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Study Of Immunochemical Marker (Pd-L1) In Colorectal Cancers And Its Correlation With Clinicopathological Features In Teritary Care Centre

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Abstract
This study aimed to evaluate PD-L1 expression in colorectal adenocarcinoma and its correlation with clinicopathological parameters. A prospective diagnostic analysis was conducted at the Department of Pathology, SBMCH, Chennai, between March and October 2021, including 48 histopathologically confirmed cases of colorectal adenocarcinoma. Immunohistochemistry (IHC) for PD-L1 was performed, and the Immunohistochemistry Score (IHS) was calculated based on staining intensity and the percentage of positive tumor cells. The majority of patients were aged 51–70 years (54.2%) with a slight male predominance (52.1%). Rectal involvement was most common (50%), and tumors were predominantly 2–5 cm in size (58.3%) and of low-grade histology (75%). PD-L1 positivity (IHS ≥ 3) was observed in 37.5% of cases, with 22.9% showing high expression (>50%). Mild to moderate staining intensity was predominant among positive cases. Although higher PD-L1 expression correlated with low-grade histology, the association lacked statistical significance ($p > 0.05$). These findings suggest that PD-L1 expression may serve as a potential biomarker for colorectal adenocarcinoma, warranting further research to establish its prognostic and therapeutic implications.

Keywords:
PD-L1 expression, colorectal adenocarcinoma, immunohistochemistry, tumor characteristics, low-grade histology, biomarker

1.Introduction

Colorectal carcinoma (CRC) is one of the most significant contributors to the global cancer burden, ranking as the third most common cancer worldwide and the second leading cause of cancer-related deaths. Accounting for approximately 10% of all new cancer cases, CRC represents a substantial public health challenge. The increasing incidence of CRC is driven by a complex interplay of genetic predisposition, environmental exposures, and lifestyle changes, including dietary patterns, physical inactivity, and obesity. Despite advancements in diagnostic techniques and therapeutic modalities, CRC continues to pose a significant challenge due to late-stage diagnosis and treatment resistance.

1.1.Pathogenesis of Colorectal Carcinoma

The development of CRC follows a multistep progression model involving a series of genetic and epigenetic changes. These alterations result in the transformation of normal colonic epithelium into adenomatous polyps and, ultimately, invasive adenocarcinoma. Key pathways implicated in CRC pathogenesis include the chromosomal instability pathway, microsatellite instability pathway, and CpG island methylator phenotype. These pathways result in mutations in critical

genes such as *APC, KRAS, TP53,* and *BRAF*, as well as alterations in DNA repair mechanisms. In addition to genetic alterations, the tumor microenvironment plays a pivotal role in CRC progression. The interplay between cancer cells and immune cells within the tumor microenvironment contributes to immune evasion, tumor invasion, and metastasis. Among the various mechanisms of immune escape, the overexpression of immune checkpoint molecules, such as programmed death-ligand 1 (PD-L1), has emerged as a critical factor in enabling tumor survival and progression. The Figure:1 shows the risk factors involved.

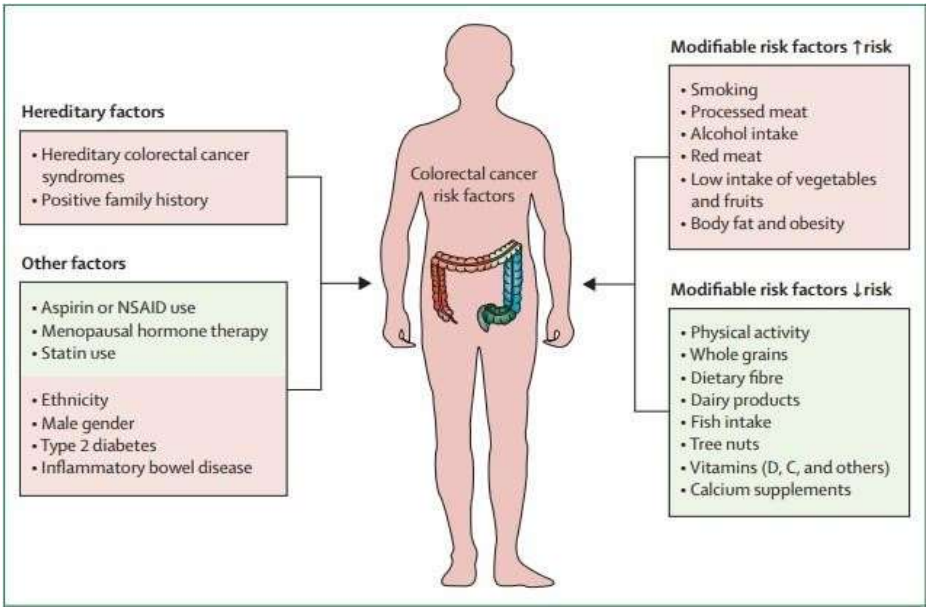

Figure:1 Risk Factors for Colorectal Cancer

1.2.Programmed Death-Ligand 1 (PD-L1): A Key Immune Checkpoint Molecule

PD-L1, also known as B7-H1, is a transmembrane protein expressed on various cell types, including tumor cells, macrophages, dendritic cells, and other immune cells. It binds to the programmed death-1 (PD-1) receptor on T-cells, leading to the suppression of T-cell activation, proliferation, and cytokine production. This interaction plays a crucial role in maintaining immune homeostasis under normal physiological conditions. However, in the context of malignancies such as CRC, PD-L1 expression is often upregulated, enabling tumor cells to evade immune surveillance. The biological significance of PD-L1 in cancer lies in its ability to create an immunosuppressive tumor microenvironment. By inhibiting T-cell-mediated cytotoxicity, PD-L1 overexpression facilitates tumor growth, invasion, and resistance to therapy. Moreover, PD-L1 expression has been associated with aggressive tumor behavior and poor clinical outcomes in various cancers, including non-small cell lung cancer, melanoma, and renal cell carcinoma.

1.3.Rationale for Studying PD-L1 in Colorectal Carcinoma

While PD-L1 expression has been extensively studied in several cancers, its role in CRC remains less well-defined. The heterogeneity of PD-L1 expression in CRC, coupled with its potential prognostic and therapeutic implications, underscores the need for comprehensive studies in this area. Immunotherapy, particularly immune checkpoint inhibitors targeting the PD-1/PD-L1 axis, has revolutionized cancer treatment by achieving durable responses in a subset of patients with advanced cancers. However, the efficacy of such therapies in CRC has been limited to specific subgroups, such as microsatellite instability-high (MSI-H) or mismatch repair-deficient (dMMR) tumors.

Given this context, evaluating PD-L1 expression in CRC is crucial for several reasons:

1. **Prognostic Marker:** PD-L1 expression may serve as a biomarker to stratify patients based on their risk of disease progression and recurrence. Identifying high-risk patients can aid in personalized treatment planning.

2. **Therapeutic Target:** Understanding PD-L1 expression patterns can help identify CRC patients who are more likely to benefit from immune checkpoint inhibitor therapies.
3. **Epidemiological Insights:** Regional studies, such as this one conducted in Chennai, India, contribute valuable data on PD-L1 expression in CRC, which may differ due to variations in genetic, environmental, and lifestyle factors.

1.4.Objectives:

1. **Primary Objective:**
    - To evaluate the expression of programmed death-ligand 1 (PD-L1), an immunohistochemical marker, in colorectal carcinoma (CRC).
2. **Specific Objectives:**
    - To analyze the histopathological features of colorectal carcinoma, including tumor site, tumor size, histological grade, and lymph node involvement.
    - To assess the correlation between PD-L1 expression and clinicopathological parameters of colorectal carcinoma.
    - To evaluate the role of PD-L1 expression as a potential prognostic biomarker in CRC by analyzing its relationship with disease aggressiveness and patient outcomes.
    - To identify CRC cases with high PD-L1 expression as potential candidates for immune checkpoint inhibitor therapy.
    - To contribute region-specific data on PD-L1 expression in CRC from a tertiary care center in India and compare it with global trends.
    - To provide insights into the utility of PD-L1 as a therapeutic target for immunotherapy in CRC.

These objectives can seamlessly integrate into the introduction by providing a roadmap for the study and highlighting its relevance in CRC research.

1.5.Scope of the Study

This study evaluates PD-L1 expression in histopathologically confirmed cases of colorectal adenocarcinoma and explores its correlation with clinicopathological features, including tumor site, size, histological grade, and lymph node involvement. The findings aim to bridge the gap in understanding the role of PD-L1 in CRC and its potential as a biomarker for prognosis and therapeutic targeting. Several studies have suggested a potential association between PD-L1 expression and various clinicopathological parameters in CRC. For instance, tumors with high PD-L1 expression have been linked to specific histological subtypes, tumor sizes, and lymph node involvement. However, the prognostic value of PD-L1 in CRC remains a subject of debate, with some studies reporting poor outcomes in PD-L1-positive cases, while others indicate no significant correlation. The immunohistochemical scoring system used in this study provides a standardized approach to evaluate PD-L1 expression based on the intensity and percentage of positive tumor cells. By stratifying cases into categories of low, moderate, and high PD-L1 expression, this study seeks to clarify the prognostic implications of PD-L1 in CRC and its relationship with tumor aggressiveness.

Therapeutic Relevance of PD-L1 in CRC

The advent of immune checkpoint inhibitors has transformed the therapeutic landscape of oncology. Agents targeting the PD-1/PD-L1 pathway, such as pembrolizumab and nivolumab, have shown remarkable efficacy in several cancers. However, in CRC, the clinical benefit of these therapies has been predominantly observed in MSI-H/dMMR tumors, which account for approximately 15% of CRC cases. The identification of PD-L1 as a therapeutic target in CRC could expand the scope of immunotherapy to a broader patient population. By identifying tumors with high PD-L1 expression, this study provides insights into potential candidates for immune checkpoint inhibitor therapy. Additionally, the findings may pave the way for combination therapies that enhance the efficacy of immunotherapy in PD-L1-positive CRC cases.

Regional Insights and Global Relevance

The epidemiological and clinicopathological characteristics of CRC can vary significantly across different populations. In India, CRC is increasingly being recognized as a major health concern, with rising incidence rates attributed to urbanization, dietary shifts, and sedentary lifestyles. However, data on the molecular and immunohistochemical features of CRC in the Indian population remain limited. This study, conducted in a tertiary care center in Chennai, provides valuable regional insights into PD-L1 expression in CRC. By adding to the growing body of literature on CRC, the findings contribute to a more comprehensive understanding of the disease in diverse populations. This is particularly relevant in the context of globalization, as insights from regional studies can inform global strategies for cancer diagnosis, prognosis, and treatment.

