Original Article

PD-L1 expression in immune cells is a favorable prognostic factor for nasopharyngeal carcinoma

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Abstract

Background: Programmed death-ligand 1 (PD-L1) has been determined as a reliable prognostic factor for various malignancies. In this study, we aimed to determine the prognostic effect of PD-L1 expression in tumor-infiltrating immune cells (TIICs) of nasopharyngeal carcinoma (NPC) patients.

Methods: Seventy patients diagnosed with non-metastatic NPC were included in the study. PD-L1 expression on immune cells was analyzed by immunohistochemical method. Patients were categorized into two groups according to the PD-L1 expression level in TIICs (level of PD-L1 staining ≥5% positive vs <5% negative).

Results: Median follow-up period was 34 months (range = 1 - 188). 1 and 2 years survival rate were found as 75% and 63% in PD-L1 negative TIICs group (47%), and 85% and 83% in PD-L1 positive TIICs group (53%), respectively. PD-L1 positivity in immune cells (ICs) was detected in 53% of the patients. The survival rate was found better in the PD- L1 positive group compared to the negative group ($P = 0.049$).

Discussion: In conclusion, the survival rate was found significantly better in the PD-L1 positive TIICs group, compared to the negative group.

Keywords:
Nasopharyngeal carcinoma, prognosis, programmed death ligand-1

Introduction

Nasopharyngeal cancer (NPC) is an Epstein-Barr virus (EBV)-associated tumor originating from the epithelial surface of the nasopharynx with different etiology and geographic distribution. The annual incidence in endemic areas such as Southern China, Hong Kong, and Taiwan has reached up to 25–50/100000. It is estimated that 50,000 deaths are observed worldwide annually.[1] Nasopharyngeal carcinoma is chemosensitive carcinoma and, the 5-year survival rate is 80% in patients with stage I and II disease, whereas it is less than 10% in stage IV disease.[2]

Although it is sensitive to chemotherapy, resistance may develop; therefore, new treatments are needed.[3,4]

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Significant immune infiltrates are observed in primary tumor tissue of patients with NPC consisting of T-cells, B-cells, dendritic cells, monocytes, and eosinophils.[5-8] Programmed death-1 (PD-1) is an immunosuppressive receptor expressed on T-cells. T-cell activation decreases after binding of the PD1 ligand (PD-L1) to PD-1 causing tumor immune escape.[9,10] PD-L1 is expressed on antigen-presenting cells such as dendritic cells and macrophages and, tumor cells.[11]

The PD-1/PD-L1 pathway plays an important role in the cancer-specific immune response. The presence of tumor-infiltrating lymphocyte (TIL) CD8 is known to be a predictive marker in response to anti-PD-1 therapy in solid tumors.[12,13] There is a positive correlation between positive PD-L1 expression in tumor-infiltrating immune cells and the number of tumor-infiltrating immune cells such as CD4 T-lymphocytes and CD8 T-lymphocytes.[14,15]

Previous studies focused exclusively on PD-L1 expression in tumor cells. However, recent studies revealed that PD-L1 expression in tumor-infiltrating immune cells plays an important role in tumor immune escape.[16-19] Those studies demonstrated that PD-L1 expression in immune cells may be used as a prognostic biomarker and may also provide more information on responses to anti-PD-1/PD-L1 therapy. Mention companion diagnostic of PD-L1 count of tumor-infiltrating immune cells (TIICs) in triple negative breast cancer (TNBC).

In this study, we aimed to determine the relationship between PD-L1 expression in immune cells and prognosis in patients with nasopharyngeal cancer.

Patients and Methods

Seventy nonmetastatic nasopharyngeal carcinoma patients diagnosed via tumor biopsy between January 2008 and December 2016 were included in this study. This is a multi-center study. Formalin-fixed paraffin-embedded tissues were obtained from the first biopsy specimens. Age, gender, tumor stage, tumor histology, and smoking history of the patients were obtained from registered files of patients. This study protocol was approved by the Ethics Committee of Ankara Numune Teaching Hospital.

Immunohistochemical method

Immunohistochemical staining was performed 4 μm-sections obtained from formalin-fixed and paraffin-embedded tissue of nasopharyngeal biopsies or excision specimens of the tumor with the antibody anti-PD-L1 (SP263 Ventana Medical Systems) using the standard protocol for routine diagnostic specimens. The percentage of positive immune cells (ICs) for PD-L1 was evaluated.

