Print ISSN: 2321-6379
Online ISSN: 2321-595X

Original Article

Human Papilloma Virus, Programmed Death Ligand-1, and Tumor Microenvironment: Implications for the Patients with Head-and-Neck Squamous Cell Carcinoma

Asttha Kapoor[1], Pinki Pandey[2], Alok Dixit[3], Savita Agarwal[4], Udbhav Tewari[5], Kailash Mittal[6], Jitendra Pratap[7], Suha Akhtar[8]

[1]Senior Resident, Department of Pathology, Uttar Pradesh University of Medical Sciences, Etawah, Uttar Pradesh, India, [2]Professor and Head, Department of Pathology, Uttar Pradesh University of Medical Sciences, Etawah, Uttar Pradesh, India, [3]Professor and Head, Department of Pharmacology, Uttar Pradesh University of Medical Sciences, Etawah, Uttar Pradesh, India, [4]Professor, Department of Pathology, Uttar Pradesh University of Medical Sciences, Etawah, Uttar Pradesh, India, [5]Senior Resident, Department of Psychiatry, Uttar Pradesh University of Medical Sciences, Etawah, Uttar Pradesh, India, [6]Professor and Head, Department of Radiation Oncology, Uttar Pradesh University of Medical Sciences, Etawah, Uttar Pradesh, India, [7]Professor, Department Of ENT, Uttar Pradesh University of Medical Sciences, Etawah, Uttar Pradesh, India, [8]3rd Year Post Graduate Student, Department of Pathology, Uttar Pradesh University of Medical Sciences, Etawah, Uttar Pradesh, India

Abstract

Objectives: To investigate the relationship among the immunohistochemical expression of programmed death ligand 1 (PD-L1), CD8, and human papillomavirus (HPV) with a variety of clinicopathological parameters.

Purpose: The study of PD-L1, HPV, and CD8 expression may provide clinicians with more exact information to appraise tumor aggressiveness and treatment modalities.

Methods: In this prospective study, a total of 100 head-and-neck squamous cell carcinoma (HNSCC) cases were investigated. The tumor was graded according to World Health Organization criteria. PD-L1, CD8, and HPV expression were determined by immunohistochemical staining. The obtained results were assessed using the Chi-square test.

Results: Out of 100 cases, 73% of patients were positive for PD-L1, 90% of cases were positive for HPV expression, and 94% of cases were positive for CD8. There was a statistically significant link between PD-L1 and drinking alcohol, abnormal sexual behavior, having multiple sexual partners, and the grade of the tumor. CD8 expression was also associated with gender and smoking. In contrast to this, HPV expression is associated with aberrant sexual behavior. The connection between the expression of PD-L1 and CD8 was found to be substantial ($P = 0.002$).

Conclusion: HNSCC with high CD8+ TIL infiltration and PD-L1 expression may have a better outcome, superior survival, and cancer endurance.

Key words: CD8, Head-and-neck squamous cell carcinoma, Human papillomavirus, PD- L1, TIL

INTRODUCTION

Head-and-neck carcinomas rank sixth among all malignancies worldwide, with almost 90% of head and neck cancers attributable to the 60,000 cases reported yearly. In India, it is one of the top three cancer types.[1] Squamous cell carcinoma of the upper aerodigestive tract typically occurs in older patients in their 5th–7th decades of life.[2-5] The most common triggering factors for head-and-neck squamous cell carcinoma (HNSCC) to be known for decades are paan chewing, cigarette smoking, alcohol abuse, and poor oral hygiene, but human papillomavirus (HPV) is now recognized as one of the primary causes of oropharyngeal squamous cell cancers.[6] HPV-associated cancers are caused by the expression of HPV's E6 and E7 proteins that bind to and inactivate tumor suppressor

Access this article online

www.ijss-sn.com

Month of Submission : 12-2023
Month of Peer Review : 01-2024
Month of Acceptance : 02-2024
Month of Publishing : 02-2024

Corresponding Author: Dr. Udbhav Tewari, Uttar Pradesh University of Medical Sciences, Saifai, Etawah, India.

proteins p53 and retinoblastoma protein, respectively, leading to malignant transformation of HPV-infected cells.[7] programmed death ligand 1 (PD-L1) expression is a potential predictive biomarker of the response to anti-PD-L1/anti-programmed cell death-1 (PD-1) therapy in multiple cancers, including HNSCC.

