

Correlation of Programmed Death Ligand-1 (PD-L1) Expression with Clinicopathological Features in Non-Small Cell Lung Carcinoma: Experience from a Tertiary Cancer Care Center in India

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Abstract

Background: According to the GLOBOCAN 2018 report, the estimated incidence of lung cancer in India was 67,795 in both sexes. The treatment of advanced Non-small cell lung cancer (NSCLC) saw a major paradigm shift with recent advances in molecular-targeted therapy. Immune checkpoint blockade therapy is one such novel strategy with promising clinical benefits in advanced NSCLC. Programmed cell death receptor-1/Programmed cell death ligand-1 (PD-1/PD-L1) pathway is one such checkpoint and has thus become the current area of interest in the treatment of lung carcinoma. The PD-1/PD-L1 pathway is under active investigation as it represents a promising therapeutic target in NSCLC. The expression of PD-L1 in tumor cells has been suggested as a predictive marker of the clinical response to PD-1/PD-L1-targeted therapy. **Methods:** This study was carried out at the Department of Pathology, Health Care Global Specialty Hospital, Bangalore from May 2018 to May 2019. In this study, we analyzed pattern of PD-L1 expression by immunohistochemistry testing using our own Laboratory Developed Test (LDT) in NSCLC patients. **Results:** Our study group comprised 50 pa-

tients of NSCLC. Among our study population, 40% of the patients exhibited PD-L1 immunopositivity ($\geq 1\%$) with 28% of them having any expression (TPS 1% - 49%) and 12% of them having a high expression (TPS $\geq 50\%$) of PD-L1. Majority of them exhibited adenocarcinoma type of NSCLC under which the solid subtype showed a direct correlation with PD-L1 positivity (p-value: 0.004) with a poorly differentiated tumor histology being common in our population in relation to PD-L1 positivity (p-value: 0.043). PD-L1 expression did not correlate with age, gender, smoking status or clinical stage in our study. No association was found between tumor histology (SCC or AC) and driver mutation status with expression of PD-L1 in the present study.

Conclusions: In our study, PD-L1 immunopositivity was found in 40% of patients and majority of them exhibited adenocarcinoma type of NSCLC. There was no correlation of PD-L1 expression with age, gender, clinical stage, smoking status and tumor histology.

Keywords

Lung Cancer, PD-L1, IHC, Biomarker, Immunotherapy

1. Introduction

Lung cancer, especially non-small cell lung cancer (NSCLC), accounts for more than 1.5 million deaths per year and is the leading cause of cancer deaths in men and women worldwide [1]. According to the GLOBOCAN 2012 report, the estimated incidence of lung cancer in India was 70,275 in all ages and both sexes. More than 70% of patients are diagnosed with advanced disease, which may not be amenable to curative therapy [2].

Histologically, lung cancer is further divided into 3 major pathologic subtypes: adenocarcinoma, squamous cell carcinoma, and large cell carcinoma [3]. Adenocarcinoma accounts for 38.5% of all lung cancer cases, with squamous cell carcinoma accounting for 20% and large cell carcinoma accounting for 2.9%. The incidence of adenocarcinoma has increased greatly in the past several decades, replacing squamous cell carcinoma as the most prevalent type of NSCLC [3] [4]. Although cigarette smoking is associated with all histologic types of lung cancer, in smokers the association is stronger for small cell lung cancer (SCLC) and for squamous cell carcinoma. Adenocarcinoma of the lung, on the other hand, is more common in never smokers (62% vs 18%, based on 5144 cases) compared with smokers (19% vs 53% based on 21,853 cases) [5] [6]. However, even among smokers, adenocarcinoma is becoming more common. An increased frequency of Epidermal Growth Factor Receptor (EGFR) mutations in lung adenocarcinomas of never smokers, especially in Asian cohorts has been reported recently [7]. Lung adenocarcinomas harbor EGFR mutations and Anaplastic Lymphoma Kinase (ALK) rearrangements almost exclusively and the identification of these molecular abnormalities is clinically relevant [8]. To im-

prove the survival rate in NSCLC patients, novel treatment strategies or agents are needed. Immune checkpoint blockade therapy is one such novel and exciting strategy with promising clinical benefits in advanced NSCLC [9] [10]. The immune checkpoint mechanism has a critical role to play in suppressing the anti-tumor T-cell mediated immune response in the tumor microenvironment. Immunotherapy targeting the cellular immune checkpoints has thus become the current area of interest in lung carcinoma [11], one such checkpoint is the Programmed cell Death receptor-1/Programmed cell Death Ligand-1 (PD-1/PD-L1) pathway.

