Original Article

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Immunohistochemical Expression of PD-L1 in Core Biopsy Samples of Non Small Cell Lung Cancer and its Association with Histopathological and Clinicoradiological Parameters

Pathology Section

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ABSTRACT

Introduction: Lung cancer is one of the most common causes of cancer-related mortality worldwide. In addition to chemotherapy, immunotherapy has yielded favourable outcomes in advanced, surgically non resectable Non Small Cell Lung Carcinoma (NSCLC). The inhibition of immune checkpoints by monoclonal antibodies targeting Programmed Death 1 (PD-1) and Programmed Death Ligand 1 (PD-L1) plays a pivotal role in preventing the downregulation of antitumour immunity.

Aim: To study the expression of PD-L1 in NSCLC cases from core biopsy specimens and its association with histological types, grades of tumour along with clinicoradiological parameters (age, sex, smoking status, and radiological location of the tumour).

Materials and Methods: This ambispective study was done in the Pathology Department of Kalinga Institute of Medical Sciences, Bhubaneshwar, Odisha, India, over two years from October 2020 to August 2022. The authors studied 35 cases of histopathologically and Immunohistochemically (IHC) diagnosed NSCLC. Partial or complete membranous staining of any intensity in ≥1% of tumour cells was considered positive in IHC. The Tumour Proportion Scoring (TPS) used for PD-L1 reporting was as follows: TPS <1%: negative, TPS 1-49%: low positive expression, and TPS ≥50%: high positive expression. Microsoft excel and Statistical Package for Social Sciences (SPSS) version 25.0 software were used for statistical analysis.

Results: The most common age group was 61-70 years (12 cases; 34.29%), with a male predominance 24 (68.60). Chronic smokers (16 cases; 45.71%) were commonly affected. Squamous Cell Carcinoma (SCC) was the common histological type (21 cases; 60%). Overall, 14 cases (40.0%) showed low (1-49%) and 5 cases (14.30%) showed high (≥50%) positive TPS of PD-L1 expression, respectively. Three cases (27.3%) of Grade 2 SCC and one case (10%) of Grade 3 SCC expressed high positivity. There was a statistical association between PD-L1 expression and male sex (p-value 0.01), positive smoking history (p-value 0.048), and SCC (p-value 0.032).

Conclusion: The PD-L1 expression is associated with increased tumour proliferation, aggressiveness, as well as shorter survival period in advanced NSCLC. The present study showed a significant association of PD-L1 expression in males, chronic smokers, and SCC. As per the existing literature, its association with clinicoradiological parameters is not clear. Future research with a larger cohort study, along with other predictive biomarkers, is highly desired to reach conclusive evidence.

Keywords: Adenocarcinoma, Immunotherapy, Programmed death ligand 1, Squamous cell carcinoma

INTRODUCTION

Worldwide, lung cancer is the most common cause of cancer-related deaths [1]. As per data from the Global Cancer Observatory (GLOBOCAN 2020), it is the 2nd common cancer, with an incidence of 2,206,771 patients per year and 1,796,144 deaths annually [2]. It represents approximately one in 10 (11.4%) cancer cases and one in 5 (18.0%) cancer-related deaths worldwide [2]. In India, the incidence is 5.9% of all cancers and 8.1% of cancer-related deaths [3].

In 2008, the World Health Organisation (WHO) classified lung carcinoma broadly into two histological types: Small Cell Lung Carcinoma (SCLC) and Non Small Cell Lung Carcinoma (NSCLC) [4]. SCLCs (15% of all lung cancer types) are neuroendocrine tumours. NSCLC is the most common type, comprising 80% of all tumour types [4]. According to the 2015 WHO classification of thoracic tumours, NSCLCs are subcategorised into Adenocarcinoma (ADC), Squamous Cell Carcinoma (SCC), Large Cell Carcinoma, and NSCLC-Not Otherwise Specified (NOS) [5]. The new WHO classification of thoracic tumours in 2021 has emphasised using Immunohistochemical (IHC) and molecular techniques for more accurate diagnosis [6].

There are many biomarkers used for the definitive diagnosis of lung cancer, such as Thyroid Transcription Factor-1 (TTF-1), Napsin-A, p63, Cytokeratin (CK 5/6), and neuroendocrine markers, along with many prognostic molecular indices like Anaplastic Lymphoma Kinase (ALK), ROS-1, Epidermal Growth Factor Receptor (EGFR), and PD-L1 [7].

