**Original Article**

# Immunohistochemical Expression of Programmed Death Ligand-1 in Colorectal Carcinoma and its Association with Clinicopathological Profile: A Cross-sectional Study

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## ABSTRACT

**Introduction:** Colorectal Carcinoma (CRC) is usually an aggressive tumour with a high mortality rate. The detection of prognostic and predictive biomarkers for CRC is very important in providing personalised treatment. Checkpoint blockade therapy targeting Programmed Cell Death-1 (PD-1) and its ligand, Programmed Death Ligand-1 (PD-L1), using anti-PD-1 and anti-PD-L1 monoclonal antibodies is under evaluation for treating cancers of the gastrointestinal tract.

**Aim:** To evaluate the Immunohistochemical (IHC) expression of PD-L1 in CRC and its association with the clinicopathological profile of patients with CRC.

**Materials and Methods:** This cross-sectional study was conducted at the Department of Pathology, Mahatma Gandhi Medical College and Research Institute, Puducherry, India, on 39 cases of resected CRC specimens received over a period of one year from January 2023 to January 2024. The IHC expression of PD-L1 in tumour cells and Tumour Infiltrating Lymphocytes (TILs) was analysed and assessed using Tumour Proportion Score (TPS) and Combined Positive Score (CPS). The association between PD-L1 expression and TPS and CPS assessment, as well as the clinicopathological profile of patients, was analysed. Data were presented as frequency and percentage. Categorical variables were compared using the Pearson Chi-square test. Significance was defined by p-values <0.05 using a two-tailed test.

**Results:** PD-L1 expression in the tumour cells was observed in 11 cases (28.21%), while 15 cases (38.46%) showed positivity only in TILs and 11 cases (28.21%) showed positivity in both. Upon correlating PD-L1 expression with clinicopathological parameters, there was a statistically significant association between PD-L1 expression in tumour cells and the histological type of carcinoma (p-value=0.038), Lymphovascular Invasion (LVI) (p-value=0.016), Perineural Invasion (PNI) (p-value=0.05) and tumour budding (p-value=0.046). Additionally, when assessing the association of TPS with the clinicopathological profile, a statistically significant association was found between TPS and histological type (p-value=0.038), LVI (p-value=0.024), PNI (p-value=0.05) and mucin pools (p-value=0.039). A statistically significant association was established between CPS assessment and tumour size (p-value=0.050), histological type (p-value=0.007), LVI (p-value=0.08), PNI (p-value=0.09) and tumour budding (p-value=0.028).

**Conclusion:** PD-L1 expression, along with TPS and CPS assessment, showed a strong association with tumour type, size, LVI, PNI, tumour budding and mucin pools, which are individual prognostic variables in CRC. Thus, PD-L1 is an independent prognostic biomarker in cases of CRC. In developing countries where molecular phenotyping is challenging, PD-L1 IHC can be useful in predicting prognosis and identifying patients who require anti-PD-L1 targeted therapies.

## INTRODUCTION

The incidence of CRC in Asian countries is on the rise, with a global incidence of 19.5 per 100,000 population and 15.2 per 100,000 in India. It is currently the seventh most common cancer in India, with 65,358 new cases reported in 2021 [1]. Adenocarcinoma is the most frequent histological subtype of CRC, accounting for 90% of all cases [2]. The current modalities of treatment for CRC include surgery, chemotherapy and targeted therapy. Despite advances in the diagnosis and treatment of CRC, the mortality rate remains quite high and efforts to reduce cancer-related deaths are being evaluated. The detection of prognostic and predictive biomarkers for a tumour is crucial in selecting patients who would benefit from a particular therapy while sparing others from unnecessary treatment. Cancer immunotherapy is a novel treatment modality currently undergoing various phases of clinical trials in cancer patients and is generally more tolerable than conventional therapies. Checkpoint blockade therapy targeting PD-1 and its ligand, PD-L1 (also known as B7-H1 or CD274), using anti-PD-1 and anti-PD-L1 monoclonal antibodies is under evaluation for the treatment of cancers of the gastrointestinal tract [3].

Tumour cells employ a variety of strategies to evade the immune response, one of which is the upregulation of surface PD-L1 expression. PD-L1 is a 40 kDa transmembrane protein expressed on activated immune cell types, including B cells, natural killer cells, macrophages, myeloid dendritic cells and vascular endothelial cells, as well as on cancer cells. The physiological role of PD-L1 is to bind to PD-1 expressed on the surface of activated cytotoxic T cells. This binding inhibits Interleukin-2 (IL-2) production and T-cell activation, serving as an important regulatory checkpoint against an excessive adaptive immune response to antigens and autoimmunity [4].

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PD-L1 is emerging as a key biomarker that plays a role in immune evasion and distant metastasis. However, the role of PD-L1 as a marker of better or worse prognosis is less understood in CRC [5]. Moreover, the expression of PD-L1 in association with the clinical and pathological features of CRC is not well established.

Hence, the present study was undertaken to evaluate the IHC expression of PD-L1 in CRC and its association with clinicopathological variables like age, gender, tumour type, tumour grade, tumour size, tumour location, tumour stage, tumour budding, the presence of LVI, PNI and intratumoural and peritumoural TILs.

## MATERIALS AND METHODS

This cross-sectional study was conducted on 39 cases of resected CRC specimens received in the Department of Pathology Mahatma Gandhi Medical College and Research Institue, Puducherry, India, over a period of one year from January 2023 to January 2024, after obtaining approval from the Institutional Human Ethics Committee (MGMCRI/Res/01/2021/101/IHEC/141).

**Inclusion criteria:** All histologically diagnosed cases of colorectal adenocarcinoma in patients above 18 years were included in the study.

**Exclusion criteria:** Epithelial malignancies other than adenocarcinoma and non epithelial malignancies of the colorectal region, colorectal biopsies and patients who had received chemotherapy or radiotherapy prior to surgery were also excluded from the study.

