

Original Research Article

PD-1 expression on peripheral blood CD4+ T lymphocytes in treatment-naïve HNSCC patients: a flow cytometry analysis

Jatin Godara¹, Arti Agarwal^{2*}, Beant Kaur², Manish Kumar³

¹Department of Pathology, V.M.M.C. & Safdarjung Hospital, New Delhi, India

²Department of Pathology, UPUMS, Saifai, Etawah, Uttar Pradesh, India

³Department of Pathology, Gastroenterology, G.B. Pant Hospital New Delhi, India

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*Correspondence:

Dr. Arti Agarwal,

E-mail: artiagarwal605@gmail.com

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ABSTRACT

Background: Head and neck squamous cell carcinoma (HNSCC) is a globally prevalent malignancy with a high burden in India. Programmed death protein-1 (PD-1), an immune checkpoint receptor, is a potential biomarker in several cancers. However, its prognostic relevance on circulating CD4+ T cells in HNSCC remains uncertain. To evaluate PD-1 expression on peripheral blood CD4+ T lymphocytes in treatment-naïve HNSCC patients and determine its association with clinicopathological features.

Methods: This prospective, cross-sectional study included 32 histopathologically confirmed, treatment-naïve HNSCC patients at a tertiary care center over 18 months. Peripheral blood mononuclear cells were analyzed using flow cytometry (Cytomics FC 500, Beckman Coulter) with antibodies against CD3, CD4 and PD-1 (CD279). PD-1 expression was quantified on gated CD4+ T cells. Statistical analysis was performed using SPSS v25.0, with significance set at $p < 0.05$.

Results: The mean age was 49.94 ± 10.5 years; all patients were male. The most common tumor sites were palate (40.63%), tongue (31.25%) and oral mucosa (28.13%). The mean PD-1 expression on CD4+ T lymphocytes was $5.51 \pm 2.06\%$. PD-1 expression showed no statistically significant association with tumor site ($p = 0.843$), histological differentiation ($p = 0.363$), lymph node metastasis ($p = 0.930$) or tumor stage ($p = 0.930$).

Conclusions: PD-1 expression on peripheral CD4+ T cells does not correlate with tumor site, grade, stage or metastatic status in HNSCC patients. While flow cytometric quantification of PD-1 may offer non-invasive insights, its standalone utility as a prognostic marker in peripheral blood appears limited. Larger studies incorporating tumor microenvironment analysis and longitudinal follow-up are warranted.

Keywords: CD4+ lymphocytes, Flow cytometry, HNSCC, Immune checkpoint, Metastasis, PD-1

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer globally, contributing to approximately 6% of new cancer cases and 1–2% of cancer-related deaths.^{1,2} In India, it accounts for nearly 30% of all malignancies, with 60–80% of cases diagnosed at an advanced stage. HNSCC is the most frequent cancer in Indian males and ranks fifth among females, largely due to tobacco use, alcohol, poor oral hygiene and certain viral infections.^{3,4} Programmed death-ligand 1 (PD-L1), which

inhibits anti-tumor T cell responses, is often overexpressed in tumors. Its receptor, PD-1, is primarily found on tumor-infiltrating lymphocytes, making the PD-1/PD-L1 axis a key immunotherapy target. Elevated PD-1 expression on peripheral CD4+ lymphocytes has been linked to T-cell dysfunction and poorer outcomes in several cancers.^{5,6} Although, the PD-1/PD-L1 pathway is increasingly studied, its exact role in HNSCC remains unclear. PD-1 is expressed on activated immune cells including CD4+ and CD8+ T cells, monocytes and dendritic cells.⁷ While its presence in circulating tumor and immune cells is

established in cancers like non-small cell lung carcinoma⁵, its relevance in HNSCC is still under investigation.⁸ Thus, the present study was conducted to evaluate PD-1 expression on peripheral blood CD4+ lymphocytes in patients with HNSCC. The findings may provide insight into the immunopathology of HNSCC and contribute toward optimizing immunotherapeutic strategies to improve patient outcomes.

Aim

To evaluate expression of programmed cell death protein-1 (PD-1) by flow cytometry on CD4+ lymphocytes in peripheral blood in patients of head and neck squamous cell carcinoma (HNSCC).

