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Original Article

Immunohistochemical expression of PD-L1 in esophageal carcinoma and its correlation with histopathological features

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Keywords:

Esophageal carcinoma; Immunohistochemistry; Immunotherapy; PD-L1

ABSTRACT

Background: Despite advances in screening and multimodal management of this disease, overall survival remains poor. There is an utmost need to find a marker for the establishment of targeted immunotherapy. One immunohistochemical marker that has surfaced with the advent of anti-PDL1 immunotherapy is PD-L1 which has been proven to be advantageous. This study aimed to determine PD-L1 expression and its correlation with the grade of tumor and other histopathological parameters in 50 patients.

Materials and Methods: A cross-sectional study was conducted on 50 cases of histopathologically proven esophageal carcinoma. Antibody used for PD-L1 was rabbit monoclonal (CAL10) Biocare. Correlation between PD-L1 expression and clinicopathological parameters was calculated.

Results: Out of 50 cases, 46 cases were of squamous cell carcinomas (SCC). The PD-L1 positivity was observed in 22 cases comprising 44 % of the total esophageal cases. The percentage of positive cells varied from 5% - 66% with mild, moderate, and strong staining intensity. PD-L1 positivity was observed in poorly differentiated tumors constituting 83.3% (5/6 cases) while moderately differentiated and well-differentiated tumors showed positive expression in 46.8% (15/32 cases) and 12.5% (1/8) cases respectively. Only one case of adenocarcinoma was positive out of 4 cases of adenocarcinoma (4 cases, 25%).

Conclusions: The expression of PD-L1 increases with grade and shows more positive expression in poorly differentiated tumors. This indicates that PD-L1 overexpression is associated with aggressive disease. Targeted therapy against PD-L1 may prove to be beneficial in such patients.

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INTRODUCTION

Esophageal carcinoma (EC) is one of the deadliest and seventh most common cancers worldwide with developing nations making up more than 80% of total cases and deaths.[1] It has an extremely aggressive course and poor survival rate and ranks sixth among all cancers in mortality. Esophageal squamous cell carcinomas (ESCC) comprise 90% of esophageal cancers in high-risk areas (including northern Iran, Central Asia, and North-central China) and are thought to be related to poor nutritional status, low intake of vegetables and fruits, and high-temperature beverage drinking. Esophageal adenocarcinoma (EAC) is more

prevalent among esophageal tumors in the U.S. and Europe, where its incidence has increased significantly in the last 30 years, in parallel with obesity and gastro-esophageal reflux disease. Due to the dismal prognosis of patients with advanced esophageal cancers, there is an urgent need for innovative therapeutics.[2] Programme death ligand – 1 (PDL-1) has emerged as a promising marker in determining overall prognosis. PDL-1 is an immune checkpoint inhibitor that has transpired as a potential predictive and prognostic marker and has revolutionized cancer treatment. PD-L1 expression and its association with clinicopathological, prognostic, and immunological factors have been seen in several human malignancies such as breast, ovarian, cervical, head-neck, brain, lung, nasopharyngeal, esophageal, gastric, liver, colorectal, pancreatic renal, urothelial, skin and blood cancers.[2]

This study aimed to determine PD-L1 expression and its correlation with the grade of tumor and other histopathological parameters of a total of 50 patients.

MATERIAL AND METHODS

This cross-sectional study was conducted on 50 cases of esophageal biopsies received as 43 biopsies and 7 esophagectomy specimens in the Department of Pathology, Sri Guru Ram Das Institute of Medical Sciences And Research, Sri Amritsar (A tertiary care center). The tissue was formalin-fixed and paraffin-embedded and was then stained for haematoxylin and eosin for histopathological typing and grading. 3–5 µm thick sections were cut and mounting was done on poly–l–lysine coated slides. All the cases of esophageal carcinomas were further subjected to immunohistochemistry for PD-L1 expression. The antibody used for PD-L1 was rabbit monoclonal (CAL10) Biocare. PD-L1 expression and staining patterns were analyzed, and the expression pattern of PD-L1 and its correlation with the grade of tumor was established. For PD-L1, membranous or cytoplasmic staining was taken into consideration. PD-L1 expression in greater than 5% of tumor cells was considered positive.[3-6]

For PD-L1 Expression: Tumor cells showing either partial or complete membrane or cytoplasmic staining (brown) were considered to be positive for PD-L1.