### Study Procedure

Specimens were fixed overnight in 10% neutral buffered formalin. Gross examination was performed and tumour size and type were noted. Haematoxylin and Eosin (H&E) sections were prepared using standard protocols. The histological type, histological grade, LVI, PNI, tumour budding, intratumoural and peritumoural TILs and pathological TNM staging were noted [6]. The formalin-fixed paraffin-embedded sections of CRC were stained with PD-L1 immunostain. The monoclonal rabbit anti-human PD-L1 antibody (Biocare Medical, USA) was used as the primary antibody. Four µm sections were cut from paraffin-embedded blocks and placed on poly-L-lysine coated slides. The sections were deparaffinised with xylene and rehydrated using a series of alcohol and water. The sections were treated in an epitope retrieval solution (EDTA buffer, pH 8.0) and placed in a decloaking chamber at 110°C for 30 minutes. IHC staining was performed using an automated stainer (Autostainer Intelipath, Biocare) using primary antibody, MACH1 horse radish peroxidase polymer, diaminobenzidine chromogen and haematoxylin counterstain. Tonsil was used as a positive control.

Tumour cells showing membranous and cytoplasmic staining with PD-L1 were indicative of positive staining. Peritumoural and intratumoural immune cells (lymphocytes and macrophages) showing membranous and cytoplasmic staining with PD-L1 were also indicative of positive staining. Recently, two scoring systems have been developed to assess PD-L1 expression: the TPS and the CPS [7,8]. Varying results among previous PD-L1 scoring methods in other studies involving CRC arise from a lack of a uniform scoring system. The role of TPS and CPS has yet to be standardised for CRC; thus, the present study evaluated the role of PD-L1 expression in terms of TPS and CPS in CRC, using a scoring system approved by the FDA for other tumours [9].

TPS is the percentage of tumour cells showing PD-L1 positivity and is calculated as follows [10]:

TPS=(Number of positive tumour cells/Number of viable tumour cells)×100

TPS <1% (Score 0), 1 to 10% (Score 1), 11 to 50% (Score 2) and >50% (Score 3). Score 0 is considered negative, while Scores 1, 2, or 3 are considered positive.

CPS is the percentage of both tumour and immune cells (lymphocytes and macrophages) showing PD-L1 positivity and is calculated as follows [10]:

CPS={Number of all positive cells (tumour cells, lymphocytes and macrophages)/Number of viable tumour cells}×100

CPS <1% (Score 0), 1 to 5% (Score 1), 6 to 10% (Score 2) and >10% (Score 3). Score 0 is considered negative, while Scores 1, 2, or 3 are considered positive.

PD-L1 expression was evaluated independently by two pathologists who were blinded to the clinical details [9] of the cases and 100% interobserver agreement was achieved for both positive and negative results as well as for the scoring system of all cases.

## STATISTICAL ANALYSIS

Data were presented as frequencies and percentages. Categorical variables were compared using the Pearson Chi-square test. Significance was defined by p-values <0.05 using a two-tailed test. Data analysis was performed using IBM SPSS version 21.0 (IBM Statistical Package for the Social Sciences (SPSS) Science Inc., Chicago, IL).

## RESULTS

The clinicopathological profile of 39 cases of resected specimens of CRC was analysed. The median age was 62 years, with ages ranging from 41 to 92 years. There was a slight male preponderance, with a male-to-female ratio of 1.2:1. A family history of CRC in first- or second-degree relatives was present in five cases (12.82%). Conventional adenocarcinoma and mucinous adenocarcinoma were the two histological types encountered, with 30 cases (76.92%) having conventional type adenocarcinoma and nine cases (23.08%) having mucinous type adenocarcinoma. The site of carcinoma was predominantly in the rectosigmoid colon with 12 cases (30.77%), followed by ascending colon with nine cases (23.08%), sigmoid colon with seven cases (17.95%), descending colon with six cases (15.38%) and the cecum with five cases (12.82%).
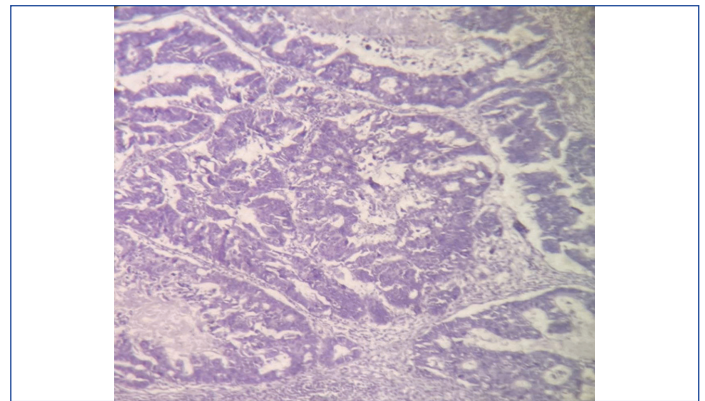
On gross examination of the specimens, 27 cases (69.23%) presented as ulceroproliferative growths, while 12 cases (30.77%) presented as polypoidal growths. Other clinicopathological parameters such as tumour size, tumour grade, presence of LVI, PNI, tumour budding, mucin pools and intratumoural and peritumoural TILs are depicted in [Table/Fig-1]. In the present study, no metastasis was identified in 12 cases (30.77%) and the metastatic status was unknown in the remaining 27 cases (69.23%).

| Clinicopathological parameter | N (%) | PD-L1 expression in tumour cells N (%) | | p-value |
|---|---|---|---|---|
| | | PD-L1 (+) | PD-L1 (-) | |
| **Age (years)** | | | | |
| <50 | 9 (23.08) | 2 (22.22) | 7 (77.78) | |
| 51-60 | 12 (30.77) | 3 (25) | 9 (75) | 0.89 |
| 61-70 | 14 (35.90) | 5 (35.72) | 9 (64.28) | |
| >70 | 4 (10.25) | 1 (25) | 3 (75) | |
| **Tumour size (cm)** | | | | |
| <5 | 23 (58.97) | 4 (17.39) | 19 (82.61) | |
| 6-10 | 15 (38.46) | 7 (46.67) | 8 (53.33) | 0.12 |
| >10 | 1 (2.57) | 0 | 1 (100) | |
| **Histologic type** | | | | |
| Conventional adenocarcinoma | 30 (76.92) | 6 (20) | 24 (80) | 0.038 |
| Mucinous adenocarcinoma | 9 (23.08) | 5 (55.56) | 4 (44.44) | |
| **Tumour grade** | | | | |
| Well differentiated | 3 (7.69) | 0 | 3 (100) | |
| Moderately differentiated | 36 (92.31) | 11 (30.56) | 25 (69.44) | 0.258 |
| Poorly differentiated | 0 | 0 | 0 | |

