ORIGINAL ARTICLE

# IMMUNOHISTOCHEMICAL EXPRESSION OF PD-L1 IN NON-SMALL CELL LUNG CARCINOMA

**Muhammad Umair Khan, Hafeez ud Din, Wajahat Ahmed Khan**

**Armed Institute of Pathology, NUMS, Rawalpindi-Pakistan**

**Background:** Lung cancer is a leading cause of death related to cancer worldwide, surpassing the number of deaths caused by breast, colon and prostate cancers. Objective of the study was to determine the frequency immunohistochemical expression of PD-L1 in non-small cell lung carcinoma (NSCLC). It was a descriptive cross-sectional study, carried out at the Department of Histopathology, Armed Forces Institute of Pathology, Rawalpindi, from January 2020 to July 2021. M**ethods:** We inducted total of 145 diagnosed cases of NSCLC irrespective of age, gender, histological grade and stage of carcinoma. Immunohistochemical staining of PD-L1 was done using Leica kit. Patients with no expression for PD-L1 were considered negative, whereas immunohistochemical expression of ≥1% is considered positive. PD-L1 statuses of all patients was determined. The data was analyzed by using SPSS version 25. **Results:** In our study age ranges from 31 to 85 years with mean age of 64.20±10.90 years. Out of the 145 patients, 126 (86.90%) were male and 19 (13.10%) were females. 108 (74.50%) were squamous cell carcinoma and 37 (25.50%) were adenocarcinoma. Most of them were histological grade II, 88 (60.70%), followed by grade I in 26 (17.9%). Immunohistochemical expression of PD-Ll in NSCLC was found in 35 (24.13%) patients. Of all the positive cases, PD-L1 expression was slightly higher in adenocarcinoma 27.02% as compared to squamous cell carcinoma 23.14%. **Conclusion:** PD-L1 expression is associated with increased aggressiveness, enhanced tumor progression and shorter survival in patients of NSCLC. This study concluded that frequency of positive immunohistochemical expression of PD-L1 in NSCLC is though low, yet significant in adenocarcinoma as compared to squamous cell carcinoma.

**Keywords:** Non-small cell lung carcinoma (NSCLC); Programmed death 1; Programmed death ligand 1

**Citation:** Khan MU, Din HU, Khan WA. Immunohistochemical expression of PD-L1 in non-small cell lung carcinoma. J Ayub Med Coll Abbottabad 2024;36(3):611–5.

**DOI:** 10.55519/JAMC-03-13135

## INTRODUCTION

Lung cancer is a leading cause of death related to cancer worldwide, surpassing the number of deaths caused by breast, colon and prostate cancers.[1] Of all lung cancers non-small cell lung cancer comprises 80% with majority of them diagnosed as either adenocarcinoma or squamous cell carcinoma.[2] Over the period of time, increased awareness and owing to advances leading to targeted therapies, studies report slight betterment in prognosis of even metastasized cases. Advanced immunotherapy via antibodies works against checkpoint regulating proteins like PD-L1.[3]

Programmed death ligand 1 (PD-L1), also known as CD-274, is considered an immune checkpoint facilitating anti-tumor suppression of the immune pathway. Various cells exhibit expression of PD-L1 on their surface. Expression of PD-L1 in macrophages, helper B and T lymphocytes, epithelial cells, muscles cell and endothelial cells has been known. It is also copiously found on neoplastic cells like lung, ovarian and breast carcinomas, squamous cell carcinoma and glioblastoma.[4] PD-1 is mainly expressed in cytotoxic T lymphocytes. PD-L1 is also known as an "immune checkpoint protein" in literature as it regulates and is involved in anti-tumor suppression. Binding of PD-L1 to PD-1 inhibits immune response. During inflammation this association of PD-L1 and PD-1 prevents an autoimmune response in peripheral cells. This inhibition of immune response is carried out via two methods. One is inhibition of synthesis of interleukin-2, and second method is inhibitory effect on T-cell receptors eliciting a stop signal.[5] It might alter the contact between T-cells, target cells and antigen presenting cells.[6] Studies depict increased levels of PD-L1 expression on cell surface of various cancer cell in NSCLC. Giving rise to this apprehension that PD-L1 expression enables cancer cells to escape immune response. The use of monoclonal antibodies is considered successful in inhibiting the contact between PD-L1 receptor and PD-1 receptor. These monoclonal antibodies competitively bind to PD-1 receptor thus inhibiting contact between PD-L1 and