Recent studies advocate that PDL1-expressing cancer cells have the capacity to expeditiously elude the host immune system. Aberrant expression of programmed death ligand 1 (PD-L1) enables tumor cells (TC) to elude the host's immune system and is reckoned to be a manner of adaptive immune resistance.[8] Inhibition of this immune checkpoint offers a promising new therapeutic approach.[9] PD-L1 is expressed on TCs and is able to bind to PD-1 on T-lymphocytes, thereby terminating their role. It is also expressed on tumor-infiltrating lymphocytes (TILs) and macrophages.[10] CD8-positive T-lymphocytes, also called cytotoxic T-cells, are the most powerful anticancer cells of the immune system and kill cells by direct lysis. Patients diagnosed with HNCC cancer whose tumor was highly infiltrated by CD8+T cells showed comparatively better outcomes than those who had minimal or no infiltration, thus confirming that the cytotoxic T-cells are associated with a favorable outcome. A retrospective evaluation of patients diagnosed with HNSCC treated with anti-PD-1/PD-L1 agents showed that increased tumor infiltration by CD8+ T cells and an increased ratio of CD8+ T cells/Tregs were positively correlated with treatment response, indicating their potential role as a predictive biomarker.[11]

In our study, PD-L1, CD8, and HPV expression in HNSCC were correlated with each other and with clinicopathological parameters.

MATERIALS AND METHODS

Sample Collection

In the current study, 100 biopsies and neck dissection specimens provided by the Department of Oncosurgery and Otorhinolaryngology were screened and histopathologically determined to be squamous cell carcinoma. Individuals with malignancies other than HNSCC, including adenocarcinoma, melanoma, sarcoma, and metastases, were excluded from the study. The sample size was calculated using the formula $n = Z^2_{\alpha/2} P(1–P)/d^2$, where n is a sample size, $Z\alpha_{/2}$ (1.96) is statistic for a level of confidence, $P = 0.07$) is expected prevalence or proportion, and d (0.05) is precision or error. By putting values in the above formula, the sample size came out to be 99.99, i.e., 100. Our study was approved by the institutional ethical committee, and informed consent was taken from all the patients.

Histopathological Examination of the Tissue Specimens

Prognostic characteristics such as histological grade, tumor size, and lymph node metastasis were evaluated using hematoxylin and eosin staining. Tumors were histologically graded as per the 2005 World Health Organization classification of head and neck tumors.[12]

Immunohistochemistry

PD-L1, HPV, and CD8 immunostaining was used to examine the immunohistochemical profile by staining a representative section of tumor with mouse monoclonal antibody Mob572, mouse monoclonal antibody Mob394, and rabbit monoclonal antibody RMAB012 (concentrated antibodies for use with diagnostic biosystems PolyVue tm plus-two-step detection system), respectively. Clinicopathological characteristics, including age, gender, smoking, alcohol intake, paan chewing, aberrant sexual practices, numerous sexual partners, histological grade, and lymph node status, were correlated with IHC expression.

Pathological assessment

The tonsil was taken as a positive control for PD-L1 immunostaining. A field with a minimum of 100 viable TCs was selected for evaluation, and the necrotic areas were avoided. Staining was seen in both immune cell (IC) and TC components. In TCs, both partial or complete membrane staining was assessed, excluding cytoplasmic staining, whereas in ICs, both membrane and cytoplasmic staining of inflammatory cells are included because they are often indistinguishable due to the high N: C ratio. Expression was determined using the combined positive score (CPS), which is the number of PDL1-staining cells (macrophages, TCs, and lymphocytes) divided by the total number of viable TCs multiplied by 100. Cells in the numerator include all viable invasive TCs and mononuclear IC directly associated with the response to the tumor (lymphocytes and macrophages within tumor nests and adjacent supporting stroma); adjacent mononuclear IC are defined as being within the same 20× field as the tumor.

$$\text{CPS} = \frac{\text{Number of PDL1}^{st}\text{aining cells (tumor cells, lymphocytes, macrophages)}}{\text{Total number of viable tumor cells}} \times 100$$

Based on the CPS value, PDL-1 expression was determined as follows: CPS <1: no PDL1expression; CPS ≥1: PDL1 expression; CPS ≥20: PDL1 expression.[13]

Immunohistochemical analyses of CD8 were used to measure the expression in the cytoplasm and membrane of lymphocytes. CD8+ lymphocyte content was counted using ten representative high-power microscopic fields (40× magnifications) of the intra-tumor region and invasive

border of the tumor. The average cell numbers for each area were scored as follows: Grade 0 = average number 0, Grade I = average numbers 1–19, Grade II = average numbers 20–49**,** and Grade III = average numbers 50. This formulated the CD8+lymphocyte infiltration score (CD8+TILscore).[14]

Membranous and/or cytoplasmic HPV IHC staining was scored [score 0–2] as: 0 = no staining, 1=focal: focally stained cells (i.e., limited staining of some [≤5] cells restricted to the upper quarter of the epithelium); 2=extensive: (i.e., widespread) staining in the upper one-third of the epithelium and/or below.[15]

Statistical Analysis

The statistical analysis was performed using the Statistical Package for Social Science version 23.0 statistical analysis software. The values were represented in number (%) and mean±standard deviation. A chi-square test was used, and "*P*" values were calculated using the following levels of significance: $P > 0.05$ (not significant), $P < 0.05$ (significant), $P < 0.01$ (highly significant), and $P < 0.001$ (very highly significant).