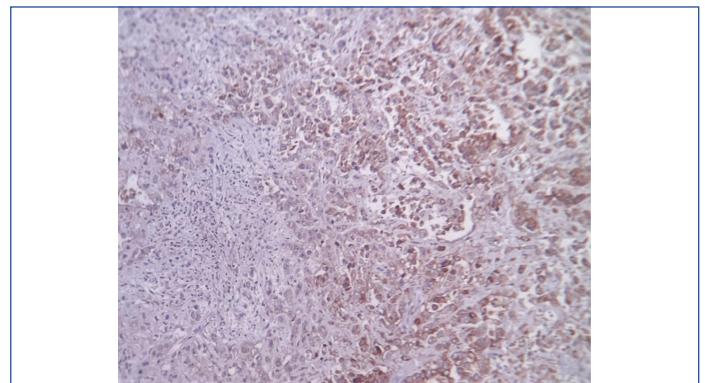| Pathological stage | | | | |
|---|---|---|---|---|
| pT1 | 1 (2.56) | 0 | 1 (100) | |
| pT2 | 9 (23.08) | 2 (22.22) | 7 (77.78) | 0.584 |
| pT3 | 22 (56.41) | 8 (36.36) | 14 (63.64) | |
| pT4 | 7 (17.95) | 1 (14.29) | 6 (85.71) | |
| Lymph node status | | | | |
| N0 | 19 (48.72) | 5 (26.32) | 14 (73.68) | |
| N1 | 11 (28.21) | 2 (18.18) | 9 (81.82) | 0.206 |
| N2 | 3 (7.69) | 1 (33.33) | 2 (66.67) | |
| Nx | 6 (15.38) | 3 (50) | 3 (50) | |
| Lymphovascular Invasion (LVI) | | | | |
| Present | 16 (41.02) | 6 (37.5) | 10 (62.5) | 0.016 |
| Absent | 23 (58.98) | 5 (21.74) | 18 (78.26) | |
| Perineural Invasion (PNI) | | | | |
| Present | 6 (15.38) | 1 (16.67) | 5 (83.33) | 0.05 |
| Absent | 33 (84.62) | 10 (30.31) | 23 (69.69) | |
| Tumour budding | | | | |
| Present | 18 (46.15) | 5 (27.78) | 13 (72.22) | 0.046 |
| Absent | 21 (53.85) | 6 (28.58) | 15 (71.42) | |
| Mucin pools | | | | |
| Present | 9 (23.08) | 4 (44.44) | 5 (55.56) | 0.217 |
| Absent | 30 (76.92) | 7 (23.33) | 23 (76.67) | |
| Intratumoural TILs | | | | |
| Mild | 36 (92.31) | 11 (30.56) | 25 (69.44) | 0.258 |
| Marked | 3 (7.69) | 0 | 3 (100) | |
| Peritumoural TIL | | | | |
| Mild | 23 (58.98) | 5 (21.74) | 18 (78.26) | 0.282 |
| Marked | 16 (41.02) | 6 (37.5) | 10 (62.5) | |

[Table/Fig-1]: Clinical characteristics of 39 patients of Colorectal Carcinoma (CRC) and association of PD-L1 expression in tumour cells with clinicopathological profile.
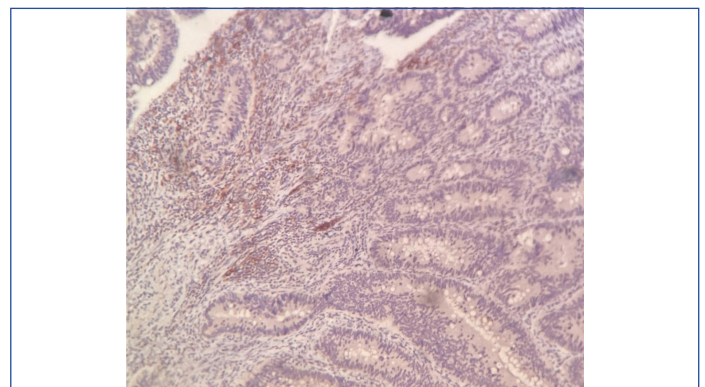
**PD-L1 expression:** The incidence of PD-L1 expression in tumour cells was 11 cases (28.21%), while 15 cases (38.46%) showed positivity only in TILs and 11 cases (28.21%) showed combined positivity in both tumour cells and TILs, as depicted in [Table/Fig-2]. A TPS of zero (less than 1%) was observed in 27 cases, a score of 1 (1 to 10%) in six cases, a score of 2 (11 to 50%) in three cases and a score of 3 (more than 50%) was observed in three cases. CPS of less than 1% was observed in 24 cases; two cases had a score of 1 to 5%, seven cases had a score of 6 to 10% and six cases had a score of more than 10%, as shown in [Table/Fig-2]. Immunohistochemical expression and scoring of the cases are illustrated in [Table/Fig-3-7].

**Association of PD-L1 expression in tumour cells with clinicopathological profile:** On assessing the association of PD-L1 expression with clinicopathological parameters, there was a statistically significant association between PD-L1 expression in tumour cells and the histological type of carcinoma (p-value=0.038), LVI (p-value=0.016), PNI (p-value=0.05) and tumour budding (p-value=0.046). However, no significant association was observed with other clinicopathological parameters such as age, tumour size, tumour grade, peritumoural and intratumoural TILs, pathological TNM staging and mucin pools [Table/Fig-1].

**Association of expression of PD-L1 as TPS and CPS with clinicopathological profile:** The association between TPS and CPS



[Table/Fig-3]: Negative PD-L1 immunohistochemical expression in tumour cells of CRC (TPS <1%) (40×).



[Table/Fig-4]: Immunohistochemical expression of PD-L1 in tumour cells of CRC (TPS >50%) (40×).



[Table/Fig-5]: Immunohistochemical expression of PD-L1 in tumour cells and immune cells of CRC (CPS >10%) (10×).



