PROGRAMMED DEATH LIGAND-1 AND MUTL HOMOLOG-1 EXPRESSION IN COLORECTAL CANCER AND ITS CORRELATION WITH CLINICOPATHOLOGICAL PARAMETERS

BY
DR. QUEEN MARY. A

DISSERTATION SUBMITTED TO
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TAMAKA, KOLAR, KARNATAKA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF MEDICINE
IN
PATHOLOGY

UNDER THE GUIDANCE OF

DR. T.N. SURESH MD, DNB, MNAMS
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DEPARTMENT OF PATHOLOGY

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SRI DEVARAJ URS MEDICAL COLLEGE, KOLAR
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This study is carried out by **Dr. A Queen Mary, Dr. T. N, Suresh[1]** and **Dr. P.N. Sreeramulu[2]** in the Department of Pathology[1] and Surgery[2] at Sri Devaraj Urs Academy of Higher Education and Research Tamaka, Kolar.

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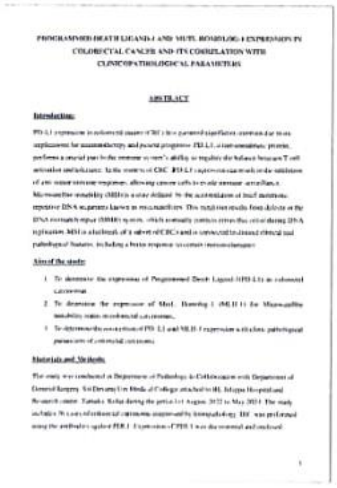

PROGRAMMED DEATH LIGAND-1 AND MUTL HOMOLOG-1 EXPRESSION IN COLORECTAL CANCER AND ITS CORRELATION WITH CLINICOPATHOLOGICAL PARAMETERS

ABSTRACT

Introduction:

Aims of the study:

1. To determine the expression of Programmed Death Ligand-1(PD-L1) in colorectal carcinomas.
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3. To determine the association of PD- L1 and MLH 1 expression with clinicopathological parameters of colorectal carcinoma.

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PROGRAMMED DEATH LIGAND-1 AND MUTL HOMOLOG-1 EXPRESSION IN COLORECTAL CANCER AND ITS CORRELATION WITH CLINICOPATHOLOGICAL PARAMETERS ABSTRACT Introduction: PD-L1 expression in colorectal cancer (CRC) has garnered significant attention due to its implications for immunotherapy and patient prognosis. PD-L1, a transmembrane protein, performs a crucial part in the immune system's ability to regulate the balance between T cell activation and tolerance. In the context of CRC, PD-L1 expression can result in the inhibition of anti-tumor immune responses, allowing cancer cells to evade immune surveillance. Microsatellite instability (MSI) is a state defined by the accumulation of brief mutations; repetitive DNA sequences known as microsatellites. This condition results from defects in the DNA mismatch repair (MMR) system, which normally corrects errors that occur during DNA replication. MSI is a hallmark of a subset of CRCs and is connected to distinct clinical and pathological features, including a better response to certain immunotherapies. Aim of the study: 1. To determine the expression of Programmed Death Ligand-1(PD-L1) in colorectal carcinomas. 2. To determine the expression of MutL Homolog-1 (MLH-1) for Microsatellite instability status in colorectal carcinomas. 3. To determine the association of PD-L1 and MLH-1 expression with clinic pathological parameters of colorectal carcinoma. Materials and Methods: The study was conducted in Department of Pathology in Collaboration with Department of General Surgery, Sri Devaraj Urs Medical College attached to RL Jalappa Hospital and Research centre, Tamaka, Kolar during the period of August 2022 to May 2024. The study includes 76 cases of colorectal carcinoma diagnosed by histopathology. IHC was performed using the antibodies against PDL1. Expression of PDL1 was documented and analysed. Statistical analysis was performed using Chi-square test or Fischers exact test. A p value of less than 0 .005 was considered statistically significant. Results: Peak incidence was seen in the 60-69 years age group (38.2%). Most frequent side of tumor was on the Left side (75%). Majority of the cases showed Moderate differentiated Adenocarcinoma (51.3%) and majority of the patients were belonging to T3 stage of the tumor (53%). TNM Stage II

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Date: Signature of the Candidate
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LIST OF ABBREVIATIONS

CRC - Colo Rectal Carcinoma

IHC - ImmunoHisto Chemistry

EMT – Epithelial Mesenchymal Transition

CSC – Cancer Stem Cells

CIN – Chromosomal Instability

ACF – Aberrant Crypt Focus

KRAS – Kirsten Rat Sarcoma Viral oncogene

APC – Adenomatosis Polyposis Coli

CIMP – CpG Island Methylator Phenotype

MSI – Micro Satellite Instability

CDK8 - Cyclin Dependant Kinase 8

RAS – Rat Sarcoma Virus

COX 2 - Cyclooxygenase 2

SMAD - Suppressor of Mothers Against Decapentaplegic

MLH 1 - Mutl Homolog 1

P13k/Akt – Phosphoinositide-3-Kinase Ak strain transforming

TGF – Tumor Growth Factor

H&E - Hematoxylin and Eosin

HPF - High Power Fields

AJCC - American Joint Committee on Cancer

PNI -Perineural Invasion

LVI - Lympho Vascular Invasion

TSR – Tumor stroma ratio

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ABSTRACT

Background:

Programmed death-ligand 1 (PD-L1) expression in colorectal cancer (CRC) has garnered significant attention due to its implications for immunotherapy and patient prognosis. PD-L1, a transmembrane protein, performs a crucial part in the immune system's ability to regulate the balance between T cell activation and tolerance. In the context of CRC, PD-L1 expression can result in the inhibition of anti-tumor immune responses, allowing cancer cells to evade immune surveillance. Microsatellite instability (MSI) is a state defined by the accumulation of brief mutations; repetitive DNA sequences known as microsatellites. This condition results from defects in the DNA mismatch repair (MMR) system, which normally corrects errors that occur during DNA replication. MSI is a hallmark of a subset of CRCs and is connected to distinct clinical and pathological features, including a better response to certain immunotherapies.

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Results:

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(53%). TNM Stage II (28%) had more cases followed by TNM Stage II (28%). TILs was graded according to ITWG Methodology: The percentage of TILs was categorized into 3 groups: low (0-10%), intermediate (15-50%) and high (55-100%). Majority of the cases were of Low TILs (52.6%). Most of the cases for Tumor stroma ratio in colorectal cancer were of ≤50% (61.8%). 20.3% showed PDL1 expression and 44.8% showed MLH 1 expression. PDL1, MLH1 and TSR showed significant association with TILs. Significant association was noted between PDL1 and TILs with a *p* value of 0.012. MLH1 also showed significant association with TILs with a *p* value of 0.041. On comparing TILs and TSR the p value was 0.001 which was statistically significant.

Conclusion:

Study of 76 cases of Colorectal cancer showed PDL1 expression in 20.3% and MLH 1 expression in 44.8% cases. PDL1 and MLH1 showed a significant association on comparing with TILs in colorectal carcinoma. Also MLH1 showed significant association with TNM staging. Study of PDL1 and MLH1 helps in prognostification and management of Colorectal carcinoma.

Key words: Colorectal carcinoma, PDL1 expression, MLH1 expression,TILs

INTRODUCTION

Introduction:

Colorectal cancer (CRC), a malignant neoplasm affecting the colon system, is among the most prevalent neoplasm globally. The mechanisms underlying colorectal cancer often commences with development of a polyp, a benign growth along the lining the rectum or colon. These polyps have the potential to develop into malignant tumors when risk elements are present and genetic mutations which are inherited or acquired.[1]

Epidemiology studies showed difference in the CRC prevalence. Colorectal cancer is causing over 930,000 deaths in 2020. [2,3]Colorectal cancer is a significant health concern in India, with varying incidence rates across different regions.[3] It's important to note that the age-adjusted incidence rates of colorectal cancer in all Indian cancer registries are very close to the lowest rates in the world. However, the incidence rates for rectal cancer are higher than colon cancer in all parts of India.[4,5] These findings stress the need of early detection and intervention tactics, as well as the need for more comprehensive and region-specific epidemiological data on colorectal cancer in India.[5,6]

The pathogenesis of CRC is typically begun with the growth of polyps, which can advance to invasive tumor through various pathways. The adenoma-carcinoma sequence is a reputable model describing the progression of CRC. It starts with the aberrant crypt focus, the earliest dysplastic lesion, which develops into a benign polyp and eventually into malignancy. This sequence is driven by the accumulating mutations in key genes that are involved in cellular growth and differentiation.[7] A major route linked to the development of colorectal cancer is the chromosomal instability (CIN) pathway. Changes to the APC gene are often the initial step in this pathway, followed by changes in other critical genes such as KRAS, TP53, and SMAD4.[8] Another significant pathway is the microsatellite instability (MSI) pathway, which involves defects in DNA mismatch repair genes. This results in a high mutation rate, particularly in regions of DNA known as microsatellites. Tumors with MSI are characterised by a distinct molecular profile and often have a better prognosis than CIN tumors. The CpG island methylator phenotype (CIMP) pathway involves the hypermethylation of DNA, resulting in the silencing of genes that decrease tumour growth. This pathway is associated with specific clinical and pathological features and may overlap with MSI tumors. [7,9,10]

Programmed death-ligand 1 (PD-L1) expression in colorectal cancer (CRC) has garnered significant attention due to its implications for immunotherapy and patient prognosis. PD-L1, a transmembrane protein, performs a crucial part in the immune system's ability to regulate the balance between T cell activation and tolerance. In the context of CRC, PD-L1 expression can result in the inhibition of anti-tumor immune responses, allowing cancer cells to evade immune surveillance.[11,12] Microsatellite instability (MSI) is a state defined by the accumulation of brief mutations, repetitive DNA sequences known as microsatellites. This condition results from defects in the DNA mismatch repair (MMR) system, which normally corrects errors that occur during DNA replication. MSI is a hallmark of a subset of CRCs and is connected to distinct clinical and pathological features, including a better response to certain immunotherapies.[13,14]

The connection between PD-L1 expression and MSI in CRC is particularly noteworthy. The PD-L1 upregulation is thought to be an adaptable reaction to the heightened immune activity typically seen in MSI-high tumors, which usually possess a more robust infiltration of cytotoxic T lymphocytes. The increased mutational burden in MSI-high tumors leads to the production of neoantigens, that is identified by immune cells as foreign, thereby eliciting an immune response.[12,13,15]

Furthermore, PD-L1 expression is linked to other molecular and clinicopathologic features. It has also been correlated with a worse outcome within the microsatellite-unstable tumor cohort. The connection between MSI and PD-L1 expression in CRC is complex and multifaceted. PD-L1 expression serves as a mechanism of immune escape in MSI-high CRC, contributing to tumor progression despite a microenvironment with an active immune system. [15–18] Ongoing projects of PD-L1 expression in CRC will continue to refine our understanding of its biological significance and inform the development of personalized treatment approaches for CRC patients.

Need for the study:

Routine screening is crucial in managing colorectal carcinoma (CRC) due to the significant difference in survival outcomes between early and late-stage CRCs. Early-stage CRCs generally has a favourable outcome, a 5-year survival percentage of 72–91%. However, advanced groups has the worst survival rates. Approximately 59% of them can achieve a

disease-free state through surgery alone.[19] Therefore, the standard treatment is curative surgery followed by adjuvant chemotherapy. Supplemental chemotherapy enhances the three-year interval without illness rate to 78.2% in these groups, but a certain percentage of cases do not advantage of supplemental chemotherapy. [20] The precise identification of patients who require adjuvant chemotherapy remains a challenge.

PD-L1, also referred to as CD274, is a checkpoint protein that resides on the membrane of a variety of immune and cancer cells. The PD-L1/PD-1 pathway has an impact in reducing immune cell function during inflammation, contributes to adaptive immune resistance in cancer. Despite these inconsistencies, there are standardized guidelines for the staining protocol and interpretation criteria in PD-L1 immunohistochemistry.[21–23] PD-L1 positivity, interpreted by immunohistochemistry, has a key role in CRC. PD-L1 positivity is relevant to the prognosis of CRC and for further CRC therapies, understanding PD-L1 expression in CRC is vital for effective treatment decisions. [24]

PD-L1 staining serves as a prognostic indicator that is both convenient and reasonably priced. The regulation of PD-L1 expression is complex, involving genomic, epigenetic, transcriptional, and post-transcriptional levels. However, the specific mechanisms within the CRC microenvironment are still to be fully understood.[25] Further understanding of the mechanisms that regulate PD-L1 expression in the tumor microenvironment could help clarify the clinical significance of PD-L1 expression and potentially the use of immunotherapy-based treatments in CRC. Hence, the study is done to assess the prevalence of PD-L1 positive CRCs and its correlation to the clinical staging in rural population.

Tumors deficient in mismatch repair (dMMR) carry imperfections in key genes of the DNA MMR system, such as MLH1, MSH2, MSH6, and PMS2. The MMR proteins form heterodimers when they are functional. MLH1 and PMS2 are a functional complex known as MutL alpha, whereas MSH2 dimerizes with MSH6 to form MutS alpha. These tumors exhibit a molecular phenotype marked by the genetic instability of numerous microsatellite repeat sequences throughout the genome, a condition known as microsatellite instability (MSI).[26] MSI holds independent prognostic value in a number of primary tumours and may be linked to a different treatment response.

Immunohistochemistry is a cost-effective and time-efficient method commonly employed in pathology departments. Unlike MSI testing which is a molecular technique to detect mutation in all 4 genes, immunohistochemistry detects the mutated gene, thereby focusing just on one gene while analysing germline mutations and avoiding the needless examination of additional mismatch repair genes. Immunohistochemistry is trustworthy for screening the affected gene that lead to protein destruction.

When all four MMR proteins are examined with IHC, there's an excellent association between MSI testing and the decline in MMR protein expression. To cut costs, some researchers suggest using only MSH6 and PMS2, with additional staining of their partner if either is absent, a method known as the two-stain method. Loss of MLH1 protein expression in immunohistochemistry can be due to epigenetic, biallelic silencing of MLH1 expression by de novo methylation of its promoter. Loss of MLH1 protein expression can be observed in both Lynch syndrome and sporadic colon tumors. Therefore, this project assess The frequency with which MLH1 expression CRCs and its correlation to the clinical staging in rural population.

Along with clinicomorphological features of colonic cancer, new biomarkers are identified to predict the survival and treatment response in newly diagnosed patients as well as treatment failure population. Development multiple targeted therapy leads to more precise therapy with minimal cancer treatment related side effects and less failure to the therapy. PD L1 blockers development revolutionised treatment of skin cancer.

PDL1 expression and MLH1 expression were studied using molecular genetic techniques by multiple researchers. Their utility in general clinical practice is not yet analysed. Immunohistichemistry technique of above antigen detection validated in research but not utilised clinical to validate their usefulness on routine clinical practice.

AIM AND OBJECTIVES

Aim And Objectives:

1. To determine the expression of Programmed Death Ligand-1(PD-L1) in colorectal carcinomas.
2. To determine the expression of MutL Homolog-1 (MLH-1) for Microsatellite instability status in colorectal carcinomas.
3. To determine the association of PD- L1 and MLH-1 expression with clinic pathological parameters of colorectal carcinoma.

REVIEW OF LITERATURE

Review of literature:

1. Anatomy of the large intestine

The large intestine runs from the ileocecal junction to the anus, measures approximately 1.5meters in length.[27]

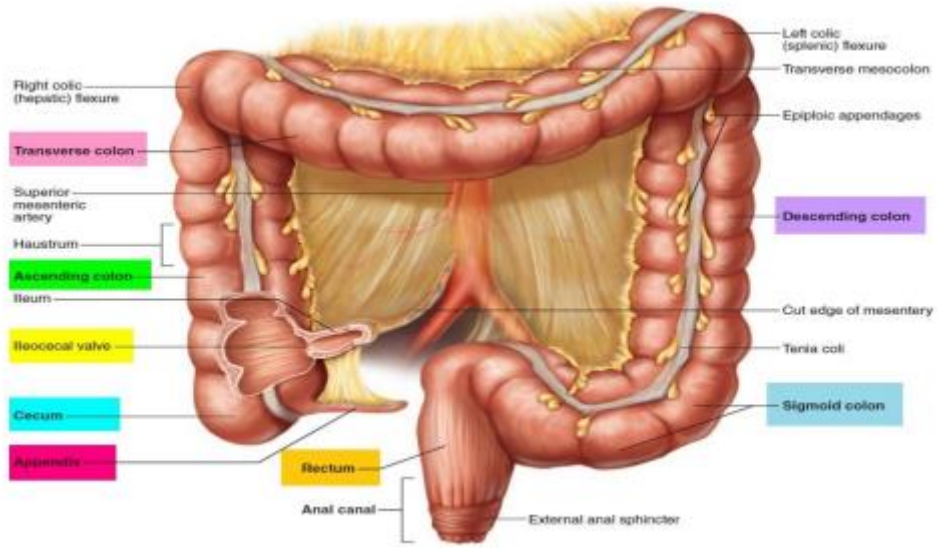


Figure1: showing parts of Large Intestine

1.1 CEACUM

The cecum, a blind pouch in the large intestine, extends downward from the beginning of the ascending colon. Typically covered by peritoneum, it measures 6 cm in length and 7.5 cm in width. Positioned in the right iliac fossa above the lateral half of the inguinal ligaments, it lies over the ileum, psoas fasciae, and nerves such as the femoral and lateral femoral cutaneous nerves. The longitudinal muscle fibers coalesce into three flat bands known as the taeniae coli—one anterior, one posteromedial, and one posterolateral.

1.2 ASCENDING COLON

Measuring approximately 15 cm, this segment extends from the cecum to the right colic (hepatic) flexure. It lies in a retroperitoneal position, and the taeniae coli extend from the cecum.

1.3 TRANSVERSE COLON

The transverse colon, measuring approximately 45 cm, extends from the hepatic flexure to the splenic flexure. It lies almost entirely within the peritoneum and is suspended freely by the transverse mesocolon. This mesocolon attaches to the inferior pole of the right kidney, the

second part of the duodenum, and the pancreas, while its connection to the inferior pole of the left kidney occurs. Additionally, the transverse mesocolon is linked to the greater curvature of the stomach via the greater omentum.[28]

1.4 **DESCENDING COLON**

The descending colon, approximately 30 cm in length, extends from the splenic flexure to the pelvic brim. It lies against the lumbar fascia and iliac fascia, terminating at the pelvic brim. The taeniae coli continue seamlessly from the transverse colon.[27]

1.5 **SIGMOID COLON**

The sigmoid colon, measuring approximately 45 cm, is entirely enveloped by the peritoneum and suspended freely by the sigmoid mesocolon. It typically resides in the pelvic cavity, coiled in front of the rectum, and rests against the peritoneal surface of the bladder (and uterus).[27,28]

1.6 RECTUM

The rectum, the distal-most part of the large intestine, measures approximately 12 cm in length. It extends from the sigmoid colon at the third part of the sacrum to the anal canal. Positioned in the posterior part of the pelvis, the rectum consistently lies in front of the sacrum and coccyx. It terminates by seamlessly connecting with the anal canal at the ano-rectal junction. The rectum follows an anteroposterior and lateral curvature, and unlike other segments of the large intestine.[27,28]

1.7 LYMPH NODES

Epicolic nodes: These nodes are located near the gut wall. **Paracolic nodes**: Found on the medial side of the ascending and descending colon, as well as near the mesocolic border of the transverse and sigmoid colon. **Intermediate nodes:** Positioned near the main branches of blood vessels. **Terminal nodes:** These nodes are close to the superior and inferior mesenteric vessels.[29]

1.8 ARTERIAL SUPPLY

The large intestine receives blood supply from the superior mesenteric and inferior mesenteric arteries. The rectum is nourished by the superior rectal artery (a branch of the inferior mesenteric artery), the middle rectal artery (from the anterior division of the internal iliac artery), and the median rectal artery (arising near the lower end of the aorta). Venous drainage corresponds to the arterial supply: the superior and inferior mesenteric veins lead to the portal

vein. In the rectum's distal portion, two drainage pathways exist: the middle and inferior hemorrhoidal veins drain into the pelvic veins, ultimately reaching the inferior vena cava, while the superior hemorrhoidal vein connects to the portal circulation via the inferior mesenteric vein.[27–29]

1.9 LYMPHATIC DRAINAGE

Intramural lymphatics within the large bowel originate as a plexus just below the lamina propria, superficial to the muscularis mucosa. These lymphatics follow blood capillaries into the submucosa. Efferent lymphatic vessels connect with an intramuscular and subserosal lymphatic plexus, radiating outward through the circular and longitudinal muscle layers. Most extramural lymphatics traverse the mesentery and converge on major artery trunks, passing through para-aortic nodes and the superior and inferior mesenteric nodes. A significant portion of lymphatic drainage from the rectum occurs along the superior hemorrhoidal artery trunk, passing through para-rectal and sigmoid nodes before reaching the inferior mesenteric artery. Lymphatics from the lower portion of the rectum travel through the middle rectal veins to reach the internal iliac nodes.[27–29]

1.10 NERVE SUPPLY

The parasympathetic supply originates partly from the vagus nerve and the pelvic splanchnic nerves. The sympathetic nerve supply arises from the T10–L2 segments.[29]

2. MICROSCOPIC ANATOMY

The large bowel wall comprsises of 6 layers.

i. Mucosa,
ii. Muscularis mucosa,
iii. Submucosa,
iv. Mucularis propria,
v. Subserosal fat and serosa.
vi. The rectum has similar histological features but lacks serosa.

The mucosa, the innermost layer of the intestine, lacks villi and contains crypts of Lieberkuhn. The muscularis mucosa forms an external longitudinal layer. External to this, three longitudinal bands—each 5 to 10 mm thick—are known as taeniae coli. At the level of each taenia, there is an exchange of muscle bundles between the circular and longitudinal layers.

The submucosa lies between the circular muscle and the muscularis mucosa, housing a rich network of blood vessels and the autonomic nervous plexus of Meissner. The muscularis propria consists of an inner circular layer and an outer longitudinal layer. Finally, the outermost layer of the large intestine is the serosa, which develops from the visceral peritoneum. [30]

3. ETIOLOGY:

The intricate aetiology of colorectal cancer involves multiple interrelated elements, such as age, gender , chronic inflammation, lifestyle, genetics, environment, and so forth.

a. Age: About 90% of adults over 50 are affected by colorectal cancer. The age range of 60 to 79 is when the incidence increases. Moreover, colorectal carcinoma ranks in the top 10 most prevalent cancers in adults aged 20 to 49.

b. Gender: There is no preference for either sex, yet men are marginally more likely.

c. Diet: The formation of colorectal cancer is significantly influenced by diet. A diet rich in fat promotes the development of bacteria that transform bile salts into potentially cancer-causing N-nitroso composed of. A high consumption of red meat is also associated with the development of colorectal cancer. Lowering your intake of fibre and eating a diet low in fruits and vegetables can raise your chance of developing colorectal cancer.[31]

d. Lifestyle: Two modifiable risk factors linked to colorectal cancer include obesity and physical inactivity. There is a connection between colorectal cancer developing early in life and smoking and heavy alcohol consumption. Alcoholics are more likely to have loss of MTHFR [5,10-Methylene Tetrahydrofolate Reductase] heterozygosity and loss of aldehyde dehydrogenase 2 phenotypic loss.[32]

e. Chronic inflammation: Inflammatory bowel disease may be the cause of persistent inflammation, which may contribute to the emerging of colorectal cancer. Those with ulcerative colitis are more susceptible. The majority of colorectal cancers that result from inflammatory bowel disease either do not include KLF 6 (kruppel-like factor 6) or have a mutation in it. [33]

f. Environmental factors: Exogenous carcinogens that enter the colon must be broken down by the colon. Thus, the detoxification of these carcinogens depends on the activity of many metabolic enzymes. Cancer can be brought on by chemical carcinogens that bind to DNA and are metabolically activated. Cytochrome P450 1A2 enhances the activation of polycyclic aromatic hydrocarbons, which is an risk factor of colon cancer. The activation also involves additional enzymes including arylamine N-acetyl transferase and cytosolic glutathione S-transferases. [34]

g. Genetic factors: A number of hereditary cancer syndromes, such as Gardner Syndrome, Turcot Syndrome, Birt Hogg Dube Syndrome, Peutz-Jeghers Syndrome, Cowden Syndrome, MYH-Adenomatous Polyposis Syndrome, Familial Adenomatous Polyposis, and Juvenile Polyposis, have been related to colorectal carcinoma.[35]

4. COLORECTAL CARCINOGENESIS:

The average adult colon epithelium is composed of three distinct cell types: goblet cells, enteroendocrine cells, and absorptive epithelial cells. Multipotent stem cells give rise to these cells. The neoplastic transformation most likely starts in stem cells or their early descendants, departing from the typical maturation phase. A number of sequential genetic changes must occur for colorectal cancer to develop. Colorectal cancers develop in phases, starting with normal epithelium and going on to more severe dysplasia including carcinoma, adenoma, and aberrant crypt foci.

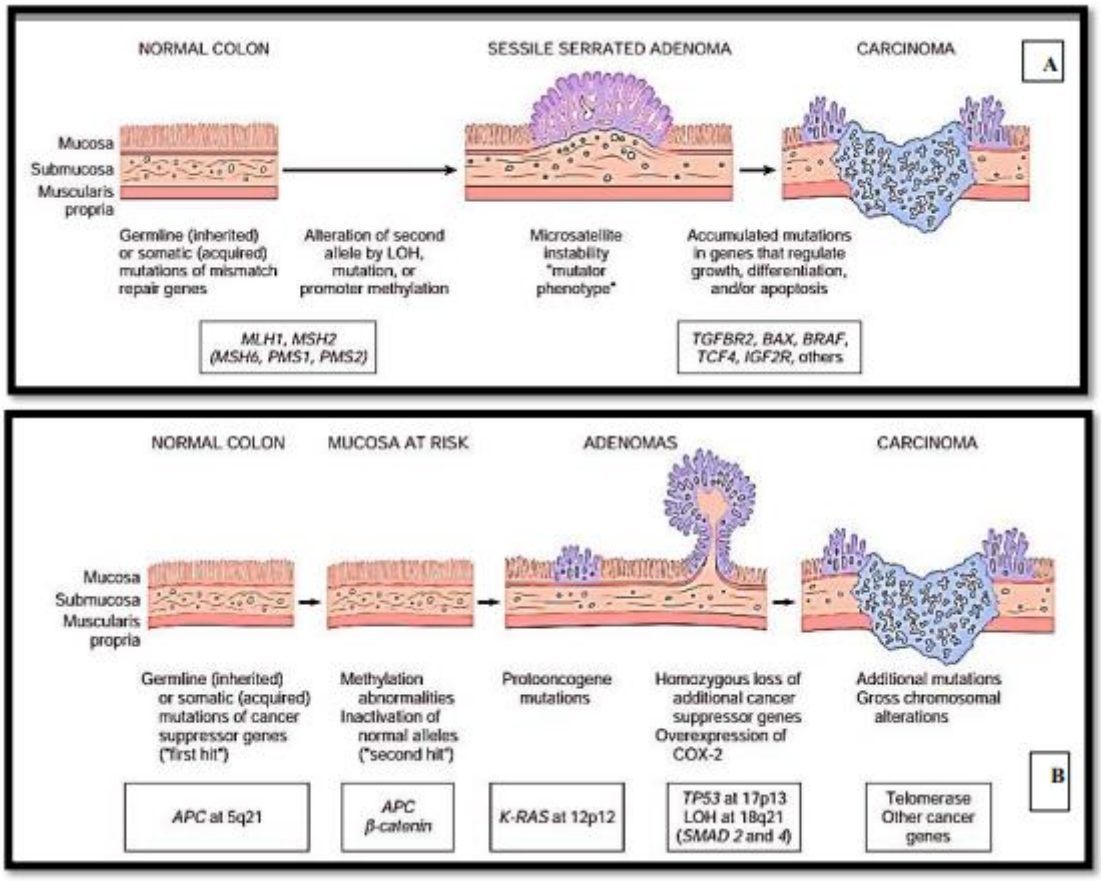


Figure 2: Pathogenesis of Colorectal cancer (Images from Robbins and Cotran Pathologic Basis of Disease. 10th ed.

Just 5–10% of occurrences of colorectal carcinoma are caused by inherited mutations in cancer-related genes; the majority of cases occur spontaneously.[36] The mechanisms behind the beginning and development of colorectal cancer can be used to identify three primary molecular pathways.

a. CpG island methylator phenotype (CIMP) pathway
b. The Chromosomal instability (CIN) pathway
c. Microsatellite Instability (MSI) pathway

The main cause of colorectal carcinomas that arise in HNPCC and through the CIN pathway is adenomatous polyps. The primary pathophysiological precursors of colorectal carcinomas that develop through the CIMP pathway are sessile serrated adenomas.[37] Inflammation and microRNAs have recently been identified as probable causes of colorectal cancer. Numerous genetic and epigenetic changes affect a patient's prognosis and likelihood of survival.

1. **Chromosomal Instability (CIN):**

The most commonest genomic instability is chromosomal instability, is present in 70–85% of colorectal cancer cases. Numerous chromosome structural abnormalities or numerical chromosome variations are defined as aneuploidy or polyploidy, both of which are signs of chromosomal instability.[38] This group includes chromosomal rearrangements, gene deletions, and duplications. Several methods including (1) DNA flowcytometry (2) comparative genomic hybridization (3) whole exome sequencing, and (4) high-density SNP arrays, can be used to evaluate these. Chromosome abnormalities have been discovered in colon adenomas, indicating that the transition from polyp to colon cancer, CIN, may take place sooner. The dysplastic aberrant crypt focus (ACF), a small mucosal lesion that is seen before polyp formation.[37]

APC is a crucial tumour suppressor gene in the CIN pathway that leads to colorectal cancer. It is the "key" first mutation that causes spontaneous CIN and all germline FAP27 mutations.

2. **The WNT Signalling Pathway**:

Villi and crypts are both present in the gastrointestinal epithelium. The crypts are the sites of cell differentiation. As the cells grow, they finally pass through the walls of the crypts and reach the villi. WNT signalling along the crypt-to-villus axis preserves crypt progenitor compartments and take care of cell cycle during differentiation. The binding of APC to Beta-

catenin reduces the activity of the WNT signalling pathway. Transformation in APC cause the protein to shorten, the interferes with the protein's ability to bind to beta-catenin. The cytoplasmic build-up of beta-catenin, which facilitates beta-catenin translocation into the nucleus and activates the T-cell factor targets, causes colorectal cells to proliferate, differentiate, migrate, and adhere more readily.[39,40]

In the initial step to colorectal carcinoma, APC mutations can even be detected in the absence of Beta-Catenin mutations. In the early stages, APC mutations might potentially be replaced by beta-catenin mutations. In almost 60% of cases of colorectal cancer, the CDK8 gene at 13q12.13 acts as an oncogene by boosting beta-catenin and Notch 1, which speeds up transcription and cell differentiation.[40]

3. RAS Pathway:

Nearly 40% of colon cancers are caused by point mutations that activate the Ras oncogene (often K-Ras, rarely N-Ras, and never H-Ras). A mutation in K-Ras(12p12) results in the loss of natural GTPase activity in the GTP-binding protein it encodes. This leads to constitutive signalling via the RAS-RAF-MEK-ERK pathway. The propagation and transmission of extracellular signals depend on this protein. Activation in K-Ras often occurs when the coding properties of codons 12 or 13 change. Codon 61 may also be affected. Glycine is changed to valine at codon 12 in the RAS G12V mutation, which is associated with an aggressive course of disease and a high likelihood of recurrence. A persistently active state brought about by K-Ras mutations allows the cell to evade apoptosis and acquire an edge in proliferation. RAS transmits FGFR signals. Mutations that activate the FGFR 3 gene have been connected to colorectal cancer and may cause an increase in RAS activity.[41,42]

4. **p53 Pathway**:

The tumour suppressor gene p53 is present on chromosome 17p. The p53 protein has three main functions: it increases the synthesis of genes participating in the cell cycle, slows down the cell cycle, and gives DNA repair ample time. While benign tumours sporadically exhibit mis-sense mutations in the remaining p53 allele, about 75% of colorectal carcinomas exhibit this loss of chromosome 17p. This implies that p53 loss plays a part in colorectal carcinogenesis's later phases. High levels of proliferative activity are induced by p53 mutations because there is no control over the cell cycle or cell death. [43].[44]

5. Other pathways involved in chromosomal Instability:

Along with APC gene modifications there is a mutation in the PI3KCA gene, which stimulates cell proliferation and the formation of FAS in the AKT pathway. mTOR is a crucial regulator of metabolism and cell development. Mutations in PI3KCA also interact with K-Ras. Chromosome 18q, which is responsible for encoding the SMAD 2, DCC, and SMAD 4 genes, is deleted. This molecular change often happens in tandem with p53 loss. A poor prognosis for colon cancer is highly associated with deletion of 18q, likely due to the substantial potential for metastasis.[45,46]

The CIN pathway is completed by HIF-1 and HIF-2. Through mTOR, they up regulate genes involved in formation of blood vessels, cell longevity, and glucose metabolism, and they influence the biological response to hypoxia. Over expressed HIF 1 and HIF 2 subunits directly promote the synthesis of COX-2 in colorectal cancer by bounding to it. This increase of HIF1 resulted in shorter survival duration, specifically for patients with colorectal cancer.[47,48]

6. CPG Island Methylator Phenotype (CIMP) Pathway:

CIMP is present in 20–30% of colorectal carcinomas. Malignancy exhibiting the CpG island methylator phenotype has increased levels of CpG island hypermethylation in DNA repair genes such as p16 and MLH1. Promoter hypermethylation is typically caused by mutations involving K-Ras and TGF-R-II. The absence of TGF-control is the main defect in the CpG island methylator phenotype.

