“ASSOCIATION BETWEEN THE IMMUNOHISTOCHEMISTRY EXPRESSION OF E-CADHERIN, BETA-CATENIN AND CD-44 IN COLORECTAL ADENOCARCINOMA.”

BY

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DISSERTATION SUBMITTED TO

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TAMAKA, KOLAR, KARNATAKA

IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

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IN

PATHOLOGY

UNDER THE GUIDANCE OF

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SRI DEVARAJ URS MEDICAL COLLEGE, KOLAR

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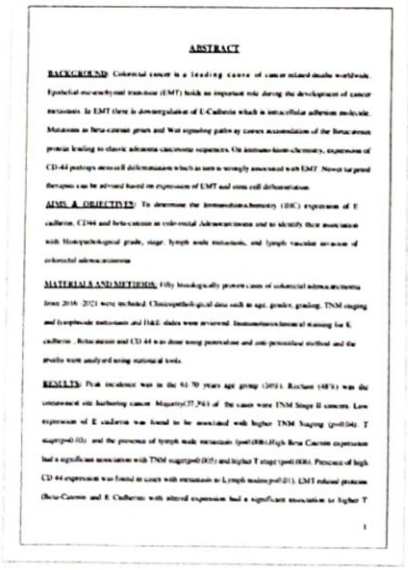

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Date: *Signature of the Candidate*

Place: Kolar ***Dr.T Amrutha***

LIST OF ABBREVATIONS

CRC - Colo Rectal Carcinoma

IHC - Immuno Histo 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 – Microsatillite Instability

CDK8 - Cyclin Dependant Kinase 8

RAS – Rat Sarcoma Virus

COX 2 - Cycloxigenase 2

SMAD - Suppressor of Mothers Against Decapentaplegic

MLH - Multi 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 -Peri Neural Invasion

LVI - Lympho Vascular Invasion

ABSTRACT

BACKGROUND: Colorectal cancer is a leading cause of cancer related deaths worldwide. Epithelial-mesenchymal transition (EMT) holds an important role during the development of cancer metastasis. In EMT there is downregulation of Ecadherin which is intracellular adhesion molecule. Mutations in Beta-catenin genes and Wnt signalling pathway causes accumulation of the Betacatenin protein leading to classic adenoma-carcinoma sequences. On immuno-histo-chemistry, expression of CD-44 portrays stem cell differentiation which in turn is strongly associated with EMT. Newer targeted therapies can be advised based on expression of EMT and stem cell differentiation.

AIMS & OBJECTIVES: To determine the Immunohistochemistry (IHC) expression of E cadherin, beta-catenin, and CD44 in colorectal Adenocarcinoma and To find the association of the IHC expression of E cadherin, beta-catenin, and CD44 with Histopathological grade, stage, lymph node metastasis, and lymphovascular invasion of colorectal adenocarcinoma

MATERIALS AND METHODS: Fifty histologically proven cases of colorectal adenocarcinoma from 2016 -2021 were included in this study. Clinicopathological data such as age, gender,grading, TNM staging and lymphnode metastasis were collected and H&E slides were reviewed. Immunohistochemical staining for E cadherin, Betacatenin ,and CD 44 was done for all cases using peroxidase and anti-peroxidase method and the results were analysed.

RESULTS: Peak incidence was in the 61-70 years age group (36%). Most common site of the tumor was rectum area (48%). Majority of cases were in TNM Stage II (37.3%). Low expression of E cadherin was found to be associated with higher T stage(p=0.03) , TNM Staging (p=0.04) and the presence of lymph node metastasis (p=0.006).High Beta Catenin

expression was noticed to have significant correlation with higher T stage (p=0.006) and TNM staging (p=0.005).High CD 44 expression was found to be associated with Lymph node metastasis (p=0.01).Altered expression of EMT related proteins (E Cadherin and Beta-Catenin) showed significant correlation with higher T stage(p=0.03), TNM staging(p=0.016) and Lymph-node metastasis(0.04)

CONCLUSION: These EMT markers (E Cadherin and Beta-Catenin) and Cancer stem cell marker (CD -44) can be used as prognostic markers for predicting tumour growth and lymph-node metastasis. Hence EMT & Cancer Stem Cell Immunohistochemistry markers are possible biomarkers for aggressive tumor behaviour and lymph-node metastasis.

KEYWORDS: Colorectal carcinoma, Epithelial-Mesenchymal Transition, Cancer Stem Cells , Immunohistochemistry.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

INTRODUCTION

Colorectal carcinoma (CRC) is a serious health problem worldwide. It is responsible for many deaths worldwide.[1.] In India the incidence rates among women are 3.9 per 1,00,000 and among men are 4.4 per 1,00,000 population annually.[2] Colorectal cancer ranks 3rd amongst all cancers afflicting Indian men and women.[1]

Genetic and epigenetic abnormalities lead to a combination of molecular events that lead to colonic adenocarcinoma. The two main genetic pathways are the APC/Beta-catenin pathway and MSI pathway. APC protein binds and promotes the degradation of Beta-catenin, a component of Wnt signaling pathway. This also has a role in promoting Epithelial-Mesenchymal Transition (EMT)

EMT has an important role to play during the development of embryos, cancer metastasis and in the process of fibrosis. Activation of EMT during cancer progression empowers cancer cells to acquire the ability to migrate, become invasive, and gain stem-like features.[3]

One of the major factors involved in the tumor progression is the pro-inflammatory cytokine (TNFα) which also has an important role in EMT. It is triggered by different signalling pathways through regulation of various EMT Transcription factors and microRNA (miRNA).[3]

E Cadherin is a transmembrane protein that links plasma membrane of two cells together. In EMT there is downregulation of E cadherin which is a strong intracellular adhesion molecule and Autocrine Motility factor (AMFR).[1]

Beta-catenin which is a glycoprotein, a central component of adhesion junctions as it has ability of binding to the E cadherin in epithelial cells thereby stabilizing the cytoskeleton

of cells and preventing any abnormal cell growth.[4] Mutations in Beta catenin causes its accumulation finally leading to classic adenoma-carcinoma sequences.[5]

EMT activation has been found to lead to generation of cancer stem cells (CSCs). In transformed epithelial cells, EMT induction culminated by enhancing cells with stem-like traits which in turn are responsible for primary tumor initiation and its accelerated metastasis.[6.] In colorectal carcinoma, the stem cell differentiation is by the expression of CD 44, which is very strongly associated with the EMT and represents target treatment.[7]

Available literature contains only a few studies pertaining to the Indian population studying these markers. This study aims to look for association between the IHC expression of E-cadherin, Beta-catenin and CD44, histopathological grade and stage of colorectal adenocarcinoma. The results of this study could probably help in decision making for targeted therapy of colorectal carcinoma.

AIMS & OBJECTIVES

AIMS AND OBJECTIVES

1. To determine the Immunohistochemistry (IHC) expression of E cadherin, beta-catenin, and CD44 in colorectal Adenocarcinoma.
2. To find the association of the IHC expression of E cadherin, beta-catenin, and CD44 with Histopathological grade, stage, lymph node metastasis, and lympho-vascular invasion of colorectal adenocarcinoma

REVIEW OF LITERATURE

REVIEW OF LITERATURE

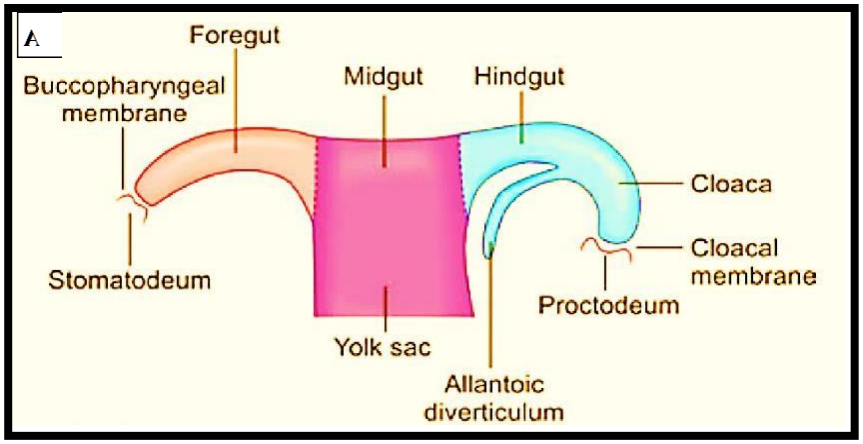
EMBRYOLOGY -LARGE INTESTINE[8]

The epithelium and parenchyma of the digestive system and its derivatives originate from the endoderm. During 5th week of the embryo, the primitive gut is divided further into foregut, midgut, and hindgut.

The embryological midgut develops into the future 3rd and 4th part of duodenum till the junction the of proximal 2/3rd of transverse colon with the distal 3rd.The hindgut forms the distal third of transverse colon till upper part of anal canal.

The midgut opens ventrally into the yolk sac. The embryologic process of rotation of the gastrointestinal tract includes 3 stages- (1) Physiological herniation of the primitive digestive tube. (2) Return of midgut to abdomen (3) Fixation of midgut. The entire length of the midgut is supplied by the superior mesenteric artery.

The major intestinal loop, which contains cranial and caudal limbs, is formed when the midgut develops as a result of the lengthening of the gut and mesentery.This is suspended by the mesentery in the abdominal cavity. Cranial limb grows and forms intestinal loops. The caudal limb forms the lower ileum, caecum, appendix, ascending colon, and proximal two-thirds of transverse colon.



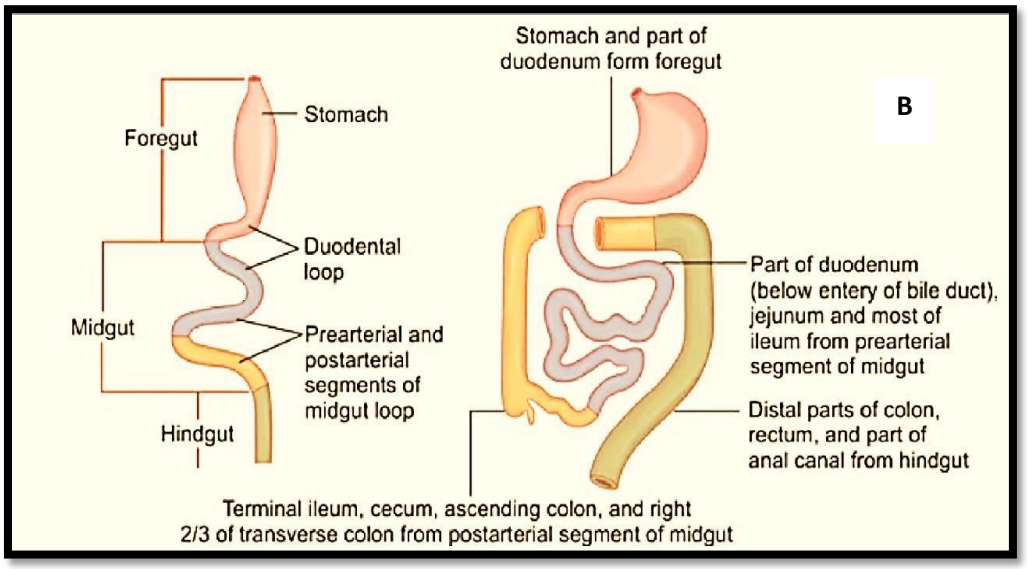


Figure 1A and 1B: Primitive Gut and its Derivatives. (Image from Inderbir singh's Human Embryology 11th edition[8])

Hindgut forms left one-third of transverse colon, descending colon, sigmoid colon, and portion of anal canal. The site of the junction the of transverse colon developed from the midgut and hindgut is assessed by the blood supply. i.e the part of the transverse colon derived from the midgut is supplied by branch of the Superior mesenteric artery whereas the part of the transverse colon derived from the hindgut is supplied by branch of Inferior mesenteric artery with corresponding venous and lymphatic drainage.

As mesentery fuses with the parietal peritoneum descending colon become retroperitoneal. The terminal part of the hindgut forms the cloaca which plays an vital role in the development of the Anal canal and rectum.

ANATOMY:[9]

The large intestine comprises of distal 1-1.5 meters of the digestive tract. It consists of caecum, the transverse colon, the descending colon, the sigmoid colon, and the rectum.

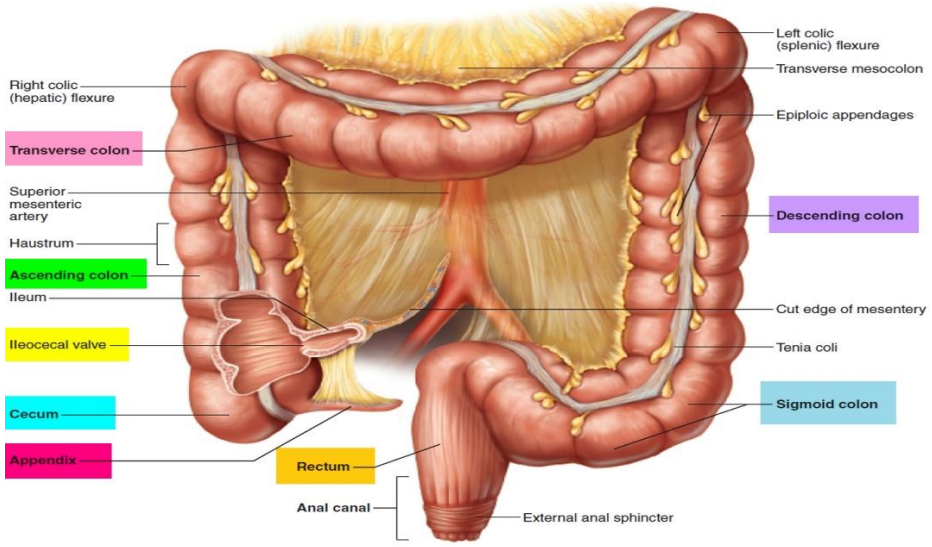


Figure 2 :Parts of Large Intestine (Image downloaded from https://healthjade.com/large-intestine)[10]

Ascending colon which begins in the caecum and ascends along the right side before joining the transverse colon. The transverse colon continues to run across the upper abdomen and links the ascending and descending colons. The descending colon is situated on the left side of the body, between the transverse and sigmoid colons. On the left side, the sigmoid colon links the descending colon with the rectum.

The confluence of the ascending and transverse colons is known as the hepatic flexure. The transverse and descending colons meet at the splenic flexure. Rectum is about 8-15cm and ends at anal canal, it has no peritoneal covering.

GROSS APPEAREANCE:

The large intestine is identified by the external surface examination. The identification points include:

1) The presence of caecum and appendix
2) Taenia coli – which are the 3 longitudinal smooth muscle bands over the surface
3) The contractions in these smooth muscles causes bulges known as Haustrations
4) The accumulation of adipose tissue on the visceral surface is called Epiploid appendages.

HISTOLOGY:[11]

The wall of large intestine has 4 layers- mucosa, submucosa, muscularis externa and serosa.

1) MUCOSA: This is the inner lining which comprises of several layers including innermost epithelium, middle lamina propria (connective tissue) and then the Muscularis mucosa. The innermost layer i.e the epithelium is made up of a single layer of cuboidal to low columnar cells. The crypts of Lieber Kuhn open into grooves or onto the epithelial surface directly.

   Epithelial lining is composed of two types of cells, namely absorptive cells and goblet cells. The function of Goblet cells is to produce, store and subsequently secrete mucin. Crypts lie parallel to each other and are tubular in shape. In addition, crypts also contain Paneth cells, immature precursor cells and endocrine cells.

   Eosinophilic secretory granules, which contain epidermal growth factors, lysozyme and other products are abundantly found in the Paneth cells. These cells are usually confined to the proximal ascending colon and the caecum. The Lamina propria

comprises of a network of collagen fibres, vessels, smooth muscle and nerves along with different cells such as lymphocytes, histiocytes, mast cells and plasma cells.