Future Directions and Research Implications

The findings of this study serve as a foundation for future research on the role of PD-L1 in CRC. Potential areas of investigation include:

- **Molecular Mechanisms:** Understanding the regulatory pathways underlying PD-L1 expression in CRC and their interaction with other immune checkpoint molecules.
- **Biomarker Validation:** Conducting large-scale studies to validate PD-L1 as a prognostic and predictive biomarker in CRC.
- **Therapeutic Strategies:** Exploring combination therapies that integrate PD-L1 inhibitors with other treatment modalities, such as chemotherapy, radiotherapy, or targeted therapies.
- **Longitudinal Studies:** Evaluating the dynamic changes in PD-L1 expression during disease progression and treatment response.

Colorectal carcinoma remains a major public health challenge, necessitating the exploration of novel biomarkers and therapeutic targets. This study highlights the significance of PD-L1 as a key immune checkpoint molecule in CRC, with potential implications for prognosis and immunotherapy. By providing insights into the clinicopathological correlation of PD-L1 expression, this study contributes to the growing body of evidence supporting its role in cancer diagnosis and treatment. The findings underscore the need for continued research on PD-L1 in CRC, with the ultimate goal of improving patient outcomes through personalized medicine.

2.Literature Review

Colorectal carcinoma (CRC) is among the most prevalent and fatal malignancies worldwide, contributing significantly to the global cancer burden. The incidence of CRC has been on the rise due to lifestyle and dietary changes, especially in urbanized regions (Chen et al., 2016; Siegel et al., 2017). As the second leading cause of cancer-related deaths globally, it necessitates continuous exploration of diagnostic and therapeutic advancements. CRC typically develops through genetic mutations, epigenetic alterations, and interactions with the tumor microenvironment (Kuipers et al., 2015). Adenocarcinoma remains the most common histological subtype of CRC, with tumors often progressing undetected until advanced stages. Recent research has focused on understanding the immune evasion strategies employed by CRC, with programmed death-ligand 1 (PD-L1) emerging as a critical factor. PD-L1, expressed on tumor and immune cells, binds to its receptor PD-1 on T-cells, inhibiting their activation and promoting immune tolerance (Keir et al., 2006). This mechanism allows tumor cells to escape immune surveillance, enabling unchecked growth and metastasis. Xiao and Freeman (2015) emphasized the potential of checkpoint inhibitors targeting the PD-1/PD-L1 pathway, particularly in microsatellite instability-high (MSI-H) CRC, which shows a heightened immune response. The prognostic and therapeutic value of PD-L1 in CRC has been extensively investigated. Yang et al. (2019) conducted a meta-analysis involving 12 studies and 4344 cases, revealing that PD-L1 expression correlates with adverse clinicopathological features and poor prognosis in CRC. Their findings underscore PD-L1's role as a significant biomarker for patient stratification and therapy selection. Similarly, Akhmaltdinova et al. (2020) reported elevated serum PD-L1 levels in CRC patients

compared to controls, highlighting its potential as a non-invasive biomarker for early detection. The immune microenvironment in CRC is diverse, as evidenced by Valentini et al. (2018), who categorized CRC tumors into three subsets based on PD-L1 expression patterns. They observed that PD-L1 expression was not restricted to tumor cells but also present on inflammatory cells, indicating its role in shaping the tumor immune landscape. These findings are further supported by Huang et al. (2018), who demonstrated that PD-L1 expression correlates with tumor grade and increased CD8+ T-cell infiltration, suggesting its dual role in immune suppression and tumor progression. Liu et al. (2018) explored the prognostic implications of PD-L1 expression and the Immunoscore in metastatic CRC patients with different RAS statuses. They observed that a higher Immunoscore and PD-L1 expression were associated with improved outcomes following palliative operations. These findings suggest that PD-L1 expression can serve as both a prognostic biomarker and a guide for personalized therapy. Wang et al. (2018) corroborated these findings, demonstrating that PD-L1, along with other markers such as p53 and Ki-67, holds prognostic value in advanced-stage CRC. Molecular features of CRC have also been linked to PD-L1 expression. Rosenbaum et al. (2016) and Li et al. (2016) found associations between PD-L1 expression and microsatellite instability, BRAF mutation, and medullary morphology. These molecular alterations are often indicative of aggressive tumor behavior, further establishing PD-L1 as a marker of poor prognosis. Saigusa et al. (2016) highlighted the role of PD-L1 in tumor recurrence and prognosis following neoadjuvant chemoradiotherapy in rectal cancer, suggesting that PD-L1 inhibition could enhance therapeutic efficacy and reduce recurrence rates. The therapeutic implications of PD-L1 expression in CRC are profound. Immune checkpoint inhibitors targeting the PD-1/PD-L1 axis have revolutionized cancer treatment, particularly in MSI-H and mismatch repair-deficient (dMMR) CRC (Lynch & Murphy, 2016). However, Tariq and Ghias (2016) noted that the variability in PD-L1 expression and its regulation within the tumor microenvironment poses challenges for uniform therapeutic response. This underscores the need for comprehensive studies to identify factors that modulate PD-L1 expression and its predictive value in immunotherapy. Emerging research has also delved into the interplay between PD-L1 expression and the immune system. Keir et al. (2006) described how PD-L1 mediates peripheral T-cell tolerance, effectively dampening immune responses against tumors. Furthermore, the activation of PD-L1 in response to inflammatory signals such as IFN-γ adds another layer of complexity. Xiao and Freeman (2015) emphasized that the microsatellite instability subset of CRC, with high mutational loads, exhibits increased responsiveness to checkpoint blockade due to heightened PD-L1 expression. Valentini et al. (2018) and Huang et al. (2018) have advanced our understanding of PD-L1's role in the tumor microenvironment. Valentini et al. observed that PD-L1-positive tumors showed distinct immune microenvironment subsets, including varying levels of CD8+ T-cell infiltration. Huang et al. noted that PD-L1 expression correlated with high tumor grades and increased infiltration of cytotoxic lymphocytes, further emphasizing the interplay between tumor cells and immune components. Akhmaltdinova et al. (2020) provided clinical evidence of elevated serum PD-L1 levels in CRC patients, suggesting its utility in non-invasive diagnostics. Similarly, Liu et al. (2018) identified a positive relationship between PD-L1 expression and improved outcomes in specific subgroups of metastatic CRC patients, demonstrating its prognostic relevance. Wang et al. (2016, 2018) highlighted the synergistic value of combining PD-L1 with other markers, such as p53 and Ki-67, to enhance prognostic accuracy. Rosenbaum et al. (2016) explored the molecular features of PD-L1 expression in CRC and found its association with aggressive molecular phenotypes, including MSI and BRAF mutation. This highlights the potential for integrating PD-L1 expression into molecular profiling strategies to refine patient stratification. Li et al. (2016) further supported this, demonstrating that high PD-L1 expression was associated with improved outcomes in patients with higher immune activity, suggesting its utility in immunotherapy response prediction. Finally, Saigusa et al. (2016) provided valuable insights into the therapeutic implications of PD-L1 in rectal cancer, particularly in the context of neoadjuvant chemoradiotherapy. Their findings suggest that targeting PD-L1 could improve therapeutic outcomes by reducing recurrence and enhancing immune response. In summary, PD-L1 has emerged as a critical biomarker with prognostic and therapeutic implications in CRC. While its role as a marker of immune evasion and poor prognosis is well-established, its variability across patient

populations and tumor subtypes necessitates further exploration. The integration of PD-L1 expression with other molecular and clinicopathological factors holds promise for refining prognostic accuracy and guiding personalized therapy. As immunotherapy continues to evolve, understanding the nuances of PD-L1 expression in CRC will be pivotal for optimizing patient outcomes.

3.Methodology

3.1.Study Design

This study was a prospective diagnostic analysis designed to evaluate the expression of programmed death-ligand 1 (PD-L1) in colorectal carcinoma (CRC) and correlate its expression with various clinicopathological parameters. The study aimed to provide insights into the immunohistochemical characterization of CRC, particularly examining the relationship between PD-L1 expression and clinical features such as tumor size, site, histological grade, and lymph node involvement. The study was conducted in the Department of Pathology at Sri Balaji Medical College and Hospital (SBMCH), Chennai, over an eight-month period, from March 2021 to October 2021. The prospective nature of the study allowed for the collection of primary data at the time of patient diagnosis, ensuring the accuracy and relevance of the information for subsequent analysis. The study's objective was to explore the potential role of PD-L1 as a biomarker for CRC prognosis and its possible clinical applications in guiding therapeutic decisions, such as immune checkpoint inhibitor therapy. The prospective design facilitated the direct observation and documentation of clinical outcomes associated with PD-L1 expression, offering real-time insights into its diagnostic and prognostic value.

3.2.Study Setting

The research was carried out in a tertiary care teaching hospital, SBMCH, which serves as a major referral center for a diverse demographic population. The hospital provides specialized medical care, including advanced diagnostic and therapeutic services, which made it an ideal setting for the study of CRC cases. The patient population was heterogeneous, encompassing individuals from various age groups, socioeconomic backgrounds, and ethnicities. This diversity ensured that the study's findings would be more generalizable to a broader spectrum of patients. SBMCH's pathology department is equipped with state-of-the-art facilities for conducting immunohistochemical (IHC) assays, histopathological evaluations, and other diagnostic procedures. This enabled the study to employ robust techniques for assessing PD-L1 expression in tissue samples, which is essential for generating high-quality data on the biomarker's clinical significance.

3.3.Study Population

The study population comprised patients diagnosed histopathologically with colorectal adenocarcinoma during the study period at SBMCH. Colorectal adenocarcinoma was chosen as the focus due to its high prevalence and significance as the most common form of CRC. All patients included in the study had undergone surgical resection of their tumors, providing tissue samples that could be used for subsequent histopathological and immunohistochemical analysis.

Inclusion Criteria:

- Specimens diagnosed as colorectal adenocarcinoma based on histopathological examination (HPE).
- Formalin-fixed paraffin-embedded (FFPE) tissue samples suitable for immunohistochemical staining.

Exclusion Criteria:

- Non-neoplastic conditions of the colon and rectum.
- Poorly processed or inadequate biopsies.
- Carcinomas of the colon or rectum other than adenocarcinoma, including squamous cell carcinoma and neuroendocrine carcinoma.

The inclusion criteria were carefully defined to ensure that only patients with confirmed CRC were included, eliminating potential biases from non-neoplastic lesions or other forms of carcinoma. The FFPE tissue samples were selected as they offer the advantage of long-term storage and preservation while maintaining the integrity of antigenic sites required for IHC analysis.

3.4.Sample Size

A total of 48 colorectal carcinoma cases were included in the study. The sample size was determined based on the availability of adequately processed tissue samples during the study period, as well as the feasibility of performing immunohistochemistry (IHC) on all samples within the study timeline. While the sample size was relatively small, it was sufficient to provide meaningful insights into the association between PD-L1 expression and clinicopathological variables in CRC. The study aimed to strike a balance between statistical power and practicality, given the resources available.