Two forms of CIMP-positive tumours exist: I CIMP-high and (ii) CIMP-low and KRAS mutations are caused by BRAF mutations with MLH1 methylation. CIMP-negative, TP53-mutant tumours continue to stabilise microsatellite architecture. BRAF V600E is highly prevalent, yet it is not associated with K-RAS. [36]

7. Microsatellite Instability (MSI) Pathway:

This pathway is responsible for around 95% of HNPCC symptoms and about 15% of spontaneous CRC cases. Numerous nucleotides repeat sequences known as microsatellites are found throughout the genome.

It is defined as the presence of at least 30% non-stable microsatellite loci in a panel of 5–10 loci composed of mono- and di-nucleotide tracts. Malignancy with an MSI of 10 to 29% have fewer unstable loci.[36] Mismatch repair goes awry in MSI because DNA polymerase is more prone to errors while copying these little repetitive sequences. The MMR system is comprising of 7 proteins: PMS1, PMS2, MSH2, MSH3, MSH6, and MLH1. When these proteins bind to specific partners, they form functional heterodimers. The necessary heterodimeric proteins for

function are MLH 1 - PMS1, MLH 1 - PMS 2, MSH 2-MSH 3, MSH 2 - MSH 6, and MLH 1 - MLH 3."[37]

One of the mechanisms causing MSI is aberrant DNA methylation, which renders the MMR family genes inactive. Sporadic MSI colorectal carcinomas, on the other hand, are caused by aberrant DNA methylation, which impairs MMR function and silences MLH 1. In sporadic MSI-high cases of colorectal cancer, the RAS-RAF-MAP kinase pathway is implicated in modulating the cellular response to growth signal. The V600E mutation is known to be seen in the BRAF oncogene.[36]

When colorectal tumours arise through the MSI pathway, they usually start in the proximal colon, have a poorly differentiated histology (mucinous or medullary), and exhibit considerable intratumoral and peritumoral lymphocyte infiltrations. Those with MSI-high CRC had a longer survival time and a better prognosis than those with chromosomal instability in their colorectal cancer.[49]

8. **MICRO RNA (miRNA):**

MiRNAs are a class of 20–25 nucleotide non-coding RNAs. By blocking the mRNA translation involved in cell development, differentiation, proliferation, and death, they regulate the expression of proteins. As more and more miRNAs are found, the number of miRNAs linked to the aetiology of CRC is constantly increasing. They operate similarly to oncogenes and tumour suppressor genes. It is regulated either up or down.[50]

9. Inflammatory Pathway:

Given the strong link between inflammatory bowel illness, long-term NSAID use, and colorectal cancer, prolonged inflammation has a important part in the onset and progression of CRC. Activation of mutagenic reactive oxygen and nitrogen species can lead to increased DNA damage, which can cause carcinogenesis in chronic inflammation. Other mechanisms include increased growth of anti-apoptotic cells, increased production of angiogenic and lymphangiogenic GF, and modifications to membrane systems that alter cell adhesion and promote invasion.

When levels of the pro-inflammatory cytokine TNF are continuously elevated, tumour formation is encouraged. The cytokine IL-6 stimulates the transcription of STAT 3 during the acute phase of inflammation.[51]

6. PROGNOSTIC FACTORS IN COLORECTAL CANCER:

a) TNM Stage:

Clinical and pathological staging is important to ascertain the local extentsion and distant extentsion of colorectal cancer (CRC) post diagnosis. For predicting the prognosis of newly diagnosed colorectal cancer, the AJCC-UICC Tumour Node Metastasis (TNM) staging system (8th edition, 2017) is still considered the best method. The TNM staging system was first created in 1968 to forecast prognosis. Since then, its application has grown to include management guidance, as seen by the development of multiple international standards.[52] However, there is considerable variation in the prognosis and results for patients with stage II and III illness.[53]

i) Tumour (T) Staging:

It has been demonstrated that tumour stage in colorectal cancer significantly affects survival on its own [54]. A increased T stage is linked to a poor 5-year overall survival (OS) (T3 87.5%, T4 71.5%) in several population-based studies.[55] For T4b tumours, the OS drops to 46%. Relapse and worse disease-free survival (DFS) are also lined with advanced T stages. Tsikitis et al. in his study, T4 stage malignancy had a three times increased chance of recurrence than T3 tumours [29]. An increased risk of nodal metastasis, distant metastases, and detection in an emergency situation is linked to higher T stages.[56]

ii) Nodal (N) Staging:

After distant metastatic dissemination, local lymph node involvement is thought to be the second best indicator of prognosis in colorectal cancer.[57] The initial tumours histological grade and T stage are correlated with regional lymph node involvement.[58] Nodal positive patients had a five-year OS of 30–60%, while node negative patients have an OS of 70–90%. In cases with nodal-positive colorectal cancer, recurrence rates range from 30% to 35%.[59] Most recurrences happens in first three years after surgical resection.

Although there is growing evidence that lymph node harvesting, apical lymph node, and lymph node ratio are becoming more important, nodal staging does not presently take these factors into account. Adjuvant therapy is indicated when there is nodal involvement in order to lower the risk of distant metastases [52]. In node-positive illness, adjuvant chemotherapy decreases the chance of recurrence by 40% and absolute risk of mortality by 10%–20%.[58]

iii) **Metastasis (M) Staging:**

The best indicator is still the presence of distant metastases at diagnosis (stage IV). Between 35 and 50 percent of patients have distant metastases upon diagnosis, which results in a 5-year of fewer than 10 percent.[52] Chemotherapy extends median survival from 5 to 18 months and is primarily used with palliative aim. Because of the digestive tract's portal venous drainage, the liver is frequently affected site of distant dissemination; the lungs, bone, and other sites are next in line.[57]

iv) Molecular Biomarkers

BRAF:

Encoding the B-RAF protein kinase, an essential part of the mitogen-activated protein kinase (MAPK) pathway, is BRAF, a proto-oncogene. In turn, the MAPK pathway is crucial for cell longevity, differentiation, multiplication, and apoptosis.[60] About 11% of all CRC cases have a BRAF mutation (BRAF-mt), which is crucial to the development of tumours. Though there are about thirty distinct BRAF mutations, 90% of BRAF mutations are caused by the V600E mutant, which is the most prevalent.[61] The impact of BRAF status on colorectal cancer prognosis is still debatable, however the available data points to a poor prognosis. Patients diagnosed with BRAF-mt CRC are typically older and female. Its predictive value varies based on stage and could be impacted by the MSI status.[62]

KRAS:

The K-Ras protein, an essential part of the mitogen-activated protein kinase (MAPK) pathway, is encoded by the proto-oncogene KRAS. About 40% of all CRC cases have the KRAS mutation (KRAS-mt), however this frequency is lower in the African population (about 21%).[63] Mutations in KRAS causes unrestrained cell proliferation, which in turn promotes the production of cancer cells.[64]

Although KRAS mutations strongly indicate resistance to anti-EGFR therapy their significance in prognostication is still unknown, particularly with regard to advanced stage of CRC. Individuals with KRAS-mt CRCs typically have feminine genders, mucinous histology, and a higher likelihood of right-sided tumours.[65] According to certain data, the MSI status may have an impact on the propensity for poor prognosis. Overall, there is still inconsistent data in the non-metastatic setting.

According to Nash et al., patients with MSS KRAS-mt had a 5-year OS of 55% compared to 68% in KRAS-wt, a considerably higher mortality rate. But only in stages I and II of the

disease did this connection become substantial; in stages III and IV, it became insignificant. The results of investigations by Eklof et al. and Taieb et al., who discovered decreased CSS in the KRAS-mt MSS group, corroborate these findings.[66] On the other hand, a study by de Cuba et al. found the opposite, indicating that patients with MSI-H KRAS-mt CRC had considerably lower CSS.

v) **MSI:**

Although MSI was not thought to be a significant prognostic factor in 1999, multiple metanalysis have demonstrated that it is linked to a better prognosis and plays a important role in CRC prognostication, especially in the early stages of the disease (primarily in stage II).[67] 1277 MSI-H CRC patients in all stages were included in the Popat et al. meta-analysis, which found a 35% lower risk of overall survival (HR 0.65, 95% CI 0.59–0.71).[68]Less is known about the prognostic impact of MSI-H in mCRC, as multiple studies have shown that the prognosis is poorer in the metastatic scenario. While some studies demonstrate a worse prognosis, several have revealed no influence of MSI in prognostication.[69]

c) **Histological Features:**

i. Tumour Size:

In colorectal cancer, tumour size is defined as the largest diameter of the tumour sample.[70] Its ability to predict outcome in colorectal cancer (CRC) is still debatable, despite being well-established and included in T staging for tumours such as breast, lung, and thyroid. Rather than tumour size, the current AJCC-UICC T staging for colorectal cancer (CRC) is based on tumour depth.[71]

Research has indicated a correlation between larger tumour size and a worse prognosis. Poorly differentiated grade, Tumor stage, nodal involvement, and tumour necrosis are among the additional poor prognostic characteristics that have been linked to greater tumour sizes. After controlling for grade, nodal status, sex, and age, Saha et al. discovered that individuals with a tumour size >6 cm had a 46% greater risk of overall death compared to a tumour size of <2 cm in a large population-based analysis on patients with colon cancer (n = 300,386).[72]

A larger tumour size increased the hazard ratio of death, decreasing both cancer-specific survival (CSS) (HR: 1.037; 95% CI: 1.032–1.463; $p < 0.05$) and overall survival (OS) (HR: 1.026; 95% CI: 1.022–1.030; $p < 0.05$) in another sizable population-based study of colon cancer patients (n = 128,369), according to Feng et al.[73] The inability to achieve thorough resection margins in bigger tumours or the malignancies' vertical invasion mechanics could be contributing factors to the variation in survival depending on the size of the tumour.[74]

However, many studies discovered that tumour dimension doesn't continue to be an independent predictor of prognosis. Larger tumours are not the only ones that can have negative characteristics; smaller tumours that include lymph node metastases and/or T4b infiltration may also have a worse prognosis.[70]

ii. **Tumour Budding:**

A histological feature known as "tumour budding" denotes the separation of cancerous cells from the invasive front of the tumour. [75]

A thorough research conducted in 2020 by Lugli et al. showed that in the context of advanced stage tumour budding, there was a worse prognosis in analysis (5-year DSS 89–98% vs. 52–80% in low-grade vs. high grade BD1 vs. BD2–3. Tumour budding is linked to worse OS and DFS after curative surgery for stage II CRC, as shown by Koelzer et al..[75]

The poor prognosis is applicable to all stages of CRC.[76] Nagata et al. concluded that the 5-year survival rate for BD3 was 18.4% in the metastatic scenario, while it was 40.5% for BD 1 or 2.[77]

The International Tumour Budding Consensus Conference (ITBCC) in 2016.[75] classified into three groups based on the criteria: BD1 (low, 0–4 buds), BD2 (intermediate, 5–9 buds), and BD3 (high, ≥10 buds). The relationship between intermediate/high grade tumour budding and bad clinicopathological characteristics and worse RFS and OS has since been confirmed by the literature.[78]

iii. **Tumour Location:**

The clinical and biochemical features of CRC on the right and left sides differ. While the left-sided colon and rectum are developed from the hind gut, the right colon is derived from the embryonic mid-gut. Compared to patients with left-sided CRC, those with right-sided CRC are most likey female, and has a higher median age at diagnosis, and had higher tumour stages and high-grade histology at first presentation. [79]Additionally, it seems that metastasis patterns vary by location: A higher percentage of left-sided CRC has a propensity to metastasis to the liver and lung, whereas right-sided CRC tends to spread to the peritoneum. [80]

In initial stage of the malignancy, right-sided CRC has a better prognosis. [81]Weiss et al. observed that stage on the right side II CRC had a lower death rate than the stage 2 on the left side CRC (HR 0.92, $p = 0.001$), but higher mortality in stage III cancer (HR 1.12, $p < 0.001$) among a sample of 53,801 CRC patients. In line with Weiss et al., a 2019 Japanese population-based study showed that the prognosis for right-sided colon cancer (CRC) is poorer than that of left-sided CRC for stages III and IV of the disease, although it is better for stage I.[82]

iv. **TILS:**

TILs are a histological observation that indicates an individual's immunogenicity and is thought to provide protection against the advancement of tumours. TILs facilitate the maturation, activation, and recruitment of immune cells that inhibit the growth of tumours. Natural killer (NK) cells, macrophages, and T lymphocyte subtypes (CD3, CD4, CD8, CD45R0, and FoxP3 cells) have all been linked to an impact on CRC outcomes. Research has demonstrated that TILs, regardless of conventional histologic tumour grading, are a favourable prognostic factor in colorectal cancer. Prolonged OS, CSS, and DFS are linked to high density TILs.[83] TILs with a higher density are also linked to beneficial tumour features, including decreased rates of lymphatic, vascular, perineural, lymph node, and distant metastases. It has been demonstrated that TILs improve prognosis and survival. It has been demonstrated that TILs with the CD3, CD8, and FoxP3 subtypes offer the best prognostication.[84]

Idos et al. carried out a meta-analysis of 43 research studies in 2020, and the results showed that an improved OS (HR = 0.65; 95% CI, 0.58–0.77), CSS (HR = 0.58; 95% CI, 0.46–0.73), and DFS (HR = 0.72; 95% CI, 0.60–0.88) was linked to a greater generalised TIL density[85]. Additionally, distinct subsets of lymphocytes (such as CD3, CD4, CD8, CD45R0, and FoxP3 cells) inside the tumor's invasive margin, tumour centre, and stroma were examined.[86]

In sequence to enhance the Reliability and uniformity of TIL readings for upcoming diagnostic investigations, a systematic approach to TIL evaluation is necessary.[87]

v. **Lymph Node Yield:**

A robust prognostic indicator, lymph node yield (LNY) is the number of lymph nodes recovered after gross inspection, especially in cases of non-metastatic colorectal cancer.[88]

Increased LNY was linked to better survival in stage II and III CRC, according to a 2007 systematic study by Chang et al.[89] A lymph node yield of 20 was linked to better disease-free survival (DFS) (HR 0.358, $p = 0.007$) and 5-year OS (78.9% vs. 68.2%, LNY > 20 vs. LNY < 20 respectively, $p = 0.036$),[90] according to a retrospective analysis by Foo et al. that looked at 659 stage I and II CRC patients. Additionally, Foo et al. demonstrated that the stage II cohort exhibited the greatest improvement in survival with greater LNY. According to Backes et al., in T1 CRC, a lower risk of recurrence (HR 0.2, $p = 0.009$) was linked to an LNY of ≥10. Additionally, there is growing evidence that better survival in synchronous CRC is linked to an increased LNY.[91,100]

Right now, neoadjuvant therapy is the accepted course of management for stage III rectal cancer, and it is widely acknowledged that radiation therapy reduces lymph node yield. [100,101,102]

While some studies have shown lower survival, evidence suggests that a lower yield in this situation may not always translate into a worse prognosis.[103,104,105]

The exact mechanism by which higher LNY enhances CRC outcomes is still unknown. [106]

vi. Perineural Invasion:

As a means of cancer dissemination, neoplastic tumour cell invasion of nerves is referred to as perineural invasion, or PNI. All three of the nerve layers are capable of harbouring tumour cells [92]. Perineural invasion has a documented incidence in colorectal cancer (CRC) ranging from 9% to 30%. It is more common in advanced stages of the disease. According to studies, PNI can occur in 10% of cases of stage I–II disease, 30% of cases of stage III disease, and 40% of cases of stage IV disease. There is proof that PNI is a separate indicator of a worse result and a lower chance of survival. [93]

Knijn el at conducted a meta-analysis and systematic review. 58 trials involving 22,900 CRC patients at all stages were examined. Reduced 5-year OS (HR 1.85, 95% CI 1.63–2.12), CSS (HR 1.91, 95% CI 1.56–2.42), and DFS (HR 2.35, 95% CI 1.97–308) were all linked to PNI. Furthermore, it was discovered that the predictive significance of PNI was comparable to other recognised prognostic variables, including extramural invasion, tumour grade, lymph node metastasis, and depth of invasion.[94] A sizable population analysis of 41,000 CRC patients, based on Surveillance, Epidemiology, and End Results (SEER), supports these conclusions. PNI was linked to a lower 3-year OS and CSS (HR 1.24 and HR 1.28, respectively, $p < 0.001$), regardless of the tumor's location, grade, T and N stages.[95]

Standardised reporting criteria and standards for PNI are lacking. With detection rates ranging from 9% to 42%, PNI is typically underreported. Numerous research employs different definitions of PNI. Within the literature, one of the more widely used classifications is tumour cells encircling more than 33% of the nerve circumference.[96]

vii. Lymphovascular Invasion:

Lymphovascular invasion (LVI) is used to describe the histologically apparent blood or lymphatic system involvement by a cancer cells. LVI is thought to be a crucial stage in the growth of lymph node metastases. [97]Reports of LVI in colorectal cancer range from 4.1% to 63.8%, most likely as a result of various study populations and diagnostic methods. In CRC, LVI has become a widely accepted, stage-independent marker of a poor prognosis.

LVI-positive cases experience up to a 55% drop in OS and considerably lower DFS (HR 1.73 CI 1.50–1.99 $p < 0.01$), according to multiple comprehensive analyses and extensive population research. [98]A increased tumour stage, lymph node positivity, distant metastatic deposit, bad differentiation, large sized tumour, neural invasion, tumour budding, and KRAS positivity are

among the additional unfavourable characteristics that are linked to LVI. All stages of CRC are affected by the poor prognostication of LVI.[99]

viii. **Circumferential Resection Margin:**

The radial margin, mesenteric margin, and non-peritonealised margin are other names for CRM. It is measured, expressed in millimetres, between the specimen's surgical cut end and deepest site of tumour invasion.[100] This circumferential, non-peritonealised edge entirely encloses low rectal tumours located below the peritoneal reflection, whereas upper rectal tumours have a peritonealised surface anteriorly and a non-peritonealised margin posterolaterally. [101]

The standards by which a positive CRM is defined are still up for debate. Tumour less than 1 millimetre from the tumor-free margin is the most widely used criterion of CRM positive. 5.3% to 20.5% of colon cancers and 7.3% to 25% of rectal malignancies are CRM positive. Advanced stage, higher tumour grade, penetrating the tumour boundary, and perineural and lymphatic invasion are connected with it. CRM positive in rectal cancer, regardless of TNM staging, is a powerful predictor of recurrence and worse survival. A positive CRM is linked to higher odds of distant metastasis (HR 2.95), local recurrence (HR 4.67 95% CI 2.51–4.15), OS (HR 3.21), and DFS (HR 3.63) [252]. CRM has a greater prognostic impact on patients receiving neo-adjuvant radiation before surgery than in patients receiving surgery alone, most likely because tumours that respond poorly to radiation are biologically undesirable.[101]

Although there has been less research on the importance of CRM positive in colorectal cancer, current findings indicate that the poor prognostication associated with this marker in rectal cancer extends to colon cancer. Patients with a CRM value of 0–30 mm benefited most from treatment, according to study conducted in 2020 by Tang et al.. There is conflicting information regarding the ideal CRM for colon and rectal cancer. There have been several suggested CRM clearance thresholds.[101] Kelly et al. (2011) suggested a CRM clearance of 5 mm or more in rectal cancer, while Beaufrere et al. (2017) suggested a clearance of less than four millimetres. Liu et al. separated CRM groups in rectal cancer patients into 0–1 mm, 1.1–2.0 mm, 2.1–5 mm, 5.1–10 mm, and >10 mm and looked at survival results between the subgroups in a sizable population study from 2018. The CRM 5.1–10–mm group showed a survival advantage over the 1.1–5–mm group; however, this difference was not statistically significant. Tang and colleagues (2019) discovered that patients with CRM-negative colon cancer who had a margin greater than 30 mm had better results.[100,101]

ix. **Tumour Grade:**

The absence of widely accepted reporting system and the substantial interobserver heterogeneity in tumour grading assessments are the primary constraints. Whether it should be based on the

area with least differentiation, or the prevailing pattern of differentiation is a matter of debate. The majority of cancer grade classifications rely on the proportion of gland formation; the incorporation of cytologic or other criteria in the grade estimation process is not always consistent.[116, 102]

A four-tiered grading system for CRC is used by the College of American Pathologists (CAP), and it is exclusively dependent on the degree of gland formation. Well-differentiated (>95% gland formation) is graded as grade 1, moderately-differentiated (50–95% gland formation) as grade 3, poorly-differentiated (<50% gland formation), and undifferentiated (no gland or mucin formation) as grade 4 were described. [103]

7. **PDL-1 Review of literature:**

The study by Pallavi Srivatsava et al (2021) investigated PD-L1 expression in colorectal carcinoma and its correlation with clinicopathological parameters, microsatellite instability, and BRAF mutation. They evaluated 110 cases and found that tumor cells showed PD-L1 positivity in 40% and tumor infiltrating lymphocytes in 45.4% of cases at a cut-off of ≥1%. The study found a significant association between tumor proportion score and increasing age, histological type, grade, tumor size, higher T stage, tumor infiltrating lymphocytes, lymph vascular invasion, and perineural invasion. PD-L1 also correlated with BRAF expression and microsatellite instability. The author concluded that the overall survival was significantly higher in cases with negative PD-L1 expression. This suggests that PD-L1 expression could be a potential prognostic marker in colorectal carcinoma.[17]

A study on PD-L1 as a prognostic factor in early-stage colon carcinoma was carried out by Pablo Azcue et al in 2021. The goal of the research was to develop a more specialised treatment strategy for colorectal cancer (CRC), a diverse illness. The study examined the potential of PD-1 ligand (PD-L1) expression as a biomarker and its integration with the Consensus Molecular Subtype (CMS) allowed for the identification of individuals who were more likely to have a poor prognosis and would benefit from early and aggressive therapy. Based on immunohistochemical assessment, the findings imply that PD-L1 is a separate prognostic factor in the early-stage context. Furthermore, patients in the CMS (CMS2/CMS3) without a certain prognosis can be distinguished by PD-L1 expression. This work adds to the 1qacontinuing attempts to identify useful biomarkers to characterise colorectal cancer..[104]

A study on PD-L1 Expression in High-Risk Early-Stage Colorectal Cancer was done by Bing Svuan Chung et al in 2022. The purpose of the study was to look into the connection

between PD-L1 expression and CRC survivorship. An independent prognostic predictor, prolonged recurrence-free survival, was linked to high PD-L1 expression (CPS ≥ 5), which was assessed in a Taiwanese CRC population. Additionally, the study discovered that six immune-related gene profiles were associated with increased PD-L1 expression, with CXCL9 being the gene that was most significantly overexpressed. Increased immune cell infiltration levels in the tumour microenvironment, particularly CD8+ T lymphocytes and M1 macrophages, were linked with high CXCL9 expression. These results imply that elevated PD-L1 expression is a predictor of early-stage colorectal cancer (CRC), and that CXCL9 may be a major modulator of PD-L1 expression.[105]

In 2024, PD-L1 expression, clinicopathological variables, and metastatic risk in patients with colorectal cancer were studied by Alireza Zarbakhsh and colleagues. The objective of the study was to investigate the correlation among PD-L1 expression, metastatic incidence, and survival rates in individuals diagnosed with colorectal cancer (CRC). The study found that there was no association between PD-L1 expression and mortality, disease-free survival, or overall survival. Regarding the presence of metastases, there was a discernible difference between patients with positive PD-L1 testing results and those with negative results, with the PD-L1 positive group exhibiting a greater incidence. The findings suggest that although PD-L1 expression may have an impact on CRC patients' risk of metastasis, overall survival does not seem to be impacted.[106]

8. MLH-1 Review of literature:

A study on MLH1 Promotor Hypermethylation in Colorectal and Endometrial Carcinomas from Patients with Lynch Syndrome was carried out by Noah C. Helderman et al in 2023. The study used immunohistochemical labelling of mismatch repair proteins to screen patients with colorectal and endometrial cancer for Lynch syndrome. Testing for MLH1 promotor hypermethylation was done in the event of MLH1 protein loss. Six novel MLH1-PM CRCs and 86 previously documented endometrial malignancies in LS patients were reported by the study. There have been reports of 30 MLH1, 6 MSH2, 6 MSH6, and 3 PMS2 variant carriers with methylation of the MLH1 gene promotor C region. The study's conclusion was that when MLH1-PM is found, a diagnosis of LS should not be ruled out, and doctors should think about doing additional genetic MMR gene testing.[107]

9. SURGICAL AND PATHOLOGICAL STAGING

1. DUKE'S CLASSIFICATION

Dukes A: Confined to the bowel wall.

Dukes B: through the bowel wall but not involving the free peritoneal surface.

Dukes C: Involvement of nodes.

Dukes D: added as modified Dukes – presence of metastases (or) advanced loco – regional disease.

Figure 3: showing the Dukes classification of colorectal carcinoma

2. TNM CLASSIFICATION (THE UICC AND THE AJCC STAGING SYSTEM)[46]

***T* – Primary tumour**

Tis - Carcinoma in situ: intraepithelial or invasion of lamina propria

T1 - Tumour invades the submucosa.

T2 - Tumours invades the muscularis propria.

T3 - Tumour invades through the muscularis propria into the subserosa or into non peritonealised, Pericolic (or) perirectal tissues.

T4 – Tumour directly invades other organs or structures and/or perforates visceral peritoneum.

N – Regional lymph nodes

NX - Regional lymph nodes cannot be assessed.

N0 - No regional lymph node metastasis

N1 – Metastasis in 1 to 3 pericolic (or) perirectal lymph nodes

N2 – Metastasis in 4(or) more pericolic (or) perirectal lymph nodes

N3 – Metastasis in any central lymph nodes (along the course of a named vascular tree)

Metastasis

Mo – No metastasis

M1 – Metastasis

Lymphatic invasion

L0 – No lymphatic involvement.

L1 – Lymphatics involved.

Venous invasion

V0 – No vessel involvement.

V1 – Vessels involved.

Figure 4: showing the TNM classification of colorectal carcinoma

MATERIALS AND METHODS

10. MATERIALS AND METHODS

STUDY DESIGN – Cross sectional Analytical Study

SOURCE OF DATA: Surgical resected specimens of colorectal carcinoma received from Department of Surgery in RL Jalappa Hospital and Research Institute affiliated to Sri Devaraj URS Academy of Higher Education and retrieval of data and paraffin blocks from the archives of Department of Pathology

DURATION OF STUDY – 18 months (Aug 2022 to March 2024)

ELIGIBILITY CRITERIAS AND METHOD OF COLLECTION OF DATA

INCLUSION CRITERIA

1. All cases with histological diagnosis of colorectal carcinoma admitted and undergone surgical resection in RL Jalappa Hospital and Research Institute affiliated to Sri Devaraj URS Academy of Higher Education from August 2022 to March 2024.

EXCLUSION CRITERIA

1. Recurrence case of colorectal cancer
2. Post treatment cases (chemo & radiation therapy)
3. Samples with inadequate tissue

SAMPLE SIZE ESTIMATION

Sample size: 76

Sample size estimated by expression of PD-L1 in Colorectal carcinoma was 40 % in a study by ***Pallavi Srivastava et al*** with 95% confidence interval and an absolute error of 11%

Formula to be used: **$n = Z(1-\alpha)2\ (p)(1-p)/d2$**

Here,

n = sample size;

Z = standard normal variant (1.96);

p = prevalence (40)

d = absolute error (11%) •

Z (1-α) = 1.96 (95% confidence interval)

n = 1.962 x 40 x 60/ 11

n = 76 (Final Sample Size)

11. METHODOLOGY

1. Specimens fixed in formalin will be taken.
2. Grossing and sampling will be done according to standard operative protocols.
3. The tumors were staged according to TNM classification proposed by American Joint Committee on Cancer (AJCC, 8th edition, 2017).
4. All diagnostic slides will be reviewed and tumour block on standard H&E staining will be selected for PDL1 and MSI (MLH 1) immunohistochemistry.

IMMUNO-HISTOCHEMISTRY STAINING PROCEDURE (PD- L1)

1. De-wax and bring sections to distilled water.
2. Wash briefly in distilled water 1 – 2 minutes.
3. Antigen retrieval 15-20 minutes according to the standardization protocol to the particular antibody in citrate buffer pH 6.0, /TRISEDTA pH 9 then cool for 5-10minutes.
4. Wash in distilled water; do not let the section dry out.
5. Endogenous Peroxidase the section in 3% H_2O_2 for 10 minutes
6. Wash in tris buffered solution (TBS) pH 7.4 for 2 minutes.
7. The sections are then covered with individual primary antibodies CLONE: SP263 for 45 mins to 1 hour based on validation min at room temperature.
8. Wash the slides for two times with TBS for 2 minutes.
9. The sections are then covered with secondary antibody (HRP) for 30 minutes.
10. Wash the slides for two times in TBS for 2 minutes.
11. Tetrahydrochlodide (DAB) chromogen for 5 minutes (R1-1ml, R2-30UL)
12. Wash with distilled water.
13. The sections are then covered with haematoxylin for 30 seconds.
14. Wash the slides with TBS followed by distilled water 2 times in 2 changes.
15. The sections are dehydrated by 3 changes of absolute alcohol & cleared with 2 changes of Xylene for 2 minutes.
16. Mount with DPX.

GRADING OF PD- L1 STAINING

- PD-L1 expression on tumour cells was evaluated using a three-tiered grading system.
  - 0 = < 5% of tumour cells
  - 1 = 5 – 49% of tumour cells
  - 2 = ≥ 50 % of tumour cells with membranous staining of any intensity
- Cytoplasmic staining was not considered in this study.
- Scores of 1 and 2 were considered to be positive for PD-L1 expression. [53]

IMMUNO-HISTOCHEMISTRY STAINING PROCEDURE (MLH- 1)

1. De-wax and bring sections to distilled water.
2. Wash briefly in distilled water 1 – 2 minutes.
3. Antigen retrieval 15-20 minutes according to the standardization protocol to the particular antibody in citrate buffer pH 6.0, /TRISEDTA pH 9 then cool for 5-10minutes.
4. Wash in distilled water; do not let the section dry out.
5. Endogenous Peroxidase the section in 3% H2O2 for 10 minutes
6. Wash in tris buffered solution (TBS) pH 7.4 for 2 minutes.
7. The sections are then covered with individual primary antibodies CLONE: ES05 for 45 mins to 1 hour based on validation min at room temperature.
8. Wash the slides for two times with TBS for 2 minutes.
9. The sections are then covered with secondary antibody (HRP) for 30 minutes.
10. Wash the slides for two times in TBS for 2 minutes.
11. Tetrahydrochloride (DAB) chromogen for 5 minutes (R1-1ml, R2-30UL)
12. Wash with distilled water.
13. The sections are then covered with hematoxylin for 30 seconds.
14. Wash the slides with TBS followed by distilled water 2 times in 2 changes.
15. The sections are dehydrated by 3 changes of absolute alcohol & cleared with 2 changes of Xylene for 2 minutes.
16. Mount with DPX.

GRADING OF MLH-1 STAINING

- At least 5 high power fields were evaluated for each tumour and the staining rate of the tumour cells was calculated.
- A mean percentage of stained tumour cells was determined and graded into three categories which is as follows:
  - <1% of positive tumour cells – Negative
  - 1 – 50 % of positive tumour cells – score 1.
  - 51 – 100% of positive tumour cells – score 2.
  - Scores of 1 and 2 were considered to be positive for MLH-1 expression [54].

12. STATISTICAL ANALYSIS

All the data will be coded and entered in Microsoft excel sheet. Quantitative data will be presented as mean ±SD or median with range. A threshold of $P < 0.05$ was considered statistically significant. Qualitative data will be analysed with Chi- Square test. P value <0.05 will be considered statistically significant. Sensitivity, specificity, PPV & NPV will be represented in comparison with gold standard test. SPSS 24 version will be used for analysing the data.