PD-L1 is a type 1 transmembrane protein (B7-H1) that belongs to the B7 ligands family and may be expressed both on hematopoietic cells and non-hematopoietic cells, including endothelial, epithelial and tumor cells. PD-L1 is the principal membrane inhibitory ligand belonging to the B7 family, and the most widely studied in NSCLC [12]. Analyzing expression of PD-L1 by immunohistochemistry (IHC) staining has been the core strategy to select NSCLC patients for PD-L1 inhibitors. Overexpression of PD-L1 has been associated with higher response rates and better outcomes to several checkpoint inhibitors like Pembrolizumab (anti-PD-1), Nivolumab (anti-PD-1), Atezolizumab (anti-PD-L1), Durvalumab (anti-PD-L1) and Avelumab (anti-PD-L1) [8]. The overexpression of PD-L1 by tumor cells in NSCLC has been demonstrated in several large retrospective studies. The largest of these studies examined archived tumor tissue from 458 patients with stage I–IV NSCLC across all histologies using quantitative immunofluorescence (QIF) to detect PD-L1 expression on the tumor cell surface [15]. This study revealed that 32% of these samples expressed elevated PD-L1. Similar smaller retrospective studies in NSCLC using both QIF and immunohistochemistry (IHC) have reported rates of PD-L1 expression by tumor cells ranging from 27% to 58% [16] [17]. Smoking status has also been correlated with elevated PD-L1 expression. However, association between overall survival (OS) and PD-L1 expression remains unclear with reports of both an associated improvement and decrease in OS [18] [19] [20]. In a study conducted by Archana GV *et al.* in 2017, 89 cases of resected NSCLC were studied and immunopositivity for PD-L1 in tumor cells (TC) was found to be 27%.

In the present study, we analyzed the immunohistochemistry (IHC) expression of PD-L1 in tumor cells (TC) of Squamous cell carcinoma (SCC), Adenocarcinoma (AC) and other NSCLC histological subtypes. This study also correlates the PD-L1 expression in association with the baseline clinical data of the patients and with different histopathological characteristics of the tumor.

2. Materials and Methods

This was a hospital based study approved by Institutional Ethics Committee, which was carried out at the Department of Pathology, Health Care Global Specialty Hospital, Bangalore from May 2018 to May 2019. The study group comprised of 50 patients with Non-Small Cell Lung Carcinoma presenting to HCG

Hospital, Bangalore. Patients included in this study were at least 18 years of age, histologically confirmed for NSCLC, those who were untreated and newly diagnosed. An ideal biopsy sample in this study was defined as a minimum of 100 tumor cells which were required for the interpretation of the IHC in terms of Tumor Proportion Score (TPS) for PD-L1 expression. Hence, too tiny biopsies (<100 tumor cells) were excluded from this study. Also, post-chemotherapy/radiation cases, Fine Needle Aspiration Cytology (FNAC) samples, decalcified tissues, specimens from the metastatic sites (ex: lymph nodes) and those with Small Cell Lung Carcinoma (SCLC) or Large Cell Neuroendocrine Carcinoma (LCNEC), were not considered for this study.

A detailed history including age, gender, clinical symptoms, radiological findings, PET scan and data of other relevant investigations were collected from patient case files. For the retrospective study, formalin fixed paraffin embedded tissue blocks and/or slides (Hematoxylin and Eosin and IHC) were recovered and reviewed for the final diagnosis of Non-Small Cell Lung Carcinoma & its subtypes. For the prospective cases, small biopsies (CT guided core biopsy) and resected (lobectomy) specimens from patients with lung carcinoma were processed and studied. These were stained with Hematoxylin & Eosin and analyzed for subtyping of the tumor using morphology and relevant immunohistochemistry, if necessary. For small biopsies, histopathological characteristics like tumor histological type, grade of differentiation, and subtype (for adenocarcinoma cases) were determined. For resections, similar histopathological characteristics including tumor size were analyzed and tabulated. Those cases which fulfilled the inclusion criteria were subjected to immunohistochemistry testing for PD-L1.

Immunohistochemistry (IHC)

IHC was performed on 3 - 5 micron sections of formalin fixed and paraffin embedded (FFPE) tissues using Rabbit monoclonal antibody (Clone CAL10) by BIOCARE. Due to the scarcity of FDA approved testing kits in India, our own Laboratory Developed Test (LDT), tested and validated in our laboratory was used. Tonsillar tissue or PD-L1 positive lung adenocarcinoma tissue was used as positive control and was taken on the same slide as patient sample (away from marking area). Single negative control was run by omitting primary antibody and replacing diluent solution (BSA) in which the primary antibody was diluted (**Figure 1**).

Tissues sectioned and attached to slides, were dewaxed if paraffin embedded, treated with an antigen retrieval solution [EDTA buffer (1 Mm, pH 8)] if required, blocked with a proteinaceous blocking solution (3% BSA) and then incubated with the primary antibody. The bound primary antibody was detected by the addition of secondary antibody conjugated with horseradish peroxidase polymer and DAB substrate. When adequate colour developed, the slides were washed in water to stop the reaction, counterstained with Harris Haematoxylin, and covered with a mounting medium (DPX). Semi-automated method, Intellipath autostainer was used to stain the IHC. The slides were scanned using Philips Digital Ultrafast Scanner and whole slide images were generated. Digital

