ORIGINAL ARTICLE

Immunohistochemical Expression Of Programmed Death Ligand 1 In Squamous Cell Carcinoma Of Head And Neck.

Maria Khan, Ayesha Safdar, Raazia Mahmood, Aisha Jamil, Faryal Javaid, Iqbal Muhammad

Department of Histopathology, Rehman Medical Institute, Peshawar, Pakstan.

Abstract

Background: Head and neck squamous cell carcinoma (HNSCC) represents one of the most prevalent malignancies originating from the squamous cells of the nasal cavity, lip, paranasal sinuses, oral cavity, pharynx, larynx, and salivary glands.

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

Materials and Methods: This cross-sectional study was conducted at the Department of Histopathology, Rehman Medical Institute, Peshawar, from September 2023 to December 2024. Patients of either gender with histologically confirmed head and neck squamous cell carcinoma were included in the study. Formalin fixed tissue blocks were subjected to immunohistochemical staining with PD-L1 antibody, followed by analysis. The demographic characteristics and histopathological parameters were recorded.

Result: 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. PD-L1 positivity was observed in 84 patients (91.3%). The most common site of the tumor was the oral cavity in 56 (60.9%) patients followed by the larynx in 28 (30.4%) and hypopharynx in 8 patients (8.7%). We observed that lymphovascular and perineural invasion was not significantly associated with PD-L1 positivity. The T-stage of the tumors revealed that most tumors had T1 stage followed by T2 stage, while N-staging revealed most specimens 39 (46.4%) had N1 stage, followed by N0, N3, and N2 stages with a significant correlation to PD-L1 positivity. Tumors with extranodal extension and moderate tumor-infiltrating lymphocytes histologically revealed PD-L1 expression in 30 (35.7%) and 59 (70.2%) with p-1.000 and p-0.255respectively.

Conclusions: 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 suggest that PD-L1 may have a crucial role in the biology and progression of disease. Consequently, these findings may lead to the development of immunotherapeutic strategies and aid in identification of patients who may benefit from PD-L1 targeted therapies.

Keywords: *Immunohistochemistry; PD-L1; Squamous Cell Carcinoma of Head and Neck; Immunotherapy*

Introduction

Head and neck squamous cell carcinoma (HNSCC) represents one of the most prevalent malignancies originating from the squamous cells of the nasal cavity, lip, paranasal sinuses, oral cavity, pharynx, larynx, and salivary glands.[1] It constitutes a significant public health concern due to its increasing incidence, with approximately 890,000 new cases and a mortality rate of 450,000 annually worldwide as according to GLOBOCAN data.[2] Approximately 90% of HNSCCs are squamous cell carcinoma, and the limited treatment options, necessitates early diagnosis prompt intervention.[2]

Several risk factors, including tobacco use, human papillomavirus infection, and low socioeconomic status, are established risk factors to the development of HNSCC.[3] The increasing incidence and elevated mortality rates associated with this condition are of significant concern to healthcare professionals. Multimodal treatment strategies including radiotherapy, chemotherapy, and surgical interventions are employed in the management of 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 within cancerous cells which may be used for initiating targeted therapies against cancerous cells for better outcomes.

Novel therapies including immunotherapies are emerging techniques for modifying/modulating and utilizing the immune system of the human body. Programmed death ligand 1 (PD-L1) is a protein that interacts with the protein Programmed death 1 (PD1) receptors on T cells, and this interaction inhibits the T cells ability to eliminate 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

Corresponding Author
Maria Khan
Department of Histopathology, Rehman Medical Institute, Peshawar, Pakstan.
Email: mskhan.maryia@gmail.com

Received: October 02, 2025
Revised: November 29, 2025
Accepted: December 3, 2025
DOI: https://doi.org/10.52442/jrcd.v6i04.186

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 investigating PD-L1 expression in HNSCC highlights an existing gap in research.

This paper aims to assess the expression of PD-L1 expression and its clinicopathological significance in HNSCC. This investigation aims to explore the potential of PD-L1 as a predictive biomarker for targeted treatment, thereby improving patient outcomes in individuals diagnosed with HNSCC. Furthermore, this research is anticipated to facilitate future studies that may initiate immunotherapies targeting PD-L1 or develop combination immunotherapies addressing additional checkpoints.

