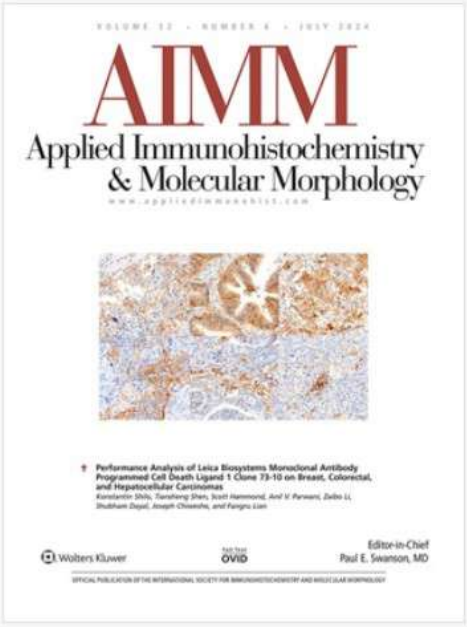


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PD-L1 and PD-L2 Expression in Different Tumor Stages and Types of Malignant Salivary Gland Neoplasms: A Single-center Experience

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Abstract: There is a limited amount of data on the role of programmed cell death ligand (PD-L) -1 and PD-L2 in salivary gland carcinomas. We aimed to evaluate the prognostic value of PD-L1 and PD-L2 expressions, which are closely related to immune mechanisms, with respect to salivary gland tumor types and stages. Data from patients with salivary gland masses surgically removed between 2006 and 2021, diagnosed with a malignant salivary gland neoplasm, were retrospectively analyzed. Immunoreactivity for PD-L1 and PD-L2 was performed on resection materials. The mean age of 90 patients was 52.1 ± 18.8 and 46.7% were male. Overall, 55.6% of patients were diagnosed with adenoid cystic carcinoma (ACC), 23.3% with mucoepidermoid carcinoma (MEC), 16.7% with acinic cell carcinoma (AciCC), 3.3% with ductal carcinoma (DC), and 1 patient with pleomorphic adenoma ex carcinoma (PA-ex-CA). In all, 52% of ACC, 12% of AciCC, 24% of MEC, and 12% of DC cases were at stage IV. The tumor diameter, frequencies of lymphovascular invasion, metastasis, positive surgical margin, recurrence, and mortality rates of patients at stages III and IV were significantly larger than those at stages I and II ($P < 0.05$). The percentages of tumor cell score (TCS) and immune cell score (ICS) for PD-L1 were significantly higher among patients with MEC compared with those with other types of tumors ($P = 0.0011$). However, the percentages of combined score (CS) for PD-L1 and tumor cell score for PD-L2 were comparable among tumor types ($P > 0.05$). No significant difference was found in these scores for PD-L1 between tumor stages ($P > 0.05$), but for PD-L2, all patients at stage I had TCS $< 1\%$ for PD-L2, while all patients at stages II and III, and 92% of patients at stage IV had TCS $\geq 1\%$ ($P < 0.0001$). High expression of PD-L1 was mostly observed in MEC cases ($P = 0.0016$), while all patients with AciCC had a low PD-L1 expression level ($P = 0.0206$). The mean tumor diameter, rate of lymphovascular invasion, perineural invasion, metastasis, positive surgical margin, recurrence, type of treatment, mortality, and TILs ratio did not differ significantly according to PD-L1 expression level ($P > 0.05$). The percentage of tumor-infiltrating lymphocytes was comparable among negative and positive PD-L1 scores according to both 1% and 5% threshold values ($P > 0.05$). High PD-L1 expression is rare in AciCC, while PD-L1 expression is high in MEC. Our findings underline the importance of future screening for PD-L1 and PD-L2 before patients undergoing immunotherapies in all salivary gland tumors.

Key Words: immune checkpoints, immunotherapies, malign salivary gland carcinoma, PD-L1, PD-L2

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Salivary gland carcinomas are rare neoplasms, accounting for ~0.5% of all malignancies and 5% of all head and neck cancers. Regardless of the histologic subtype, curative treatment consists of surgical resection with or without postoperative adjuvant radiotherapy, but other adjuvant treatment options, including chemoradiation, have no detectable effect on survival rates among highly aggressive subtypes such as salivary duct carcinomas. In addition, there is no standard treatment with confirmed efficacy for patients with unresectable primaries, relapses, or distant metastases.[1,2]

The immune system is one of the main defenses in the antitumor response; however, tumor cells stem from the patient's own cells, enabling the tumor to maintain several innate autoimmune defense mechanisms that can prevent its self-destruction by the immune system.[3,4] Programmed cell death ligand (PD-L) is a glycoprotein expressed in inflammatory cells and tumor cell membranes, aiming to evade the lethal effects of inflammatory cells. When cells expressing PD-L encounter cytotoxic T lymphocytes, they escape from the cytotoxic effects of these cells by using this protein. Targeted immune therapy using PD-1/PD-L1 inhibitors (eg, pembrolizumab, atezolizumab) and PD-L2 can