RESULTS

The study included a hundred patients, with an age range of 25 to 75 years and a mean of 48.40 ± 12.093 years. The majority of the patients (31%) belonged to the 31–40 age group. Fifty-six of them were <50 years of age, of which 55 (98%) cases had positive CD8 expression, while forty-four were >50 years of age, of which 39 (88.63%) cases were CD8 positive. Six cases had CD-8 Grade 0, whereas CD-8 Grades I, II, and III comprised 18, 33, and 43 cases, respectively [Figure 1a-c]. HPV Grade 0 was observed in 10 cases, of which 5 were <50 years and the remaining 5 were >50 years old. Whereas HPV Grade 1 was seen in 22 cases (14 cases belonged to the age group <50 years and 8 cases >50 years) and HPV Grade 2 in 68 cases (37 cases were <50 years and 31 cases >50 years) [Figure 1d-f].

PD-L1 expression was seen such that 27 cases had CPS <1, CPS ≥1, and CPS >20 seen in 36 and 37 cases, respectively. Out of 27 patients with CPS <1, 24 (88.88%) cases were <50 years of age, and 3 (11.11%) cases were >50 years of age. CPS ≥1 was seen in 36 patients; 21 (58.33%) of them were <50 years of age. Whereas CPS > 20 was seen in 37 patients, out of them, 26 (70.27%) cases were >50 years of age. [Figure 1g-i]. A statistically significant direct association was observed between CD8 and age ($P = 0.036$) and PD-L1 and age ($P = 0.002$), whereas no such associations were observed with HPV.

The entire cohort of 100 patients consisted of 89% males and 11% females. Among 89 males, 86 (96.62%) and 11 females, 8 (72.72%) cases expressed CD8 positivity. A statistically significant association was observed between CD-8 expression and gender propensity ($P = 0.008$), whereas no significant relationship was observed between HPV ($P = 0.519$) and PD-L1 expression ($P = 0.396$).

The most common site of involvement was the oral cavity (77%), followed by the face and neck (11%), oropharynx (9%), hypopharynx (3%), and larynx (2%) [Figure 2a-c]. No significant relationship was seen between site and CD-8 ($P = 0.085$), HPV ($P = 0.093$), and PD-L1 ($P = 0.352$) expression. 55% of cases were graded as well differentiated (Grade 1), whereas moderately differentiated (Grade 2), and poorly differentiated (Grade 3) constitute 40%–50%, respectively [Figure 2d-f].

Eighty-eight patients were paan chewers, of which 80 (90.90%) cases had positive HPV expression, while 10 (83.33%) cases out of 12 non-paan chewers had positive HPV expression. No statistically significant association was observed between the habit of paan chewing and CD-8 ($P = 0.754$), HPV ($P = 0.654$), and PD-L1 ($P = 0.909$) expression.

The habit of smoking was seen in 65 patients, of whom 63 (96.92%) cases had positive CD8 expression, while 31 (88.5%) cases out of 35 non-smoker patients also had positive CD8 expression. A statistically significant correlation was observed between smoking and CD-8 expression ($P = 0.003$), whereas no such association was observed with HPV ($P = 0.195$) and PD-L1 ($P = 0.250$).

A history of alcohol abuse was present in 34 patients, all of whom had positive CD8 expression. 66 patients had a negative history of alcohol abuse, of which 60 (90.90%) had positive CD8 expression. Out of 34 patients who had a positive history of alcohol abuse, 16 (47.05%) cases had CPS <1, 13 (38.23%) cases had CPS ≥1, and 5 (14.70%) cases had CPS >20. Whereas, 66 patients did not have a history of alcohol abuse, of which 11 (16.66%) cases had CPS <1 expression, 23 (34.84%) cases had CPS ≥1, and 32 (48.48%) cases had CPS >20. A statistically significant direct association was observed between the history of alcohol abuse and CD-8 ($P = 0.014$) and PD-L1 ($P = 0.001$) expression. Abnormal sexual habits were present in 19 patients, all of whom had positive CD8 as well as HPV expression. PD-L1 expression amongst these patients was such that 9 (47.36%) cases showed CPS <1 expression, 7 (36.84%) cases CPS ≥1, and 3 (15.78%) cases CPS >20. Whereas, 81 patients had a negative history of abnormal sexual habits, of which 75 (92.59%) cases had positive CD8 expression and 71 (87.65%) cases had positive