[Table/Fig-6]: Immunohistochemical expression of PD-L1 in peritumoural TILs (10×).

| N=39 | PD-L1 positivity in tumour cells | PD-L1 positivity in TILs | PD-L1 positivity in both tumour cells TILs | TPS | | | | CPS | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 |
| | | | | <1% | 1-10% | 11-50% | >50% | <1% | 1-5% | 6-10% | >10% |
| Number of cases N (%) | 11 (28.21) | 15 (38.46) | 11 (28.21) | 28 (71.79) | 6 (15.38) | 3 (7.69) | 2 (5.13) | 25 (64.10) | 2 (5.13) | 7 (17.95) | 5 (12.82) |

[Table/Fig-2]: PD-L1 expression in tumour cells, TILs and in both tumour cells and TILs along with TPS and CPS.
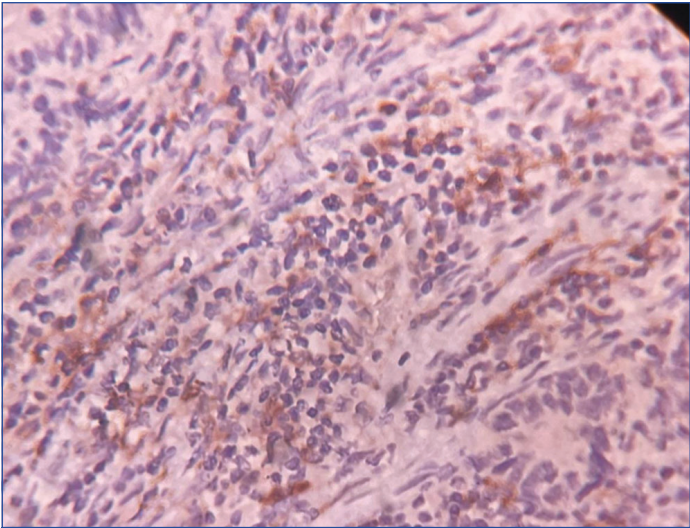
[Table/Fig-7]: Immunohistochemical expression of PD-L1 in TILs (40×).

of PD-L1 expression and various clinicopathological parameters is shown in [Table/Fig-8,9]. In correlating TPS assessment with the clinicopathological profile, there was a statistically significant association between TPS and histological type (p-value=0.038), LVI (p-value=0.024), PNI (p-value=0.05) and mucin pools (p-value=0.039). A statistically significant association was also established between CPS assessment and tumour size (p-value=0.050), histological type (p-value=0.007), LVI (p-value=0.08), PNI (p-value=0.09) and tumour budding (p-value=0.028).

| Clinicopathological parameter | Number of cases N (%) | N (%) of cases according to TPS | | | | p-value |
|---|---|---|---|---|---|---|
| | | <1% | ≥1-10% | ≥10-50% | ≥50% | |
| Overall | 39 | 28 (71.79) | 6 (15.38) | 3 (7.69) | 2 (5.13) | |
| **Age (years)** | | | | | | |
| <50 | 9 (23.08) | 7 (77.78) | 1 (11.11) | 0 | 1 (11.11) | 0.848 |
| 51-60 | 12 (30.77) | 9 (75) | 1 (8.33) | 1 (8.33) | 1 (8.33) | |
| 61-70 | 14 (35.90) | 9 (64.29) | 3 (21.42) | 2 (14.29) | 0 | |
| >70 | 4 (10.25) | 3 (75) | 1 (25) | 0 | 0 | |
| **Tumour size (cm)** | | | | | | |
| <5 | 23 (58.97) | 19 (82.61) | 3 (13.04) | 1 (4.35) | 0 | 0.438 |
| 6-10 | 15 (38.46) | 8 (53.33) | 3 (20) | 2 (13.33) | 2 (13.33) | |
| >10 | 1 (2.57) | 1 (100) | 0 | 0 | 0 | |
| **Histologic type** | | | | | | |
| Conventional adenocarcinoma | 30 (76.92) | 24 (80) | 2 (6.67) | 2 (6.67) | 2 (6.67) | 0.038 |
| Mucinous adenocarcinoma | 9 (23.08) | 4 (44.44) | 4 (44.44) | 1 (11.11) | 0 | |
| **Tumour grade** | | | | | | |
| Well differentiated | 3 (7.69) | 3 (100) | 0 | 0 | 0 | |
| Moderately differentiated | 36 (92.31) | 25 (69.44) | 6 (16.67) | 3 (8.33) | 2 (5.56) | 0.735 |
| Poorly differentiated | 0 | 0 | 0 | 0 | 0 | |
| **Pathological tumour stage (T)** | | | | | | |
| pT1 | 1 (2.56) | 1 (100) | 0 | 0 | 0 | |
| pT2 | 9 (23.08) | 7 (77.78) | 1 (11.11) | 0 | 1 (11.11) | |
| pT3 | 22 (56.41) | 14 (63.63) | 4 (18.18) | 3 (13.63) | 1 (4.54) | 0.89 |
| pT4 | 7 (17.95) | 6 (85.71) | 1 (14.29) | 0 | 0 | |
| **Pathological lymph node (N)** | | | | | | |
| pN0 | 19 (48.72) | 14 (73.68) | 2 (10.53) | 2 (10.53) | 1 (5.26) | |
| pN1 | 11 (28.21) | 9 (81.81) | 1 (9.09) | 0 | 1 (9.09) | 0.21 |
| pN2 | 3 (7.69) | 2 (66.67) | 1 (33.33) | 0 | 0 | |
| pNx | 6 (15.38) | 3 (50) | 2 (33.33) | 1 (16.67) | 0 | |
| **Lymphovascular Invasion (LVI)** | | | | | | |
| Present | 16 (41.02) | 10 (62.5) | 2 (12.5) | 2 (12.5) | 2 (12.5) | 0.024 |
| Absent | 23 (58.98) | 18 (78.26) | 4 (17.39) | 1 (4.34) | 0 | |
| **Perineural Invasion (PNI)** | | | | | | |
| Present | 6 (15.38) | 5 (83.33) | 0 | 1 (16.67) | 0 | 0.05 |
| Absent | 33 (84.62) | 23 (69.69) | 6 (18.18) | 2 (6.06) | 2 (6.06) | |
| **Tumour budding** | | | | | | |
| Present | 18 (46.15) | 13 (72.22) | 3 (16.67) | 2 (11.11) | 0 | 0.52 |
| Absent | 21 (53.85) | 15 (71.42) | 3 (14.28) | 1 (4.76) | 2 (9.52) | |
| **Mucin pools** | | | | | | |
| Present | 9 (23.08) | 5 (55.56) | 4 (44.44) | 0 | 0 | 0.039 |
| Absent | 30 (76.92) | 23 (76.66) | 2 (6.66) | 3 (10) | 2 (6.66) | |
| **Intratumoural TILs** | | | | | | |
| Mild | 36 (92.31) | 25 (69.44) | 6 (16.67) | 3 (8.33) | 2 (5.56) | 0.735 |
| Marked | 3 (7.69) | 3 (100) | 0 | 0 | 0 | |
| **Peritumoural TILs** | | | | | | |
| Mild | 23 (58.98) | 18 (78.26) | 2 (8.69) | 2 (8.69) | 1 (4.34) | 0.553 |
| Marked | 16 (41.02) | 10 (62.5) | 4 (25) | 1 (6.25) | 1 (6.25) | |