Material and Methods

This cross-sectional study was conducted at Rehman Medical Institute, Peshawar, from September 2023 to December 2024 , following the approval of the ethical review committee under **ERC # 42** dated September 06 2023.The Sample size (n=81) was calculated using the WHO sample size calculator, with an 8% level of significance and 95% power of the test, based on reported prevalence of head and neck squamous cell carcinoma of 16%.[6] Sampling was performed using a non-probability consecutive technique. Keeping a 15% drop out rate, a total of 93 specimens were included and one was excluded due to inadequacy of the specimen.

The study included Patients of both genders with a confirmed biopsy-proven diagnosis of head and neck squamous cell carcinoma. Patients with suspected HNSCC, however with inadequate biopsy specimens were excluded from the study.

A total of 92 samples were included in the research, and the demographic characteristics of all the patients were documented. Tissue blocks which were formalin fixed and embedded in paraffin (FFPE blocks) from histopathologically confirmed cases of squamous cell carcinoma head and neck were collected. Tissue sections of 5 micrometer thickness were prepared and mounted onto glass slides. These slides were subsequently heated at 60 °C to obtain optimum adhesion of the section. The staining protocol involved deparaffinization using xylene, followed by rehydration via a series of alcohol solutions with decreasing concentrations. 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 PD-L1 Antibody Clone 28-8 by Cell Marque which was incubated for a period of one hour. Following rinsing with Phosphate Buffer Saline, the secondary conjugated antibody was applied to visualize the color change, Diaminobenzidine substrate was utilized. The final steps included counterstaining with Hematoxylin, dehydration and mounting with a coverslip. Tonsillar tissue was taken as control. PD-L1 identification was achieved 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 less than 1 was recorded as PD-L1 negative while a CPS of greater than 1 was considered PD-L1 positive. Specimens with a CPS greater than 1 are shown in Figure 2.

The results were independently analyzed by two classified histopathologist. The initial findings by one of the histopathologist were recorded and then confirmed by a second histopathologist to reach a conclusion. The recorded results were based on a consensus diagnosis. The clinicopathological parameters of each specimen, along with PD-L1 expression were systematically documented on a predesigned Performa.

Data entry and analysis were conducted using the Statistical Package for the Social Sciences (SPSS) software, version 23. For qualitative variables, frequencies and percentages were calculated. The Fisher's exact test was employed for analyzing the association between PD-L1 and qualitative variables such as tumor site, lymphovascular invasion, perineural invasion, N-stage, T-stage, grade of tumor, extra nodal extension and tumor infiltrating lymphocytes. For continuous variables, such as age, the distribution of the data was analyzed using the Shapiro-Wilk and Kolmogorov-Smirnov tests. In cases where the data did not follow a normal distribution, the median and interquartile range were calculated and Mann-Whitney U-test was employed for analysis of significance. A p-value of less than 0.05 was considered statistically significant.

Results

Among 92 participants a predominance of male was observed, 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%) individuals. It was concluded that the frequency of PD-L1 positivity was observed in 84 (91.3%) patients. The demographic characteristics of patients are shown in Table I. The most common site of the tumor was the oral cavity in 56 (60.9%)cases, followed by the larynx in 28 (30.4%) cases and the hypopharynx in 8 (8.7%) cases (p = 0.084). Oral squamous cell carcinoma is shown in Fig-1. When analyzing lymphovascular invasion, we observed that 84/92 (91.3%) were PD-L1 positive, 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 = 0.194). The T-stage of the tumors revealed that most tumors had T1, followed by T2 stage, and the correlation with PD-L1

positivity was not significant (p=0.213) . N-staging of tumors revealed most specimens 39 (46.4%) were at N1 stage followed by N0, N3 and N2 stages 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 (70.2%) with p-0.920 and p-0.239 respectively. The correlation between PD-L1 expression and clinicopathological parameters is shown in Table II.

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

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

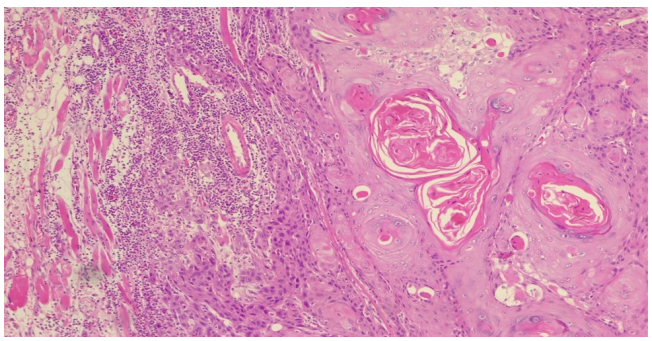

Fig-1. Photomicrograph depicting an Oral Squamous cell carcinoma, well-differentiated (H&E; Mag: 100x)