A thin layer of smooth muscle known as Muscularis mucosae lies between the mucosa and submucosa.

2) SUBMUCOSA: It is made up of loose connective tissue, blood vessels and Meissner's plexus

3) MUSCULARIS PROPRIA: This layer is made up of a longitudinal outer layer and circular inner layer of muscle fibres. Auerbach's plexus or the myenteric plexus of parasympathetic nerves lies within this layer.

4) SEROSA : Flattened to cuboidal mesothelial cells and fibro elastic tissue make up the outermost layer, the serosa. The large intestine absorbs water, vitamins K, B12, thiamine, and riboflavin, and compacts and lubricates faeces with mucus.

The structure of the rectum is similar to colon except for the following.

1) A continuous layer of longitudinal muscle is present, there are no taenia.

2) Peritoneum covers the anterior surface and sides of the upper one third of the rectum and only anterior surface of the middle third. The rest of the rectum is free of a serous covering.

3) There are no appendices epiploicae.

EPIDEMOLOGY OF COLORECTAL CARCINOMA:

CRC is the third most common cancer and stands second in cancer-relatedths in the world. It is estimated 1.9 million incidence cases along with 0.9 million deaths worldwide .[1] . Almost 60% of cases are encountered in developed countries. In India, the annual incidence rates (AARs) for CRC in women is 3.9 per 100000. The AAR for CRC

cancer in men are 4.4 per 100000, respectively. In India, CRC ranks 2nd in terms of incidence and prevalence and 3rd in mortality according to the recent GLOBOCON 2020.

ETIOLOGY:

The etiology of colorectal cancer is very complicated, including the interplay of several elements including Age, Sex, Chronic Inflammation, Lifestyle, Environmental Factors, Genetic Factors, etc.

1. Age: Colorectal cancer affects approximately 90% of people over the age of 50. The incidence rises between the ages of 60 and 79. Further, colorectal carcinoma is among the top 10 most common malignancies in adults 20-49 years old.[12]
2. Gender: There is no sex preference, but males are slightly more likely
3. Diet: Diet has a significant impact on colorectal cancer development. A high-fat diet encourages the growth of bacteria that converts bile salts into possibly cancerous N-nitroso composed of. The development of colorectal cancer is likewise linked to a high red meat intake.[13] Reduced fibre intake, together with a diet poor in fruits and vegetables, can increase the risk of colorectal cancer development.
4. Life-style: Obesity and physical inactivity are two modifiable risk factors associated with colorectal cancer.[14] Smoking and drinking a lot of alcohol have been linked to colorectal cancer developing earlier in life.[15] Aldehyde dehydrogenase 2 phenotypic loss and loss of MTHFR [5,10-Methylene Tetrahydrofolate Reductase] heterozygosity both increase the risk in alcoholics.
5. Chronic inflammation: It's possible that chronic inflammation brought on by inflammatory bowel disease has a role in the development of colon cancer. A higher risk exists for those who have ulcerative colitis. Most colorectal carcinomas arising

from inflammatory bowel illness lack or have a mutation of the ubiquitous zinc finger tumour suppressor KLF 6 (kruppel-like factor 6).[16]

6. Environmental factors: The colon is crucial in breaking down exogenous carcinogens that enter the colon. Therefore, the function of several metabolic enzymes is crucial for the detoxification of these carcinogens. Metabolically activated chemical carcinogens that attach to DNA can cause cancer. The metabolic activation of polycyclic aromatic hydrocarbons, which is linked to an elevated risk of colon cancer, is mediated by cytochrome P450 1A2. The activation also involves other enzymes including arylamine N-acetyl transferase and cytosolic glutathione S-transferases. Rapid acetylators and those with high cytochrome P450 1A2 activity are more likely to acquire colorectal cancer.[17]
7. Genetic factors: Colorectal carcinoma has been linked to a number of hereditary cancer syndromes, including Juvenile polyposis, Hereditary Non-Polyposis Colorectal Cancer, MYH-Adenomatous Polyposis Syndrome, Familial Adenomatous Polyposis, Gardner Syndrome, Turcot Syndrome, Birt Hogg Dube Syndrome, Peutz-Jeghers Syndrome, and Cowden Syndrome.

COLORECTAL CARCINOGENESIS

Absorptive epithelial cells, enteroendocrine cells, and goblet cells are the three differentiated cell types that make up the typical adult colon epithelium. These cells develop from a multipotent stem cell. Stem cells or their early progeny are most likely where the neoplastic transformation begins, deviating from the usual maturation process. In order for colorectal cancer to develop, several genetic alterations must take place in succession. Colorectal tumours grow in stages, beginning with normal epithelium and progressing to increasingly severe types of dysplasia, such as aberrant crypt foci, adenoma, and carcinoma.

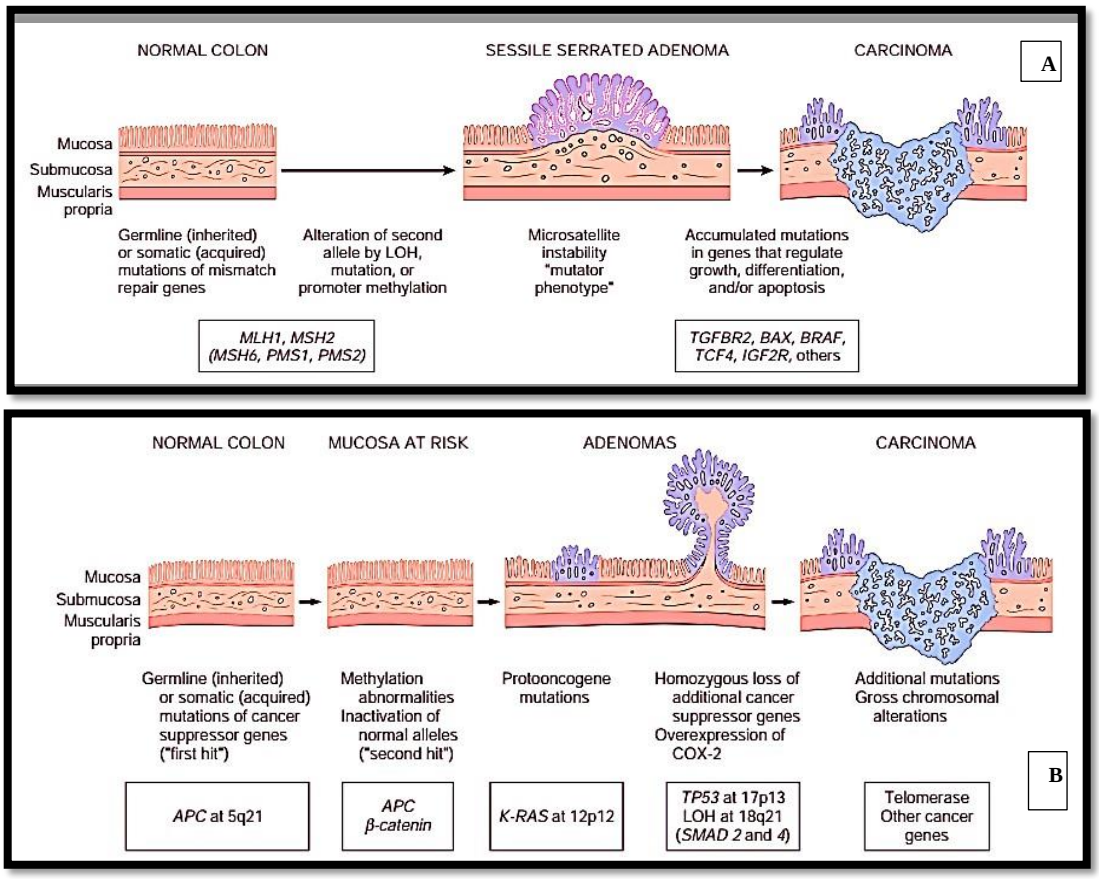


Figure 3A and 3B: Pathogenesis of Colorectal cancer (Images from Robbins and Cotran Pathologic Basis of Disease. 10th ed.)

The majority of colorectal carcinoma cases are spontaneous, and only 5–10% of them are caused by inherited mutations in genes relevant to cancer. [18] Three main molecular pathways can be seen from the processes underlying the onset and progression of CRC.

- "CpG island methylator phenotype (CIMP) pathway
- The Chromosomal instability (CIN) pathway
- Microsatellite Instability (MSI) pathway"

Adenomatous polyps are the primary source of colorectal carcinomas that develop through the CIN route and in HNPCC. Sessile serrated adenomas are the principal pathophysiological antecedents for colorectal carcinomas that grow via the CIMP pathway.[19] Colorectal malignancy has recently been linked to inflammation and microRNAs as potential causes. A

patient's prognosis and the chance of survival depend on a number of genetic and epigenetic modifications.

1. **Chromosomal Instability (CIN):** Chromosomal instability is seen in around 70-85% of colorectal carcinomas, which is most prevalent kind of genomic instability. Aneuploidy or polyploidy, both of which are indicative of chromosomal instability, are defined as the existence of numerous chromosome structural aberrations or numerical chromosome changes.[20] Gene duplications, deletions, and chromosomal rearrangements fall under this category. Several methods including (1) DNA flow cytometry (2) comparative genomic hybridization (3) whole exome sequencing, and (4) high-density SNP arrays, can be used to evaluate these. Colon adenomas have been found to contain chromosomal aberrations, which suggests that the progression from polyp to colon cancer, CIN may occur at an earlier stage.

   The first observable lesion in this route is the dysplastic aberrant crypt focus (ACF), a small mucosal lesion that develops before the formation of a polyp. The CIN pathway includes the KRAS oncogene mutation, loss of 18q, deletion of 17p, which contains the tumour suppressor gene TP53, and loss of 5q, which contains the APC gene. The CIN route to colorectal cancer includes APC as a key tumour suppressor gene. It is the "key" initial mutation responsible for all germline FAP27 mutations as well as spontaneous CIN. Under chromosomal instability, the three most often involved pathways in colorectal carcinogenesis are the WNT Signalling Pathway, the RAS Pathway, and the P53 Pathway."[19]

The WNT Signalling Pathway

Both crypts and villi can be found in the gastrointestinal epithelium. Cell differentiation takes place in the crypts, and as the cells develop, they eventually travel through the crypt walls to

the villi. The crypt-to-villus axis's WNT signalling maintains crypt progenitor compartments and regulates cell fate during differentiation.

The Wnt signalling pathway is reduced thanks to APC's attachment to Beta-catenin. The binding of APC to Beta-catenin is disrupted by mutations in APC, which result in a shortened APC protein. Colorectal cells proliferate, differentiate, migrate, and adhere more readily as a result of the Beta-catenin cytoplasmic build-up, which promotes translocation of beta-catenin into the nucleus and activates the T-cell factor targets.

APC mutations without Beta-Catenin mutations can also found in the initial stages of colorectal pathogenesis. Beta-catenin mutations may even take the place of APC mutations in the starting phases.[21] By promoting both -catenin and Notch 1, the CDK8 gene at 13q12.13 functions as an oncogene in around 60% of instances of colorectal cancer, accelerating transcription and cell differentiation.[22]

Ras Pathway

"Ras oncogene activation by point mutations (usually K-Ras, seldom N-Ras, and never H-Ras) is the underlying cause of nearly 40% of colon cancers. When K-Ras(12p12) is mutated, the GTP-binding protein it encodes loses its natural GTPase activity, causing constitutive signalling to take place through the subsequent RAS-RAF-MEK-ERK pathway. This protein is important in the transmission and propagation of extracellular signals. In K-Ras, activation normally happens when codons 12 or 13's coding characteristics change.[23] Also potentially impacted is codon 61. The G12V mutation of RAS, which changes glycine to valine at codon 12, is linked to an aggressive illness and a significant probability of recurrence. K-Ras mutations result in a persistently active state that gives the cell the ability to avoid apoptosis and gain a growth advantage. FGFR signals are sent by RAS. The FGFR 3 gene activating mutations are linked to colorectal cancer and can result in increased RAS activation."[24]

P53 Pathway

On chromosome 17p, the tumor suppressor gene P53 can be found. Functions of P53 protein are Increased production of cell cycle genes, slowing down the process of cell cycle, and providing enough time for DNA repair About 75% of colorectal carcinomas have a loss of chromosome 17p, which is linked to mis-sense mutations in the remaining p53 allele, whereas benign lesions only sometimes do. This suggests that loss of p53 has role in the late stages of colorectal carcinogenesis. Due to the lack of cell cycle regulation and cell death, p53 mutations induce high levels of proliferative activity.[25] 40% of p53 mutations in CRC occur in five hot areas (codons 175, 248, 282, 273, 245). The most frequent p53 gene mutations in both germline Li-Fraumeni syndrome and colon tumors are C-T transitions at CpG locations. P53 also interacts with COX-2, which is involved in colorectal carcinoma's promotion of inflammation and cell proliferation.[26]

Other pathways involved in chromosomal Instability:

The alteration in the PI3KCA gene, which promotes cell proliferation and the generation of FAS via the AKT pathway, is a mutation that co-occurs with APC gene alterations. mTOR, an important regulator for growth of cell and metabolism. K-Ras also interact with PI3KCA mutations.[27,28]

The deletion of chromosome 18q, which encodes the SMAD 2, DCC and SMAD 4 genes, is a molecular alteration that frequently occurs in conjunction with the loss of p53. Due to the significant potential for metastasis, loss of 18q is strongly related to a poor prognosis for colon cancer.[29]

HIF-1 and HIF-2 complete the CIN pathway. They mediate the biological response to hypoxia and upregulate genes that are involved in angiogenesis, survival of cell and glucose metabolism through mTOR. By attaching to it, a HIF 1 and HIF 2 subunit that is

overexpressed directly increases COX-2 production in colorectal cancer. A poorer survival time for those specifically with colorectal cancer has been linked to this HIF1 upregulation.[30]

CTSB which is a lysosomal cysteine protease, has shown to be overexpressed in every stage of CRC. It has a substantial link with an increase in the chance of dying from CRC.[28,30]

2. Cpg Island Methylator Phenotype (CIMP) Pathway

About 20 to 30 percent of colorectal carcinomas include CIMP. High levels of CpG island hypermethylation in DNA repair genes like p16 and MLH1 are seen in tumors with the CpG island methylator phenotype.

K-Ras and TGF-R-II mutations frequently lead to promoter hypermethylation. The primary flaw in the CpG island methylator phenotype is the lack of TGF- control.

CIMP-positive cancers have two types: I BRAF mutations with MLH1 methylation cause CIMP-high (ii) CIMP-low and KRAS mutations. TP53-mutated, CIMP-negative cancers maintain microsatellite stability. BRAF V600E is not linked to K-RAS, despite its high prevalence.[18]

3. MICROSATELLITE INSTABILITY(MSI) PATHWAY

About fifteen percent of spontaneous CRC and more than 95% of HNPCC syndromes are derived from the microsatellite instability pathway. The genome contains many nucleotides repeat sequences called microsatellites.