3.5.Ethical Considerations

Ethical approval for the study was obtained from the institutional ethics committee prior to the commencement of data collection. The approval process ensured that the study adhered to ethical guidelines, particularly with regard to patient confidentiality, informed consent, and data privacy. Written informed consent was obtained from all participants or their legal representatives before tissue samples were used for research purposes. The consent process included detailed explanations regarding the study's aims, procedures, and potential risks, ensuring that participants understood their rights and the use of their biological samples. Patient confidentiality was a key consideration throughout the study. Data were anonymized by assigning unique identification codes to each participant, preventing any direct identification from the collected data. The study team adhered to strict confidentiality protocols, and all patient-related information was securely stored in a password-protected database accessible only to authorized personnel.

3.6.Specimen Processing

The processing of tissue samples followed a standardized protocol to ensure the accuracy and reproducibility of the histopathological and immunohistochemical analyses. The primary steps involved fixation, embedding, sectioning, and staining of tissue specimens.

Fixation and Gross Examination:

Immediately after surgical excision, tissue specimens were fixed in 10% neutral buffered formalin to preserve tissue morphology and antigenicity. Proper fixation is crucial for maintaining the integrity of tissue structures, which is essential for downstream histological and immunohistochemical analysis. The fixation process typically took 24 to 48 hours to ensure thorough penetration of the fixative into the tissue. The gross examination of specimens followed established protocols, with detailed documentation of tumor dimensions, margins, and lymph node retrieval. Tumor size was measured in centimeters, and the presence of lymph nodes was recorded for further examination during histopathological evaluation.

Tissue Embedding and Sectioning:

After fixation, tissue samples were processed in an automated tissue processor to remove water and replace it with paraffin wax, which helps preserve the tissue architecture. Once embedded in paraffin, tissue blocks were sectioned into 3–4 µm thick slices using a rotary microtome. These thin sections were then placed on glass slides for subsequent histopathological and immunohistochemical staining.

Hematoxylin and Eosin (H&E) Staining:

Routine histopathological evaluation was performed using H&E staining. This stain is widely used in clinical pathology to assess tissue morphology and identify abnormalities such as tumor grade, size, and lymph node involvement. The H&E stain provided essential information for confirming the diagnosis of adenocarcinoma and evaluating histological features, such as glandular differentiation and the extent of tumor invasion. The below Figure:2 Shows the entire workflow.

3.7.Histopathological Examination

The histopathological parameters were carefully evaluated to provide a comprehensive analysis of the tumor's characteristics and its potential correlation with PD-L1 expression. Key features assessed included the tumor site, size, histological grade, and lymph node involvement.

Tumor Site: Tumors were classified into three main sites: right-sided (caecum, ascending colon, transverse colon), left-sided (splenic flexure, descending colon, sigmoid colon), and rectal. This classification allowed for the identification of potential site-specific differences in PD-L1 expression, which could have implications for prognosis and treatment.

Tumor Size: Tumor size was categorized into two groups: ≤5 cm and >5 cm. Tumor size has long been recognized as an important prognostic factor in CRC, with larger tumors often associated with more advanced stages of disease and poorer clinical outcomes.

Histological Grade: The histological grade of tumors was assessed based on glandular differentiation and nuclear features. Tumors were classified as low-grade (well/moderately differentiated) or high-grade (poorly differentiated). High-grade tumors generally have a more aggressive clinical course, and this grading system provided useful insights into the potential relationship between PD-L1 expression and tumor aggressiveness.

Lymph Node Involvement: The presence of metastatic deposits in lymph nodes was evaluated as an indicator of disease progression and spread. Lymph node involvement is a critical factor in determining the stage of CRC and can significantly influence treatment decisions. The Figure:2 shows the entire workflow.

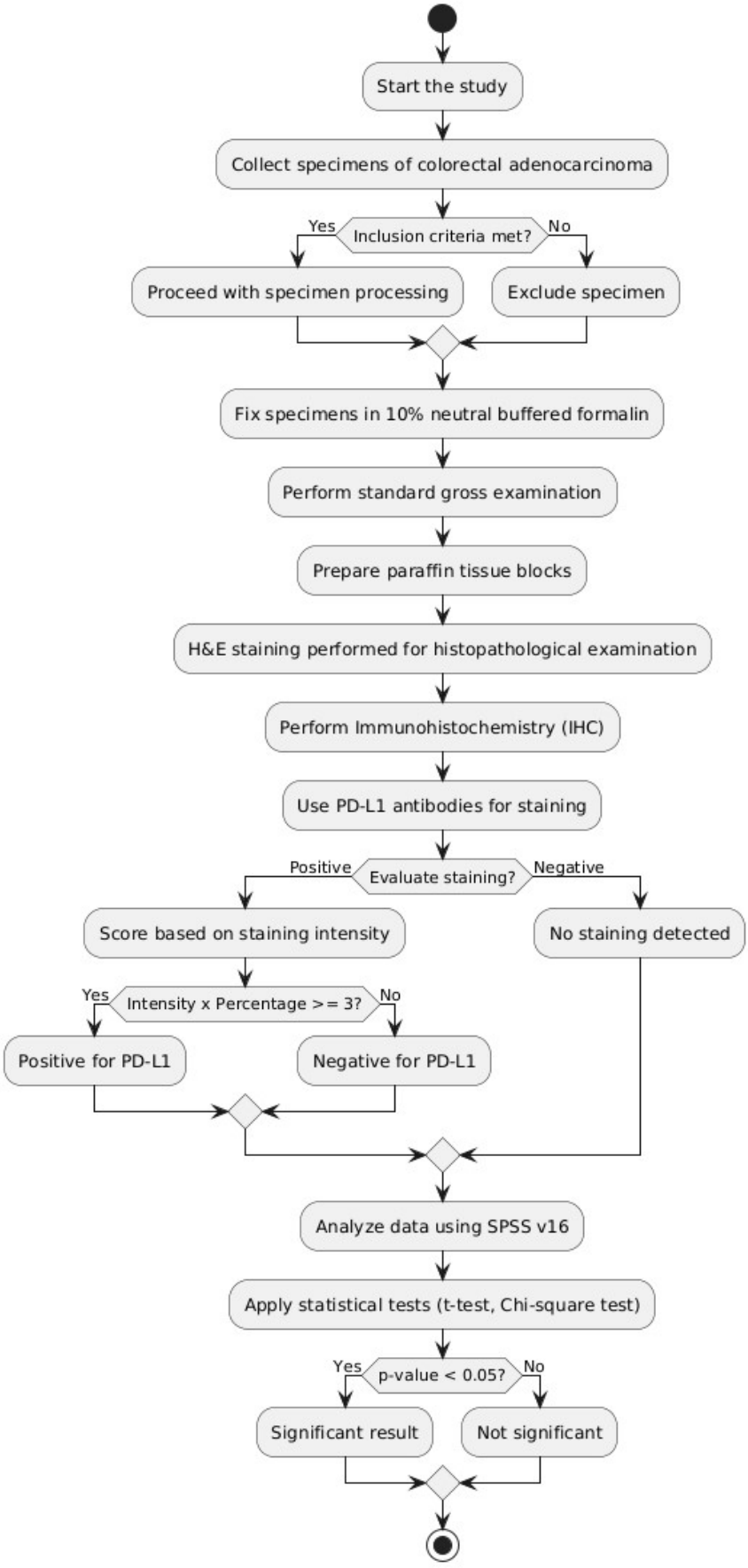
Start the study
Collect specimens of colorectal adenocarcinoma
Inclusion criteria met?
Yes
No
Proceed with specimen processing
Exclude specimen
Fix specimens in 10% neutral buffered formalin
Perform standard gross examination
Prepare paraffin tissue blocks
H&E staining performed for histopathological examination
Perform Immunohistochemistry (IHC)
Use PD-L1 antibodies for staining
Evaluate staining?
Positive
Negative
Score based on staining intensity
No staining detected
Intensity x Percentage >= 3?
Yes
No
Positive for PD-L1
Negative for PD-L1
Analyze data using SPSS v16
Apply statistical tests (t-test, Chi-square test)
p-value < 0.05?
Yes
No
Significant result
Not significant

Figure:2 Methodology Workflow

3.8.Immunohistochemistry (IHC) Procedure

The immunohistochemical (IHC) technique was used to assess the expression of PD-L1 in colorectal cancer tissue samples. IHC is a widely accepted method for detecting specific proteins in tissue sections and provides detailed information about protein localization and expression levels.

Antigen Retrieval: To unmask the PD-L1 epitopes in the tissue sections, heat-induced antigen retrieval was performed using a citrate buffer (pH 6.0) in a microwave oven. This step is crucial for optimizing the binding of the antibody to its target protein.

Blocking: Endogenous peroxidase activity was blocked using 3% hydrogen peroxide to prevent non-specific binding. Additionally, a blocking solution containing 5% bovine serum albumin was applied to minimize non-specific antibody interactions.

Primary Antibody Incubation: The tissue sections were incubated with a PD-L1 monoclonal antibody, which specifically targets the PD-L1 protein. The recommended dilution and incubation conditions provided the optimal conditions for antibody binding.

Secondary Antibody and Detection: Following primary antibody incubation, a biotinylated secondary antibody was applied to bind to the primary antibody. A streptavidin-HRP conjugate was then used to visualize the antibody-antigen complex, and a diaminobenzidine (DAB) chromogen was used to generate a brown precipitate at the site of PD-L1 expression.

Counterstaining and Mounting: After the signal was visualized, the tissue sections were counterstained with hematoxylin to highlight cellular details. The slides were then dehydrated and mounted with a coverslip for final microscopic evaluation.

IHC Scoring and Interpretation

PD-L1 expression was quantified by evaluating the staining intensity and the percentage of positive tumor cells. Only membrane staining of tumor cells was considered positive, with cytoplasmic staining excluded from the analysis.

Scoring System:

- **Intensity:** The intensity of staining was graded as 0 (negative), 1+ (weak), 2+ (moderate), and 3+ (strong).
- **Percentage of Staining:** The percentage of PD-L1-positive tumor cells was categorized into four groups: 0% (negative), 1–10%, 11–50%, and >50%.

The combined percentage and intensity score were used to classify cases as PD-L1-negative or PD-L1-positive, with the latter indicating a potential role for immunotherapy.

3.9.Statistical Analysis

Data were analyzed using SPSS software (version 26.0). Descriptive statistics were used to summarize patient demographics, tumor characteristics, and PD-L1 expression. The chi-square test was applied to assess the association between PD-L1 expression and various clinicopathological features, such as tumor size, grade, site, and lymph node involvement. A p-value of <0.05 was considered statistically significant.