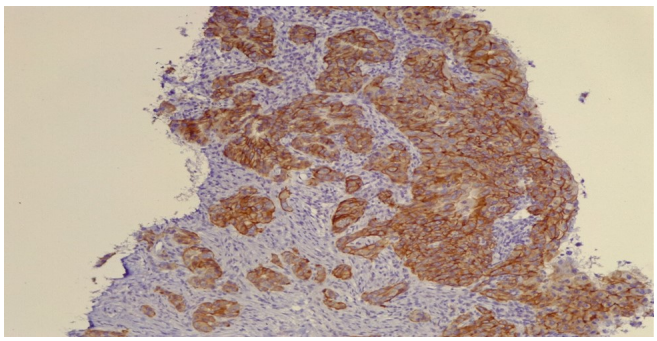

Fig-2. Image showing Positive PD-L1 Immunostain; CPS > 1 (IHC; Mag: 100x)

Discussion

The present cross-sectional study aimed 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 for 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 for HNSCC observed in 23(62.2%) 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 with HNSCC. It was observed that patients with a history of smoking abstinence exceeding 10 years exhibited superior overall survival compared to current smokers, with an adjusted hazard ratio (aHR) of - 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 comparable 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% of cases.[11]

We assessed the correlation of PD-L1 with various clinicopathological parameters and used a combined positive scoring (CPS) to classify the specimens as PD -L1 positive or PD-L1 negative. Interpretation of PD-L1 positivity was determined using the on CPS and a score of >1 was taken as PD-L1 positive. However, further categorization into specimens of CPS scores 1-20 or >50 was not performed. In contrast to our study, several trials have 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 that reported in an another study revealing a PD-L1 positivity in 59 (63.4%) speicmens.[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 patients with HNSCC, with no significant association 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

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

Parameters		PD-L1 positive (n=84)	PD-L1 Negative (n=08)	p-value
Age in years Median (IQR)		59.73 (56.58-62.16)	59.06 (57.17-61.06)	0.603^
Age Groups	<55	11(13.1%)	0 (0%)	0.589*
	≥55	73 (86.9%)	8 (100%)	
Tumor site n(%)	Oral cavity	54 (64.3%)	2 (25%)	0.063*
	Larynx	23 (27.4%)	5 (62.5%)	
	Hypopharynx	7 (8.3%)	1 (12.5%)	
Lymphovascular invasion n(%)	Yes	13 (15.5%)	0 (0.0%)	0.595*
	No	71 (84.5%)	8 (100.0%)	
Perineural invasion n(%)	Yes	23 (27.4%)	0 (0.0%)	0.194*
	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%)	
T-stage	T1	40 (47.6%)	3 (37.5%)	0.148*
	T2	28 (33.3%)	1 (12.5%)	
	T3	7 (8.3%)	2 (25.0%)	
	T4	9 (10.7%)	2 (25.0%)	
Grade of Tumor	Well-differentiated	11 (13.1%)	3 (37.5%)	0.077*
	Moderately differentiated	58 (69%)	3 (37.5%)	
	Poorly differen-tiated	15 (17.9%)	2 (25.0%)	
Extranodal extension n(%)	Yes	30 (35.7%)	3 (37.5%)	1.000*
	No	54 (64.3%)	5 (62.5%)	
Tumor-infiltrating lympho-cytes	Mild	25 (29.8%)	4 (50.0%)	0.255*
	Moderate	59 (70.2%)	4 (50.0%)	