RESULTS

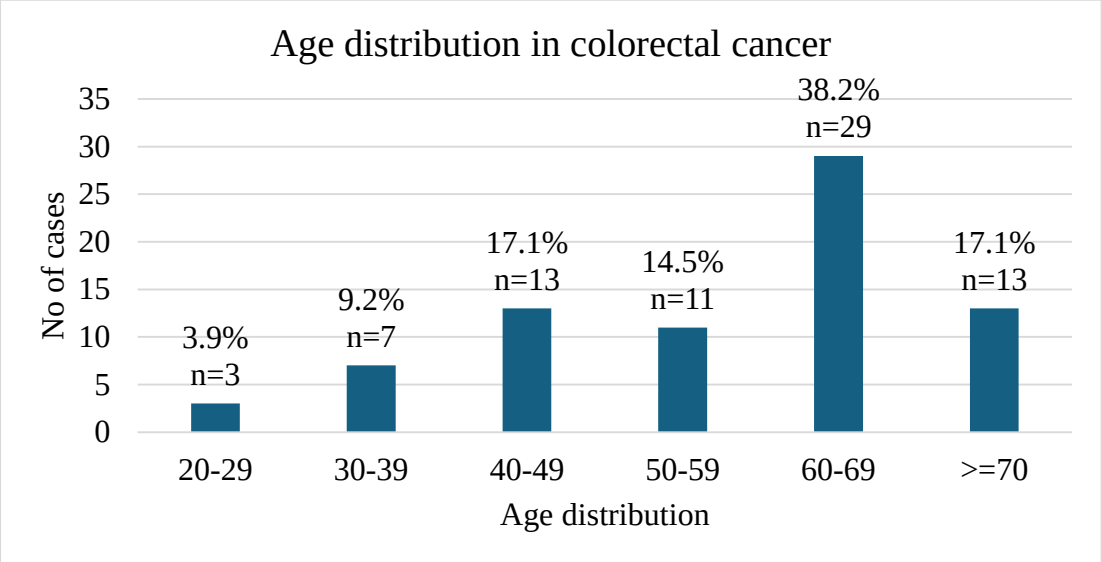
RESULTS:

1.1 Age

Table 1: Distribution of colorectal cancer cases in various age groups:

Age Group	Frequency	Percent
20-29	3	3.9
30-39	7	9.2
40-49	13	17.1
50-59	11	14.5
60-69	29	38.2
>=70	13	17.1
Total	76	100

Chart 1: Distribution of colorectal cancer cases in various age groups:



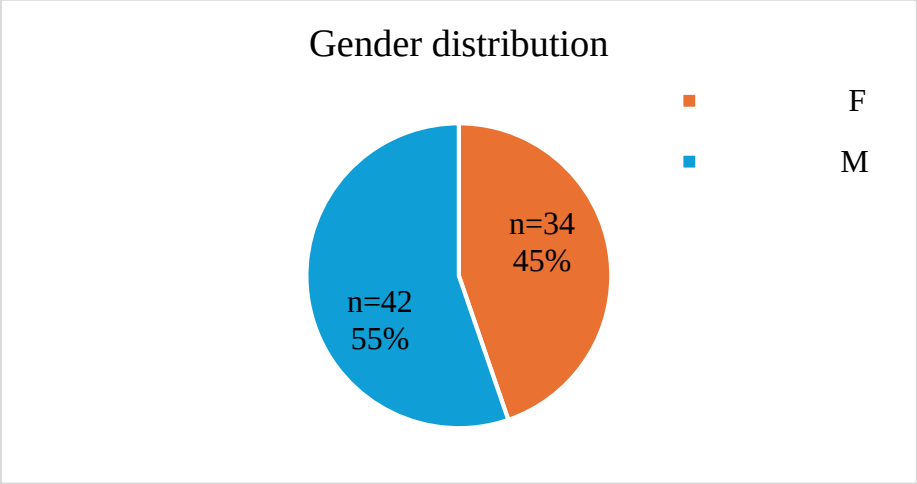
Mean age distribution of our study is 56.5 years. More than 50 years of age patients composed 68.9% in our study. The majority of the patients is seen in the age group 60 - 69 years. Even though colorectal cancer is diseases of six decade, our study has 3 patients in third decade.

1.2 Sex:

Table 2 : Distribution of colorectal cancer cases in gender:

Sex	Frequency	Percent
F	34	45
M	42	55
Total	76	100

Chart 2 : Distribution of colorectal cancer cases in gender in Pie chart:



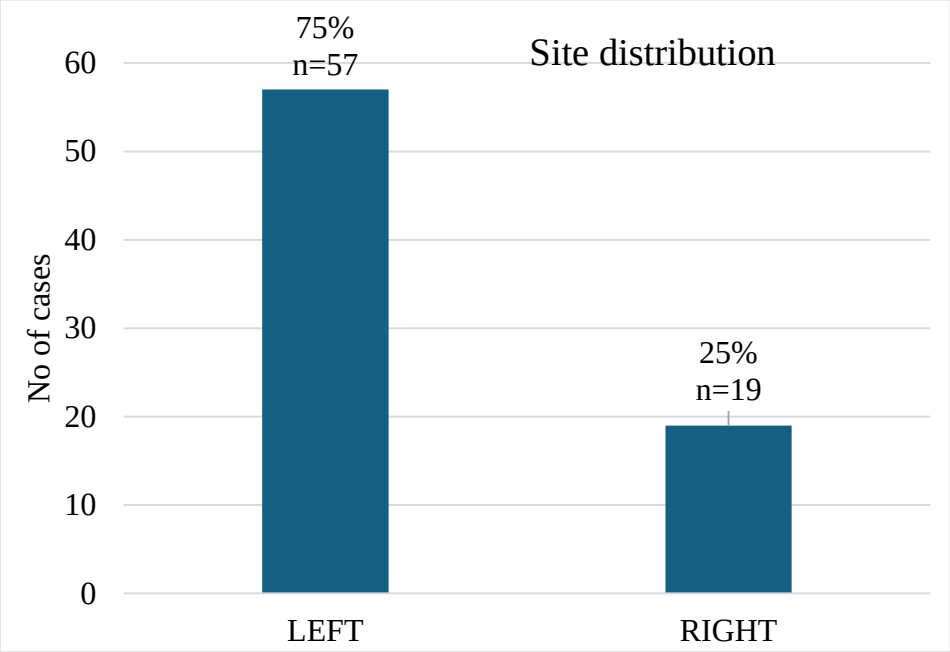
The highest number of colorectal cancer (CRC) were observed in male patients 42 (55%) with M:F ratio of 1.24:1.

1.3 Laterality of the tumor

Table 3 : Distribution of colorectal cancer cases with respect to laterality of tumor:

	Frequency	Percent
LEFT	57	75
RIGHT	19	25
Total	76	100

Chart 3 : Distribution of colorectal cancer cases with respect to laterality of tumor in Bar diagram:



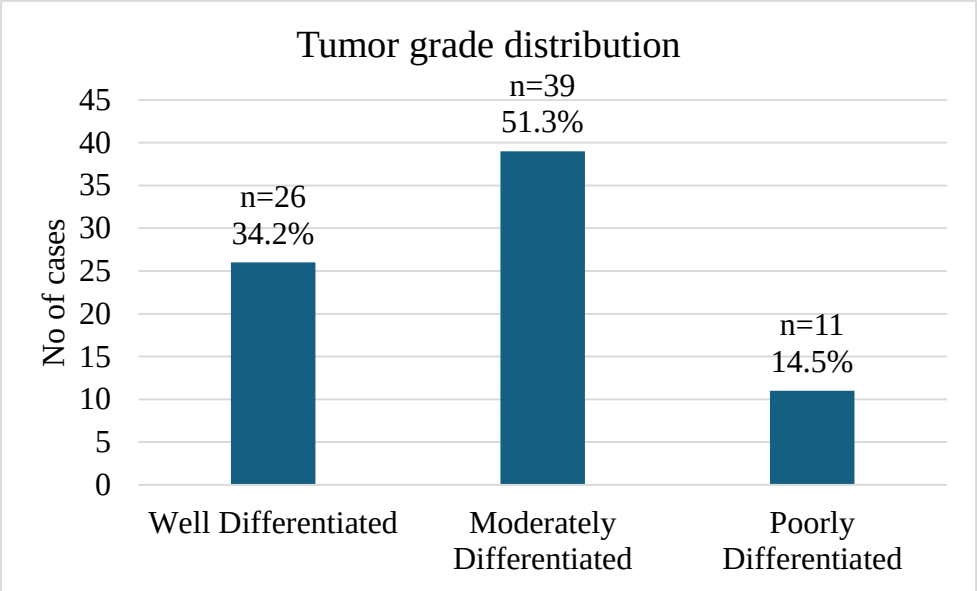
The majority of cases was observed to have left side of colon involvement 57 (75%) in our study and 19 cases was seen on the right side which is 25% of total cases.

1.4 Histological grading

Table 4 : Distribution of colorectal cancer cases in different grades of tumor:

	Frequency	Percent
Well Differentiated	26	34.2
Moderately Differentiated	39	51.3
Poorly Differentiated	11	14.5
Total	76	100

Chart 4 : Distribution of colorectal cancer cases in different grades of tumor in Bar diagram:



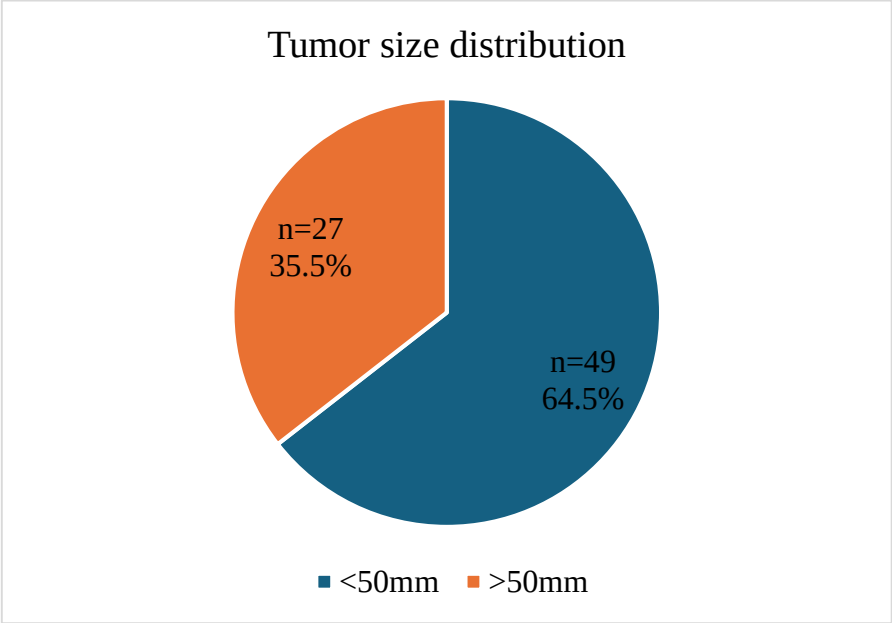
The highest number of the 76 cases fell into the category of moderate differentiation, accounting for 39 cases (51.3%), well-differentiated tumors, 26 cases (34.2%), and poorly differentiated tumors, 11 cases (14.5%).

1.5 Tumor size:

Table 5 : Distribution of colorectal cancer cases in tumor size:

	Frequency	Percent
<50mm	49	64.5
≥50mm	27	35.5
Total	76	100

Chart 5 : Distribution of colorectal cancer cases in tumor size using pie chart:



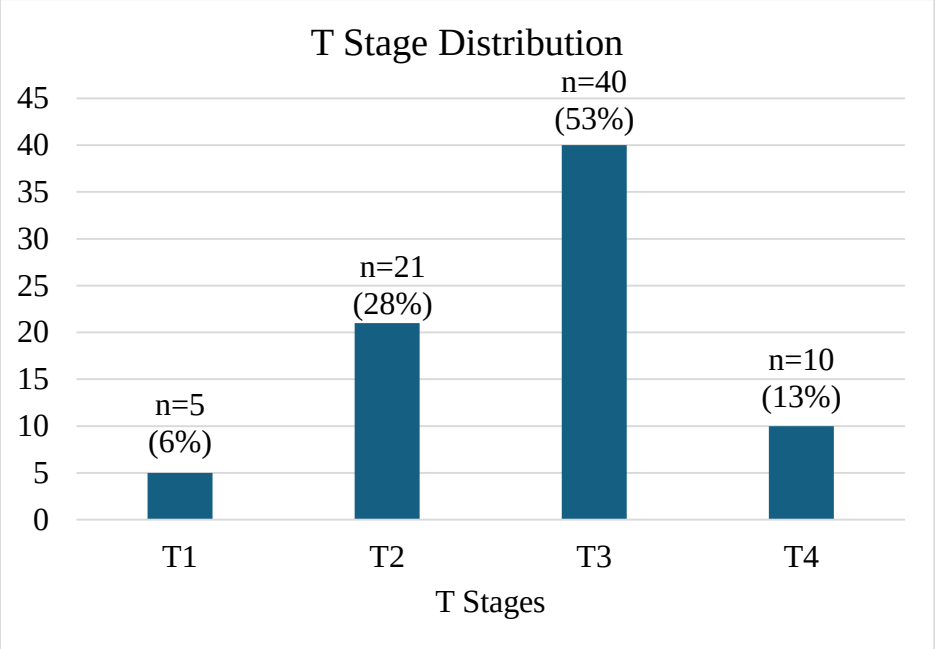
Most of the tumors in our study is <50mm size. Out of the 76 cases, <50mm size tumor observed in 49 (64.5%) remaining are ≥50mm size tumour.

1.6 T staging:

Table 6 : Distribution of colorectal cancer cases with respect to T stage:

T stage	Frequency	Percentage
T1	5	6%
T2	21	28%
T3	40	53%
T4	10	13%
Total	76	100%

Chart 6 : Distribution of colorectal cancer cases with respect to T stage in bar diagram:



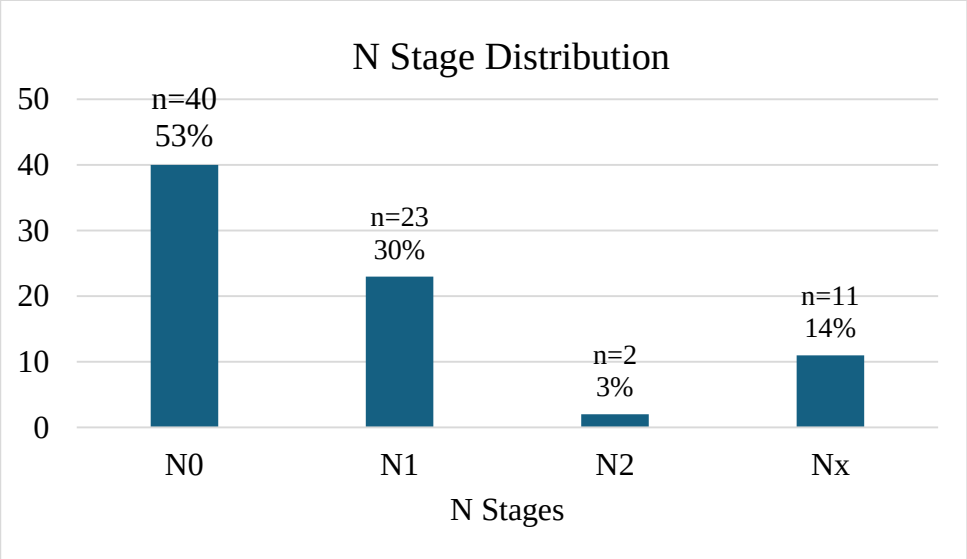
Majority of cases were seen in the T3 stage of the tumor with a percentage of 53% followed by T2 stage with a percentage of 28%.

1.7 N staging:

Table 7: Distribution of colorectal cancer cases with respect to N stage:

N Stage	Frequency	Percent
N0	40	53%
N1	23	30%
N2	2	3%
Nx	11	14%
Total	76	100%

Chart 7: Distribution of colorectal cancer cases with respect to N stage using a Bar diagram:



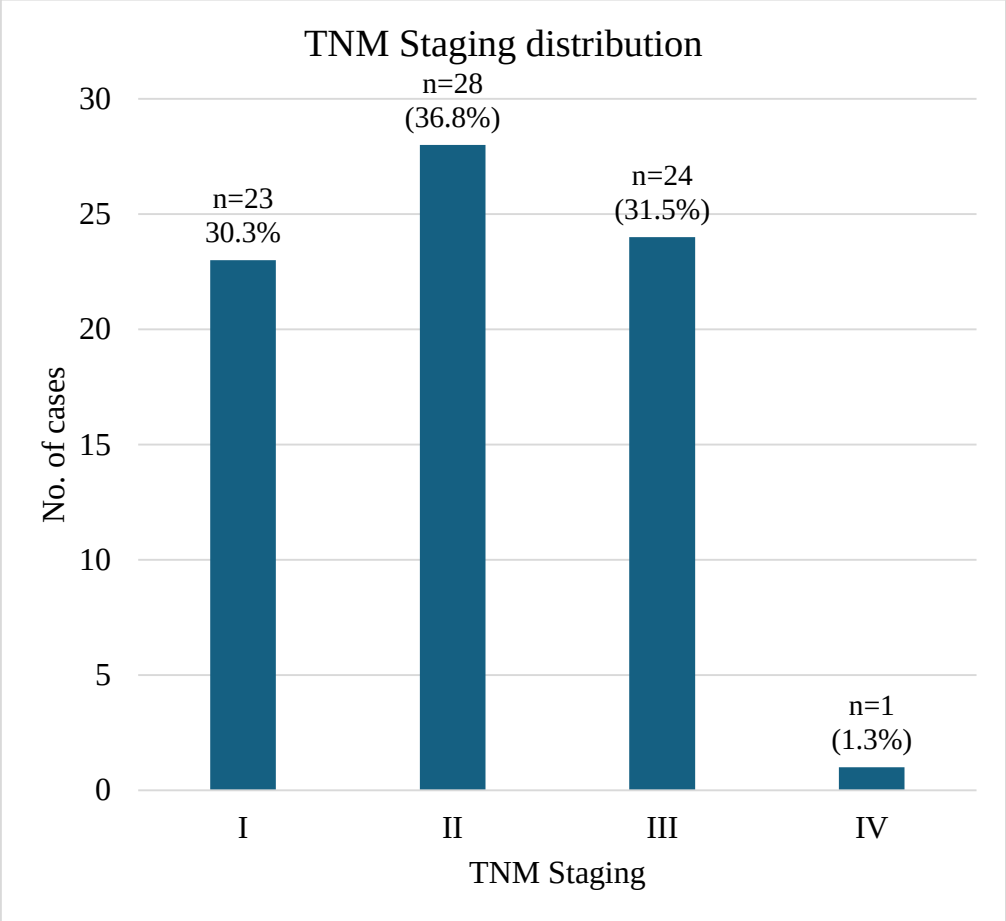
With respect to N stage of the tumor maximum no. of cases were seen in the N0 stage -40 (53%) followed by N1 Stage with a percentage of 30%. In the N stage majority of cases fell into the N0 category with 40% followed by N1 category which showed 30% of nodal involvement.

1.8 TNM staging

Table 8 : Distribution of colorectal cancer cases with respect to TNM stage:

TNM Stage	Frequency	Percent
I	23	30.3%
II	28	36.8%
III	24	31.5%
IV	1	1.3%
Total	76	100%

Chart 8 : Distribution of colorectal cancer cases with respect to TNM stage using a bar diagram



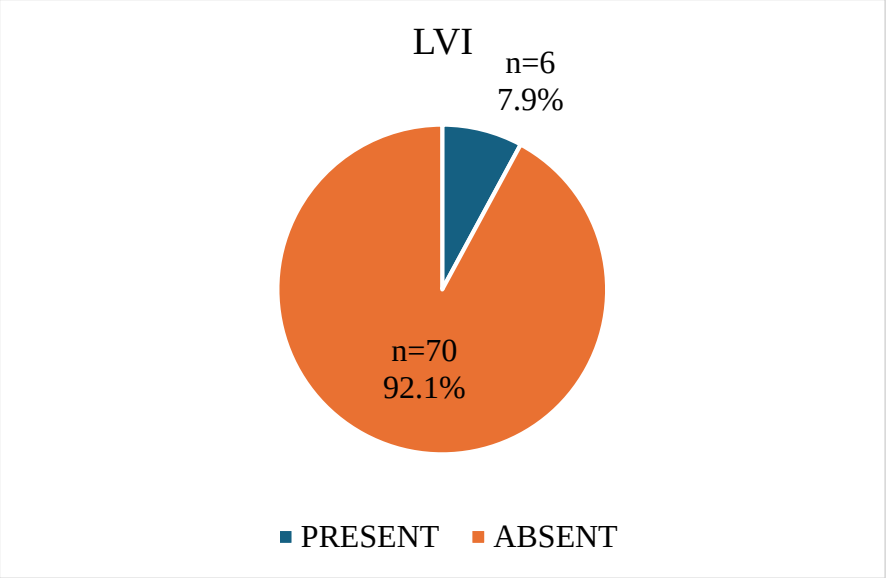
With respect to TNM stage of the tumor maximum no. of cases were seen in the Stage II of the disease 28 (36.8%) followed by Stage III with a percentage of 31.5%.

1.9 LVI:

Table 9 : Distribution of colorectal cancer cases with Lymphovascular invasion:

	Frequency	Percent
PRESENT	6	7.9
ABSENT	70	92.1
Total	76	100

Chart 9 : Distribution of colorectal cancer cases with Lymphovascular invasion using a Pie chart:



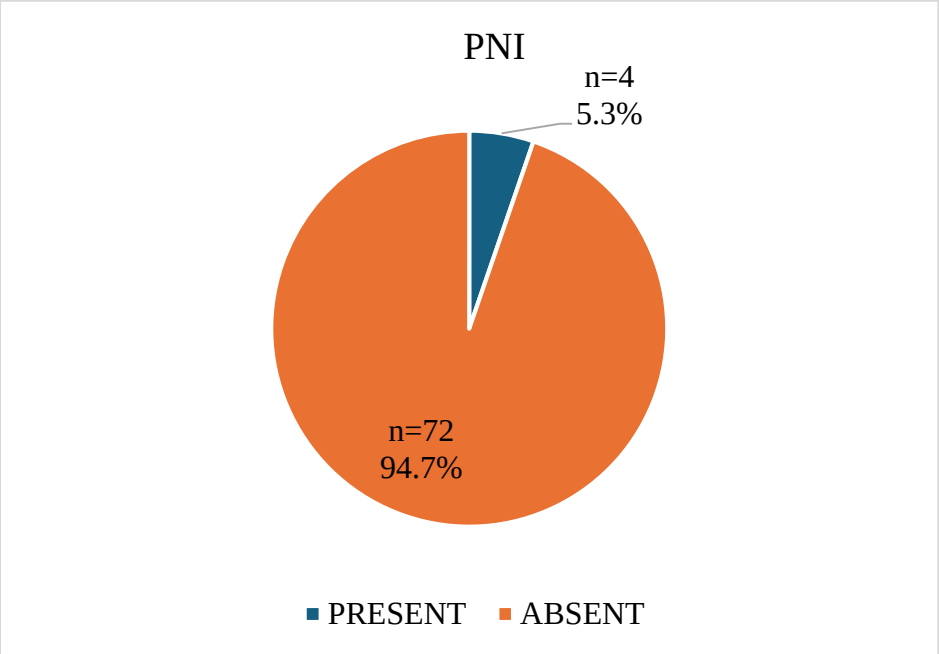
Out of the 76 cases 7.9% of tumor case had lymphovascular invasion whereas 92.1% showed no lymphovascular invasion.

1.10 PNI:

Table 10 : Distribution of colorectal cancer cases with Perineural invasion:

	Frequency	Percent
PRESENT	4	5.3
ABSENT	72	94.7
Total	76	100

Chart 10 : Distribution of colorectal cancer cases with Perineural invasion using a Pie chart:



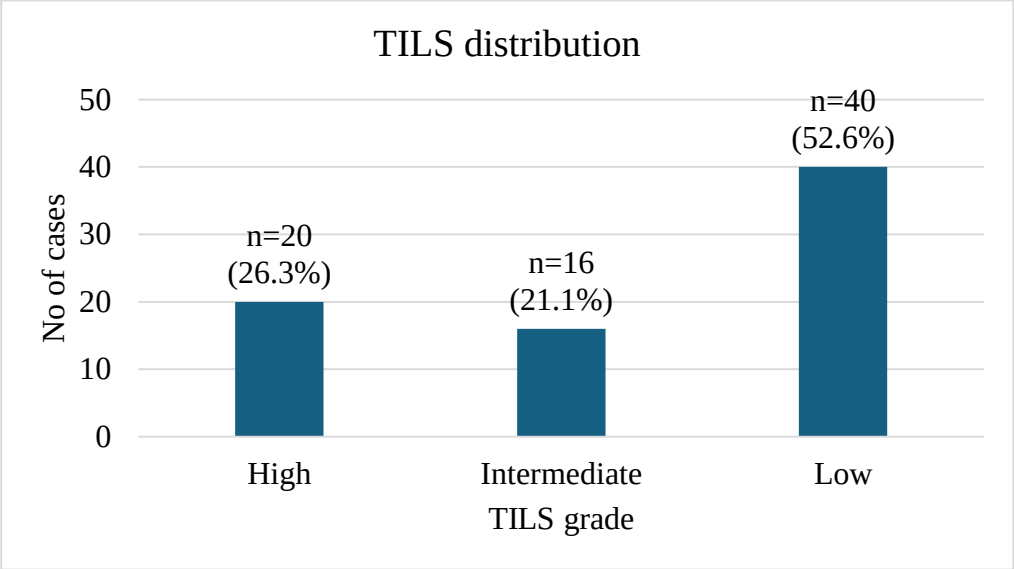
Out of the 76 cases 5.3% of tumor case had perineural invasion whereas 94.7% showed no perineural invasion.

1.11 TILS:

Table 11: Distribution of colorectal cancer cases with Tumor infiltrating lymphocytes:

	Frequency	Percent
High	20	26.3
Intermediate	16	21.1
Low	40	52.6
Total	76	100

Chart 11: Distribution of colorectal cancer cases with Tumor infiltrating lymphocytes using Bar diagram:



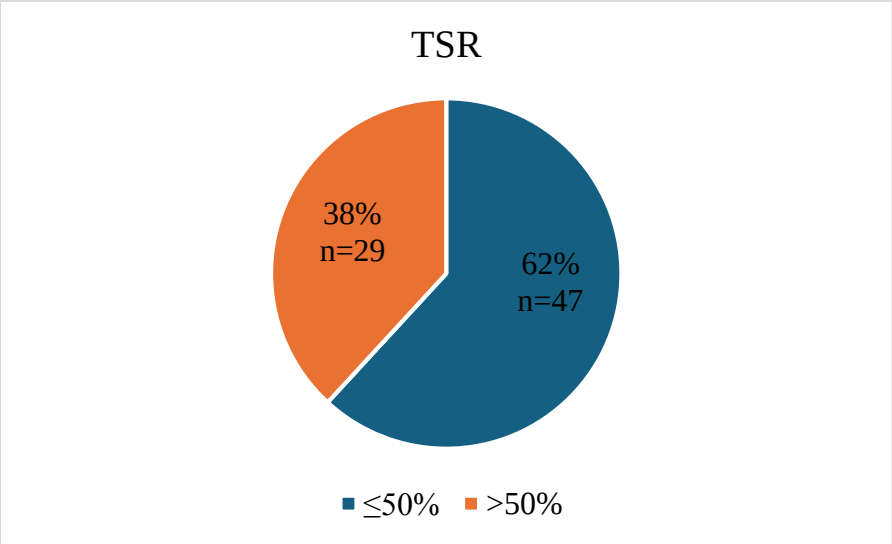
TILS was graded according to the ITWG Methodology: The percentage TILs' score was categorized into 3 groups: low (0% to 10%), intermediate (15% to 50%), and high (55% to 100%). 52.6% showed Low TILS followed by High TILS in 26.3% and Intermediate TILS in 21.1%

1.12 TSR:

Table 12 : Distribution of colorectal cancer cases with Tumor stroma ratio:

	Frequency	**Percent**
≤ 50%	47	61.8
>50%	29	38.2
Total	76	100

Chart 12 : Distribution of colorectal cancer cases with Tumor stroma ratio using a Pie chart:

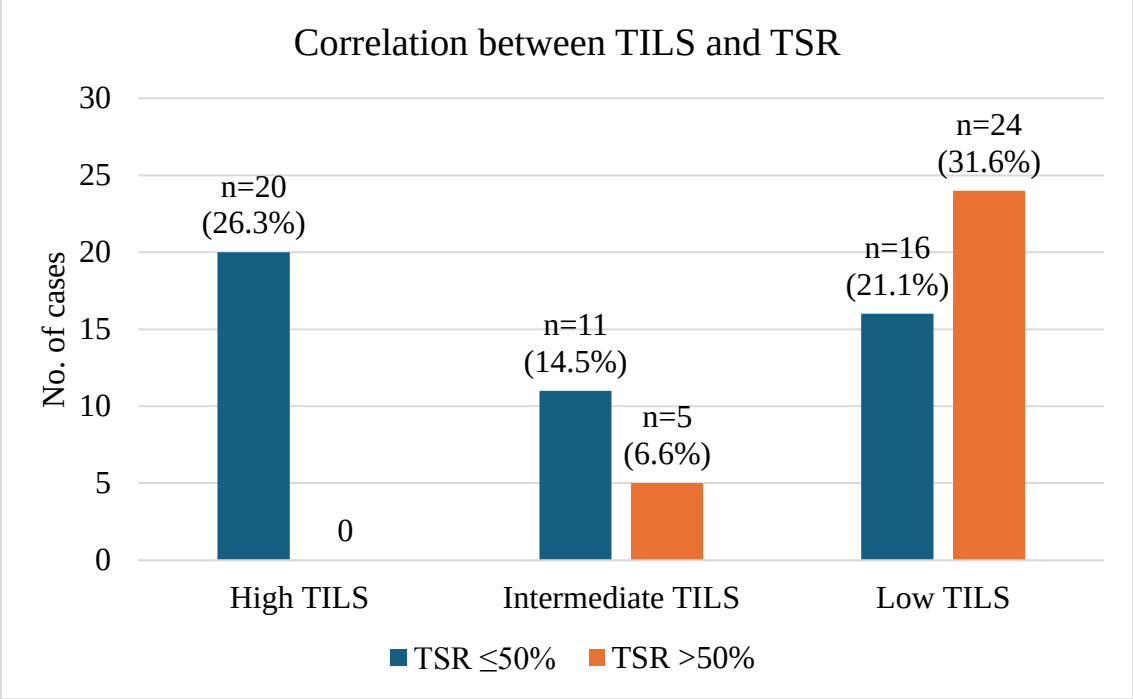


Tumor stroma was divided into Stroma-High >50% and Stroma-low ≤50% in the histological section and maximum cases was seen in ≤50% with a percentage of 62% and >50% had a percentage of 38%.

1.13 Table 13: Distribution of TILS with respect to Tumor stroma ratio:

		TSR		Total	*p* value
		≤50%	>50%		
TILS	H	20 (26.3%)	0 (0.0%)	20 (26.3%)	**0.001**
	I	11 (14.5%)	5 (6.6%)	16 (21.1%)	
	L	16 (21.1%)	24 (31.6%)	40 (52.6%)	
Total		47	29	76	

Chart 13: Distribution of TILS with respect to Tumor stroma ratio using Bar diagram:



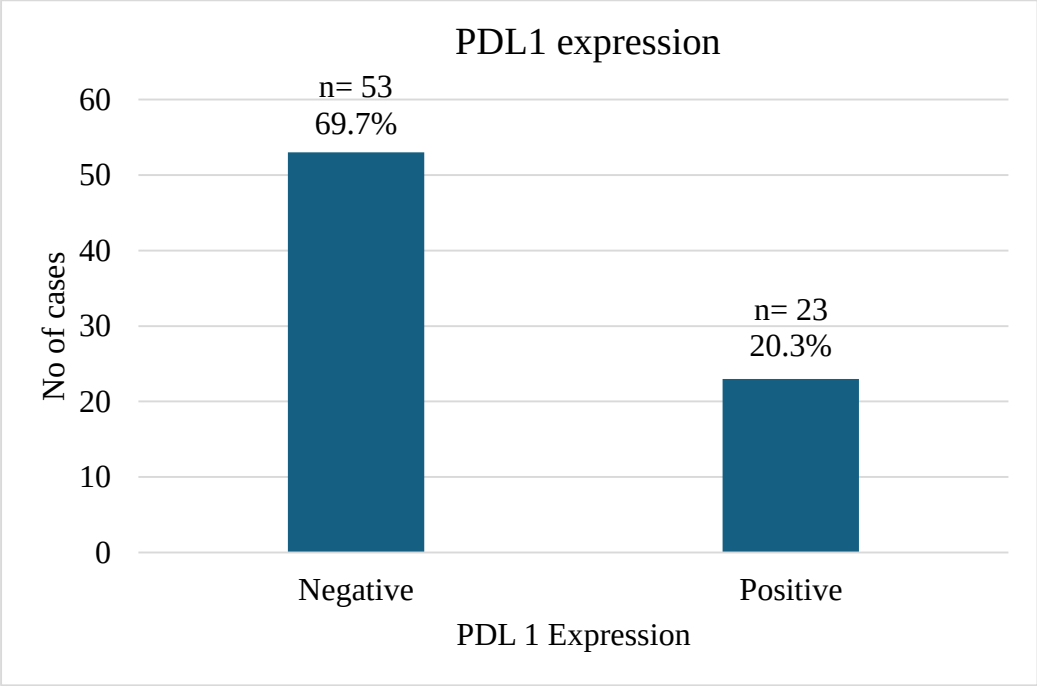
The table illustrates the distribution of tumor stromal ratio (TSR) in relation to tumor infiltrating lymphocytes (TILs) across 76 cases. TILs are categorized into high (H), intermediate (I), and low (L) levels, while TSR is divided into two groups: less than 50% (≤50%) and greater than 50% (>50%). Among cases with high TILs (H), 20 cases (26.3%) have a TSR ≤50% and none have a TSR >50%, totalling 20 cases (26.3%). For cases with intermediate TILs (I), 11 cases (14.5%) have a TSR ≤50% and 5 cases (6.6%) have a TSR >50%, summing up to 16 cases (21.1%). Among cases with low TILs (L), 16 cases (21.1%) have a TSR ≤50% and 24 cases (31.6%) have a TSR >50%, making a total of 40 cases (52.6%). The overall distribution shows that 47 cases have a TSR ≤50% and 29 cases have a TSR >50%, leading to a total of 76 cases. The statistical analysis reveals a significant association between TILs and TSR with a *p* value of 0.001. This suggests that there is a strong correlation between the levels of tumor infiltrating lymphocytes and the tumor stromal ratio in the examined sample, with higher TILs levels being associated with a lower TSR and vice versa.