Meanwhile, ICs in which the membrane or cytoplasm was immunostained at ≥5% staining intensity were considered positive for PD-L1. The majority of PD-L1 positive ICs were macrophages and lymphocytes—(Natural killer cells, cancer-associated fibroblasts,vascular endothelial cells). All immune cells were evaluated. Immune cells were quantified by evaluating the ratio of the area covered by stained ICs to the tumor area (IC scores), as described in previous reports.[20,21] The necrotic areas were excluded from the scoring area. Negative reagent controls were evaluated in each case by confirming the acceptable level of background staining. The immunohistochemistry (IHC) assay was considered positive if ≥5% of immune cells were stained [Figure 1].

Statistical analysis

The relationship between PD-L1 expression in immune cells and clinicopathological characteristics was evaluated by Pearson's X^2 test. The association of demographic, clinical and histopathological parameters with a prognosis of nasopharyngeal carcinoma were evaluated. Survival curves were depicted using the Kaplan-Meier method and compared using the log-rank test. Multivariate Cox regression analysis was done with factors that may affect survival. Overall survival (OS) of patients following treatment was calculated by considering all death events. All analyses were performed with SPSS 20 version program (SPSS Inc., Chicago, IL). *P* value <0.05 was considered statistically significant.

Results

Seventy patients with nonmetastatic NPC were included in the study. The median was 52 years (18-76 years). 14 patients were female, and 56 patients were male. The median follow-up period was 34 months (1–188 months). During follow-up, 31.4% (*n* = 22) of the patients died. The 2-year survival rate was 80%. Median OS was not reached. 1 and 2-year survival rates were 75% and 63% in PD-L1 negative ICs group; 85% and 83% in PD-L1 positive ICs group, respectively. Programmed death ligand-1 (PD-L1) positivity in immune cells was detected in 53% (*n* = 37) of the patients.

The relationship between PD-L1 expression in immune cells and clinicopathological characteristics (age, sex, smoking history, histology, treatment, and TNM stage) was evaluated in patients with nasopharyngeal cancer. No significant relationship was found between the level of PD-L1 in immune cells and clinicopathological characteristics [Table 1].

The relationship between clinicopathological variables and survival was analyzed with log-rank test. No significant relationship was found between survival and age (<50 years vs ≥50 years; *P* = 0.332),

Key Message

PD-L1 expression in immune cells can be used as a good prognostic marker in patients with nasopharyngeal cancer.

Table 1: Associations between PD-L1 expression and the clinicopathological characteristics of nasopharyngeal carcinoma

Feature	Number of patients	Immune cells (negative)	Immune cells (positive)	*P*
Gender				
Female	14	6 (18)	8 (22)	0.719
Male	56	27 (82)	29 (78)	
Age				
<50	27	11 (33)	16 (43)	0.395
≥50	43	22 (67)	21 (57)	
Histology				
Differentiated	27	13 (39)	14 (38)	0.894
Undifferentiated	43	20 (61)	23 (62)	
N classification				
N_{0-1}	26	11 (39)	15 (43)	0.775
N_{1-2}	27	17 (61)	20 (57)	
T classification				
T_{1-2-3}	32	20 (45,5)	24 (54,5)	0.973
T_4	29	9 (45)	11 (55)	
Stage				
1 and 2	15	8 (53)	7 (47)	0.574
3 and 4	51	23 (45)	28 (55)	
Smoking Status				
Yes	45	21 (64)	24 (65)	0.915
No	25	12 (36)	13 (35)	
Definitive therapy				
RT	7	2 (6)	5 (14)	0.299
CRT	63	31 (94)	32 (86)	

CRT: Chemoradiotherapy; RT: Radiotherapy; N classification: Node stage; T classification: Tumor stage