Primary objective

To assess PD-1 expression in CD4+ lymphocytes in peripheral blood in patients of HNSCC by flow cytometry

Secondary objective

To compare PD1 expression in HNSCC with and without metastasis. To correlate PD1 expression with site of lesion, grade of tumor.

METHODS

This cross-sectional study was conducted over a period of 18 months, from January 2021 to June 2022, in the Departments of Pathology and Otorhinolaryngology at V.M.M.C and Safdarjung Hospital, New Delhi, after obtaining clearance from the Institutional Ethics Committee and review board. Informed written consent was obtained from each patient prior to inclusion. All patients with histopathologically confirmed cases of head and neck squamous cell carcinoma (HNSCC) were included. Patients were excluded if they had a prior history of any other malignancy, had received chemotherapy or radiotherapy or had cutaneous squamous cell carcinoma of the head and neck region. Detailed history and clinical examination were performed for all enrolled patients. Information collected included age, gender, duration of symptoms and chief complaints. Tumor characteristics such as site, stage, grade and presence of metastasis were recorded. Diagnosis, staging and grading of HNSCC were performed according to the TNM staging system of the American Joint Committee on Cancer (AJCC), 8th edition.⁸

Sample size determination

As per the study done by Chang et al on prognostic role of pretreatment circulating tumor cells, circulating cancer stem cell-like cells and programmed cell death-1 expression on peripheral lymphocytes in patients with initially unresectable, recurrent or metastatic head and neck cancer (HNSCC) patients applying the formula to calculate sample size.⁹

$$n = (Z\alpha/2)^2 \times P \times Q / L^2$$

n=Sample size=33, Z=Standard deviate

α =95% CI=0.05

P=Proportional population=53 (PD-1) Q=100-P, L=Allowable error=5%

Taking finite population as: $SS/1+SS/Population$

SS=Sample size=32.9

Thus, a total of 32 cases were taken for the study as convenient sampling.

For flow cytometry 2 ml blood sample was taken in EDTA vial from patients of HNSCC. Flow cytometer- cytomics fc 500 (beckman and coulter) was used.

Step 1: preparation of the sample and acquiring of the events

Blood incubated with antibodies (targeted with different fluoro-chromes) target at different cellular antigen – for 20 minutes. Blood was lysed with optilyse for 20 minutes and centrifuge at 1700 RPM for 08 minutes, discard the supernatant. The cell pellet was washed with sheath fluid twice. Make up the volume with sheath fluid up to 500 microlitre, the events were acquired up to 10 lacs.

Step 2: analysis single tube analysis was done using CD3/CD45/CD4/CD8/and CD279 (PD-1).

On CD45 versus side scatter, the leucocytes were differentiated into neutrophils, monocytes and lymphocytes. On CD3+ versus CD4+ gating, T-cells lymphocytes and gated were isolated and quantitative expression of PD1 on T lymphocytes were noted. Comparison and correlation if any between PD-1 expression in T lymphocytes in primary and metastatic head and neck squamous cell carcinoma and stage and grade of tumor were noted.

Statistical analysis

The presentation of the categorical variables was done in the form of number and percentage (%). On the other hand, the quantitative data were presented as the means $\pm$ SD and as median with 25th and 75th percentiles (interquartile range). The data normality was checked by using Kolmogorov-Smirnov test. The cases in which the data was not normal, we used non parametric tests. The association of the variables which were quantitative and not normally distributed in nature were analyzed using Mann-Whitney Test (for two groups) and Kruskal Wallis test (for more than two groups). The data entry was done in the Microsoft EXCEL spreadsheet (Annexure IV) and the final analysis was done with the use of Statistical Package for Social Sciences (SPSS) software, IBM

manufacturer, Chicago, USA, version 25.0. For statistical significance, p value of less than 0.05 was considered statistically significant.

RESULTS

The mean age of the patients was 49.94 ± 10.5 years, with a median of 46 years. All 32 patients were male. Smoking was reported in 84.38%, while 93.75% consumed alcohol. The most common cancer site was the palate (40.63%), followed by the tongue (31.25%) and oral mucosa (28.13%).