1.14 PD L1:

Table 14 : Distribution of colorectal cancer cases with PDL 1 expression:

PDL 1 score	Frequency	Percent
0	53	69.7
1	11	14.5
2	12	15.8
Total	76	100

Chart 14 : Distribution of colorectal cancer cases with PDL 1 expression using Bar diagram:



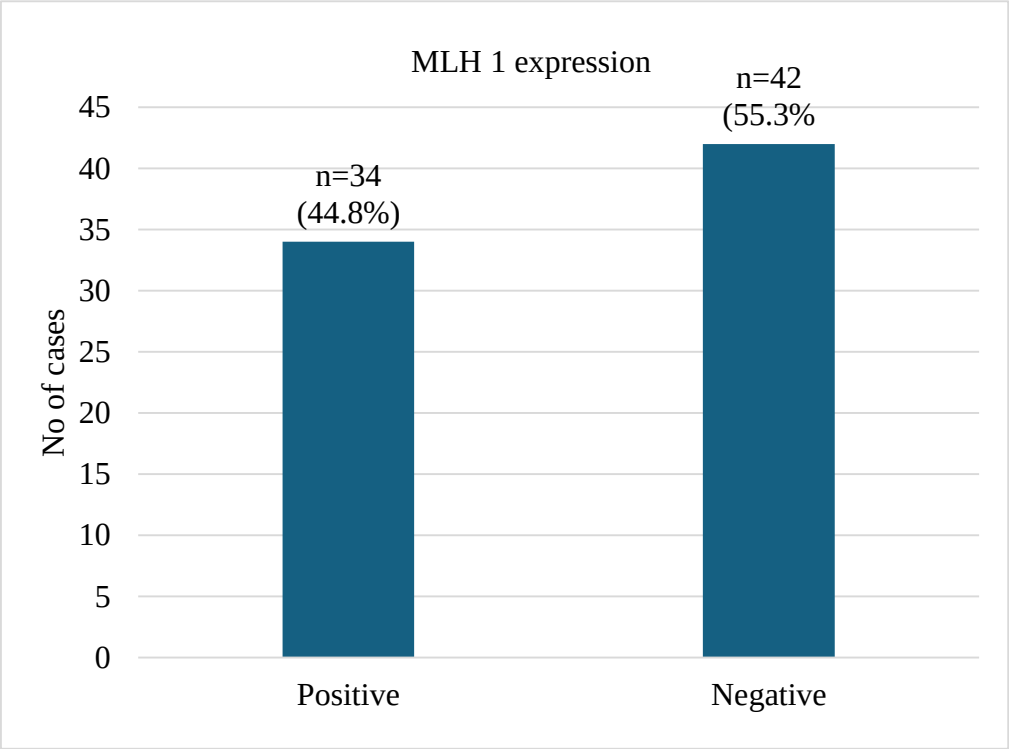
PD-L1 expression on tumor cells was evaluated using a three-tiered grading system: 0 = <5% of the tumor cells; 1 = 5–49% of tumor cells; and 2 =≥ 50% tumor cells with membranous staining of any intensity. Cytoplasmic staining was not considered in this study. Scores of 1 and 2 were considered to be positive for PD-L1 expression. In our study, PDL 1 positive was seen in 30.3% and PDL 1 negative is seen in 69.7% cases.

1.15 MLH 1:

Table 15: Distribution of colorectal cancer cases with MLH 1 expression:

MLH 1 score	Frequency	Percent
0	42	55.3
1	11	14.5
2	23	30.3
Total	76	100

Chart 15: Distribution of colorectal cancer cases with MLH 1 expression using Bar diagram:

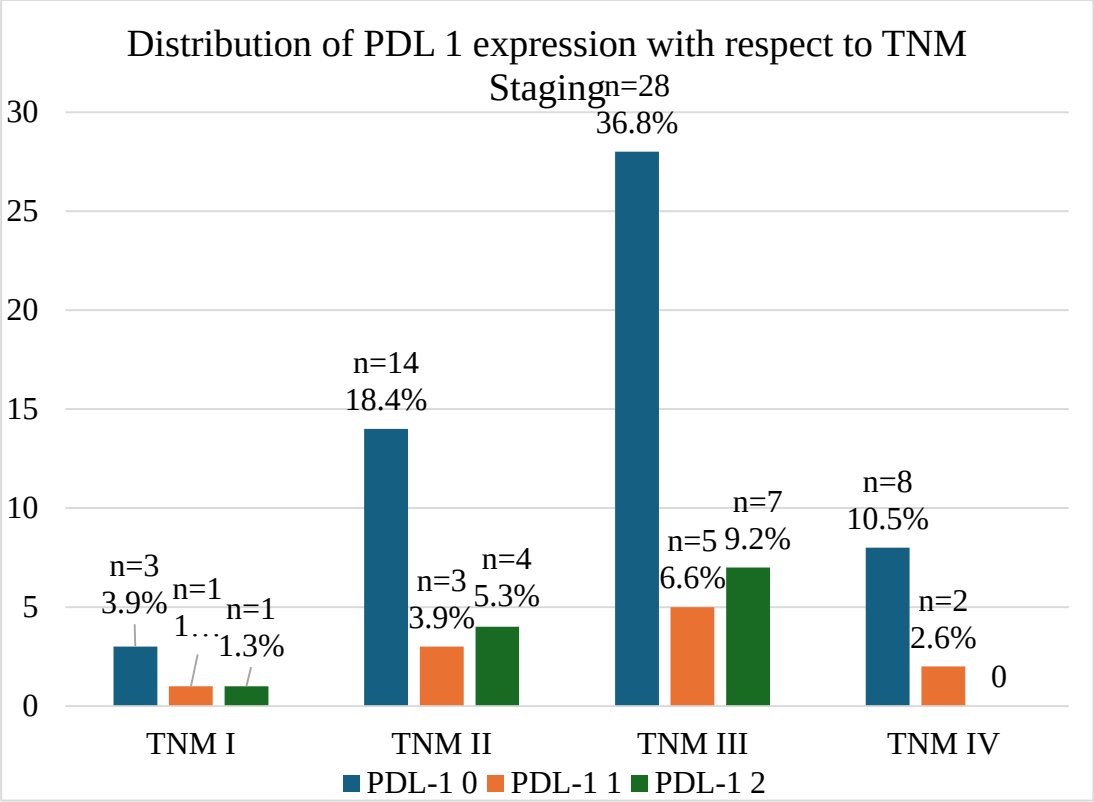


A mean percentage of stained tumor cells was determined and graded into three categories, <1% of the positive tumor cells- negative, 1-50% of the positive tumor cells- score 1, 51-100% of the positive tumor cells- score 2. Scores of 1 and 2 were positive for MLH-1 expression. In our study, MLH 1 positive was seen in 44.8% and MLH 1 negative is seen in 55.3% cases.

1.16 Table 16: Distribution of PDL 1 expression with respect to TNM Staging:

		PDL-1			Total	*p* value
		0	1	2		
TNM	I	3 (3.9%)	1 (1.3%)	1 (1.3%)	5 (6.6%)	0.866
	II	14 (18.4%)	3 (3.9%)	4 (5.3%)	21 (27.6%)	
	III	28 (36.8%)	5 (6.6%)	7 (9.2%)	40 (52.6%)	
	IV	8 (10.5%)	2 (2.6%)	0 (0.0%)	10 (13.2%)	
Total		53	11	12	76	

Chart 16: Distribution of PDL 1 expression with respect to TNM Staging using Bar diagram:



The table shows the number of patients with each combination of PD-L1 expression and TNM stage.

Stage I: Most tumors (3 out of 5) have no PD-L1 expression (0), with the remaining two tumors showing low expression (1).

Stage II: The majority of tumors (14 out of 21) have no PD-L1 expression (0), with a smaller number showing low (3 out of 21) or high (4 out of 21) expression.

Stage III: There is a more even distribution of PD-L1 expression across all three categories (0, 1, and 2), although no expression (0) is still the most common.

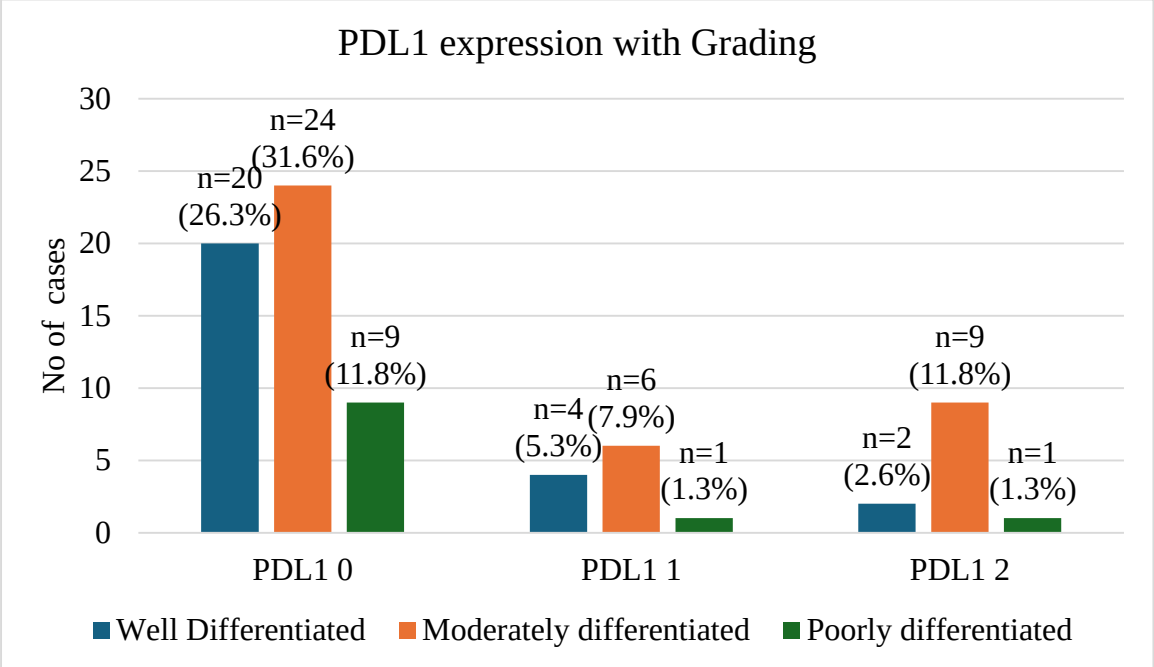
Stage IV: Most tumors (8 out of 10) have either no (0) or low (2 out of 10) PD-L1 expression, with none showing high expression (2).

In the present study there is a trend towards higher PD-L1 expression with higher TNM stage, it is not statistically significant (p-value = 0.866). This means that the association between PDL-1 and TNM Stage is not statistically significant.

Table 17: comparing PDL1 with malignancy grade:

		Malignancy Grading			Total	*P* value
		Well-Differentiated	Moderately Differentiated	Poorly Differentiated		
PDL-1	0	20 (26.3%)	24 (31.6%)	9 (11.8%)	53 (69.7%)	0.439
	1	4 (5.3%)	6 (7.9%)	1 (1.3%)	11 (14.5%)	
	2	2 (2.6%)	9 (11.8%)	1 (1.3%)	12 (15.8%)	
Total		26	26	11	76	

Chart 17: comparing PDL1 with malignancy grade:



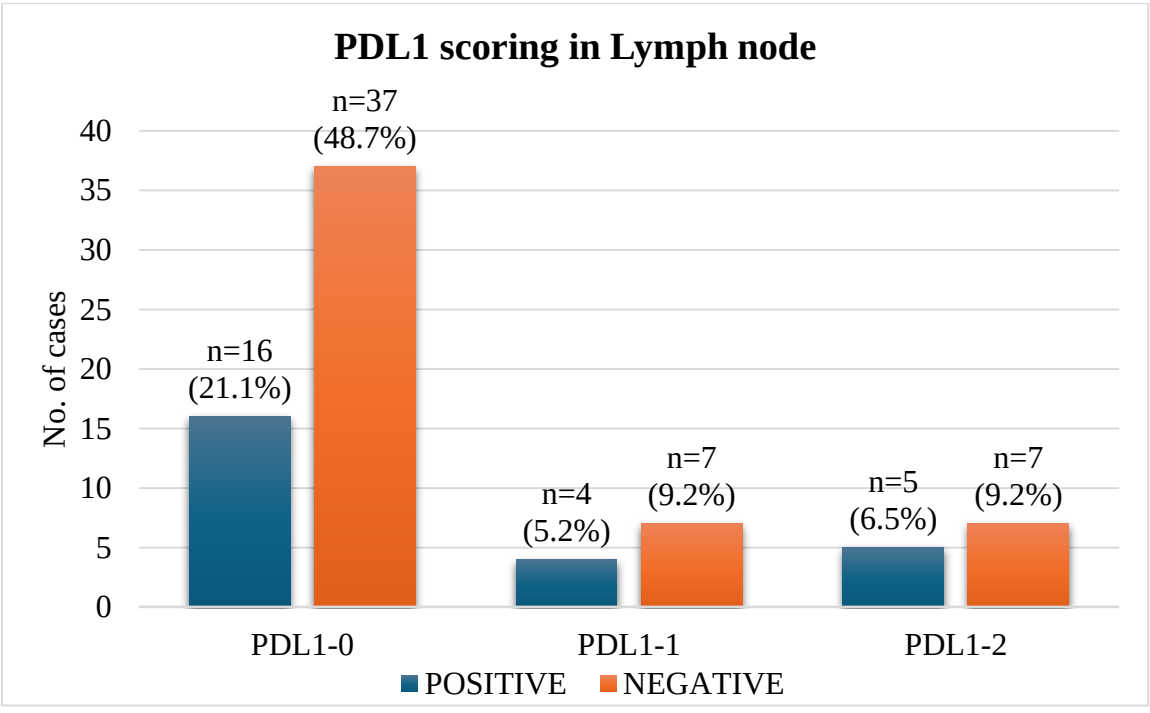
This table shows the percentages of patients with different grades of colorectal carcinoma, stratified by PDL1 expression (0, 1, and 2). PDL-1. The majority of tumors (69.7%) have positive PD-L1 expression (1 or 2). Patients with moderately or poorly differentiated tumors are more likely to have PD-L1 expression compared to patients with well-differentiated tumors. Specifically, 81.8% of patients with poorly differentiated tumors and 61.5% of patients with

moderately differentiated tumors have PD-L1 expression, whereas only 31.6% of patients with well-differentiated tumors have PD-L1 expression. Possible explanation for this observation is PD-L1 expression is associated with tumorogenesis and immune escape. As tumors become more poorly differentiated, they may upregulate the PD-L1 expression to evade immune detection.

1.18 Table 18 : Distribution of PDL 1 expression with respect to Lymph nodes:

Lymph nodes Status	**PDL 1**			*p* value
	0	1	2	
POSITIVE	16(21.1%)	4(5.2%)	5(6.5%)	0.249
NEGATIVE	37(48.7%)	7(9.2%)	7(9.2%)	

Chart 18 : Distribution of PDL 1 expression with respect to Lymph nodes using Bar diagram:

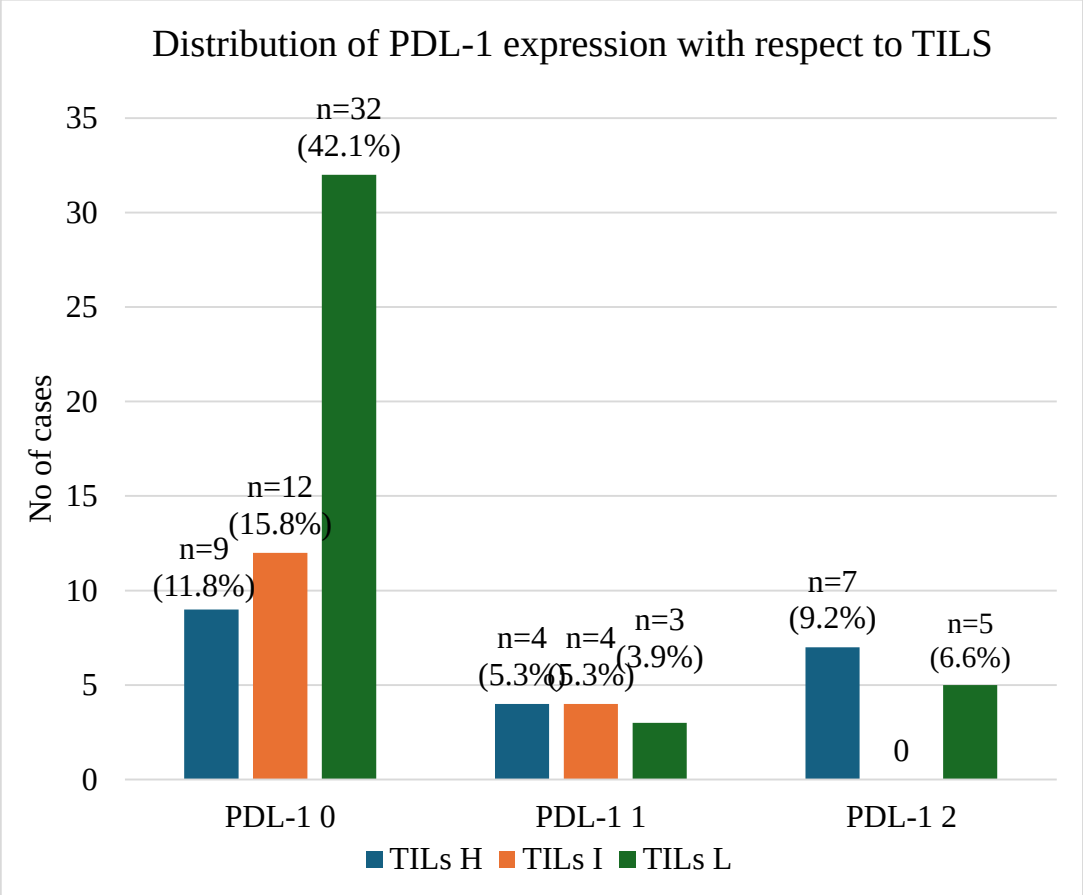


This table on PDL1 expression in colorectal cancer and its correlation with lymph node tumor positivity compared. Observation suggests that higher PDL1 expression is associated with positive lymph node status in colorectal cancer. However, the *p* value indicates that this association is not statistically significant ($p > 0.05$).

Table 19 : Distribution of PDL 1 expression with respect to TILS:

		TILS			Total	*p* value
		H	I	L		
PDL-1	0	9 (11.8%)	12 (15.8%)	32 (42.1%)	53 (69.7%)	**0.012**
	1	4 (5.3%)	4 (5.3%)	3 (3.9%)	11 (14.5%)	
	2	7 (9.2%)	0 (0.0%)	5 (6.6%)	12 (15.8%)	
Total		20	16	40	76	

Chart 19 : Distribution of PDL 1 expression with respect to TILS using a Bar diagram:



This table shows the relationship between PD-L1 expression and TILs (tumor-infiltrating lymphocytes) in colorectal carcinoma.

TILs:

High infiltration (2) is present in 20 out of 76 (26.3%) of the samples.
Low infiltration (1) is present in 16 out of 76 (21.1%) of the samples.
No infiltration (0) is present in 40 out of 76 (52.6%) of the samples.

PD-L1 expression:

High expression (2) is present in 12 out of 76 (15.8%) of the samples.
Low expression (1) is present in 11 out of 76 (14.5%) of the samples.
No expression (0) is present in 53 out of 76 (69.7%) of the samples.

Most of the samples (52.6%) have no TIL infiltration (0). Most of the samples (69.7%) also do not show PD-L1 expression (0). There are a few samples (12 out of 76) that have high PD-L1 expression (2) and no TIL infiltration (0). This suggests that these tumors may be able to evade the immune system.

The table shows that there is a statistically significant association between PD-L1 expression and TILs (*p*-value = 0.012), which means this observation is unlikely to be due to chance.

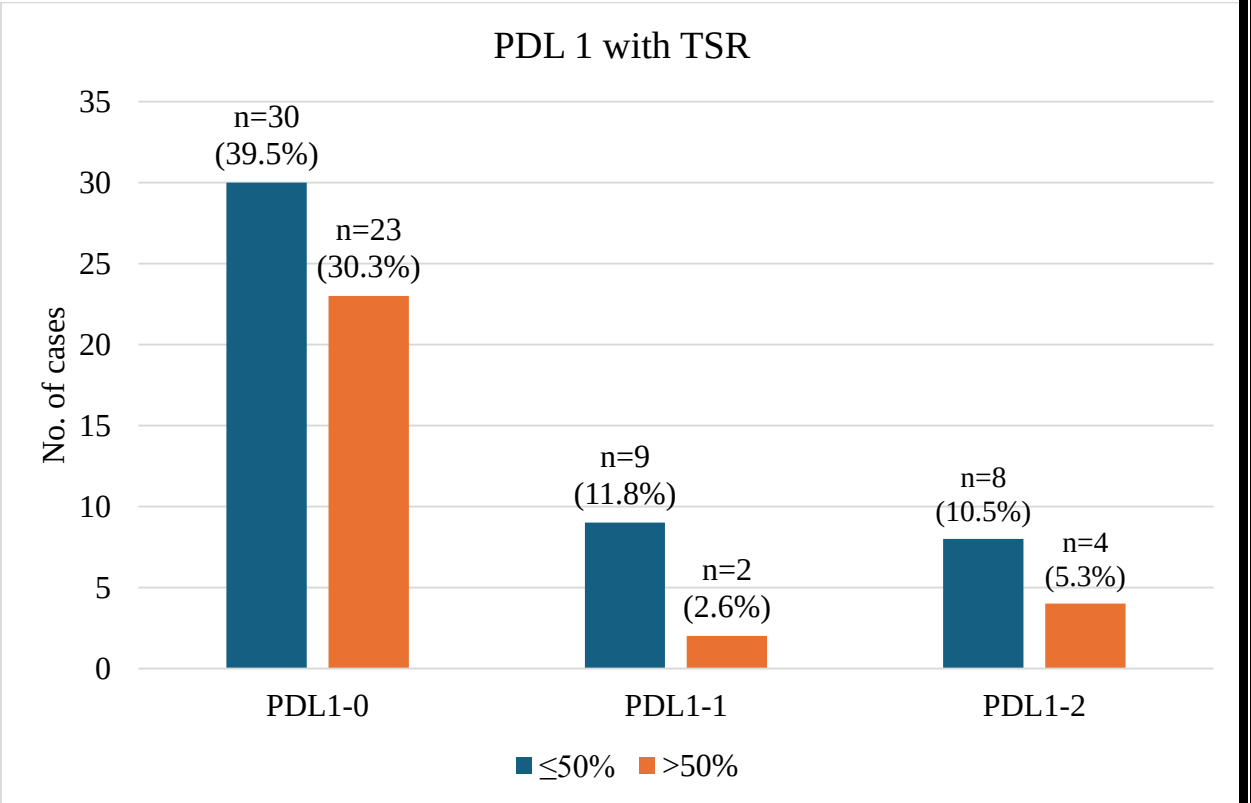
Overall, present study observation suggests that there is a relationship between PD-L1 expression and TILs in colorectal carcinoma. Tumors with high PD-L1 expression may be less infiltrated by TILs.

It is important to note that our study has small sample size (n=76) and more research is needed to confirm these findings. Limitation of this study is retrospective cross-sectional study. There can be change in TILs and PDL1 expression as the disease progress in patients.

Table 20 : Distribution of PDL 1 expression with respect to Tumor stroma ratio:

		TSR		Total	*p* value
		≤50%	>50%		
PDL-1	0	30 (39.5%)	23 (30.3%)	53 (69.7%)	0.273
	1	9 (11.8%)	2 (2.6%)	11 (14.5%)	
	2	8 (10.5%)	4 (5.3%)	12 (15.8%)	
Total		47	29	76	

Chart 20 : Distribution of PDL 1 expression with respect to Tumor stroma ratio using a Bar diagram:

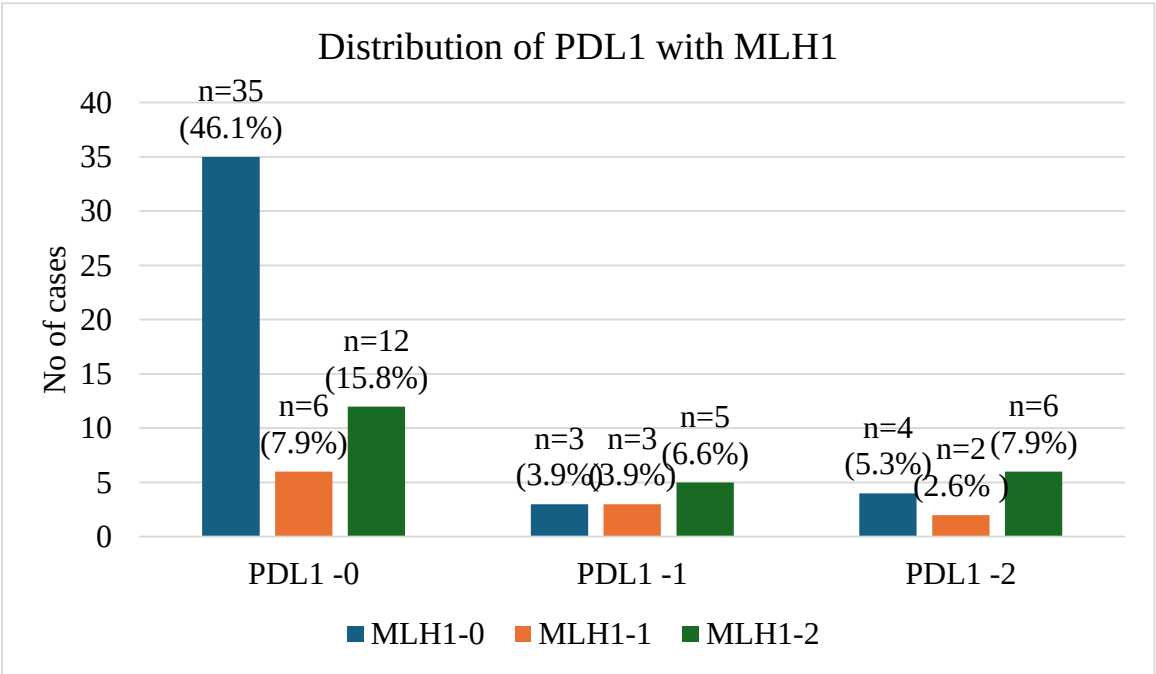


This graph explores the relationship between Programmed Death-Ligand 1 (PDL1) expression levels and the Tumor Stromal Ratio (TSR) in a cohort of cancer cases. The key findings are PDL1-0 (Nil Expression) represents 39.5% of the cases, with most cases exhibiting less than 50% TSR (n=30) and a remaining are (n=23) demonstrating more than 50% TSR. PDL1-1 (Low Expression) accounts for 14.3% of cases, distributed across TSR levels—9 cases with less than 50% TSR and 2 case with more than 50% TSR. PDL1-2 (High Expression) comprises 10.5% of cases, with less than 50% TSR and 4 case with more than 50% TSR. This observation shows when PDL1 expression increases, there is a gradual decrease in cases with high TSR.

Table 21 : Distribution of PDL-1 expression with respect to MLH-1:

		MLH-1			Total	*p* value
		0	1	2		
PDL -1	0	35 (46.1%)	6 (7.9%)	12 (15.8%)	53 (69.7%)	0.067
	1	3 (3.9%)	3 (3.9%)	5 (6.6%)	11 (14.5%)	
	2	4 (5.3%)	2 (2.6%)	6 (7.9%)	12 (15.8%)	
Total		42	11	23	76	

Chart 21 : Distribution of PDL-1 expression with respect to MLH-1 using Bar diagram:

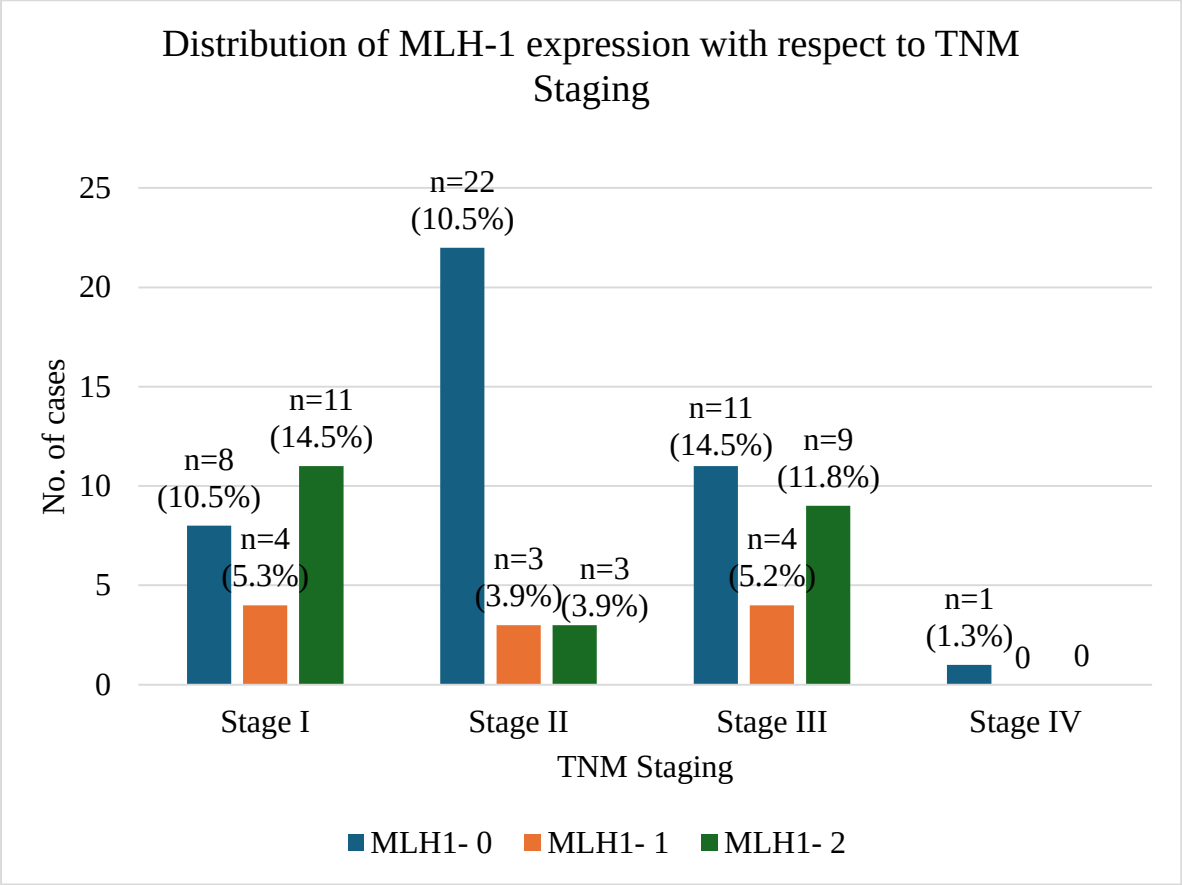


This table depicts the co-expression of PD-L1 and MLH1 in colorectal carcinoma (CRC) patients. MLH1 and PD-L1 are evaluated on a scale of 0 (no expression), 1 (low expression), and 2 (high expression). A total of 76 patients were included in the analysis. The majority of patients (69.7%) exhibited PD-L1 expression (scores 1 or 2). There is a statistically marginally significant association (p-value = 0.067) between PD-L1 expression and MLH1 expression levels. Patients with high MLH1 expression (score 2) tended to have lower PD-L1 expression levels (0 or 1) compared to patients with low or no MLH1 expression (scores 0 or 1). Mismatch repair deficiency (MMR-D), which can be caused by mutations in MLH1 and other genes, can lead to increased tumor mutational burden (TMB). TMB is a measure of the number of mutations within a tumor's genes. Tumors with high TMB may be more readily recognized by the immune system and respond favourably to immunotherapy. However, MMR-D can also lead to the production of immunosuppressive factors by tumor cells, potentially reducing the effectiveness of immunotherapy. The sample size in this study is relatively small (n=76). Larger studies are required to confirm the observed trends.

