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Evaluation of the Presence and Importance of PD-L1 Expression in Head-neck Squamosus Cell Carcinomas and Premalign Lesions

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Abstract

Background: Programmed Death-Ligand 1 (PD-L1) is a cell membrane protein found on the surface of cancer cells, immune system cells in the tumor microenvironment, and various healthy tissues. Moreover, it plays a key role in suppressing the immune system.
Objectives: We aim to explain the presence of PD-L1 in Head-Neck Squamosus Cell Carcinomas (HNSCC) and premalign lesions by immunohistochemical method.
Methods: Our retrospective study included 22 patients with HNSCC (15(68.1%) Oral cavity and 7(31.9%) Oropharynx), 20 patients with oral lichen planus, and 14 patients with normal oral cavity mucosa. In the evaluation of PD-L1 antibodies applied immunohistochemically in patients with HNSCC, the percentage of tumor cells showing membranous staining with PD-L1 antibodies was calculated.
Results: The mean age of HNSCC patients participating in the study was 52.24 ± 11.7 years, the mean age of oral lichen planus patients was 34.10 ± 9.8 years, and the control patients' mean age was 31.42 ± 10.6 years. The rate of PD-L1 staining of tumor cells of HNSCC patients was significantly higher than the control group (P=0.001).
Conclusion: The importance of PD-L1 expression in HNSCC and precancerous lesions of the oral cavity is remarkable, and the values may be related to the pathophysiology of these diseases.

Keywords: Carcinoma, Head and neck cancer, Oral lichen planus, PD-L1, Precancerous lesions

1. Background

Squamous cell carcinoma may arise from different head and neck region localizations. These include the oral and nasal cavity, upper aerodigestive tract, pharynx, and larynx. It is known that smoking and alcohol use play a role in the etiology of these tumors. In addition, some oropharyngeal tumors develop from a history of Human PapillomaVirus (HPV) infection (1-2). While stage 1 and 2 tumors are treated with surgical resection or radiotherapy, the prognosis for stage 3 and 4 tumors is poor despite multimodal combined surgery, radiotherapy, and systemic treatments.

Programmed Death-Ligand 1 (PD-L1) is a cell membrane receptor and it is a member of the immunoglobulin superfamily located on the surface of antigen-presenting cells and T cells. Except for the tonsil crypt epithelium, it is not found in normal epithelial cells (3). In the literature, it has been shown that PD-L1 gene polymorphism is associated with autoimmune diseases as systemic erythematosus and rheumatoid arthritis (4-5), it has been shown that PD-L1 gene polymorphism is associated with autoimmune diseases. One of the ways that cancer cells escape from the immune system is to have PD-L1 densely on the cell surface. This cell membrane receptor is found in many different tumors. Pancreatic, renal cell, lung carcinoma, and lymphoma are cancers in which PD-L1 positivity is detected. Because PD-L1 is a factor that stops cancer-fighting immune cells, PD-L1 positivity is associated with a poor prognosis (6-7).

The purpose of immune checkpoint inhibitors, which are new-generation cancer immunotherapies, is to activate the immune system against cancer by preventing the interactions of molecules such as PD-L1 and PD-1. This can be achieved with drugs in the structure of monoclonal antibodies that will prevent the work of molecules that inactivate the immune system. In animal studies (8), PD-L1 blockade has produced an antitumor immune response. However, healthy cells may also be partially affected by this increased immune response (9).

2. Objectives

Therefore, new drugs are producing promising results in treatment (10-11). Immune checkpoint inhibitors such as pembrolizumab and nivolumab are used in managing PD-L1 antibody-positive tumors (12). These drugs are especially preferred in treating recurrent and metastatic tumors.

In the related literature, although PD-L1 expression has been examined in various tumors, few studies compared benign, premalignant and malignant lesions in the head and neck region (3, 11-12). In addition, this study aims to use immune checkpoint inhibitor therapies in premalignant and malignant lesions in the correct patient selection and prevent conversion to malignancy by increasing their effective use in premalignant lesions.

The presence of PD-L1 in various tumors has been reported in the literature; however, only few studies invetigated the presence of PD-L1 in malignant and premalignant lesions in the head and neck region (3,11-12). Therefore, the present study aimed to show the presence and importance of PD-L1 in squamous cell cancers and precancerous lesions in the head and neck region.