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The study protocol was approved by the Ethical Committee of Noninvasive Clinical Research of Kocaeli University (Date: 2022/11/10, Decision No: KUGOKAEK-2022/18.42 Project No: 2022/314).
B.Y.B., I.C., A.H.C., E.B.T., B.C.O., Y.K.A., H.M.D., C.V., and M.O. collected the data. B.Y.B. and E.B.T. drafted the manuscript. B.Y.B. edited the manuscript, participated in the study design, and coordination. All authors read and approved the final manuscript.
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disrupt these pathways and increase the antitumor activity of the immune system.[4–6]

There is a limited amount of data on the role of PD-L1 and PD-L2 in salivary gland carcinomas. However, the efficacy of chemotherapeutic agents is quite limited in these tumors, making it advantageous to identify factors predicting prognosis depending on patient-specific treatment.[1–6]

In this retrospective study, we aimed to investigate tumor types, tumor stages, and the expression of PD-L1 and PD-L2 in patients with salivary gland masses detected, resected, and diagnosed as malignant salivary gland tumors, as well as the effects of variations in these expression levels on the clinical and prognosis of these patients.

MATERIALS AND METHODS

Study Group and Data Collection

The present retrospective cross-sectional study included the patients with a salivary gland mass surgically removed between 2006 and 2021 December and diagnosed with a malignant salivary gland neoplasia in our pathology laboratory. The data was collected from a hospital management information system. Data including the pathology reports, medical records, age, sex, clinical findings, and radiologic findings of 90 cases were reviewed retrospectively. Age, sex, clinical history, pathologic diagnoses, tumor type, tumor stages, macroscopic and microscopic findings, treatment management, recurrence, and mortality rates were determined in all patients.

The slides of all these cases were re-examined, and the rates of lymphocyte infiltrating the tumor were recorded. PD-L1 and PD-L2 immunoreactivity was applied to the paraffin blocks of the slides representing the tumor and tumor periphery. Expressions of these immune markers were evaluated.

Histopathologic Analysis

The diagnosis of lesions, tumor diameter, lymphovascular and perineural invasion, LN metastasis, positive surgical margin was determined in selected cases. In addition, tumor areas and tumor-infiltrating lymphocytes (TILs) rates were determined as 0% to 100% according