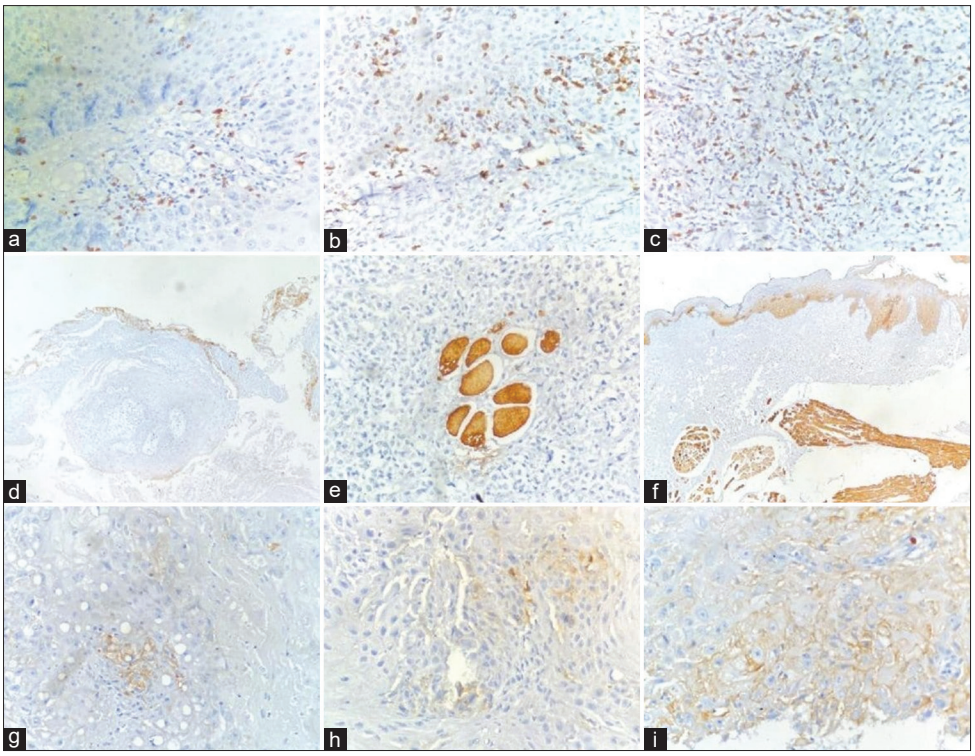

Figure 1: Immunohistochemistry images of: (a) CD-8 Grade I (average number 1–19 cells) (×400), (b) CD-8 Grade II (average number 20–49 cells) (×400), (c) CD-8 Grade III (average number 50 cells) (×400), (d) HPV Grade 1 (Focally stained cells [i.e., limited staining of some cells restricted to the upper quarter of the epithelium]) (×100), (e) HPV Grade 1 (focally stained cells) (×400), (f) HPV Grade 2 (Extensively stained cells [i.e., widespread staining in the upper one- third of the epithelium and/or below]) (×100), (g) PD-L1 CPS <1 (×400), (h) PD-L1 CPS ≥1 (×400), (i) PD-L1 CPS ≥20 (×400). PD-L1: Programmed death-ligand 1

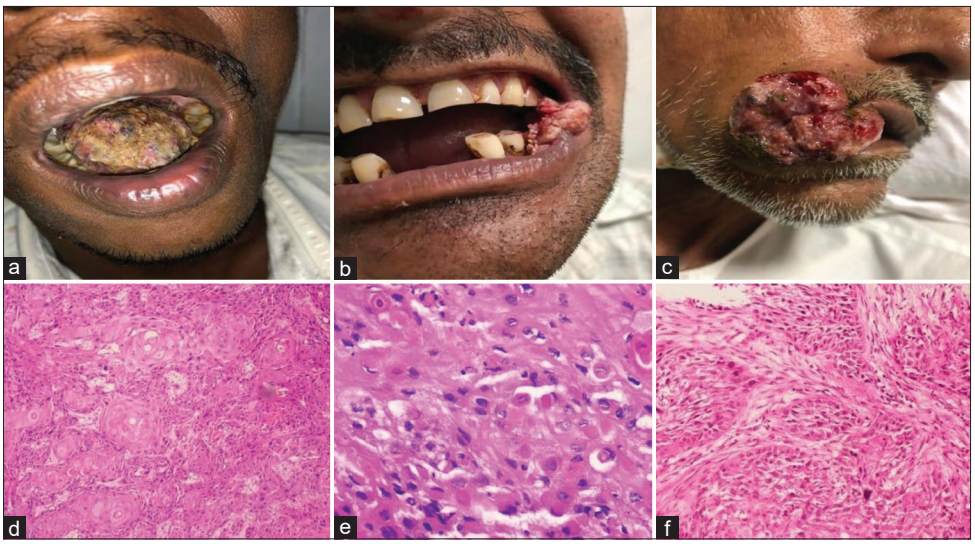

Figure 2: Gross image of (a) an ulcer proliferative growth involving whole tongue. (b) exophytic growth involving buccal mucosa and lower lip. (c) exophytic growth involving buccal mucosa and upper lip. Haematoxylin and eosin-stained slides of (d) Well differentiated squamous cell carcinoma showing keratin pearls and intracytoplasmic keratinization (×100) (H&E). (e) Moderately Differentiated Squamous cell carcinoma showing intracytoplasmic keratinization and nuclear pleomorphism (×400) (H&E). (f) Poorly differentiated squamous cell carcinoma showing clusters of highly pleomorphic tumour cells with hyperchromatic nucleus. (×400) (H&E)