Despite advanced treatment modalities, the prognosis of NSCLC remains poor. Immune checkpoint blockade has recently emerged as a promising and novel treatment for cancer. This has led to the development of immunotherapies, particularly antibodies targeting the PD-1 receptor and its ligand PD-L1 [8,9]. These have been extensively studied in lung cancer [8,9]. The primary role of PD-L1 is to mediate innate and adaptive immune resistance in tumour cells. CD8+ T-cells release Interferon Gamma (IFN-γ), which plays a major role in inducing PD-L1 expression [10,11]. Upregulation of PD-L1 expression can also occur due to oncogenic signaling by tumour cells, as evidenced by the high expression of PD-L1 in some cancers, despite a lack of Tumour-infiltrating Lymphocytes (TILs). Activation of T cells leads to the inhibition of the PD-1/PD-L1 interaction, allowing tumour cells to bypass immune surveillance. Thus, enhancing the active immune response against tumours can be achieved by blocking the PD-1/PD-L1 axis. Inhibitors of PD-1/PD-L1 have shown improvement in patients with PD-L1 overexpression compared with those with low or no expression of PD-L1 in lung cancer [12,13].

The rationale for the present study was to observe PD-L1 overexpression in NSCLC cases where patients may benefit from immunotherapy and to assess its association with various histological types and grades of tumours, along with clinicoradiological parameters.

The aim of this study was to analyse PD-L1 expression in NSCLC cases from core needle biopsy samples and its association with histological types, grades, and clinical parameters like age, sex, smoking status, and location of tumour by imaging studies.

MATERIALS AND METHODS

This ambispective study was done in the Pathology Department of Kalinga Institute of Medical Sciences, Bhubaneshwar, Odisha, India, over a period of two years (October 2020 to August 2022). A total of 14 cases from January 2019 to September 2020 were archived, and 21 cases were prospective from 2020 to 2022. Informed consent was obtained from all the patients before the procedures and also before IHC analysis in both prospective and retrospective cases. Approval by the Ethics Committee of the Institution, bearing IEC no. KIIT/KIMS/IEC/459/2020, was obtained before conducting the study.

Initially, 47 cases of lung mass lesions identified in radiology were selected for the present study. Three cases were excluded as they were diagnosed as small cell carcinoma on IHC. Out of the remaining 44 cases, four cases were proven to be metastatic carcinoma on IHC, three cases had extensive tumour necrosis which was unsuitable for IHC, and two were non representative biopsy samples. Therefore, 12 biopsy samples were excluded from the present study. Consequently, a total of 35 cases of clinically suspected and radiologically detected lung mass lesions were selected. These cases were histologically diagnosed and IHC confirmed to be NSCLC with subtyping.

Inclusion criteria: Core needle biopsies were taken from radiologically detected mass lesions in the lungs by various procedures like Computed Tomography (CT) guided Percutaneous Transthoracic Lung Biopsy (PTLB), flexible bronchoscopy-assisted Transbronchial Lung Biopsy (TBLB), and Endobronchial Ultrasound Bronchoscopy (EBUS). Cases that were histologically diagnosed and IHC confirmed to be NSCLC were included in the present study.

Exclusion criteria:

- Other types of lung cancer;
- Metastatic lesions;
- Non-representative biopsy samples;
- Tumours with extensive necrosis;
- Crushing artifacts.

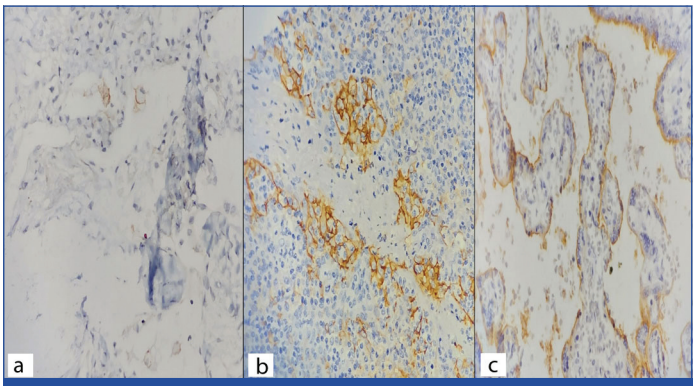
Study Procedure

A Baxter Deerfield IL Trucut biopsy needle was used for collecting PTLB samples from peripherally located lung masses. These samples were obtained from the Department of Radiodiagnosis. TBLB and EBUS samples were received from the Department of Pulmonary Medicine. The samples obtained by the aforementioned procedures were immediately fixed in 10% Neutral Buffered Formalin (NBF) and sent to the Pathology Department. Clinical data like age, sex, smoking status, and radiological findings were recorded.