*** Fischer exact test**

^Mann-Whitney U-test

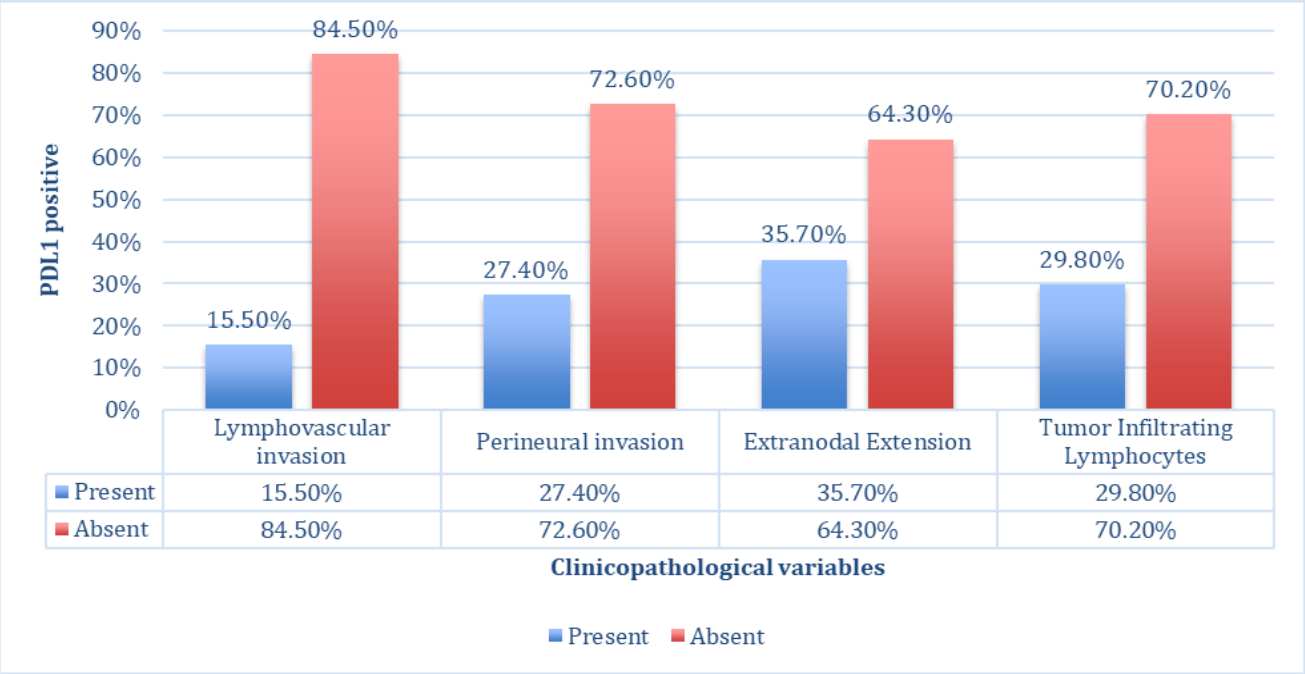

Fig-3. PD-L1 positive cases in relation to various clinicopathological parameters

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 serve as a prognostic indicator for HNSCC. However, further research incorporating other immune markers may help predict the nature of the tumor.[18] This approach could facilitate the development of targeted therapies aimed at reducing morbidity and mortality associated with HNSCC.

Limitation

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

Conclusion

We conclude PD-L1 positivity predominance in HNSCC, with a significant correlation between PD-L1 positivity and nodal metastasis. These 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 the identification of patients who may benefit from PD-L1 targeted therapies.

CONFLICT OF INTEREST: None

FUNDING SOURCES: None

Reference

1. Barsouk A, Aluru JS, Rawla P, Saginala K, Barsouk A. Epidemiology, risk factors, and prevention of head and neck squamous cell carcinoma. Medical Sciences. 2023 Jun 13;11(2):42. https://doi.org/10.3390/medsci11020042
2. Johnson DE, Burtness B, Leemans CR, Lui VW, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma. Nature reviews Disease primers. 2020 Nov 26;6(1):92. https://doi.org/10.1159/000446117
3. Sun Z, Sun X, Chen Z, Du J, Wu Y. Head and neck squamous cell carcinoma: risk factors, molecular alterations, immunology and peptide vaccines. International Journal of Peptide Research and Therapeutics. 2022 Jan;28:1-8. https://doi.org/10.1007/s10989-021-10334-5
4. Muzaffar J, Bari S, Kirtane K, Chung CH. Recent advances and future directions in clinical management of head and neck squamous cell carcinoma. Cancers. 2021 Jan 18;13(2):338. https://doi.org/10.3390/cancers13020338
5. Wu J, Wang N. Current progress of anti PD 1/PD-L1 immunotherapy for glioblastoma. Molecular Medicine Reports. 2024 Sep 30;30(6):221. https://doi.org/10.3892/mmr.2024.13344
6. Unnikrishnan A, Basavaraj V. Evaluation of programmed death-ligand 1 (Pd-L1) expression in head and neck squamous cell carcinoma. Iranian Journal of Pathology. 2023;18(2):193. https://doi.org/10.30699/ijp.2023.1988955.3057
7. Wolfer S, Kunzler A, Foos T, Ernst C, Leha A, Schultze-Mosgau S. Gender and risk-taking behaviors influence the clinical presentation of oral squamous cell carcinoma. Clinical and Experimental Dental Research. 2022 Feb;8(1):141-51.