[Table/Fig-8]: Association of TPS with clinicopathological parameters.

| Clinicopathological parameter | Number of cases N (%) | N (%) of cases according to CPS | | | | p-value |
|---|---|---|---|---|---|---|
| | | <1% | 1-5% | 6-10% | >10% | |
| Overall | 39 | 25 (64.10) | 2 (5.13) | 7 (17.95) | 5 (12.82) | |
| **Age (Years)** | | | | | | |
| <50 | 9 (23.08) | 7 (77.78) | 0 | 2 (22.22) | 0 | |
| 51-60 | 12 (30.77) | 7 (58.33) | 0 | 4 (33.33) | 1 (8.33) | 0.351 |
| 61-70 | 14 (35.90) | 8 (57.14) | 2 (14.28) | 1 (7.14) | 3 (21.42) | |
| >70 | 4 (10.25) | 3 (75) | 0 | 0 | 1 (25) | |
| **Tumour size (cm)** | | | | | | |
| <5 | 23 (58.97) | 18 (78.26) | 2 (8.69) | 2 (8.69) | 1 (4.34) | |
| 6-10 | 15 (38.46) | 7 (46.66) | 0 | 4 (26.66) | 4 (26.66) | 0.05 |
| >10 | 1 (2.57) | 0 | 0 | 1 (100) | 0 | |
| **Histologic type** | | | | | | |
| Conventional adenocarcinoma | 30 (76.92) | 22 (73.33) | 1 (3.33) | 6 (20) | 1 (3.33) | 0.007 |
| Mucinous adenocarcinoma | 9 (23.08) | 3 (33.33) | 1 (11.11) | 1 (11.11) | 4 (44.44) | |
| **Tumour grade** | | | | | | |
| Well differentiated | 3 (7.69) | 3 (100) | 0 | 0 | 0 | |

| | | | | | | p-value |
|---|---|---|---|---|---|---|
| Moderately differentiated | 36 (92.31) | 22 (61.11) | 2 (5.56) | 7 (19.44) | 5 (13.89) | 0.611 |
| Poorly differentiated | 0 | 0 | 0 | 0 | 0 | |
| **Pathological tumour stage (T)** | | | | | | |
| pT1 | 1 (2.56) | 1 (100) | 0 | 0 | 0 | |
| pT2 | 9 (23.08) | 7 (77.78) | 1 (11.11) | 1 (11.11) | 0 | |
| pT3 | 22 (56.41) | 13 (59.09) | 1 (4.54) | 4 (18.18) | 4 (18.18) | 0.891 |
| pT4 | 7 (17.95) | 4 (57.14) | 0 | 2 (28.57) | 1 (14.28) | |
| **Pathological lymph node (N)** | | | | | | |
| pN0 | 19 (48.72) | 12 (63.15) | 1 (5.26) | 3 (15.78) | 3 (15.78) | |
| pN1 | 11 (28.21) | 8 (72.72) | 0 | 3 (27.27) | 0 | |
| pN2 | 3 (7.69) | 2 (66.67) | 0 | 1 (33.33) | 0 | 0.117 |
| pNx | 6 (15.38) | 3 (50) | 1 (16.67) | 0 (0) | 2 (33.33) | |
| **Lymphovascular Invasion (LVI)** | | | | | | |
| Present | 16 (41.02) | 9 (56.25) | 1 (6.25) | 3 (18.75) | 3 (18.75) | 0.08 |
| Absent | 23 (58.98) | 16 (69.56) | 1 (4.34) | 4 (17.39) | 2 (8.69) | |
| **Perineural Invasion (PNI)** | | | | | | |
| Present | 6 (15.38) | 4 (66.66) | 0 | 1 (16.66) | 1 (16.66) | 0.09 |
| Absent | 33 (84.62) | 21 (63.63) | 2 (6.06) | 6 (18.18) | 4 (12.12) | |
| **Tumour budding** | | | | | | |
| Present | 18 (46.15) | 13 (72.22) | 2 (11.11) | 0 | 3 (16.67) | 0.028 |
| Absent | 21 (53.85) | 12 (57.14) | 0 | 7 (33.33) | 2 (9.52) | |
| **Mucin pools** | | | | | | |
| Present | 9 (23.08) | 4 (44.44) | 1 (11.11) | 1 (11.11) | 3 (33.33) | 0.129 |
| Absent | 30 (76.92) | 21 (70) | 1 (3.33) | 6 (20) | 2 (6.67) | |
| **Intratumoural TILs** | | | | | | |
| Mild | 36 (92.31) | 22 (61.11) | 2 (5.56) | 7 (19.44) | 5 (13.89) | 0.611 |
| Marked | 3 (7.69) | 3 (100) | 0 | 0 | 0 | |
| **Peritumoural TIL** | | | | | | |
| Mild | 23 (58.98) | 17 (73.91) | 1 (4.34) | 2 (8.69) | 3 (13.04) | 0.31 |
| Marked | 16 (41.02) | 8 (50) | 1 (6.25) | 5 (31.25) | 2 (12.5) | |

[Table/Fig-9]: Association of CPS with clinicopathological parameters.

## DISCUSSION

The CRC is one of the most frequent malignancies worldwide. In the present study, we analysed PD-L1 expression in 39 cases of CRC. The median age for CRC was 62 years, with a slight male preponderance. This aligns with studies by Sekhar G et al., and Peedikayil MC et al., which reported a mean age of 55±7.8 years and 58.4 years, respectively [11,12]. Equal gender distribution was observed in studies by Peedikayil MC et al., Bhattacharya S et al., and Tadachina S et al., [12-14].