Histology: All the core biopsy samples received in the Pathology Department were subjected to routine processing for 16 hours in a Leica automated tissue processor. Haematoxylin and Eosin (H&E)-stained slides were prepared from Formalin-fixed Paraffin-embedded (FFPE) blocks of tissue. Tumour subtyping and grading were done according to the WHO classification of Thoracic Tumours (5th edition, 2021) [6].

Immunohistochemistry (IHC): Rabbit antihuman monoclonal antibody (clone QR001) from BIOCYC, Germany, was used for the PD-L1 study. IHC was done according to standard protocols.

Quality Control [Table/Fig-1a-c]: External positive and negative control slides were used with each batch of IHC staining. For the positive external control of PD-L1, tonsil and placental tissue were used. Strong membranous staining of the tonsillar epithelium and trophoblastic cells in the placenta were considered positive external controls. The omission of the primary antibody and the incubation of the test slide in Phosphate Buffer Saline (PBS) for each batch of staining served as the negative control. Membranous staining of alveolar macrophages acted as a positive internal control.



[Table/Fig-1]: Positive internal and external control of PDL1 expression by IHC. a) Positive internal control (membranous staining of alveolar macrophages, IHC 200x); b)Positive external control (membranous staining of epithelial cells of tonsil, IHC 400x); c) Positive external control (membranous staining of trophoblastic cells of placenta, IHC 400x)

Scoring Pattern: A minimum of 100 viable tumour cells were counted per core biopsy specimen, and the Tumour Proportion Score (TPS) was measured. Complete or partial membranous pattern with or without cytoplasmic staining of any intensity, was considered positive. Only cytoplasmic staining was not considered to be positive. PD-L1 expression was assigned scores based on the following TPS system [12]:

- TC0 (Negative): Membranous staining in less than 1% of tumour cells.
- TC1 (Low positive): Membranous staining in 1% to less than 50% of tumour cells.
- TC2 (High positive): Membranous staining in 50% or more of tumour cells.

STATISTICAL ANALYSIS

Microsoft excel and SPSS version 25 software were used for statistical analysis. A p-value of less than 0.05 using the Chi-Square test was considered significant.

RESULTS

Out of 35 cases in the present study, 20 (57.14%) cases were TBLB, 10 (28.57%) cases were PTLB, and 5 (14.29%) cases were EBUS.

Clinical Parameters: Of the 35 cases of NSCLC included in the present study, the most commonly affected age group was 61-70 years 12 (34.29%) cases, with a mean age of 57.74 years. There was a male predominance 24 (68.60%) cases over females 11 (31.40%) cases, with an M:F ratio of 2.18:1. Chronic smokers 16 (45.71%) cases were more affected, followed by non smokers 15 (42.86%) cases and exsmokers 4 (11.43%) cases. The right lung was a more common location 25 (71.43%) cases, and the upper lobes were most frequently involved in both lungs 17 (48.57%) cases [Table/Fig-2].

Association between PD-L1 expression and clinicoradiological parameters [Table/Fig-2]: In the present study, a statistically significant correlation was observed between PD-L1 expression and male gender (p-value 0.019) as well as the smoking status of the patients (p-value 0.046). Radiologically, 25 (71.43%) cases had right lung involvement and 10 (28.57%) cases had left lung involvement. Of the right lung NSCLC cases, 10 (40%) cases showed negative, 11 (44%) cases showed low positive, and 4 (16%) cases showed

Parameters	Category	No. of cases (%)	PD-L1 expression {n (%)}			p-value
			Negative	Low positive	High positive	
Age (years)	<60	19 (54.3%)	7 (36.8%)	8 (42.1%)	4 (21.1%)	0.179
	≥60	16 (45.7%)	9 (56.3%)	6 (37.5%)	1 (6.3%)	
Sex	Male	24 (68.6%)	8 (33.3%)	11 (45.8%)	5 (20.8%)	0.019
	Female	11 (31.4%)	8 (72.7%)	3 (27.3%)	00	
Smoking status	Chronic	16 (45.7%)	5 (31.3%)	8 (50%)	3 (18.8%)	0.046
	Ex-smoker	04 (11.4%)	1 (25%)	2 (50%)	1 (25%)	
	Nonsmoker	15 (42.9%)	10 (66.7%)	4 (26.7%)	1 (6.7%)	
Location of tumour	Right lung	25 (71.43%)	10 (40%)	11 (44%)	04 (16%)	0.5613
	Left lung	25 (71.43%)	06 (60.0%)	03 (30.0%)	1 (10.0%)	

[Table/Fig-2]: Association of PD-L1 expression with clinical parameters. (N=35)

high positive PD-L1 expression. Of the left lung NSCLC cases, 6 (60%) cases, 3 (30%) cases, and 1 (10%) cases showed negative, low positive, and high positive PD-L1 expression, respectively. The location of NSCLC and PD-L1 expression was not statistically significant (p-value=0.5613).

