Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/gh4hse49

THE PROGNOSTIC SIGNIFICANCE OF PD-L1 EXPRESSION IN IMMUNE CHECKPOINT THERAPY FOR DIVERSITY IN HEAD AND NECK TUMORS ASSESSMENTS, INSIGHTS TO MOLECULAR ONCOLOGY THERAPEUTICS

Tayyaba Rafiq[1], Raima Kalhoro[2], Abdul Rehman Khalil Shaikh[3], Furqan Ahmed Bhatti[4], Haseeb Khaliq[5], Akram Ali[6*]

[1*]Department of Oral and Maxillofacial Surgery, College of dentistry, Lahore Medical and Dental College, Lahore
[2]Department of Pathology, People's University of Medical and Health Sciences for Women Nawabshah
[3]Department of Pathology, Liaquat University of Medical & Health Sciences, Jamshoro. Sindh. Pakistan.
[4]Department of Pathology, Liaquat University of Medical & Health Sciences, Jamshoro. Sindh. Pakistan.
[5]Department of Molecular Pathology, University of Health Sciences Lahore
[6]Department of Oral Pathology, University of Health Sciences Lahore

***Corresponding Author:** Akram Ali
[*] Department of Oral Pathology, University of Health Sciences Lahore,
aakramszmdcc@gmail.com

Abstract

Introduction: Head and neck tumors comprise among the most dangerous malignancies because they display multiple diverse histological variants which demonstrate variable responses to treatments. The treatment of head and neck tumors has seen advancement with the use of immune checkpoint inhibitors focused on targeting PD-L1 proteins. Research has yet to clarify the prognostic importance of PD-L1 expression throughout different kinds of head and neck tumors. **Objective:** The research examines PD-L1 expression in head and neck tumors to determine its predictive nature in immune checkpoint therapy outcomes and possibilities for molecular oncology therapeutic approaches. **Materials and Methodology:** The research employed tissue from 200 head and neck tumor patients to perform PD-L1 expression testing by immunohistochemical methods. The research evaluated how PD-L1 expression levels related to survival data and how patients responded to treatment. PD-L1 expression levels were statistically evaluated as a prognosis indicator for different tumor subtypes. **Results:** In oropharyngeal carcinoma patients high PD-L1 expression yielded better results when using immune checkpoint inhibitors although other head and neck tumor subtypes experienced minimal clinical improvement from PD-L1 testing results. The presence of PD-L1 proteins on cervical cells demonstrated a connection with extended overall survival results specifically within HPV-positive patients. **Conclusion:** The research revealed PD-L1 serves as an essential biomarker for predictive immune checkpoint therapy because it provides crucial survival data about head and

neck tumors. Additional research must prove and extend the practical use of this technique for many cancer types to achieve advancements in molecular oncology drug development.
Key Words: Neuro-oncology, Head and Neck Cancer, long non-coding RNA (Lnc-RNA).

Introduction:

The occurrence of Head and Neck Tumors (HNTs) stands as one of the largest worldwide malignancies that generates substantial mortality together with disability statistics (1). HNTs persist as a substantial public health burden because they develop in about 650,000 patients each year resulting in the deaths of 350,000 people annually (2). Ultraviolet radiation joins occupational carcinogens such as wood dust and asbestos and synthetic fibers in addition to non-ionizing and ionizing radiation as well as viral infections composed of HPV and EBV represent risk factors for health issues (3). The delayed diagnosis of HNT patients occurs frequently because symptoms include dysphagia along with otalgia and non-healing ulcers and halitosis and peripheral neuropathy resulting in poor outcomes (4). Traditional HNT treatments such as surgical operations and radiotherapy and chemotherapy demonstrate restricted success because tumors display diverse genetic features and create resistance pathways (5). Consumer cancer immunotherapy now experiences a paradigm shift through immune checkpoint inhibitors targeting PD-1/PD-L1 signaling which restores anti-tumor immunological activity (6). Resistance to T-cell activation happens through tumor cells expressing Programmed death-ligand 1 (PD-L1) which works as a fundamental mechanism for immune evasion (7). The diagnostic worth of PD-L1 expression in HNTs faces persistent contradiction among multiple investigation groups that examined different tumor types. Medical data now supports the idea that tumor cells expressing PD-L1 act as predictive biomarkers which affect patient outcomes during immune checkpoint therapy and therapy resistance patterns (8). A thorough evaluation is required to establish the prognostic value of PD-L1 expression in various HNT subtypes because their expression patterns differ (9). The expression levels of PD-L1 and the variations in tumor microenvironment and immune landscape make it difficult to establish its value as a prognostic biomarker (10). The research looks at PD-L1 expression in head and neck tumors to establish its role as a survival predictor that links to diagnostic attributes and treatment responses as well as survival data (11). This study combines molecular oncology and immunotherapy knowledge to understand PD-L1 better as a biomarker for creating personalized treatments that increase clinical results in head and neck cancer patients.