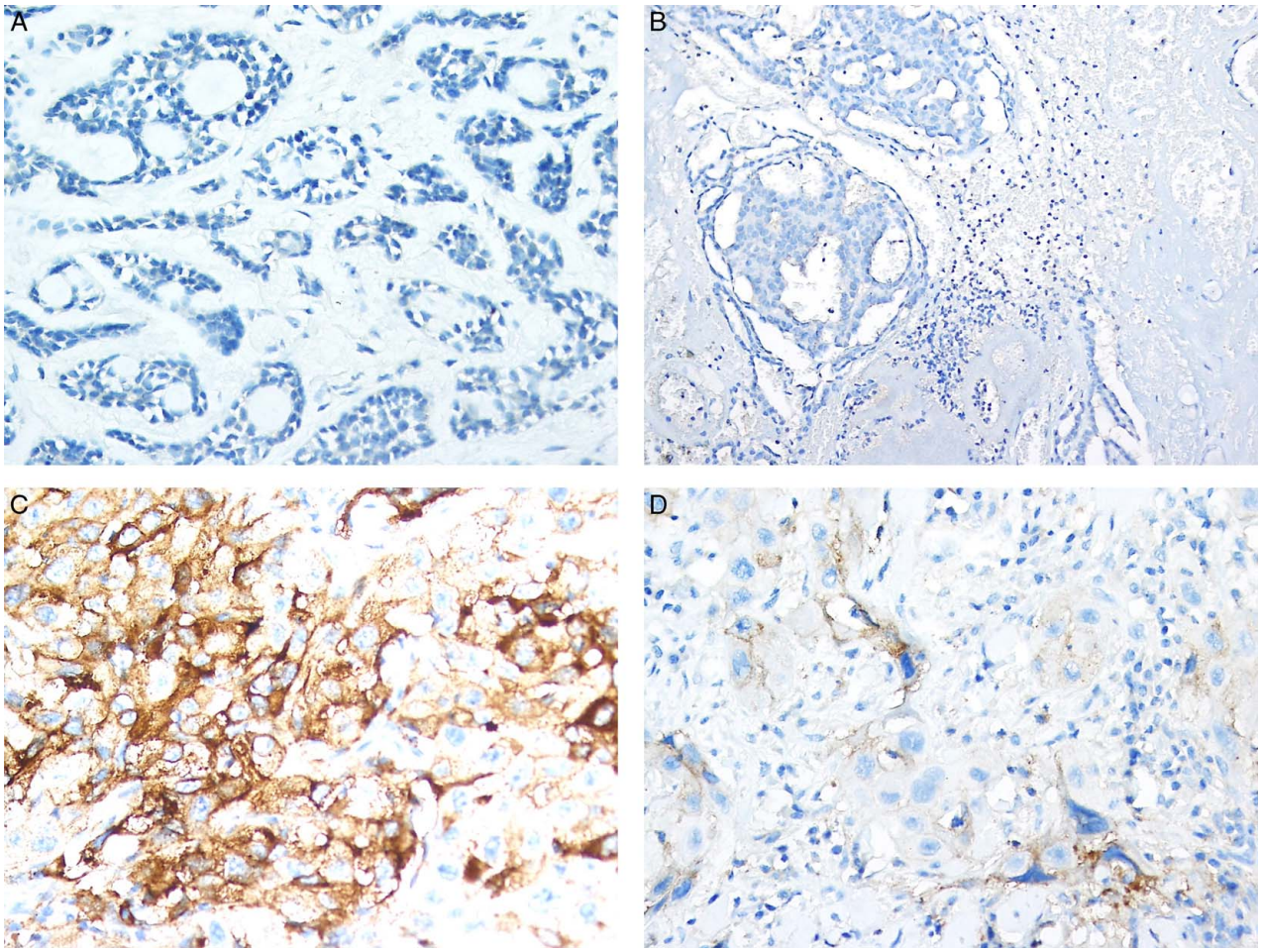


FIGURE 1. A and B, Negative reaction with PD-L1 and PD-L2 in malignant salivary gland neoplasia (x100). C and D, Positive reaction with PD-L1 and PD-L2 in malignant salivary gland neoplasia (x200).

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to the literature.[1,7,8] To determine TILs rate, the inflammatory cells infiltrating the tumor and in the narrow stroma adjacent to the tumor in infiltrative tumors were evaluated. Inflammatory cells located in the lamina propria, not adjacent to the tumor, were not evaluated. After determining the ratio of TILs, they were grouped according to their percentages (group 1: $\leq 5\%$, group 2: 10%, and group 3: $\geq 20\%$).

Immunohistochemical Analysis

PD-L1 (Cell signaling; E1L3N) and PD-L2 (Cell signaling; D7U8C) antibodies on 4 μm thick sections from paraffin blocks containing salivary gland resection materials was performed. Staining was conducted on a Ventana automatic device (Roche Ventana), using Ventana Optiview and Ultraview DAB kits. Tonsil tissue served as a positive external control for both markers.

Scoring System

Samples were evaluated by 2 pathologists under an OLYMPUS BX50 model double-headed binocular microscope. PD-L1 and PD-L2 tumor cell scoring (TCS) were determined by dividing the number of positively stained tumor cells at ×100 magnification by the total number of tumor cells. Similarly, the percentage of staining in TILs was determined and recorded as immune cell scoring (ICS) (Figs. 1, 2). In addition, the percentage of the total number of cells stained in this area (TCS and TILs) compared with the total tumor cell was recorded as the combined score (CS).

TCS for PD-L1 were grouped as follows: group 0: $<1\%$, group 1: 1% to 4%, group 2: 5% to 49%, group 3: $\geq 50\%$. ICS for PD-L1 were also grouped as follows: group 0: $<1\%$, group 1: 1% to 4%, group 2: 5% to 9%, and group 3: $\geq 10\%$. Combined score was determined as group 0: $<1\%$, group 1: 1% to 9%, group 2: 10% to 49%, and group 3: $\geq 50\%$.[1,7,8] The tumor score and immune score determined for PD-L2 were grouped according to the percentage of staining as follows: group 0: $<\%1$, group 1: 1% to 19%, group 2: 20% to 49%, and group 3: $\geq 50\%$.[9,10]

Statistical Analysis

Statistical analysis was performed using the Graph Pad Instat program. Descriptive statistics were determined for continuous variables (mean, SD, median, minimum, and maximum values) and categorical variables (%). Normality of variable distribution and variance homogeneity were tested using the Kolmogorov-Smirnov test. Parametric analysis of variance tests (Student t test) or nonparametric tests (Mann-Whitney U test) were employed based on test assumptions. Multiple groups were compared using the Kruskal-Wallis test (nonparametric analysis of variance) followed by Dunn multiple comparisons test. Categorical variables were compared using the χ^2 test for Independence. A significance level of 95% ($P<0.05$, <0.01, and <0.001) was considered statistically significant.

RESULTS

The mean age of 90 patients with malign tumor in salivary gland was 52.1 ± 18.8 years. Overall, 5.5% of patients was at stage I of salivary glands tumor, 48.9% was at stage II, 17.8% was at stage III, and 27.8% was at stage IV. As depicted in Table 1, 73.3% of patients were under age of 65 years which was also notable in groups of stages without significant difference ($P=0.411$). The distribution of sex did not differ among groups of stages ($P=0.818$). The most frequent type of tumor was adenoid cystic carcinoma (ACC) (55.6%) and mucoepidermoid carcinoma (MEC) (23.3%). The tumor diameter of patients at stage III and IV were significantly larger than those at stage I ($P<0.0001$). Two of patients at stage III and 4 of patients at stage IV had lymphovascular invasion ($P=0.0487$). The frequency of LN and distant metastasis among patients at stage IV was significantly higher than those at lower stages ($P<0.0001$). Since invasion of the surrounding tissues was wider in high-stage tumors, the frequency of positive surgical margin was higher than those in tumors at lower stages ($P=0.0438$). Therefore, the frequency of chemotherapy and radiotherapy application to this group was significantly highest ($P=0.0006$). The recurrence rate (48%) and mortality rate (28%) were significantly highest in stage IV tumor compared with other stage groups ($P=0.0026$ and 0.0013, respectively). The frequency of patients with TILs ratio $\geq 20\%$ was significantly highest in all patients and stage groups, compared with the patients with TILs ratio $<20\%$ ($P=0.447$).