A family history of colon cancer was present in five cases (12.82%), which was similar to the study by Deo SVS et al., which found a family history in 14.7% of cases [15]. Tumours were predominantly located on the left side in 25 cases (64.10%) and most of the tumours (27 cases or 69.23%) exhibited an ulceroproliferative growth pattern, which was consistent with the findings of Tadachina S et al., [14]. In the present study, most tumours were less than 5 cm in size (58.97%), which aligns with the study by Gupta M et al., where 56.25% of tumours measured 2 to 5 cm, while Tadachina S et al., reported that 58.83% of tumours were more than 5 cm [14,16]. Histologically, 30 cases (76.92%) were classified as conventional adenocarcinoma, consistent with findings in most other studies [9,12,14,15]. Moderately differentiated adenocarcinoma was the most common histological grade, found in 36 cases (92.31%), which was concordant with most studies [16,17].

LVI was present in 16 cases (41.02%) of CRC, while other studies reported LVI in approximately 65% of cases [14,17]. PNI was observed in six cases (15.38%), which aligns with studies by Tadachina S et al., and Elfishawy M et al., where PNI was reported at 18.3% and 17.6%, respectively [14,17]. Mucin pools were noted in nine cases (23.08%), which was lower than reported in another study that found a prevalence of 38.32%. The presence of peritumoural and intratumoural TILs was similar to the findings of Tadachina S et al., [14]. Regarding tumour stage, pT3 was observed in 22 cases (56.41%), which was in concordance with a study by Elfishawy M et al., [17]. Lymph node involvement was noted in 14 cases (35.90%), while other studies indicated that 35 to 45% of cases had no lymph node involvement [16,17].

**PD-L1 expression in Colorectal Carcinoma (CRC):** In the present study, the immunohistochemical expression of PD-L1 was evaluated in tumour cells, TILs and in both, in cases of CRC and TPS and CPS were derived. The study analysed the association between PD-L1 expression in tumour cells and the clinicopathological profile, including age, tumour size, tumour type, tumour grade, LVI, PNI, mucin pools, tumour budding, pathological stage and intratumoural and peritumoural TILs. The study also investigated the association between TPS and CPS with patient demographic details and tumour characteristics.

In the present study, PD-L1 expression was observed in 11 cases (28.21%) in tumour cells, in 15 cases (38.46%) in TILs and in 11 cases (28.21%) in both tumour cells and TILs. PD-L1 expression in other studies ranged from 5-50%, as shown in [Table/Fig-10] [14,16-19]. These variations could be attributed to differences in immunostaining techniques, the use of microarray versus whole slide staining and the scoring systems employed.

| S. No. | Study (year) | PD-L1 expression | | |
|---|---|---|---|---|
| | | Tumour cells | TILs | Tumour cells and TILs |
| 1 | Present study (2025) | 28.21% | 38.46% | 28.21% |
| 2 | Tadachina S et al., (2024) [14] | 17.65% | 17.65% | - |
| 3 | Gupta M et al., (2020) [16] | 50% | 57.50% | 30% |
| 4 | ELfishawy M et al., (2020) [17] | 25% | 38.30% | 26.70% |
| 5 | Inaguma S et al., (2017) [18] | 12% | - | - |
| 6 | Lee LH et al., (2016) [19] | 5% | 19% | - |

[Table/Fig-10]: Studies showing PD-L1 expression in cases of Colorectal Carcinoma (CRC) [14,16-19].

**Relationship between PD-L1 expression and clinicopathological features:** PD-L1 expression in tumour cells did not show any association with age or gender in the present study, which was consistent with most other studies [14]. Yamashita K et al., and Masugi Y et al., found a significant association between PD-L1 expression and age, which could be an incidental finding [7,20]. There was no association between PD-L1 expression and tumour size, while several other studies established a significant association of PD-L1 with tumour site and tumour size [19,21,22]. This discrepancy could be attributed to the small sample size in the present study.

During the study period, moderately differentiated adenocarcinoma was most frequently encountered. The study showed no significant association between PD-L1 expression and tumour grade. Shi SJ et al., found a strong association between well-differentiated adenocarcinoma and PD-L1 expression [23], while Inaguma S et al., demonstrated a significant association between PD-L1 expression and poor differentiation [18].

There was a statistically significant association between the expression of PD-L1 in tumour cells and histological type (p-value=0.038), which was in concordance with the study by Gupta M et al., [16]. A significant association was also noted with the presence of LVI (p-value=0.016) and PNI (p-value=0.05). These results were comparable to those found by Tadachina S et al., Kim JH et al., and Droeser RA et al., who also found a significant association with LVI and PNI [14,24,25]. PD-L1 expression is strongly associated with TNM stage, lymph node metastasis and distant metastasis and the study by Yamano T et al., concluded that elevated PD-L1 expression in tumour cells is a poor predictive indicator on its own [26].

The present study found a significant association between PD-L1 expression and tumour budding (p-value=0.046). Kim JH et al., also identified an association between PD-L1 expression and tumour budding [24]. They further established an association between PD-L1 expression, decreased differentiation and reduced mucin component. However, the present study showed no association with mucin pools. In Mismatch Repair (MMR)-competent CRC, Droeser RA et al., discovered a link between mucinous histology and PD-L1 expression [25].

The present study showed no significant association between PD-L1 expression and pathological tumour and nodal stage, which was similar to the findings of Tadachina S et al., [14]. Masugi Y et al., and Droeser RA et al., found a significant association between tumour stage and PD-L1 expression, establishing that PD-L1 is an independent risk factor. Droeser RA et al., also identified a significant association between PD-L1 expression and nodal stage. Their study indicated that poor tumour differentiation, lymph node metastases and positive PD-L1 expression all impacted the prognosis of CRC [20,25].

**Relationship between TPS and CPS with clinicopathological characteristics:** TPS and CPS assessment was also correlated with other clinicopathological features in CRC. In the present study, nine cases (23.07%) showed a TPS score of 1 or 2, which was similar to the study by Frančina M et al., which indicated that statistically significant cases had a TPS of less than 1%. Fourteen cases (35.88%) showed CPS positivity with scores of 1, 2, or 3 (1 to 5%, 6 to 10% and more than 10%). However, Frančina M et al., reported CPS positivity in around 95% of cases, which could be attributed to a larger sample size [9].