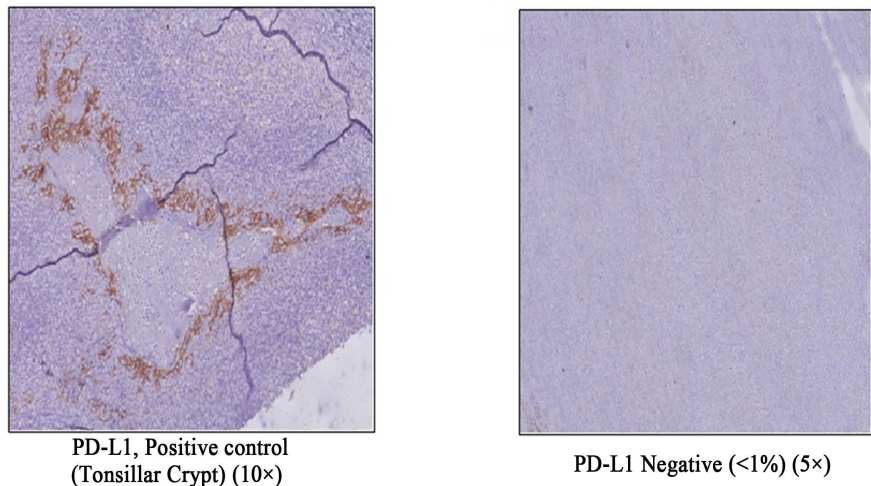


Figure 1. PD-L1 positive control (Tonsillar tissue) and PD-L1 negative control.

images of the selected image histology were then photographed using the snapshot tool in the image management system software.

Immunohistochemical Staining Analysis

PD-L1 immunopositivity was determined and interpreted using the Tumor Proportion Score (TPS) of <1% (no expression), 1% - 49% (any expression) and >50% (high expression) (**Figure 2**). Any membrane (complete circumferential or partial linear plasma membrane) or cytoplasmic staining, at any intensity was considered positive for PD-L1.

Statistical Analysis

Descriptive statistics were used to describe data such as patient demographics and tumor-specific variables. Continuous variables were expressed as Mean and Standard deviation and categorical variables were expressed as Frequency and Percentage.

Statistical analysis was done to assess correlation of PDL-1 expression with clinicopathological and histopathological features. In these inferential statistics, Chi-square test/Fisher's Exact test was used to associate between the categorical variables. All statistical analyses were tested at 95% confidence interval and a P-value of <0.05 was considered as statistically significant. SPSS Software, Version 23 (2015) was used for the analysis.

3. Results

Patient Characteristics

A total of 50 patients were included in our study and all of the patients' data in this study were analyzed for various demographic, clinicopathological and histopathological characteristics as mentioned in **Table 1**.

Of the 50 patients included in the study, 11 patients (22%) were aged below 50 years, 29 patients (58%) were between 50 - 70 years, and 10 patients (20%) were aged above 70 years. The mean age for lung carcinoma was found to be 60.92 ± 13.46 years, with the youngest patient being 21 years old and the oldest being 93

Table 1. Patient & tumor characteristics.

Characteristics	Number (N) [Total = 50]	Percentage (%)	Mean ± SD (Min - Max)
Age			
<50 Years	11	22.0	60.92 ± 13.46 (21 - 93)
50 - 70 Years	29	58.0	
>70 Years	10	20.0	
Gender			
Male	31	62.0	NA
Female	19	38.0	
Smoking History			
Yes	13	26.0	NA
No	37	74.0	
Pack Years			
<20 Years	2	15.4	NA
20 - 40 Years	7	53.8	
>40 Years	4	30.8	
Tumor Size			
<3 cm	7	14.0	NA
3 - 7 cm	41	82.0	
>7 cm	2	4.0	
Metastasis			
Yes	45	90.0	NA
No	5	10.0	
Clinical Stage			
Stage I	2	4.0	NA
Stage II	3	6.0	
Stage III	7	14.0	
Stage IV	38	76.0	
Histological Type			
Squamous Cell Carcinoma (SCC)	7	14.0	NA
Adenocarcinoma (AC)	43	86.0	
Adenocarcinoma Subtypes			
Acinar	19	44.18	NA
Lepidic	1	2.32	
Papillary	4	9.30	
Mucinous	1	2.32	
Solid	18	41.86	
Tumor Differentiation Grade			
Well (G1)	6	12.0	NA
Moderate (G2)	21	42.0	
Poor (G3)	23	46.0	
Sampling Method Used			
Core Biopsies	48	96.0	NA
Resections	2	4.0	