Table 2 presented the comparison of patients' data according to the tumor type. 56.2% of patients was diagnosed as ACC, 23.6% as MEC, 16.7% as acinic cell carcinoma (AciCC), 3.4% as ductal carcinoma (DC), 1 patient as pleomorphic adenoma ex carcinoma (PA-ex-CA) who was excluded from the analysis given in Table 2. The percentages of TCS, ICS, and CS for PD-L1 $<1\%$ were higher than those $\geq 1\%$ for all patients and types of tumors. The percentages of TCS and ICS for PD-L1 were significantly higher among patients with MEC compared with those with other types of tumors ($P=0.0011$). However, the percentage of CS for PD-L1 was comparable among tumor types ($P>0.05$). The percentage of TCS for PD-L2 between 1% and 19% were higher than those $<1\%$ and $\geq 20\%$ for all patients and types of tumors but no significant difference was found in TCS for PD-L2 between tumor types ($P=0.66$).

Table 3 presented the comparison of patients' data according to the tumor stage. In all, 5.6% of patients was at stage I of malign salivary gland tumor, 49.4% at stage II, 16.9% at stage III, and 28.1% at stage IV. The percentages of TCS, ICS, and CS for PD-L1 $<1\%$ were higher than those $\geq 1\%$ for all stages of tumors but no significant difference was found in these scores for PD-L1 between tumor stages ($P>0.05$). All of 5 patients at stage I had TCS $<1\%$ for PD-L2, while all patients at stage II and III and 92% of patients at stage IV had TCS $\geq 1\%$ for PD-L2 ($P<0.0001$).

Demographic, clinical, and histopathologic data of the patients was also compared according to PD-L1 expression level given in Table 4. The majority of tumor cells

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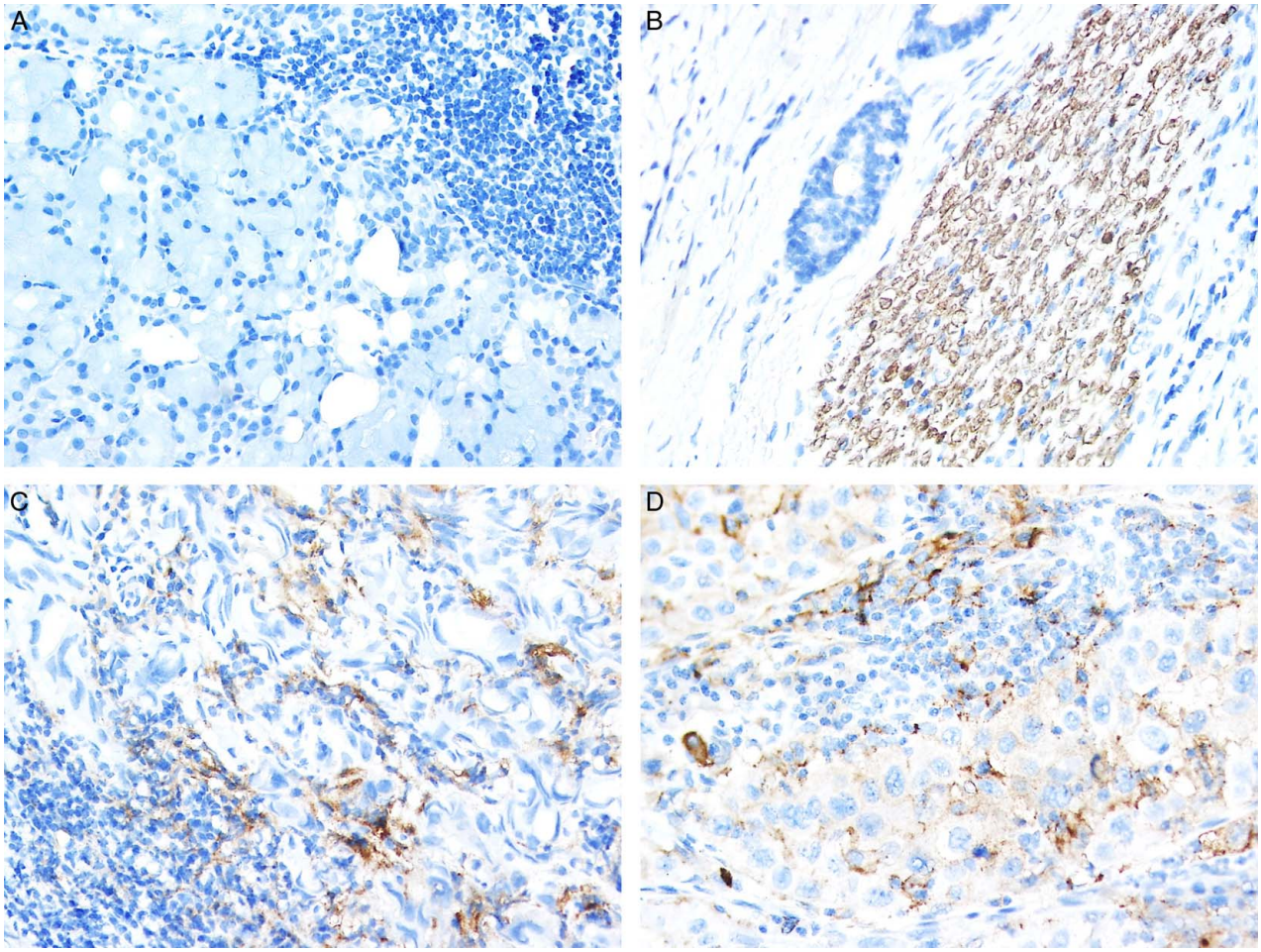


