)	2 (25%)	0.084
	Larynx	23 (27.4%)	5 (62.5%)	
	Hypopharynx	7 (8.3%)	1 (12.5%)	
Lymphovascular invasion n (%)	Yes	13 (15.5%)	0 (0.0%)	0.230
	No	71 (84.5%)	8 (100.0%)	
Perineural invasion n (%)	Yes	23 (27.4%)	0 (0.0%)	0.087
	No	61 (72.6%)	8 (100.0%)	
N-Stage n (%)	N0	22 (26.2%)	8 (100%)	0.000
	N1	39 (46.4%)	0 (0.0%)	
	N2	11 (13.1%)	0 (0.0%)	
	N3	12 (14.3%)	0 (0.0%)	
	T1	40 (47.6%)	3 (37.5%)	0.213
T-stage	T2	28 (33.3%)	1 (12.5%)	

Grade of Tumor	T3	7 (8.3%)	2 (25.0%)	0.125
	T4	9 (10.7%)	2 (25.0%)	
	Well-differentiated	11 (13.1%)	3 (37.5%)	
	Moderately differentiated	58 (69%)	3 (37.5%)	
Extranodal extension (%)	Poorly differentiated	15 (17.9%)	2 (25.0%)	0.920
	Yes	30 (35.7%)	3 (37.5%)	
Tumor-infiltrating lymphocytes	No	54 (64.3%)	5 (62.5%)	0.239
	Mild	25 (29.8%)	4 (50.0%)	
	Moderate	59 (70.2%)	5 (50.0%)	

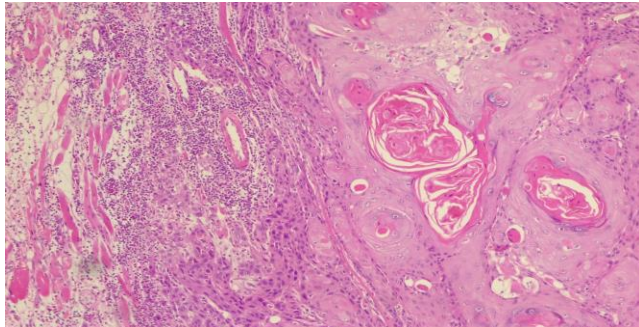


Figure-I: Photomicrograph depicting an Oral Squamous cell carcinoma, well-differentiated (H&E; Mag: 100x).

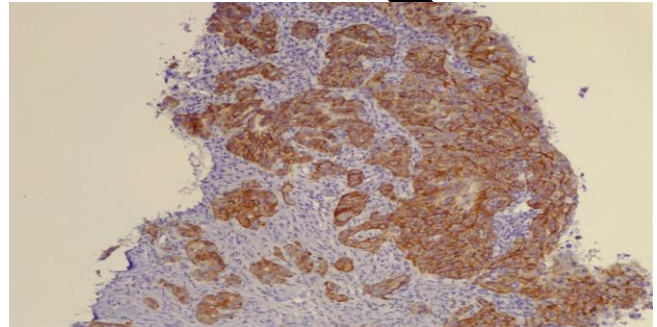


Figure-II: Immunohistochemical image showing Positive PD-L1 Immunostain; CP (IHC; Mag: 100x).

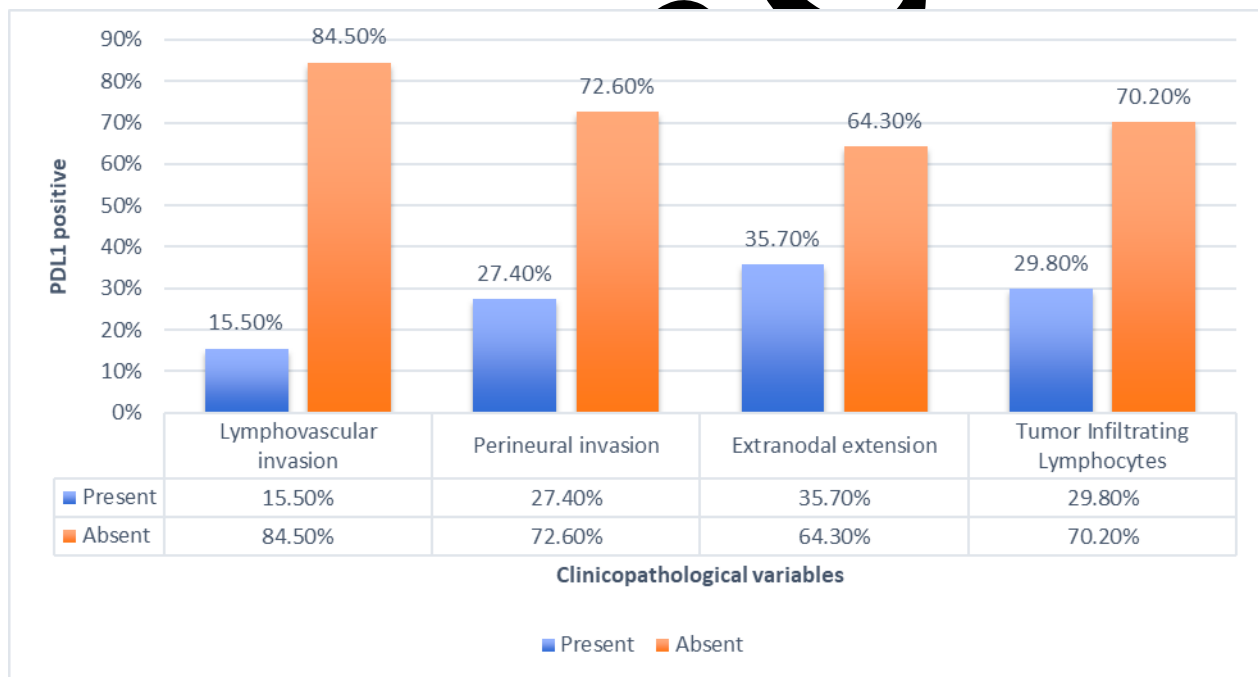


Figure-III: PD-L1 positive cases in relation to various clinicopathological parameters.