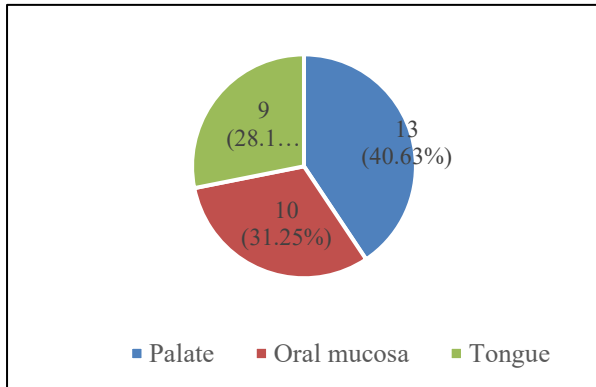


Figure 1: Distribution of cancer sites.

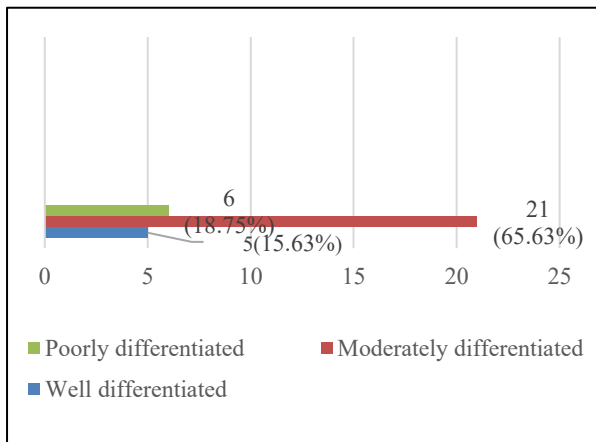


Figure 2: Tumor differentiation pattern.

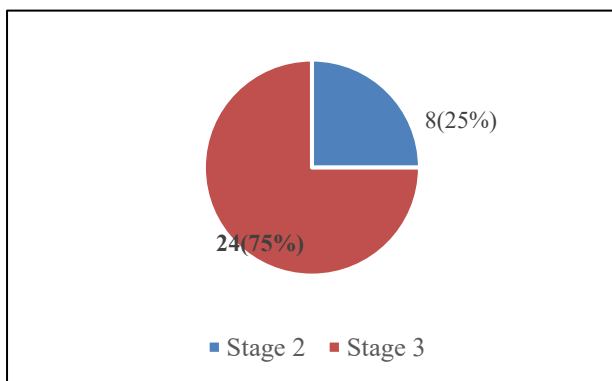


Figure 3: Distribution of stages.

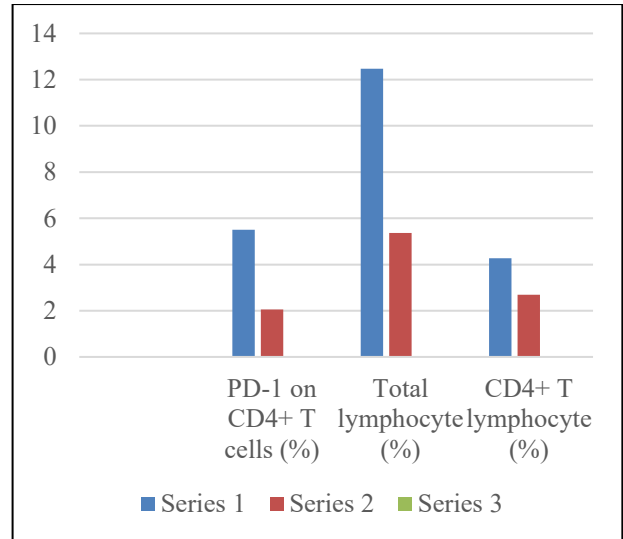


Figure 4: Descriptive statistics of immune markers.