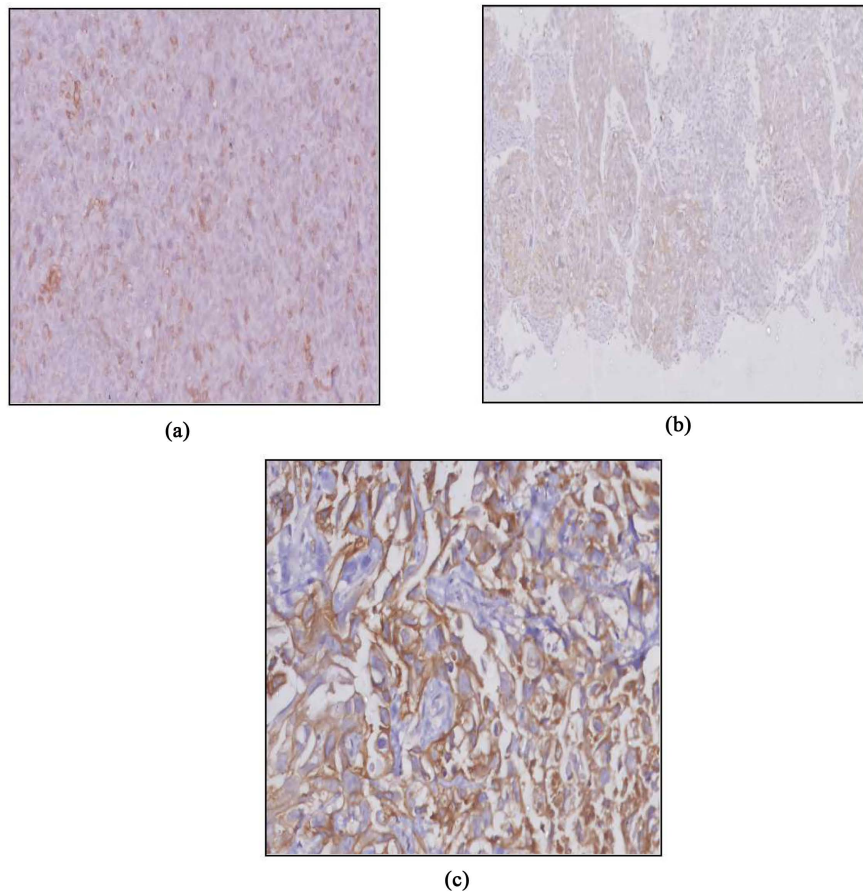


Figure 2. PD-L1 positive expression (a) 20%, any expression (10×); (b) 30%, any expression (10×); (c) 50%, high expression (40×).

years of age. Out of these patients, 62% were males ($n = 31$) and 38% were females ($n = 19$), with a male to female ratio of 1.6:1. The total number of smokers in this study was 13 (26%) and 37 patients (74%) were non-smokers. Among smokers, all were males (no female smokers) and among these, 9 patients were ex-smokers and the other 4 were current/active smokers. 15.4% ($n = 2$) of the patients had a smoking history of <20 pack years, 53.8% ($n = 7$) had a history of 20 - 40 pack years of smoking, and 30.8% ($n = 4$) had smoked for >40 pack years in their lifetime.

Histopathological Features of the Tumor among Our Study Group

Among the 50 cases, 12% ($n = 6$) had well differentiated tumors and 42% ($n = 21$) had moderately differentiated tumors. Majority of the cases (46%, $n = 23$) exhibited a poorly differentiated histology. Maximum number of patients (76%, $n = 38$) belonged to clinical Stage IV. 14% of the patients ($n = 7$) belonged to Stage III and a minority of patients, *i.e.* 6% ($n = 3$) and 4% ($n = 2$) had Stage II and Stage I disease, respectively. Majority (90%) of the patients in this study presented with metastatic tumor ($n = 45$), with only 10% ($n = 5$) of the patients having a tumor confined to the lung. The various sites of metastasis were lymph nodes, axial skeleton, liver, adrenal gland and colon in decreasing order of fre-

quency. There were 43 cases of adenocarcinoma (86%), while only 7 patients (14%) had squamous cell carcinoma. Adenocarcinoma was the most common histological type of NSCLC in our cohort. Among the adenocarcinoma cases, acinar predominant subtype was more common ($n = 19$, 44.18%), closely followed by solid predominant subtype ($n = 18$, 41.86%). There were 4 cases of papillary predominant adenocarcinoma (9.3%), while lepidic and mucinous adenocarcinoma each constituted only 2.32% ($n = 1$) of the cases, respectively. Representative H & E images of squamous cell carcinoma and adenocarcinoma subtypes are depicted in **Figure 3**.

Frequency of EGFR, ALK and ROS Mutations

Data collected on EGFR mutation, ALK rearrangements and ROS1 mutations are summarized in **Table 2**. Among our cases, 18% tested positive for EGFR mutation and 64% were tested negative for EGFR mutation. For ALK, data was available for 32 patients among whom 2 patients (6%) demonstrated ALK rearrangement. Data was not available for 18 (36%) of the cases. Whereas, mutations in ROS1 gene were tested in 46% of the patients ($n = 23$) with all cases being negative, while no data on ROS1 gene was available in 54% of the patients ($n = 27$).