FIGURE 2. A, Negative reaction with PD-L in both tumor and tumor-infiltrating lymphocytes. B, Positive reaction with PD-L in tumor-infiltrating lymphocytes. C and D, Positive reaction with PD-L in both tumor and tumor-infiltrating lymphocytes. full color online

(72.2%) showed low PD-L1 expression, and 27.8% had high PD-L1 expression. The distribution of age and sex did not differ according to the PD-L1 expression level. Depending on the type of tumor, the high expression level of PD-L1 was mostly observed in patients with MEC compared with those with low expression level ($P = 0.0016$). All patients with AciCC had low PD-L1 expression level ($P = 0.0206$). The mean of tumor diameter, rate of lymphovascular invasion, perineural invasion, LN and distant metastasis, positive surgical margin, recurrence, type of treatment, mortality, and the TILs ratio did not differ significantly according to the PD-L1 expression level ($P > 0.05$).

When the TILs ratio was analyzed according to the 1% threshold value of the PD-L1 tumor score in all patients (Table 5), this ratio was found to be between 10-100% in the majority of patients with a negative and positive PD-L1 tumor score (81.5% vs. 92.0%, respectively) but the difference was not statistically significant ($P = 0.367$). TILs ratio was found to be between 10% and 100% in the majority of patients with a negative and positive PD-L1 immune score (84.6% vs. 84.0%, respectively) but the difference was not significant ($P = 0.943$). When the TILs ratio was analyzed according to the 5% threshold value of the PD-L1 tumor score in all patients (Table 5), this ratio was found to be between 10% and 100% in the majority of patients with a negative and positive PD-L1 tumor score (83.9% vs. 88.9%, respectively) but the difference was not statistically significant ($P = 0.698$). TILs ratio was found to be between 10% and 100% in the majority of patients with a negative and positive PD-L1 immune score (84.6% vs. 83.3%, respectively) but the difference was not significant ($P = 0.909$).

DISCUSSION

The present study investigated PD-L1 and PD-L2 expressions in different tumor stages and types of malignant salivary gland neoplasms, with data collected retrospectively from a single-center experience. In comparisons according to the tumor stage, it was observed that demographic data were homogeneously distributed, but the majority of the histopathologic data, morbidity, and mortality rates worsened as the stage increased. According

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TABLE 1. Demographic, Clinical, and Histopathologic Data of the patients, and Comparison of These Data According to the Tumor Stage

	n (%)					
Parameters	**Total (N = 90)**	**Stage I (n = 5)**	**Stage II (n = 44)**	**Stage III (n = 16)**	**Stage IV (n = 25)**	***P***
Age (y)						
< 65	66 (73.3)	4 (80.0)	32 (72.7)	14 (87.5)	16 (64.0)	0.411
≥ 65	24 (26.7)	1 (20.0)	12 (27.3)	2 (12.5)	9 (36.0)	
Sex						
Male	42(46.7)	2 (40.0)	21 (47.7)	6 (37.5)	13 (52.0)	0.818
Female	48 (53.3)	3 (60.0)	23 (52.3)	10 (62.5)	12 (48.0)	
Type of tumor						
ACC	50 (55.6)	2 (40.0)	28 (63.6)	7 (43.8)	13 (52.0)	0.443
AciCC	15 (16.7)	2 (40.0)	8 (18.2)	2 (12.5)	3 (12.0)	0.453
MEC	21 (23.3)	1 (20.0)	8 (18.2)	6 (37.5)	6 (24.0)	0.478
DC	3 (3.3)	0 (0.0)	0 (0.0)	0 (0.0)	3 (12.0)	**0.0446**
PA-ex-CA	1 (1.1)	0 (0.0)	0 (0.0)	1 (6.2)	0 (0.0)	0.197
Tumor diameter (mean ± SD) (cm)	3.2 (± 1.8)	1.5 (± 0.5)	2.2 (± 0.5)	4.5 (± 1.8)*	4.4 (± 2.2)*	< 0.0001
Lymphovascular invasion	6 (6.7)	0 (0.0)	0 (0.0)	2 (12.5)	4 (16.0)	0.0487
Perineural invasion	25 (27.8)	2 (40.0)	11 (25.0)	4 (25.5)	8 (32.0)	0.843
LN metastasis	15 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	15 (60.0)	**< 0.0001**
Distant metastasis	18 (20.0)	0 (0.0)	4 (9.1)	0 (0.0)	14 (56.0)	**< 0.0001**
Positive surgical margin	23 (25.6)	1 (20.0)	6 (13.6)	5 (31.3)	11 (44.0)	**0.0438**
Recurrence	21 (23.3)	2 (40.0)	6 (13.6)	1 (6.2)	12 (48.0)	**0.0026**
Treatment						
Chemotherapy	13(14.4)	0 (0.0)	3 (6.8)	2 (12.5)	8 (32.0)	**0.0006**
Radiotherapy	21 (23.3)	0 (0.0)	7 (15.9)	3 (18.8)	11 (44.0)	
Mortality	8 (8.9)	0 (0.0)	1 (2.3)	0 (0.0)	7 (28.0)	**0.0013**
TILs ratio						
Group 1: ≤ 5%	13 (14.4)	0 (0.0)	8 (18.2)	2 (12.5)	3 (12.0)	0.447
Group 2: 10%	11 (12.3)	1 (20.0)	8 (18.2)	1 (6.2)	1 (4.0)	
Group 3: ≥ 20%	66 (73.3)	4 (40.0)	28 (63.6)	13 (81.3)	21 (84.0)	