"When there are at least 30% of unstable microsatellite loci in a panel of 5–10 loci made up of mono- and di-nucleotide tracts, the term microsatellite instability is used. MSI - low tumours are those with just 10 to 29% of unstable loci.[18] Because DNA polymerase is particularly prone to mistakes while duplicating these little repetitive sequences, MSI is caused by malfunctioning mismatch repair. The seven proteins that make up the MMR

system are MLH1, MLH3, MSH2, MSH3, MSH6, PMS1, and PMS2. These proteins create functional heterodimers when they associate with certain partners. 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."[19]

The inactivation of MMR family genes by abnormal DNA methylation is one of the processes driving MSI. The lack of MMR function caused by abnormal DNA methylation, which silences MLH 1, results in sporadic MSI colorectal carcinomas, on the other hand. The RAS-RAF-MAP kinase pathway is implicated in mediating the cellular response to growth signal in sporadic MSI-high colorectal cancer cases. The BRAF oncogene is known to have the V600E mutation.[18]

Colorectal cancers that originate via MSI pathway are situated in the proximal colon, frequently characterized by a poorly differentiated, mucinous or medullary histology, and are characterized by significant intratumoral and peritumoral lymphocytic infiltrations. In contrast to individuals with colorectal cancer that has chromosomal instability, those with MSI-high CRC had a better outlook and a prolonged survival time.[31]

MICRO RNA (miRNA):

A group of 20–25 nucleotide non-coding RNAs is known as miRNAs. They control the expression of proteins by preventing the mRNA translation that is involved in cell growth, differentiation, proliferation, and death. The number of miRNAs that are implicated in etiology of CRC is continually growing as more and more are being discovered. They function similarly to tumor suppressor genes and oncogenes. It can be up- or down-regulated.[32]

INFLAMMATORY PATHWAY

Prolonged inflammation serves a key role in the beginning and advancement of CRC since there has been a clear correlation between inflammatory bowel disease and chronic NSAID usage and colorectal cancer. Carcinogenesis can occur in chronic inflammation via various mechanisms such as "anti-apoptotic cell growth, increased DNA damage caused by the activation of mutagenic reactive oxygen and nitrogen species, increased production of angiogenic and lymphangiogenic growth factors, and changes to the membrane systems that promote invasion and change cell adhesion".

The pro-inflammatory cytokine TNF - promotes tumor development when levels are persistently raised. In the acute stage of inflammation, the cytokine IL-6 increases STAT 3 transcription. [33]

WHO CLASSIFICATION OF COLORECTAL CARCINOMA[34]

"BENIGN EPITHELIAL TUMOURS AND PRECURSORS:

- Serrated dysplasia, low grade
- Serrated dysplasia, high grade
- Hyperplastic polyp, microvesicular type
- Hyperplastic polyp, goblet cell
- Adenomatous polyp, low-grade dysplasia
- Adenomatous polyp, high-grade dysplasia
- Tubular adenoma, low grade
- Tubular adenoma, high grade
- Villous adenoma, low grade
- Villous adenoma, high grade
- Tubulovillous adenoma, low grade
- Tubulovillous adenoma, high grade
- Advanced adenoma

- **Glandular intraepithelial neoplasia, low grade**
- **Glandular intraepithelial neoplasia, high grade**

MALIGNANT EPITHELIAL TUMOURS:

- **Adenocarcinoma NOS**
  - Serrated adenocarcinoma
  - Adenoma like adenocarcinoma
  - Micropapillary adenocarcinoma
  - Mucinous adenocarcinoma
  - Poorly cohesive carcinoma
  - Signet ring cell carcinoma
  - Medullary adenocarcinoma
  - Adenosquamous carcinoma
  - Carcinoma undifferentiated, NOS
  - Carcinoma with sarcomatoid component

- **Neuroendocrine tumour, NOS**
  - Neuroendocrine tumour, grade 1
  - Neuroendocrine tumour, grade 2
  - Neuroendocrine tumour, grade 3
  - L cell tumour
  - Glucagon- like peptide -producing tumour
  - PP/PYY-producing tumour
  - Enterochromaffin –cell carcinoid
  - Serotonin-producing tumour

- **Neuroendocrine carcinoma NOS**
  - Large cell neuroendocrine carcinoma
  - Small cell neuroendocrine carcinoma

- **Mixed neuroendocrine–non–neuroendocrine neoplasm (MiNEN)"**

TUBULAR ADENOMAS: Adenomatous polyps, sometimes referred to as tubular adenomas, are typically evenly distributed throughout the whole large intestine but less frequently in the rectum. These are often asymptomatic and occasionally lead to changes in bowel habits. They can be sessile or pedunculated and are typically smaller than 1 cm in size. These adenomas are composed of tubular crypts that are closely spaced apart and only include 20% villous tissue. These exhibit cellular crowding and glandular hyperplasia, as well as possible abnormal nuclear characteristics. Carcinoembryonic antigen (CEA) immunostaining demonstrates increased positivity, especially in unusual places.[35]

VILLOUS ADENOMA: These are often seen alone and in older age groups. Rectum and recto sigmoid regions are the most frequent locations, although because of the lesions' very soft nature, even a digital inspection often misses them. More than 80% of the components in these adenomas are villous. They have a broad base from which finger-like villi emerge. Long papillary structures and a crown-like pattern may be visible under a light microscope. Treatment varies based on the size and severity of lesion. A 29%–70% chance to malignant transformation exists.[35]

TUBULO-VILLOUS ADENOMA: These typically consist of a mix of villous and tubular elements, with 20 to 80 percent of the villous element present.[35]

SERRATED ADENOMAS: These are typically sessile and tiny, no larger than 5 mm. These adenomas are called serrated because they exhibit saw-toothed architecture under a microscope. These have a distinctive infolding of the glands into the lumen. Additionally, there could be an increase in mitotic activity. Sessile, conventional, and hyperplastic polyp serrated adenomas are the three types of serrated adenomas.[35]

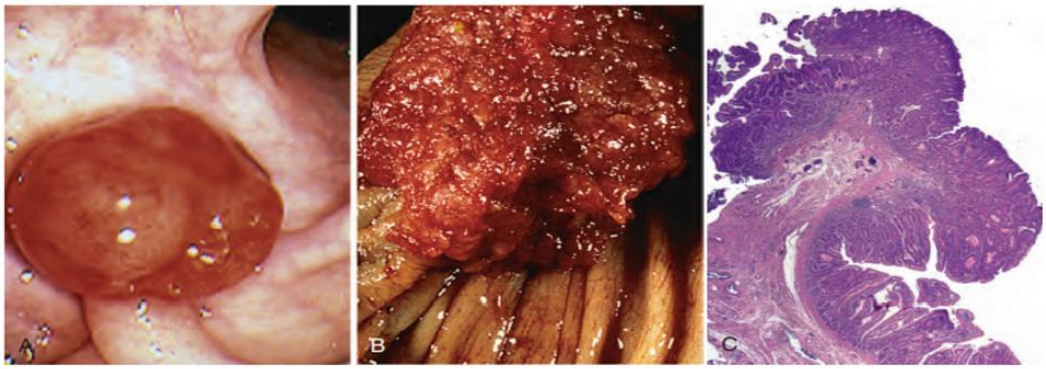


Figure 4: Colonic adenomas. (A) Pedunculated adenoma (endoscopic view). (B) Adenoma with a velvety surface. (C) Low-magnification photomicrograph of a pedunculated tubular adenoma. (Images from Robbins and Cotran Pathologic Basis of Disease. 10th ed.)

ADENOCARCINOMA: The tumor cells must completely penetrate the muscular mucosae into the submucosa in order to be classified as a carcinoma. They often exhibit no symptoms, and the most typical form of presentation is a change in bowel habits, hemochromatosis, or anemia. Early diagnosis could be helped by colonoscopy. Exophytic with intraluminal growth, diffusely infiltrative/linitis plastic type with endophytic development, or with total circumferential involvement are possible growth patterns.[35]

MUCINOUS CARCINOMA: Over 50% of malignant cells have extracellular mucin accumulations. Typically connected to microsatellite instability[35]

SIGNET RING CELL CARCINOMA: More than 50% of the cell makeup should be tumour cells, and the cells' nucleus should be eccentrically positioned with intracellular mucin.[35]

ADENOSQUAMOUS CARCINOMA: Squamous cell carcinoma and adenocarcinoma components should both be present in the entity. There should be more than one component and convincing foci of squamous cell carcinoma.[35]

MEDULLARY CARCINOMA: A solid pattern of cells with a vesicular nucleus, large nucleoli, and eosinophilic cytoplasm are seen in this uncommon tumor, which has a favorable prognosis.[35]

TNM CLASSIFICATION OF COLORECTAL TUMORS[35]:("AJCC Cancer Staging Manual, ed 8, New York, Springer, 2017")

TNM	
Tumor	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ, intramucosal carcinoma
T1	Tumor invades the submucosa
T2	Tumor invades the muscularis propria
T3	Tumor invades through the muscularis propria into pericolorectal tissues
T4	Tumor invades visceral peritoneum or invades or adheres to adjacent organ or structure
T4a	Tumor invades visceral peritoneum
T4b	Tumor directly invades or adheres to adjacent organs or structures
Regional Lymph Nodes	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in one to three regional lymph nodes
N1a	Metastasis in one regional lymph node
N1b	Metastasis in two to three regional lymph nodes
N1c	Tumor deposit(s) in subserosa or in non-peritonealized pericolic or perirectal soft tissue without regional nodal metastasis
N2	Metastasis in four or more regional lymph nodes
N2a	Metastasis in four to six regional lymph nodes
N2b	Metastasis in seven or more regional lymph nodes
Distant Metastasis	
M0	No distant metastasis
M1	Distant metastasis to one or more distant sites or organs or peritoneal metastasis
M1a	Metastasis to one site or organ without peritoneal metastasis
M1b	Metastases to two or more sites or organs without peritoneal metastasis
M1c	Metastasis to the peritoneal surface, alone or with other site or organ metastases

Figure 5: TNM CLASSIFICATION OF COLORECTAL TUMORS (Image used from Amin MB, Edge SB, Greene FL, et al, editors: AJCC Cancer Staging Manual, ed 8, New York, Springer, 2017.)

TNM STAGING:

Stage	T	N	M
0	Tis	N0	M0
I	T1–T2	N0	M0
IIA	T3	N0	M0
IIB	T4a	N0	M0
IIC	T4b	N0	M0
IIIA	T1–T2 T1	N1/N1c N2a	M0 M0
IIIB	T3–T4a T2–T3 T1–T2	N1/N1c N2a N2b	M0 M0 M0
IIIC	T4a T3–T4a T4b	N2a N2b N1/N2	M0 M0 M0
IVA	Any T	Any N	M1a
IVB	Any T	Any N	M1b
IVC	Any T	Any N	M1c

Figure 6: TNM STAGING OF COLORECTAL TUMORS (Image used from Amin MB, Edge SB, Greene FL, et al, editors: AJCC Cancer Staging Manual, ed 8, New York, Springer, 2017.)

PROGNOSTIC FACTORS OF COLORECTAL TUMORS:[34,35]

- The prognosis is bad for those at the extremes of age (young and old).
- Gender: Males often have worse prognoses than females.
- CEA levels: more than 5 ng/dl are linked to a bad prognosis.
- Tumor budding: A bad prognosis is associated with presence of the lone cancer cells or a cluster of greater than five cells at invasive front.
- Vascular invasion: A bad prognosis is linked to tumor cells invading the vascular wall.

- Perforation: Large tumors that cause perforation are more advanced and have a poor prognosis.
- Involvement of lymph nodes is linked to a poor prognosis.
- Higher stages are linked to a bad prognosis.
- Locations: Studies have shown that cancers on the left side of the colon had a good prognosis.
- Inflammation: A better prognosis is linked to tumors with extensive inflammation in the tissue-tumor interphase.
- Grade: Grade I cancers have a higher survival rate than grade III tumors due to their well-differentiated nature.

"EPITHELIAL-MESENCHYMAL TRANSITION (EMT):

In fibrosis, cancer metastasis, and embryonic development, epithelial-mesenchymal transition (EMT) plays a crucial role. Cancer cells can become more migratory, invasive, and stem-like when EMT is activated throughout the development of the disease. The vital connection between EMT and cancer stemness is supported by an increasing amount of research. Contradictory findings, on the other hand, have shown that blocking EMT also enhances cancer stemness and that mesenchymal-epithelial transition, the reversal of EMT, is linked to the tumor-initiating capacity necessary for metastatic colonization. A possible explanation for these contradicting findings is the idea of "intermediate-state EMT." Additionally, recent research has shown that the emergence of "hybrid" epithelial-mesenchymal cells is advantageous for the development of metastasis".[36]

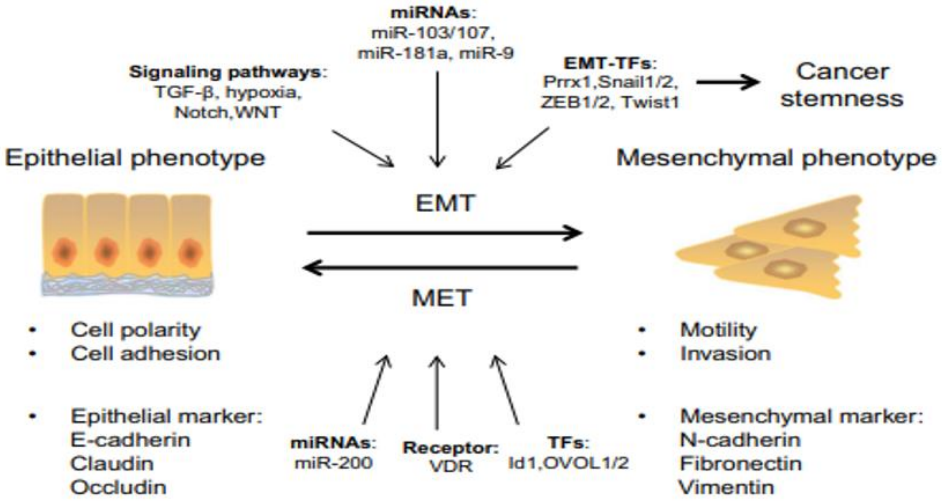


Figure 7: Dynamic Change Between The Epithelial And Mesenchymal Phenotype In Cancer Cells During Metastasis.[36]

Cancer cells change from having an epithelial phenotype to having a mesenchymal phenotype as a result of EMT triggering events, like the activation of signalling pathways of TGF-b, hypoxia, Notch, Wnt) or the expression of EMT-TFs and miRNAs. An EMT program's activation leads to the acquisition of migratory and invasion skills that aid in the spread of malignancy. EMT-TFs also encourage cancer cells to develop stem cell characteristics. Cancer cells of the mesenchymal type undergo a transformation into epithelial cells by MET, which is essential for cancer colonization when they reach metastatic locations. Some transcriptional factors, such as Id1 and OVOL1/2, miRNAs, such as miR-200, and receptors are among the effectors of MET (VDR).[36]

The majority of experimental models look at morphological alterations and markers of epithelial and mesenchymal tissue (E-cadherin, N cadherin, Beta-catenin, and vimentin) to determine whether or not EMT has occurred.[36]

E CADHERIN:

E-cadherin is an adhesion molecule and one of the potential protein biomarkers for the prediction of tumor development. One of the factors underpinning cancer invasion and

progression is the lack of cell adhesion. The type-I or "classical" cadherin subfamily includes E cadherin. This subfamily has a total of 16 molecules, each weighing around 120 kDa. It is a transmembrane glycoprotein that is calcium-dependent and confined to adherens junctions on the basolateral surface of epithelial cells. It participates in cell-cell interactions, including those that occur during cancer.[37]

E-cadherin has three domains: cytoplasmic, single-pass transmembrane, and extracellular with five cadherin-motif tandem repeat subdomains with likely calcium binding sites. Beta-catenin and plakoglobin (also known as ɣ-catenin) attach to cytoskeleton actin filaments through the cytoplasmic domain."[38]

According to the study by Van Roya F et al. Tumor progression, loss of differentiation, invasion, and metastasis are some of the features of cancer that are associated to E-cadherin downregulation. Through processes including mutations, epigenetic silencing, enhanced endocytosis, and proteolysis, E-cadherin may be rendered inactive in cancer.[38]

The study by Gomma et al. explained that while intercellular communication is compromised and cell-cell interactions are reduced as a result of E-cadherin loss, there is no direct transition of the tumor into another kind of cell. Cell-cell interaction, which is linked to membrane localization, affects the E-cadherin subcellular distribution. This study found a correlation between loss of immunostaining in primary CRC and the occurrence of disease recurrence, as well as that immunostaining loss was a separate predictor of disease relapse.[39]

It was observed that E cadherin showed decreased expression with the increase of grade, local invasiveness, metastasis, and relapsed tumor after therapy.[7] According to Briede I et all study results the overall E cadherin score was 1.86 with a p-value of 0.001 in colorectal adenocarcinoma. It showed statistically significant differences by grade.