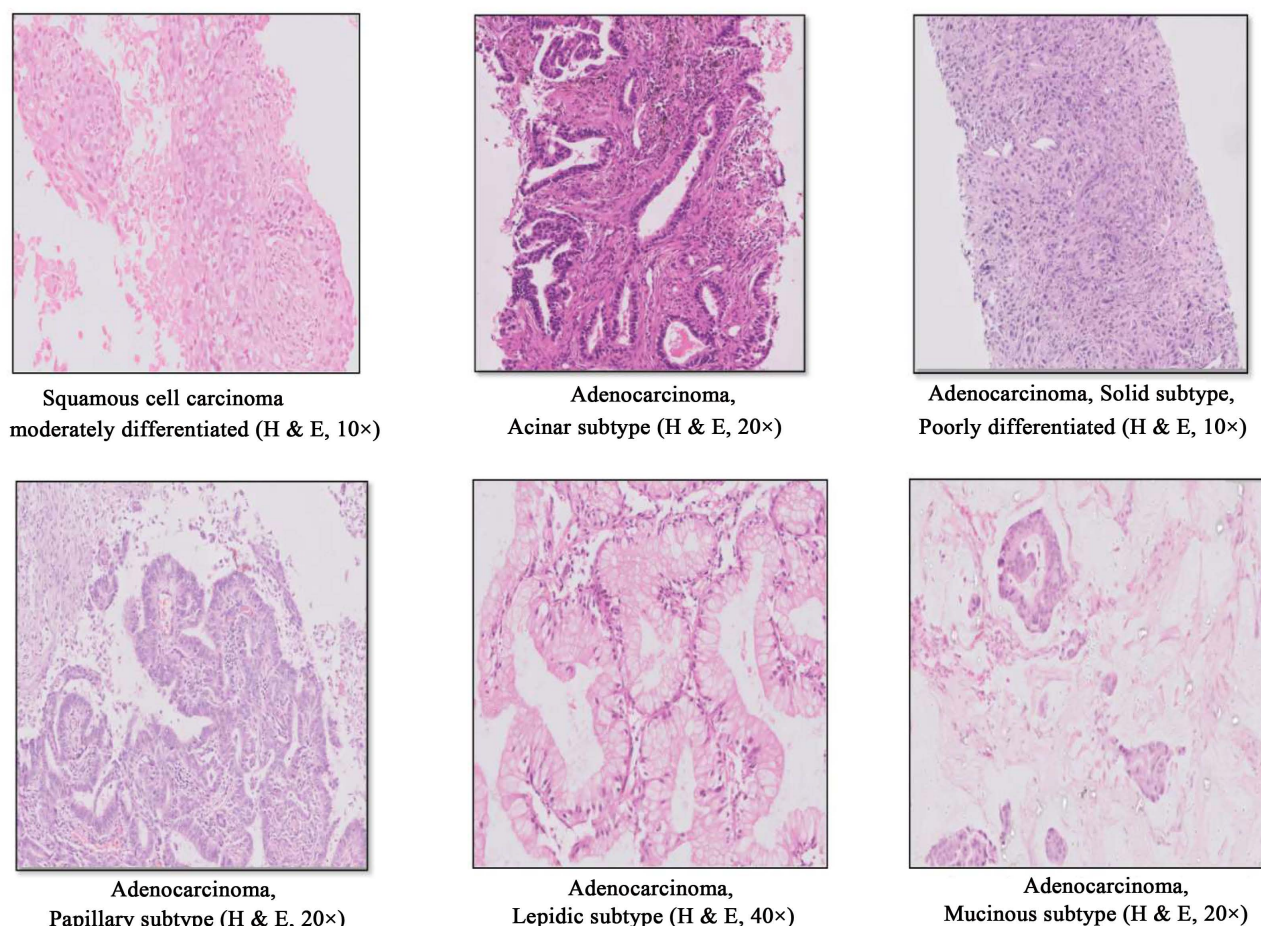


Figure 3. Representative H & E images of Squamous cell carcinoma and Adenocarcinoma subtypes.

Table 2. Frequency of EGFR, ALK and ROS1 mutations among the study group.

Driver Mutation	Number (N)	Percentage (%)
EGFR Mutation		
Exon 19 Deletion	6	12.0
Exon 21 Mutation	3	6.0
Negative	32	64.0
Data Not Available	9	18.0
ALK Rearrangement		
Positive	2	4.0
Negative	30	60.0
Data Not Available	18	36.0
ROS1 Mutation		
Positive	0	0.0
Negative	23	46.0
Data Not Available	27	54.0

PD-L1 Expression Profile

PD-L1 positivity in our cohort was 40% (n = 20/50) with 30% of the PD-L1 positive patients showing > 50% or high PD-L1 expression (6/20), while the remaining 70% of PD-L1 positive patients showing an expression between 1% and 49% (14/20). Overall, 28% of the patients (14/50) showed PD-L1 expression of 1% - 49%, while 12% of the patients (6/50) showed a high or >50% PD-L1 expression (**Table 3**).