3. Methods

3.1. Study Design

This retrospective study was conducted with the tissues of a total of 57 patients diagnosed with head and neck region squamous cell carcinomas (22 sample), oral lichen planus (OLP) as precancerous lesion (20 sample), and 15 normal mucosal epithelium between 2010-2020 at Alanya Alaaddin Keykubat University Medical Faculty Hospital.

3.2. Inclusion and exclusion criteria

Head-Neck Squamosus cell carcinomas (HNSCC) patients who did not receive radiotherapy, chemotherapy, or immunotherapy treatment before surgery were included in the study. The HNSCC patients with autoimmune disease were not included in the study.

3.3. Ethics Committee Approval

Ethics committee approval was obtained for the study from Alanya Alaaddin Keykubat University Ethics Committee (Date: 28/04/2021 no:2021/08-04). Since our study was retrospective, patient consent was not required.

3.4. Immunohistochemical Staining

The preparations containing the highest number of tumors were used for the study when selecting tumor blocks. There is usually a single block in benign and premalignant cases, and the entire tissue was included in the study. To apply immunohistochemical PD-L1 antibodies, sections were taken from patients' paraffin blocks. In our study, we used SP263 clones, which are stated to be more stained in the literature. Ventana PD-L1 (SP 263) monoclonal antibodies were applied to these sections by fully automated Ventana Ultra Benchmark XT instrument.

3.5. Evaluation of Immunohistochemical Staining

To evaluate the results, 100 cells were counted from the region of tumor density under the light microscope. Among these cells, cells showing membranous staining with PD-L1 antibodies were counted, and the percentage was calculated. An accurate assessment of the role of PD-L1 in malignant tissues requires the exclusion of PD-L1 inflammatory cells in scoring. Therefore, PD-L1 expression of immune cells was also counted and scored separately. İn patients with oral lichen planus and normal oral cavity mucosa, PD-L1 positive lymphocytes, and macrophages were counted.

3.6. Statistical Analysis

The SPSS (version 22, SPSS Inc, Chicago, IL, USA) was used for all statistical analyses. The assumption of normality was evaluated with the Shapiro-Wilk test. The chi-square and Kruskal-Wills tests were applied to compare gender, age, and PD-L1 staining rate among groups. Receiver operating characteristic (ROC) and area under the curve (AUC) analyses were used for Cut-off values for PD-L1 staining. A $P < 0.05$ was considered statistically significant.

4. Results

The mean age of HNSCC patients participating in the study was 52.24 ± 11.7 years, the mean age of oral lichen planus patients was 34.10 ± 9.8 years, and the control patients' mean age was 31.42 ± 10.6 years (Table 1). The rate of PD-L1 staining of tumor cells of HNSCC patients was significantly higher than the control group [P<0.001, Figure 1a]. Although the rate of PD-L1 staining in epithelial cells of oral lichen planus patients was higher than in the control group, it was not statistically significant [P=0.223, Figure 1b]. The PD-L1 staining of lymphocyte was statistically significantly higher in the SCC and oral lichen planus (OLP) group than in the control group [P=0.001, Figures 2a, 2b, 3, and 4]. In the ROC analysis, the cut-off value for tumoral PD-L1 staining in the SCC group was ≥ 5 (AUC:720 P=0.004) and ≥ 3 (AUC:0.832 P<0.001) for lymphocyte. In Spearman's correlation test, no negative or positive correlation was found between tumor cell PD-L1 satination and lymphocyte PD-L1 staining.

Table 1. Comparison of age, gender, and distribution and PD-L1 staining percentages among groups

		HNSCC group (n=22)	Oral lichen planus (n=20)	Control group (n=15)	*P*-value
Gender	Male	17 (77%)	8 (40%)	10 (67%)	0.458*
	Female	5 (23%)	12 (60%)	5 (33%)	
Age, year		52.24±11.7	34.10± 9.8	31.42 ± 10.6	0.254**
PD-L1-positive tumors %		82	65	33	<0.001**
PD-L1-positive T lymphocytes%		45	90	7	0.223**

* Chi square test, ** Kruskal-Wallis test
HNSCC: Head-Neck Squamosus cell carcinoma