Bold values are statistical significant $P < 0.001$.

ACC indicates adenoid cystic carcinoma; AciCC, acinic cell carcinoma; DC, ductal carcinoma; LN, lymph node; MEC, mucoepidermoid carcinoma; PA-ex-CA, pleomorphic adenoma ex carcinoma; TIL, tumor-infiltrating lymphocytes.

*$P < 0.001$ versus stage I [Kruskal-Wallis test (nonparametric analysis of variance) and Dunn multiple comparisons test].

TABLE 2. Comparison of Patients' Immunohistochemical Data According to the Tumor Type

	n (%)					
Parameters	**Total (N = 89)***	**ACC (n = 50)**	**AciCC (n = 15)**	**MEC (n = 21)**	**DC (n = 3)**	***P***
PD-L1						
TCS						
Group 0: < 1%	65 (73.1)	41 (82.0)	14 (93.3)	7 (33.4)	3 (100.0)	**0.0011**
Group 1: 1%-4%	6 (6.7)	3 (6.0)	1 (6.7)	2 (9.5)	0 (0.0)	
Group 2: 5%-49%	16 (18.0)	6 (12.0)	0 (0.0)	10 (47.6)	0 (0.0)	
Group 3: ≥ 50%	2 (2.2)	0 (0.0)	0 (0.0)	2 (9.5)	0 (0.0)	
ICS						
Group 0: < 1%	65 (73.1)	38 (76.0)	15 (100.0)	9 (42.9)	3 (100.0)	**0.003**
Group 1: 1%-4%	4 (4.5)	4 (8.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Group 2: 5%-9%	10 (11.2)	3 (6.0)	0 (0.0)	7 (33.4)	0 (0.0)	
Group 3: ≥ 10%	10 (11.2)	5 (10.0)	0 (0.0)	5 (23.7)	0 (0.0)	
CS (%)						
Group 0: < 1%	62 (69.7)	36 (72.0)	14 (93.3)	9 (42.9)	3 (100.0)	0.056
Group 1: 1%-9%	18 (20.2)	10 (20.0)	1 (6.7)	7 (33.4)	0 (0.0)	
Group 2: 10%-49%	7 (7.9)	4 (8.0)	0 (0.0)	3 (14.2)	0 (0.0)	
Group 3: ≥ 50%	2 (2.2)	0 (0.0)	0 (0.0)	2 (9.5)	0 (0.0)	
PD-L2						
TCS (%)						
Group 0: < 1%	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.660
Group 1: 1%-19%	87 (97.8)	48 (96.0)	15 (100.0)	21 (100.0)	3 (100.0)	
Group 2: 20%-49%	2 (2.2)	2 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Group 3: ≥ 50%	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	

Bold values are statistical significant $P < 0.001$.

*Since there is only 1 pleomorphic adenoma ex carcinoma, it was not included in this table.

ACC indicates adenoid cystic carcinoma; AciCC, acinic cell carcinoma; CS, combined score; DC, ductal carcinoma; ICS, immune cell scoring; MEC, mucoepidermoid carcinoma; TCS, tumor cell scoring.

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TABLE 3. Comparison of Patients' Immunohistochemical Data According to the Tumor Stage

	n (%)				
Parameters*	**Stage I (N = 5)**	**Stage II (n44)**	**Stage III (n = 15)**	**Stage IV (n = 25)**	***P***
PD-L1					
TCS (%)					
Group 0: < 1%	4 (80.0)	35 (79.5)	11 (73.3)	15 (60.0)	0.714
Group 1: 1%-4%	0 (0.0)	3 (6.8)	0 (0.0)	3 (12.0)	
Group 2: 5%-49%	1 (20.0)	5 (11.4)	4 (2.7)	6 (24.0)	
Group 3: ≥ 50%	0 (0.0)	1 (2.3)	0 (0.0)	1 (4.0)	
ICS (%)					
Group 0: < 1%	4 (80.0)	34 (77.2)	11 (73.3)	16 (64.0)	0.528
Group 1: 1%-4%	0 (0.0)	1 (2.3)	0 (0.0)	3 (12.0)	
Group 2: 5%-9%	1 (20.0)	4 (9.1)	1 (6.7)	4 (16.0)	
Group 3: ≥ 10%	0 (0.0)	5 (11.4)	3 (20.0)	2 (8.0)	
CS (%)					
Group 0: < 1%	4 (80.0)	33 (75.0)	11 (72.3)	14 (56.0)	0.325
Group 1: 1%-9%	1 (20.0)	7 (15.9)	1 (6.7)	9 (36.0)	
Group 2: 10%-49%	0 (0.0)	3 (6.8)	3 (20.0)	1 (4.0)	
Group 3: ≥ 50%	0 (0.0)	1 (2.3)	0 (0.0)	1 (4.0)	
PD-L2					
TCS (%)					
Group 0: < 1%	5 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	**< 0.0001**
Group 1: 1%-19%	0 (0.0)	44 (100.0)	15 (100.0)	23 (92.0)	
Group 2: 20%-49%	0 (0.0)	0 (0.0)	0 (0.0)	2 (8.0)	
Group 3: ≥ 50%	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	