NSCLC subtyping and grading [Table/Fig-3,4]: Histomorphologically, the most frequent type was squamous cell carcinoma (SCC) 21 (60%) cases, followed by Adenocarcinoma (ADC) 13 (37.14%) cases, and adenosquamous carcinoma 1 (2.86%) cases. Of the 13 ADC cases, 6 (17.14%) cases each were Grade 1 and Grade 2, while only one case belonged to Grade 3 (2.86%). Of the 21 SCC cases, Grade 2 was the most common 11 (31.43%) cases, followed by Grade 3 10 (28.57%) cases. There were no Grade 1 SCC cases. The single case (2.86%) of adenosquamous cell carcinoma was Grade 3.

Parameters		No. of cases (%)	PD-L1 expression n (%)			p-value
			Negative	Low positive	High positive	
Subtypes	ADC	13 (37.14%)	7 (53.8%)	6 (46.2%)	00	0.032
	SCC	21 (60.0%)	9 (42.9%)	8 (38.1%)	4 (19.0%)	
	Adenosquamous	1 (2.86%)	00	00	1 (100%)	
Grades	Grade 1	6 (17.14%)	3 (50%)	3 (50%)	00	0.065
	Grade 2	17 (48.57%)	5 (29.4%)	9 (52.9%)	3 (17.6%)	
	Grade 3	12 (34.29%)	8 (66.7%)	2 (16.7%)	2 (16.7%)	

[Table/Fig-3]: Association of PD-L1 expression with histopathological types and grades of tumours (N=35).

Tumour Proportion Score (TPS)	PD-L1 expression	Number of cases	Percentage (%)
<1%	Negative	16	45.70
1-49%	Low positive	14	40.00
≥50%	High positive	05	14.30
Total cases		35	100

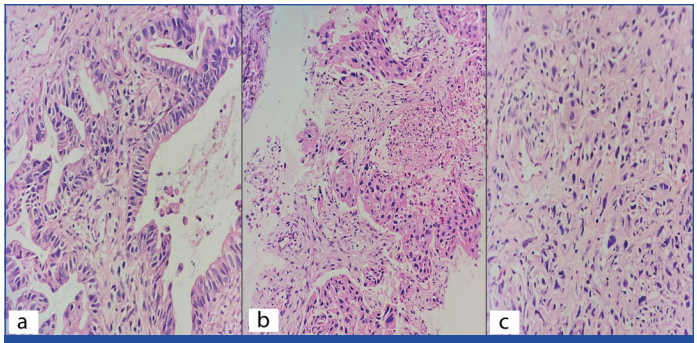
[Table/Fig-4]: PD-L1 expression and tumour proportion score (TPS) in NSCLC (n=35).

Association between PD-L1 expression and histopathological types and grades [Table/Fig-3]: A significant association of PD-L1 expression with SCC (p=0.032) was found, but there was no significant association with the grades of tumours (p-value=0.065).

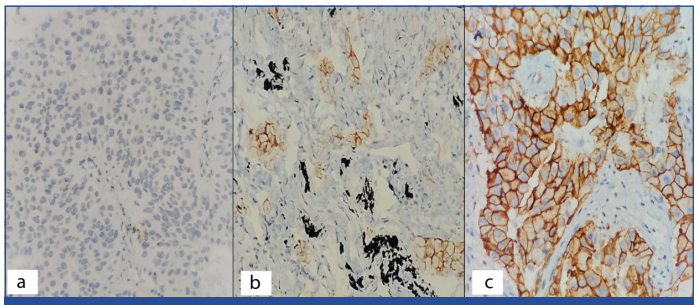
PD-L1 expression in histological types and grades of NSCLC [Table/Fig-4-7]: Of the 35 NSCLC cases, 16 (45.70%) cases showed negative PD-L1 expression, 14 (40.0%) cases showed low positive expression, and 5 (14.30%) cases expressed high positivity.