PD-1 receptor.[7] This inability of cancer cells to alter the function of T cells aids in active immune response.

Emergence of PD-L1 directed therapies have evolved the modalities of treatment in oncology field. Monoclonal antibodies have been known to markedly increase anti-tumor immune response as per literature.[7] The identification of elements contributing to good response to immunotherapy is considered helpful and important specifically in cases of NSCLC.[8] However, the link between PD-L1 expression and clinicopathological parameters is still unclear. Owing to room for research in this aspect, we analyzed the association between PD-L1 expression and clinicopathological parameters to study prognostic and predictive impact in NSCLC in our population.

## MATERIAL AND METHODS

In this cross-sectional study 145 patients were included using non-probability consecutive sampling technique. All newly diagnosed cases of NSCLC irrespective of age, gender, histological grade and histological type, received at Department of Histopathology, Armed Forces Institute of Pathology Rawalpindi over a period of 24 months from January 2020 to December 2021. WHO sample size calculator was used to calculate the sample size, with a confidence interval of 95%, margin of error 5% and taking into consideration the probability of 10% in previous studies; sample size of 140 came to be adequate.[9] Formalin-fixed, paraffin-embedded tissues were selected. 3–5µm tissue sections are cut with semi-automated rotary microtome. After sectioning, tissues are mounted on Dako FLEX IHC microscope slides and then placed in a 58±2 ºC oven for 1 hour. Deparaffinization, rehydration and target retrieval are done by heat-induced epitope retrieval method. 3,3N-Diaminobenzidine Tetrahydrochloride (DAB) and substrate-chromogen solution are used. Leica Bond Autostainer is used for staining preprogrammed with Dako Link software.

Immunohistochemical staining for PD-L1 was done using Leica kit, clone 22-C3 on separate sections as per manufacturer's instructions. Results were studied on high power field objective lens. The results were verified by 2 different consultant histopathologists. Viable tumor cells exhibiting complete circumferential or partial linear plasma membrane staining at any intensity were graded as positive in the study. Immunohistochemical expression of PD-L1 in <1% was considered negative and ≥1% was considered positive, which was further subdivided into weak positive, when staining is between 1–49% while >50% was considered strong positive.[10] Statistical data analysis was done using SPSS version 25.0. (IBM Corp., Armonk, NY). Frequency and percentages were calculated for age, gender, histological grade and immunohistochemical expression of PD-L1.

## RESULTS

Age range in our study was from 31–85 years with mean age of 64.20±10.90 years. Majority of the patients 111 (76.55%) were between 50–75 years of age. Out of the 145 patients, 126 (86.90%) were male and 19 (13.10%) were females with male to female ratio of 6.63:1 as shown in figure 1. When stratification was done according to histological type, there were 108 (74.48%) cases of squamous cell carcinoma and 37 (25.52%) cases of adenocarcinoma as shown in figure 2. Most common histological grade of the tumors was moderately differentiated with 88 (60.90%) cases, followed by poorly differentiated with 31 (21.40%) cases and then well differentiated with 26 (17.90%) cases as shown in figure 3.

Immunohistochemical expression of PD-Ll in NSCLC was found in 35 (24.13%) patients as shown in figure 4. When stratification of immunohistochemical expression of PD-Ll was done on age groups, gender, histological subtypes and histological grades, it was found that there was no significant difference between different age groups, gender, histological subtypes and grade and stage of tumor as illustrated in table I.