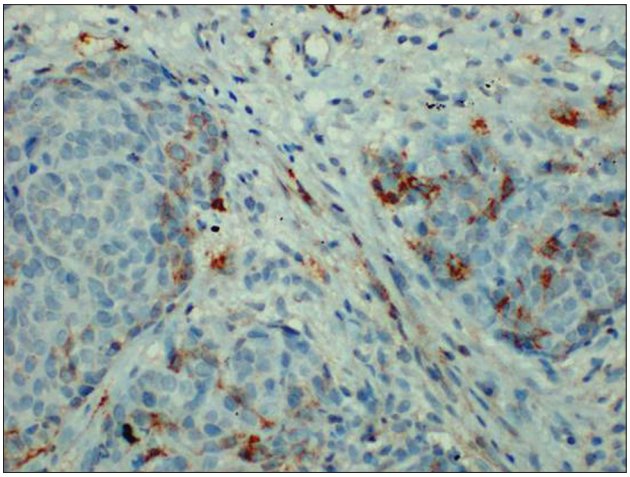

Figure 1: High level of programmed death-ligand 1 (PD-L1) expression in a biopsy of a nasopharyngeal carcinoma patient. PD-L1 expression in tumor-infiltrating lymphocytes is also present. (IHC, ×200)

gender (male vs female; *P* = 0.260), smoking status (smoking vs. no smoking; *P* = 0.762), N stage (N0/1 vs N2/3; *P* = 0.564), pathology (differentiated vs undifferentiated; *P* = 0.256), stage (1/2 vs 3/4; *P* = 0,206), and type of therapy (radiotherapy vs. chemoradiotherapy; *P* = 0.702). A significant relationship was found between survival rate and T-stage (T 1/2/3 vs 4; *P* = 0.009) [Table 2].

Results of Kaplan-Meier analysis and log-rank test demonstrated that survival was significantly better in PD-L1 positive immune cell group compared to the PD-L1 negative group (*P* = 0.049) [Table 2 and Figure 2].

T stage (Hazard ratio (HR) = 0.250, 95% confidence interval (CI): 0.084–0.748; *P* = 0.013) and PD-L1 positivity of immune cells (HR = 0.328, 95%CI: 0.122–0.881; *P* = 0.027) were found as independent factors affecting prognosis by performing multivariate Cox regression analysis [Table 3].

Discussion

Immunotherapy is an effective treatment strategy for head and neck tumors. Therefore, the specification of immune-related biomarkers is important. Although the association of TILs such as CD3 and CD8 with

Table 2: Univariate analysis to assess the association of clinical parameters with prognosis of nasopharyngeal carcinoma

Items	Cases (70)	Univariate analysis *P*
Age		
≥50	42	0.322
<50	28	
Gender		
Male	56	0.260
Female	14	
Smoking status		
Yes	45	0.762
No	25	
TNM stage		
Stage 1 and 2	15	0.206
Stage 3 and 4	51	
T stage		
T_{1-2-3}	32	0.009
T_4	29	
N stage		
N_{0-1}	26	0.564
N_{2-3}	27	
Pathology		
Differentiated	26	0.256
Undifferentiated	44	
PDL-1		
Positive	37	0.049
Negative	33	
Definitive therapy		
RT	7	0.702
CRT	63	

CRT: Chemoradiotherapy; RT: Radiotherapy; TNM stage: Tumor, node, metastasis stage; TIICs: Tumor infiltrating-immune cell

Table 3: Multivariate Cox regression analyses demonstrating relationship between some variables and survival

	P	HR (Hazard ratio)	95% CI for HR	
			Lower	Upper
TIICs PD-L1 expression	0.027	0.328	0.122	0.881
T stage	0.013	0.250	0.084	0.748
N stage	0.949	1.033	0.378	2.823
Histology	0.053	2.550	0.988	6.582
TNM stage	0.776	0.773	0.131	4.550
RT vs. CRT	0.238	2.532	0.541	11.849

CRT: Chemoradiotherapy; RT: Radiotherapy; T stage: Tumor stage; N stage: Node stage; TNM stage: Tumor, node, metastasis stage; TIICs: Tumor infiltrating-immune cell; PD-L1: Programmed death ligand-1; HR: Hazard ratio CI: Confidence interval

prognosis is known in patients with head and neck cancer, most of the immune-related biomarkers are unknown. PD-L1 expression in tumor cells has been shown to be a predictive marker in response to anti-PD1/PD-L1 treatment in many studies.[22]

Also, PD-L1 expression has been shown to be a good prognostic factor in metastatic melanoma,