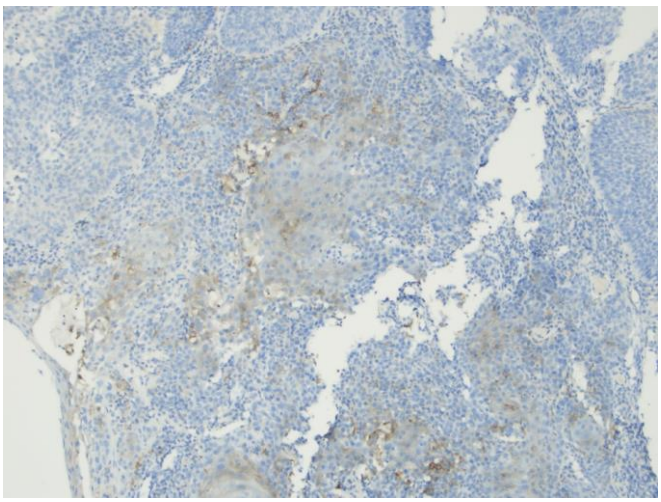

Figure 1a. PD-L1 expression in SCC (10x)

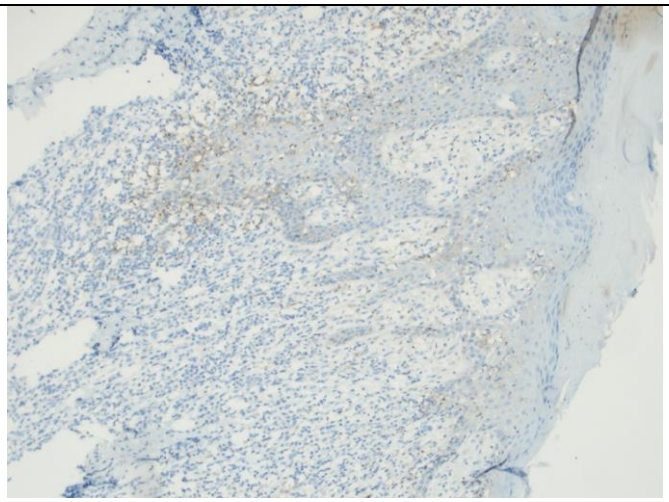

Figure 1b. PD-L1 expression in oral lichen planus (10x)

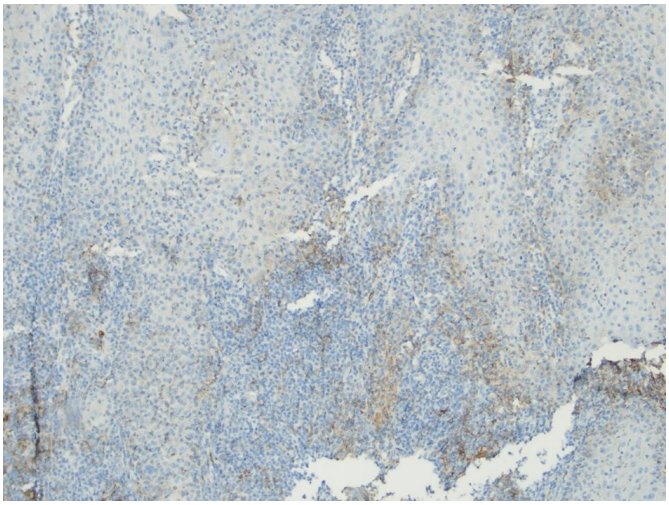

Figure 2a. PD-L1 expression by lymphocytes in the SCC area (10x)

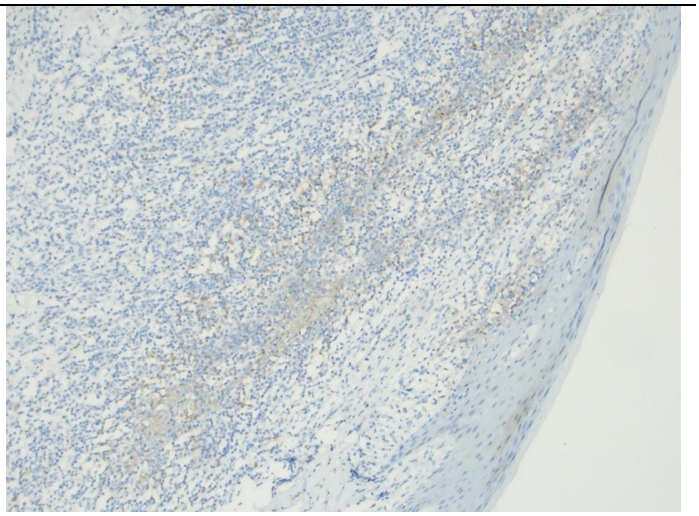

Figure 2b. PD-L1 expression by lymphocyte in oral lichen planus (10x)