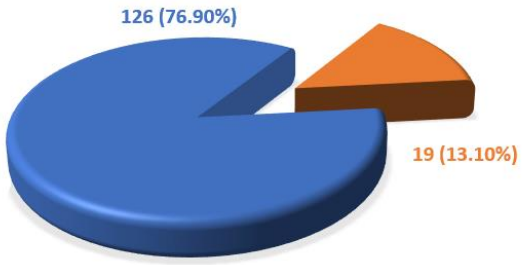


**Figure-1: Distribution of patients according to gender (n=145).**

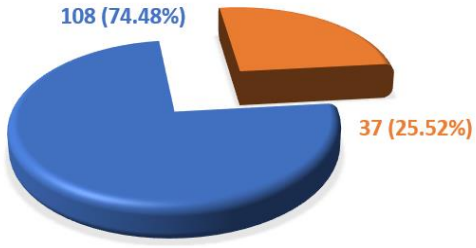


**Figure-2: Distribution of patients according to histological type (n=145).**

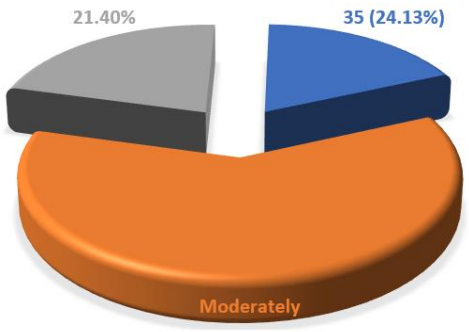


**Figure-3: Frequency of histological grade (n=145)**

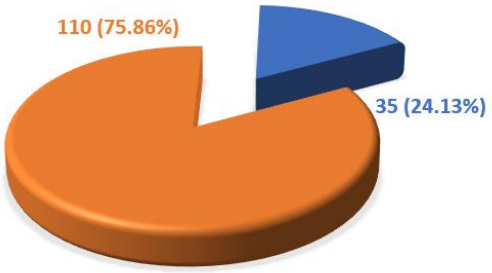


**Figure-4: Frequency of immunohistochemical expression of PD-L1 in non-small cell lung carcinoma (n=145).**

**Table-1: Stratification of immunohistochemical expression of PD-L1 (n=145).**

| Variables | Negative, n (%) | Positive, n (%) |
|---|---|---|
| **Age** | | |
| 20-45 years | 32 (74.42) | 11 (25.58) |
| 46-70 years | 78 (76.47) | 24 (23.53) |
| **Gender** | | |
| Male | 88 (77.20) | 26 (22.80) |
| Female | 22 (70.96) | 9 (29.04) |
| **Histological grade** | | |
| Well (I) | 18 (69.24) | 8 (30.76) |
| Moderate (II) | 66 (75) | 22 (25) |
| Poor (III) | 26 (83.87) | 5 (16.13) |
| **Histological subtype** | | |
| Squamous cell carcinoma | 83 (76.86) | 25 (23.14) |
| Adenocarcinoma | 27 (72.98) | 10 (27.02) |

## DISCUSSION

Programmed death ligand 1 (PD-L1) is regarded as an immunological checkpoint that enables the immune system to be suppressed against tumors. The crucial immune checkpoint proteins programmed death protein 1 (PD-1) and programmed death ligand 1 (PD-L1) are in responsible for controlling the stability and consistency of T-cell immunological activity negatively. Anti-PD-1/PD-L1 medications have been created for immune checkpoint blocking and can cause clinical responses in a variety of cancer types, offering fresh hope for the treatment of cancer. However, the existing anti-PD-1 or anti-PD-L1 medicines have a low incidence of patient response, and many initial responders eventually acquire resistance to them.[11] Adenocarcinoma and squamous cell carcinoma are the two primary histological subtypes of NSCLC, and the identification and characterization of criteria to define individuals with favorable response to immunotherapy appears to be of utmost importance in these particular instances.[12]