Association of PD-L1 Expression with Age, Gender and Smoking Status

In our study, the mean age of patients with PD-L1 positivity was 59.15 years while PD-L1 negative patients had a mean age of 62.10. In the group of patients < 50 years of age, 6 cases (54.5%) tested PD-L1 positive, while 9 patients (31.0%) in the age group of 50 - 70 years showed PD-L1 positivity. 50% of the patients (n = 5) in the age group of >70 years showed positive PD-L1 expression. However, the association between age and PD-L1 was not found to be statistically significant (p-value 0.308). The overall positivity of PD-L1 among females was 26.3% (5/19) and 48.4% (15/31) among males. No significant statistical association between gender and PD-L1 expression was found in our study (p-value 0.122). Our study did not show any significant association of age and sex with PD-L1 expression. The total number of smokers in this study was 13 (26%), all of which were males, and 37 (74%) patients were non-smokers. All the women in the present study were non-smokers. 46.2% of smokers (6/13) were PD-L1 positive while 53.8% (7/13) were PD-L1 negative. The association between smoking status and PD-L1 expression was not statistically significant in our study (p-value 0.236) (**Table 4**).

PD-L1 Expression across Various Tumor Sizes and Clinical Stages

Table 3. PD-L1 expression among the study population.

PD-L1 Expression	Number (n)	Percentage (%)
Positive	20	40.0
Negative	30	60.0
Tumor Proportion Score (TPS)		
<1%	30	60.0
1% - 49%	14	28.0
>50%	6	12.0

Table 4. Association between PD-L1 expression and different patient characteristics.

Patient Characteristics			PD-L1		Total	p-Value
			Negative	Positive		
Age	<50 years	Count	5	6	11	0.308 (NS)
		% within <50 yrs	45.5%	54.5%	100.0%	
	50 - 70 years	Count	20	9	29	
		% within 50 - 70 yrs	69.0%	31.0%	100.0%	
	>70 years	Count	5	5	10	
		% within >70 yrs	50.0%	50.0%	100.0%	
Gender	FEMALE	Count	14	5	19	0.122 (NS)
		% within females	73.7%	26.3%	100.0%	
	MALE	Count	16	15	31	
		% within males	51.6%	48.4%	100.0%	
	Yes	Count	7	6	13	
		% within smokers	53.8%	46.2%	100.0%	
Smoking Status	No	Count	23	14	37	0.236 (NS)
		% within non-smokers	37.9%	62.1%	100.0%	
	<3 cm	Count	5	2	7	
		% within <3 cm	71.4%	28.6%	100.0%	
	3 - 7 cm	Count	24	17	41	
		% within 3 - 7 cm	58.5%	41.5%	100.0%	
Tumor Size	>7 cm	Count	1	1	2	0.778 (NS)
		% within >7 cm	50.0%	50.0%	100.0%	
	STAGE I	Count	1	1	2	
		% within Stage I	50.0%	50.0%	100.0%	
	STAGE II	Count	1	2	3	
		% within Stage II	33.3%	66.7%	100.0%	
	STAGE III	Count	4	3	7	
		% within Stage III	57.1%	42.9%	100.0%	
	STAGE IV	Count	24	14	38	
		% within Stage IV	63.2%	36.8%	100.0%	
	G1, G2	% within G3	43.5	56.5	100.0%	
		Count	20	7	27	
	G1, G2	% within G1 and G2	74.1	25.9	100.0%	

28.6% of patients who had a tumor < 3 cm were PD-L1 positive ($n = 2$), whereas 71.4% of these patients were PD-L1 negative ($n = 5$). Among patients whose tumor size varied between 3 - 7 cm, 41.5% ($n = 17$) showed PD-L1 expression while 58.5% ($n = 24$) showed no expression of PD-L1. 50% of patients with a tumor > 7 cm in size tested positive for PD-L1 ($n = 1$) while the remaining 50% were negative ($n = 1$). Tumor size and PD-L1 expression showed no significant association (p-value 0.778) (**Table 4**).

Majority of the patients ($n = 38$) belonged to Stage IV in the present study. Among these Stage IV patients, 36.8% ($n = 14$) showed PD-L1 expression, while 63.2% ($n = 24$) showed no expression of PD-L1. Out of 7 patients who belonged to clinical Stage III, 42.9% ($n = 3$) were PD-L1 positive while 57.1% ($n = 4$) were negative. Among Stage II patients, 66.7% ($n = 2$) and 33.3% patients ($n = 1$) were PD-L1 positive and negative, respectively. 50% of Stage I patients ($n = 1$) showed PD-L1 expression. The association of PD-L1 with clinical stage was not significant statistically (p-value 0.764) (**Table 4**).

Association of PD-L1 with Tumor Histology

Out of 20 patients with PD-L1 positive expression, 17 patients (85%) had adenocarcinoma while 3 patients (15%) had squamous cell carcinoma histology. 60.5% ($n = 26$) of adenocarcinoma patients were PD-L1 negative and 57.1% ($n = 4$) of squamous cell carcinoma patients were PD-L1 negative. However, no significant association was found between tumor histologic type and PD-L1 expression (p-value 0.868) (**Figure 4**).