Of the 13 ADC cases, 7 (53.8%) cases and 6 (46.2%) cases showed negative and low positive expression, respectively, with no cases showed high positive expression. Among the 21 SCC cases, 9 (42.9%) cases, 8 (38.1%%) cases, and 4 (19.0%) cases showed negative, low, and high positive expression, respectively.

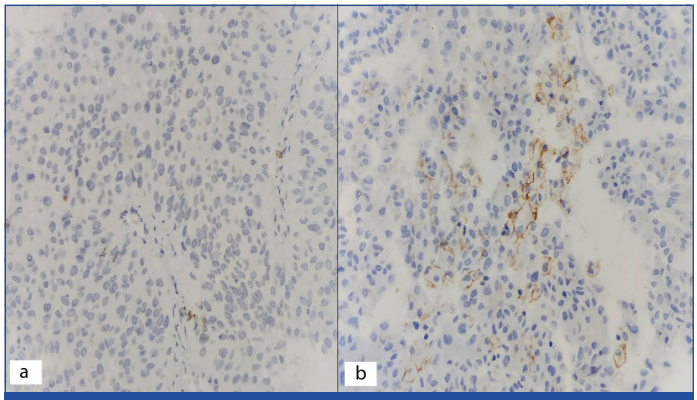
In terms of overall grading, 6 (17.14%) cases were Grade 1, 17 (48.57%) cases were Grade 2, and 12 (34.29%) cases were Grade 3. Of the Grade 1 cases, 3 (50%) cases each were negative and low positive. There were no high positive ADC cases. Of the Grade 2 cases, 5 (29.5%) cases, 9 (52.9%) cases, and 3 (17.6%) cases showed negative, low positive, and high positive PD-L1 expression, respectively. Of the 12 Grade 3 cases, 8 (66.6%) cases exhibited negative expression, while 2 (16.7%) cases each expressed low and high positive PD-L1 expression.



[Table/Fig-5]: Histological types of NSCLC. a) ADC- Tumour cells are arranged in glandular pattern (H&E 400x); b) SCC- Pleomorphic tumour cells are arranged in nests and irregular islands (H&E 200x); c) ADSC- Highly pleomorphic tumour cells in diffuse pattern (H&E 400x).



[Table/Fig-6]: PDL1 expression in SCC by IHC. a) Negative (200x); b) Low positive (200x); c) High positive (400x).



[Table/Fig-7]: PDL1 expression in ADC by IHC. a) Negative (400x); b) Low positive (400x).

Of the 13 cases of ADC, 6 (46.15%) cases each were classified as Grade 1 and Grade 2, while 1 (7.7%) case was Grade 3. The single case of Grade 3 ADC showed negative PD-L1 expression. Out of the six cases of both Grade 1 and Grade 2 ADC, three cases each (50%) showed negative and low positive expression.

Of the 21 cases of SCC, there were no Grade 1 cases. Among the 11 cases in Grade 2, 2 (18.1%) cases displayed negative PD-L1 expression, 6 (54.5%) cases showed low positive expression, and 3 (27.2%) cases exhibited high positive expression. Of the 10 cases of Grade 3 SCC, 7 (70.0%) cases expressed negative PD-L1, 2 (20.0%) cases had low positive expression, and 1 (10.0%) case showed high positive expression. One case of adenosquamous carcinoma demonstrated high positive PD-L1 expression [Table/Fig-8].

DISCUSSION

In the present era of personalised medicine, small biopsies and cytology samples play a crucial role in the diagnosis, management,

Subtype	Tumour grade	Pd-L1 expression		
		Negative n (%)	Low positive n (%)	High positive n (%)
ADC	Grade 1	3 (50%)	3 (50%)	00
	Grade 2	3 (50%)	3 (50%)	00
	Grade 3	1 (100%)	00	00
SCC	Grade 1	00	00	00
	Grade 2	2 (18.1%)	6 (54.5%)	3 (27.2%)
	Grade 3	07 (63.6%)	3 (27.2%)	1 (9.1%)
ADSC	Grade 3	00	00	1 (100%)

[Table/Fig-8]: PD-L1 expression according to grades of subtypes of NSCLC (n=35).

as well as prioritisation of tumour material for multiple predictive biomarker testing in NSCLC.