Table 22: Distribution of MLH-1 expression with respect to TNM Staging:

		MLH-1			Total	*p* value
		0	1	2		
TNM	1	8 (10.5%)	4 (5.3%)	11 (14.5%)	23 (30.3%)	**0.044**
	2	22 (10.5%)	3 (3.9%)	3 (3.9%)	28 (36.8%)	
	3	11 (14.5%)	4 (5.2%)	9 (11.8%)	24 (31.5%)	
	4	1 (1.3%)	0 (0%)	0 (0%)	1 (1.3%)	
Total		42	11	23	76	

Chart 22: Distribution of MLH-1 expression with respect to TNM Staging using a Bar diagram:



This table shows the number of patients with each combination of MLH1 expression and TNM stage.

MLH1 expression:

- High expression (2) is present in 23 out of 76 (30.3%) of the samples.
- Low expression (1) is present in 11 out of 76 (14.5%) of the samples.
- No expression (0) is present in 42 out of 76 (55.3%) of the samples.

TNM stage:

- Stage I: Most tumors (8 out of 23) have high MLH1 expression (2), with some tumors showing low expression (4 out of 23) and no expression (11 out of 23).
- Stage II: The majority of tumors (22 out of 28) have high MLH1 expression (2), with a few tumors showing low expression (3 out of 28) and no expression (3 out of 28).

- Stage III: There is a more even distribution of MLH1 expression across all three categories (0, 1, and 2), but high expression (2) is still the most common (9 out of 24).
- Stage IV: Only one tumor (out of 1) shows high MLH1 expression (2), with the remaining tumors showing no expression (0).

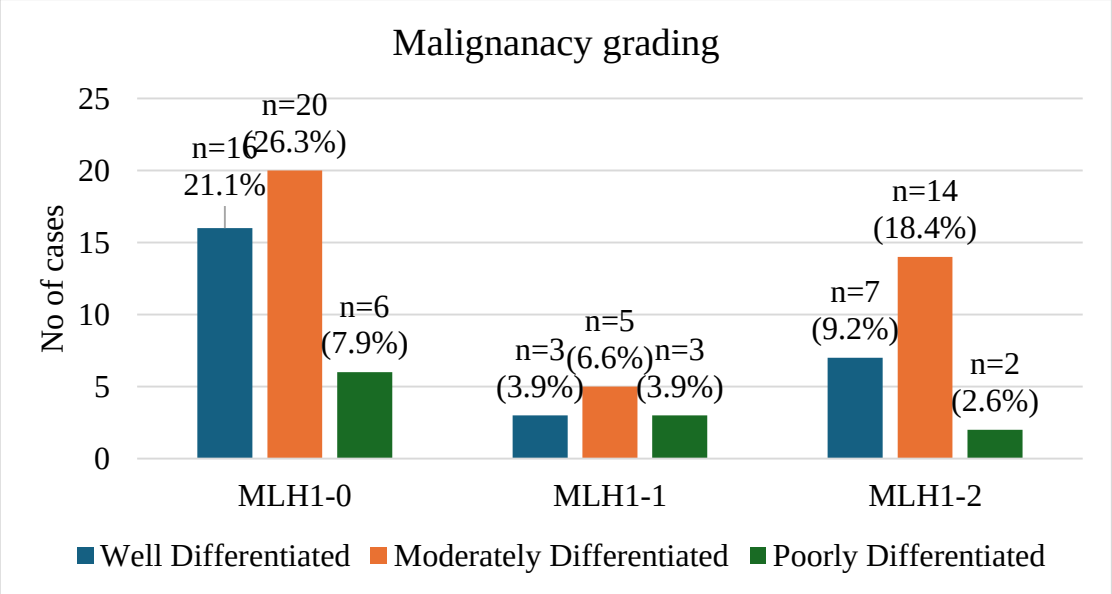
A higher proportion of tumors with lower TNM stages (I and II) have high MLH1 expression (2) compared to tumors with higher TNM stages (III and IV). This observation suggests that colorectal tumors with lower TNM stages tend to have higher MLH1 expression.

There is a statistically significant association between MLH1 expression and TNM stage in colorectal carcinoma (p-value = 0.044).

Table 23: comparing MLH1 with malignancy grade:

		Malignancy Grading			Total	*p* value
		Well Differentiated	Moderately Differentiated	Poorly Differentiated		
MLH1	0	16(21.1%)	20 (26.3%)	6 (7.9%)	42 (55.3%)	0.591
	1	3 (3.9%)	5 (6.6%)	3 (3.9%)	11 (14.5%)	
	2	7 (9.2%)	14 (18.4%)	2 (2.6%)	23 (30.3%)	
Total		26	39	11	76	

Chart 23: comparing MLH1 with malignancy grade using Bar diagram:

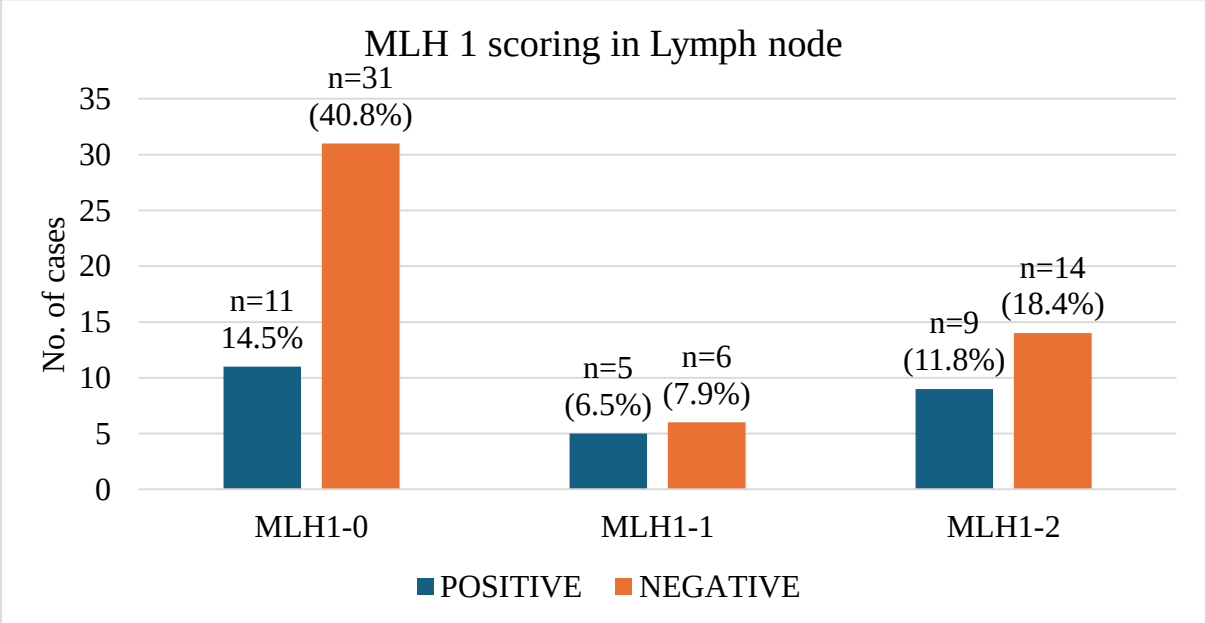


The table compares MLH1 expression (0, 1, or 2) with tumor grade (well-differentiated, moderately differentiated, or poorly differentiated) in colorectal carcinoma patients. The majority of tumors (55.3%) have no MLH1 expression (0). Higher MLH1 expression (1 or 2) in well-differentiated tumors compared to moderately or poorly differentiated tumors. 42.3% of well-differentiated tumors have high MLH1 expression (2), whereas only 13.6% of moderately differentiated tumors and 9.1% of poorly differentiated tumors have high MLH1 expression.

Table 24 : Distribution of MLH-1 expression with respect to Lymph nodes:

Lymph nodes Status	**MLH 1**			*p* value
	0	1	2	
POSITIVE	11(14.5%)	5(6.5%)	9(11.8%)	0.207
NEGATIVE	31(40.8%)	6(7.9%)	14(18.4%)	

Chart 24 : Distribution of MLH-1 expression with respect to Lymph nodes using a Bar diagram:

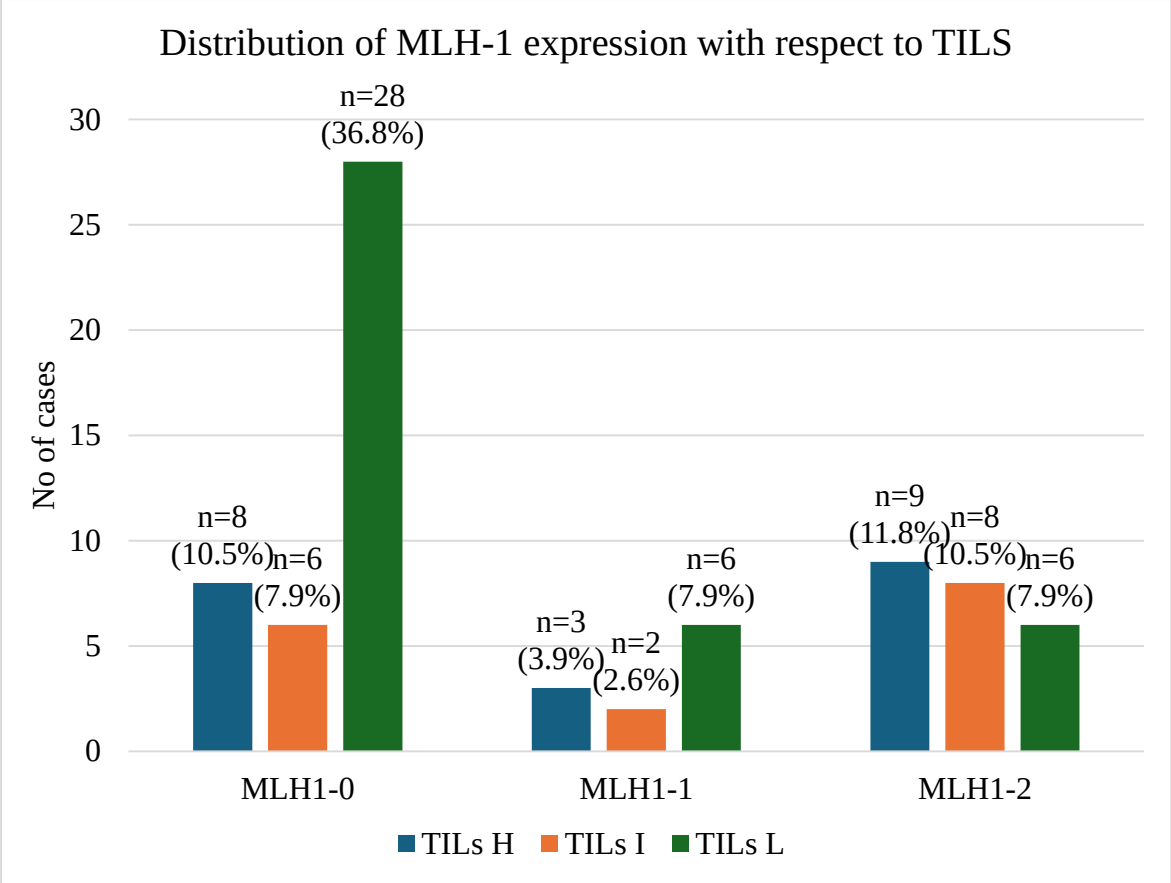


This table on MLH1 expression levels in colorectal cancer based on lymph node status analysis showed varying expression. Grade 2 (high) MLH1 expression is more prevalent in positive lymph nodes, but the association is not statistically significant ($p > 0.05$).

Table 25 : Distribution of MLH-1 expression with respect to TILS:

		TILS			Total	*p* value
		H	I	L		
MLH 1	0	8 (10.5%)	6 (7.9%)	28 (36.8%)	42 55.3%	**0.041**
	1	3 (3.9%)	2 (2.6%)	6 (7.9%)	11 (14.5%)	
	2	9 (11.8%)	8 (10.5%)	6 (7.9%)	23 (30.3%)	
Total		20	16	40	76	

Chart 25 : Distribution of MLH-1 expression with respect to TILS using Bar diagram:

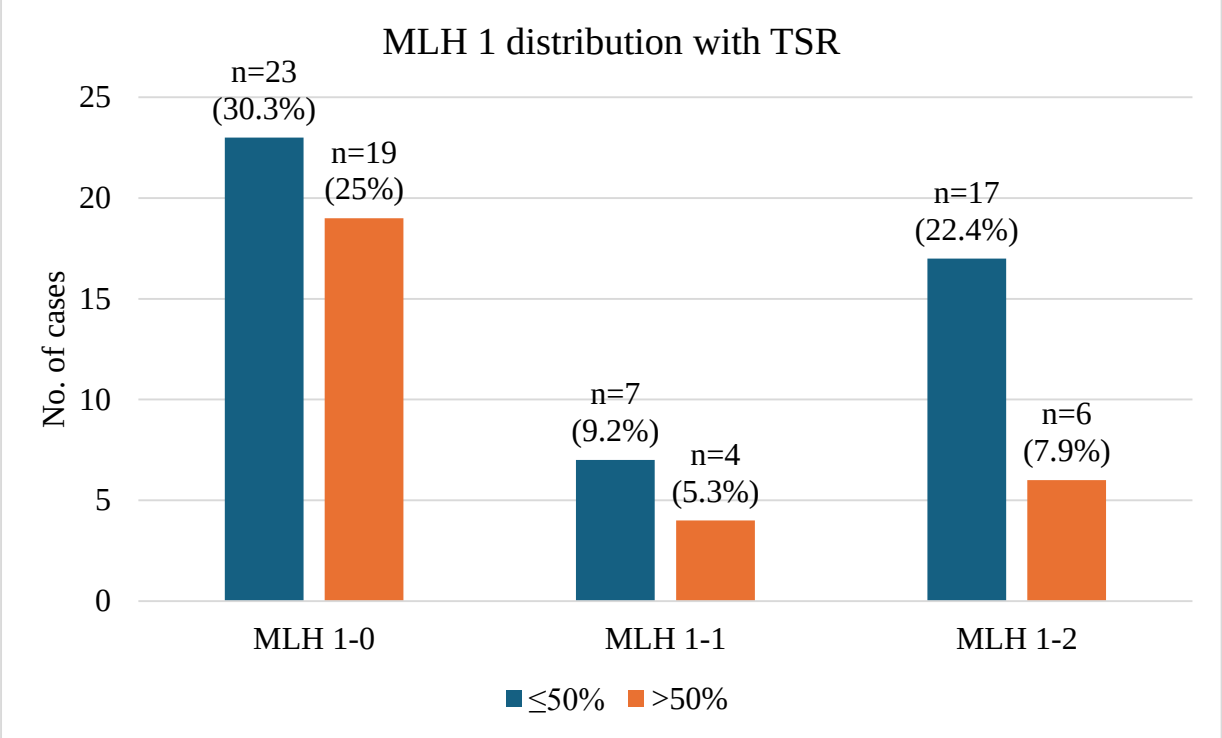


The graph presents a distribution of tumor infiltrating lymphocytes (TILs) in relation to MLH1 expression levels in a sample of 76 cases. The TILs are categorized into three groups: high (H), intermediate (I), and low (L), while MLH1 expression is classified into nil (0), low (1), and high (2). For MLH1-0, there are 8 cases with high TILs (10.5%), 6 cases with intermediate TILs (7.9%), and 28 cases with low TILs (36.8%), total 42 cases (55.3%). For MLH1-1, there are 3 cases with high TILs (3.9%), 2 cases with intermediate TILs (2.6%), and 6 cases with low TILs (7.9%), summing up to 11 cases (14.5%). For MLH1-2, there are 9 cases with high TILs (11.8%), 8 cases with intermediate TILs (10.5%), and 6 cases with low TILs (7.9%), making a total of 23 cases (30.3%). The total cases for each TILs category are 20 for high, 16 for intermediate, and 40 for low, culminating in 76 cases. The statistical analysis reveals a significant association between MLH1 expression and TILs with a *p* value of 0.041, indicating a potential correlation between higher MLH1 expression and increased presence of TILs.

Table 26: Distribution of MLH-1 expression with respect to Tumor stroma ratio:

		TSR		Total	*p* value
		≤50%	>50%		
MLH-1	0	23 (30.3%)	19 (25.0%)	42 (55.3%)	0.312
	1	7 (9.2%)	4 (5.3%)	11 (14.5%)	
	2	17 (22.4%)	6 (7.9%)	23 (30.3%)	
Total		47	29	76	

Chart 26: Distribution of MLH-1 expression with respect to Tumor stroma ratio using Bar diagram:



The table displays the distribution of tumor stromal ratio (TSR) in relation to MLH1 expression levels across 76 cases. MLH1 expression is categorized into nil (0), low (1), and high (2), while TSR is divided into two groups: less than 50% (≤50%) and greater than 50% (>50%). For

MLH1-0, there are 23 cases with TSR ≤50% (30.3%) and 19 cases with TSR >50% (25.0%), totalling 42 cases (55.3%). For MLH1-1, there are 7 cases with TSR ≤50% (9.2%) and 4 cases with TSR >50% (5.3%), summing up to 11 cases (14.5%). For MLH1-2, there are 17 cases with TSR ≤50% (22.4%) and 6 cases with TSR >50% (7.9%), making a total of 23 cases (30.3%). The overall distribution of cases shows that 47 cases have a TSR ≤50% and 29 cases have a TSR >50%, leading to a total of 76 cases. The statistical analysis indicates no significant association between MLH1 expression and TSR, with a *p* value of 0.312. This suggests that variations in MLH1 expression do not have a statistically significant correlation with the tumor stromal ratio in the examined sample.

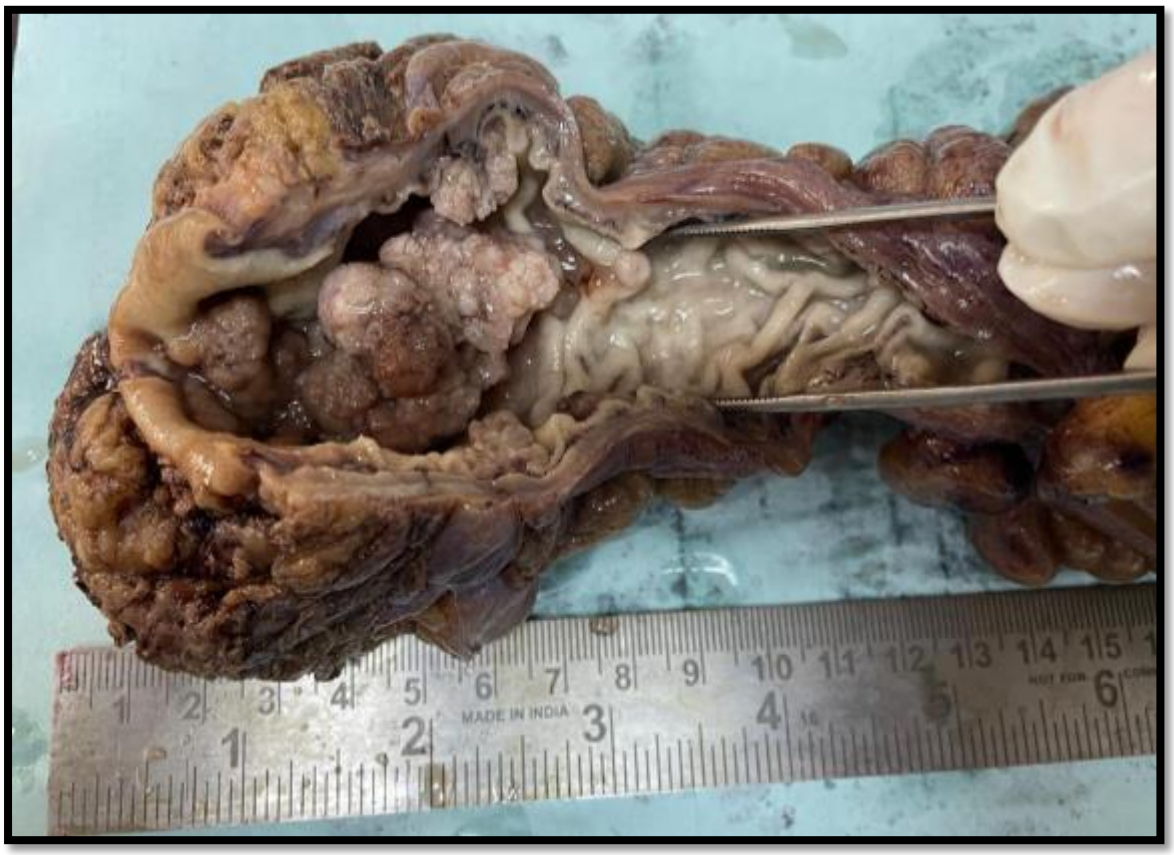


Figure 5: Gross photograph showing ulcero proliferative growth in the rectum

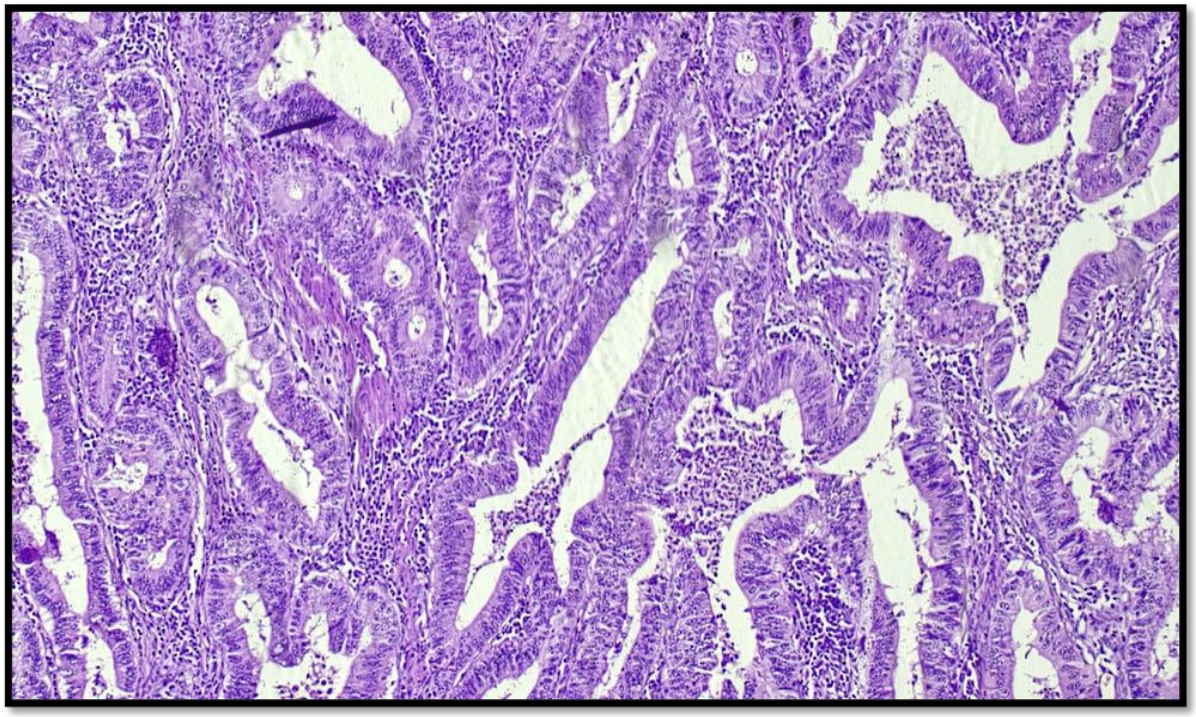


Figure 6: Microphotograph of H and E-stained section showing Well differentiation of Colorectal Adenocarcinoma (Original magnification, x400)

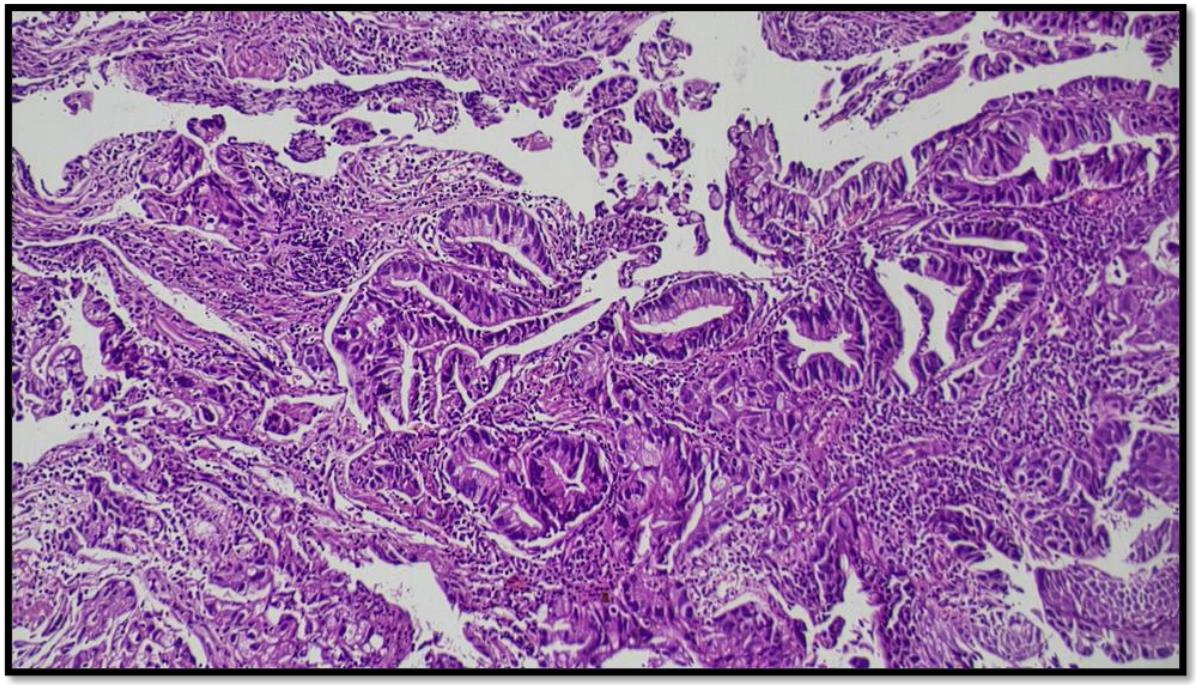


Figure 7: Microphotograph of H and E stained section showing Moderate differentiation of Colorectal Adenocarcinoma(Original magnification, x100)

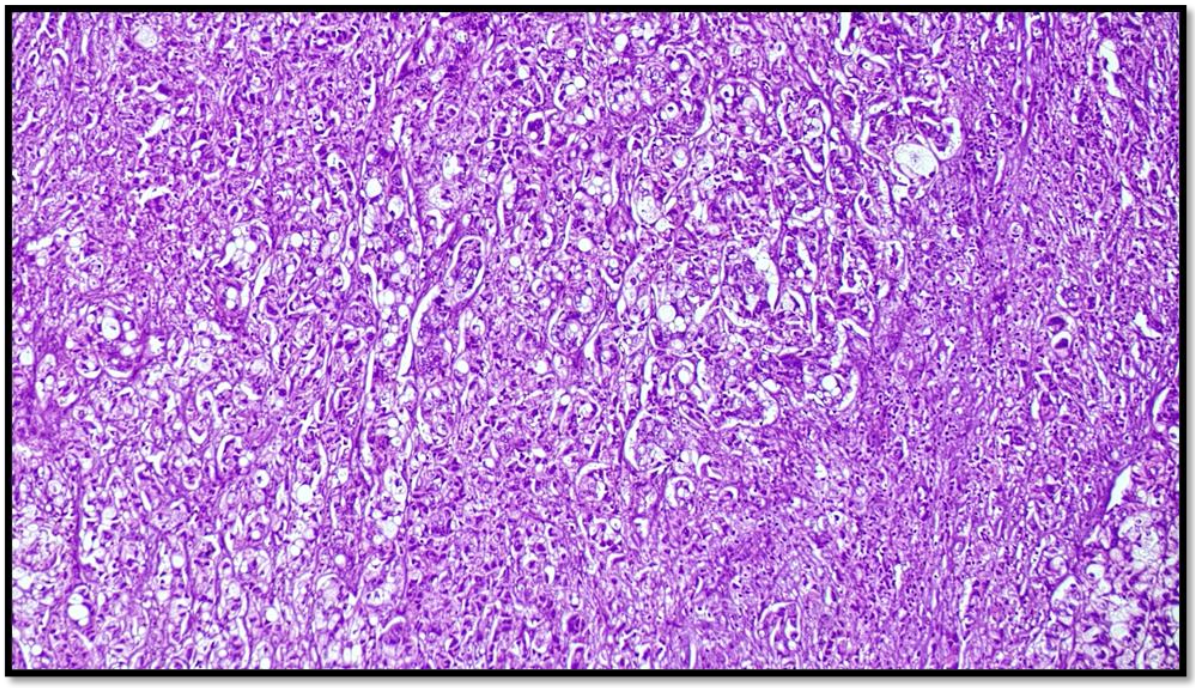


Figure 8: Microphotograph of H and E-stained section showing Lymph node metastasis by Poorly differentiated Adenocarcinoma colon (Original magnification, x400)

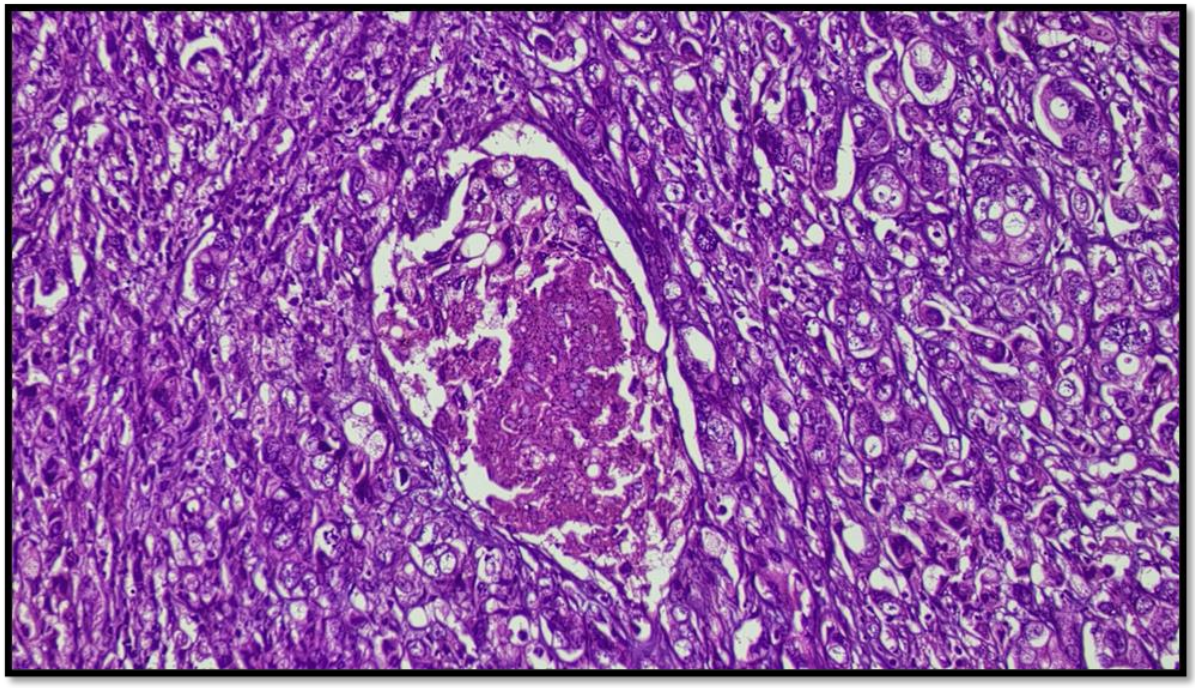


Figure 9: Microphotograph of H and E-stained section showing vascular invasion by Moderate differentiation of Colorectal Adenocarcinoma (Original magnification, x100)