PD-L1 scoring algorithm for esophageal carcinoma: It was done according to studies conducted before by Dereks S et al, Zhu Y et al, Tsutsumi S et al and Chen K et al who took PD-L1 positive cut-off as 5% in tumor cells.[4,7,8]

Tumour cell staining assessment	PD-L1 expression
Histological evidence of cell membrane staining or cytoplasmic staining in 5% or more cells with variable intensity	Positive
Histological evidence of cell membrane or cytoplasmic staining in <5% of cells	Negative

Proper informed consent was taken from patients. The findings of the study were statistically analyzed using Statistical Package for the Social Sciences (SPSS) software version 21.0, a statistical program for Microsoft Windows.

RESULTS

Most of the patients belonged to the age group of 41-60 years with the oldest patient being 83 years old whereas the youngest was 30 years of age. The majority of the patients were females constituting 64% of the total with a male-to-female ratio of 1:1.6. The majority of patients presented with a multitude of complaints. Amongst these, the most common complaint was dysphagia. The middle segment of the esophagus was involved in 58% of cases, followed by the lower segment (34%) and the upper segment showing the least involvement (8%). The most common type of growth observed was the ulcerated type, constituting 48% of cases followed by proliferative and polypoidal growth in 30% and 22% respectively. Out of 50 cases, 46 cases were of squamous cell carcinomas (SCC). Most of the SCC cases were reported as moderately differentiated tumors constituting approximately 70 % of all cases, followed by well differentiated (16%) and poor differentiated (12%). The PD-L1 positivity was observed in 22 cases comprising 44 % of the total esophageal cases. The percentage of positive cells varied from 5% - 66% with mild, moderate, and strong staining intensity. Maximum PD-L1 positive cases were seen within the 41-60 year age group (12 cases; 54.5%). The 22 cases which showed PD-L1 expression consisted of 12 female patients and 10 males. Out of these, PD-L1 positive expression was seen in 21 cases of Squamous cell carcinoma (21 cases, 45.65%) and only one case of adenocarcinoma was positive out of 4 cases of adenocarcinoma (4 cases, 25%). (Table 1) In squamous cell carcinomas, maximum PD-L1 positivity was observed in poorly differentiated tumors constituting 83.3% (5/6 cases) while moderately differentiated and well-differentiated tumors showed positive expression in 46.8% (15/32 cases) and 12.5% (1/8) cases respectively. PD-L1 expression was statistically significant with histological grade (p=0.0030). So it was deduced from the study that as the grade increases, PD-L1 positivity increases. There was only one PD-L1 positive expression seen in adenocarcinoma out of 4 cases which was reported as moderately differentiated. No correlation was found between PD-L1 positivity and age, sex, lymph node status, and tumor invasion (Table 1 and Table 2, fig. 2)