A study by Nikzami et al. proved that associated with the development of cancer As metastasis and invasion increase, mesenchymal marker expression rises, which might be a useful indicator of the progression of clone cancer since it changes inversely with E-cadherin expression.[40]

BETA-CATENIN

Depending on its cellular localization, "Beta-catenin has a dual role in the growth of tumors and is an important part in maintaining the integrity of the epithelium. In order to sustain cell-cell adhesion, membrane-catenin interacts with the intracellular domain of E-cadherin. Thus, the expansion and mobility of tumor cells are constrained. When E-cadherin is lost, there is an increase in cell motility because there is less cell adhesion. This leads to the release of Beta-Catenin into the cytoplasm, where it accumulates and is transported into the cell nucleus, where it activates the downstream target genes that lead to abnormal cell proliferation, migration, invasion, and metastasis.[41]

In addition, Beta-catenin is a key player in the canonical Wnt signaling pathway in both the cytoplasm and the nucleus. Adenomatous polyposis coli (APC), casein kinase 1 (CK1), and glycogen synthase kinase 3 (GSK3) are members of a protein complex that phosphorylates cytoplasmic ß -catenin in the absence of Wnt signals.[42]

Numerous publications in the literature show that immunohistochemical analysis might be utilised to identify the expression of Beta-catenin as a marker for disease progression and a poor prognosis in CRC. Overexpression of nuclear Beta-Catenin has been linked in recent studies to distant metastasis, lymph node metastases, and unfavorable outcomes.[41]

Beta-catenin exhibits membrane positivity in both normal mucosa and tumor cells, as well as an increase in cytoplasm positivity in tumor cells as compared to normal mucosa, and nuclear

switch in the invasion front in approximately 80% of instances. Therefore, nuclear positivity should be taken into account as an EMT indication."[6]

Beta-catenin demonstrated statistically significant changes in subcellular localization and an increase in beta-catenin score across benign and malignant neoplasms in the study by Bhattacharya I et al. (p=0.001).[5]

CD 44

A common potential cancer stem cell (CSCs) marker for colorectal cancer is CD44. "Hyaluronic acid (HA) has been found to stimulate a variety of biological characteristics in tumors, including proliferation, differentiation, invasion, and motility. An essential membrane receptor for HA, CD44, has been identified.[43]

According to previous studies, CD44 stimulates the MAPK, PI3K/Akt, and Wnt signaling pathways among other signaling pathways. Tumor growth, migration, EMT, chemoresistance, and apoptotic resistance are all associated with the activation of these pathways. Additionally, it has been demonstrated that CD44 promotes tumor development and metastasis by bringing MMP-9 activity to the cell surface."[44]

Several studies have shown that the prognosis and clinicopathological characteristics of various cancers, including CRC, were related to the overexpression of CD44 and its variants.[45]

According to the study by Wang Z et al, there was a strong correlation between high CD44 expression & low overall survival (OS). Poor differentiation, lymph node metastases, and far-reaching metastasis were also linked to high CD44 expression.[46]

Activation of EMT is also linked to the development of cancer stem cells. Few studies have shown a connection between EMT, stemness, and the capacity of tumour cells to metastasise.

The colorectal stem cell marker CD44 is closely related to EMT. Additionally statistically significant (p=0.026) was the CD44 expression. No relationship existed between the tumor grade, tumor stage, or invasive growth symptoms and CD44 expression.[46]

Prognostic indications allow for the matching of companion diagnostics to certain therapies and the customization of treatment intensity based on the likelihood of tumor progression or recurrence. Numerous studies have suggested that certain EMT indicators (such as "decreased E cadherin expression and increased vimentin expression") may be used to predict the treatment and survival results of colorectal cancer patients.[47]

According to a study by Choi JE et al., an Invasive colorectal cancer multi-marker study of "Altered expression of E-cadherin, Beta-catenin, vimentin, Snail, and CD133 revealed a higher connection with disease-free survival (66.2 months vs 84.6 months) and overall survival (60.8 months vs 77.9 months) than individual protein analysis."[47]

Similar to this, adding EMT markers such as E-cadherin, Beta -catenin, Snail enhances predictive performance, according to longitudinal follow-up research.[48] Three general treatment approaches might be used for medications meant to suppress EMT. To overcome pharmacological resistance in advanced illness, combine traditional anticancer medications first.

Second, it's possible that medications that block EMT could be used as adjuvant therapy to lessen recurrence after resection of metastatic disease, where the event rate is higher than after resection of primary tumors and where postoperative chemotherapy is not generally accepted to play a role. EMT inhibitors may be created as chemopreventive medicines, which is the third, but most difficult, therapeutic approach, if medications with the right safety profile were found.[49]

MATERIALS AND METHODS

MATERIALS AND METHODS

STUDY DESIGN: Laboratory Based Observational Study

SOURCE OF DATA: Surgical resected specimens of colorectal carcinoma received from R.L.Jalappa Hospital and Research Center in the Department of Pathology attached to Sri Devaraj Urs Medical College, Tamaka, Kolar from 2019 to 2021 and also retrieved the data along with paraffin blocks of all cases of Colorectal carcinoma from the Archives of Department of Pathology from the period of 2012 to 2019.

DURATION OF STUDY: Two years

METHOD OF COLLECTION OF DATA: All the cases of Colorectal carcinoma from 2012 to 2021 along with clinical details were collected from the archives of the Departmentof Pathology.

INCLUSION CRITERIA:

All patients who were diagnosed with colorectal adenocarcinoma and have been surgically operated for the same at R L Jalappa Hospital & Research Centre between 2012-2021.

EXCLUSION CRITERIA:

1. Metastatic tumor from other sites.
2. Recurrent lesion.
3. Patients subjected to chemotherapy and Radiotherapy.

SAMPLE SIZE

The sample size was estimated based on the statistically positive correlation observed between Beta-catenin and cytoplasmic, nuclear scores with staging in a study of "Assessment

of beta-catenin expression by immunohistochemistry (IHC) in colorectal neoplasms and its role as an additional prognostic marker in colorectal adenocarcinoma." [5]

Considering an α error of 5% with 80% power. **Sample size is 50.**

Calculation : $\frac{Z_{\alpha}^2 (p)(1-p)}{d^2}$

"Here , Z = Standard normal variant(1.96)

p = Expected proportion in population-based on previous study(80%)

d = Absolute error of 5%"

METHODOLOGY:

All the clinicopathological data of colorectal carcinoma cases such as age, sex, histological grading, lymph node status, and staging were collected. The resected specimens of all colorectal carcinoma which were confirmed histopathologically were included in the study. H & E Slides of all cases were reviewed, then selected the tumor tissue and performed immunohistochemistry against E Cadherin, Beta Catenin, and CD44 (Rabbit monoclonal antibody, prediluted, PathnSitu) for all cases of colorectal carcinoma by following the peroxidase and antiperoxidase method . For all cases positive and negative controls were performed.

IMMUNOHISTOCHEMISTRY STAINING:

Protocol:

IHC staining was performed on 10% Neutral formalin-fixed sections which are cut at 3-4 µm, floated on positively charged slides, incubated at 37degrees 1 day, and further at 58 degrees overnight. Deparaffinization using xylene-I And Xylene –II using both for about 15

minutes each. Rehydration was done using Absolute alcohol-I and Absolute Alcohol –II each for 5 minutes. These slides then taken for antigen retrieval under high steam pressure, followed by a wash in distilled water after allowing them to cool for 10 mins.

Following this blocking of endogenous peroxidase activity with Peroxidase blocking reagent for about 20 mins and incubated at ambient temperature. the slides are rinsed with Tri buffered saline (TBS) 3-5 times and then incubated with E cadherin(EP6), Beta-catenin (EP35), and CD 44(SP37) Primary prediluted rabbit monoclonal antibody (PathnSitu) for 1 hour at room temperate. The slides were then rinsed with TBS for five times and incubated with Secondary reagent 2: a conjugated goat anti-mouse polymer horseradish peroxidase(HRP) secondary antibody for 30 mins at room temperature. DAB was applied for 5 mins. For counterstaining the slides were then rinsed with deionized water, incubated for 2-5 mins with Hematoxylin, and rinse with TBS Buffer for 1 minute. Mounting was done with DPX.

IHC METHODOLOGY FOR E CADHERIN AND ß CATENIN:[50,51]

"The staining of E cadherin and Beta catenin was scored according to the proportion and intensity categories proposed by Allred et al. The staining intensity was estimated in a 4-step scale :

0- no staining

1- weak intensity

2- moderate intensity

3- strong intensity.

The fraction of the stained cells was scored according to the following criteria:

Score 0- <10% positive cancer cells

Score1- 11-33% positive cancer cells

Score2- 33-66% positive cells

Score3- >67% positive cancer cells

The final staining score was assigned based on the multiplication of the staining intensity and the percentage of the positive cells and graded as follows:

0 :0; 1: 1-3; 2: 4-6; 3: 7-9. Low expression – 0 or 1; High expression – 2 or 3."[50,51]

IHC METHODOLOGY FOR CD44:[52]

"The staining of CD44 was scored according to the proportion and intensity categories proposed by Fang Y J et al The staining intensity was estimated in a 4-step scale :

0- no staining

1- weak intensity

2- moderate intensity

3- strong intensity.

The fraction of the stained cells was scored according to the following criteria:

Score 0- <10% positive cancer cells

Score1- 11-50% positive cancer cells

Score2- 51-80% positive cells

Score3- >80% positive cancer cells

The final staining score was assigned based on the multiplication of the staining intensity and the percentage of the positive cells and graded as follows:

0 :0; 1: 1-3; 2: 4-6; 3: 7-9. Low expression – 0 or 1; High expression – 2 or 3"

Epithelial-Mesenchymal Transition (EMT) : All the cases showing altered protein expression of EMT markers were regarded positive for Epithelial-Mesenchymal transition .[53] In the current study low expression of E cadherin and high expression of Beta-catenin, together were taken as positive for Epithelial-Mesenchymal Transition .

STATISTICAL ANALYSIS:

The Data - entry was done using MS Excel and statistically analysed using Statistical package for social sciences (SPSS Version 22). Categorical variables were summarized with n%. All results were presented in tabular form and are shown graphically using bar diagram or pie diagram as appropriate. The groups were tested for statistical significance using "Chi square test. p-value less than 0.05 considered to be statistically significant".

OBSERVATION AND RESULTS

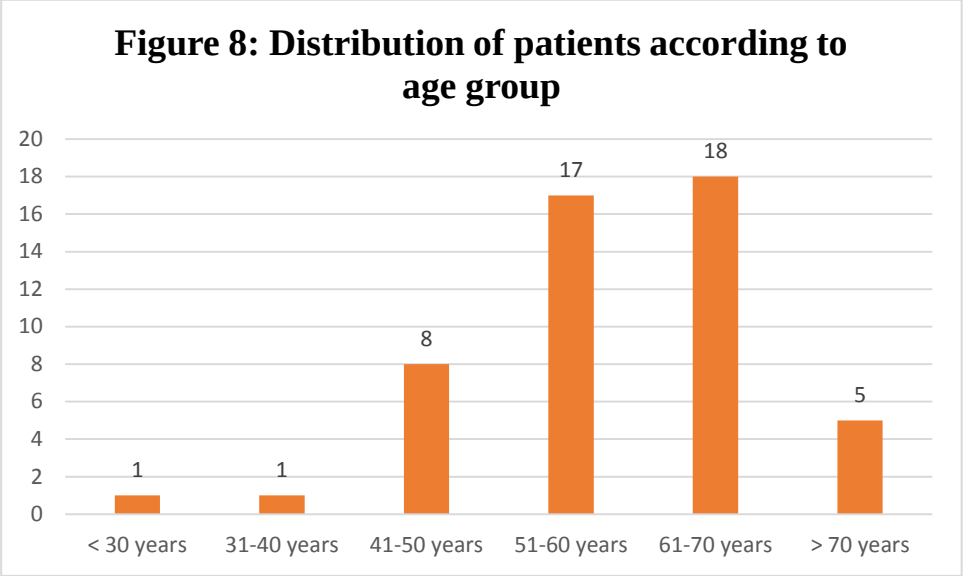
RESULTS

Total number of patients taken in this study are 50.

Age Distribution:

Table 1. Distribution of patients according to age group.

Age Group	Frequency (%)
≤ 30 years	1 (2)
31-40 years	1 (2)
41-50 years	8(16)
51-60 years	17(34)
61-70 years	18(36)
> 70 years	5 (10)



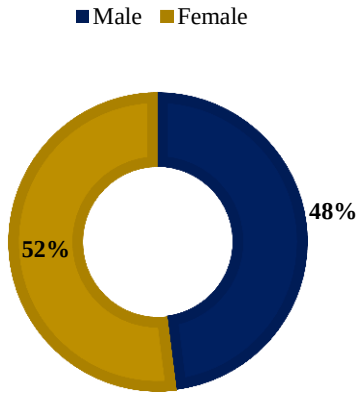
In the current study, the majority of patients were in age group of 61-70 years (36%), followed by 51-60 years (34%), 41-50 years (16%), >70 years (10%) and the least in ≤ 30 years and 31-40 years (2%).

Gender:

Table 2. Distribution of patients according to Gender.

Sex	Frequency (%)
Male	24 (48)
Female	26 (52)

Figure 9: Distribution of Patients according to Gender.

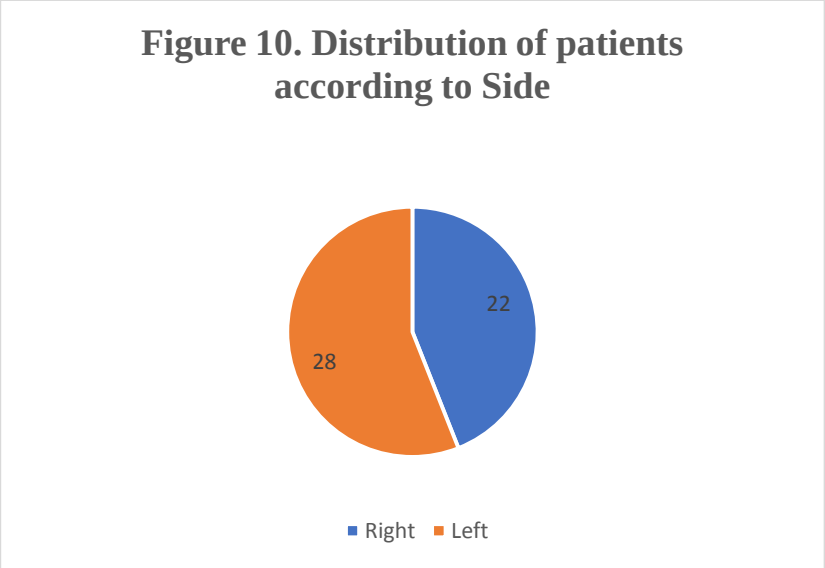


In the current study, most patients were females (52%) and 48% were male, with male to female ratio of 0.92:1

Distribution of patients according to affected side:

Table 3. Distribution of patients according to the affected side.