DISCUSSION

The cross-sectional study was conducted to assess the immunohistochemical expression of and clinicopathological features associated with Programmed Death Ligand 1 in squamous cell carcinoma of the head and neck. We found male predominance in our study with 73 (79.3%)

males and 19 (20.7%) females with a median age of 59.62 (56.85-62) years. Similar to our results, another study revealed that the median age of patients diagnosed with HNSCC was 58 (35-93) years.⁷ Chauhan R *et al.* conducted a retrospective study, which concluded that males were predominantly affected as compared to

females with a male-to-female ratio of 8:1 [8]. Smoking is a known risk factor leading to HNSCC and we concluded in our study that smoking is a leading cause of HNSCC. The percentage of smokers in our study was 65 (70.7%). Similarly, another cross-sectional study revealed that smoking was a major risk factor leading to HNSCC observed in 23 (62.2%) of patients [9]. We were unable to record the rate of survival and the factors affecting it. However, another prospective study revealed that abstinence from smoking for 10 years or greater has survival benefits in patients of HNSCC. It was observed that patients who had a history of abstinence from smoking for a period of greater than 10 years had better overall survival as compared to current smokers with adjusted hazard ratio aHR- 0.72; 95% CI, 0.56–0.93; ($p = 0.001$) [10]. Our results revealed that the oral cavity was the most common site of HNSCC observed in 54 (64.3%) patients. A similar locoregional study conducted in Rawalpindi, Pakistan revealed similar results, and the oral cavity was the most common site in HNSCC as seen in 37.9% [11].

We assessed the correlation of PD-L1 with various clinicopathological parameters and used a combined positive scoring (CPS) to label the specimens as PD-L1 positive or PD-L1 negative. Interpretation of PD-L1 positivity was based on CPS and a score of ≥ 1 was given as PD-L1 positive, however, further categorization into specimens of CPS scores 1- 20 or >50 was not done. In contrast to our study, several trials conducted on the subject used a quantitative value of CPS and its association with clinicopathological parameters [12]. Our results showed a PD-L1 positivity in 84 (91.3%) specimens, which was higher than another study revealing a PD-L1 positivity in 59 (63.4%) [13]. Our results were inconclusive in terms of statistical significance when PD-L1 positivity was correlated with tumor T-stage and grade and these findings were in concordance with a similar study conducted by Mishra PS *et al.*¹³ Similarly another cross-sectional study revealed a higher PD-L1 positivity seen in 72% of the patients with HNSCC and no significant

association was observed with tumor grading and staging [14].

Our results revealed a higher frequency of PD-L1 positivity in specimens exhibiting a moderate degree of tumor-infiltrating lymphocytes seen in 59 (70.2%) as compared to 25 (29.8%) specimens with a mild degree of tumor-infiltrating lymphocytes without reaching a significant level. Higher PD-L1 positivity with tumor-infiltrating lymphocytes was observed in the study done by Lenouvel *et al* [15]. This may imply that the microenvironment and the tissue response of the tumor by infiltrating lymphocytes affects PD-L1 positivity and requires further research for the guidance of immunotherapies. We could not record the stage of the disease, however, a similar trial revealed a significant correlation of PD-L1 expression with tumor stage with a p -value of 0.022.¹⁶ Our results revealed a significant association of the nodal status with PD-L1 positivity revealing most tumors having N1 status in 39 (46.4%). These results were favored by other studies revealing a significant correlation between the nodal stage of the tumor and PD-L1 positivity [16,17]. Our study reveals that PD-L1 positivity and its significant correlation with the nodal stage of the tumor may be beneficial to predict the prognosis of HNSCC, however, further research in conjunction with other immune markers may help predict the nature of the tumor [18]. This will pave the way to targeted therapies for reducing morbidity and mortality in HNSCC.

LIMITATIONS

A multicenter study with analysis of additional immunomarkers and prognostic evaluation of patients with HNSCC would have been more conclusive.

CONCLUSION

We conclude that there is a predominance of PD-L1 positivity in HNSCC with a significant correlation between PD-L1 positivity and nodal metastasis. The findings imply that PD-L1 may have a crucial role in disease biology and progression. These findings may lead to the development of immunotherapeutic strategies and identification

of patients who may benefit from PD-L1 targeted therapies.

ACKNOWLEDGMENTS

We are thankful to our histopathology staff for their continuous help and support. The constant guidance of our seniors throughout the research was praiseworthy.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Maria Khan: Study design, acquisition of data, data analysis, data interpretations, final approval, accountable for all aspects of the work

Ayesha Safdar: Acquisition of data, data analysis, final approval, accountable for all aspects of the work

Raazia Mahmood: Manuscript writing and review, final approval, accountable for all aspects of the work

Aisha Jamil: Literature search, final approval, accountable for all aspects of the work

Faryal Javaid: Data analysis, final approval, accountable for all aspects of the work

Iqbal Muhammad: Main conception, study design, final approval, accountable for all aspects of the work

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