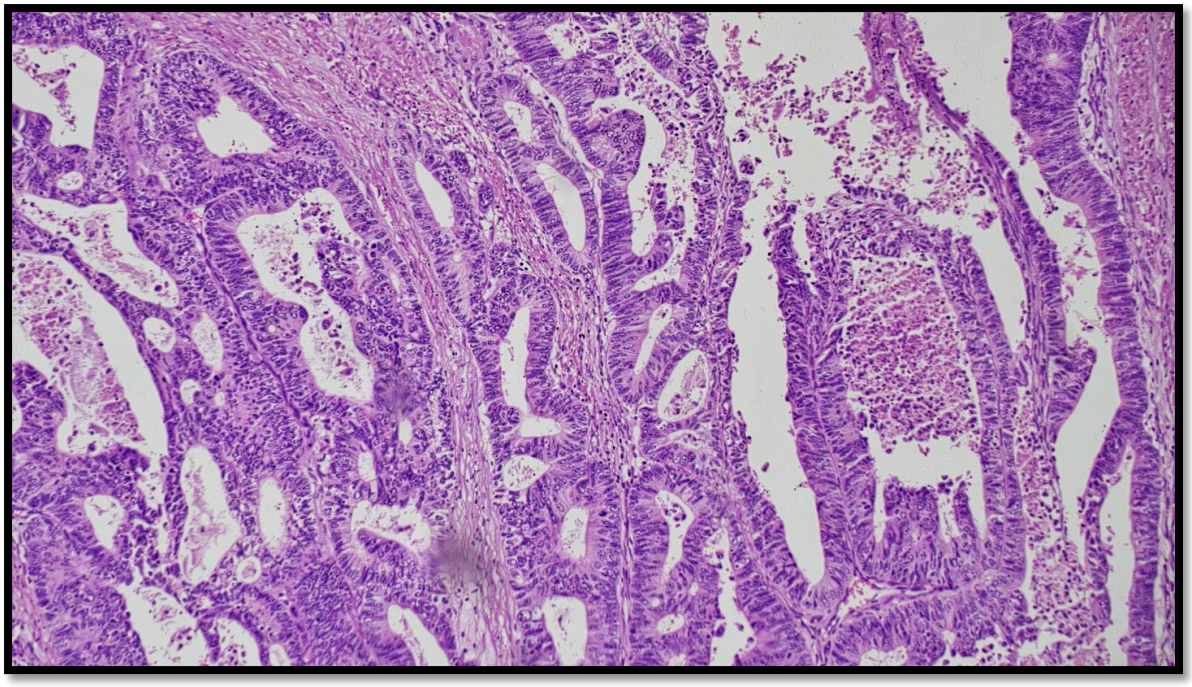


Figure 10: Microphotograph of H and E-stained section showing Low TILS in Colorectal Adenocarcinoma (Original magnification, x200)

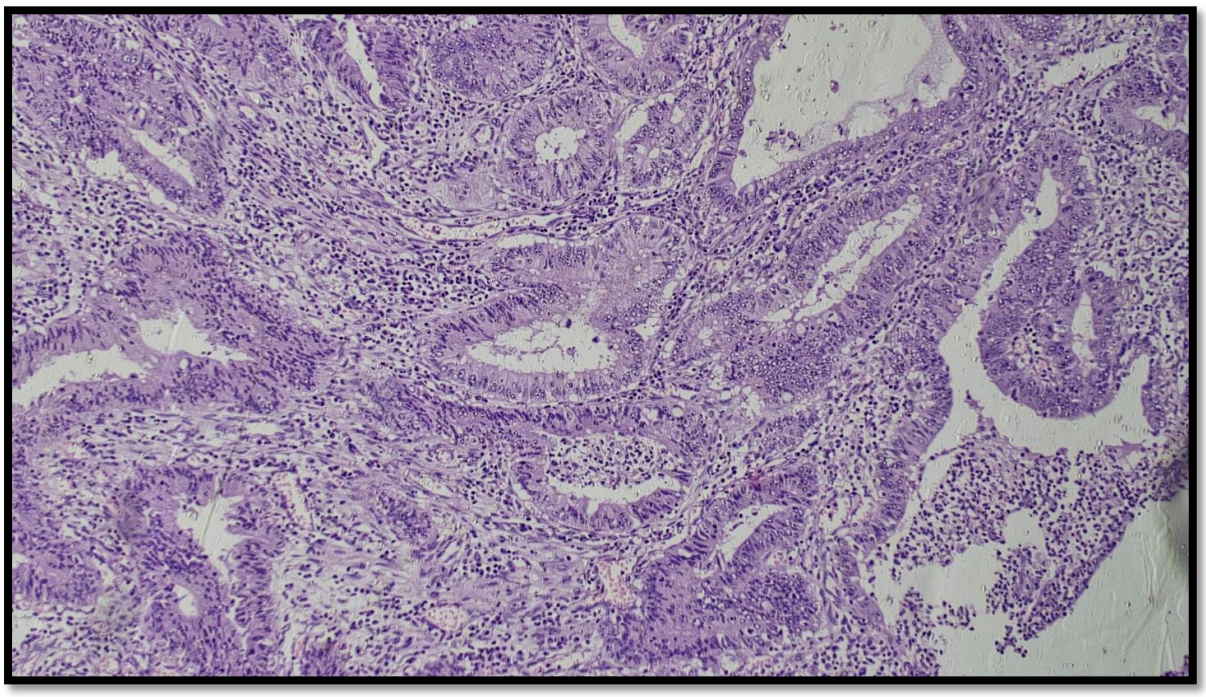


Figure 11: Microphotograph of H and E-stained section showing Intermediate TILS in Colorectal Adenocarcinoma (Original magnification, x200)

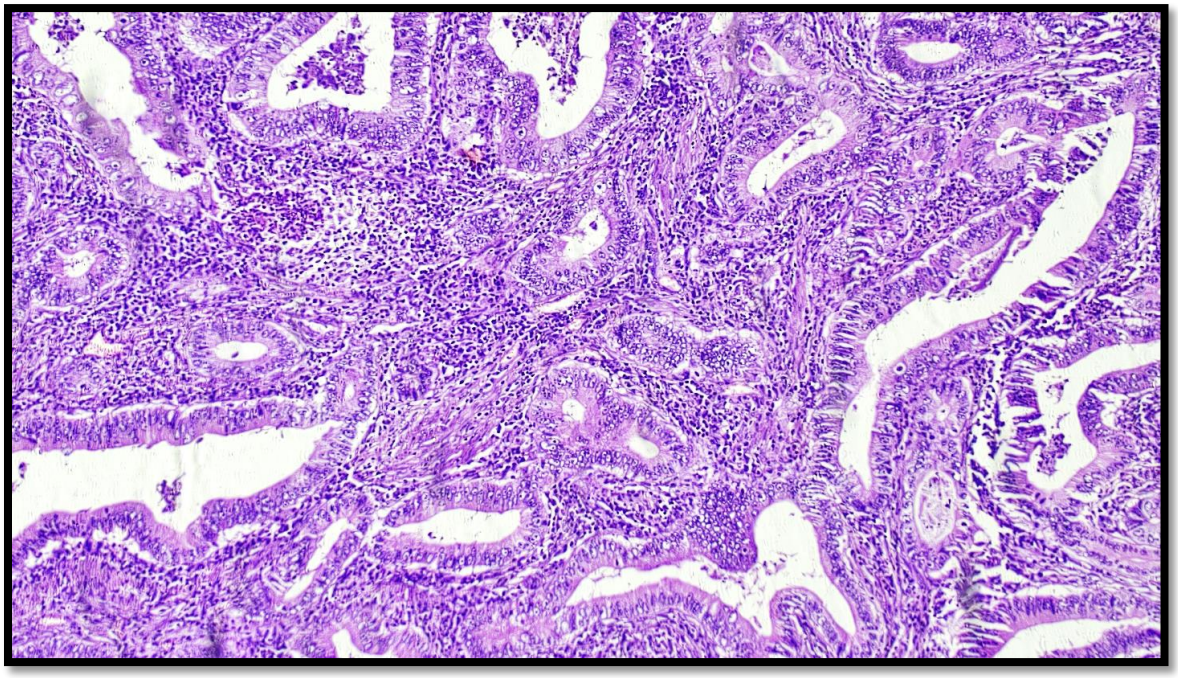


Figure 12: Microphotograph of H and E-stained section showing High TILS in Colorectal Adenocarcinoma (Original magnification, x200)

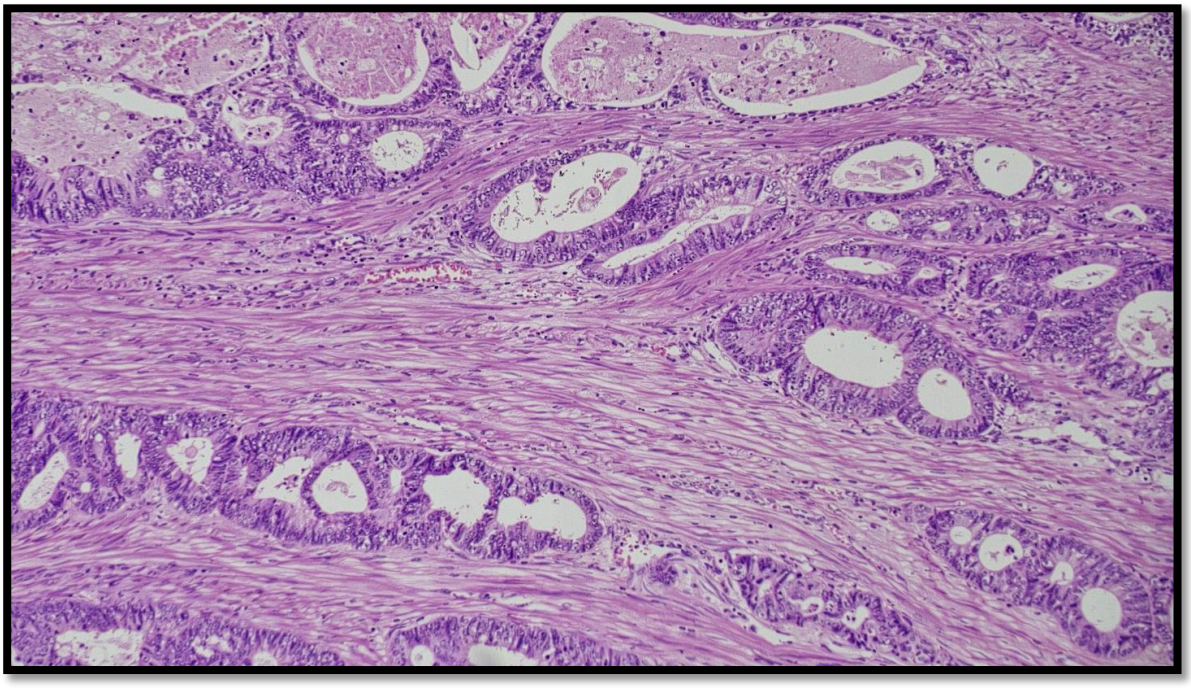


Figure 13: Microphotograph of Hand E-stained section showing High Tumor stroma in Colorectal Adenocarcinoma (Original magnification, x200)

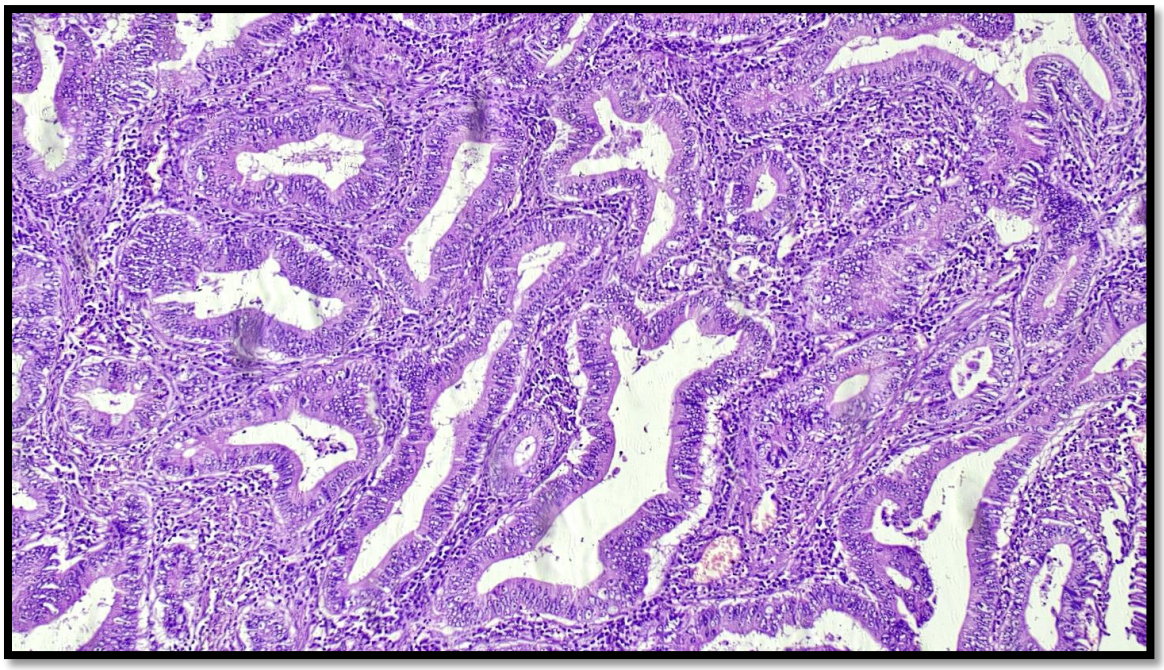


Figure 14: Microphotograph of Hand E-stained section showing Low Tumor stroma in Colorectal Adenocarcinoma (Original magnification, x200)

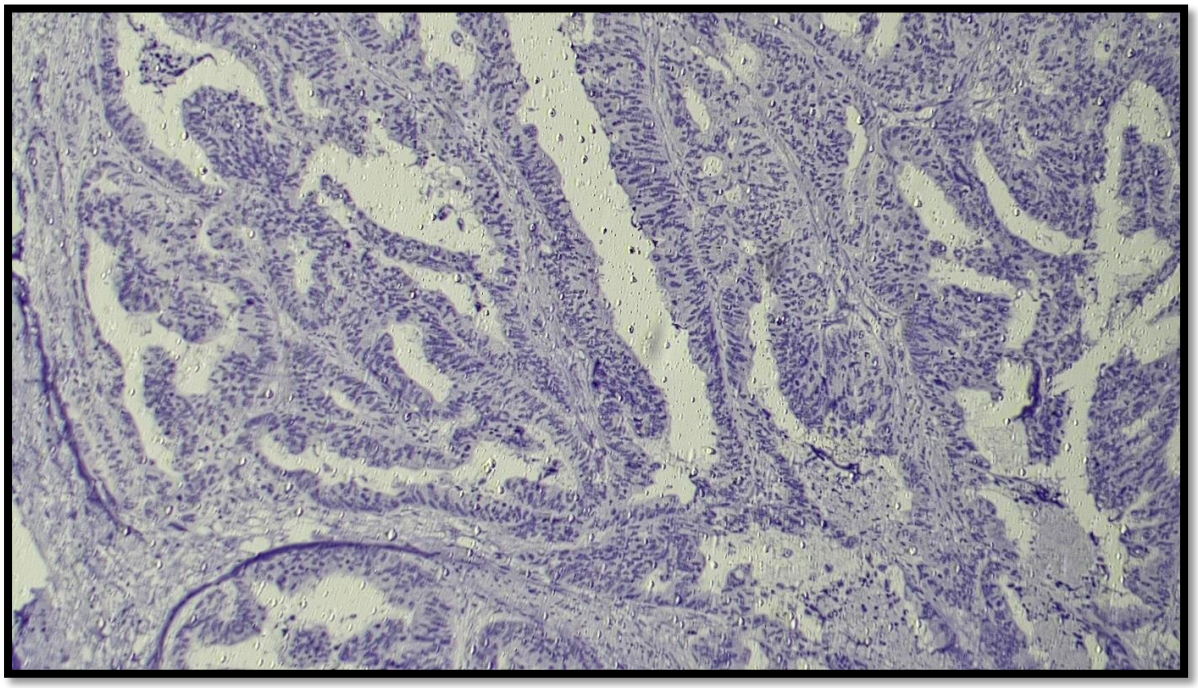


Figure 15: Microphotograph of PDL1 IHC staining showing no expression of PDL1-Score 0 in Colorectal Adenocarcinoma (Original magnification, x200)

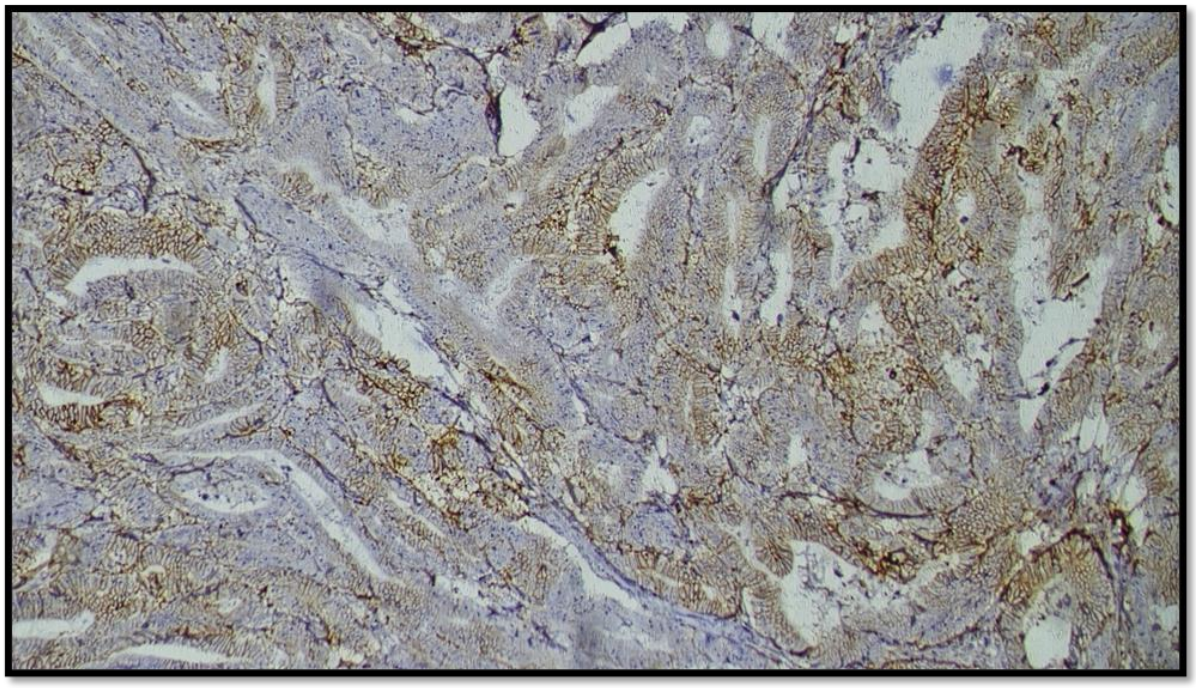


Figure 16: Microphotograph of PDL1 IHC staining showing expression of PDL1- Score 1 in Colorectal Adenocarcinoma (Original magnification, x200)

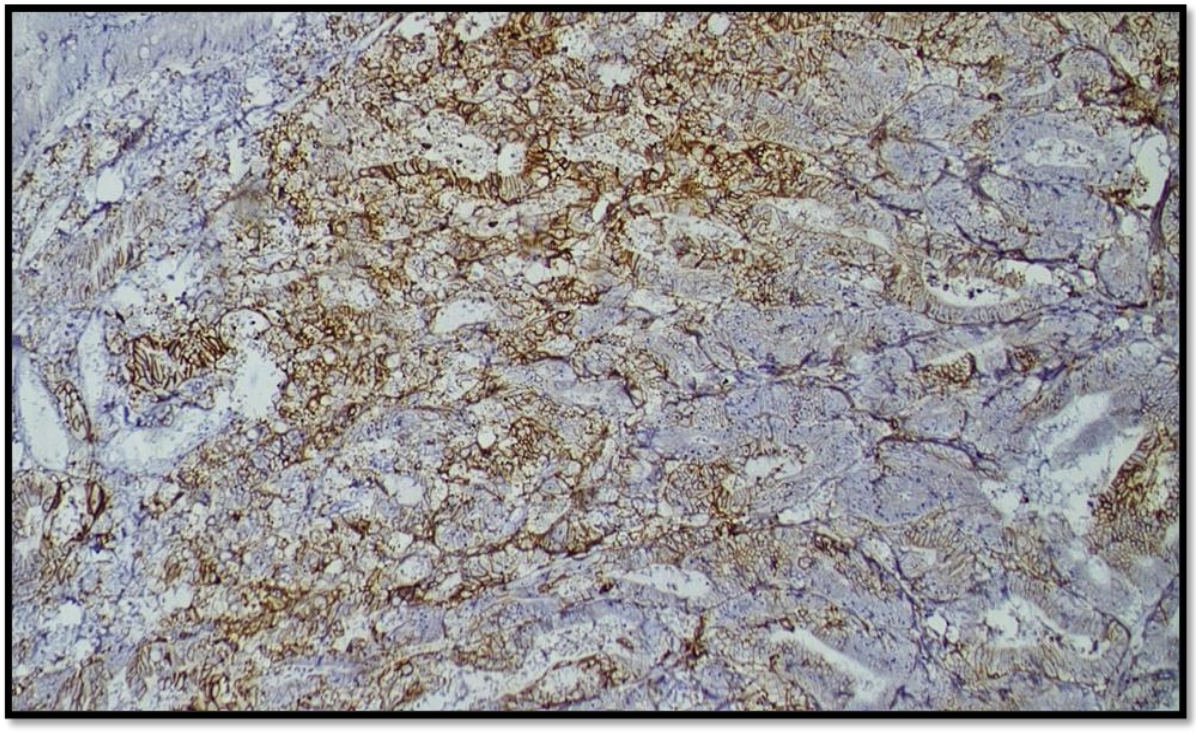


Figure 17: Microphotograph of PDL1 IHC staining showing expression of PDL1- Score 2 in Colorectal Adenocarcinoma (Original magnification, x200)

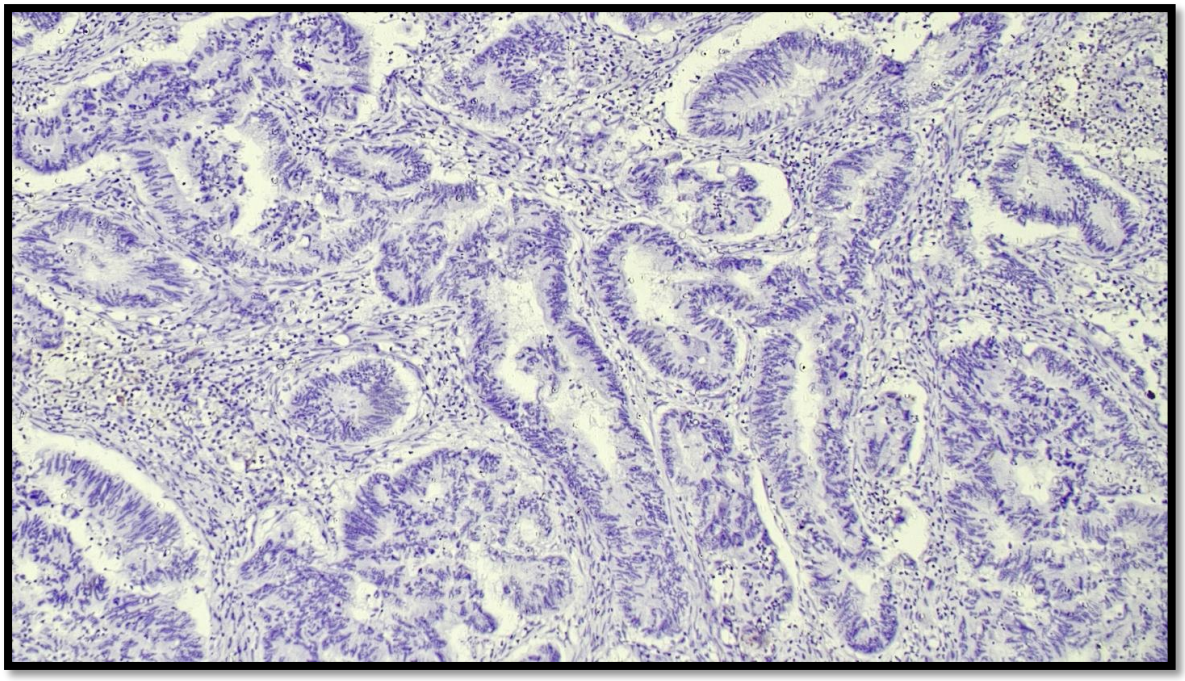


Figure 18: Microphotograph of MLH1 IHC staining showing no expression of MLH1 - Score 0 in Colorectal Adenocarcinoma (Original magnification, x200)

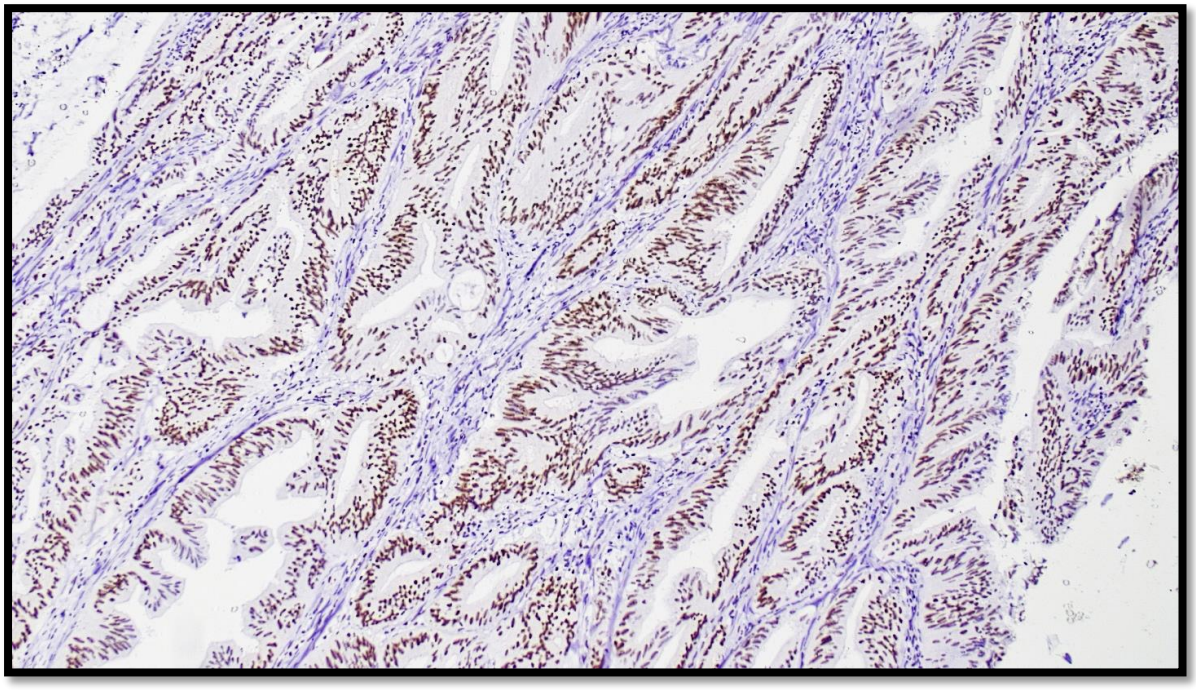


Figure 19: Microphotograph of MLH1 IHC staining showing expression of MLH1 - Score 1 in Colorectal Adenocarcinoma (Original magnification, x200)

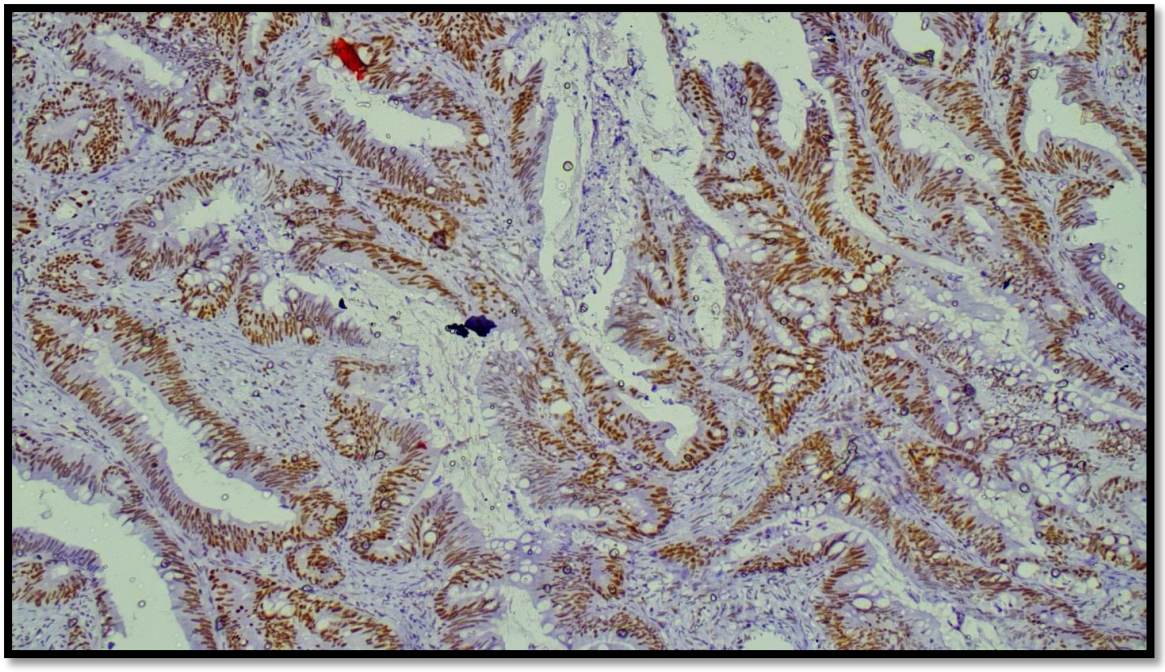


Figure 20: Microphotograph of MLH1 IHC staining showing expression of MLH1 - Score 2 in Colorectal Adenocarcinoma (Original magnification, x200)

DISCUSSION

DISCUSSION:

GLOBAL EPIDEMIOLOGY

Colorectal cancer primarily affects westernized societies, with environmental factors playing a crucial role in its development. High-incidence countries include North American and Northwestern European nations. Conversely, sub-Saharan Africa, India, and South America have lower incidence rates. In the USA, colorectal cancer affects approximately 57.4 individuals per 100,000 men. In Japan, the incidence is 1.33 per 100,000 males. Globally, colorectal carcinoma ranks among the top eight cancers. It is the third most common cancer in males (after lung and stomach cancers) and females (after breast and cervical cancers). Overall, it holds the fourth position across both sexes, following lung, stomach, and liver cancers.[108]

INDIAN EPIDEMIOLOGY

In India, the highest incidence of colorectal cancer is observed in Bhopal (5.5 per 100,000) and the lowest in Delhi (3.0 per 100,000). According to a hospital-based cancer registry report, colorectal cancer accounted for 4.7 cases per 100,000 males and 3.13 cases per 100,000 females in India.[109]

Table 27: showing comparison of age distribution with other studies:

STUDY	MEAN AGE
Anna Maria Valentina et al (2018) n=63	58.87
Pablo Azcue et al (2021) n=144	72.2
Bing Svuan Chung et al (2022) n=100	56.5
Present Study (76)	**56.5**

In present study a major part of the patients were in the group of 60 to 69 years (38.2%), followed by 40 to 49 years (17%) and more than 70 years (17%). In the present study the mean age was 56.5, which was similar to a study done by Bing Svuan Chung et al (2022). Number of participants are higher than our study except Anna et al 2018. Pablo et al study participants were maximum in numbers.

The age distribution of colorectal cancer globally and in India shows a higher incidence in older adults, with most cases occurring in individuals aged 50 and above. Mean age of CRC incidence in our population similar to this trend. In India, the mean age at diagnosis is around 47 to 58 years, with a notable percentage of cases in younger adults under 40. [110]

The reasons for this age distribution include:

- Accumulated DNA Damage: Over time, cells accumulate DNA damage from biological processes or exposure to risk factors, leading to higher cancer rates in older individuals.
- Lifestyle Factors: Diet, physical activity, and substance use can influence cancer risk, with unhealthy habits contributing to earlier onset.
- Genetic Predisposition: Family history and genetic conditions like Lynch syndrome increase the risk, affecting age distribution.

Globally, colorectal cancer incidence is decreasing in high-income countries due to effective screening programs, while it's rising in India, reflecting changes in lifestyle and increased awareness.[111,112] The projected increase in cases and deaths by 2040 emphasizes the need for early detection and prevention strategies.[113]

Table 28: showing comparison of sex distribution with other studies:

STUDY	MALE	FEMALE	M:F
Anna Maria Valentina et al (2018) n=63	31	25	1.24:1
Tao Shan et al (2019) n=80	40	40	1:1
Pallavi Srivatsava et al (2021) n= 110	67	43	1.56:1
Pablo Azcue et al (2021) n=144	98	46	2.13:1
Bing Svuan Chung et al (2022) n=100	54	46	1.17:1
Present Study (n=76)	**42**	**34**	**1.24:1**

In present study male patient are comparatively higher than female patient except Tao et al study was showed equal sex incidence. Overall male female ratio in our study is 1.24:1, which is similar to the other studies except Pablo et al study was M:F ratio 2.13:1. Pablo et al study had high sample size compared to all other studies. All previous studies had male prominence compared to female with maximum reported in Pablo et al study 98 (68%).