Side	Frequency (%)
Right	22(44)
Left	28 (56)

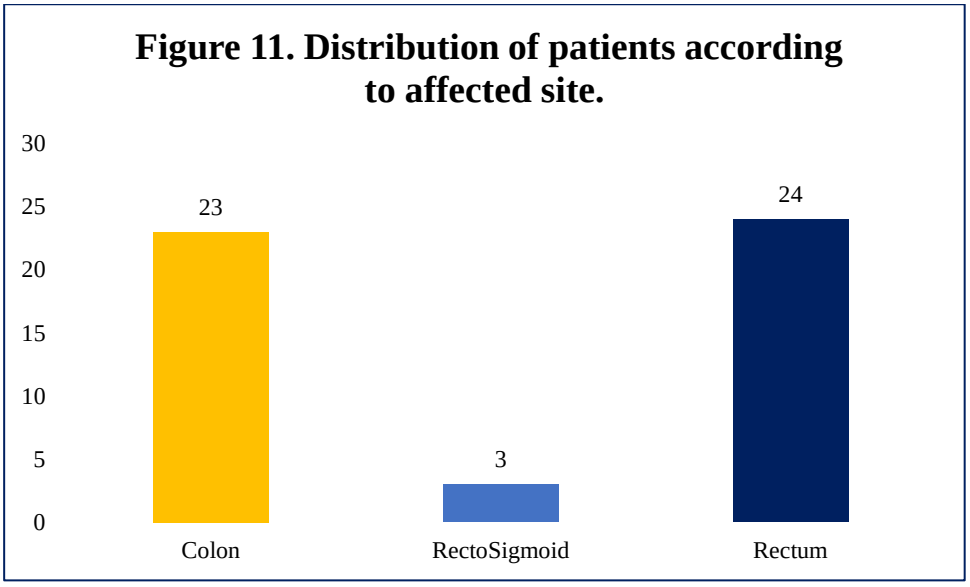


In the current study, most patients had left-side tumors (56%), and 44% had a tumor on the right side

Affected site:

Table 4. Distribution of patients according to the affected site.

Affected site	Frequency (%)
Colon	23 (46)
Rectosigmoid	3 (6)
Rectum	24 (48)

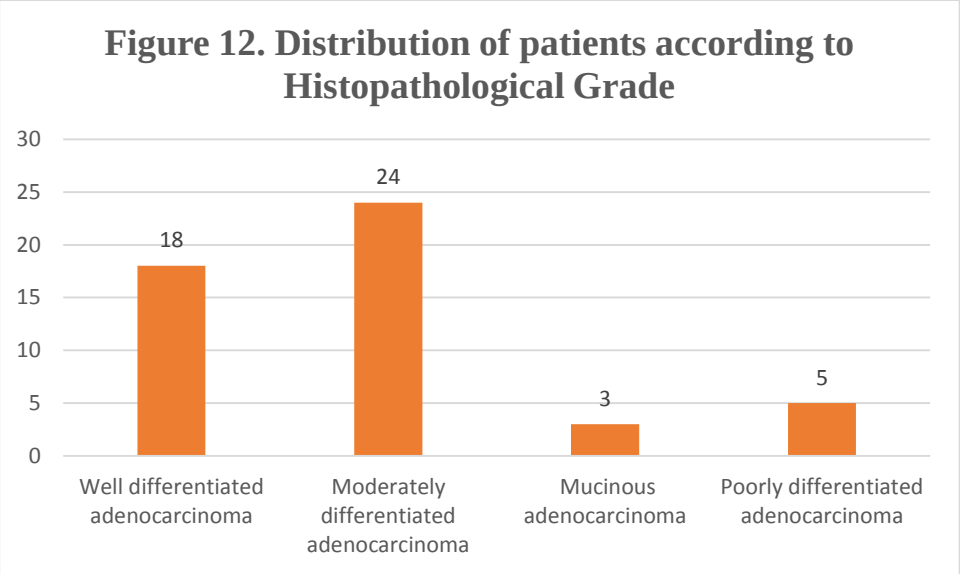


In the current study, the rectum was affected in 48% of the patients, followed by the colon (46%) and rectosigmoid (6%). (Refer Figure 31,32).

Histopathological Grade:

Table 5. Distribution of patients according to Histopathological Grade.

Histopathological Grade	Frequency (%)
Well-differentiated adenocarcinoma	18 (36)
Moderately differentiated adenocarcinoma	24(48)
Mucinous adenocarcinoma	3(6)
Poorly differentiated adenocarcinoma	5(10)



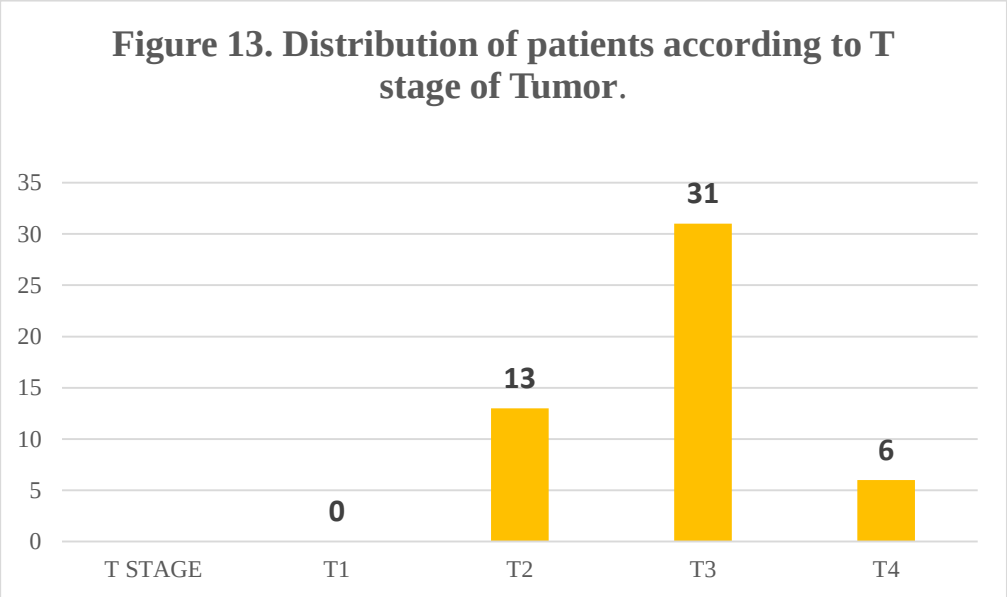
In the current study, most of the participants had moderately differentiated adenocarcinoma (48%), followed by Well-differentiated adenocarcinoma (36%), Poorly differentiated adenocarcinoma (10%), and Mucinous adenocarcinoma (6%). (Refer to Figures 33,34,35,36).

In this study, we have considered all the cases of Well-differentiated adenocarcinoma, moderately differentiated adenocarcinoma, and Mucinous adenocarcinoma as Low grade and poorly differentiated adenocarcinoma as High grade. Most of the patients (90%) were Low grade and high grade was observed in a few patients (10%).

T STAGING:

Table 6. Distribution of patients according to T Stage of Tumor.

T Stage	Frequency (%)
T1	0(0)
T2	13(26)
T3	31(62)
T4	6(12)



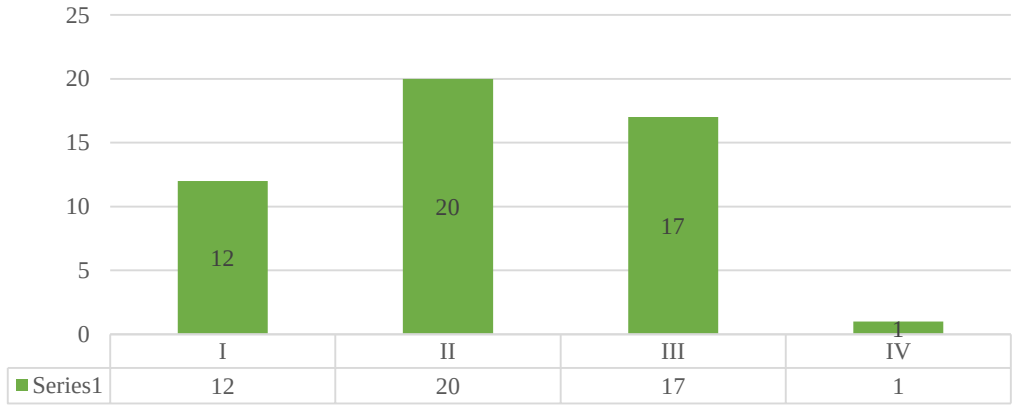
The majority of the participants in the current study had a T staging of T3 (62%), followed by T2 (26%) and T4 (12%).

TNM Stage of Tumor:

Table 7. Distribution of patients according to TNM Stage of Tumor.

TNM Stage of tumour	Frequency (%)
I	12 (24)
II	20(40)
III	17(34)
IV	1(2)

Figure 14. Distribution of patients according to TNM Staging.

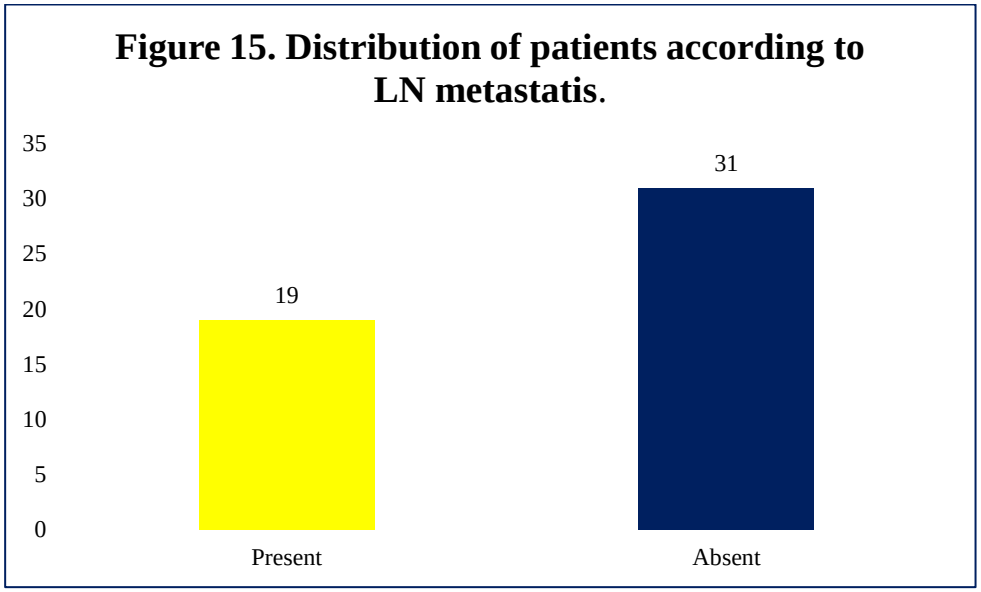


40% of the patients were in stage II, followed by stage III and stage I with 34% and 24%, the least was seen in stage IV (2%).

LN metastasis:

Table 8. Distribution of patients according to LN metastasis.

LN metastasis	Frequency (%)
Present	19(38)
Absent	31(62)



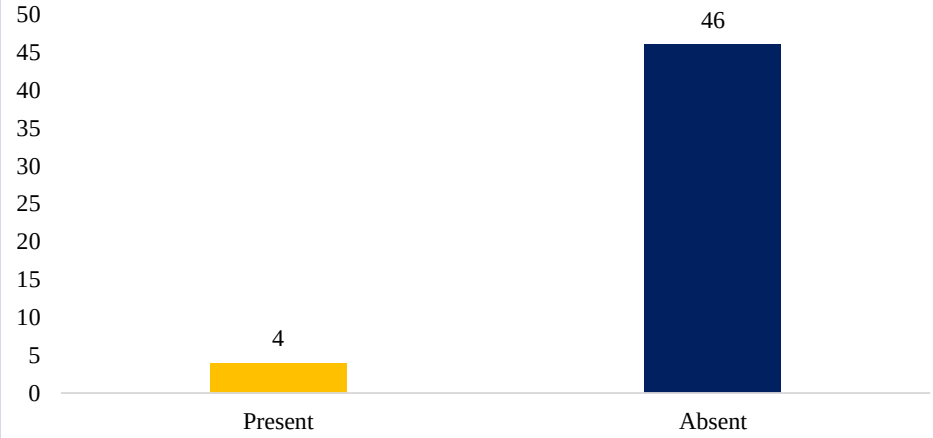
In the current study, among the study participants, LN metastasis was absent in 62% of the participants and was present in 38%. (Refer Figure 37)

Peri Neural Invasion(PNI):

Table 9. Distribution of patients according to PNI.

PNI	Frequency (%)
Present	4 (8)
Absent	46 (92)

Figure 16. Distribution of patients according to PNI.

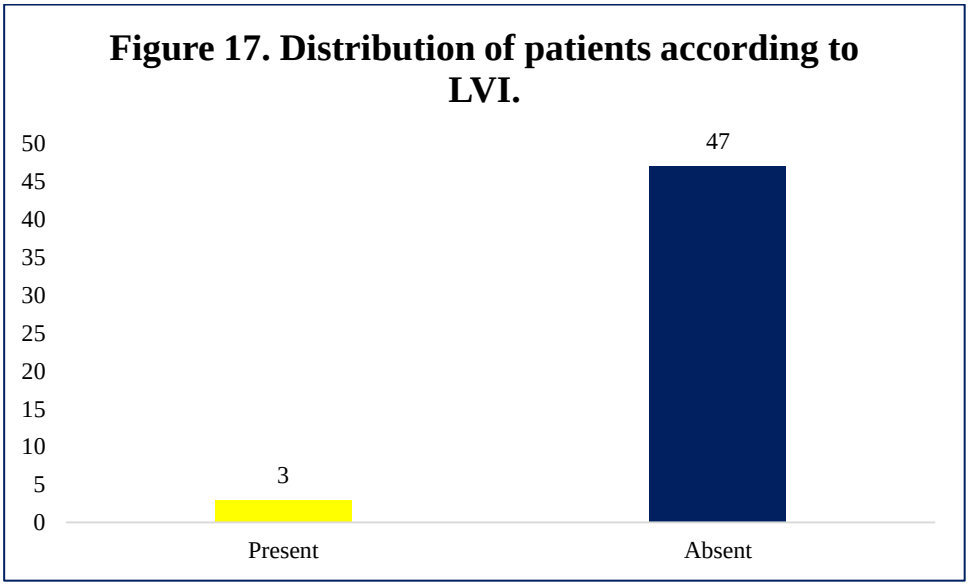


In the current study, among the study participants, PNI was absent in 92% of the participants, and it is present in only 8%

Lympho-vascular Invasion (LVI):

Table 10. Distribution of patients according to LVI.

LVI	Frequency (%)
Present	3 (6)
Absent	47 (94)



In the current study, among the study participants, LVI was absent in 94% of the participants, and it is present in only 6%. (Refer figure 38)

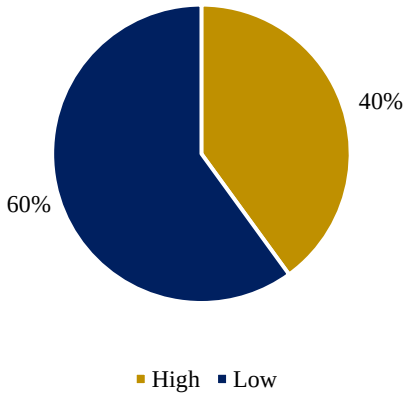
IHC RESULTS:

E cadherin level:

Table 11. Distribution of patients according to E cadherin level.

E cadherin level	Frequency (%)
High	20 (40)
Low	30 (60)

Figure 18. Distribution of patients according to E cadherin level.



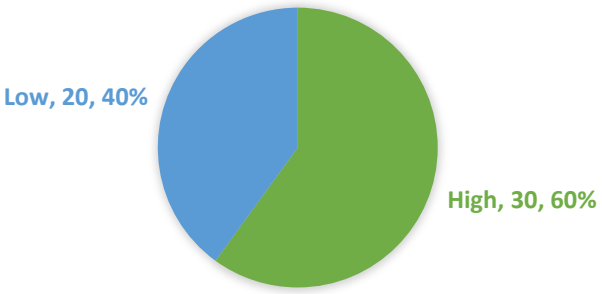
In the current study, most patients had low expression (60%) of E-cadherin levels, and 40% had high expression of E-cadherin. (Refer Figure 39,40)

Beta catenin level:

Table 12. Distribution of patients according to Beta catenin level.