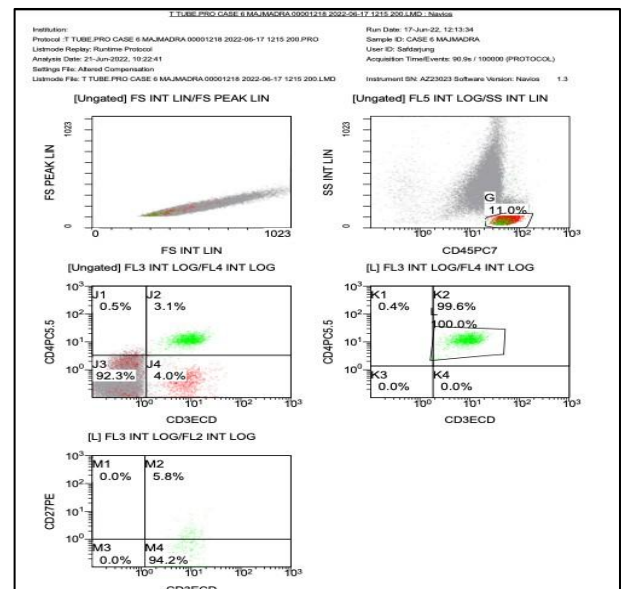


Figure 5: Flow cytometry scatter plot.

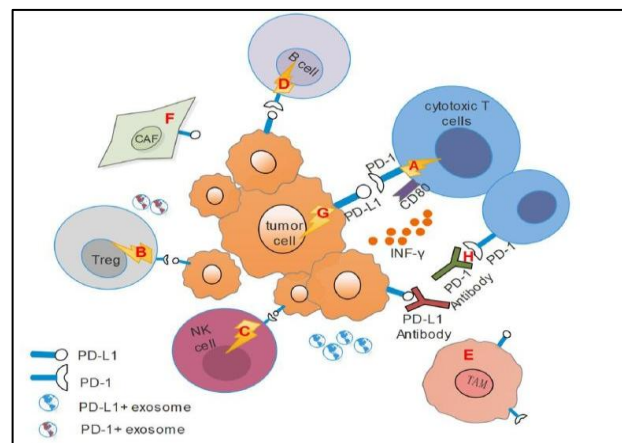


Figure 6: PD-1/PD-L1-mediated immunosuppressive mechanisms in HNSCC.³⁷

Hematological parameters revealed. Mean haemoglobin: 12.61 g/dl. Mean total leukocyte count: $12.91 \times 10^3/\text{mm}^3$ Mean platelet count: $135.97 \times 10^3/\text{mm}^3$ Most patients (65.63%) had moderately differentiated carcinoma; 18.75% were poorly differentiated and 15.63% were well-

differentiated. Correspondingly, 65.63% had Grade 2 tumors, 18.75% Grade 3 and 15.63% Grade 1. Tumor staging showed stage 3 in 75% and stage 2 in 25% of patients.

Table 1: Demographic profile of study subjects.

Parameter	Value
Number of patients	32
Gender	All Male
Mean Age (years)	49.94±10.5
Median Age (years)	46
Smokers	27 (84.38%)
Alcohol Users	30 (93.75%)

Table 2: PD-1 expression based on clinicopathological features.

Feature	Subgroup	Median PD-1 expression	P value	Significance
Tumor site	Oral mucosa/tongue/palate	5.2 / 5.5 / 5.2	0.843	Not Significant
Metastasis	Absent/present	5.5 / 5.2	0.930	Not Significant
Histological grade	Well/moderate/poor	5.2 / 5.2 / 5.8	0.363	Not Significant
Tumor stage	stage 2/stage 3	5.5 / 5.2	0.930	Not Significant

Table 3: Summary of PD-1 Expression Findings.

Tumor parameter	Median PD-1 expression	P value
Site (palate, tongue, mucosa)	5.2–5.5	0.843
Metastasis (present/absent)	5.2 vs. 5.5	0.930
Differentiation	5.2–5.8	0.363
Stage (II vs III)	5.5 vs. 5.2	0.930

These results confirm that peripheral blood PD-1 expression on CD4+ T cells in HNSCC is not significantly influenced by key clinicopathological parameters.

DISCUSSION

The progression and prognosis of head and neck squamous cell carcinoma (HNSCC) are influenced by tumor grade, histological differentiation and disease stage.¹⁰ This study evaluated the expression of Programmed Cell Death Protein-1 (PD-1) on peripheral blood CD4+ T lymphocytes in relation to these clinicopathological factors using flow cytometry. PD-1, a co-inhibitory receptor of the CD28 family, is expressed on activated T cells, regulatory T cells, NK cells, B cells and macrophages.