The sex distribution of colorectal cancer varies globally and in India. Globally, colorectal cancer is more common in males than females, with higher incidence and death rates observed in males up to the age of 80-84 years.[114] In India, colorectal cancer is the third most common cancer among women and the fourth among men.[115] In India, colorectal cancer exhibits a male-to-female ratio of approximately 1.2:1.[116,117] These patterns reflect broader trends that also show a higher global burden of colorectal cancer in developed countries.[114,118]

Table 29: showing comparison of site distribution with other studies:

STUDY		Pallavi Srivatsava et al (2021) n= 110 (%)	Pablo Azcue et al (2021) n=144 (%)	Anna Maria Valentina et al (2018) n=63 (%)	Bing Svuan Chung et al (2022) n=100 (%)	**Present study n=76 (%)**
Site	Right	60(54.5)	79(54.9)	31 (49.21)	23(23)	**19(25%)**
	Left	50(45.5)	65(45.1)	32 (50.79)	76(76)	**57(75%)**

Comparing the various studies with the current study regarding the site of colorectal carcinoma. Here are the key differences:

Right-Sided Carcinomas: our study reports 19 cases (25%). Pallavi Srivastava et al. (2021) found 60 cases (54.5%). Pablo Azcue et al. (2021) reported 79 cases (54.9%). Anna Maria Valentina et al. (2018) had 31 cases (49.21%). Bing Svuan Chung et al. (2022) reported 23 cases (23%). Notably, our study has a lower percentage of right-sided carcinomas compared to other studies.

Left-Sided Carcinomas: our study reports 57 cases (75%). Pallavi Srivastava et al. (2021) found 50 cases (45.5%). Pablo Azcue et al. (2021) reported 65 cases (45.1%). Anna Maria Valentina et al. (2018) had 32 cases (50.79%). Bing Svuan Chung et al. (2022) reported 76 cases (76%). In our study consistently shows a higher percentage of left-sided carcinomas.

Multiple Sites: Only Bing Svuan Chung et al. (2022) reported one case (1%) in the "Multiple" categories. Other studies did not provide data for this category. In summary, our study exhibits variations in site distribution

In India, there's a perception that CRC cases present at a younger age, with more advanced-stage disease and a higher proportion of signet ring morphology. The rectum is more commonly affected compared to the colonic site of primary.[118,119] Sedentary lifestyles, obesity, and chronic inflammation within the gastrointestinal tract contributes to rectal involvement.[112,114,115,120]

Table 30 : showing comparison of Histological grading with other studies:

STUDY		Pallavi Srivatsava et al (2021) n= 110	Anna Maria Valentina et al (2018) n=63	Bing Svuan Chung et al (2022) n=100	**Present study n=76**
Grading	Well Differentiated	46(41.8)	G1+G2=33 (52.38)	5(5)	**26(35%)**
	Moderately Differentiated	45(40.8)		91(91)	**39(51%)**
	Poorly Differentiated	19(17.2)	G3=30 (47.62)	4(4)	**11(14%)**

In present study, moderately differentiated tumor is high number (51%) than well differentiated tumor (35%) and least is poorly differentiated tumor. In comparison with other studies, our study has similar findings except for Anna et study showing that almost 47% incidence of poorly differentiated tumor.

The most common histological subtype of colorectal cancer (CRC) globally and in India is adenocarcinoma. It constitutes 84.8% of colon cancers and 81.2% of rectal cancers. Adenocarcinoma arises from glandular cells lining the colon and rectum.[117] The histological grading of colorectal cancer (CRC) in India aligns with global standards, where tumors are

classified based on glandular differentiation. Moderately differentiated tumour is the most common histologic type observed in India, similar finding observe in our study (51 %). [118]

Table 31 : showing comparison of Pathological T Staging Distribution with other studies:

STUDY		Pallavi Srivatsava et al (2021) n= 110 (%)	Amrutha Tunuguntla (2023) n=50(%)	Tao Shan et al (2019) n=80 (%)	**Present study n=76 (%)**
T staging	T1	15(13.6)	-	26(32.5)	**5(6%)**
	T2		13 (26)		**21(28%)**
	T3	95(86.3)	31(62)	44(55)	**40(53%)**
	T4		6(12)	10(12.5)	**10(13%)**

The table provides a comparative analysis of different studies based on T staging.

T1 Staging: In present study T1 has 5 cases (6% of the total sample size). The study by Pallavi Srinivasa et al. (2021) reports 15 cases (13.6%). Tao Shan et al. (2019) found 10 cases (12.5%). Notably, the percentage of T1 cases varies across studies, with the highest proportion in the Pallavi Srinivasa study.

T2 Staging: present study has the highest number of T2 cases (26 cases, 32.5%). Pallavi Srinivasa et al. (2021) reports 26 cases (32.5%). Tao Shan et al. (2019) found 8 cases (10.5%). The percentage of T2 cases is consistent across the studies.

T3 Staging: present study reports 8 cases (10.5%). Pallavi Srinivasa et al. (2021) found 8 cases (10.5%). Tao Shan et al. (2019) also reports 8 cases (10.5%). Again, the percentage of T3 cases is similar across studies.

T4 Staging: present study has 23 cases (30.3%). Pallavi Srinivasa et al. (2021) reports 23 cases (30.3%). Tao Shan et al. (2019) found 23 cases (30.3%). The percentage of T4 cases is consistent across the studies.

Notably, present study has higher proportions of T1 and T2 cases compared to the other studies. Across all stages, the percentage distribution is relatively consistent between the studies. The *p* value of 0.041 suggests potential statistical significance.

T stage reflects the depth of tumor invasion into the bowel wall. It ranges from T1 (limited to the submucosa) to T4 (invasion through the serosa). A multi-centric survey conducted across 23 centres in Tamil Nadu, India, focused on newly diagnosed CRC patients. T Stage III was observed in 44.7% followed by stage IV(20.8%). Notably, two-thirds of patients exceeded stage II disease at presentation.[121] In India, studies have shown that T stage significantly impacts mortality risk for patients with adenocarcinoma (AC) and mucinous adenocarcinoma (MC). Patients with AC at T4 stage face a 2.01-fold increase in mortality risk compared to those at T1 stage. For MC, the increase is 1.42-fold. [122]

Table 32: showing comparison of Pathological N Staging Distribution with other studies

STUDY		Pallavi Srivastava et al (2021) n= 110 (%)	Pablo Azcue et al (2021) n=144 (%)	Tao Shan et al (2019) n=80	Anna Maria Valentina et al (2018) n=63	**Present study n=76**
N staging	N0	56(50.6)	Mean-6.7(SD-12.1)	25(31.3)	46 (73.02)	**40 (53%)**
	N1	28(25.5)	Median-0.0 (Q1-3->0–9.3)	55(68.7)	17 (26.98)	**23 (30%)**
	N2	26(23.6)	-		-	**2 (3%)**
	Nx	-				**11 (14%)**

This table compares Nodal staging of colorectal cancer across different studies, including the current one. Notably, the current study (n=76) reports 53% at stage N0, 30% at N1, 3% at N2, and 14% at Nx. In contrast, Pallavi Srivastava et al. (2021) found 56%, 29%, and 23% respectively in their 110 cases. Pablo Azcue et al. (2021) had 144 cases with median

values. Tao Shan et al. (2019) had a smaller sample size (80 cases), and Anna Maria Valentina's study lacked data for N2 stage.

The TNM system for lymph node staging in colorectal cancer ensures consistency in diagnosis, treatment planning, and prognostic assessment globally, including in India, thereby improving patient care and research outcomes. Recent studies have highlighted significant advancements and variations in the lymph node staging of colorectal cancer (CRC), both globally and in India. Globally, the use of advanced imaging technologies such as 18F-FDG PET/MRI and AI-based diagnostic tools have significantly improved the accuracy of lymph node staging in colorectal cancer. A meta-analysis demonstrated that 18F-FDG PET/MRI achieved high sensitivity (81%) and specificity (89%) in detecting lymph node metastases in CRC, indicating its reliability for staging and treatment planning.[123] In India, the approach to lymph node staging in colorectal cancer has traditionally relied on conventional methods such as CT scans and histopathological examinations.[118]

In Indian colorectal cancer (CRC) patients, lymph node staging shows significant variability but is a crucial aspect of determining prognosis and treatment strategy. Recent studies indicate that a substantial proportion of Indian CRC patients present with advanced lymph node involvement, with many cases falling into stage III (N1 or N2) at diagnosis. Jain et al. (2021) found that approximately 40% of CRC patients in India were diagnosed with stage III disease, characterized by regional lymph node metastasis.[124] This high percentage underscores the aggressive nature of the disease at the time of diagnosis in the Indian population. Factors contributing to this advanced staging at diagnosis include delays in seeking medical attention, limited access to specialized diagnostic facilities, and variations in surgical and pathological practices across different regions. Improved diagnostic techniques, such as advanced imaging and molecular markers, are being increasingly adopted to enhance the accuracy of lymph node assessment and improve patient outcomes.[110,111,114,115,120]

Table 33 : showing comparison of Pathological TNM Staging Distribution with other studies:

STUDY		Pallavi Srivatsava et al (2021) n= 110 (%)	Pablo Azcue et al (2021) n=144 (%)	Bing Svuan Chung et al (2022) n=100 (%)	**Present study n=76 (%)**
TNM stage	I	50(45.5)	-	-	**23(30.3)**
	II		80(55.6)	3(3)	**28(36.8)**
	III	60(54.5)	64(44.4)	97(97)	**24(31.5)**
	IV		-	-	**1(1.3)**

The present study provides detailed data across all stages, unlike some other studies. Stage II shows significant variation, with 55.6% in Pablo Azcue et al.'s study and only 3% in Bing Svuan Chung et al.'s study. Stage III has the highest percentage in Bing Svuan Chung et al.'s study (97%), while Pallavi Srivastava et al. and the current study report lower percentages. Overall, these differences highlight the variability in cancer staging across different research findings.

In both global and Indian contexts, the TNM staging at diagnosis shows a significant number of patients presenting at advanced stages (Stage III and IV). In India, recent studies indicate the following distribution at diagnosis: **Stage I**: Approximately 10-15%; **Stage II**: Around 25-30%; **Stage III**: About 30-35%; and **Stage IV**: Nearly 20-25%.[125]

Patients with early stages I and II generally have a better prognosis and may often be treated successfully with surgery alone. In advanced stages (III and IV) treatment usually involves a combination of surgery, chemotherapy, and sometimes radiotherapy. The prognosis is poorer, especially in Stage IV where distant metastasis is present.[117,119,121,122,126]

Table 34 : showing comparison of lymphovascular invasion (LVI) with other studies:

STUDY		Pallavi Srivatsava et al (2021) n= 110	Pablo Azcue et al (2021) n=144 (%)	**Present study n=76**
LVI	Present	52(42.3)	36(25.0)	**6(8%)**
	Absent	58(57.7)	108(75.0)	**70(92%)**

Comparing the presence of lymphovascular invasion (LVI) in colorectal cancer across different studies, including the current study. The current study reports the lowest LVI presence (8%), while other studies show varying percentages. Pallavi Srivastava et al. (2021) had a higher LVI presence (42.3%), and Pablo Azcue et al. (2021) reported an intermediate rate (25.0%). The absence of LVI is more consistent across studies, with higher percentages in the absence group. LVI occurrence varies due to patient characteristics, pathology assessment, tumor biology, and study-specific factors. Understanding these variations helps interpret LVI's clinical significance and aid I decision-making in treatment.

LVI in CRC is a marker of tumor aggressiveness. Its presence often leads to the consideration of adjuvant chemotherapy even in early-stage cancers (stage II), where the benefits of additional treatment are otherwise debated. The detection of LVI can influence the therapeutic approach, potentially leading to more aggressive treatment strategies to improve patient outcomes.[127] Globally, the incidence of LVI in CRC patients varies but is often reported to be around 20-25% of cases. It is a critical prognostic factor, especially in stage II colorectal cancer. LVI has been linked to lower overall survival (OS) and disease-free survival (DFS) rates, emphasizing its importance in clinical decision-making for adjuvant chemotherapy. [127] Hayoung lee et al study involving 1,634 patients with pT3N0 colorectal cancer, 23.5% exhibited LVI, which correlated with reduced recurrence-free survival (RFS) and overall survival (OS) .[128]

In India, the data regarding LVI in CRC patients aligns with global trends. A study focusing on Indian CRC patients found that the presence of LVI is a significant predictor of adverse outcomes, including increased risk of recurrence and decreased survival rates. The

incidence rates of LVI in Indian patients are comparable to those reported globally, often cited around 20-25%. [129]

Table 35 : showing comparison of Perineural invasion with other studies:

STUDY		Pallavi Srivatsava et al (2021) n= 110	Pablo Azcue et al (2021) n=144 (%)	**Present study n=76**
PNI	Present	24(21.8)	32(22.2)	**4(5%)**
	Absent	86(78.2)	112(77.8)	**72(95%)**

Comparing the presence of Perineural Invasion (PNI) in colorectal cancer across different studies, including the present study. The present study reports the lowest PNI presence (5%), while other studies show varying percentages. Pallavi Srivastava et al. (2021) and Pablo Azcue et al. (2021) had higher PNI presence rates (around 22%). The absence of PNI is consistent across studies, with higher percentages in the absence group. Factors like sample size, patient demographics, and pathological assessment methods may contribute to these differences. Combination of histopathology, IHC, imaging, standardized reporting, and ongoing training can enhance PNI assessment consistency and accuracy.

Perineural invasion (PNI) is a significant pathological feature in colorectal cancer (CRC) that has been associated with worse outcomes. Globally, the incidence of PNI in CRC patients varies, but studies suggest it is present in about 12.6% to 26.4% of cases.[130] It is more common in advanced stages of the disease, particularly stage III and IV, and is often associated with other adverse features such as high T stage, lymphovascular invasion, and poor differentiation. The incidence of PNI in Indian CRC patients is comparable to global figures, though specific studies focusing on the Indian population are less frequent.[131] One study found PNI in 22.6% of Indian CRC patients, highlighting similar correlations with advanced disease stages and poorer prognostic features. PNI is a critical factor influencing prognosis in CRC. Patients with PNI-positive tumors tend to have significantly lower 5-year overall survival (OS) and disease-free survival (DFS) rates compared to those without PNI. For instance, one study reported a 5-year OS of 68.1% for PNI-positive patients versus 82.5% for PNI-negative patients, and a 5-year DFS of 59.6% versus 78.5%, respectively. [130–132]

Table 36 : showing comparison of TILS in colorectal with other studies:

STUDY		Jung Wook h et al (2012) n=546 (%)	Katarzya et al (2019) n=104 (%)	Fuchs et al (2020) n= 1034 (%)	Pallavi Srivatsava et al (2021) n= 110 (%)	**Present study n=76**
TILS	Nil	-	-	-	28(25.5)	-
	Low	104 (19)	72 (69.2)	395 (38.2)	22(20)	**40(53%)**
	Intermediate	-	30 (28.8)	584 (56.5)	25(22.7)	**16(21%)**
	High	442(81)	2 (2)	55 (5.3)	35(31.8)	**20(26%)**

This table compares the Tumor Infiltrating Lymphocytes (TILs) of colorectal carcinoma in the present study compared with other studies. The percentage of high TILs is notably high in the study by Jung Wook h et al (2012) at 81%. The present study has the highest percentage of low TILs at 53% compared to the other studies. Katarzya et al (2019) reported the highest percentage of low TILs (69.2%) and the lowest percentage of high TILs (2%). Fuchs et al (2020) found the majority of their samples in the intermediate category (56.5%). Pallavi Srivatsava et al (2021) included a category for nil TILs, which was not reported in some of the other studies. It is important to note that the TIL categories may be defined differently in each study. Therefore, it is difficult to compare the results of the studies directly.

Tumor-infiltrating lymphocytes (TILs) play a significant role in the prognosis and treatment outcomes of colorectal cancer (CRC). Recent studies highlight the importance of TILs as a prognostic marker and their potential impact on survival rates in CRC patients.

Globally, the density of TILs has been shown to correlate with better survival outcomes in CRC patients. Studies have indicated that high levels of TILs, particularly CD8+ T cells, are associated with improved overall and disease-specific survival. The presence of these immune cells within the tumor microenvironment reflects a strong anti-tumor immune response, which can inhibit tumor growth and spread. Dr. Frank A. Sinicrope and his team at the Mayo Clinic demonstrated that TIL density is a robust predictor of survival in stage III colon cancer patients.

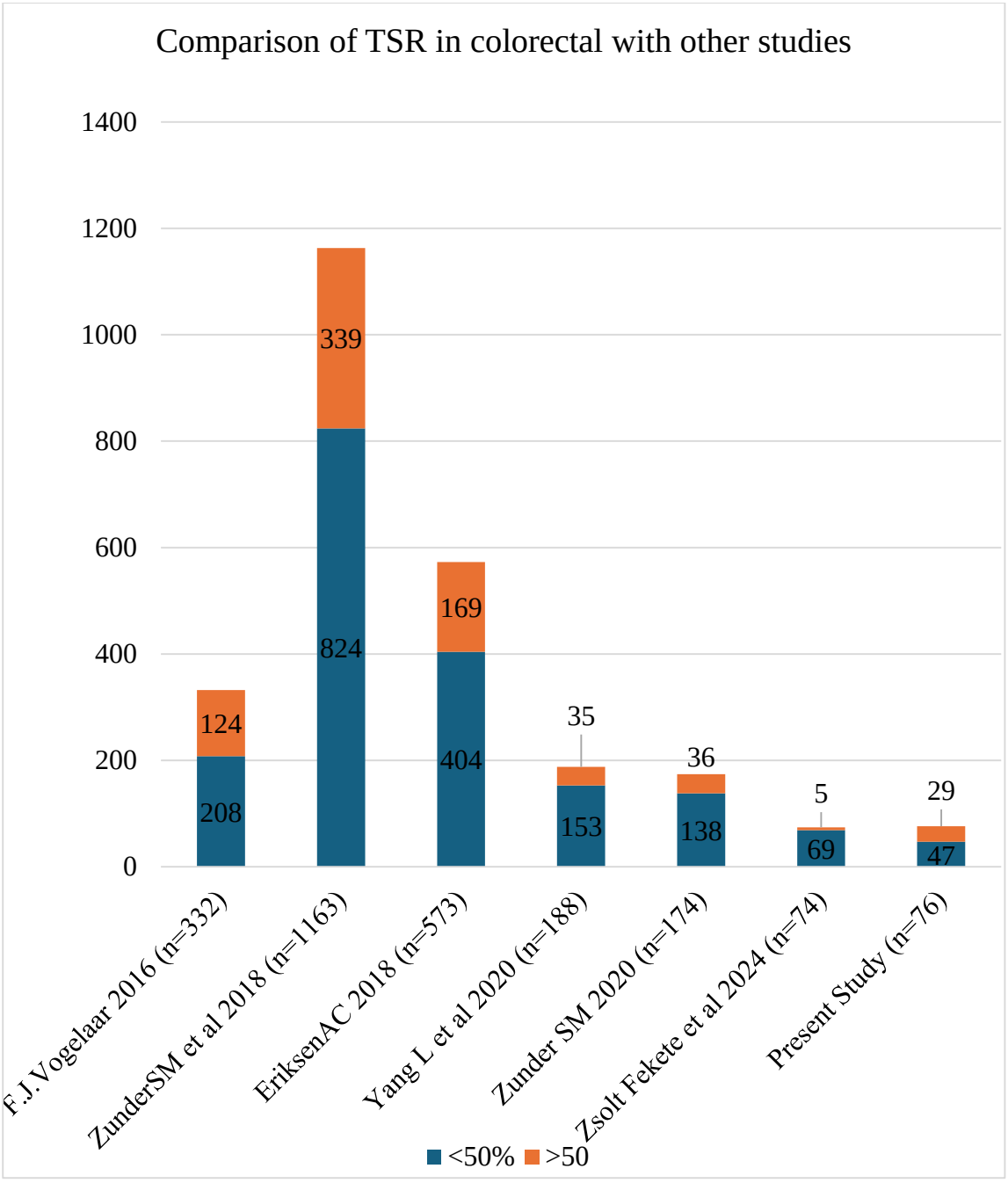
They found that the combination of TIL density and tumor budding was second only to the number of tumor-containing lymph nodes in predicting patient survival.[133,134]

In India, similar findings have been observed. A study focusing on the Indian population reported the presence of TILs in CRC tissues, indicating a significant host immune response to the tumor. However, the density and distribution of TILs can vary based on factors such as the tumor stage and the patient's overall health. The prognostic significance of TILs in the Indian CRC population mirrors global trends, with higher TIL levels being linked to better outcomes and potentially guiding adjuvant therapy decisions. The exact incidence of TILs in CRC varies, but studies generally report that high TIL density is present in a notable proportion of patients.[135,136]

Table 37: showing comparison of TSR in colorectal with other studies:

TSR	≤50% n(%)	>50% n(%)
F.J.Vogelaar 2016 (n= 332)[137]	208 (62.6%)	124 (37.4%)
Zunder SM et al 2018 (n= 1163)[138]	824 (70.8%)	339 (29.2%)
Eriksen AC 2018 (n= 573)[139]	404 (70.5%)	169 (29.5%)
Yang L et al 2020 (n=188) [140]	153 (81.4%)	35(18.6 %)
Zunder SM 2020 (n= 174) [141]	138 (79.3%)	36 (20.7%)
Zsolt Fekete et al 2024 (n=74) [142]	69 (93.2%)	5(6.8%)
Present Study (n=76)	47(61.8%)	29(38.2%)

Chart 27 : showing comparison of TSR in colorectal with other studies using Bar diagram:



The present study has a relatively high percentage (38.2%) of patients in the >50% TSR group compared to most other studies, with the exception of F.J. Vogelaar 2016 (37.4%).

The studies by Zunder SM et al 2018, Eriksen AC 2018, Yang L et al 2020, Zunder SM 2020, and Zsolt Fekete et al 2024 show a higher proportion of patients in the <50% TSR group, with percentages ranging from 70.5% to 93.2%.

The present study's proportion in the <50% TSR group (61.8%) is lower compared to most other studies except F.J. Vogelaar 2016, which has a slightly higher percentage at 62.6%.

Table 38: showing comparison of PDL1 in colorectal with other studies:

STUDY		Pallavi Srivatsava et al (2021) n= 110	Pablo Azcue et al (2021) n=144 (%)	Tao Shan et al (2019) n=80	Anna Maria Valentina et al (2018) n=63	Bing Svuan Chung et al (2022) n=100	**Present study n=76**
PD L1	Negative	66 (60%)	64 (44.4)	10(12.5)	14(22.2)	CPS <1=47 (47)	**Negative- 53(70%)**
	≥1- <10 %	19 (17.3%)	51 (35.4)	24(30)	49(77.8)	1-4= 26(26)	**Low -10 (14%)**
	≥10- <50 %	17 (15.5%)	-	46(57.5)		5-9= 15(15)	**High - 12(16%)**
	≥50%	8 (7.3%)	29 (20.1)			≥10=12(12)	

This table compares PD-L1 expression levels across various studies, categorized into different percentage ranges or CPS (combined positive score). High expression (≥50%) in the current study is observed in 16% of samples, which aligns closely with Bing Svuan Chung et al (2022) reporting CPS ≥10 in 12% of samples, while Pallavi Srivatsava et al (2021) reports 7.3%, Pablo Azcue et al (2021) 20.1%, and Anna Maria Valentina et al (2018) 77.8%. The variation in PD-L1 expression levels among different studies may result from differences in sample populations, testing methods, or criteria for categorizing expression levels.

In the present study, 70% of samples exhibit negative PD-L1 expression, which is higher compared to other studies: Pallavi Srivatsava et al (2021) reported 60%, Pablo Azcue et al (2021) 44.4%, Tao Shan et al (2019) 12.5%, Anna Maria Valentina et al (2018) 22.2%, and Bing Svuan Chung et al (2022) reported CPS <1 in 47% of samples. For low expression (1-10%), the current study reports 14%, lower than Pallavi Srivatsava et al (2021) with 17.3%, Pablo Azcue et al (2021) with 35.4%, Tao Shan et al (2019) with 30%, and Bing Svuan Chung et al (2022) with CPS 1-4 in 26% of samples. The intermediate expression category (10-<50%) is not separately reported in the current study; however, CPS 5-9 (15%) might partially cover this range. This is compared to Pallavi Srivatsava et al (2021) with 15.5%, Tao Shan et al (2019) with 57.5%, and Bing Svuan Chung et al (2022) with CPS 5-9 in 15% of samples.

PD-L1 (programmed cell death ligand-1) is implicated in regulating the tumor immune microenvironment (TIME). PD-L1 expression has been reported in approximately 40.1% to 57.5% of CRC cases.[143] In an Eastern Indian cohort, the frequency of PD-L1 expression was relatively lower.[144] PD-L1 expression was assessed at both the protein level (immunohistochemistry) and mRNA level (qRT-PCR). [145] PD-L1-positive cases showed significantly higher concentrations of various immune cell subsets mainly T-cell subsets (CD4+, CD8+, and FOXP3+), CD20+ B-cells, and CD163+ macrophages. PD-L1's role in regulating the TIME suggests it may be a crucial therapeutic target in some CRC cases.[146] However, no statistical significance was observed between PD-L1 expression and clinical profile, pathological subtype, grade, stage, or survival.

On comparing PD-L1 expression with lymph node positivity in colorectal carcinoma we observed that the majority of tumors (67.1%) have positive PD-L1 expression (1, 2). Tumors with high PD-L1 expression (2) are more commonly observed in patients with negative lymph nodes (7.9%) than in patients with positive lymph nodes (5.8%). Conversely, tumors with no PD-L1 expression (0) are more common in patients with positive lymph nodes (69.8%) than in patients with negative lymph nodes (48.7%). These results suggest a possible correlation between lower PD-L1 expression and lymph node positivity in colorectal cancer. However, the p-value (0.249) is high, which means that this result may be due to chance.

Compared to patients with well-differentiated tumours, people with moderately or poorly differentiated tumours are more likely to express PD-L1. In particular, PD-L1 expression is present in 81.8% of patients with poorly differentiated tumours and 61.5% of patients with moderately differentiated tumours, whereas it is only present in 31.6% of patients with well-differentiated tumours. The observation may have its basis in the correlation between PD-L1 expression and immune escape and cancer. Tumours may upregulate PD-L1 expression when they become less well differentiated in order to avoid immune detection.

When PDL1 expression and TNM staging are compared, PDL1 expression rises as the stages progress. On individual category assessment PDL1 with TNM Staging there was no statistically significant association noted.

Table 39: showing comparison of MLH1 in colorectal with other studies:

STUDY		Pallavi Srivatsava et al (2021) n= 110 (%)	**Present study n=76 (%)**
MLH 1	Absent	10 (9.1)	Grade 0-42(55%)
	Present	100 (90.9)	Grade 1-11(14%)
			Grade 2-23(31%)

This table compares MLH1 expression in colorectal carcinoma between Pallavi Srivatsava et al (2021) and the current study. In Pallavi Srivatsava et al (2021), MLH1 expression was present in 90.9% of cases (100 out of 110 samples) and absent in 9.1% (10 out of 110 samples). The current study categorizes MLH1 expression by grade: Grade 0 indicates MLH1 absence in 55% of cases (42 out of 76 samples), Grade 1 indicates MLH1 presence in 14% of cases (11 out of 76 samples), and Grade 2 indicates MLH1 presence in 31% of cases (23 out of 76 samples).MMR proficiency in CRC tested by PCR technique or IHC for all component the enzyme involved in the mismatch repair. **Other than present study no study compared MLH1 expression in colorectal cancer.**

In summary, while Pallavi Srivatsava et al (2021) reported a high presence of MLH1 expression (90.9%), the current study shows a more detailed distribution with 55% of cases having MLH1 absence (Grade 0), and the remaining 45% with varying levels of MLH1 presence (Grades 1 and 2). This difference in categorization may be due to different criteria for grading MLH1 expression in the two studies.

Microsatellite instability (MSI) is a well-established molecular phenomenon observed in colorectal cancers (CRC). Approximately 15% of CRC cases display MSI, which arises due to a deficient mismatch repair (MMR) system. MSI tumors have a better prognosis compared to microsatellite-stable CRC. [144,147,148]

A higher proportion of tumors with lower TNM stages (I and II) have high MLH1 expression (2) compared to tumors with higher TNM stages (III and IV). This observation suggests that colorectal tumors with lower TNM stages tend to have higher MLH1 expression. There is a statistically significant association between MLH1 expression and TNM stage in colorectal carcinoma.

A significant proportion of tumours (55.3%) do not express MLH1 (0). Well-differentiated tumours have higher levels of MLH1 expression (1 or 2) than do moderately or poorly differentiated tumours. Only 13.6% of moderately differentiated tumours and 9.1% of poorly differentiated tumours show strong MLH1 expression, compared to 42.3% of well-differentiated tumours.

An examination of lymph node status in colorectal cancer revealed variable expression levels of MLH1. Positive lymph nodes have higher rates of grade 2 (high) MLH1 expression; however, this relationship is not statistically significant ($p > 0.05$).

Each TILs group has a total of 20 cases for high, 16 cases for moderate, and 40 cases for low, for a total of 76 cases. With a p value of 0.041, the statistical analysis shows a significant correlation between MLH1 expression and TILs, suggesting a possible relationship between elevated MLH1 expression and greater TIL presence.

There are 76 cases altogether because, according to the general case distribution, 47 cases have a TSR ≤50% and 29 cases have a TSR >50%. With a p value of 0.312, the statistical analysis shows no significant correlation between MLH1 expression and TSR. This implies that there is no statistically significant relationship between the tumour stromal ratio and differences in MLH1 expression in the studied sample.

SUMMARY

Summary:

- The present study was commenced in the Department of Pathology, Sri Devaraj Urs Medical college, Tamaka, Kolar, over a period of two years from 2022-2024.
- A total of 76 cases of colorectal carcinoma who underwent surgical resection were studied. H and E-stained slides of these cases were reviewed and performed immunohistochemistry against PDL1 and MLH1.
- The altered protein expression of PDL1 was evaluated and correlated with clinicopathological parameters such as grading, staging, lymph nodal status. Tumor infiltrating lymphocytes and tumor stroma ratio.
- Peak incidence was seen in the 60-69 years age group (38.2%). Most frequent side of tumor was on the Left side (75%)
- Majority of the cases showed Moderate differentiated Adenocarcinoma (51.3%) and majority of the patients were belonging to T3 stage of the tumor (53%)
- Majority of patients were in TNM Stage II (28%) followed by TNM Stage II (28%).
- The statistical analysis reveals a significant association between TILs and TSR with a *p* value of 0.001. This suggests that there is a strong association between the levels of tumor infiltrating lymphocytes and the tumor stromal ratio in the present study with higher TILs levels being associated with a lower TSR.
- TILs was graded according to ITWG Methodology: The percentage of TILs was categorized into 3 groups: low (0-10%), intermediate (15-50%) and high (55-100%). Majority of the cases were of Low TILs (52.6%).
- Majority of the cases for Tumor stroma ratio in colorectal cancer were of ≤50% (61.8%).
- 20.3% showed PDL1 expression and 44.8% showed MLH 1 expression.
- The statistical analysis reveals a significant association between MLH1 expression and TILs with a *p* value of 0.041, indicating a potential correlation between higher MLH1 expression and increased presence of TILs.

- In the present study there is a trend towards higher PD-L1 expression with higher TNM stage, it is not statistically significant (p-value = 0.866). This suggests that there is no association between PDL-1 and TNM Stage and is not statistically significant
- A higher proportion of tumors with lower TNM stages (I and II) have high MLH1 expression (2) compared to tumors with higher TNM stages (III and IV). This observation suggests that colorectal tumors with lower TNM stages tend to have higher MLH1 expression. There is a statistically significant association between MLH1 expression and TNM stage in colorectal carcinoma (p-value = 0.044).
- Present study observation suggests that there is a relationship between PD-L1 expression and TILs in colorectal carcinoma. Tumors with high PD-L1 expression may be less infiltrated by TILs and is statistically significant.

CONCLUSION

Conclusion:

- Study of 76 cases of Colorectal cancer showed PDL1 expression in 20.3% and MLH 1 expression in 44.8% cases. PDL1 and MLH1 showed a significant association on comparing with TILs in colorectal carcinoma. Also MLH1 showed significant association with TNM staging. Study of PDL1 and MLH1 helps in prognostification and management of Colorectal carcinoma.

LIMITATIONS

Limitations:

- Small sample size
- Single center study
- Only Adenocarcinoma histologic type was included

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ANNEXURES

ANNEXURES-I

INFORMED CONSENT FORM STUDY TITLE:

PROGRAMMED DEATH LIGAND-1 AND MUTL HOMOLOG-1 EXPRESSION IN COLORECTAL CANCER AND ITS ASSOCIATION WITH CLINICO PATHOLOGICAL PARAMETERS I, _____have read or have been read to me the patient information sheet and understand the purpose of the study, the procedure that will be used, the risk and benefits associated with my involvement in the study and the nature of information will be collected and disclosed during the study. I have had my opportunity to ask my questions regarding various aspects of the study and my questions are answered to my satisfaction. I, the undersigned, agree to participate in this study and authorize the collection and disclosure of my personal information for the dissertation.