https://doi.org/10.1002/cre2.523

8. Chauhan R, Trivedi V, Rani R, Singh U. A study of head and neck cancer patients with reference to tobacco use, gender, and subsite distribution. South Asian journal of cancer. 2022 Jan;11(01):046-51. https://doi.org/10.1055/s-0041-1740601
9. Michaelraj MJ, Kuttiappan K, Ramasamy S, Rodrigues FA, Govindaraj S. Demographic profile and risk factors of head-and-neck squamous cell carcinoma in west Tamil Nadu: A cross-sectional observational study. Cancer Research, Statistics, and Treatment. 2023 Apr 1;6(2):215-23. https://doi.org/10.4103/crst.crst_364_22
10. Lee JJ, Kunaratnam V, Kim CJ, Pienkowski M, Hueniken K, Sahovaler A et al. Cigarette smoking cessation, duration of smoking abstinence, and head and neck squamous cell carcinoma prognosis. Cancer. 2023 Mar 15;129(6):867-77. https://doi.org/10.1002/cncr.34620
11. Malik IS, Asif M, Bashir N, Ara N, Rashid F, Din HU et al. Frequency of expression of PD-1 and PD-L1 in head and neck squamous cell carcinoma and their association with nodal metastasis: a cross-sectional study. Asian Pacific Journal of Cancer Prevention: APJCP. 2022 Feb;23(2):467. https://doi.org/10.31557/APJCP.2022.23.2.467
12. Paolino G, Pantanowitz L, Barresi V, Pagni F, Munari E, Moretta L et al. PD-L1 evaluation in head and neck squamous cell carcinoma: Insights regarding specimens, heterogeneity, and therapy. Pathology-Research and Practice. 2021 Oct 1;226:153605. https://doi.org/10.1016/j.prp.2021.153605
13. Mishra PS, Sidhu A, Dwivedi G, Mulajker DS, Awasthi S. Determining PD-L1 expression in head and neck squamous cell carcinoma using immunohistochemistry. Indian Journal of Cancer. 2022 Oct 1;59(4):474-9. https://doi.org/10.4103/ijc.IJC_920_19
14. Kilaru S, Panda SS, Moharana L, Mohapatra D, Mohapatra SS, Panda A et al. PD-L1 expression in head and neck squamous cell carcinoma and its clinical significance: A prospective observational study from a tertiary care center. Journal of Cancer Research and Therapeutics. 2024 Jan 1;20(1):46-51.https://doi.org/10.4103/jcrt.jcrt_1239_22
15. Lenouvel D, González-Moles MÁ, Ruiz-Ávila I, Chamorro-Santos C, González-Ruiz L, González-Ruiz I et al. Clinicopathological and prognostic significance of PD-L1 in oral cancer: A preliminary retrospective immunohistochemistry study. Oral diseases. 2021 Mar;27(2):173-82.https://doi.org/10.1111/odi.13509
16. Wusiman D, Guo L, Huang Z, Li Z, Liu S, Ying J, Li W, An C. The clinicopathological significance of PD-L1 expression was assessed by the combined positive score (CPS) in head and neck squamous cell carcinoma. Pathology-Research and Practice. 2022 Aug 1;236:153934. https://doi.org/10.1016/j.prp.2022.153934
17. Schneider S, Kadletz L, Wiebringhaus R, Kenner L, Selzer E, Füreder T et al. PD-1 and PD-L1 expression in HNSCC primary cancer and related lymph node metastasis–impact on clinical outcome. Histopathology. 2018 Oct;73(4):573-84. https://doi.org/10.1111/his.13646
18. Jiang S, Li X, Huang L, Xu Z, Lin J. Prognostic value of PD-1, PD-L1 and PD-L2 deserves attention in head and neck cancer. Frontiers in Immunology. 2022 Sep 2;13:988416. https://doi.org/10.3389/fimmu.2022.988416

How to cite this article?

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. J Rehman Coll Dent 2025; 6(4): 138-143

Author Contributions

1. **Maria Khan:** Conception ; Design; Materials; Data Collection; Analysis; Writer
2. **Ayesha Safdar:** Data Collection and Processing
3. **Raazia Mahmood:** Data Analysis and Interpretation
4. **Aisha Jamil:** Literature review
5. **Faryal Javaid:** Data collection and processing, Literature review
6. **Iqbal Muhammad:** Supervision; Critical review