Correlation of PD-L1 with Adenocarcinoma Subtype and Tumor Differentiation

Among the adenocarcinoma group of patients ($n = 43$), 18 patients had solid predominant subtype. Among these 18, 12 patients showed a positive PD-L1 expression (66.6%) and the remaining 6 were PD-L1 negative (33.4%). The remaining subtypes of adenocarcinoma (acinar, papillary) ($n = 25$) showed PD-L1 positivity in only 5 cases (20%) while the rest were PD-L1 negative (80%, $n = 20$) (**Figure 5**). There was a highly significant statistical association between adenocarcinoma subtype and PD-L1 expression, with the solid subtype correlating with positive PD-L1 expression (p-value 0.004) (**Table 5**).

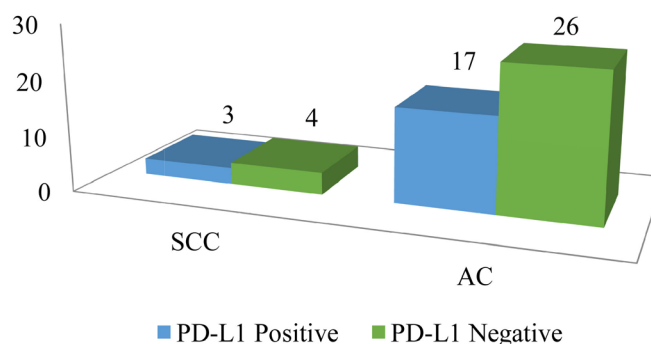


Figure 4. Association between PD-L1 expression with histologic type; Squamous Cell Carcinoma (SCC) and Adenocarcinoma (AC).

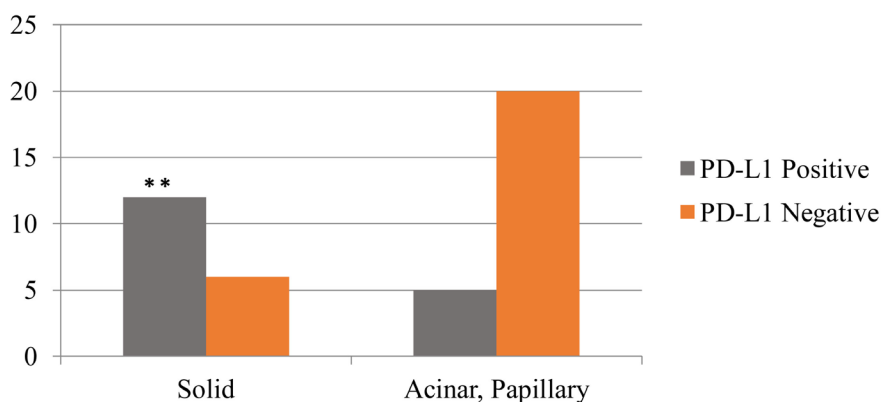


Figure 5. PD-L1 expression among histological subtypes of Adenocarcinoma.

Table 5. Association between PD-L1 expression with Adenocarcinoma subtype and Tumor Differentiation.

Tumor Characteristics			PD-L1		Total	p-Value
			Negative	Positive		
Adenocarcinoma Subtype	Solid	Count	6	12	18	0.004*
		% within Solid Subtype	33.4%	66.6%	100.0%	
	Acinar, Papillary	Count	20	5	25	
		% within Other Subtypes	80%	20%	100.0%	
Tumor Differentiation	G3	Count	10	13	23	0.043*
		% within G3	43.5	56.5	100.0%	
	G1,G2	Count	20	7	27	
		% within G1 and G2	74.1	25.9	100.0%	

Among 27 tumors belonging to well (G1) and moderately differentiated (G2) categories, 25.9% showed PD-L1 expression ($n = 7$) and 74.1% showed no expression of PD-L1 ($n = 20$). However, 56.5% of the tumors ($n = 13$) having poorly differentiated histology (G3) were positive for PD-L1 while 43.5% ($n = 10$) had <1% PD-L1 expression (**Figure 6**). This correlation was found to be statistically significant (p-value 0.043) (**Table 5**).

PD-L1 and its Correlation with Driver Mutations

PD-L1 expression was >1% in 3 cases (50%) out of 6 cases with Exon 19 mutations. Out of 3 cases with Exon 21 mutation, 1 case (33.3%) showed PD-L1 expression while the remaining 2 (66.7%) showed no expression. Among the 32 cases that tested negative for EGFR mutations, 43.8% ($n = 14$) showed positivity for PD-L1 while 56.2% ($n = 18$) were negative. There was no statistically significant association between EGFR mutations and PD-L1 expression (p-value 0.642) (**Table 6**).

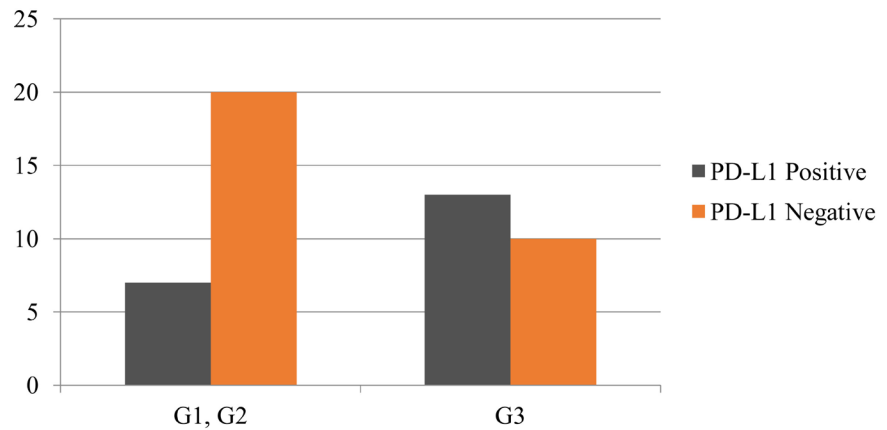


Figure 6. Association between PD-L1 and tumor differentiation.