4.Results

4.1.Demographic Characteristics

The demographic characteristics of the study cohort are summarized in Table 5, which provides detailed information on the age and gender distribution of the patients.

Age Distribution

A total of 48 patients were included in this study, and their age distribution is shown in Table 5. The majority of patients were aged between 51 and 70 years (54.2%), indicating that colorectal cancer (CRC) predominantly affects middle-aged individuals. Patients aged 51–70 years were followed by the age group of ≤50 years, which accounted for 29.2% of the total cohort. The age group of ≥71 years had the least representation, comprising only 16.6% of the patients.

Table 1: Distribution of age among the study population (n=48)

Age (Years)	Number (n)	Percentage (%)
≤50	14	29.2
51–70	26	54.2
≥71	8	16.6
Total	**48**	**100**

Mean ± SD: 58.39 ± 9.84 years

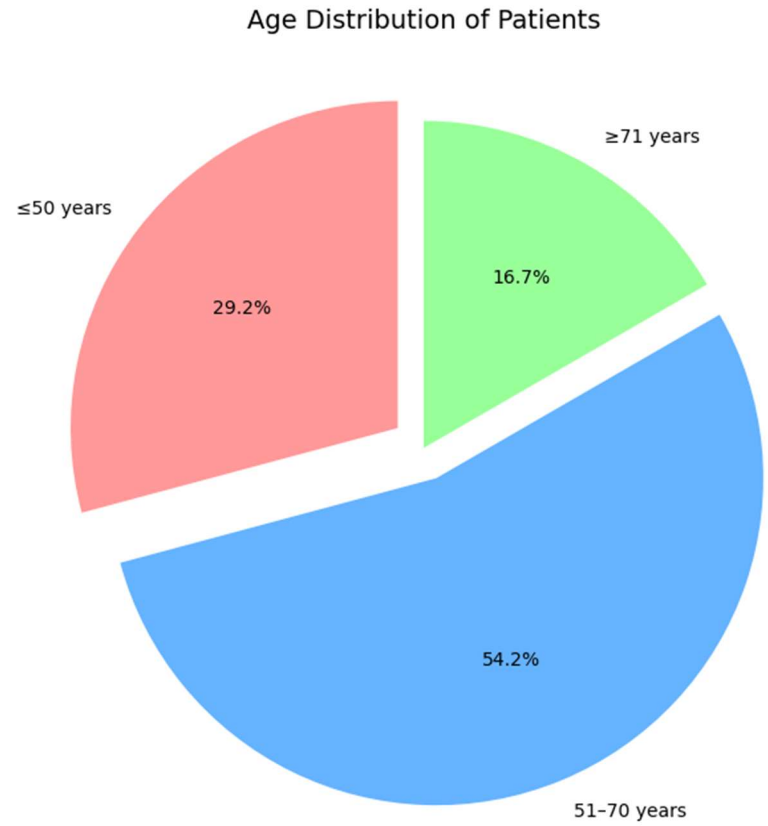

Figure:3 Age Distribution of patents

Table:1 shows the age distribution highlights the predominance of CRC in middle-aged individuals, with the peak incidence occurring between 51 and 70 years as shown in figure:1.

4.2.Gender Distribution

Table 2 presents the gender distribution of the cohort. Of the 48 patients, there was a slight male predominance, with 52.1% of the patients being male and 47.9% being female. The relatively balanced gender distribution reflects the overall epidemiology of CRC, which affects both genders, though with slight variation in certain populations.

Table 2: Distribution of gender among the study population (n=48)

Gender	Number (n)	Percentage (%)
Male	25	52.1
Female	23	47.9

Total	**48**	**100**

This table:2 shows the distribution aligns with the typical gender ratio observed in CRC, where a slightly higher incidence is seen in males. The below Figure:4 shows the Gender distribution.

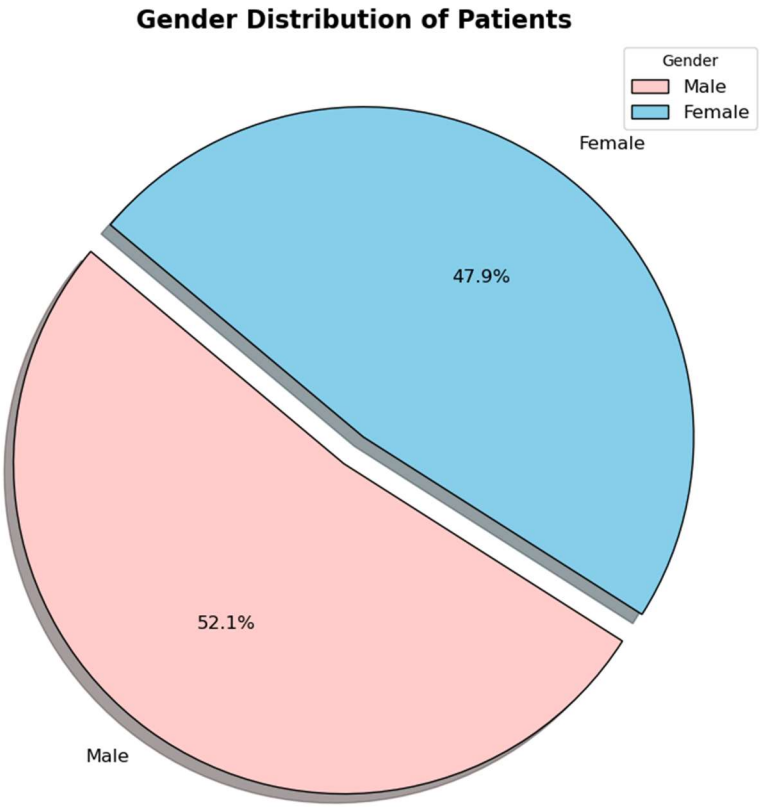

Figure:4 Gender Distribution of Patents

4.3.Tumor Characteristics

The tumor characteristics assessed include the site of the tumor, tumor size, and histological grade. These factors are crucial in understanding the clinical presentation of CRC and their relationship with PD-L1 expression.

Tumor Site Distribution

The distribution of tumor sites is presented in Table 3. The rectum was the most commonly involved site, accounting for 50% of the cases. This finding is consistent with the known epidemiology of CRC, where rectal cancer is often as prevalent as colon cancer. The left side of the colon, including the splenic flexure to the sigmoid colon, was the second most common tumor site, representing 35.4% of cases. The right side of the colon, including the caecum, ascending, and transverse colon, was the least commonly involved site, accounting for only 14.6% of cases as shown in figure:5 and figure:6.

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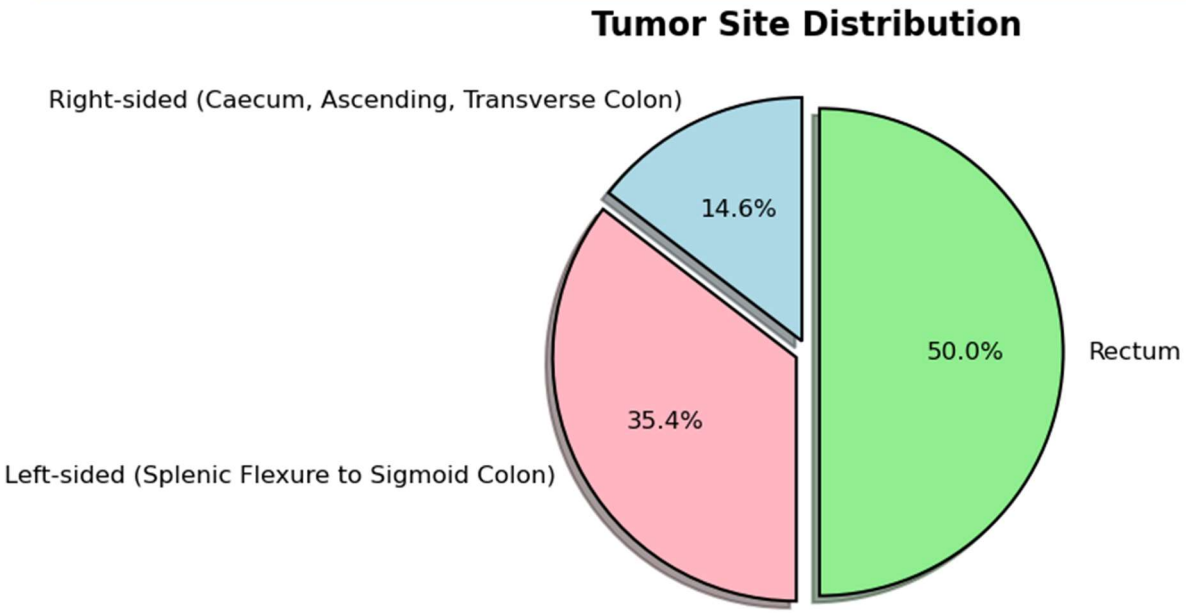

Figure:5 Tumor site distribution Pie Chart

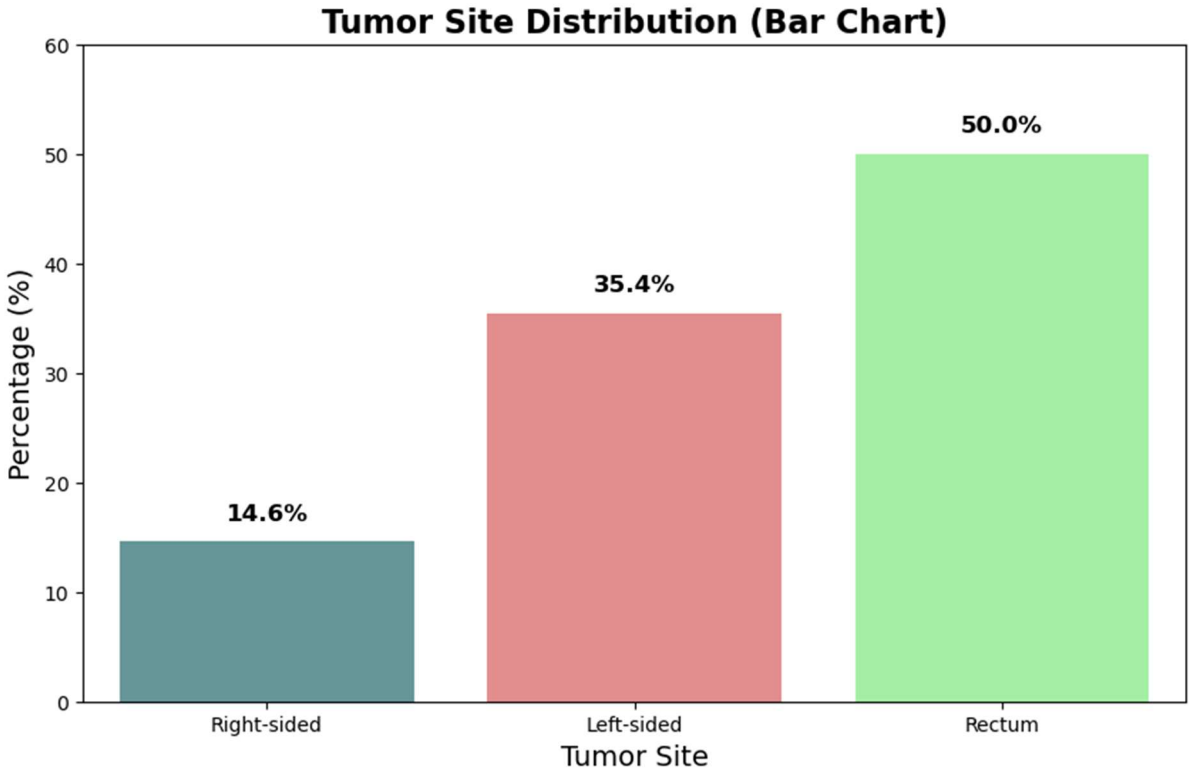

Figure:6 Tumor Size Distribution Bar chart

Table 3: Distribution of tumour site among the study population (n=48):

