

Expression Of Pd-1 And Pd-L1 In Head And Neck Squamous Cell Carcinoma And Its Association With Lymphnodal Metastasis

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ABSTRACT

Introduction Head and Neck Squamous Cell Carcinoma (HNSCC) is a highly aggressive cancer in which several key biological processes become altered such as mechanisms of cell differentiation, regulation of the cell cycle, epithelial–mesenchymal interactions, programmed cell death, formation of new blood vessels and the ability of tumor cells to invade and metastasize.

Aim & Objectives To assess PD-1 and PD-L1 expression in tumor-infiltrating lymphocytes (TILs) and tumor cells of Head and Neck Squamous Cell Carcinoma (HNSCC) cases and to determine its association with nodal involvement and clinicopathological features.

Methodology This is a cross-sectional study done on 53 diagnosed cases of HNSCC who underwent composite dissection with lymph node resection from January 2022 to September 2024. Immunohistochemical expression of PD-1 and PD-L1 was done. Tonsil tissue was used as a positive control for both markers. Immunoreactivity score was done using cell staining intensity as score 0 = <1%, score 1 = 1-5%, score 2 = 5-50% and score 3 = >50%. Statistical analysis was carried out using the chi-square test, and a p-value ≤0.05 was considered statistically significant.

Results In the present study, 36 patients (67.9%) were male and 17 (32.1%) were female with a mean age of 30 years. The buccal mucosa was the most frequently involved site, accounting for 27 cases (50.9%). Among the 53 patients, 24 (45.3%) exhibited lymph node metastasis, which showed positive expression of both PD-1 and PD-L1 in the primary tumor cells. A statistically significant association was observed between PD-1 and PD-L1 expression in the primary tumor and lymph node metastasis, with p-values of <0.01 and <0.02 respectively.

Conclusion A significant correlation was observed between the immunohistochemical expression of PD-1 and PD-L1 and lymph node metastasis in HNSCC. Evaluation of PD-1 and PD-L1 expression in OSCC offers important prognostic insights and may guide the design of targeted immunotherapies to enhance patient outcomes.

KEYWORDS: Squamous cell carcinoma, PD-1, PD-L1, Tumor infiltrating lymphocytes (TILs), Lymph node metastasis.

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INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) encompasses a diverse group of malignancies arising from the mucosal epithelium of the oral cavity, oropharynx, larynx and hypopharynx. It remains one of the most prevalent cancers globally and poses a significant clinical challenge due to its aggressive behavior and high propensity for regional lymph node metastasis, which is a key determinant of prognosis and therapeutic planning ^[1,2].

In recent years, increasing attention has been directed toward the tumor immune microenvironment, particularly the role of immune checkpoint pathways in tumor progression and immune evasion. One such pathway involves the interaction between programmed death-1 (PD-1), a receptor expressed on activated T cells and its ligand PD-L1, which is frequently upregulated on tumor cells and tumor-infiltrating immune cells ^[3]. The PD-1/PD-L1 axis plays a critical role in suppressing antitumor immunity by promoting T-cell exhaustion, thereby enabling tumor cells to escape immune surveillance ^[4].

Immunohistochemical (IHC) assessment of PD-1 and PD-L1 has become increasingly important in HNSCC cases as it not only helps predict responsiveness to immune checkpoint therapies but also provides potential prognostic information [5]. Previous research has indicated that higher PD-L1 expression is often linked to unfavorable histopathological features, including poor tumor differentiation, lymphovascular invasion and cervical lymph node metastasis [6,7]. However, the association between PD-1 and PD-L1 expression with nodal status has shown considerable variation across different populations and tumor subsites highlighting the need for continued investigation.

The purpose of this study is to examine PD-L1 expression in primary tumor cells and PD-1 expression in tumor-infiltrating lymphocytes (TILs) and to analyze their relationship with lymph node metastasis and other clinicopathological parameters. Findings from this study may provide valuable support to clinicians and oncologists by improving prognostic assessment for guiding treatment planning, and to facilitate more accurate understanding of disease progression at the time of initial diagnosis.