Its upregulation is well documented in tumor-infiltrating and peritumoral immune cells in HNSCC, especially at the invasive front.^{11,12} Compared to conventional therapies, anti-PD-1/PD-L1 agents have shown better efficacy and lower toxicity in advanced HNSCC, underscoring the clinical importance of PD-1 expression. While intratumoral PD-1 expression has been extensively studied, data on peripheral expression remain limited.^{11,12} Our study addresses this gap and aligns with findings by Chang et al, who reported no prognostic impact of PD-1 expression on peripheral CD4+ or CD8+ T cells in HNSCC.⁸

In our cohort, PD-1 expression on peripheral CD4+ T cells showed no significant correlation with tumor stage, site, histological grade or metastatic status. The mean expression was 5.51 ± 2.06 and median values across subgroups (site, metastasis, differentiation, staging) did not differ significantly. These negative associations may reflect the small sample size and inter-laboratory variability in flow cytometry. To date, only Chang et al, have reported similar findings in a comparable cohort (n=34), consistent with our sample size (n=32).⁸

Demographic and clinical characteristics

Head and neck squamous cell carcinoma (HNSCC) primarily affects older adults. In our study, the mean age was 49.94 ± 10.5 years, comparable to findings by Gilyoma et al (42 years) and Liu et al, (52.9 years), though slightly lower than other studies, possibly due to regional and lifestyle variations.¹⁵⁻¹⁷ All 32 patients in our cohort were male, reflecting the well-established male predominance in HNSCC. Similar gender distributions have been reported with male-to-female ratios ranging from 2:1 to 4:1, largely attributed to higher rates of tobacco and alcohol use among men.^{13-15,18}

The most common presenting complaints included growths or ulcers in the palate (40.63%), tongue (31.25%) and buccal mucosa (28.13%), consistent with known clinical features of oral cavity cancers. Similar patterns were noted by Carpen et al, McIlwain et al and Molina et al, Tumor sites in our study were predominantly the palate, tongue and oral mucosa.¹⁹⁻²¹ Oral squamous cell carcinoma (OSCC) constitutes over 90% of oral malignancies.¹⁸ High incidences of tumors in the buccal mucosa, tongue and larynx have also been reported by Alam et al, Liu et al and Basumatari et al.^{14,15,22}

Tobacco and alcohol use were prevalent in our cohort, with 84.38% of patients being smokers and 93.75% reporting alcohol consumption. These findings are in line with established literature identifying tobacco (smoking and chewing) and alcohol as major risk factors for HNSCC, particularly in South Asia.^{13,17,22-24}

Among investigations, the mean haemoglobin level was 12.61 ± 1 g/dl. Anemia is common in cancer due to chronic inflammation, nutritional deficiency or marrow suppression.²⁵ Although we did not assess treatment response, previous studies suggest that low pre-treatment haemoglobin levels are associated with poorer outcomes and increased recurrence rates.²⁵⁻²⁹

In terms of tumor staging, eight patients were in stage II and 24 in stage III. Histological grading revealed a mix of well, moderately and poorly differentiated carcinomas. This distribution is consistent with findings by Lin et al, and Hong et al although variations are noted based on patient demographics and tumor biology.^{12,30} Lymph node metastases were observed in 24 patients, aligning with reports by Lin et al and Chang et al, where nodal involvement was seen in 38–60% of cases.^{8,12} Distant metastasis was not assessed in this study but remains a critical determinant of HNSCC prognosis.

Programmed death-1 receptor: its role as an emerging molecular marker for prognosis

Programmed Death-1 receptor (PD-1) is “an inhibitory receptor on hematopoietic cells”, which regulates immune responses negatively, specifically response towards tumors that usually upregulates PD-1 ligand. PD-1/PD-1 ligand blocking antibodies can reverse the inhibition and demonstrate significant treatment outcomes in the treatment of RCC, lung carcinoma and melanoma.³¹