HPV expression. A statistically significant direct association was observed between abnormal sexual habits and CD8 (P = 0.018), HPV (P = 0.020), and PD-L1 (P = 0.040) [Table 1].

A history of multiple sexual partners was present in 16 patients; all of them had positive CD8 and HPV expression. PD-L1 expression in these cases was such that 9 (56.25%) cases had CPS <1, 5 (31.25%) cases.

Table 1: Correlation of CD-8, HPV and PD-L1 grading with various clinical factors and risk factors

Clinical factors and risk factors	CD-8 expression				Results (*n*=100)	HPV expression			Results (*n*=100)	PD-L1 expression			Results (*n*=100)
	Grade 0 (*n*=6)	Grade I (*n*=18)	Grade II (*n*=33)	Grade III (*n*=43)		Grade 0 (*n*=10)	Grade 1 (*n*=22)	Grade 2 (*n*=68)		CPS<1 (PD-L1 expression) (*n*=27)	CPS≥1 (PD-L1 expression) (*n*=36)	CPS>20 (PD-L1 expression) (*n*=37)	
Age													
<50 years	1	7	25	23	X^2=30.200	5	14	37	X^2=8.059	24	21	11	X^2=30.951
>50 years	5	11	8	20	*P*=0.036*	5	8	31	*P*=0.781	3	15	26	*P*=0.002*
Gender													
Male	3	15	30	41	X^2=11.805	8	19	62	X^2=1.313	25	30	34	X^2=1.853
Female	3	3	3	2	*P*=0.008*	2	3	6	*P*=0.519	2	6	3	*P*=0.396
Site													
Oral cavity	3	16	26	32		10	21	46		21	27	29	X^2=6.671
Oropharynx	2	1	1	5	X^2=15.232	0	0	9	X^2=10.862	2	2	5	
Hypopharynx	1	0	0	2		0	0	3	*P*=0.093	0	1	2	*P*=0.352
Larynx	0	0	0	0	*P*=0.085	0	0	0		0	0	0	
Face and neck	0	1	6	4		0	1	10		4	6	1	
Paan													
Chewing	6	15	29	38	X^2=1.195	8	19	61	X^2=0.849	24	31	33	X^2=0.191
Present	0	3	4	5		2	3	7	*P*=0.654	3	5	4	
Absent					*P*=0.754								*P*=0.909
Smoking													
Present	2	6	26	31	X^2=14.287	4	14	47	X^2=3.272	21	21	23	X^2=2.772
Absent	4	12	7	12	*P*=0.003*	6	8	21	*P*=0.195	6	15	14	*P*=0.250
Alcohol abuse													
Present	0	2	16	16	X^2=10.576	2	8	24	X^2=0.979	16	13	5	X^2=14.668
Absent	6	16	17	27	*P*=0.014*	8	14	44	*P*=0.613	11	23	32	*P*=0.001*
Abnormal													
Sexual													X^2=6.461
Habits	0	2	12	5	X^2=10.119	0	1	18	X^2=7.798	9	7	3	
Present absent	6	16	21	38	*P*=0.018*	10	21	50	*P*=0.020*	18	29	34	*P*=0.040*
Multiple sexual partners													
Present	0	1	11	4	X^2=11.416	0	2	14	X^2=3.751	9	5	2	X^2=9.245
Absent	6	17	22	39	*P*=0.010*	10	20	54	*P*=0.153	18	31	35	*P*=0.010*

(* signifies statistical significance), PD-L1: Programmed death-ligand 1

CPS ≥1, and 2 (12.50%) cases of CPS >20. Eighty-four patients had a negative history of abnormal sexual habits; 78 (92.85%) cases out of them showed positive CD8 expression, while 74 (88.09%) had positive HPV expression. A statistically significant direct association was observed between the history of multiple sexual partners and CD8 ($P = 0.010$) and PD-L1 ($P = 0.010$).

Expression of these markers was correlated with each other such that a statistically significant correlation was observed between PDL1 expression and CD8 expression ($P = 0.002$) [Table 2]. No significant correlation was observed in CD8 expression between HPV-positive and HPV-negative cases ($P = 0.260$) [Table 3]. No significant correlation was observed between PD-L1 expression and HPV expression ($P = 0.774$).