Tumor Site	Number (n)	Percentage (%)
Caecum, Ascending, Transverse Colon (Right)	7	14.6

Splenic Flexure to Sigmoid Colon (Left)	17	35.4
Rectum	24	50.0
Total	**48**	**100**

This table:3 shows the distribution reflects the well-established fact that CRC is more common in the rectum and the left colon, especially in the age group studied.

4.4.Tumor Size

Tumor size, as summarized in Table 4, is another important characteristic of CRC that can influence prognosis. The majority of tumors in the cohort were between 2 and 5 cm in size (58.3%). Tumors larger than 5 cm were observed in 41.7% of the cases. This indicates that while a substantial portion of the tumors were relatively small, a significant proportion were larger, which may be associated with more advanced disease and poorer prognosis.

Table 4: Distribution of tumour size among the study population (n=48):

Tumor Size (cm)	**Number (n)**	**Percentage (%)**
2–5	28	58.3
>5	20	41.7
Total	**48**	**100**

- **Mean ± SD:** 4.63 ± 1.99 cm

This table:4 suggests that the cohort had a mixture of both early-stage and more advanced CRC, with a relatively higher proportion of intermediate-sized tumors.

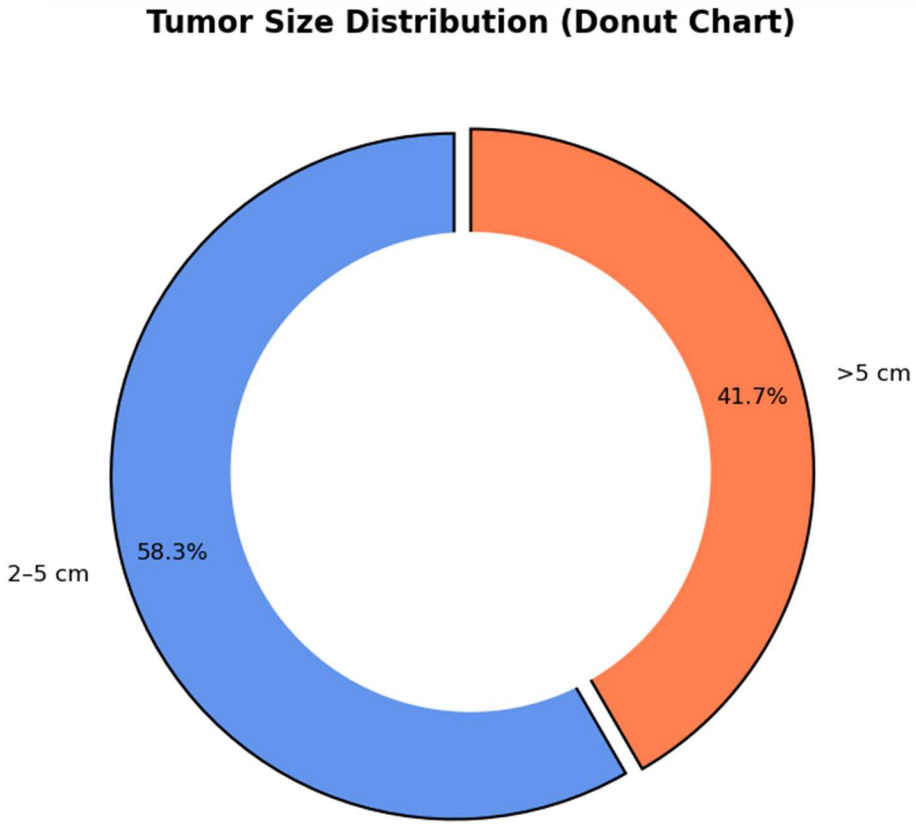

Figure:7 Tumor Size Distribution Donut Chart

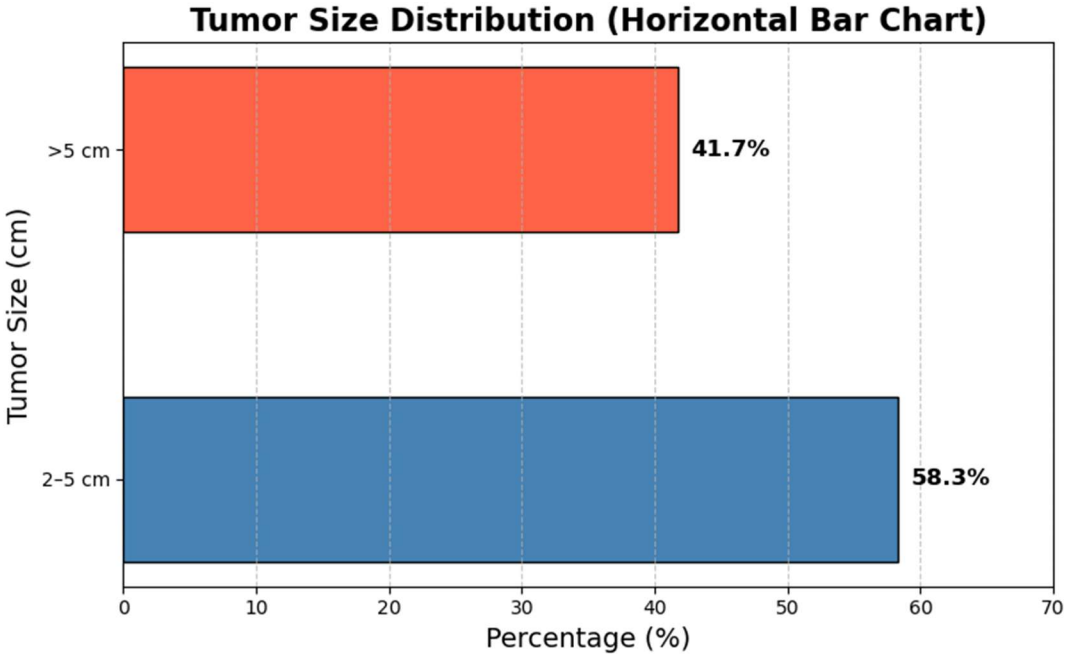

Figure:8 Tumor Size Distribution Bar Chart

The above figure:7 and figure:8 shows the Tumor size distribution visualization in bar and donut chart.

4.5.Histological Grade

Histological grading, as summarized in Table 5, helps assess the aggressiveness of the tumor. Most of the tumors in the cohort were low-grade (75%), which suggests that the majority of cases were less aggressive. High-grade tumors, which typically indicate a more aggressive form of cancer, were seen in 25% of the cases as shown in figure:9. Low-grade tumors are often associated with a better prognosis and may be less likely to exhibit aggressive biological behavior.

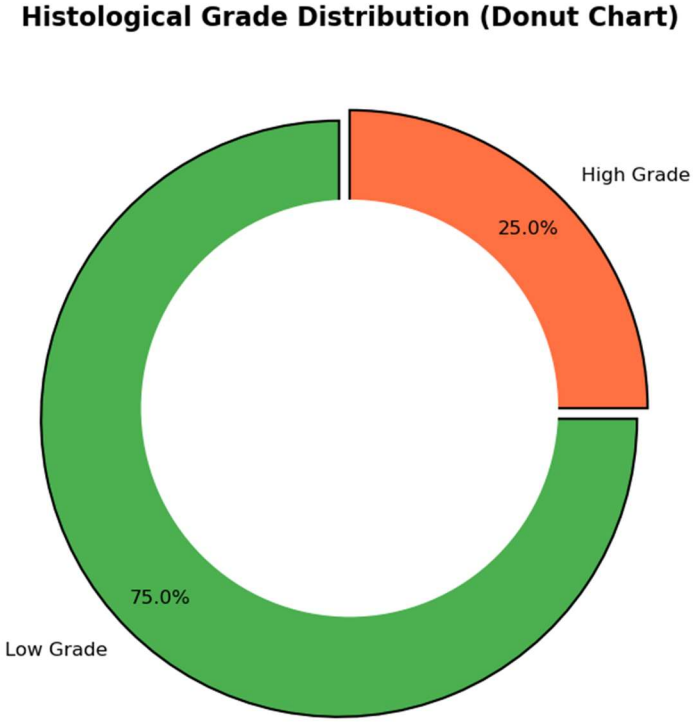

Figure:9 Historical grade Donut Chart

Table:5 Histological Grade

Histological Grade	Number (n)	Percentage (%)
Low Grade	36	75.0
High Grade	12	25.0
Total	**48**	**100**

This distribution shown in table:5 is typical for CRC, where the majority of cases are low grade but still pose a significant clinical burden.

4.6.PD-L1 Expression

PD-L1 expression is a critical marker in cancer immunotherapy, particularly in assessing the potential efficacy of immune checkpoint inhibitors. The results of PD-L1 expression in the cohort are summarized in the following tables.

Expression Levels

Table 6 summarizes the PD-L1 expression levels. PD-L1 expression was negative in 41.7% of the cases, which suggests that these patients are unlikely to benefit from PD-L1-targeted therapies. Expression levels of <10% were seen in 10.4% of the cases, while 25% of the tumors exhibited 10%–50% PD-L1 positivity. Interestingly, 22.9% of the tumors showed >50% PD-L1 positivity, indicating a potential for immunotherapy in these patients.

Table:6 Expression levels

PD-L1 Expression	Number (n)	Percentage (%)
Negative	20	41.7
<10%	5	10.4
10%–50%	12	25.0
>50%	11	22.9
Total	**48**	**100**

This distribution indicates that a significant proportion of CRC cases could benefit from immunotherapy, especially those with high PD-L1 expression as shown in figure:10 and figure:11.