Bold values are statistical significant $P < 0.001$.
*Since there is only 1 pleomorphic adenoma ex carcinoma, it is not included in this table.
CS indicates combined score; ICS, immune cell scoring; TCS, tumor cell scoring.
χ^2 test for independence of the variables.

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to the type of malignant salivary gland tumor, the frequency of PD-L1 positivity was significantly higher in tumor and immune cells of patients diagnosed with MEC, compared with other tumor types. There was no significant relationship between PD-L1 positivity and higher stage in tumor and immune cells, but PD-L2 positivity in tumor cells was associated with stage. A high PD-L1 expression level was found especially in patients with MEC, while a low PD-L1 expression level was detected in all patients with AciCC. In malignant salivary gland tumors, regardless of tumor type, there was no statistical significance in the distribution of TILs, PD-L1 tumor, and immune scores according to 1% and 5% threshold values in all patients.

There are promising results from checkpoint inhibitor therapy including PD-L1 and PD-L2 in malignant salivary gland tumors.[1,2,4] It has been reported that blocking the interactions between PD-1 and PD-L1 can inhibit antitumor immunity, enhance T-cell-mediated immune function, and promote the antitumor activity of therapeutic agents in preclinical models and in vitro.[5] The expression of PD-L1 and PD-L2 has been reported in tumor cells of both malignant and benign tumors but PD-L1 expression was found to be significantly higher in malignant tumor cells than benign tumor cells.[11,12] However, there is limited data on PD-L1 and PD-L2 scoring in these tumors, and no objective scoring criteria has, so far, been evaluated. While most previous studies focused on the prognostic value of PD-L1 expression, the aim of the present study was to compared PD-L1 and PD-L2 expressions in tumor and immune cells according to the type of malign tumors in salivary glands and stage of tumor.

PD-L1 are present in normal tissues, and suggested to participate in immunosurveillance, immunoregulation, and homeostasis.[13–15] The conditions of cancer microenvironment including inflammation, especially those processes with high IFN-γ expression, activates the expression of PD-L1 in tumor tissues.[13] In a survey conducted in October 2016 at the Service of the US National Institute of Health[16] identified ~60 clinical trials using PD-L1 inhibitory drugs in patients with head and neck cancers. In one of these tumors, Witte et al[4] found that tumor scores in MEC expressed PD-L1 higher than tumor scores in ACC, in consistent with our findings showing that the highest tumor and immune scores for PD-L1 was found in MEC compared with other types of salivary gland tumors. Increased expression of PD-L1 in MEC was also reported by Mosconi et al,[14] which reflects increased proportions of epidermoid and intermediate cells. We found that 57% (12/21) of MECs had PD-L1 positivity over 1% which was considered as high PD-L1 expression level, while Mukaigawa et al[7] demonstrated that only 17.6% (3/31) of MECs showed positivity for PD-L1. These controversial findings of our study and literature suggest that PD-L1 expression needs more attention especially in MECs.

Immunotherapy as a new therapeutic approach, combined with other conventional therapies, has been proposed as a more effective treatment emphasizing the role of tumor microenvironment.[17] This is probably due to

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TABLE 4. Demographic, Clinical, and Histopathologic Data of the Patients According to PD-L1 Expression Level

	n (%)		
Location	**Low PD-L1 expression (N = 65)**	**High PD-L1 expression (N = 25)**	***P***
Age (y)			
< 65	48 (73.8)	18 (72.0)	0.859
≥ 65	17 (21.2)	7 (28.0)	
Sex			
Male	26 (40.0)	16 (64.0)	0.071
Female	39 (60.0)	9 (36.0)	
Type of tumor			
ACC	38 (58.5)	12 (48.0)	0.511
AciCC	15 (23.1)	0 (0.0)	**0.0206**
MEC	9 (13.8)	12 (48.0)	**0.0016**
DC	3 (4.6)	0 (0.0)	0.662
PA-ex-CA	0 (0.0)	1 (4.0)	0.618
Tumor diameter (mean ± SD) (cm)	3.2 (± 1.9)	3.3 (± 1.4)	0.317
Lymphovascular invasion	4 (6.2)	2 (8.0)	0.753
Perineural invasion	21 (32.3)	4 (16.0)	0.199
LN metastasis	8 (12.3)	7 (28.0)	0.908
Metastasis	14 (21.5)	4 (16.0)	0.769
Positive surgical margin	20 (30.8)	3 (12.0)	0.119
Recurrence	15 (23.1)	6 (24.0)	0.926
Treatment			
Chemotherapy	8 (12.3)	5 (20.0)	0.460
Radiotherapy	17 (21.2)	4 (16.0)	
Mortality	4 (6.2)	4 (16.0)	0.291
TILs ratio			
Group 1: ≤ 5%	10 (15.4)	3 (12.0)	0.651
Group 2: 10%	9 (13.8)	2 (8.0)	
Group 3: ≥ 20%	46 (70.8)	20 (80.0)	

Bold values are statistical significant $P < 0.001$.