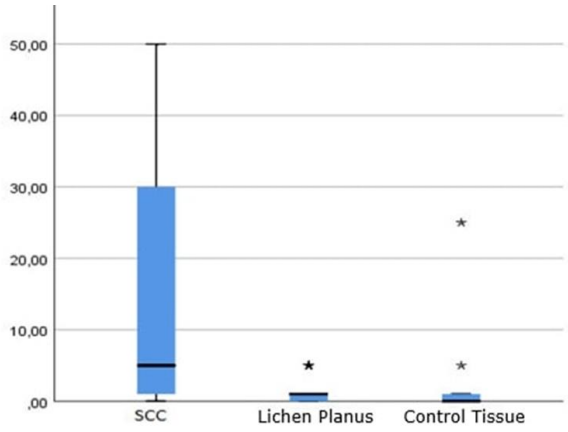

Figure 3. Kruskal-Wallis test for PD-L1 staining of SCC, oral lichen planus, and control Epithelial tissue

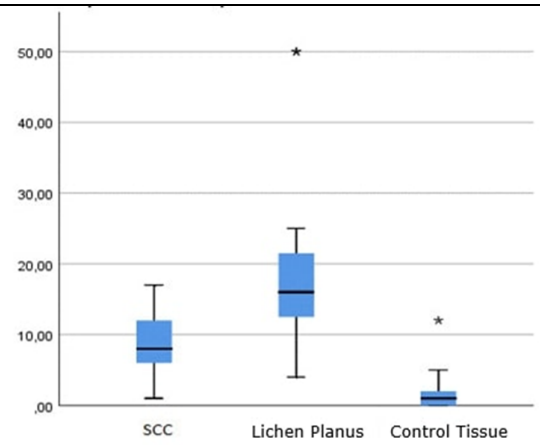

Figure 4. Kruskal-Wallis test for PD-L1 staining of lymphocytes in SCC, oral lichen planus, and control epithelial tissue

5. Discussion

In our study, we showed that PDL-1 excretion is increased in HNSCC. This result supports the view that PD-L1 affects cancer development by stopping cancer-fighting immune cells. In addition, PD-L1 was

found to be increased in precancerous tissues. Therefore, PD-L1 can be used as a potential marker in precancerous lesions.

The PD-1 is a member of the normal immune system and is a predictive biomarker. The PD-L1 is a glycoprotein cell membrane receptor, and it is expressed by antigen-presenting and tumor cells. The presence of PD-L1 is usually determined by immunohistochemical staining (IHC). Different clones and scoring methods may cause difficulties in detecting and interpreting the presence of PD-L1. Various parameters play a role in determining the presence of PD-L1 in tissues in the best way. Different companies have antibodies and subclones that vary depending on the tumor. There are also differences when evaluating the staining pattern in these clones. While only membranous staining is accepted in Dako subclones, both membranous and cytoplasmic staining are considered positive in Ventana subclones (13). Due to these differences, selecting the appropriate dye and clone for the tumor type is necessary. Different cut-off values have also been reported in the literature in evaluating the immunohistochemical expression of PD-L1 (14). Various cut-off values as low as 1% or as high as > 20% have been used in various studies to score PD-L1 expression in HNSCC (3, 15-17). The most common cut-off value was reported as 5% in previous studies. Variable cut-off levels may result in different results in the interobserver evaluation. Some studies suggest that the intensity and extent of staining is sufficient to show the presence of staining. But it is important that the determining of the cut-off value for standart and more objective results. (18). In our study, the cut-off value in tumor and premalignant epithelial cells was 5%, the most reported in the literature. It is seen that there are many technical differences in the evaluation of PD-L1 in the literature. However, the technical equipment and dyes used in immunohistochemical staining are standard, and the interpretation of the results according to certain cut-off values will make the obtained data more valid.