The body's immune system plays an essential role in regulating tumour growth. Immunotherapy harnesses the specificity and long-term memory of the adaptive immune response, which causes durable regression of tumour cells and potentially achieving a cure [13]. Immunocompromised patients are at an increased risk of developing cancer [14]. The emergence of immune checkpoint blockers has revolutionised the treatment of various advanced malignancies. The treatment of inoperable and locally advanced NSCLC had not seen significant improvements for decades until, in 2016, the phase III trial led to the FDA's approval of Pembrolizumab as a frontline treatment for NSCLC with high positive PD-L1 expression [15]. Subsequently, the PACIFIC trial for stage III NSCLC has revolutionised the use of Durvalumab, an anti-PD-L1 antibody, as maintenance therapy after coeval chemoradiotherapy [16,17]. This has spurred great interest in research into the PD-L1 pathway [16,17].

The PD-L1 pathway is a crucial mechanism for immune escape by Tumour-infiltrating Lymphocytes (TILs) in NSCLC [18,19]. The blockade of immune checkpoints is the most commonly used predictor, assessed by PD-L1 expression in tumour cells for advanced NSCLC. The correlation between PD-L1 expression and clinicoradiological parameters is little known [20,21].

Most patients in the present study were under the age of 60 (54.3%). Sahin S et al., found similar results in their study, with 51% of cases being below 60 years of age. The present study identified 68.6% of cases as male [22]. Sahin S et al., and Kim H et al., also found similar findings in their studies, with 88.5% and 62.4% male patients, respectively [22,23]. The authors found a statistically significant association between PD-L1 expression and male sex (p=0.019).

In the present study, the association of NSCLC was more prevalent in chronic smokers (45.7%) followed by non smokers (42.9%). The correlation between PD-L1 expression and smoking status was statistically significant, with a p-value of 0.046. The present study revealed that SCC was the most common subtype with 21 cases (60.0%), followed by adenocarcinoma (ADC) with 11 cases (37.14%), and only one case of adenosquamous carcinoma. Studies by Sahin S et al., and Schmidt LH et al., showed that the majority of cases were SCC (51.4%) followed by ADC (46.0%) [22,24].

Overall, PD-L1 expression was observed in 19 cases (54.3%), out of which five cases (14.3%) showed high positivity (≥ 50%) and 16 cases (45.7%) were negative. This is consistent with findings by Jain E et al., who found the majority of cases (53.2%) to have positive PD-L1 expression [25]. In the present study, 57.1% of SCC cases showed positive PD-L1 staining, similar to the studies Sahin S et al., [22]. We found a significant correlation between PD-L1 expression and SCC, with a p-value of 0.032.

For ADC, 53.8% showed negative PD-L1 expression, and 46.2% showed low positive expression, with no cases exhibiting high positivity. In contrast, in SCC, 42.9% were negative, 38.1% had low positive expression, and 19.0% had high positive expression. The single case of adenosquamous carcinoma expressed high positivity. These findings are in line with those of Kim H et al., who noted that the majority of ADC cases (83.8%) were negative, followed by 10.1% with low positivity and only 6.1% with high positivity [23]. They also found that the majority of SCC cases (74.8%) were negative, with 15.3% being low positive and 9.9% high positive.

Regarding the overall grading among all NSCLC subtypes, the authors observed 6 (17.14%) cases in Grade 1, 17 (48.57%) cases in Grade 2, and 12 (34.29%) cases in Grade 3. This distribution is similar to the findings of Dix Junqueira Pinto G et al., who reported the most cases (45.8%) in Grade 2, followed by (28.2%) in Grade 3, and the fewest cases (2.8%) in Grade 1 [26]. Of the 13 ADC cases in the present study, 6 (46.15%) cases were Grade 1, 6 (46.15%) cases were Grade 2, and 1 (7.7%) case was Grade 3. For SCC, 11 (52.4%) cases were Grade 2, 10 (47.6%) cases were Grade 3, with no cases in Grade 1.

In the present study, the authors did not find a significant correlation between PD-L1 expression and other parameters like age, tumour location, and tumour grade.

Limitation(s)

The present study was limited by the small number of cases. A further prospective study with a larger sample size, along with analysis of other molecular indices like ALK, ROS-1, EGFR, etc., using an independently collected cohort, is necessary to validate the authors approach.

CONCLUSION(S)

The PD-L1 expression by different tumours, including NSCLC, is one of the most predictive biomarkers investigated in the present era. This has benefited the use of immune checkpoint blockers in advanced NSCLC, causing higher treatment response rates and increased disease-free survival. The present study showed significant association of PD-L1 expression with male sex, chronic smoking, and SCC. There was no significant association with other clinicopathological parameters like age, tumour location, histological types, and grades. Additional studies with larger sample sizes are needed to establish this as a reliable and dynamic predictive biomarker.

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