TPS and CPS positivity were predominantly observed in the age group of 61 to 70 years, with a slight male predominance, which aligns with findings from Francina M et al., White A et al., and Shen Z et al., [9,27,28]. There was a statistically significant correlation between TPS score and CPS score with tumour type (p-value=0.038 and p=0.007, respectively). Twenty percent of cases of conventional adenocarcinoma showed positivity on TPS assessment, while 55.5% of mucinous adenocarcinomas exhibited positivity on TPS assessment. Regarding CPS, 26.6% of conventional adenocarcinoma was positive and 66.7% of mucinous adenocarcinoma was positive. These findings were concordant with those of Francina M et al., [9]. Additionally, TPS demonstrated a significant correlation with mucin pools (p=0.039). These results suggest that immune checkpoint inhibitors could be a viable treatment option for mucinous adenocarcinoma.

The study showed a significant association between PD-L1 assessment by TPS and LVI and PNI. Similarly, a significant association of CPS with LVI, PNI and tumour budding was noted in present study, which was in concordance with the findings of Tadachina S et al., Droeser RA et al., and Huang CY et al., [14,25,29]. These studies indicated that PD-L1 expression was strongly associated with lymph node status and distant metastasis. Thus, PD-L1 expression in the tumour serves as an independent predictor of prognosis in CRC [14,25,29].

TPS and CPS did not associate with age, histological grade, pathological tumour (T) and lymph node stage (N), or intratumoural and peritumoural TILs. However, PD-L1 expression, as measured by TPS and CPS, associated with tumour type (mucinous/non-mucinous), tumour size, LVI, PNI and tumour budding. Therefore, PD-L1 expression and scoring have significant association in predicting tumour prognosis, establishing PD-L1 as an independent prognostic factor. This was consistent with the study by Li Y et al., which similarly discussed this in CRC patients [30]. Tadachina S et al., found that PD-L1 could be used as a biomarker for poor prognosis, while Droeser RA et al., linked PD-L1 expression with low-grade tumours, early T stage, lack of vascular invasion and lack of lymph node metastasis, all of which correspond to better patient survival outcomes [14,25]. Inaguma S et al., demonstrated a positive correlation between PD-L1 expression and high lymph node metastasis, tumour diameter, differentiation and vascular invasion, concluding that PD-L1 expression is an independent predictor of poor prognosis, which aligns with present study findings [18].

The variation in findings from other studies could be attributed to differences in sample size, non uniform scoring methods for PD-L1, and other epidemiological factors. Therefore, PD-L1 assessment is an important biomarker for determining prognosis in CRC patients and evaluating PD-L1 expression can aid in identifying candidates for anti-PD-L1 therapy, potentially enhancing survival outcomes.

### Limitation(s)

The sample size was limited in the present study and the number of cases with lymph node and distant metastasis during the study period was also restricted. Additionally, no cases of poorly differentiated tumours were presented during this time. A follow-up of PD-L1 expression positive cases undergoing anti-PD-L1 therapy and monitoring their response would provide more reliable insights into the role of PD-L1 expression as a prognostic and predictive marker for CRC.

## CONCLUSION(S)

The CRC is usually an aggressive malignancy that requires intensive therapy. The fundamental management approach includes surgery and chemoradiation. In the present study, PD-L1 expression and the assessment of TPS/CPS showed a strong association with tumour type, size, LVI, PNI, tumour budding and mucin pools, all of which are individual prognostic variables in CRC. Thus, PD-L1 is an independent prognostic biomarker in cases of CRC. Other clinicopathological prognostic variables did not demonstrate significant associations. Hence, in developing countries where molecular phenotyping is often unattainable, PD-L1 IHC can be useful in predicting prognosis and identifying patients who may benefit from anti-PD-L1 targeted therapies.

## REFERENCES

[1] Bose B, Clarke J, Glasbey JC, Haque PD, Jolly K, Kingsley PA, et al. Catastrophic expenditure and treatment attrition in patients seeking comprehensive colorectal cancer treatment in India: A prospective multicentre study. Lancet Reg Heal Asia. 2022;6:100058.

[2] Fleming M, Ravula S, Tatishchev SF, Wang HL. Colorectal carcinoma: Pathologic aspects. J Gastrointest Oncol. 2012;3(3):153-73.

[3] Wang HB, Yao H, Li CS, Liang LX, Zhang Y, Chen YX, et al. Rise of PD-L1 expression during metastasis of colorectal cancer: Implications for immunotherapy. J Dig Dis. 2017;18(10):574-81.

[4] Schott DS, Pizon M, Pachmann U, Pachmann K. Sensitive detection of PD-L1 expression on Circulating Epithelial Tumour Cells (CETCs) could be a potential biomarker to select patients for treatment with PD-1/PD-L1 inhibitors in early and metastatic solid tumours. Oncotarget. 2017;8(42):72755-72.

[5] Rosenbaum MW, Bledsoe JR, Morales-Oyarvide V, Huynh TG, Mino-Kenudson M. PD-L1 expression in colorectal cancer is associated with microsatellite instability, BRAF mutation, medullary morphology and cytotoxic tumour-infiltrating lymphocytes. Mod Pathol. 2016;29(9):1104-12.

[6] American Joint Committee on Cancer. Chapter 20 - Colon and Rectum. In: AJCC Cancer Staging Manual. 8th ed. New York, NY: Springer; 2017.

[7] Yamashita K, Iwatsuki M, Harada K, Eto K, Hiyoshi Y, Ishimoto T, et al. Prognostic impacts of the combined positive score and the tumour proportion score for programmed death ligand-1 expression by double immunohistochemical staining in patients with advanced gastric cancer. Gastric Cancer. 2020;23(1):95-104.

[8] Mercier A, Conan-Charlet V, Quintin-Roué I, Doucet L, Marcorelles P, Uguen A. Reproducibility in PD-L1 immunohistochemistry quantification through the tumour proportion score and the combined positive score: Could dual immunostaining help pathologists? Cancers (Basel). 2023;15(10):2768.