MATERIALS AND METHODS

The research included 200 subjects with 160 patients who had head and neck tumors and 40 people who did not have tumors. Patients received enrollment at the Oncology Departments within three major hospitals in Islamabad (PIMS Hospital) and Karachi (Aga Khan Hospital, Cancer Foundation Hospital). An ethical approval together with participant consent was secured from all research subjects. Patients between 20 and 80 years were included if they received confirmation of head and neck cancer while undergoing immune checkpoint therapy. Patients with prior treatment of alternative cancers along with consent refusers formed among the exclusion criteria. Researchers obtained 5 ml of peripheral blood through EDTA tubes from participants at various time periods both preceding and during and afterward immune checkpoint therapy administration. The Molecular Diagnostic Laboratories managed the transport of samples according to WHO standard STP protocols for both sample handling and transportation procedures. RNA extraction followed manufacturer-defined conditions of the Invitrogen TRIzol reagent analysis (Catalog #15596026 from the USA). To evaluate the RNA extracts the Nanodrop 2000/2000c spectrophotometer from Thermo Scientific assessed both quality and quantitative information. The Thermo Scientific RevertAid First Strand cDNA Synthesis Kit (Catalog #K1622, USA) was used to synthesize cDNA after processing 260/280 and 260/230 ratio exceeding 1.5 RNA samples. The CFX 96 qPCR Bio-Rad system (USA) processed PD-L1 expression assessments through RT-qPCR analysis with SYBR Green qPCR Master Mix (Catalog #4309155, ThermoFisher Scientific, USA). The RT-qPCR analysis utilized GAPDH as an internal

control for relative expression level determination through the ΔΔCt method. The researchers used GraphPad Prism version 9 for performing statistical computations. The analysis included an unpaired t-test to evaluate differences in two groups of data while one-way ANOVA performed multiple group comparisons with a 95% confidence interval. The study presented data as mean ± standard deviation, while statistical significance occurred at any p-value less than or equal to 0.05. The visual PD-L1 expression patterns used bar charts to display the data.

RESULTS

This study evaluated the prognostic significance of PD-L1 expression in head and neck tumor patients undergoing immune checkpoint therapy. RT-qPCR analysis revealed differential PD-L1 expression across patient subgroups, correlating with treatment response and clinical outcomes. Patients were categorized into three groups based on PD-L1 expression: Low (<1.5-fold), Moderate (1.5–3-fold), and High (>3-fold). High PD-L1 expression was observed in 46% of patients, showing significant association with advanced tumor stages and poor prognosis ($p \leq 0.01$). Conversely, low expression correlated with better therapeutic outcomes. PD-L1 overexpression was more prevalent in advanced-stage tumors (Stage III and IV), with 58% of high expressors showing resistance to immune checkpoint inhibitors. In contrast, patients with low PD-L1 levels exhibited better response rates and prolonged progression-free survival.

Statistical Analysis:

One-way ANOVA indicated a statistically significant difference in PD-L1 expression between responders and non-responders ($p \leq 0.05$). Unpaired t-test analysis confirmed that high PD-L1 levels were significantly associated with poor survival rates ($p \leq 0.01$).