DISCUSSION

The age and gender distribution of the present study and prior studies indicate that the incidence of head-and-neck malignancies is higher in older age groups and males. This can be accredited to the practice of paan chewing, tobacco smoking, or alcohol consumption, which are more prevalent among males in our part of the world. These play an imperative role in the etiopathogenesis of HNSCC. The age and gender distribution in our study were in concordance with the research conducted by Klussmann *et al.*[16], Rischin *et al.*[17] The majority of the cases incorporated in the present research were paan chewers (88%), smokers (65%), non-alcoholics (66%), and those without multiple sexual partners (84%). Paan chewing and alcohol consumption were found to have preponderance among males. Similar results were seen in a study conducted by Alam *et al.*[18] The history of multiple sexual partners was seen in 16 (16%) cases, and that of abnormal sexual habits was seen in 19 (19%) cases. Bulks of them were male. Similar to our study, Heck *et al.*[1] also found that the majority of male cases had a history of multiple sexual partners. In our research, only a handful of subjects gave sexual history, plausible explanations, and societal and cultural stigma when discussing sexual practices and deviancies in this region of the nation.

In our study, the maximum number of cases belonged to grade I (55%). Grade II and grade III tumors constituted 40% and 5% of cases, respectively. The distribution of histologic grade was erratic in various studies. Our study corresponded to the study by Chen *et al.*[19] which also found the maximum cases (50%) during their study showing Grade I/Well-differentiated SCC. Grade III tumors were less frequently observed in our study when compared to the other studies.[20-22] That observed moderately differentiated carcinoma/grade II forming the most common group. A statistically significant association was seen between CD8 expression and age ($P = 0.036$) as well as gender ($P = 0.008$) in our study, which was in concordance with other studies like Nguyen *et al.*[23] and Balermpas *et al.*[24]

The 100 cases of head-and-neck squamous cell cancers analyzed for expression of CD8 showed a statistically significant association between CD8 expression and smoking ($P = 0.003$), alcohol abuse ($P = 0.014$), abnormal sexual habits ($P = 0.018$), and the history of multiple sexual partners ($P = 0.010$). No statistically significant association was found between CD8 expression and paan chewing ($P = 0.754$). Ward *et al.*[25] and Balermpas *et al.*[24], like our study, found a statistically significant association between CD8 expression and smoking ($P < 0.001$). Furthermore, Fang *et al.*[26] found a statistically significant association of CD8 expression with alcohol consumption ($P < 0.05$).

A statistically significant association was seen between HPV expression and abnormal sexual habits ($P = 0.020$), which is in concordance with the study conducted by Herrero *et al.*[27]. Likewise, Ajila *et al.*[28] studied a positive relationship between HPV DNA and abnormal sexual behavior such as a greater number of sexual partners, oral-genital gender, and oral-anal gender. Similar to our study, Herrero *et al.*[27] found that HPV is detected less frequently among current smokers and paan chewers as compared to non-smokers

Table 2: Correlation of PD-L1 expression with HPV and CD8 expression

HPV expression	PD-L1 expression			Results (*n*=100)
	CPS <1 (PD- L1 expression) (*n*=27)	CPS ≥1 (PD-L1 expression) (*n*=36)	CPS >20 (PD-L1 expression) (*n*=37)	
Grade 0	4	4	2	
Grade 1	6	7	9	X^2=1.790
Grade 2	17	25	26	*P*=0.774
CD-8 expression				
Grade 0	3	2	1	
Grade I	4	10	4	X^2=20.789
Grade II	15	11	7	*P*=0.002*
Grade III	5	13	25	

* signifies statistical significance, CPS: Combined positive score, PD-L1: Programmed death-ligand 1

Table 3: Correlation of CD-8 expression in HPV postive and HPV negative cases

CD-8 expression	HPV expression		Total (*n*=100)	Results
	Positive (*n*=90)	Negative (*n*=10)		
Grade 0	5	1	6	
Grade I	15	3	18	X^2=7.707
Grade II	30	3	33	*P*=0.260
Grade III	40	3	43	

* signifies statistical significance

and non-chewers. A statistically significant association was seen between PD-L1 expression and age ($P = 0.002$), and no statistically significant correlation was seen with gender ($P = 0.396$) in our study, which was in concordance with other studies like Müller *et al.*[5] However, Kouketsu *et al.*[29] showed no statistical significance in PD-L1 expression with age but a statistically significant correlation with gender, which is in discordance with our study.

In our study, no significant association was seen between PD-L1 and tumor site ($P = 0.352$), which was in concordance with the study by Kouketsu *et al.*[29] Whereas the observations of Müller *et al.*[5] were discordant with the present study, which observed that a significant association was seen between PD-L1 and tumor site. A statistically significant correlation was observed between PD-L1 expression and CD8 expression ($P = 0.002$). In concordance with our study, Kogashiwa *et al.*[30] also found a statistically significant correlation between PD-L1 expression and CD8 expression ($P <0.001$).