MATERIAL AND METHODS:

Aim & Objectives

To assess PD-L1 and PD-1 expression in tumor cells and tumor-infiltrating lymphocytes (TILs) of HNSCC and to determine their association with lymph node involvement and clinicopathological features.

Sample Collection

This cross-sectional study included 53 histologically confirmed cases of head and neck squamous cell carcinoma (HNSCC) who underwent composite resection with neck dissection between January 2022 and September 2024 in a rural tertiary hospital. Formalin-fixed, paraffin-embedded (FFPE) tissue blocks containing representative tumor sections were selected for further analysis. Patients who had received neoadjuvant chemotherapy or radiotherapy or whose tissue blocks were necrotic or insufficient were excluded from the study.

Immunohistochemistry (IHC)

Immunohistochemical staining was performed on 4-micron-thick paraffin sections using the following primary antibodies. PD-L1: Mouse monoclonal antibody (clone 2746, BioGenex, Fremont, CA), diluted 1:20, incubated for 30 minutes. PD-1: Mouse monoclonal antibody (clone IHC001, BioGenex, Fremont, CA), diluted 1:10, incubated for 30 minutes. Tonsil tissue was used as a positive control for both markers. Expression of PD-L1 was evaluated on tumor cell membranes and PD-1 was evaluated on tumor-infiltrating lymphocytes (TILs) as shown in figure 1, figure 2 and figure 3 were assessed semi-quantitatively based on membranous staining intensity, according to the method described by Subramaniam N et al^[8]. Scores were defined as the cell staining intensity as 0 = <1%; 1 = 1–5%; 2 = 5–50% and 3 = >50% for both tumor and tumour infiltrating lymphocytes.

Statistical Analysis-Data were compiled and analyzed to assess the relationship between PD-1/PD-L1 expression and clinicopathological features, with particular focus on lymph node metastasis. All the data was coded and entered in Microsoft excel sheet. Data was analyzed with Chi- Square test. SPSS 22 version was used for analyzing the data. p -value < 0.05 was considered statistically significant.

RESULTS:

Current study analyzed 53 confirmed cases of HNSCC. Among these, 36 patients (67.9%) were male and 17 (32.1%) were female, indicating a male predominance. The average age of the patients was 30 years, suggesting that the disease was common in relatively younger individuals within the study population. The buccal mucosa was the most frequently involved site, reported in 27 cases (50.9%), followed by other regions of the oral cavity and head and neck.

Out of the total cases, 24 patients (54.7%) had lymph node metastasis. Among these, high PD-L1 expression was noted in 23 cases (95.8%), and high PD-1 expression was observed in 21 cases (87.5%) as shown in Table 1. These metastatic cases showed a higher rate of positive immunohistochemical expression of PD-1 and PD-L1 in the tumor cells of their primary lesions. Statistical analysis demonstrated that PD-1 expression in the TILs had a significant correlation with nodal metastasis ($p < 0.01$). Similarly, PD-L1 expression in tumor cells was also significantly associated with lymph node involvement ($p < 0.02$). Overall, the findings indicate that patients with lymph node metastasis exhibited increased PD-1 and PD-L1 positivity in their primary tumors, suggesting a strong relationship between checkpoint molecule expression and the metastatic potential of HNSCC.

DISCUSSION:

The present study demonstrated a significant positive expression of PD-1 in 21 cases (87.5%) and PD-L1 expression was detected in 23 cases (95.8%) out of 24 positive lymph node metastasis cases. These findings align with study done by Malik I S et al [9] reported PD-1 expression in 93% of cases and PD-L1 in 52% of cases.