In our study, 111 (76.55%) out of a total of 145 cases had a presentation at or above the age of 50. Lung cancer often manifests at a mean age of 64.5±10.3 years, with >90% of cases being over 50 years of age.[13]

Males tend to develop lung cancer more often. 86.90% of the affected individuals in our study were men. In a research by Chang et al., which included 186 patients, 89.8% of the cases of lung carcinomas were male, confirming the findings of our study that males are more likely to develop lung cancer.[14] In a different study carried out in the United States by Devesa *et al*. 83.6% of the cases were males.[15]

In our geographical region, squamous cell carcinoma predominates over adenocarcinoma. In contrast to our study, Milovinovic *et al.'s* study, adenocarcinoma is the most prevalent histologic type.[13] Squamous cell lung carcinoma has been replaced by adenocarcinoma as the most common NSCLC histologic type in recent years, especially in advanced countries.[15]

In our study, 35 (24.13%) out of 145 patients had immunohistochemistry evidence of PD-Ll expression in their NSCLC. A comparable study conducted in China found that lung cancer was present in 40 out of 104 (38.5%) participants.[16] Another study carried out in Taiwan found that 33 out of 43 (76.7%) lung cancer cases had positive PD-L1 expression.[17] Out of 186 cases of small cell lung cancer, 145 (78%) have a high rate of PD-L1 immunoexpression.[17]

In our study, higher grade lung carcinomas have immunohistochemistry expression of PD-L1 at significant rates. Igarashi et al. found a higher level of expression of PD-L1 in moderately and poorly differentiated adenocarcinomas compared to well differentiated adenocarcinomas, which is similar to our results.[18] Higher PD-L1 expression was noted in the meta-analysis conducted by Zhang et al. in cases of higher histological grade carcinoma.[19]

In our study, adenocarcinoma (27.02%) had somewhat higher immunohistochemistry expression of PDL1 than squamous cell carcinoma (23.14%). A comparable study done in China found that 38.5% of lung adenocarcinoma cases displayed PD-Ll positive.[9] Another study conducted in Taiwan found that PD-L1

was expressed in 66.8% of lung cancer cases.[20] Only 32.8% of patients in research on a cohort of 678 patients showed varied intensity membranous PD-L1 expression.[21] Several researches have produced varying findings in this area. PDL1 expression was found to be 34% in both squamous and adenocarcinoma in research by Scheel *et al*.[22] Similar to Scheel *et al*., Lin et al. evaluated PDL1 expression in NSCLC using the same standards of positive in 1% of tumor cells. In contrast, they found that squamous cell carcinoma had a greater positive rate (46%) than adenocarcinoma (27%).[23] Cooper *et al*. also reported 8% PD-L1 positivity in squamous cell carcinoma and 5% positivity in adenocarcinoma, which is in contrast to our results.[21]

PD-L1 immunohistochemistry staining intensity varies among NSCLC cells. In certain instances, NSCLC tissue samples contained cells with varying degrees of PD-L1 staining intensity. These results imply that PD-L1 expression levels should be assessed throughout a tissue segment.[18] In a study with 208 patients in China, it was discovered that PD-L1 expression was detected in 136 (65.3%) of the NSCLC cases using the immunotherapy response score (IRS) scale, when the threshold value exceeded 3.[12,24] However, in the Cooper *et al.* study, where 32% of patients had PD-L1 expression (detected by rabbit monoclonal antibody E1L3N, MA1:200), positive expression was found when more than 50% of cells were PD-L1 positive.[21] In contrast to Tang *et al*., who considered IHC to be positive when PD-L1 expression was found in more than 5% of NSCLC cells, IHC intensity was not taken into consideration in that study. In this trial, 65.9% of patients responded favorably, which was significantly higher than in Cooper's study.[21]