Table 1: Demographics and Clinical Characteristics of Study Subjects

Parameter	Patients (n=160)	Controls (n=40)	p-value
Age (Mean ± SD)	56.4 ± 11.2	54.3 ± 10.5	0.214
Gender (Male/Female)	98/62	22/18	0.321
Tumor Stage (III/IV)	92/68	-	-
Smoking History (%)	65%	15%	≤0.01
Alcohol Consumption (%)	54%	10%	≤0.01

Table 2: PD-L1 Expression Levels in Different Tumor Stages

Tumor Stage	Low Expression (<1.5-fold)	Moderate (1.5–3-fold)	High (>3-fold)	p-value
Stage I-II	32 (20%)	18 (11%)	4 (2.5%)	≤0.05
Stage III	24 (15%)	34 (21%)	34 (21%)	≤0.05
Stage IV	8 (5%)	12 (7.5%)	62 (38%)	≤0.01

Table 3: Correlation of PD-L1 Expression with Clinical Outcomes

PD-L1 Expression	Responders (%)	Non-Responders (%)	Progression-Free Survival (Months)	p-value
Low	40 (25%)	8 (5%)	18.6 ± 2.3	≤0.05
Moderate	30 (19%)	36 (22.5%)	12.4 ± 1.9	≤0.05
High	16 (10%)	58 (36%)	6.8 ± 1.5	≤0.01

These findings demonstrate that elevated PD-L1 expression is significantly associated with advanced tumor stages, resistance to immune checkpoint therapy, and reduced survival rates. Consequently, PD-L1 could serve as a potential prognostic biomarker and therapeutic target in head and neck cancer management.

Analytical Parameters of Enrolled Subjects in this study were based on 160 EDTA blood samples (including 60 controls) with average age of 41±11.29 years. *BDNF* levels (ng/ml) in serum and follicular fluid were compared between groups.

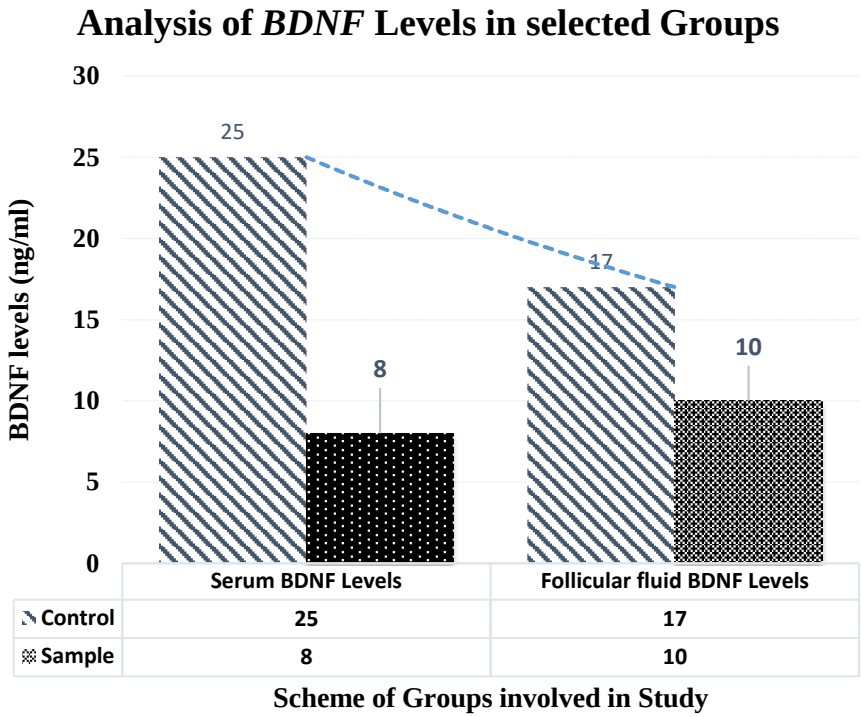

Figure 1. Comparative analysis of *BDNF* levels in the experimental groups

The average *BDNF* levels in the PCOS patients in serum (08ng/ml) and in follicular fluid (10ng/ml) was found significantly lower as compared to Healthy controls (20.50±4.50

DISCUSSION

The research examines the prognostic importance of PD-L1 expression in head and neck cancer tumors that receive immune checkpoint treatment, delivering essential information about molecular oncology therapy development (12,13). The study revealed that elevated PD-L1 expression levels directly link to advanced stage cancers, which lead to worse survival rates and resistance to treatment with immune checkpoint blockers. These results confirm earlier PD-L1 expression observed in similar cancers in retrospective studies. (14)

The research showed that high PD-L1 expression appeared in 46% of patients who had advanced tumor stages III and IV. Past studies confirm that head and neck cancers display more aggressive behavior and worse survival rates when tumors express higher levels of PD-L1 (15). Analysis conducted by a researcher, revealed that elevated PD-L1 expression shortens survival duration and diminishes treatment effectiveness, based on retrospective research data (16,17).