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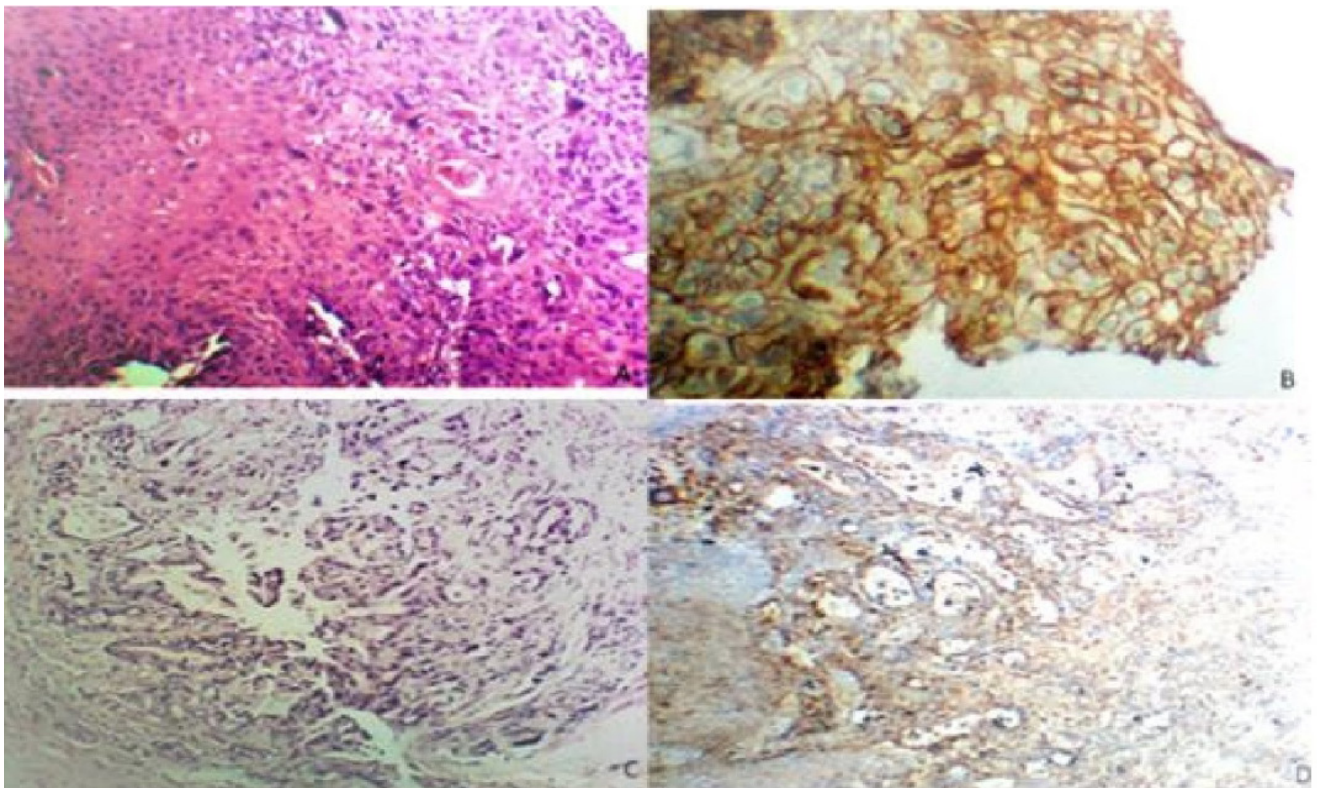

Figure 1: *Microphotographs. (A) Esophageal squamous cell carcinoma –moderately differentiated (H&E; 100X). (B) PD-L1 positivity in esophageal squamous cell carcinoma – membraneous staining with strong intensity (IHC; 100X). (C) Esophageal adenocarcinoma –moderately differentiated (H&E; 100X); (D) PD-L1 positivity in esophageal adenocarcinoma – membraneous staining with moderate intensity (IHC; 100X)*