Recent studies have utilized immunohistochemistry (IHC) to assess PD-1 and PD-L1 expression in HNSCC, linking it with patient survival. PD-L1 exists in a soluble form (sPD-L1) in serum, as seen in melanoma and lung adenocarcinoma and has also been identified in the plasma of HNSCC patients by Theodoraki et al.³³ PD-L1 binds to CD80 (B7-H1), delivering inhibitory signals to T cells. Meanwhile, PD-L2 is predominantly expressed on antigen-presenting cells such as dendritic cells,

macrophages and B cells and similarly inhibits T-cell activation and cytokine production.³⁴

Human OSCC cell lines exhibit variable PD-L1 expression, which is further upregulated upon IFN stimulation.³⁵ Chen et al, showed that IFN- γ induces PD-L1 via upregulation of protein kinase D isoform 2 (PKD2), a downstream target of the PI3K pathway, in a time- and dose-dependent manner.³⁶ PD-L1 overexpression in tumor cells is driven by both intrinsic and extrinsic mechanisms, with IFN- γ being a key cytokine mediator.

PD-1 immunostaining in HNSCC reveals elevated expression on inflammatory cells, especially at the invasive tumor front. PD-L1 demonstrates both membranous and cytoplasmic staining in tumor parenchyma and stroma, with a positive correlation between their expression levels.³⁷ Two distinct PD-L1 patterns are observed: diffuse staining throughout the tumor parenchyma and peripheral staining along tumor margins.

PD-L1/PD-1 positivity in head and neck squamous cell carcinoma: evidence from previous studies

Lyford-Pike et al evaluated 27 patients with oropharyngeal squamous cell carcinoma (SCC).³⁸ A 5% threshold of cell surface PD-L1 expression on tumor cells was used to define positivity. Overall, PD-L1 positivity was observed in 59% of cases. Among the 20 HPV-positive HNSCC samples, 70% (n=14) expressed PD-L1, with all of them showing $\geq 5\%$ tumor cell surface staining. In contrast, only 29% (n=2) of the 7 HPV-negative cases were PD-L1 positive.

Kim et al analyzed 133 patients with oropharyngeal SCC, defining PD-L1 positivity as membrane staining in $\geq 20\%$ of tumor cells. PD-L1 expression was detected in 68% of patients.³⁹ p16 positivity, used as a surrogate marker for HPV status, was seen in 67% of the cases. No significant difference in PD-L1 expression was noted between HPV-negative and HPV-positive tumors (61% vs. 71%, $p=0.274$).

Hong et al studied 99 patients with tonsillar carcinoma, defining PD-L1 positivity as any unequivocal membrane staining of tumor cells.³⁰ A significant association was observed between HPV status and PD-L1 expression, with positivity rates of 83.3% in HPV-positive and 56.9% in HPV-negative cases ($p<0.05$).

Mattox et al assessed 53 patients with tongue cancer. PD-L1 positivity was defined as $>1\%$ membranous expression by tumor and/or immune cells.⁴⁰ PD-L1 positivity was reported in 79% of samples. A positive correlation was found between PD-L1 expression and moderate-to-high levels of CD4+ and CD8+ tumor-infiltrating lymphocytes (TILs). Balermipas et al analyzed 161 patients with SCC of the oral cavity, oropharynx and hypopharynx.⁴¹ A 5% cut-off in tumor and/or stromal cells was used to define PD-L1

positivity. PD-L1 expression was positive in 39.1% of the cases. PD-L1 positivity was significantly higher in HPV-positive than in HPV-negative tumors (53% vs. 31%, $p < 0.05$). Ou et al studied 38 patients with SCC from multiple head and neck subsites.⁴² Using a 1% cut-off, 71.1% of patients showed PD-L1 positivity. When the threshold was raised to 5%, the positivity rate dropped to 50%.

A meta-analysis by Yang et al included 23 studies involving 3,105 patients to assess the prognostic value of PD-1/PD-L1 expression in HNSCC.³¹ The overall PD-L1 positivity rate in HNSCC was 42% (95% CI: 36–48%). No significant differences were found between PD-L1-positive and negative patients in overall survival (OS; HR: 0.98, 95% CI: 0.71–1.37, $p = 0.93$), disease-free survival (DFS; HR: 1.07, 95% CI: 0.68–1.70, $p = 0.76$) or disease-specific survival (DSS; HR: 0.90, 95% CI: 0.63–1.29; $p = 0.56$). However, improved progression-free survival (PFS) was observed in patients with PD-L1 positivity (HR: 0.71, 95% CI: 0.55–0.93, $p = 0.01$). Among patients with low CD8+ TILs, those with positive PD-L1 expression had poorer OS (HR: 1.90, 95% CI: 1.07–3.36; $p = 0.03$).