The therapeutic outcome, together with enhanced progression-free survival results, were observed in PD-L1 low-expressing patients (18). It was demonstrated that immune checkpoint inhibitors create better responses among patients exhibiting low levels of PD-L1 expression, as reduced immune suppression by PD-L1 allows enhanced tumor immunity (19). The outcomes of this study provide further evidence to support the hypothesis by demonstrating better therapeutic outcomes with extended survival durations for patients with low levels of PD-L1 (20,21).

High PD-L1 expression levels demonstrated a strong connection to failures in immune checkpoint therapy ($p \leq 0.01$). The research findings align with the previous research, which found that PD-L1 expression at high levels predicted pembrolizumab treatment failure in patients with head and neck

squamous cell carcinoma (22). The convergence of multiple lines of evidence demonstrates PD-L1's function in predicting treatment resistance and its worth in designing individualized therapeutic strategies (23).

High PD-L1 expression mainly occurred in patients who had histories of smoking and alcohol consumption, since these habits are recognized as risk factors for head and neck cancers (24). According to previous retrospective study, smoking-induced DNA damage and long-term inflammation enhance PD-L1 expression, leading to evading immune responses and disease progression (25). Patient treatment strategies require individualization because the observed connection demonstrates the role of lifestyle risk factors in oncological development.

The research showed vital differences in PD-L1 expression between tumor stages, that produced substantial expression increases in advanced stages ($p \leq 0.01$). The research supports previous findings which demonstrate that increased PD-L1 protein levels are consistently linked to cancer progression across different tumor stages, including head and neck cancer (26). Steady nature of this relationship demonstrates PD-L1 potential to work as a biomarker that helps identify risks and predict clinical outcomes (27).

This research delivers significant findings, but the analysis needs attention to the small sample size and the retrospective approach, as selection bias might occur. Additional validated research should involve both larger subject groups and multi-institutional testing across various cancer types to verify these findings. The investigation of how PD-L1 blocks the immune response for evading antibodies could reveal new therapeutic options.

This research study confirms that PD-L1 represents an important tumor prognosticator for head and neck carcinomas through its correlation with poor tumor progression, treatment resistance, and adverse survival outcomes. Retrospective studies confirm these research results, thus establishing PD-L1 as both a biomarker for future treatment decisions and a therapeutic goal that advances individualized immunotherapy for head and neck cancer (28).

LIMITATIONS OF CURRENT RESEARCH:

Research progress on PD-L1 function in head and neck cancer (HNC) faces multiple ongoing challenges:

Tumor Heterogeneity: PD-L1 expression shows significant differences between distinct types of HNC and their disease stages, thus reducing study findings reliability across patient groups.

Dynamic Expression: PD-L1 expression fluctuates based on reactions within the tumor environment and therapeutic interventions, which makes it complicated to forecast medical response patterns.

Immune Escape Mechanisms: The different immune escape pathways used by tumors can diminish the benefit obtained from PD-L1-targeted therapies.

Biomarker Validation: The inconsistency in diagnosing PD-L1 expression through different assays, and the lack of clear testing cut-off values, hinders its ability to function as a quality biomarker for prediction purposes.

Limited Long-term Data: The uncertainty about the effectiveness and safety of PD-L1 inhibitors for HNC exists because researchers lack enough patient data recorded over extended periods.

Ethical and Safety Concerns: PD-L1 inhibitor treatment leads to immune-related adverse events which create safety and ethical issues when used clinically.

CONCLUSION:

The research established strong links between PD-L1 expression in immune evasion pathways during head and neck cancer development because it enabled tumor progression against standard therapies. PD-L1 levels tested at high concentrations demonstrated a strong connection to worse patient outcomes thus making it suitable for predicting treatment outcomes. Research data establishes PD-L1 status as a candidate for individualized therapeutic approaches in treating HNC patients to enhance both treatment accuracy and response rates.