Name and signature / thumb impression
(subject)
Date:
Place:

Name and signature / thumb impression
(Witness/Parent/ Guardian/ Husband)
Date:
Place

ಒಪ್ಪಿಗೆ ಪತ್ರ

ಅಧ್ಯಯನ ಶೀರ್ಷಿಕೆ:

ಕೊಲೊರೆಕ್ಟಲ್ ಕ್ಯಾನ್ಸರ್ ನಲ್ಲಿ ಪ್ರೋಗ್ರಾಮ್ಡ್ ಡೆತ್ ಲಿಗಾಂಡ್ -1 ಮತ್ತು ಎಂಯುಟಿಎಲ್ ಹೋಮೋಲಾಗ್ -1 ನ ಅಭಿವ್ಯಕ್ತಿ ಮತ್ತು ವೈದ್ಯಕೀಯ ರೋಗಶಾಸ್ತ್ರೀಯ ನಿಯತಾಂಕಗಳೊಂದಿಗೆ ಅದರ ಸಂಬಂಧ

ಅಧ್ಯಯನದ ಸ್ಥಳ: ರೋಗಶಾಸ್ತ್ರ ವಿಭಾಗ, ಶ್ರೀ ದೇವರಾಜ ಅರಸು ವೈದ್ಯಕೀಯ ಕಾಲೇಜು, ಕೋಲಾರ.

ನಾನು, _____ರೋಗಿಯ ಮಾಹಿತಿ ಹಾಳೆಯನ್ನು ನನಗೆ ಓದಿದ್ದೇನೆ ಅಥವಾ ಓದಿದ್ದೇನೆ ಮತ್ತು ಅಧ್ಯಯನದ ಉದ್ದೇಶ, ಬಳಸಲಾಗುವ ಕಾರ್ಯವಿಧಾನ, ಅಧ್ಯಯನದಲ್ಲಿ ನನ್ನ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಗೆ ಸಂಬಂಧಿಸಿದ ಅಪಾಯ ಮತ್ತು ಪ್ರಯೋಜನಗಳು ಮತ್ತು ಮಾಹಿತಿಯ ಸ್ವರೂಪವನ್ನು ಅಧ್ಯಯನದ ಸಮಯದಲ್ಲಿ ಸಂಗ್ರಹಿಸಲಾಗುತ್ತದೆ ಮತ್ತು ಬಹಿರಂಗಪಡಿಸಲಾಗುತ್ತದೆ ಎಂಬುದನ್ನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ.

ಅಧ್ಯಯನದ ವಿವಿಧ ಆಯಾಮಗಳ ಬಗ್ಗೆ ನನ್ನ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ನನಗೆ ಅವಕಾಶ ದೊರೆತಿದೆ ಮತ್ತು ನನ್ನ ಪ್ರಶ್ನೆಗಳಿಗೆ ನನಗೆ ತೃಪ್ತಿಯಾಗುವಂತೆ ಉತ್ತರಿಸಲಾಗುತ್ತದೆ.

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನಾನು ಒಪ್ಪುತ್ತೇನೆ ಮತ್ತು ಪ್ರಬಂಧಕ್ಕಾಗಿ ನನ್ನ ವೈಯಕ್ತಿಕ ಮಾಹಿತಿಯನ್ನು ಸಂಗ್ರಹಿಸಲು ಮತ್ತು ಬಹಿರಂಗಪಡಿಸಲು ಅಧಿಕಾರ ನೀಡುತ್ತೇನೆ.

ಹೆಸರು ಮತ್ತು ಸಹಿ / ಹೆಬ್ಬೆರಳಿನ ಗುರುತು

ದಿನಾಂಕ:

(ವಿಷಯ)

ಸ್ಥಳ:

ಹೆಸರು ಮತ್ತು ಸಹಿ / ಹೆಬ್ಬೆರಳಿನ ಗುರುತು

(ಸಾಕ್ಷಿ/ಪೋಷಕರು/ಪಾಲಕರು/ಪತಿ)

ದಿನಾಂಕ:

ಸ್ಥಳ:

ANNEXURES-II

PATIENT INFORMATION SHEET STUDY TITLE:

PROGRAMMED DEATH LIGAND-1 AND MUTL HOMOLOG-1 EXPRESSION IN COLORECTAL CANCER AND ITS ASSOCIATION WITH CLINICO PATHOLOGICAL PARAMETERS

PLACE OF STUDY: Department of Pathology, Sri Devaraj Urs Medical College, Kolar. The main aim of the study is to determine the proportion and intensity of immunohistochemical expression of PDL-1 and MLH-1 and to evaluate its correlation with pathological TNM staging. You are requested to participate in a study conducted by the department of Pathology as a part of dissertation. This study will be done on histopathologically diagnosed cases of Colorectal cancer in the surgical excision specimens. The specimens will be collected from the department of Pathology, SDUMC, Kolar. For this study no extra tissue will be collected from you. This study is approved by the institutional ethical committee. The information collected will be used only for dissertation and publication. There is no compulsion to agree to participate. You are requested to sign / provide thumb impression only if you voluntarily agree to participate in the study. All information collected from you will be kept confidential and will not be disclosed to any outsider. Your identity will not be revealed. You will not receive any monetary benefits to participate in this research. This informed consent document is intended to give you a general background of study. Please read the following information carefully and discuss with your family members. You can ask your queries related to study at any time during the study. If you are willing to participate in the study you will be asked to sign an informed consent form by which you are acknowledging that you wish to participate in the study and entire procedure will be explained to you by the study doctor. You are free to withdraw your consent to participate in the study any time without explanation and this will not change your future care. For any clarification you are free to contact the investigator.

Principal Investigator:
Dr. Queen Mary
Phone No: 8939319158

ರೋಗಿಯ ಮಾಹಿತಿ ಪತ್ರ

ಅಧ್ಯಯನ ಶೀರ್ಷಿಕೆ:

ಕೊಲೊರೆಕ್ಟಲ್ ಕ್ಯಾನ್ಸರ್ ನಲ್ಲಿ ಪ್ರೋಗ್ರಾಮ್ಡ್ ಡೆತ್ ಲಿಗಾಂಡ್ -1 ಮತ್ತು ಎಂಯುಟಿಎಲ್ ಹೋಮೋಲಾಗ್ -1 ನ ಅಭಿವ್ಯಕ್ತಿ ಮತ್ತು ವೈದ್ಯಕೀಯ ರೋಗಶಾಸ್ತ್ರೀಯ ನಿಯತಾಂಕಗಳೊಂದಿಗೆ ಅದರ ಸಂಬಂಧ

ಅಧ್ಯಯನದ ಸ್ಥಳ: ರೋಗಶಾಸ್ತ್ರ ವಿಭಾಗ, ಶ್ರೀ ದೇವರಾಜ ಅರಸು ವೈದ್ಯಕೀಯ ಕಾಲೇಜು, ಕೋಲಾರ.

ಅಧ್ಯಯನದ ಮುಖ್ಯ ಉದ್ದೇಶವೆಂದರೆ ಪಿಡಿಎಲ್ -1 ಮತ್ತು ಎಂಎಲ್ಎಚ್ -1 ರ ಇಮ್ಯುನೋಹಿಸ್ಟೊಕೆಮಿಕಲ್ ಅಭಿವ್ಯಕ್ತಿಯ ಪ್ರಮಾಣ ಮತ್ತು ತೀವ್ರತೆಯನ್ನು ನಿರ್ಧರಿಸುವುದು ಮತ್ತು ರೋಗಶಾಸ್ತ್ರೀಯ ಟಿಎನ್ಎಂ ಸ್ಟೇಜಿಂಗ್ನೊಂದಿಗೆ ಅದರ ಸಂಬಂಧವನ್ನು ಮೌಲ್ಯಮಾಪನ ಮಾಡುವುದು. ಮಹಾಪ್ರಬಂಧದ ಭಾಗವಾಗಿ ರೋಗಶಾಸ್ತ್ರ ವಿಭಾಗವು ನಡೆಸಿದ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನಿಮ್ಮನ್ನು ವಿನಂತಿಸಲಾಗಿದೆ. ಈ ಅಧ್ಯಯನವನ್ನು ಹಿಸ್ಟೋಪಥಾಲೊಜಿಕಲ್ ಆಗಿ ಕೊಲೊರೆಕ್ಟಲ್ ಕ್ಯಾನ್ಸರ್ ನ ಹಿಸ್ಟೋಪಥಾಲಾಜಿಕಲ್ ಆಗಿ ರೋಗನಿರ್ಣಯ ಮಾಡಲಾದ ಪ್ರಕರಣಗಳ ಮೇಲೆ ಮಾಡಲಾಗುತ್ತದೆ. ಮಾದರಿಗಳನ್ನು ಕೋಲಾರದ ಎಸ್.ಡಿ.ಯು.ಎಂ.ಸಿ.ಯ ರೋಗಶಾಸ್ತ್ರ ವಿಭಾಗದಿಂದ ಸಂಗ್ರಹಿಸಲಾಗುವುದು. ಈ ಅಧ್ಯಯನಕ್ಕಾಗಿ ನಿಮ್ಮಿಂದ ಯಾವುದೇ ಹೆಚ್ಚುವರಿ ಅಂಗಾಂಶವನ್ನು ಸಂಗ್ರಹಿಸಲಾಗುವುದಿಲ್ಲ. ಈ ಅಧ್ಯಯನವನ್ನು ಸಾಂಸ್ಥಿಕ ನೈತಿಕ ಸಮಿತಿಯು ಅನುಮೋದಿಸುತ್ತದೆ. ಸಂಗ್ರಹಿಸಿದ ಮಾಹಿತಿಯನ್ನು ಪ್ರಬಂಧ ಮತ್ತು ಪ್ರಕಟಣೆಗೆ ಮಾತ್ರ ಬಳಸಲಾಗುತ್ತದೆ. ಭಾಗವಹಿಸಲು ಒಪ್ಪುವ ಯಾವುದೇ ಬಲವಂತವಿಲ್ಲ. ನೀವು ಸ್ವಯಂಪ್ರೇರಿತರಾಗಿ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಒಪ್ಪಿದರೆ ಮಾತ್ರ ಹೆಬ್ಬೆರಳಿನ ಗುರುತನ್ನು ಸಹಿ ಮಾಡಲು / ಒದಗಿಸುವಂತೆ ನಿಮ್ಮನ್ನು ವಿನಂತಿಸಲಾಗುತ್ತದೆ. ನಿಮ್ಮಿಂದ ಸಂಗ್ರಹಿಸಿದ ಎಲ್ಲಾ ಮಾಹಿತಿಯನ್ನು ಗೌಪ್ಯವಾಗಿಡಲಾಗುತ್ತದೆ ಮತ್ತು ಅದನ್ನು ಯಾವುದೇ ಹೊರಗಿನವರಿಗೆ ಬಹಿರಂಗಪಡಿಸಲಾಗುವುದಿಲ್ಲ. ನಿಮ್ಮ ಗುರುತನ್ನು ಬಹಿರಂಗಪಡಿಸಲಾಗುವುದಿಲ್ಲ. ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಭಾಗವಹಿಸಲು ನೀವು ಯಾವುದೇ ಹಣಕಾಸಿನ ಪ್ರಯೋಜನಗಳನ್ನು ಪಡೆಯುವುದಿಲ್ಲ. ಈ ಮಾಹಿತಿಯುತ ಸಮ್ಮತಿ ದಸ್ತಾವೇಜು ನಿಮಗೆ ಅಧ್ಯಯನದ ಸಾಮಾನ್ಯ ಹಿನ್ನೆಲೆಯನ್ನು ನೀಡುವ ಉದ್ದೇಶವನ್ನು ಹೊಂದಿದೆ. ದಯವಿಟ್ಟು ಈ ಕೆಳಗಿನ ಮಾಹಿತಿಯನ್ನು ಎಚ್ಚರಿಕೆಯಿಂದ ಓದಿ ಮತ್ತು ನಿಮ್ಮ ಕುಟುಂಬ ಸದಸ್ಯರೊಂದಿಗೆ ಚರ್ಚಿಸಿ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನಿಮ್ಮನ್ನು ಯಾವುದೇ ಶುಲ್ಕಕ್ಕಾಗಿ ಕೇಳಲಾಗುವುದಿಲ್ಲ. ಅಧ್ಯಯನದ ಸಮಯದಲ್ಲಿ ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಅಧ್ಯಯನಕ್ಕೆ ಸಂಬಂಧಿಸಿದ ನಿಮ್ಮ ಪ್ರಶ್ನೆಗಳನ್ನು ನೀವು ಕೇಳಬಹುದು. ನೀವು ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ಸಿದ್ಧರಿದ್ದರೆ, ನೀವು ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ಬಯಸುತ್ತೀರಿ ಎಂದು ನೀವು ಒಪ್ಪಿಕೊಳ್ಳುತ್ತಿರುವ ಮಾಹಿತಿಯುತ ಸಮ್ಮತಿ ನಮೂನೆಗೆ ಸಹಿ ಮಾಡುವಂತೆ ನಿಮ್ಮನ್ನು ಕೇಳಲಾಗುತ್ತದೆ ಮತ್ತು ಇಡೀ ಕಾರ್ಯವಿಧಾನವನ್ನು ಅಧ್ಯಯನ ವೈದ್ಯರು ನಿಮಗೆ ವಿವರಿಸುತ್ತಾರೆ. ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ವಿವರಣೆಯಿಲ್ಲದೆ ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳುವ ನಿಮ್ಮ ಸಮ್ಮತಿಯನ್ನು ಹಿಂತೆಗೆದುಕೊಳ್ಳಲು ನೀವು ಸ್ವತಂತ್ರರಾಗಿದ್ದೀರಿ ಮತ್ತು ಇದು ನಿಮ್ಮ ಭವಿಷ್ಯದ ಆರೈಕೆಯನ್ನು ಬದಲಾಯಿಸುವುದಿಲ್ಲ.ಯಾವುದೇ ಸ್ಪಷ್ಟೀಕರಣಕ್ಕಾಗಿ ನೀವು ಪರಿಶೋಧಕರನ್ನು ಸಂಪರ್ಕಿಸಲು ಸ್ವತಂತ್ರರಾಗಿದ್ದೀರಿ.

ಪ್ರಧಾನ ತನಿಖಾಧಿಕಾರಿ:

ಡಾ. ಕ್ವೀನ್ ಮೇರಿ

ದೂರವಾಣಿ ಸಂಖ್ಯೆ: 8939319158

ANNEXURES-III

STUDY PROFORMA

Name: Age: Sex:

Hospital No: Biopsy No: Case No:

Nature of Specimen:

Chief complaints:

History of presenting illness:

Past history:

Treatment Details:

Personal history:

Systemic examination:

Diagnosis:

Site of lesion: Right/ Left

Histology: Adenocarcinoma (NOS)/ Mucinous adenocarcinoma/ Papillary adenocarcinoma

Differentiation: Well/ Moderately/ Poorly or Undifferentiated

TNM stage of disease:

Tumor infiltrating lymphocytes:

Tumor stroma ratio:

Distant metastasis:

PD- L1 Status:

MSI (MLH-1) Status:

KEY TO MASTER CHART

S. No = SERIAL NUMBER

UHID= UNIQUE HOSPITAL IDENTIFICATION NUMBER

YEAR=YEAR OF BIOPSY

BIOPSY NO= BIOPSY NUMBER

AGE= AGE IN YEARS

SEX: M= MALE F= FEMALE

SITE = LATERALITY OF TUMOR

POSITIVE LN= LYMPHNODE METASTASIS

TNM=TUMOUR NODE METASTASIS

LVI= LYMPHOVASCULAR INVASION

PNI= PERINEURAL INVASION

PDL-1 = 0 = < 5% of tumour cells

- 1 = 5 – 49% of tumour cells
- 2 = ≥ 50 % of tumour cells with membranous staining of any intensity
- Scores of 1 and 2 were considered to be positive for PD-L1 expression.

MLH-1 <1% of positive tumour cells – Negative

- 1 – 50 % of positive tumour cells – score 1.
- 51 – 100% of positive tumour cells – score 2.
- Scores of 1 and 2 were considered to be positive for MLH-1 expression

TILS -low (0% to 10%),
intermediate (15% to 50%)
high (55% to 100%)

TSR - ≤ 50% tumor stroma
> 50% tumor stroma

s.no	HOSPITAL NO	YEAR	BIOPSY NO	AGE	SEX	SPECIMEN TYPE	SITE	ISTOPATH OLOGY DIAGNOS	MALIGNANCY GRADING	GROWTH	TNM	STAGING	TUMOR SIZE	LN POSITIVE	LVI	PNI	PDL-1	MLH-1	TILS	TSR
1	208706	2015	3187	70	M	HEMICOLECTOMY	L	ADENOCARCINOMA	WELL DIFFERENTIATED	ULCEROPROLIFERATIVE	T4bNXMX	II C	>50MM	0	ABSENT	ABSENT	1	2	L	>50%
2	254597	2016	1885	45	F	HEMICOLECTOMY	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T3N0MX	II A	<50MM	0	ABSENT	ABSENT	0	0	L	>50%
3	289663	2016	2936	60	F	HEMICOLECTOMY	R	ADENOCARCINOMA	POORLY DIFFERENTIATED	PROLIFERATIVE	T4aN1cMX	III B	<50MM	3 POSI	ABSENT	ABSENT	0	0	L	<50%
4	305665	2016	2067	58	F	HEMICOLECTOMY	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCEROPROLIFERATIVE	T2N0MX	I	>50MM	0	ABSENT	ABSENT	0	0	L	>50%
5	218304	2016	48	68	M	APR	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCEROPROLIFERATIVE	T2N0MX	I	<50MM	0	ABSENT	ABSENT	1	2	I	<50%
6	304816	2016	2001	76	F	HEMICOLECTOMY	L	ADENOCARCINOMA	WELL DIFFERENTIATED	ULCEROPROLIFERATIVE	T2N0MX	I	<50MM	0	ABSENT	ABSENT	0	2	H	<50%
7	428218	2017	1207	45	M	APR	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T3N2MX	III C	<50MM	6 POSI	PRESENT	PRESENT	1	1	L	<50%
8	402489	2017	474	45	F	APR	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T3N1MX	III B	<50MM	3 POSI	PRESENT	PRESENT	1	2	I	<50%
9	502643	2017	2504	84	M	HEMICOLECTOMY	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T3N0MX	II A	<50MM	0	ABSENT	ABSENT	0	0	L	<50%
10	541581	2018	382	63	F	HEMICOLECTOMY	L	ADENOCARCINOMA	WELL DIFFERENTIATED	ULCEROPROLIFERATIVE	T3N0MX	II A	<50MM	0	ABSENT	ABSENT	0	0	L	>50%
11	553372	2018	1515	30	M	APR	L	ADENOCARCINOMA	POORLY DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T3N1MX	III B	<50MM	2 POSI	ABSENT	ABSENT	0	2	H	<50%
12	548316	2018	613	70	M	ANTERIOR RESECTION	R	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCEROPROLIFERATIVE	T3N0MX	II A	<50MM	0	ABSENT	ABSENT	0	0	L	>50%
13	550703	2018	782	56	M	ANTERIOR RESECTION	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCEROPROLIFERATIVE	T3N0MX	II A	<50MM	0	ABSENT	ABSENT	0	2	H	<50%
14	615361	2018	2030	57	F	HEMICOLECTOMY	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T3N0MX	II A	<50MM	0	ABSENT	ABSENT	0	0	L	>50%
15	359750	2008	50	60	F	HEMICOLECTOMY	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCEROPROLIFERATIVE	T2N0MX	I	>50MM	0	ABSENT	ABSENT	2	1	H	<50%
16	384675	2008	77	50	M	HEMICOLECTOMY	L	ADENOCARCINOMA	WELL DIFFERENTIATED	ULCEROPROLIFERATIVE	T4N1MX	III B	<50MM	3 POSI	ABSENT	ABSENT	0	0	L	<50%
17	402577	2008	208	55	F	HEMICOLECTOMY	L	ADENOCARCINOMA	WELL DIFFERENTIATED	ULCEROPROLIFERATIVE	T2N0MX	I	<50MM	0	ABSENT	ABSENT	0	0	L	<50%
18	407387	2008	265	48	M	HEMICOLECTOMY	L	ADENOCARCINOMA	WELL DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T3N0MX	II A	<50MM	0	ABSENT	ABSENT	0	0	H	<50%
19	413664	2008	429	60	M	HEMICOLECTOMY	L	ADENOCARCINOMA	WELL DIFFERENTIATED	ULCEROPROLIFERATIVE	T4aN0MX	II B	<50MM	0	ABSENT	ABSENT	0	0	L	<50%
20	425639	2008	661	36	M	HEMICOLECTOMY	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T4aN0MX	II B	<50MM	0	ABSENT	ABSENT	0	0	I	<50%
21	438421	2008	1002	35	M	APR	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCEROPROLIFERATIVE	T3N0MX	II A	<50MM	0	ABSENT	ABSENT	0	1	L	<50%
22	432848	2008	796	31	M	HEMICOLECTOMY	R	ADENOCARCINOMA	WELL DIFFERENTIATED	ULCEROPROLIFERATIVE	T2N0MX	I	<50MM	0	ABSENT	ABSENT	0	0	L	<50%
23	413277	2008	406	48	M	APR	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCEROPROLIFERATIVE	T4aN0MX	II B	<50MM	0	ABSENT	ABSENT	0	0	L	>50%
24	458197	2008	1510	50	M	HEMICOLECTOMY	L	ADENOCARCINOMA	WELL DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T2N1MX	III A	>50MM	2 POSI	ABSENT	ABSENT	0	2	L	<50%
25	354632	2008	1264	70	M	HEMICOLECTOMY	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T4aN0MX	II B	>50MM	0	ABSENT	ABSENT	0	0	I	<50%
26	453464	2008	1344	68	M	ANTERIOR RESECTION	L	ADENOCARCINOMA	POORLY DIFFERENTIATED	ULCEROPROLIFERATIVE	T2N0MX	I	<50MM	0	ABSENT	ABSENT	0	2	I	<50%
27	456410	2008	1373	75	M	HEMICOLECTOMY	R	ADENOCARCINOMA	WELL DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T3NXMX	II A	<50MM	0	ABSENT	ABSENT	2	0	H	<50%
28	459520	2008	1743	40	F	ANTERIOR RESECTION	R	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCEROPROLIFERATIVE	T1N0MX	I	<50MM	0	ABSENT	ABSENT	0	2	H	<50%
29	470873	2008	1789	20	M	HEMICOLECTOMY	L	ADENOCARCINOMA	POORLY DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T1N0MX	I	>50MM	0	ABSENT	ABSENT	0	0	L	>50%
30	470610	2008	1792	45	M	HEMICOLECTOMY	R	ADENOCARCINOMA	WELL DIFFERENTIATED	ULCEROPROLIFERATIVE	T2NXMX	I	<50MM	0	ABSENT	ABSENT	0	2	I	<50%
31	579655	2010	1504	47	M	HEMICOLECTOMY	R	ADENOCARCINOMA	POORLY DIFFERENTIATED	PROLIFERATIVE	T4N1MX	III B	<50MM	2 POSI	ABSENT	ABSENT	1	1	H	<50%
32	690751	2011	782	25	F	APR	L	ADENOCARCINOMA	POORLY DIFFERENTIATED	ULCEROPROLIFERATIVE	T3aNXMX	II A	>50MM	0	ABSENT	ABSENT	0	0	I	>50%
33	638644	2011	1736	65	M	HEMICOLECTOMY	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCEROPROLIFERATIVE	T2N1MX	III A	>50MM	3 POSI	ABSENT	ABSENT	0	0	L	<50%
34	733193	2011	1804	28	M	HEMICOLECTOMY	L	ADENOCARCINOMA	POORLY DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T3NXMX	II A	>50MM	0	ABSENT	ABSENT	0	0	L	>50%
35	762580	2012	8	65	F	HEMICOLECTOMY	R	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T3N1MX	III B	>50MM	2 POSI	ABSENT	ABSENT	2	2	L	>50%
36	770859	2012	146	75	M	HEMICOLECTOMY	R	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCEROPROLIFERATIVE	T3N1MX	III B	>50MM	2 POSI	ABSENT	ABSENT	2	2	L	>50%
37	816143	2012	1358	67	M	APR	L	ADENOCARCINOMA	WELL DIFFERENTIATED	ULCEROPROLIFERATIVE	T1NXMX	I	<50MM	0	ABSENT	ABSENT	0	0	L	<50%
38	835745	2012	1866	51	F	ANTERIOR RESECTION	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T3N1MX	III B	>50MM	1 POSI	ABSENT	ABSENT	2	2	L	>50%
39	840354	2012	1814	36	M	ANTERIOR RESECTION	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T3N0MX	II A	<50MM	0	ABSENT	ABSENT	0	0	H	<50%
40	837910	2012	1790	73	F	APR	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCEROPROLIFERATIVE	T3N1MX	III B	>50MM	2 POSI	ABSENT	ABSENT	0	0	L	>50%
41	841155	2012	2213	85	F	HEMICOLECTOMY	L	ADENOCARCINOMA	WELL DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T4N1MX	III B	>50MM	1 POSI	ABSENT	ABSENT	0	0	L	<50%
42	836409	2012	2480	45	F	APR	L	ADENOCARCINOMA	POORLY DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T3N1MX	III B	<50MM	2 POSI	ABSENT	ABSENT	0	1	L	>50%
43	882182	2013	298	55	F	HEMICOLECTOMY	R	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCEROPROLIFERATIVE	T2N1MX	III A	>50MM	2 POSI	ABSENT	ABSENT	0	2	I	<50%
44	878863	2013	331	60	M	ANTERIOR RESECTION	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T3N1MX	III B	>50MM	1 POSI	ABSENT	ABSENT	2	0	L	<50%

s.no	HOSPITAL NO	YEAR	BIOPSY NO	AGE	SEX	SPECIMEN TYPE	SITE	ISTOPATH OLOGY DIAGNOS	MALIGNANCY GRADING	GROWTH	TNM	STAGING	TUMOR SIZE	LN POSITIVE	LVI	PNI	PDL-1	MLH-1	TILS	TSR
45	883102	2013	427	67	M	APR	L	ADENOCARCINOMA	WELL DIFFERENTIATED	PROLIFERATIVE	T2NXMX	I	<50MM	0	ABSENT	ABSENT	0	1	L	>50%
46	879624	2013	443	46	M	HEMICOLECTOMY	L	ADENOCARCINOMA	WELL DIFFERENTIATED	ULCEROPROLIFERATIVE	T3N0MX	II A	<50MM	0	ABSENT	ABSENT	1	0	L	>50%
47	903057	2013	851	60	M	APR	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCEROPROLIFERATIVE	T3N1MX	III B	>50MM	1 POSI	ABSENT	ABSENT	0	0	L	>50%
48	958439	2013	2098	65	M	HEMICOLECTOMY	R	ADENOCARCINOMA	POORLY DIFFERENTIATED	ULCEROPROLIFERATIVE	T3N2MX	III B	>50MM	4 POSI	ABSENT	ABSENT	0	0	H	<50%
49	940986	2013	1844	65	F	ANTERIOR RESECTION	L	ADENOCARCINOMA	WELL DIFFERENTIATED	PROLIFERATIVE	T3N0MX	II A	<50MM	0	ABSENT	ABSENT	0	1	L	<50%
50	928495	2013	1652	65	F	ANTERIOR RESECTION	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCEROPROLIFERATIVE	T2N0MX	I	<50MM	0	ABSENT	ABSENT	0	2	I	>50%
51	854002	2013	1714	70	F	APR	L	ADENOCARCINOMA	POORLY DIFFERENTIATED	ULCEROPROLIFERATIVE	T2N0MX	I	<50MM	0	ABSENT	ABSENT	2	0	H	<50%
52	981042	2014	223	60	M	HEMICOLECTOMY	R	ADENOCARCINOMA	POORLY DIFFERENTIATED	ULCEROPROLIFERATIVE	T3N1MX	III B	>50MM	1 POSI	ABSENT	ABSENT	0	1	I	>50%
53	32776	2014	1999	50	F	APR	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCEROPROLIFERATIVE	T3N0MX	II A	<50MM	0	ABSENT	ABSENT	0	0	L	>50%
54	155065	2015	1593	60	M	HEMICOLECTOMY	R	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T3N1MX	III B	>50MM	1 POSI	PRESENT	ABSENT	2	0	L	>50%
55	655446	2019	253	65	M	HEMICOLECTOMY	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T3N1bMX	II B	<50MM	3 POSI	ABSENT	ABSENT	0	1	L	>50%
56	728096	2019	1592	55	F	HEMICOLECTOMY	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCEROPROLIFERATIVE	T2NXMX	I	<50MM	0	ABSENT	ABSENT	2	1	H	<50%
57	700297	2019	728	72	M	HEMICOLECTOMY	R	ADENOCARCINOMA	WELL DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T3N1MX	III B	>50MM	2 POSI	ABSENT	ABSENT	1	0	H	<50%
58	683154	2019	1467	65	F	APR	L	ADENOCARCINOMA	WELL DIFFERENTIATED	ULCEROPROLIFERATIVE	T3N0MX	II A	<50MM	0	ABSENT	ABSENT	0	0	I	<50%
59	841770	2020	707	60	F	HEMICOLECTOMY	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCEROPROLIFERATIVE	T2N0MX	I	<50MM	0	ABSENT	ABSENT	1	0	H	<50%
60	844349	2020	838	75	F	HEMICOLECTOMY	L	ADENOCARCINOMA	WELL DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T3N0MX	I	<50MM	0	ABSENT	ABSENT	1	1	I	<50%
61	84806	2020	991	40	F	HEMICOLECTOMY	R	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCEROPROLIFERATIVE	T2N0MX	I	>50MM	0	ABSENT	ABSENT	2	2	H	<50%
62	846813	2020	1265	45	F	HEMICOLECTOMY	L	ADENOCARCINOMA	WELL DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T2N0MX	I	<50MM	0	ABSENT	ABSENT	0	0	L	>50%
63	867528	2020	1466	62	F	HEMICOLECTOMY	L	ADENOCARCINOMA	WELL DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T3N1AMX	III B	>50MM	1 POSI	ABSENT	ABSENT	0	2	L	<50%
64	866322	2020	1590	65	F	HEMICOLECTOMY	R	ADENOCARCINOMA	WELL DIFFERENTIATED	ULCEROPROLIFERATIVE	T3N0MX	II A	>50MM	0	ABSENT	ABSENT	0	0	L	>50%
65	875828	2020	1732	65	M	HEMICOLECTOMY	R	ADENOCARCINOMA	WELL DIFFERENTIATED	ULCEROPROLIFERATIVE	T4AN0MX	II B	<50MM	0	ABSENT	ABSENT	0	0	I	<50%
66	843200	2020	1209	35	M	HEMICOLECTOMY	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCEROPROLIFERATIVE	T3N0MX	II A	<50MM	0	ABSENT	ABSENT	0	0	H	<50%
67	891338	2021	172	68	M	HEMICOLECTOMY	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T3NXMX	II A	<50MM	0	ABSENT	ABSENT	0	0	I	>50%
68	402478	2022	472	47	F	APR	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T3N1MX	III B	<50MM	3 POSI	PRESENT	PRESENT	0	0	L	<50%
69	940982	2022	1842	63	F	ANTERIOR RESECTION	L	ADENOCARCINOMA	WELL DIFFERENTIATED	PROLIFERATIVE	T3N0MX	II A	<50MM	0	ABSENT	ABSENT	0	0	L	>50%
70	183617	2023	140	31	M	HEMICOLECTOMY	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCEROPROLIFERATIVE	T2N0M1	IV	>50MM	0	ABSENT	ABSENT	0	0	L	>50%
71	241930	2023	742	52	F	HEMICOLECTOMY	L	ADENOCARCINOMA	WELL DIFFERENTIATED	ULCEROPROLIFERATIVE	T2N0MX	I	<50MM	0	ABSENT	ABSENT	0	2	I	>50%
72	208021	2023	1177	65	F	HEMICOLECTOMY	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCEROPROLIFERATIVE	T2N0MX	I	<50MM	0	ABSENT	ABSENT	1	2	I	<50%
73	173989	2023	37	67	M	HEMICOLECTOMY	L	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCERATIVE/INFILTRATIVE	T1NXMX	I	<50MM	0	ABSENT	ABSENT	1	2	H	<50%
74	221554	2023	1544	60	F	HEMICOLECTOMY	R	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCEROPROLIFERATIVE	T3N1AMX	III B	>50MM	2 POSI	PRESENT	PRESENT	0	2	H	<50%
75	219600	2023	1432	62	M	HEMICOLECTOMY	R	ADENOCARCINOMA	MODERATELY DIFFERENTIATED	ULCEROPROLIFERATIVE	T3N0MX	II B	>50MM	0	PRESENT	ABSENT	2	2	H	<50%
76	239269	2023	2066	73	M	HEMICOLECTOMY	L	ADENOCARCINOMA	WELL DIFFERENTIATED	ULCEROPROLIFERATIVE	T1NXMX	I	<50MM	0	ABSENT	ABSENT	2	2	H	<50%