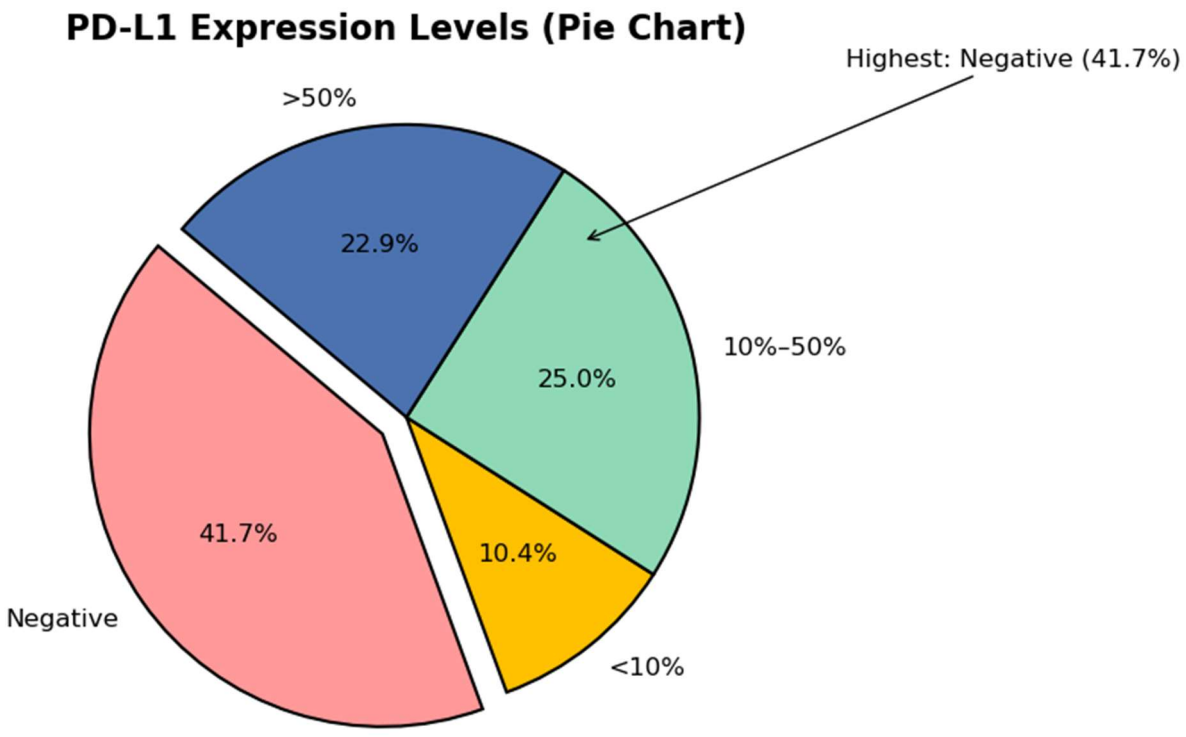

Figure:10 PD-L1 Expression level pie chart

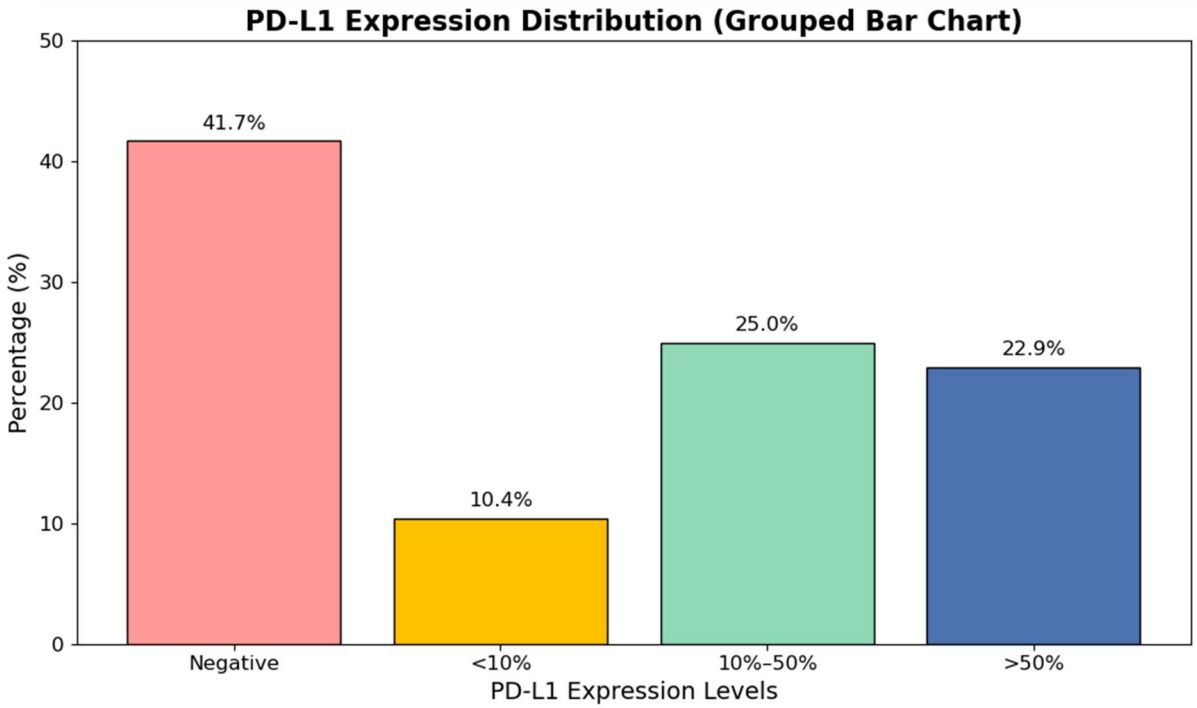

Figure:11 PD-L1 Expression level Grouped Bar chart

Staining Intensity

Table:7 presents the staining intensity of PD-L1 in the tumor samples. Most positive cases exhibited mild to moderate intensity, with 29.2% of the cases showing mild intensity and 25% exhibiting moderate intensity. Only 4.1% of the cases showed strong staining intensity, indicating that while PD-L1 expression is present in a large proportion of the cohort, it is generally of mild to moderate intensity as shown in figure:12.

Table:7 Staining Intensity

Staining Intensity	**Number (n)**	**Percentage (%)**
Nil	20	41.7
Mild	14	29.2
Moderate	12	25.0
Strong	2	4.1
Total	**48**	**100**

This distribution suggests that the overall PD-L1 expression is relatively low in intensity, with only a small fraction of cases showing high-intensity staining.

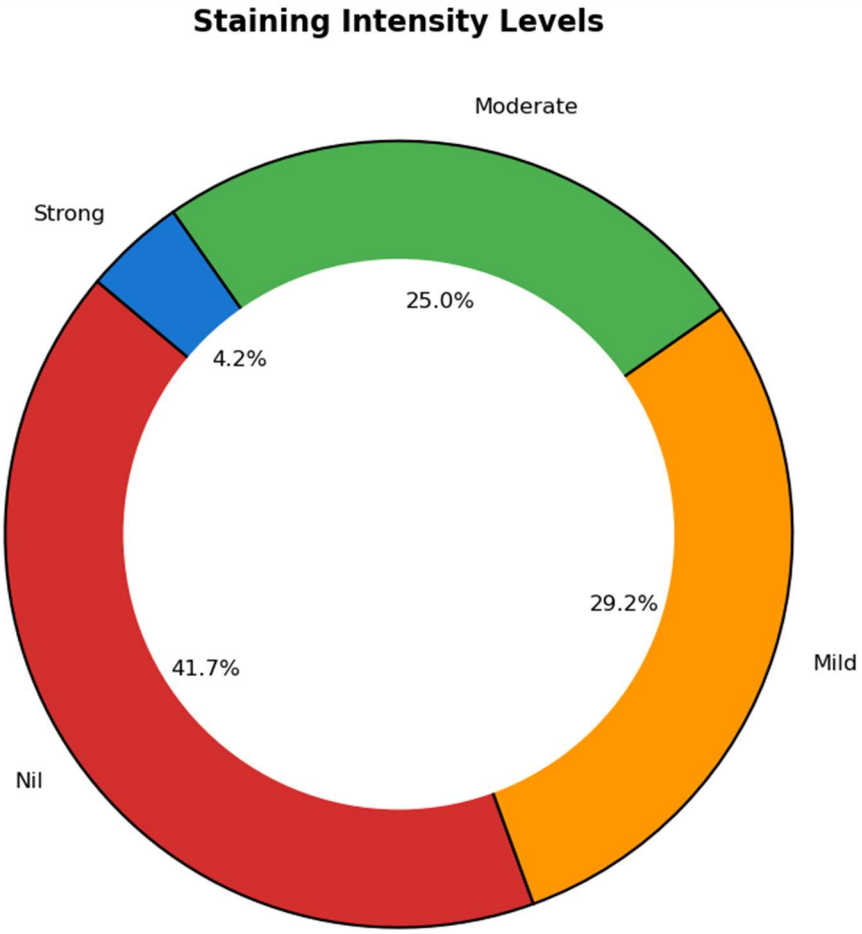

Figure:12 Staining Intensity levels Donut chart

4.7.IHS Score

The immunohistochemical (IHS) scores, which combine both intensity and percentage of PD-L1 staining, are summarized in Table 8. An IHS score of ≥3, which is considered positive, was observed in 37.5% of the cases. The remaining 62.5% had an IHS score of <3, indicating low or no PD-L1 expression as shown in figure:13 and Figure:14.

Table:8 HIS Score

IHS Score	**Number (n)**	**Percentage (%)**
<3	30	62.5
≥3	18	37.5
Total	**48**	**100**

This result table:8 suggests that while a significant portion of the tumors showed low PD-L1 expression based on IHS scoring, there is still a considerable group with a positive IHS score, warranting further investigation into the potential for immunotherapy in these patients.