CONCLUSION AND FUTURE IMPLICATIONS

The presence of HPV DNA, together with the expression of HPV E6 and/or E7 RNA has often been deemed the "golden standard" to assess if a tumor is caused by HPV or not. Findings like the high incidence of high-risk HPV offer support for a vaccination program for this risk group. Informed education of patients and their close contacts (like sexual partners and family members) will also require knowledge of HPV status. In our study, we verified a positive correlation between HPV and abnormal sexual habits. PD-L1 expression is a potential predictive biomarker of the response to anti-PD-L1/anti-PD-1 therapy in multiple cancers, including HNSCC. Superior survival and cancer endurance were linked with a greater CPS, such that immunotherapy was drastically effectual as a first-line treatment in metastatic and non-resectable cases of HNSCC if the CPS was 20 or greater. A prognostic benefit of high CD8+TIL has been described, but the majority of reports illustrate no significant association of high CD8+TIL with OS or DSS. These differing observations can be elucidated by the relative amounts of CD8+TILs to other TILs affecting their function. A significant association was found between PD-L1, HPV, CD8, and various clinicopathological parameters. Thus, the association of these markers with other well-established prognostic markers needs to be evaluated for any inconsistent outcome. Hence, the study of PD-L1, HPV, and CD8 expression may provide clinicians with more exact information to appraise tumor aggressiveness and treatment modalities.

ACKNOWLEDGMENT

I would like to express my sincere gratitude to all the respected faculty members and colleagues who contributed to the successful completion of this study. Their dedication, expertise, and commitment were instrumental in the realization of our study objectives. I am thankful for their valuable insights, collaborative spirit, and unwavering support throughout the project. I am grateful to the technician staff for their constant support; without their invaluable contributions, this study would not have been possible.

REFERENCES

1. Heck JE, Berthiller J, Vaccarella S, Winn DM, Smith EM, Shan'gina O, *et al.* Sexual behaviours and the risk of head and neck cancers: A pooled analysis in the International Head and Neck Cancer Epidemiology (INHANCE) consortium. Int J Epidemiol 2010;39:166-81.
2. El-Mofty SK. Human papillomavirus related carcinomas of the upper aerodigestivetract. Head Neck Pathol 2007;1:181-5.
3. Murthy V, Calcuttawala A, Chadha K, d'Cruz A, Krishnamurthy A, Mallick I, *et al.* Human papillomavirus in head and neck cancer in India: Current status and consensus recommendations. South Asian J Cancer 2017;6:93-8.
4. Schulz D, Stancev I, Sorrentino A, Menevse AN, Beckhove P, Brockhoff G, *et al.* Increased PD-L1 expression in radioresistant HNSCC cell lines after irradiation affects cell proliferation due to inactivation of GSK-3beta. Oncotarget 2019;10:573-83.
5. Müller T, Braun M, Dietrich D, Aktekin S, Höft S, Kristiansen G, *et al.* PD-L1: A novel prognostic biomarker in head and neck squamous cell carcinoma. Oncotarget 2017;8:52889-900.
6. Fregonesi PA, Teresa DB, Duarte RA, Neto CB, de Oliveira MR, Soares CP. p16(INK4A) immunohistochemical overexpression in premalignant and malignant oral lesions infected with human papillomavirus. J Histochem Cytochem 2003;51:1291-7.
7. Srivastava S. P16INK4A and MIB-1: An immunohistochemical expression in preneoplasia and neoplasia of the cervix. Indian J Pathol Microbiol 2010;53:518-24.
8. Zou W, Chen L. Inhibitory B7-family molecules in the tumour microenvironment. Nat Rev Immunol 2008;8:467-77.
9. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, *et al.* PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 2014;515:568-71.
10. Wang X, Teng F, Kong L, Yu J. PD-L1 expression in human cancers and its association with clinical outcomes. Onco Targets Ther 2016;9:5023-39.
11. Oliva M, Spreafico A, Taberna M, Alemany L, Coburn B, Mesia R, *et al.* Immune biomarkers of response to immune-checkpoint inhibitors in head and neck squamous cell carcinoma. Ann Oncol 2019;30:57-67.
12. Barnes L, Eveson J, Reichart P, Sidransky D. World Health Organization