Table 6. Association between PD-L1 expression and driver mutations.

Driver Mutation		PD-L1		Total	p-Value
		Negative	Positive		
EGFR mutation	Exon 19 deletion	Count: 3	Count: 3	6	0.642 (NS)
		%: 50.0%	%: 50.0%	100.0%	
	Exon 21 mutation	Count: 2	Count: 1	3	
		%: 66.7%	%: 33.3%	100.0%	
	No mutation	Count: 18	Count: 14	32	
		%: 56.2%	%: 43.8%	100.0%	
ALK Rearrangement	Data not available	Count: 7	Count: 2	9	0.415 (NS)
		%: 77.8%	%: 22.2%	100.0%	
	No rearrangement	Count: 16	Count: 14	30	
		%: 53.3%	%: 46.7%	100.0%	
	Data not available	Count: 13	Count: 5	18	
		%: 72.2%	%: 27.8%	100.0%	
ROS1 Mutation	Positive	Count: 1	Count: 1	2	0.487 (NS)
		%: 50.0%	%: 50.0%	100.0%	
	Positive	Count: 0	Count: 0	0	
		%: 0.0%	%: 0.0%	0.0%	
	Negative	Count: 15	Count: 8	23	
		%: 65.2%	%: 34.8%	100.0%	
	Data not available	Count: 15	Count: 12	27	
		%: 55.6%	%: 44.4%	100.0%	

50% of the patients (n = 1) having an ALK rearrangement showed PD-L1 expression, while 46.7% (n = 14) of those who did not have any ALK mutation were PD-L1 positive. No statistical association was found between PD-L1 expression and ALK mutation (p-value 0.415). None of the cases had ROS1 muta-

tion and 46.7% (n = 14) of these had positive PD-L1 expression. Data was unavailable in 18 cases. The association between ROS1 and PD-L1 expression was not statistically significant (p-value 0.487) (**Table 6**).

4. Discussion

Our study was carried out to analyze the pattern of PD-L1 expression in NSCLC patients, in a tertiary care hospital in Bangalore. To our knowledge, there are limited studies from India which have evaluated the expression profile of this important biomarker. A meta-analysis conducted by Minghui Z *et al.* included 47 different studies on PD-L1 expression and found PD-L1 positivity rates ranging from 14.3% to 75% among NSCLC patients [2]. Our study showed an overall PD-L1 positivity of 40%, which falls within this range. In a study by Archana GV *et al.*, no correlation was found between smoking and PD-L1 expression [11]. Hence, our study showed similar results as the other Indian study. No significant association was found between tumor size and PD-L1 expression among the subjects in the present study. This finding was contrary to that demonstrated by Yunjian P *et al.* who found that there was a significant association between positive PD-L1 expression (including TPS \geq 50% and TPS < 50%) in the ADC cohort with large tumor size [21]. Adenocarcinoma was the most common histological type of NSCLC in the current study. PD-L1 expression, however, showed no significant correlation with histologic type. Archana GV *et al.* found that higher PD-L1 positivity rates were shown by sarcomatoid carcinoma and solid predominant adenocarcinomas [11]. Our study is in agreement with these observations as we found a highly significant statistical association between positive PD-L1 expression (*i.e.* TPS 1% - 49% and >50%) with solid subtype of adenocarcinoma (p-value 0.004). Thus, we can conclude that patients with solid subtype of adenocarcinoma might show a positive PD-L1 expression and are likely to benefit from immune checkpoint/anti-PD-L1 therapy. Our study demonstrated that less tumor differentiation/poorly differentiated histology (G3) was significantly associated with PD-L1 positivity (p-value 0.043). This finding is in concordance with the finding by Kentaro Inamura *et al.*, who observed that PD-L1 expression was associated with less tumor differentiation [1]. In our study, we did not find any significant association between EGFR, ALK or ROS1 mutation status and PD-L1 positivity in our cohort. However, our study was limited by the small size and lack of follow-up data.

5. Conclusion

In our study, PD-L1 immunopositivity was found in 40% of patients and majority of them exhibited adenocarcinoma type of NSCLC. There was no correlation of PD-L1 expression with age, gender, clinical stage, smoking status and tumor histology. There was a highly significant statistical association between adenocarcinoma subtype and PD-L1 expression, with the solid subtype correlating with positive PD-L1 expression. Our study also demonstrated that less tumor

differentiation/poorly differentiated histology (G3) was significantly associated with PD-L1 positivity.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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