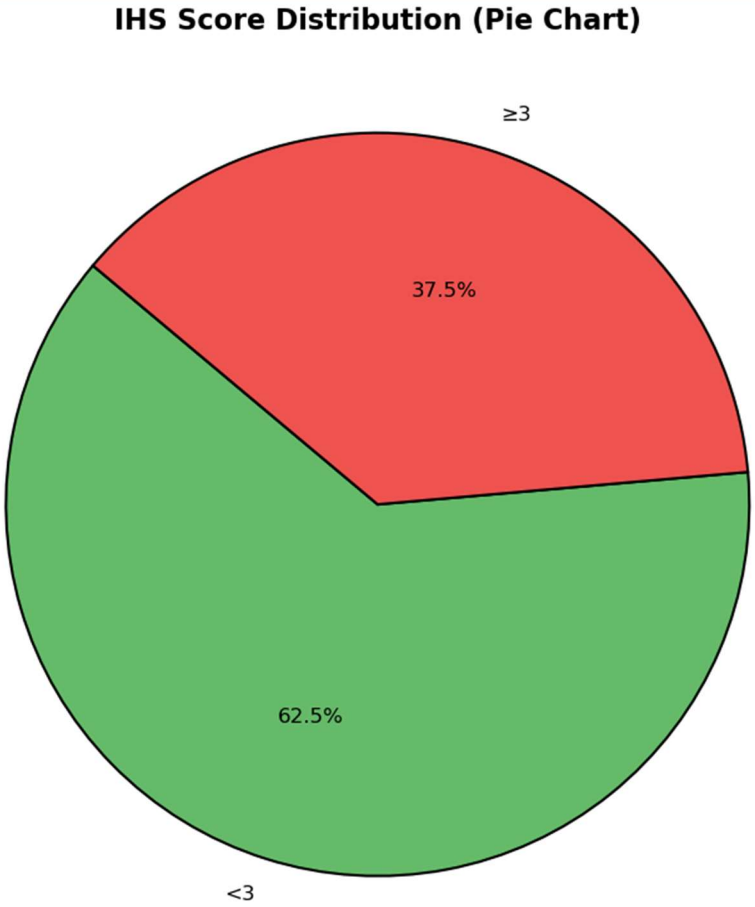

Figure:13 HIS Score distribution pie chart

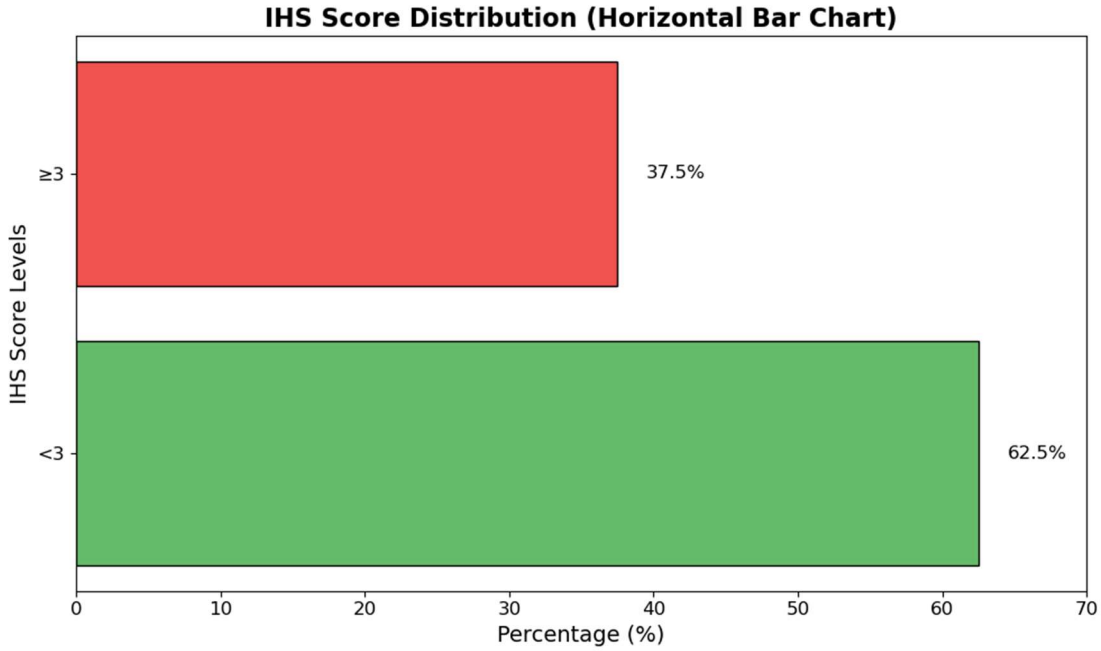

Figure:14 HIS Score Distribution Bar chart

The demographic characteristics of the study cohort show that the majority of patients were aged 51–70 years, with a slight male predominance. In terms of tumor characteristics, the rectum was the most common site of CRC, with tumors predominantly sized between 2–5 cm. Most cases were low-grade histology, indicating a relatively less aggressive tumor profile in this cohort. Regarding PD-L1 expression, 41.7% of tumors showed no PD-L1 expression, while 22.9% exhibited high PD-L1 expression (>50%). Mild to moderate staining intensity was observed in most positive cases, and 37.5% of the tumors had a positive IHS score. Higher PD-L1 expression was associated with lower histological grade, although this correlation was not statistically significant. These findings suggest that while a substantial portion of patients may benefit from PD-L1-targeted therapies, further research is needed to explore the clinical relevance of PD-L1 expression and its relationship with tumor grade and other clinical variables.

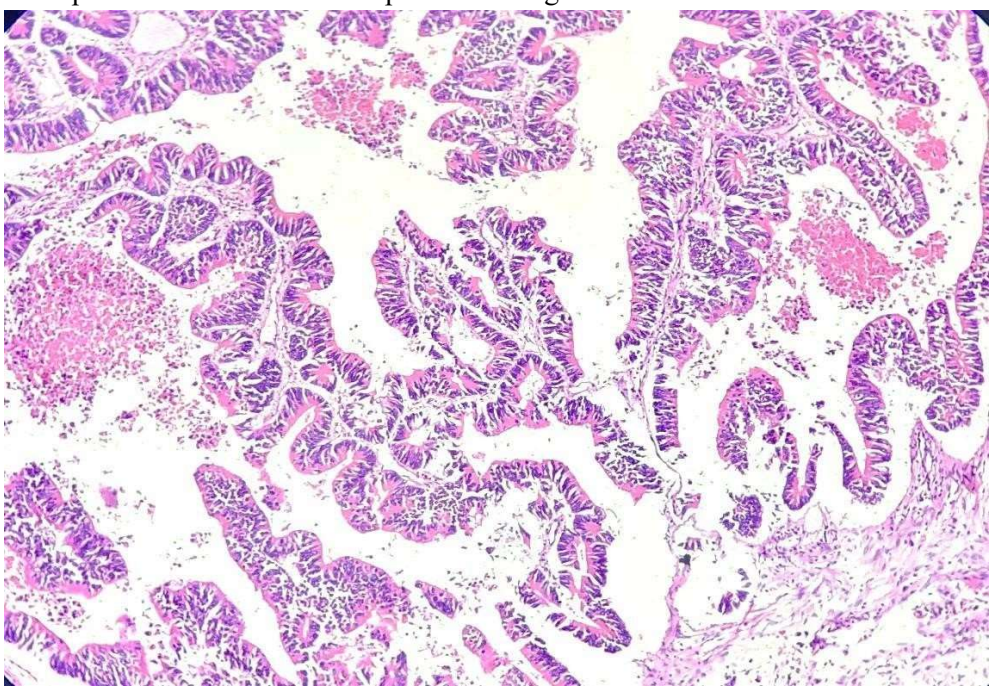

Fig 15: H & E section of low grade colorectal adenocarcinoma with >50% of glands and necrosis (Low power- 10X)

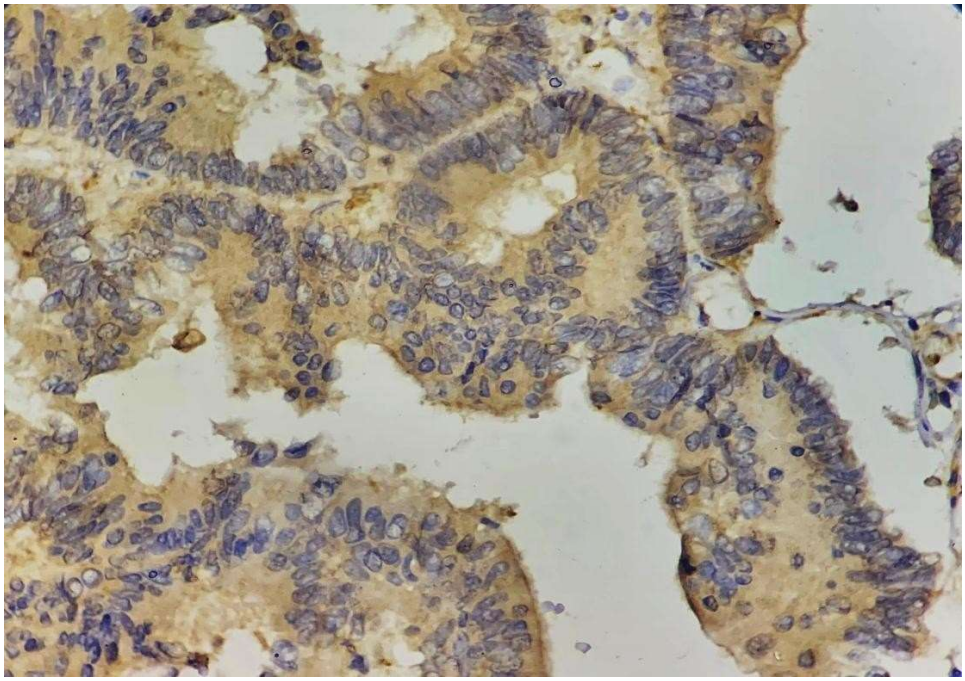

Fig 16: Low grade colorectal adenocarcinoma show moderate staining intensity for PDL1(High power- 40X)

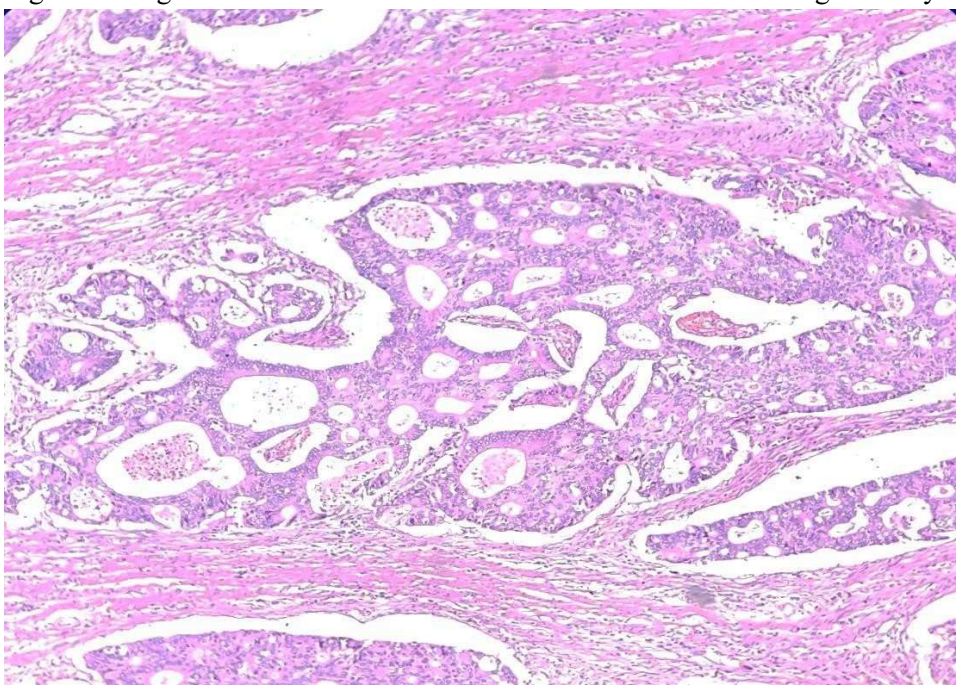

Fig 17: Low grade colorectal adenocarcinoma showing well formed glands with mucin secretions (H & E- 10X)