The impact of this protein on accelerated tumor cell proliferation is explained by the escape of cancer cells from the immune system caused by PD-L1 expression.[25] The outcome for patients with high PD-L1 levels should be worse as a result of this process, which should cause tumor growth. Patients with high PD-L1 expression frequently have large tumor size, lower histologic grade, and a high number of tumors infiltrating lymphocytes (TILs).[8]

## CONCLUSION

PD-L1 expression is associated with increased aggressiveness, enhanced tumor progression and shorter survival in patients of NSCLC. This study concluded that frequency of positive immunohistochemical expression of PD-L1 in NSCLC is though low, yet significant. Development of PD-1 and PD-L1-targeted therapies have revealed their anti-tumor effects with significant responses in patients with NSCLC. However, the relationship between the expression level of PD-L1 protein and clinicopathological factors is not clear and studies have revealed contradictory results also the inhibition of PD-1 and PD-L1 axis is a reliable medicinal target for NSCLC. However, therapeutic biomarkers in these molecular-targeted therapies remain to be established, and an actual role of PD-L1 in the progression of NSCLC is yet to be explained, further research is recommended to determine the association between PD-L1 expression and the clinicopathological factors of NSCLC.

## AUTHORS' CONTRIBUTION

MUK: Conception of study, data collection, drafting. HUD: Conception of the study, drafting. WAK: Data collection, drafting.

## REFERENCES


1. Cruz CSD, Tanoue LT, Matthay RA. Lung cancer: epidemiology, etiology, and prevention. Clin Chest Med 2011;32(4):605–44.
2. Glatzel-Plucinska N, Piotrowska A, Grzegrzolka J, Olbromski M, Rzechonek A, Dziegiel P, *et al*. SATB1 level correlates with Ki-67 expression and is a positive prognostic factor in non-small cell lung carcinoma. Anticancer Res 2018;38(2):723–36.
3. Kanitkar AA, Schwartz AG, George J, Soubani AO. Causes of death in long-term survivors of non-small cell lung cancer: A regional Surveillance, Epidemiology, and End Results study. Ann Thorac Med 2018;13(2):76–81.
4. Konishi J, Yamazaki K, Azuma M, Kinoshita I, Dosaka-Akita H, Nishimura M. B7-H1 expression on non-small cell lung cancer cells and its relationship with tumor-infiltrating lymphocytes and their PD-1 expression. Clin Cancer Res 2004;10(15):5094–100.
5. Sheppard KA, Fitz LJ, Lee JM, Benander C, George JA, Wooters J, *et al*. PD-1 inhibits T-cell receptor induced phosphorylation of the ZAP70/CD3ζ signalosome and downstream signaling to PKCθ. FEBS Lett 2004;574(1-3):37–41.
6. Parra ER, Villalobos P, Mino B, Rodriguez-Canales J. Comparison of different antibody clones for immunohistochemistry detection of programmed cell death ligand 1 (PD-L1) on non–small cell lung carcinoma. Appl Immunohistochem Mol Morphol 2018;26(2):83–93.
7. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12(4):252–64.
8. Ribas A, Tumeh PC. The future of cancer therapy: selecting patients likely to respond to PD1/L1 blockade. Clin Cancer Res 2014;20(19):4982–4.
9. Mery CM, Pappas AN, Bueno R, Colson YL, Linden P, Sugarbaker DJ, *et al*. Similar long-term survival of elderly patients with non-small cell lung cancer treated with lobectomy or wedge resection within the surveillance, epidemiology, and end results database. Chest 2005;128(1):237–45.
10. Ribas A, Hu-Lieskovan S. What does PD-L1 positive or negative mean? J Exp Med 2016;213(13):2835–40.
11. Ghosh C, Luong G, Sun Y. A snapshot of the PD-1/PD-L1 pathway. J Cancer 2021;12(9):2735–46.
12. Chen YY, Wang LB, Zhu HL, Li XY, Zhu YP, Yin YL, *et al*. Relationship between programmed death-ligand 1 and clinicopathological characteristics in non-small cell lung cancer patients. Chin Med Sci J 2013;28(3):147–51.