[9] Frančina M, Mikuš M, Mamić M, Jovanović T, Ćorić M, Lovrić B, et al. Evaluation of PD-L1 expression in colorectal carcinomas by comparing scoring methods and their significance in relation to clinicopathologic parameters. Diagnostics. 2024;14(10):1007.

[10] de Ruiter EJ, Mulder FJ, Koomen BM, Speel EJ, van den Hout MFCM, de Roest RH, et al. Comparison of three PD-L1 immunohistochemical assays in head and neck squamous cell carcinoma (HNSCC). Mod Pathol. 2021;34(6):1125-32. Doi: 10.1038/s41379-020-0644-7.

[11] Sekhar G, Menon D, Porchelvan S. A study of PDL-1 expression in colorectal carcinoma and its relationship with clinicopathologic factors- A retrospective study in a tertiary care centre. ASRO [Internet]. 2021;23(23). [cited 2024 Aug 23].

[12] Peedikayil MC, Nair P, Seena SM, Radhakrishnan L, Sadasivan S, Naryanan VA, et al. Colorectal cancer distribution in 220 Indian patients undergoing colonoscopy. Indian J Gastroenterol. 2009;28(6):212-15.

[13] Bhattacharya S, Bhattacharya S, Basu R, Bera P, Halder A. Colorectal cancer: A study of risk factors in a tertiary care hospital of north Bengal. J Clin Diagn Res. 2014;8(11):FC08-FC10.

[14] Tadachina S, Devi Shivalingaiah S, Shetty M. Immunohistochemical expression of programmed death ligand- 1 (pd-l1) in colorectal carcinoma; A cross-sectional study. Iran J Pathol. 2024;19(1):22-30.

[15] Deo SVS, Kumar S, Bhoriwal S, Shukla NK, Sharma A, Thulkar S, et al. Colorectal cancers in low- and middle-income countries—Demographic pattern and clinical profile of 970 patients treated at a tertiary care cancer center in India. JCO Glob Oncol. 2021;(7):1110-15.

[16] Gupta M, Manjari M, Kaur H. PD-L1 expression in colorectal carcinoma: Immunohistochemical study. Ann of Pathol and Lab Med [Internet]. 2020;7(6):A275-81. Available from: https://pacificejournals.com/journal/index.php/apalm/article/view/2742

[17] ELfishawy M, Abd-ELaziz SA, Hegazy A, EL-yasergy DF. Immunohistochemical Expression of Programmed Death Ligand-1 (PDL-1) in colorectal carcinoma and its correlation with stromal tumour infiltrating lymphocytes. Asian Pac J Cancer Prev. 2020;21(1):225-32.

[18] Inaguma S, Lasota J, Wang Z, Felisiak-Golabek A, Ikeda H, Miettinen M. Clinicopathologic profile, immunophenotype, and genotype of CD274 (PD-L1)-positive colorectal carcinomas. Mod Pathol. 2017;30(2):278-85.

[19] Lee LH, Cavalcanti MS, Segal NH, Hechtman JF, Weiser MR, Smith JJ, et al. Patterns and prognostic relevance of PD-1 and PD-L1 expression in colorectal carcinoma. Mod Pathol. 2016;29(11):1433-42.

[20] Masugi Y, Nishihara R, Yang J, Mima K, da Silva A, Shi Y, et al. Tumour CD274 (PD-L1) expression and T cells in colorectal cancer. Gut. 2017;66(8):1463-73.

[21] Wu Z, Yang L, Shi L, Song H, Shi P, Yang T, et al. Prognostic impact of adenosine Receptor 2 (A2aR) and Programmed Cell Death Ligand 1 (PD-L1) expression in colorectal cancer. BioMed Res Int [Internet]. 2019;2019:8014627. [cited 2024 Aug 23].

[22] Lee KS, Kim BH, Oh HK, Kim DW, Kang SB, Kim H, et al. Programmed cell death ligand-1 protein expression and CD274/PD-L1 gene amplification in colorectal cancer: Implications for prognosis. Cancer Sci. 2018;109(9):2957-69.

[23] Shi SJ, Wang LJ, Wang GD, Guo ZY, Wei M, Meng YL, et al. B7-H1 expression is associated with poor prognosis in colorectal carcinoma and regulates the proliferation and invasion of HCT116 colorectal cancer cells. PLoS One. 2013;8(10):e76012.

[24] Kim JH, Park HE, Cho NY, Lee HS, Kang GH. Characterisation of PD-L1-positive subsets of microsatellite-unstable colorectal cancers. Br J Cancer. 2016;115(4):490-96.

[25] Droeser RA, Hirt C, Viehl CT, Frey DM, Nebiker C, Huber X, et al. Clinical impact of programmed cell death ligand 1 expression in colorectal cancer. Eur J Cancer. 2013;49(9):2233-42.

[26] Yamano T, Yamauchi S, Kimura K, Babaya A, Hamanaka M, Kobayashi M, et al. Influence of age and comorbidity on prognosis and application of adjuvant chemotherapy in elderly Japanese patients with colorectal cancer: A retrospective multicentre study. Eur J Cancer. 2017;81:90-101.

[27] White A, Ironmonger L, Steele RJC, Ormiston-Smith N, Crawford C, Seims A. A review of sex-related differences in colorectal cancer incidence, screening uptake, routes to diagnosis, cancer stage and survival in the UK. BMC Cancer. 2018;18(1):906.

[28] Shen Z, Gu L, Mao D, Chen M, Jin R. Clinicopathological and prognostic significance of PD-L1 expression in colorectal cancer: A systematic review and meta-analysis. World J Surg Oncol. 2019;17(1):4.

[29] Huang CY, Chiang SF, Ke TW, Chen TW, You YS, Chen WTL, et al. Clinical significance of programmed death 1 ligand-1 (CD274/PD-L1) and intra-tumoural CD8+ T-cell infiltration in stage II–III colorectal cancer. Sci Rep. 2018;8(1):15658.

[30] Li Y, Liang L, Dai W, Cai G, Xu Y, Li X, et al. Prognostic impact of programed cell death-1 (PD-1) and PD-ligand 1 (PD-L1) expression in cancer cells and tumour infiltrating lymphocytes in colorectal cancer. Mol Cancer. 2016;15(1):55.

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