The PD-1/PD-L1 axis contributes significantly to the immunosuppressive microenvironment in HNSCC, promoting immune evasion and resistance. Inhibitory checkpoint receptors such as PD-1, CTLA-4 and Tim-3 are commonly expressed on dysfunctional T cells in HNSCC. PD-1 is also present on CD4+ and CD8+ T cells in lymph nodes and circulation. Tumor-associated macrophages (TAMs), influenced by IL-10 secreted from OSCC tumor cells, express PD-L1 and contribute to T-cell apoptosis and poor prognosis. In HPV-positive HNSCC, TAMs mediate adaptive immune resistance via PD-L1 expression at the tumor-stroma interface, suppressing tumor-specific T cell responses.³⁷

PD-L1 is also expressed on cancer-associated fibroblasts (CAFs); around 40% of HNSCC tissues show PD-L1+ CAFs, though without clear clinical significance. In HPV-positive tumors, fibroblasts enhance PD-L1 expression on tumor cells. CD44+ tumor-initiating cells also evade immune surveillance via PD-L1 expression.⁴⁴ Elevated plasma exosomes in HNSCC patients contain immunosuppressive proteins including PD-L1, CTLA-4 and COX-2, further impairing immune function.³⁷

A schematic (Figure 6) illustrates how PD-1/PD-L1 signaling drives tumor immune evasion in HNSCC: (A) PD-L1 binding to PD-1 on cytotoxic T cells induces T cell exhaustion, (B) PD-L1 inhibits CD28-CD80 co-stimulation by binding CD80, (C) PD-1/PD-L1 interaction suppresses NK cell activity, (D) PD-1 activation on B cells hinders CD4+ and CD8+ T cell proliferation, (E) IFN- γ from CD4+ T cells enhances PD-L1 on TAMs, promoting resistance, (F) Tumor cells and fibroblasts mutually upregulate PD-L1, forming an immune-evasive loop, (G) PD-L1-PD-1 interaction on tumor cells delivers anti-apoptotic signals, (H) Immune checkpoint inhibitors

targeting PD-1/PD-L1 can reverse this immunosuppression.

Biological significance and prognosis value of PD-1/PD-L1 Axis in HNSCC tumor and peripheral blood

As the PD-1/PD-L1 pathway is implicated in immune evasion as well as tumour development, several researchers have examined into whether the level of PD-1 and PDL1 protein expression in tumour tissues is associated with HNSCC clinical features as well as biological behaviour. However, the recent findings are still debatable. A correlation is reported between stronger PD-L1 immunostaining in HNSCC with distant metastases and worse outcomes, which is independent of origin of tumor.³⁷ Overall the results of the meta-analysis of Yang WF et al¹³ (2018) opened the gates for further research on PD-1 expression in various places like circulating tumor cells (CTCs), CD8 T cells and CD4 cells. Moreover, the expression was also studied in the tumor and in peripheral blood.

Previous studies have largely focused on PD-1 expression in tumor-infiltrating lymphocytes (TILs) rather than peripheral blood. Lyford-Pike et al reported higher PD-1 expression on CD4+ and CD8+ T cells in tonsil tissue compared to peripheral blood in HNSCC patients, suggesting limited clinical utility of peripheral PD-1 assessment a view supported by Chang et al, Waki et al, found that in lung cancer patients receiving peptide vaccines, a higher proportion of PD-1+CD4+ T cells before and after treatment and a reduction in PD-1+CD8+ T cells post-treatment, correlated with longer overall survival (OS).^{5,8,38,44}

Lin et al analyzed 305 OSCC cases and observed higher PD-L1 expression in females and those with distant metastasis.¹² PD-L1 was also an independent prognostic marker in males and smokers. Similarly, Hong et al, demonstrated improved OS and event-free survival in HPV-positive/PD-L1-positive tonsillar cancers.³⁰ Vassilakopoulou et al observed PD-L1 mRNA upregulation in laryngeal SCCs and found that high TIL density and PD-L1 levels were associated with better OS and disease-free survival (DFS).⁴⁵