Classification of Tumours: Pathology and Genetics of Head and Neck Tumours. Vol. 11. France. Lyon: IARC Press; 2005. p. 1-15.
13. Dako. PD-L1 IHC 22C3 pharmDx, Agilent: PD-L1 IHC 22C3 pharmDx Interpretation Manual. MSCLC. Vol. 8. Denmark: Dako; 2020. p. 17-21.
14. Mardanpour K, Rahbar M, Mardanpour S, Mardanpour N, Rezaei M. CD8+ T-cell lymphocytes infiltration predict clinical outcomes in Wilms' tumor. Tumour Biol 2020;42:1010428320975976.
15. van der Zee RP, Meijer CJ, Cuming T, Kreuter A, van de Sandt MM, Quint WG, *et al.* Characterisation of anal intraepithelial neoplasia and anal cancer in HIV-positive men by immunohistochemical markers p16, Ki-67, HPV-E4 and DNA methylation markers. Int J Cancer 2021;149:1833-44.
16. Klussmann JP, Gültekin E, Weissenborn SJ, Wieland U, Dries V, Dienes HP, *et al.* Expression of p16 protein identifies a distinct entity of tonsillar carcinomas associated with human papillomavirus. Am J Pathol 2003;162:747-53.
17. Rischin D, Young RJ, Fisher R, Fox SB, Le QT, Peters LJ, *et al.* Prognostic significance of p16INK4A and human papillomavirus in patients with oropharyngeal cancer treated on TROG 02.02 phase III trial. J Clin Oncol 2010;28:4142-8.
18. Alam MS, Siddiqui SA, Perween R. Epidemiological profile of head and neck cancer patients in Western Uttar Pradesh and analysis of distributions of risk factors in relation to site of tumor. J Cancer Res Ther 2017;13:430-5.
19. Chen ZW, Weinreb I, Kamel-Reid S, Perez-Ordoñez B. Equivocal p16 immunostaining in squamous cell carcinoma of the head and neck: Staining patterns are suggestive of HPV status. Head Neck Pathol 2012;6:422-9.
20. Solomon MC, Carnelio S, Gudattu V. Molecular analysis of oral squamous cell carcinoma: A tissue microarray study. Indian J Cancer 2010;47:166-72.
21. Marcus B, Arenberg D, Lee J, Kleer C, Chepeha DB, Schmalbach CE, *et al.* Prognostic factors in oral cavity and oropharyngeal squamous cell carcinoma. Cancer 2004;101:2779-87.
22. Pena JC, Thompson CB, Recant W, Vokes EE, Rudin CM. Bcl-xL and Bcl-2 expression in squamous cell carcinoma of the head and neck. Cancer 1999;85:164-70.
23. Nguyen N, Bellile E, Thomas D, McHugh J, Rozek L, Virani S, *et al.* Tumor infiltrating lymphocytes and survival in patients with head and neck squamous cell carcinoma. Head Neck 2016;38:1074-84.
24. Balermpas P, Rödel F, Rödel C, Krause M, Linge A, Lohaus F, *et al.* CD 8+ tumour-infiltrating lymphocytes in relation to HPV status and clinical outcome in patients with head and neck cancer after postoperative chemoradiotherapy: A multicentre study of the German cancer consortium radiation oncology group (DKTK-ROG). Int J Cancer 2016;138:171-81.
25. Ward MJ, Thirdborough SM, Mellows T, Riley C, Harris S, Suchak K, *et al.* Tumour-infiltrating lymphocytes predict for outcome in HPV-positive oropharyngeal cancer. Br J Cancer 2014;110:489-500.
26. Fang J, Li X, Ma D, Liu X, Chen Y, Wang Y, *et al.* Prognostic significance of tumor infiltrating immune cells in oral squamous cell carcinoma. BMC Cancer 2017;17:375.
27. Herrero R, Castellsagué X, Pawlita M, Lissowska J, Kee F, Balaram P, *et al.* Human papillomavirus and oral cancer: The International Agency for Research on Cancer multicenter study. J Natl Cancer Inst 2003;95:1772-83.
28. Ajila V, Shetty H, Babu S, Shetty V, Hegde S. Human papilloma virus associated squamous cell carcinoma of the head and neck. J Sex Transm Dis 2015;2015:791024.
29. Kouketsu A, Sato I, Oikawa M, Shimizu Y, Saito H, Takahashi T, *et al.* Expression of immunoregulatory molecules PD-L1 and PD-1 in oral cancer and precancerous lesions: A cohort study of Japanese patients. J Craniomaxillofac Surg 2019;47:33-40.
30. Kogashiwa Y, Yasuda M, Sakurai H, Nakahira M, Sano Y, Gonda K, *et al.* PD-L1 Expression confers better prognosis in locally advanced oral squamous cell carcinoma. Anticancer Res 2017;37:1417-24.

How to cite this article: Kapoor A, Pandey P, Dixit A, Agarwal S, Tewari U, Mittal K, Pratap J, Akhtar S Human Papilloma Virus, Programmed Death Ligand-1, and Tumor Microenvironment: Implications for the Patients with Head-and-Neck Squamous Cell Carcinoma. Int J Sci Stud 2024;11(11):22-29.

Source of Support: Nil, **Conflicts of Interest:** None declared.