ACC indicates adenoid cystic carcinoma; AciCC, acinic cell carcinoma; DC, ductal carcinoma; LN, lymph node; MEC, mucoepidermoid carcinoma; PA-ex-CA, pleomorphic adenoma ex carcinoma; TIL, tumor-infiltrating lymphocyte. χ^2 test and Mann-Whitney test of the variables.

TABLE 5. Distribution of PD-L1 Tumor and Immune Scores With TILs According to 1% and 5% Threshold Value in all Patients

	1% threshold [n (%)]		***P***
Parameter	**PD-L1 TCS negative (N = 65)**	**PD-L1 TCS positive (N = 25)**	0.367
TILs (0%-9%)	12 (18.5)	2 (8.0)	
TILs (10%-100%)	53 (81.5)	23 (92.0)	
Parameter	**PD-L1 ICS negative (N = 65)**	**PD-L1 ICS positive (N = 25)**	
TILs (0%-9%)	10 (15.4)	4 (16.0)	0.943
TILs (10%-100%)	55 (84.6)	21 (84.0)	
	5% threshold [n (%)]		
Parameter	**PD-L1 TCS negative (N = 81)**	**PD-L1 TCS positive (N = 9)**	0.698
TILs (0%-9%)	13 (16.1)	1 (11.1)	
TILs (10%-100%)	68 (83.9)	8 (88.9)	
Parameter	**PD-L1 ICS negative (N = 78)**	**PD-L1 ICS positive (N = 12)**	
TILs (0%-9%)	12 (15.4)	2 (16.7)	0.909
TILs (10%-100%)	66 (84.6)	10 (83.3)	

Bold values are statistical significant $P < 0.001$.

ICS indicates immune cell scoring; TCS, tumor cell scoring; TIL, tumor-infiltrating lymphocyte.

the promising data of publications showing that the blocking of targets such as PD-1 and PD-L1 leads to reduced progression of salivary glands and longer survival rates.[18] There are reports showing that the expression of PD-L1 in tumor and immune cells of ACC tumors, defined by treatment failure heralded by locoregional recurrence and distant metastatic disease, was significantly lower than in other malignant subtypes.[5,8] Also, Mosconi et al[8] indicated that PD-L1 expression in ACC tumors was not detected or at low level, while PD-L2 was strongly expressed. In addition, Gerdabi et al[5] reported that the PD-L1 expression was not at high levels in tumor cells of ACC type of malignant salivary gland tumors as in our patients with ACC whose TCS and ICS for PD-L1 were mostly < 1%. ACC is resistant to immune checkpoint inhibition due to low tumor immunogenicity and lack of PD-L1 expression.[19] In this background, PD-L1 expression does not necessarily correlate with effectiveness in ACC. Future studies are required to explore the extrinsic and intrinsic roles of PD-1/PD-L1 and how to best predict and promote treatment response in ACC.

In contrast to PD-L1, PD-L2 is expressed in 45% to 73% of primary and metastatic ACC cases.[9] Yearley et al[20] investigated PD-L2 expression in human tumors and found that PD-L2 expression is independently associated with clinical response to pembrolizumab (anti-PD-1), often in the absence of PD-L1 expression in a number of cancers. PD-L2 expression was also associated with improved benefit of anti-PD-L1 atezolizumab therapy in melanoma, renal cell carcinoma, urothelial carcinoma and non–small cell lung cancer.[21] In salivary gland tumors, low PD-L2 is associated with shorter relapse-free survival, suggestive of a PD-L2 survival benefit.[10] The common expression of PD-L2 highlights a possible role for PD-L2 inhibitors in the treatment of ACC.[22] A phase I clinical trial of anti-PD-1 antibody BGB-A317 is proposed, competitively inhibiting both PD-L1 and PD-L2 binding in combination with the TetMYB vaccine.[23] Our findings showed that although PD-L2 was expressed over 1% ratio in tumor cells of all types of salivary gland cancers and the expression increased significantly in higher stages, suggesting that PD-L2 may have also contribution to the prognosis of ACC.

CONCLUSIONS

Our findings suggest that high PD-L1 expression is rare in acinic cell carcinoma. It is obvious that high PD-L1 expression has a place in the differential diagnosis of MEC. Our findings underline the importance of future screening for PD-L1 and PD-L2 before patients undergoing immunotherapies in all salivary gland tumors, and their relationship deserves further study in the context of evidence from other cancers. Last, there is a need for new

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and improved therapeutic approaches against locally advanced or metastatic malignant salivary gland tumors to identify entities that might be promising targets for immuno-oncologic treatment and to provide positive treatment outcomes in patients.

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