In a study of 517 HNSCC patients, Lyu et al reported high PD-1 expression in HPV-positive tumors, with improved recurrence-free survival in those receiving radiotherapy and better response to immunotherapy in patients with high PD-L1 expression.⁴⁶ Balermipas et al found high PD-L1 expression and CD8+ TIL infiltration in HPV16-positive tumors; however, PD-L1 remained a prognostic marker only in HPV16-negative patients.⁴¹

Strati et al evaluated PD-L1 mRNA in circulating tumor cells (CTCs) of 113 patients using RT-qPCR.⁴⁷ PD-L1 overexpression at end of treatment correlated with shorter progression-free survival (PFS) and OS and absence of PD-L1 overexpression predicted complete response

(OR=16.00, $p=0.002$). Theodoraki et al, found PD-L1 in exosomes correlated with disease activity and lymph node status, suggesting its potential as a biomarker, while plasma sPD-L1 and exosomal PD-1 showed no significant correlation.³³

In RCC, increased PD-1 expression on CD4+ T cells, NK cells and effector T cells correlated with disease stage and levels decreased post-tumor resection, highlighting peripheral PD-1 as a potential biomarker.^{31,53} Similarly, Vaidaya et al found high PD-1/PD-L1 expression in cutaneous squamous cell carcinoma (CSCC), reinforcing its broader relevance in squamous neoplasms.⁵²

Moratin et al observed that PD-L1 expression in OSCC and its lymph node metastases was significantly associated with tumor size, stage, metastasis and poor OS.⁵¹ Higher PD-L1/PD-L2 scores were linked to worse survival. In high-risk CSCC and transplant-associated CSCC, PD-1/PD-L1 expression correlated with aggressive features and perineural invasion.⁵ Cemiplimab, a PD-1 inhibitor, demonstrated 47–50% response rates and >6-month durable responses in unresectable/metastatic CSCC, leading to FDA approval.^{6,44}

These findings collectively indicate that PD-1/PD-L1 expression whether in tissue, CTCs or exosomes has potential as a biomarker in HNSCC and other squamous cancers. However, the prognostic role of peripheral PD-L1 expression remains underexplored and warrants further research.

Clinical application of PD-1/PD-L1 inhibitors in head and neck squamous cell carcinoma

Currently, the FDA has approved two PD-1 inhibitors nivolumab and pembrolizumab for advanced HNSCC. PD-1/PD-L1 blockade reduces tumor burden and enables durable tumor regression. Nivolumab was approved in 2016 for recurrent/metastatic HNSCC, regardless of PD-L1 status.⁵⁰ The KEYNOTE-048 phase III trial by Burtneß et al (n=882) showed that pembrolizumab monotherapy improved OS in PD-L1-positive patients compared to standard cetuximab-based chemotherapy.⁴⁹ However, no OS benefit was seen in the entire cohort.

Durvalumab and atezolizumab are PD-L1 inhibitors not yet FDA-approved for HNSCC but have shown antitumor activity in other settings.⁴⁸ These findings emphasize the importance of PD-1/PD-L1 profiling for personalized immunotherapy in HNSCC.

The negative findings in our study and their study might be because of a small sample size and standardisation methods of flow cytometry. Follow up of the patients after treatment was not done. Association of Hb with survival analysis was not done. Tumor expression of PD-1 was not determined. Cases with only lymph node metastasis were enrolled in the study.

CONCLUSION

This study evaluated the expression of PD-1 on peripheral CD4+ T lymphocytes in patients with head and neck squamous cell carcinoma (HNSCC) using flow cytometry. The results showed no statistically significant association between PD-1 expression and clinicopathological variables including tumor stage, site, histological differentiation or lymph node metastasis. These findings suggest that PD-1 expression on circulating CD4+ T cells may not reflect tumor burden or aggressiveness in treatment-naïve HNSCC patients. Further large-scale, prospective studies incorporating longitudinal monitoring and correlation with treatment response and survival outcomes are warranted. Future research should also assess PD-1 expression within the tumor microenvironment to determine its concordance with peripheral blood levels and explore its potential as a biomarker for immunotherapy response.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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