Beta catenin level	Frequency (%)
High	30(60)
Low	20 (40)

Figure 19. Distribution of patients according to Beta catenin levels

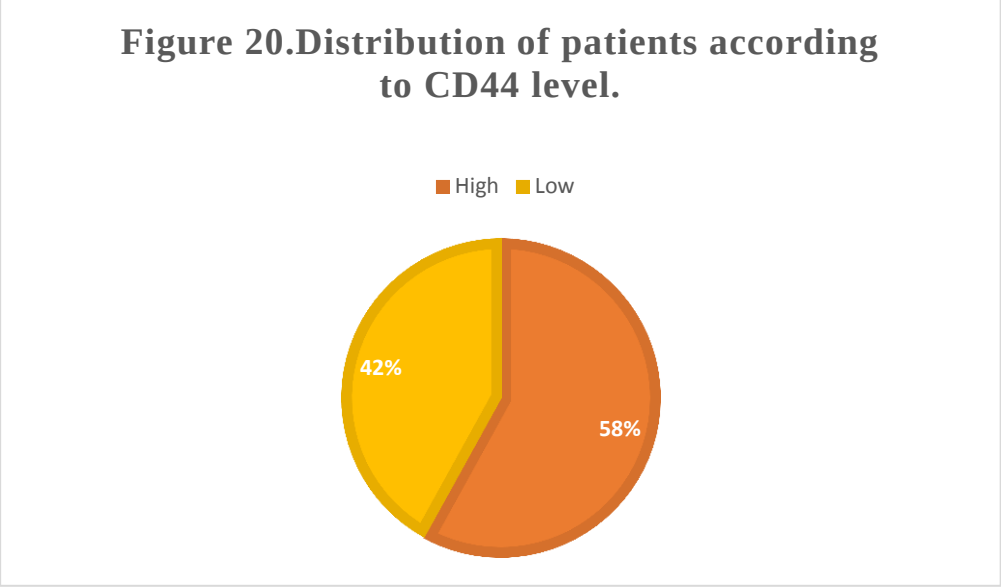


In the current study, most participants had high expression of Beta-catenin levels (60%), and 40% had low expression of Beta-catenin. (Refer Figure 41,42).

CD44 level:

Table 13. Distribution of patients according to CD44 level.

CD44 level	Frequency (%)
High	29 (58)
Low	21 (42)

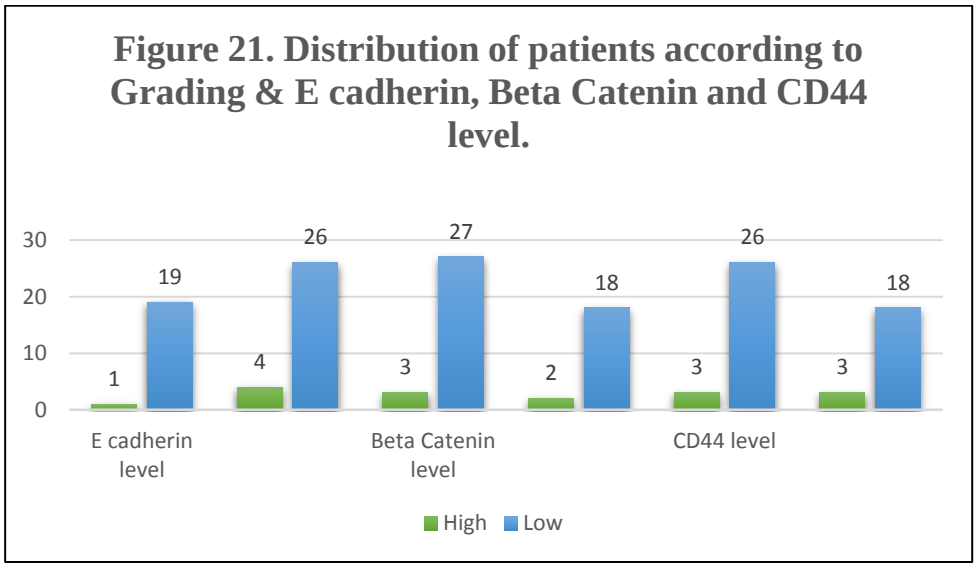


Most of the patients in the current study had high expression (58%) CD44 levels, and 42% had low expression of CD44. (Refer Figure 43,44.)

Distribution of patients according to Grading & E cadherin, Beta Catenin, and CD44 level:

Table 14. Distribution of patients according to Grading & E cadherin, Beta Catenin, and CD44 level.

Variable		Grading		p-value
		High	Low	
E cadherin level	High	1	19	0.336
	Low	4	26	
Beta Catenin level	High	3	27	1
	Low	2	18	
CD44 level	High	3	26	0.7
	Low	3	18	



In the current study, most of the patients with low expression of E cadherin have a low grading (26) with an odds ratio of 0.34. Similarly, the participants with high Beta-Catenin levels have a low grading (27) with a p-value of 1.

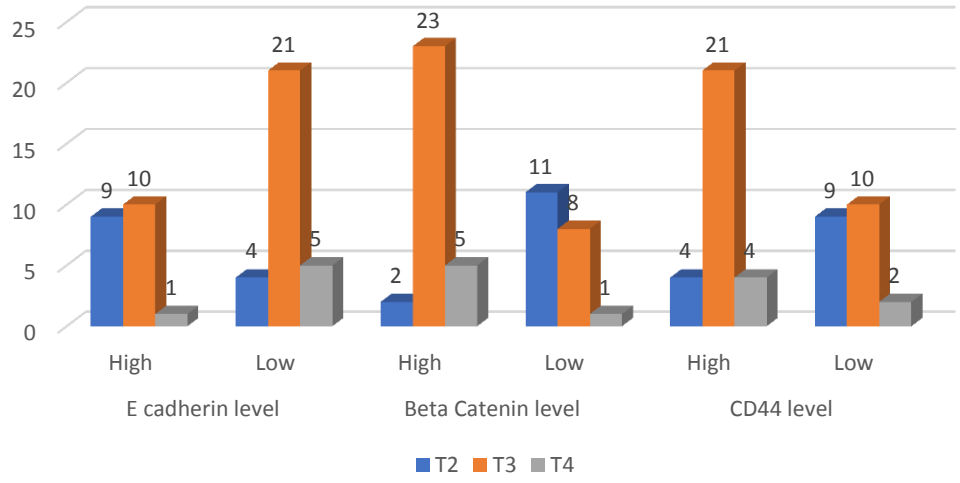
The participants with high expression of CD44 show low grading (26) with a p value of 0.7. This indicates that expression E cadherin, Beta-catenin, and CD44 have no statistical significance with respect to the Grading of the tumor.

Distribution of patients according to T staging & E cadherin, Beta Catenin and CD44 level:

Table 15. Distribution of patients according to T staging & E cadherin, Beta Catenin and CD44 level.

Variable		Tumour staging			p-value
		T2	T3	T4	
E cadherin level	High	9	10	1	0.0339
	Low	4	21	5	
Beta Catenin level	High	2	23	5	0.00062
	Low	11	8	1	
CD44 level	High	4	21	4	0.0689
	Low	9	10	2	

Figure 22. Distribution of patients according to T staging & E cadherin, Beta Catenin and CD44 level.



Most of the patients with low expression of E cadherin (21) have T3 with an odds ratio of 0.432 and a p-value of 0.03, which shows statistical significance. Similarly, the participants with high expression of Beta-catenin levels (23) have a p-value of 0.0006, which shows statistical significance. The participants with higher expression of CD44 did not show any statistical significant value (p=0.06).

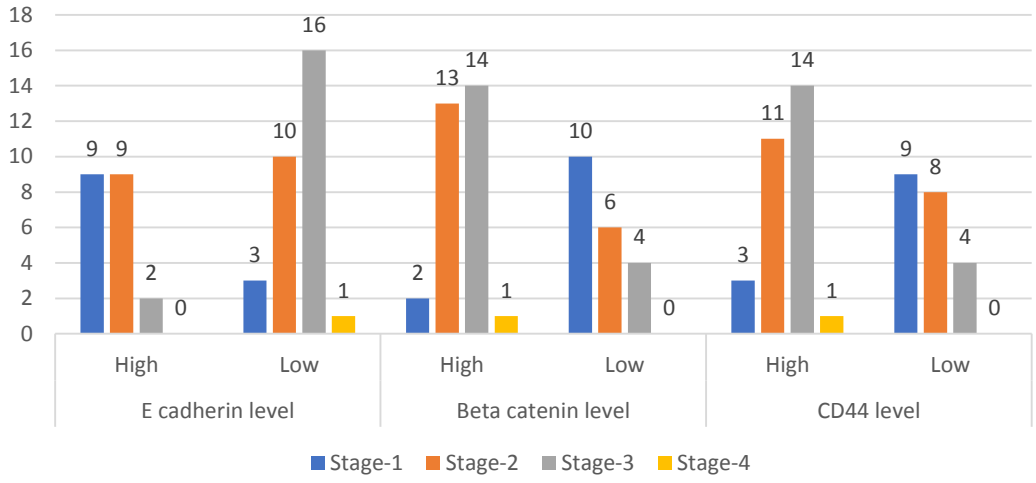
This result shows the patients having higher T stage showed low expression of E cadherin and high expression of Beta-Catenin.

TNM Staging of cancer & E cadherin, Beta Catenin and CD44 level:

Table 16. Distribution of patients according to the TNM staging of cancer & E cadherin, Beta Catenin and CD44 level.

Variable		TNM Stage of cancer				p-value
		Stage-I	Stage-II	Stage-III	Stage-IV	
E cadherin level	High	9	9	2	0	0.04
	Low	3	10	16	1	
Beta catenin level	High	2	13	14	1	0.005
	Low	10	6	4	0	
CD44 level	High	3	11	14	1	0.064
	Low	9	8	4	0	

Figure 23. Distribution of patients according to TNM staging of cancer & E cadherin, Beta Catenin and CD44 level.



Participants with low expression of E cadherin were seen in stage IV (1), a majority of stage III (16) , and stage II (10) cancer patients with a p-value of 0.04, which shows statistical significance.

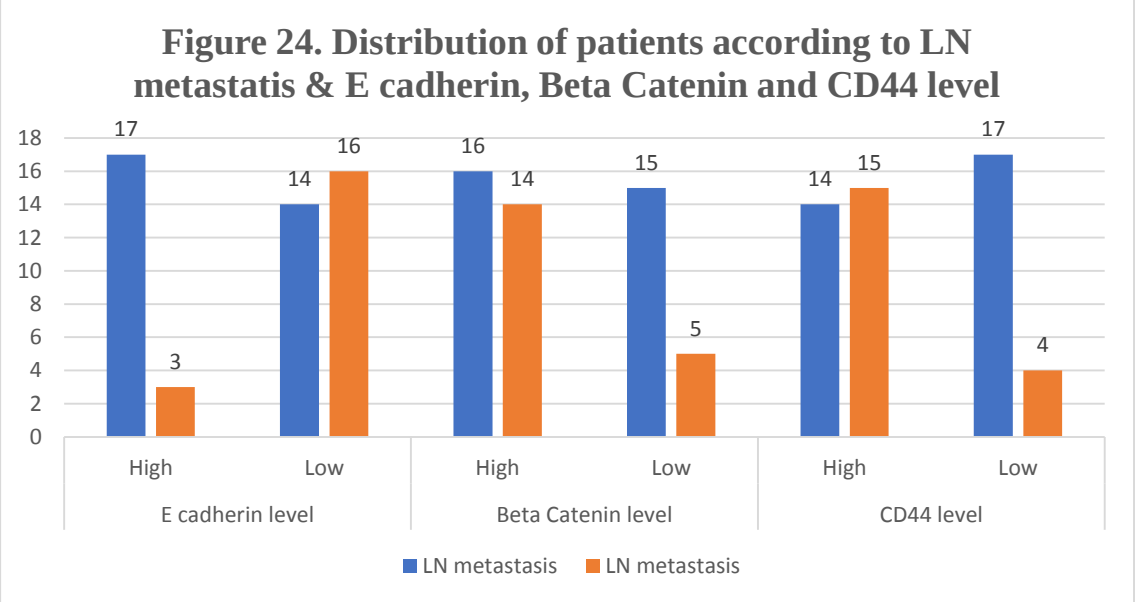
Participants with high expression of beta-catenin were seen in stage IV(1), a majority of stage III (14,) and stage II(13) cancer with an odds ratio of 0.879 and a p-value of 0.005, which shows statistical significance. This indicates that the participants with low expression of E cadherin and high expression beta-catenin are seen in higher stages.

However there was no significant association of CD 44 expression with TNM stage.

Distribution of patients according to LN metastasis & E cadherin, Beta Catenin and CD44 level:

Table 17. Distribution of patients according to LN metastasis & E cadherin, Beta Catherin and CD44 level.

Variable		LN metastasis		p-value
		Absent	Present	
E cadherin level	High	17	3	0.006
	Low	14	16	
Beta Catenin level	High	16	14	0.122
	Low	15	5	
CD44 level	High	14	15	0.018
	Low	17	4	

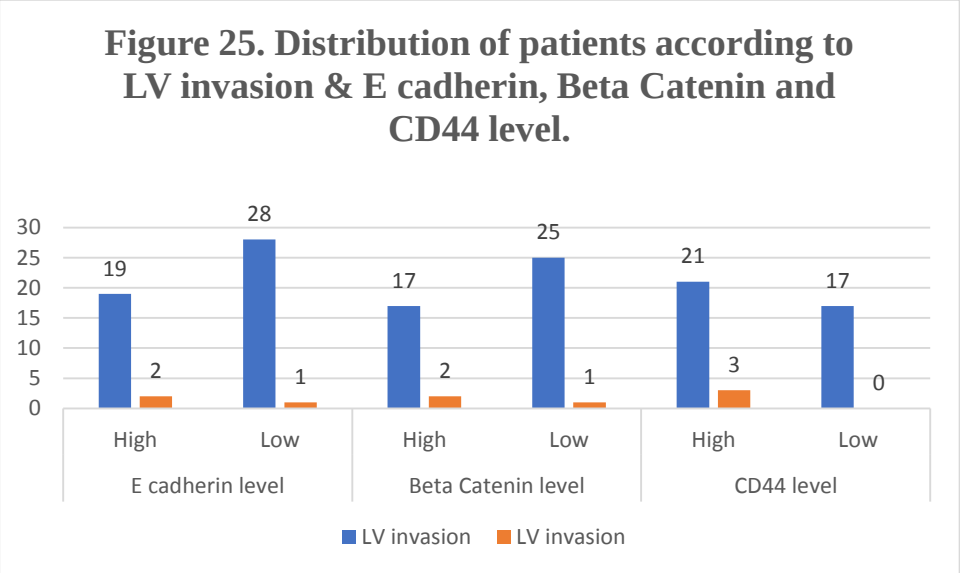


Most of the patients with low expression of E cadherin have a presence of LN metastasis (16) with a p-value of 0.006, which shows statistical significance. Participants with high beta-Catenin levels have a presence of LN metastasis (14) with a p-value of 0.122 showing no statistical significance. The participants with higher CD44 expression have the presence of LN metastasis (15) with a p-value of 0.04, which shows statistical significance.

Distribution of patients according to LV invasion & E cadherin, Beta Catenin and CD44 level:

Table 18. Distribution of patients according to LV invasion & E cadherin, Beta Catherin and CD44 level.