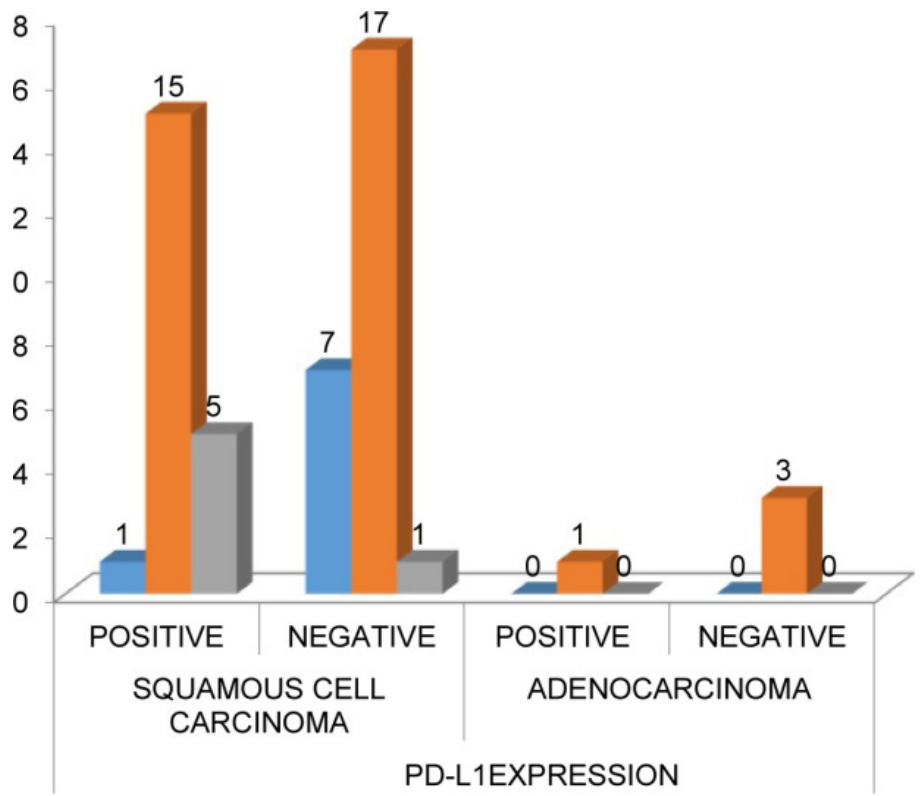

Figure 2: *Bar diagram showing correlation of PD-L1 expression in different histological types of esophageal carcinoma*

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Table 1: Correaltion of PD-L1 expression with different histological types of esophageal carcinoma

Type of Carcinoma	PD-L1 Expression		Percentage positivity
	Positive	Negative	
Squamous cell carcinoma	21	25	45.65%
Adenocarcinoma	1	3	25%
Total	22	28	

Table 2: Correlation of PD-L1 expression with grade of esophageal carcinoma

Grade of tumour	PD-L1 expression					
	Squamous cell carcinoma			Adenocarcinoma		
	Positive	Negative	Percentage positivity	Positive	Negative	Percentage positivity
Well-differentiated	1	7	12.5%	0	0	0
Moderately-differentiated	15	17	46.8%	1	3	25%
Poorly-differentiated	5	1	83.3%	0	0	0
Total	21	25		1	33	

PD-L1 expression was significantly higher in poorly differentiated tumors (83.3%) as compared to moderately differentiated tumors (44.4%) and well-differentiated tumors (12.5%.) (p=0.0030).

DISCUSSION

Esophageal cancer (EC) is one of the least studied and deadliest cancers worldwide because of its extremely aggressive nature and poor survival rate.[1,2] Despite advances in screening and multimodal management of this disease, overall survival for both ESCC and EAC remains poor. The need to identify tumor markers as prognostic indicators and as targets for new therapeutic strategies remains a major challenge in esophageal cancer research. PD-L1 has emerged as a promising prognostic marker. PD-L1 is a 290-amino-acid type transmembrane glycoprotein that belongs to the B7-CD28 family of the immunoglobulin superfamily. It is also known as cluster of differentiation 274 (CD274) or B7 homolog 1 (PD-L1). It comprises extracellular IgV and IgC domains, a transmembrane region, and an intracellular domain. It is expressed in dendritic cells (DCs), macrophages-activated T cells, and B cells, dendritic cells (DCs). Ligation with its ligands results in the formation of PD-1/T cell receptor inhibitory microclusters. SHP-2 is recruited to these sites and there occur dephosphorylation of multiple members of the T-cell receptor signaling pathway and this ultimately results in suppression of T-cell activation. Thus the whole interaction between PD-L1 and PD-1 is of prime importance in limiting the activity of T-cells in peripheral tissues at the time of an inflammatory response. PD-L1 expression is regulated by both extrinsic and intrinsic mechanisms. Extrinsic induction of PD-L1 is largely mediated by inflammatory mediators such as cytokines, interferon γ, interleukin 4 (IL-4), and interleukin 10 (IL-10), vascular endothelial growth factor and granulocyte-macrophage colony-stimulating factor. Intrinsic induction of PD-L1 is seen in transcriptional and posttranscriptional control. In addition, the activity of PI3K and mTOR pathway or epidermal growth factor receptor–mitogen-activated protein kinase pathways is also associated with overexpression of PD-L1 in some malignancies. So, as high frequency of mutations are seen in cancer tissues, biochemical signaling pathways may play a pivotal role in aberrant PD-L1 expression in cancer cells.[2] PD-L1 expression on cell surface is detected by immunohistochemistry analysis in many surgical and biopsy specimens of cancer. This implicates a mechanism in the tumour microenvironment that is required for the maintenance of PD-L1 expression in cancer tissues. Minimal expression of PD-L1 in normal tissues but the inducible expression in tumour site is the most unique feature of this pathway distinguished from other co-inhibitory pathways. This selective expression of PD-L1 allows tumor-targeted immune modulation and may be responsible for high antitumor efficacy and limited toxicity of blocking antibodies. Thus, the expression of PD-L1 on tumour site implicates not only an important mechanistic insight but also the prognostic value.[4-6]