Maruse Y et al⁽¹⁰⁾ found PD-1 and PD-L1 expression in 61.9% and 64.9% of cases respectively. The present study comparatively higher PD-L1 expression may suggest a stronger immune checkpoint activation in OSCC.

This study employed a semi-quantitative cell staining intensity scoring system for both PD-1 and PD-L1. This methodological approach is supported by Srivastava et al⁽¹¹⁾ who also integrated intensity grading (+1 to +3) with tumor proportion score (TPS) and combined positive score (CPS) demonstrating significant correlations with clinicopathologic parameters such as stage and perineural invasion. These findings underscore that intensity-based evaluation captures biologically relevant variations beyond the percentage of positive cells alone.

Moreover, Srivastava et al⁽¹¹⁾ reported notable discrepancies between PD-L1 scoring in small biopsy specimens versus resection samples with potential under or overestimation in limited tissue. Current study exclusively utilized resection specimens, this intensity scoring likely reflects a more accurate representation of PD-1/PD-L1 expression strengthening the reliability of this observed association with nodal metastasis.

While present study demonstrated a strong association between elevated PD-1 and PD-L1 expression and with lymph node metastasis, Fiedler et al⁽¹²⁾ extended these observations by linking PD-1/PD-L1 scoring metrics (TPS, immune cell score [ICS] and CPS) to specific patient subgroups such as never-smokers/never-drinkers, tumor site, tumor-infiltrating lymphocyte density and overall survival. This suggests that our findings on nodal involvement align within a broader framework of tumor immune biology.

Study done by Wang et al⁽¹³⁾ adds further information, revealing that PD-L1 expression restricted to cancer cells rather than immune cells was a key driver of poor survival in locally advanced OSCC. This raises the possibility that the high PD-L1 expression observed in metastatic cases in this cohort may be particularly adverse when localized to tumor cell membranes an aspect warranting further evaluation in future studies.

Geum D et al⁽¹⁴⁾ study done on 81 OSCC patients revealed that high PD-L1 expression was correlated with advanced clinical stage, tumor size, lymph node metastasis and reduced 5 and 10-year survival rates (53.7% vs. 78.2% for low expression; $p < 0.05$). This study also demonstrated that knocking down PD-L1 in OSCC cell lines increased apoptosis and suppressed migration and invasion suggesting PD-L1's active role in tumor aggression via the Akt–Stat3 signaling axis.

A retrospective study done by Leporace-Jiménez F et al⁽¹⁵⁾ with 65 OSCC patients used a Combined Positive Score (CPS) for PD-L1 revealed that all patients with lymph node involvement were statistically significant with PD-L1 positive p value < 0.004 . Finally all comparative studies emphasized PD-1 and PD-L1 as potential selection biomarkers for immune checkpoint inhibitor therapy, identifying subgroups such as never-smokers/never-drinkers or PD-L1 high tumors as potentially more responsive. This study observation is that high PD-1/PD-L1 expression is enriched in metastatic cases supports the notion that these patients may derive particular benefit from immunotherapeutic approaches reinforcing the clinical relevance of present study findings.

CONCLUSION:

Findings of present study demonstrate a significant association between PD-1 and PD-L1 expression and the occurrence of lymph node metastasis in head and neck squamous cell carcinoma cases. Notably high expression rates of these markers in metastatic cases suggest their involvement in facilitating tumor spread through immune evasion mechanisms. Assessing PD-1 and PD-L1 status in HNSCC provide valuable prognostic information and could support the development of targeted immunotherapeutic strategies aimed at improving patient outcomes.

LIMITATIONS:

Present study is limited by a relatively small sample size and larger, multicenter studies are warranted to validate these results and to determine whether PD-1 and PD-L1 can serve as reliable biomarkers for prognosis and therapeutic decision making in oral squamous cell carcinoma.

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ETHICAL CLEARANCE

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH SRI DEVARAJ URS MEDICAL COLLEGE Tamaka, Kolar INSTITUTIONAL ETHICS COMMITTEE	
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