Variable		LV invasion		p-value
		Absent	Presence	
E cadherin level	High	18	2	0.303
	Low	29	1	
Beta Catenin level	High	28	2	0.807
	Low	19	1	
CD44 level	High	26	3	0.25
	Low	21	0	



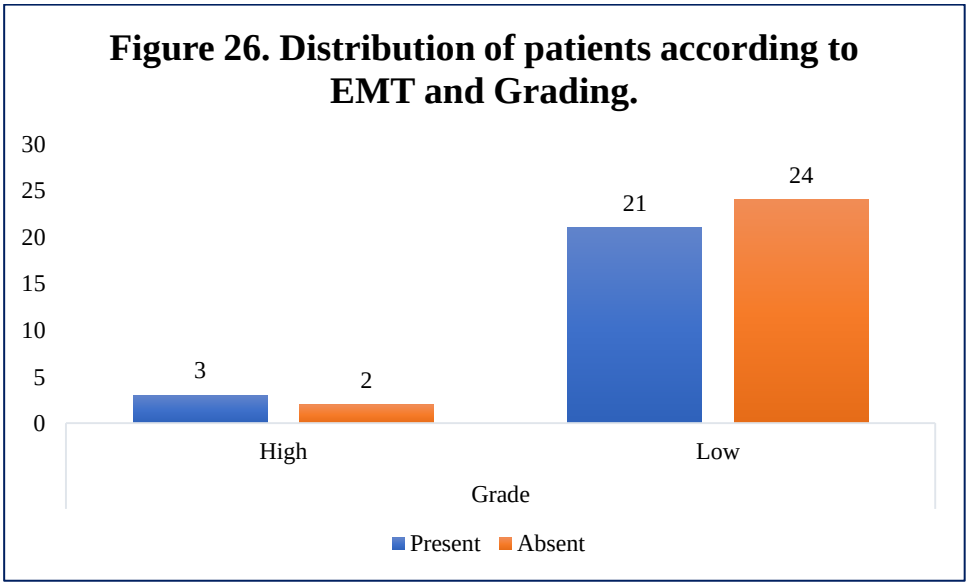
In the current study, Lympho-vascular invasion was seen in very few cases and these cases did not show any statistical association with any of the three markers.

Epithelial-Mesenchymal Transition (EMT): All the cases showing low expression of E cadherin and high expression of Beta-catenin were taken as positive for Epithelial-Mesenchymal Transition. In the current study, 24 cases showed positive for EMT (48%) and 52% showed no EMT.

EMT and Grading:

Table 19. Distribution of patients according to EMT and Grading.

EMT	Grade		p-value	Odd's Ratio
	High	Low		
Present	3	21	0.576	1.71
Absent	2	24		

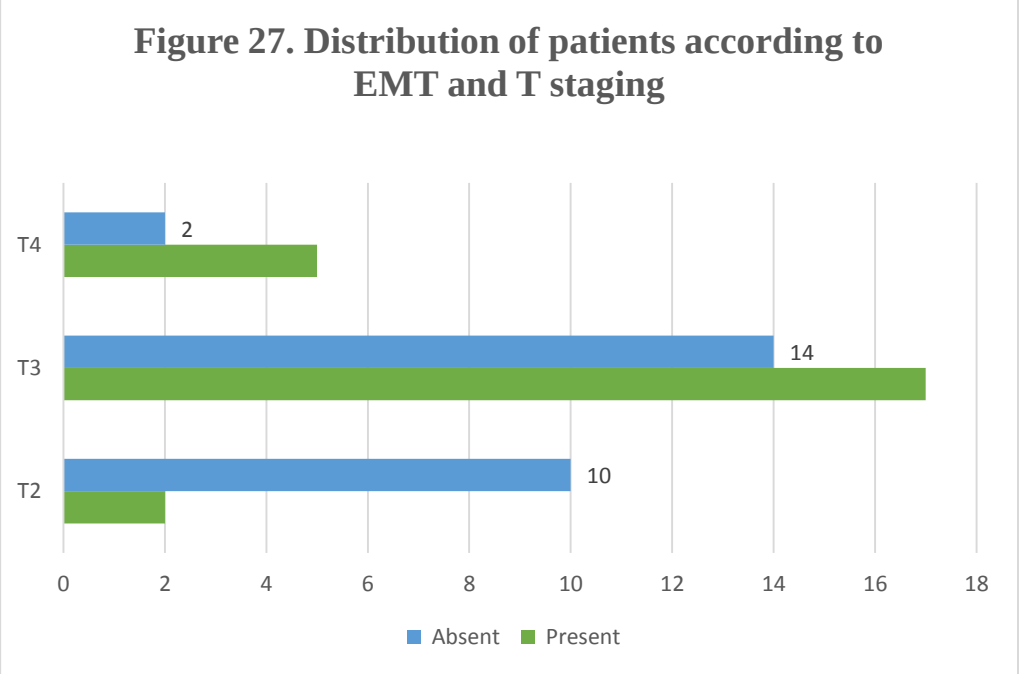


The participants with Epithelial-mesenchymal transitions (EMTs) have low grading (21) and, in the absence of Epithelial-mesenchymal transitions (EMTs), have also shown low grading (24) with an odds ratio of 1.7.

Distribution of subjects according to EMT and T Staging:

Table 20. Distribution of patients according to EMT and T Staging

EMT	Tumour staging			p-value	Odd's Ratio
	T2	T3	T4		
Present	2	17	5	0.032	0.5
Absent	10	14	2		



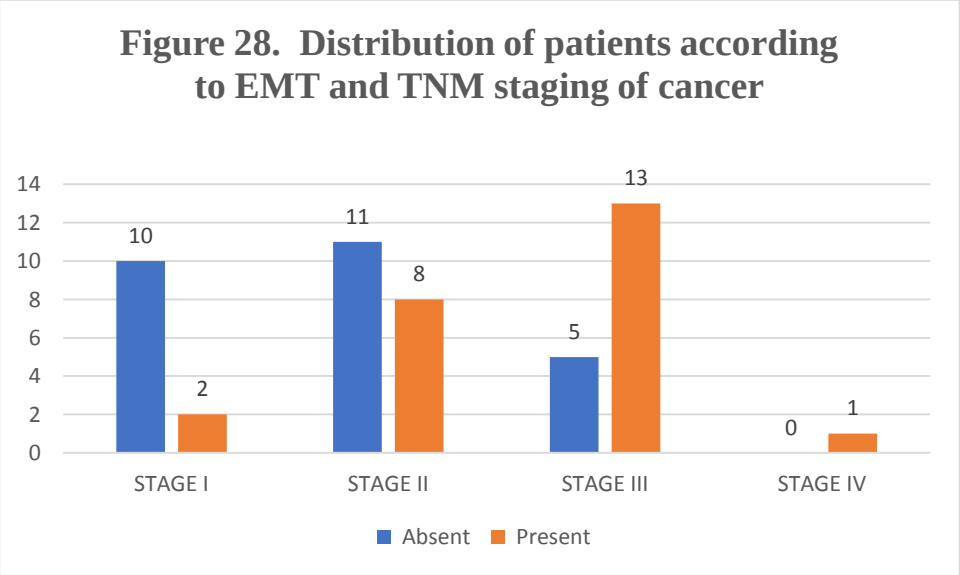
Most of the participants with Epithelial-mesenchymal transitions (EMTs) had T3staging 3(17) T4(5) with a significant p value of 0.032.

The odds ratio indicates that the participants with Epithelial-mesenchymal transitions (EMTs) have a risk of developing higher T stage carcinoma compared to others

EMT and staging of cancer:

Table 21. Distribution of patients according to EMT and TNM Staging of cancer

EMT	TNM staging				p-value	Odd's Ratio
	STAGE I	STAGE II	STAGE III	STAGE IV		
Absent	10	11	5	0	0.016	0.527
Present	2	8	13	1		



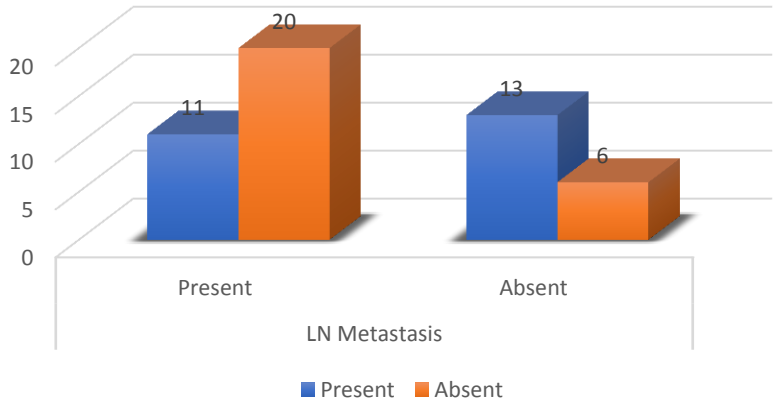
The participants with Epithelial-mesenchymal transitions (EMTs) have a majority of Stage III (13). In the absence of Epithelial-mesenchymal transitions (EMTs), the majority of the participants were of stage II(11) and stage I (10) with an odds ratio of 0.52. and a p-value of 0.016, which shows statistical significance, indicating that EMT is more present in higher TNM stages i.e Stage III and IV

Distribution of patients according to EMT and LN Metastasis:

Table 22. Distribution of patients according to EMT and LN Metastasis.

EMT	LN Metastasis		p-value	Odd's Ratio
	Absent	Present		
Present	11	13	0.040	0.317
Absent	20	6		

Figure 29. Distribution of patients according to EMT and LN Metastasis.

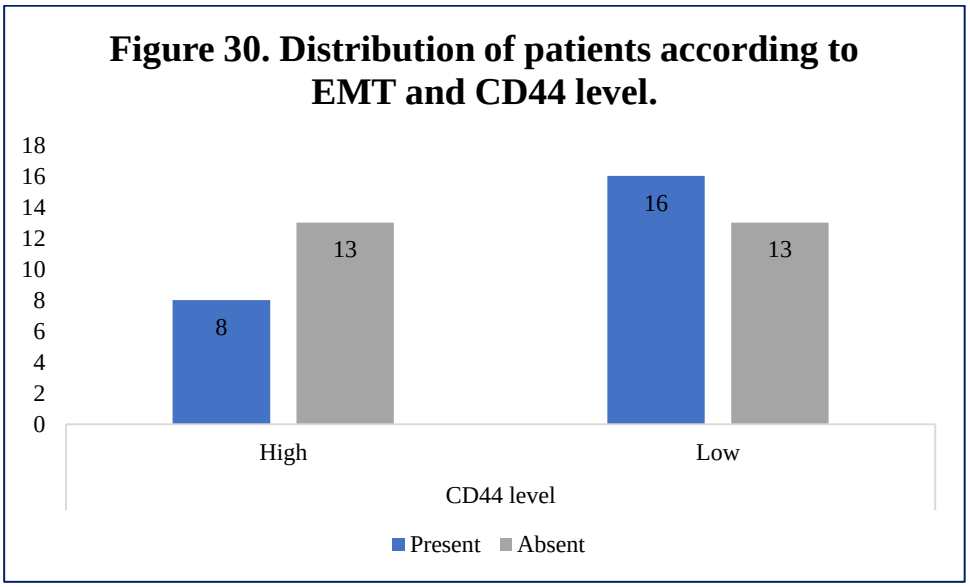


The patients with Epithelial-mesenchymal transitions (EMTs) also have LN metastasis (13) with an odds ratio of 0.31 and a significant p-value of 0.04. This indicates that the participants with Epithelial-mesenchymal transitions (EMTs) carry the risk of Lymph node metastasis.

Distribution of patients according to EMT and CD44 level:

Table 23. Distribution of patients according to EMT and CD44 level.

EMT	CD44 level		p-value	Odd's Ratio
	Low	High		
Present	8	16	0.235	2
Absent	13	13		



Most of the patients with Epithelial-mesenchymal transition (EMT) showed low expression of CD44 (16) . The results did not show any significant association between the presence of EMT and high expression of CD44 with an odds ratio of 2.

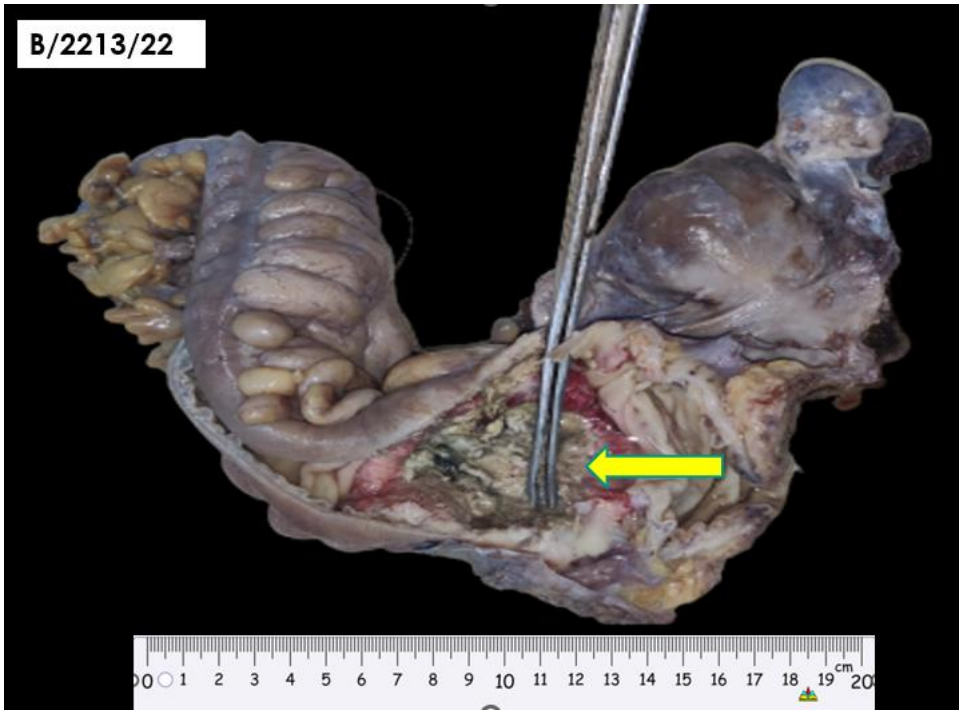


Figure 31: Gross image of Colon showing (yellow arrow) grey-white tumor on cut section and the tumor is extending uptill the serosa.