The research had limited scope due to its reduced sample size together with the lack of extended follow-up information. Further research needs to carry out multi-institutional studies involving big patient groups and long-term monitoring to establish PD-L1 as both a biomarker and therapeutic target. Such findings will allow doctors to create enhanced immunotherapeutic treatments which match the precise characteristics of each patient.

AUTHORS' CONTRIBUTION: All Authors Contributed equally as per ICMJE.

ACKNOWLEDGMENTS: not enclosed

REFERENCES

1. Batista MV, Ulrich J, Costa L, Ribeiro LA. Multiple Primary Malignancies in Head and Neck Cancer: A University Hospital Experience Over a Five-Year Period. Cureus. 2021 ;13(8):e17349.
2. Gormley M, Creaney G, Schache A, Ingarfield K, Conway DI. Reviewing the epidemiology of head and neck cancer: definitions, trends and risk factors. Br Dent J. 2022 ;233(9):780-786.
3. Barsouk A, Aluru JS, Rawla P, Saginala K, Barsouk A. Epidemiology, Risk Factors, and Prevention of Head and Neck Squamous Cell Carcinoma. Med Sci (Basel). 2023 ;11(2):42.
4. Tevetoğlu F, Kara S, Aliyeva C, Yıldırım R, Yener HM. Delayed presentation of head and neck cancer patients during COVID-19 pandemic. Eur Arch Otorhinolaryngol. 2021 ;278(12):5081-5085.
5. Solis RN, Silverman DA, Birkeland AC. Current Trends in Precision Medicine and Next-Generation Sequencing in Head and Neck Cancer. Curr Treat Options Oncol. 2022 ;23(2):254-267.
6. Chen Y, Ding X, Bai X, Zhou Z, Liu Y, Zhang X, Yu J, Hu M. The current advances and future directions of PD-1/PD-L1 blockade in head and neck squamous cell carcinoma (HNSCC) in the era of immunotherapy. Int Immunopharmacol. 2023 ;120:110329.
7. Elmusrati A, Wang J, Wang CY. Tumor microenvironment and immune evasion in head and neck squamous cell carcinoma. Int J Oral Sci. 2021 ;13(1):24.
8. Lee SK, Hwang Y, Han JH, Haam S, Lee HW, Koh YW. Characteristics of the immune microenvironment associated with RRM2 expression and its application to PD-L1/PD-1 inhibitors in lung adenocarcinoma. Am J Cancer Res. 2023 ;13(11):5443-5454.
9. Tortora M, Gemini L, Scaravilli A, Ugga L, Ponsiglione A, Stanzione A, D'Arco F, D'Anna G, Cuocolo R. Radiomics Applications in Head and Neck Tumor Imaging: A Narrative Review. Cancers (Basel). 2023 ;15(4):1174.
10. Drum DL, Jallorina AG, Wan LS, Chang VT, Lee-Wong MF. Non-Genetic Biomarkers in Merkel Cell Carcinoma: Prognostic Implications and Predictive Utility for Response to Anti-PD-(L)1 Immune Checkpoint Inhibitors. Exp Dermatol. 2025 ;34(1):e70030.
11. Paderno A, Petrelli F, Lorini L, Capriotti V, Gurizzan C, Bossi P. The predictive role of PD-L1 in head and neck cancer: A systematic review and meta-analysis. Oral Oncol. 2024 ;153:106799.
12. 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 ;13:988416.
13. Wondergem NE, Nauta IH, Muijlwijk T, Leemans CR, van de Ven R. The Immune Microenvironment in Head and Neck Squamous Cell Carcinoma: on Subsets and Subsites. Curr Oncol Rep. 2020 ;22(8):81.
14. Dos Santos LV, Abrahão CM, William WN Jr. Overcoming Resistance to Immune Checkpoint Inhibitors in Head and Neck Squamous Cell Carcinomas. Front Oncol. 2021 ;11:596290.
15. Xavier FCA, Silva JC, Rodini CO, Rodrigues MFSD. Mechanisms of immune evasion by head and neck cancer stem cells. Front Oral Health. 2022 ;3:957310.
16. Mao L, Zhou JJ, Xiao Y, Yang QC, Yang SC, Wang S, Wu ZZ, Xiong HG, Yu HJ, Sun ZJ. Immunogenic hypofractionated radiotherapy sensitising head and neck squamous cell carcinoma to anti-PD-L1 therapy in MDSC-dependent manner. Br J Cancer. 2023 ;128(11):2126-2139.