13. Milovanovic IS, Stjepanovic M, Mitrovic D. Distribution patterns of the metastases of the lung carcinoma in relation to histological type of the primary tumor: an autopsy study. Ann Thorac Med 2017;12(3):191–8.
14. Chang YL, Yang CY, Huang YL, Wu CT, Yang PC. High PD-L1 expression is associated with stage IV disease and poorer overall survival in 186 cases of small cell lung cancers. Oncotarget 2017;8(11):18021–30.
15. Devesa SS, Bray F, Vizcaino AP, Parkin DM. International lung cancer trends by histologic type: male: female differences diminishing and adenocarcinoma rates rising. Int J Cancer 2005;117(2):294–9.
16. Ma J, Chi D, Wang Y, Yan Y, Zhao S, Liu H, *et al.* Prognostic value of PD-L1 expression in resected lung adenocarcinoma and potential molecular mechanisms. J Cancer 2018;9(19):3489–99.
17. Lin SY, Yang CY, Liao BC, Ho CC, Liao WY, Chen KY, *et al*. Tumor PD-L1 expression and clinical outcomes in advanced-stage non-small cell lung cancer patients treated with nivolumab or pembrolizumab: real-world data in Taiwan. J Cancer 2018;9(10):1813–20.
18. Igarashi T, Teramoto K, Ishida M, Hanaoka J, Daigo Y. Scoring of PD-L1 expression intensity on pulmonary adenocarcinomas and the correlations with clinicopathological factors. ESMO Open 2016;1(4):e000083.
19. Zhang M, Li G, Wang Y, Wang Y, Zhao S, Haihong P, *et al*. PD-L1 expression in lung cancer and its correlation with driver mutations: a meta-analysis. Sci Rep 2017;7(1):10255.
20. Miyazawa T, Marushima H, Saji H, Kojima K, Hoshikawa M, Takagi M, *et al*. PD-L1 expression in non-small-cell lung cancer including various adenocarcinoma subtypes. Ann Thorac Cardiovasc Surg 2019;25(1):1–9.
21. Cooper WA, Tran T, Vilain RE, Madore J, Selinger CI, Kohonen-Corish M, *et al*. PD-L1 expression is a favorable prognostic factor in early stage non-small cell carcinoma. Lung Cancer 2015;89(2):181–8.
22. Scheel AH, Ansén S, Schultheis AM, Scheffler M, Fischer RN, Michels S, *et al*. PD-L1 expression in non-small cell lung cancer: correlations with genetic alterations. Oncoimmunology 2016;5(5):e1131379.
23. Lin G, Fan X, Zhu W, Huang C, Zhuang W, Xu H, *et al*. Prognostic significance of PD-L1 expression and tumor infiltrating lymphocyte in surgically resectable non-small cell lung cancer. Oncotarget 2017;8(48):83986–94.
24. Remmele W, Stegner H. Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue. Pathologe 1987;8(3):138–40.
25. Takada K, Okamoto T, Shoji F, Shimokawa M, Akamine T, Takamori S, *et al*. Clinical significance of PD-L1 protein expression in surgically resected primary lung adenocarcinoma. J Thorac Oncol 2016;11(11):1879–90.

| *Submitted: March 21, 2024* | *Revised: June 5, 2024* | *Accepted: June 5, 2024* |
|---|---|---|

## Address for Correspondence:

**Dr. Muhammad Umair Khan**, Armed Institute of Pathology, NUMS, Rawalpindi-Pakistan
**Cell:** +92 336 912 3636
**Email:** Umairkhan0953@gmail.com