When the relationship between tumor-infiltrating lymphocytes and tumoral cell PD-L1 is examined, there are discordant study results. Cho et al. (19) stated that tumor cell PD-L1 and lymphocyte PD-L1 were inversely related. İn another study, a positive correlation was observed between tumor cell PD-L1 and lymphocyte PD-L1 (20). Furthermore, a positive correlation was observed between the number of tumor cells and premalignant PD-L1 staining and the rate of PD-L1 staining in the same group of lymphocytes. The HNSCC has been defined as one of the highly lymphocyte infiltrating cancer types. The PD-L1 positivity of peritumoral T cells is an important factor in prognosis. In one study, researchers reported that the presence of peritumoral T cells PD-L1 is a major factor in predicting survival rate (21). In our study, we separately evaluated the PD-L1 expression of tumoral and precancerous epithelial cells and T lymphocytes in scoring. In our study, PD-L1 expression in lymphocyte differed in all three groups. In the present study, the rate of PD-L1 staining in lymphocyte in premalignant lesions and the SCC group was significantly higher than in the control tissue and was statistically significant. Qiao et al. (22) did not detect PD-L1 expression in oral SCC cells; however, they observed intense staining in stromal cells around cancer tissue. In the related literature, researchers believe that lymphocyte PD-L1 expression might be associated with prognosis. Taube et al. (23) examined the potential relationship between lymphocyte PD-L1 expression and clinical outcomes and found a borderline relationship. In addition, they associated a higher lymphocyte cell count in cancer tissue after treatment compared to pre-treatment in different tumor cells with a good prognosis (24, 25). Therefore, it has been reported that PD-L1 expression in lymphocyte can be used to evaluate the clinical response in anti-PD-L1-immune therapy. Since our study was cross-sectional and retrospective, we could not comment on the relationship between our lymphocyte PD-L1 expression results and clinical response.

Actinic cheilitis, oral lichen planus, and squamous dysplasia are premalignant lesions of the head and neck region. The prognostic value of PD-L1 in premalignant and malignant lesions has been demonstrated in various studies. Chena et al. (26) found more PD-L1 expression in oral lichen planus cases compared to normal tissue. Saraggi et al. (27) indicated that PD-L1 expression was higher in malignant and dysplasic lesions in gastrointestinal system. Our study also found that PD-L1 expression was higher in precancerous lesions than in control tissue; however, no statistical correlation was found. It is assumed that PD-L1 expression was induced by inflammatory cytokines released during an active immune response. It is founded in the genetic studies that the PD-L1 gene polymorphism is associated with autoimmune diseases as rheumatoid arthritis and systemic erythematosus (28, 29). Excessive increase in PD-L1 production in lymphocytes in precancerous lesions may disrupt the immune balance with a process similar to that in autoimmune diseases. This may explain the formation of precancerous lesions.

The PD-L1 expression was shown in 18-87% of squamous cell carcinomas observed in the head and neck region (13, 16, 17). Qiao et al. (22) reported that PD-L1 expression was significantly higher in advanced head and neck SCC compared to other sites SCC. In our study, the rate of PD-L1 staining in the SCC group was 55%. Although PD-L1 positivity is generally associated with a poor prognosis, it is a good prognostic indicator in oral cavity tumors. Due to the retrospective nature of our study, we could not

evaluate the prognosis of the patients. In addition, PD-L1 can be used as a biomarker to predict response to anti-PD-1/PD-L1 blocking therapy. There are different pharmacological agents used as PD-L1 immune checkpoint inhibitors. In the literature, the reults of two randomized trials involving 240 patients treated with nivolumab (anti-PDL) and 301 patients treated with pembrolizumab (anti-PDL) compared to standard therapy showed that the PD-L1 immune checkpoint inhibitors significantly prolonges the survival chance in patients with recurrent or metastatic HNSCC (30). With the determination of the presence and importance of PD-L1 in HNSCC in studies, immune checkpoint inhibitor drugs were started to be used in the treatment.

One of the limitations of our study was that our hospital was not a large center; therefore, a limited number of patients could be used in the study. In addition, the effect of PDL-1 positivity on clinical prognosis could not be investigated since our study was retrospective and patients were referred to other centers in the postoperative period.

6. Conclusion

Standard methods should be defined for the correct determination of PD-L1 expression in the tissue. In our study, the importance of PD-L1 expression in HNSCC and precancerous lesions of the oral cavity draws attention. A significant increase in PDL-1 in HNSCC in our study suggests that target drugs may play a role in the treatment of these lesions showing PD-L1 expression. Further studies with larger sample size concering this issue are required to be conducted. We hope that the results of our study will contribute to the related literature.

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None.

Footnotes

Conflicts of Interest: The authors declared no conflict of interest.

Ethical Approval: Ethical approval was obtained from the Institutional Review Board of Alanya Alaaddin Keykubat University.

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