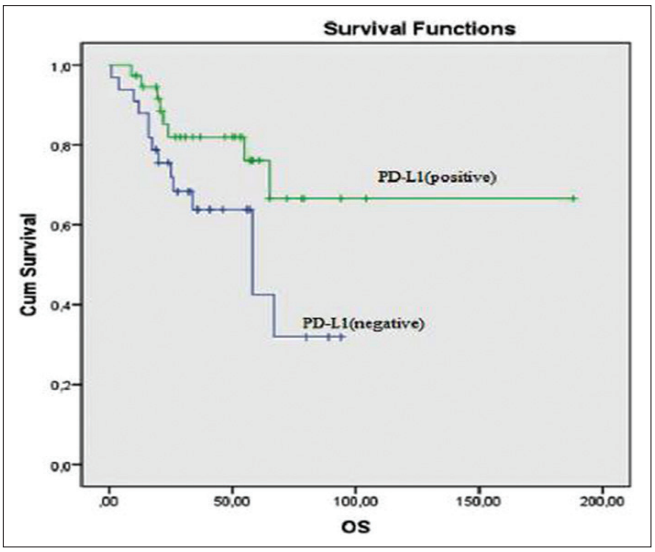

Figure 2: Kaplan-Meier survival curve for PD-L1 expression in tumor infiltrating-immune cell

non-small cell lung cancer and Merkel cell carcinoma while it was shown as a negative prognostic factor in many types of cancer such as kidney, colorectal and lung cancer.[18,23,24] In a study of patients with laryngeal squamous cell carcinoma, the presence of PD-L1 expression and abundance of TILs were found to be independent good prognostic factors.[25]

In a meta-analysis, the risk of death has been shown to decrease in patients with positive PD- L1 expression in tumor-infiltrated immune cells (HR = 0.784, 95% CI: 0.616–0.997, *P* = 0.047).[26] In a study by Kim *et al*. in patients with head and neck cancer, patients with PD-L1 positivity in ICs had significantly better 5-year relapse-free survival (RFS) (80.1% vs 69.7%) and OS (90.6% vs 75.6%) compared to PD-L1 negative patients.[27] Patients with oral cavity and oropharynx tumors were included in this study by Kim *et al*. In a study by Badoual *et al.* conducted in patients with head and neck cancer, better survival was detected in patients with human papillomavirus (HPV)-positive and expressing a high level of PD-L1 in T-cells compared to those expressing a low level of PD-L1.[28] In a study of patients with urothelial carcinoma, the relationship between PD-L1 expression in tumor-infiltrating mononuclear cells and survival was evaluated; and survival rate was found better in urothelial carcinoma patients with PD-L1 positive ICs.[25] In another study conducted in patients with NPC, no significant relationship was found between PD-L1 in immune cells and OS (*P* = 0.278) and DFS (*P* = 0.290).[29] There is a strong relationship between tumor-infiltrating immune cells expressing PD-L1 and cancer immune response. There is a positive correlation between positive PD-L1 expression in tumor-infiltrating immune

cells and the number of TILs such as CD4 and CD8 T lymphocytes in various tumors. Survival was found better in cancer patients with a high level of CD4 and CD8 T lymphocytes in studies.[14,15] TILs play a crucial role in host immune response to the tumor, and may reveal the antitumor immune response. In a study conducted in patients with melanoma, patients with a high level of TILs in the tumor tissue were found to have better survival and the risk of metastasis was found to be lower.[30] Although the expression of PD-L1 mediates the immune escape in cancer, it implies an effective immune response, particularly in the early stage of cancer.[31]

Diverse cut-off values were defined for PD-L1 in studies. In many studies, the immunohistochemical cutoff value for positive PD-L1 expression was determined as >5% and >1% of immune cells.[26] In our study, we defined the cut-off value for PD-L1 expression on >5% of immune cells. In this study, the PD-L1 positivity rate in TIICs was 53%. PD-L1 positivity rate in tumor-infiltrating immune cells was found as 46.8% in a previous study of NPC patients. In our study, a similar rate was found. A standard cut-off value is required to use PD-L1 expression in immune cells as a good prognostic marker.

The limitations of our study are small sample size and retrospective design. More prospective studies are needed with a larger number of patients.

Conclusion

A significant relationship was found between survival and positive PD-L1 expression in ICs in patients with NPC. The survival of patients with PD-L1 positive in ICs was significantly better than the negative ones. PD-L1 expression in ICs may be used as a good prognostic factor and may be useful for selecting patients who may respond to anti PD- L1 therapy as well.

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Conflicts of interest

There are no conflicts of interest.

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