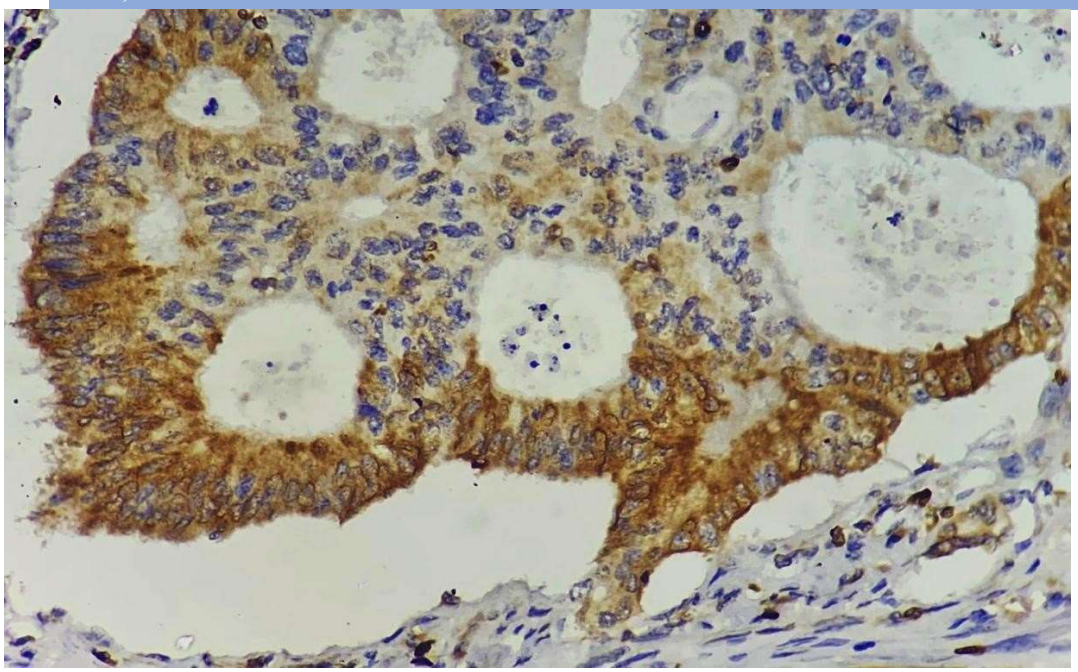

Fig 18: Low grade colorectal adenocarcinoma showing strong staining for PDL1 (high power- 40X)

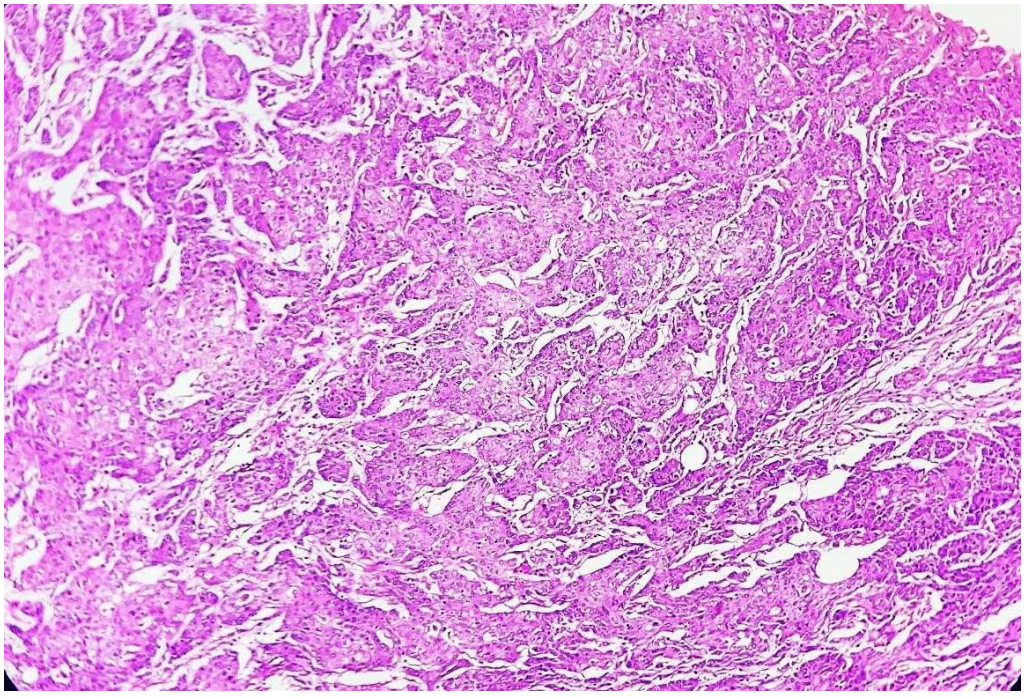

Fig 19: H & E section of high-grade colorectal adenocarcinoma composed of tumor cells in sheets (Low power- 10X)

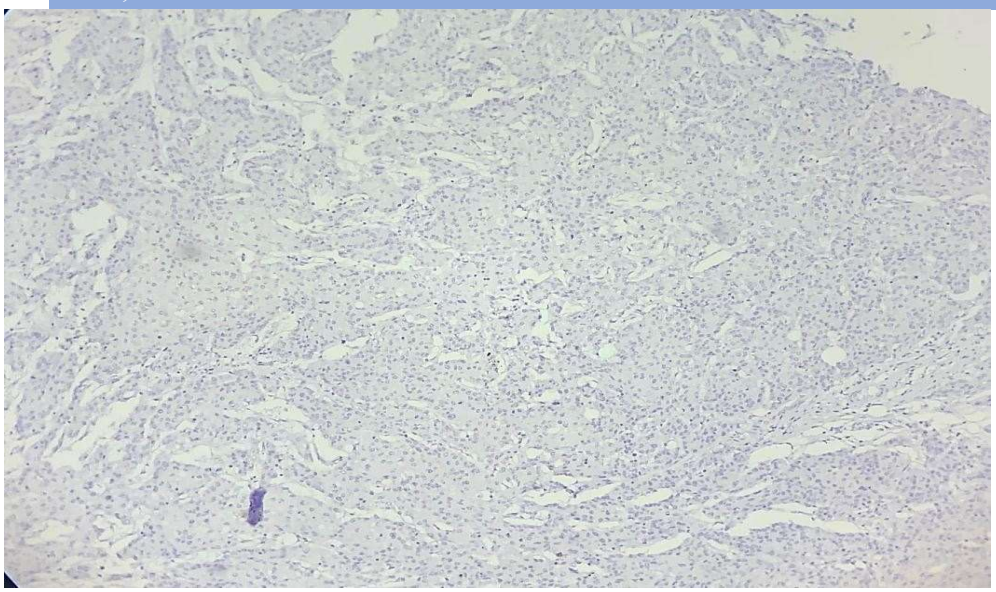

Fig 20: High-grade colorectal adenocarcinoma showing nil staining for PDL1 (Low power- 10X)

Figure:15 shows the H&E section of low-grade colorectal adenocarcinoma shows over 50% of the area occupied by well-formed glandular structures, accompanied by regions of necrosis. Observed under low power (10X), the overall architecture indicates a predominantly glandular differentiation Figure:16 shows the image highlights a low-grade colorectal adenocarcinoma with moderate staining intensity for PD-L1, as visualized under high power (40X). The partial staining suggests an intermediate level of PD-L1 expression within the tumor cells. Figure:!7 shows the H&E section reveals low-grade colorectal adenocarcinoma with well-formed glands containing mucin secretions. Under low power (10X), the glands are well-defined, reflecting the mucinous nature of the tumor. Figure:18 also shows the Low-grade colorectal adenocarcinoma demonstrates strong staining intensity for PD-L1 under high power (40X). This robust PD-L1 expression indicates significant immunogenic activity within the tumor microenvironment. Figure:19 is a High-grade colorectal adenocarcinoma is seen in this H&E section, with tumor cells arranged in disorganized sheets. Low power (10X) observation reveals a lack of glandular differentiation, characteristic of high-grade tumors. Figure:20 is The high-grade colorectal adenocarcinoma shows no detectable staining for PD-L1 at low power (10X). The absence of PD-L1 expression suggests a lack of immune checkpoint protein activity in this tumor type.

5.Conclusion

This study highlights the significance of programmed death-ligand 1 (PD-L1) expression in colorectal carcinoma (CRC) and its potential role as a prognostic biomarker and therapeutic target. Colorectal carcinoma, a leading gastrointestinal malignancy, exhibits diverse clinicopathological characteristics that influence patient outcomes. By focusing on PD-L1, this research provides insights into the tumor microenvironment's role in immune evasion, a key mechanism in cancer progression. The findings revealed that PD-L1 expression varied across the study population, with a substantial proportion exhibiting no expression, while others showed moderate to high positivity. Although there was no statistically significant correlation between PD-L1 expression and factors such as age, gender, tumor size, or histological grade, trends suggested a potential association between higher PD-L1 expression and lower tumor differentiation. This underscores the complexity of PD-L1 regulation and its interaction with tumor biology. The clinical implications of this study are substantial. High PD-L1 expression may identify a subset of CRC patients who could benefit from immune checkpoint inhibitor therapies, which have shown promising results in other malignancies. However, the variability in PD-L1 expression across cases underscores the need for further research to establish robust predictive markers for therapeutic response. Additionally, this study provides a regional perspective on CRC in India, contributing valuable data to the global understanding of this malignancy. The findings pave the way for larger, multicenter studies to validate

the role of PD-L1 in CRC and explore its utility in personalized medicine. In conclusion, while PD-L1 expression alone may not serve as a definitive prognostic marker, its evaluation in combination with other clinicopathological and molecular factors holds promise for improving prognostic accuracy and tailoring immunotherapeutic strategies in colorectal carcinoma.

6.References:

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