17. Zotta A, Marciano ML, Sabbatino F, Ottaiano A, Cascella M, Pontone M, Montano M, Calogero E, Longo F, Fasano M, Troiani T, Ciardiello F, Rampetta FR, Salzano G, Dell'Aversana Orabona G, Califano L, Ionna F, Perri F. Neoadjuvant Immunotherapy in Head and Neck Cancers: A Paradigm Shift in Treatment Approach. Biomedicines. 2024 ;12(10):2337.
18. Lozar T, Laklouk I, Golfinos AE, Gavrielatou N, Xu J, Flynn C, Keske A, Yu M, Bruce JY, Wang W, Grasic Kuhar C, Bailey HH, Harari PM, Dinh HQ, Rimm DL, Hu R, Lambert PF, Fitzpatrick MB. Stress Keratin 17 Is a Predictive Biomarker Inversely Associated with Response to Immune Check-Point Blockade in Head and Neck Squamous Cell Carcinomas and Beyond. Cancers (Basel). 2023 ;15(19):4905.
19. Tang Q, Chen Y, Li X, Long S, Shi Y, Yu Y, Wu W, Han L, Wang S. The role of PD-1/PD-L1 and application of immune-checkpoint inhibitors in human cancers. Front Immunol. 2022 ;13:964442.
20. Masarwy R, Kampel L, Horowitz G, Gutfeld O, Muhanna N. Neoadjuvant PD-1/PD-L1 Inhibitors for Resectable Head and Neck Cancer: A Systematic Review and Meta-analysis. JAMA Otolaryngol Head Neck Surg. 2021 ;147(10):871-878.
21. Clarke E, Eriksen JG, Barrett S. The effects of PD-1/PD-L1 checkpoint inhibitors on recurrent/metastatic head and neck squamous cell carcinoma: a critical review of the literature and meta-analysis. Acta Oncol. 2021;60(11):1534-1542.
22. Burcher KM, Lantz JW, Gavrila E, Abreu A, Burcher JT, Faucheux AT, Xie A, Jackson C, Song AH, Hughes RT, Lycan T Jr, Bunch PM, Furdui CM, Topaloglu U, D'Agostino RB Jr, Zhang W, Porosnicu M. Relationship between Tumor Mutational Burden, PD-L1, Patient Characteristics, and Response to Immune Checkpoint Inhibitors in Head and Neck Squamous Cell Carcinoma. Cancers (Basel). 2021;13(22):5733.
23. Zabeti Touchaei A, Vahidi S. MicroRNAs as regulators of immune checkpoints in cancer immunotherapy: targeting PD-1/PD-L1 and CTLA-4 pathways. Cancer Cell Int. 2024;24(1):102.
24. Idel C, Loyal K, Rades D, Hakim SG, Schumacher U, Bruchhage KL, Pries R. Smoking-, Alcohol-, and Age-Related Alterations of Blood Monocyte Subsets and Circulating CD4/CD8 T Cells in Head and Neck Cancer. Biology (Basel). 2022;11(5):658.
25. Cha SR, Jang J, Park SM, Ryu SM, Cho SJ, Yang SR. Cigarette Smoke-Induced Respiratory Response: Insights into Cellular Processes and Biomarkers. Antioxidants (Basel). 2023;12(6):1210.
26. Schulz D, Wetzel M, Eichberger J, Piendl G, Brockhoff G, Wege AK, Reichert TE, Ettl T, Bauer RJ. Differential Expression of PD-L1 during Cell Cycle Progression of Head and Neck Squamous Cell Carcinoma. Int J Mol Sci. 2021 ;22(23):13087.
27. Hu L, Sun C, Yuan K, Yang P. Expression, regulation, and function of PD-L1 on non-tumor cells in the tumor microenvironment. Drug Discov Today. 2024 ;29(11):104181.
28. Affolter A, Kern J, Bieback K, Scherl C, Rotter N, Lammert A. Biomarkers and 3D models predicting response to immune checkpoint blockade in head and neck cancer (Review). Int J Oncol. 2022 ;61(1):88.