In our study, histologically proven cases of esophageal carcinoma were analyzed. Most of the cases were reported as the squamous cell type constituting 92% (46/50) of the total cases with the remaining being adenocarcinoma. Out of 46 cases of ESCC in the present study, 70% (35/50) cases were reported as moderately differentiated. Well-differentiated and poorly-differentiated squamous cell carcinomas accounted for only 17% (8/50) and 13% (6/50) respectively. Four cases of adenocarcinoma were moderately differentiated. PD-L1 positivity was noted in 44% (22/50) of total cases when it was taken as ≥5% membranous/cytoplasmic positivity in tumour cells. Of the 46 cases of ESCC, PD-L1 positivity was observed in only 21 cases (45.6%). Similar observations were seen in studies conducted by Zhu et al, Tsuttsumi et al, and Chen K et al, who reported PD-L1 expression in 51.1%, 63.3%, and 41.4% respectively while taking a 5% cut-off for PD-L1 positivity in tumour cells. Other researchers have reported PD-L1 expression in ESCC ranging from 40% - 60%. Thus it was seen that the percentage

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of PD-L1 positivity varies widely depending on the assay of methodology, cut-off for positivity. In the present study, out of 4 cases of EAC, PD-L1 positivity was observed in only 1 case.[3-7] Results of the present study do not concur with the study by Dereks S et al, who reported a PD-L1 expression in 1.7% cases (6/344) of EAC as 1.[3] This disparity may be due to the low sample size in the present study. In the present study, PD-L1 expression in ESCC was statistically correlated with histological differentiation. PD-L1 expression was significantly higher in poorly differentiated tumors (83.3%) as compared to moderately differentiated tumors (44.4%) and well-differentiated tumors (12.5%.) (p=0.0030). (Table 1) Similar observations were noted by Zhu et al. who showed that poorly differentiated tumors displayed stronger PD-L1 expression than well and moderately differentiated tumors.[7] This finding also corroborated with the study done by Wang et al. which also showed the same results.[9]

Thus, in the present study, we can conclude that PD-L1 expression increases with the grade of tumour. Poorly differentiated tumors have higher expression of PD-L1, indicating an aggressive course. The overexpression of this molecule has been linked to worse prognosis and resistance to anti-cancer therapies in many of the malignancies. This indicates that they are associated with aggressive disease. Previous studies have suggested that targeted therapy against PD-L1 may be beneficial in such patients and could potentially help prevent recurrence. The treatment approach may hold value for patients undergoing treatment for metastatic esophageal carcinoma, as current therapy options are limited.

Limitations: The main limitation of the present study was the small sample size. Conducting further studies with a larger sample size may provide additional findings.

Conflict of Interest: None

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