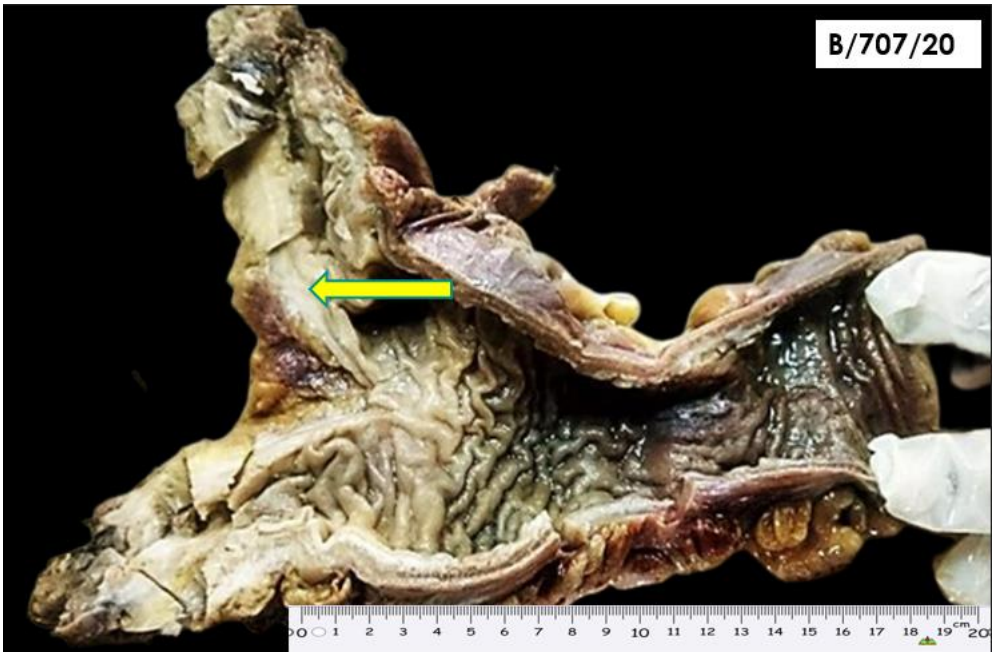


Figure 32: Gross image of Large intestine which shows (yellow arrow) grey-white tumor measuring 4.8x3.6x2.8 cm extending uptill subserosa

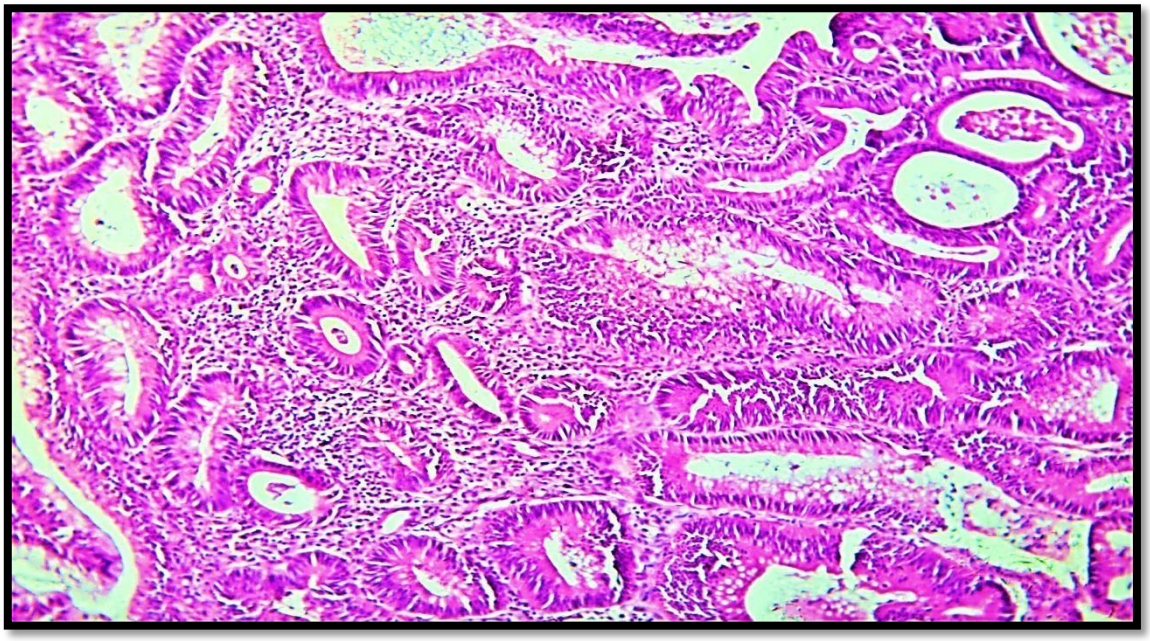


Figure 33: Microphotograph of H and E stained section showing Well Differentiated Adenocarcinoma (original magnification x100)

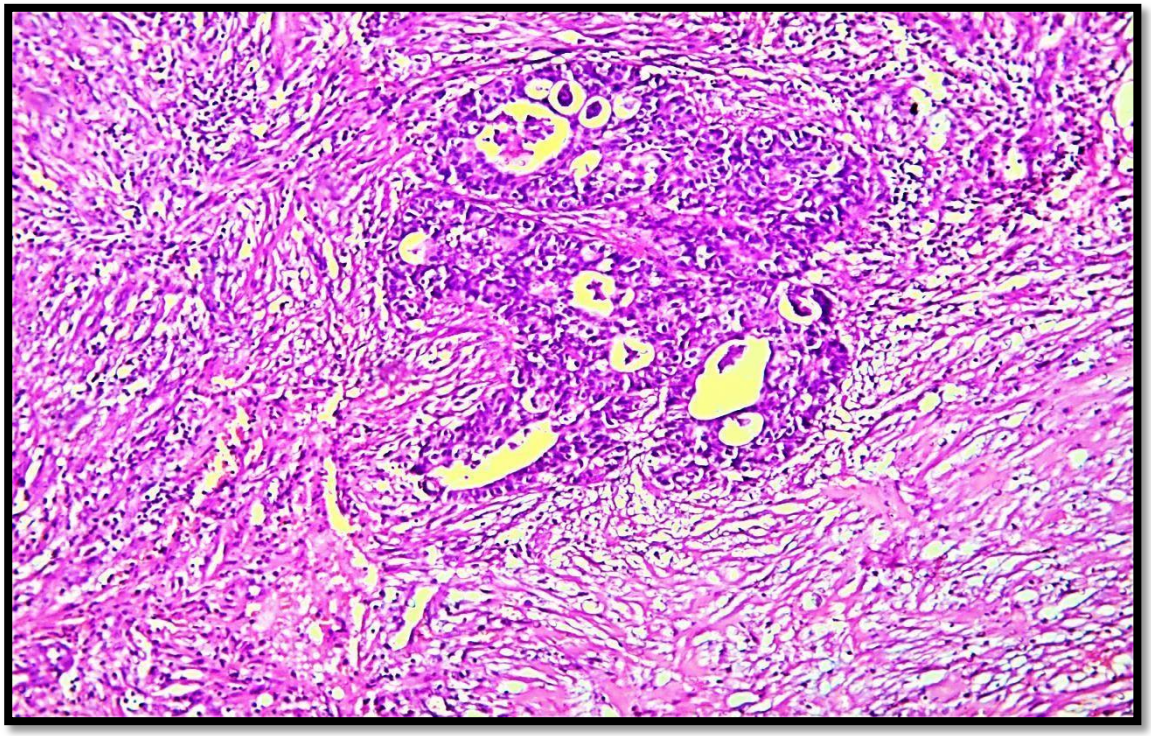


Figure 34: Microphotograph of H and E stained section showing Moderately DifferentiatedAdenocarcinoma (original magnification, x100)

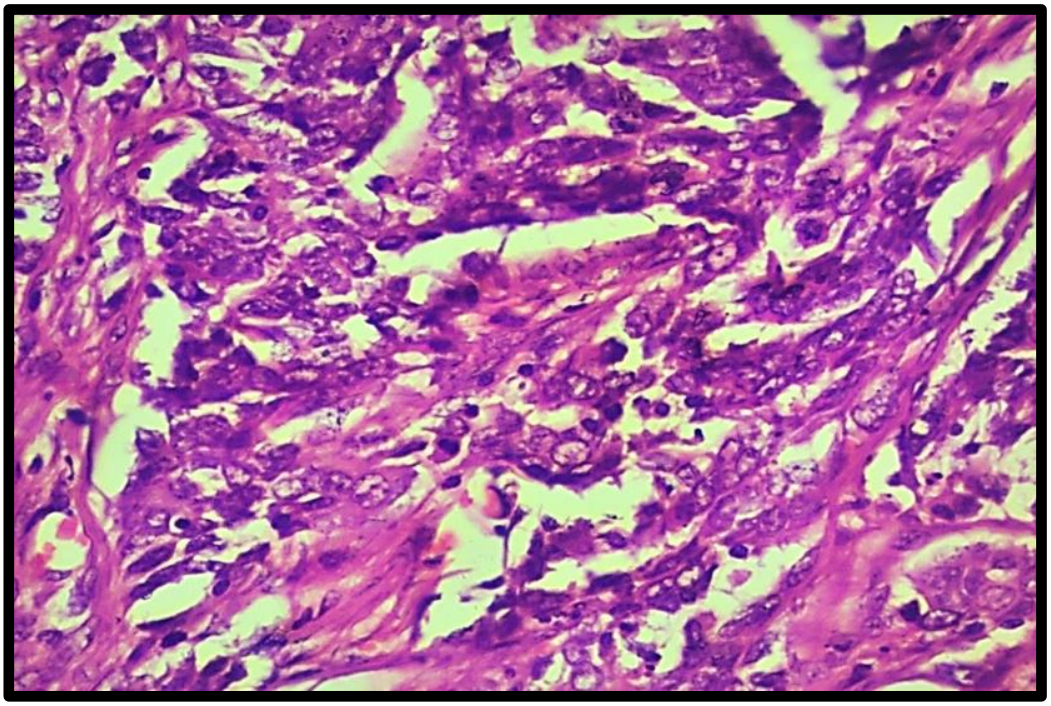


Figure 35: Microphotograph of H and E stained section showing Poorly Differentiated Adenocarcinoma (original magnification, x400).

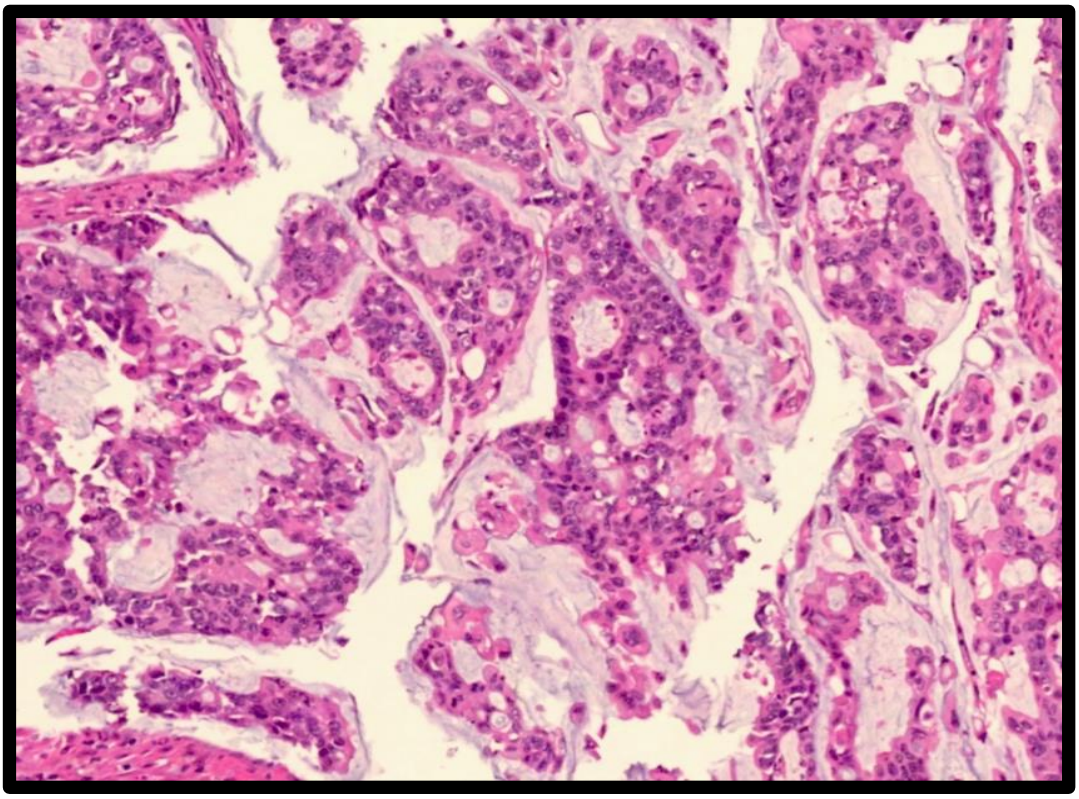


Figure 36: Microphotograph of H and E stained section showing Mucinous Adenocarcinoma (original magnification, x400)

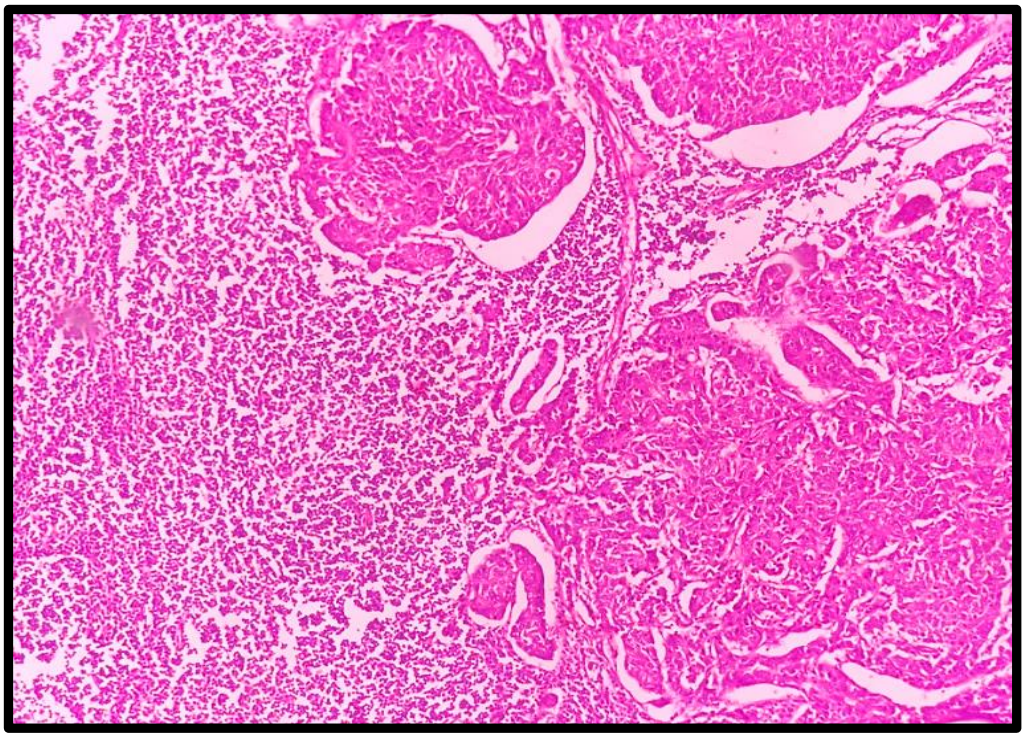


Figure 37: Microphotograph of H and E stained section showing Lymphnodal metastasis of Colorectal Adenocarcinoma (original magnification, x100).

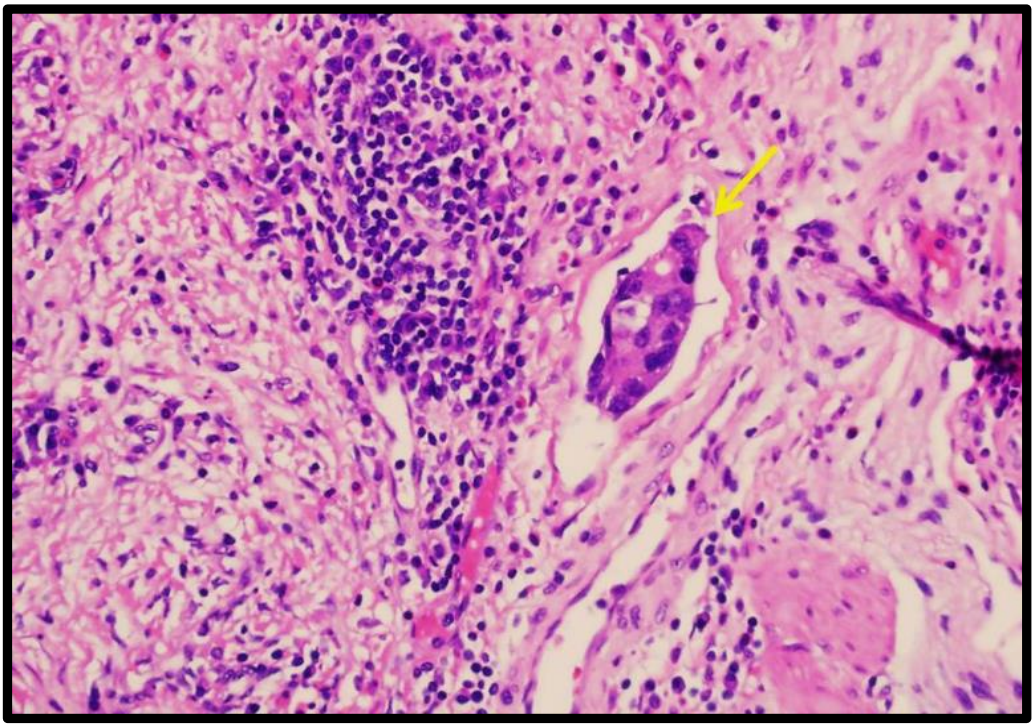


Figure 38: Microphotograph of H and E stained section showing Vascular invasion in Colorectal Adenocarcinoma. (original magnification, x400).

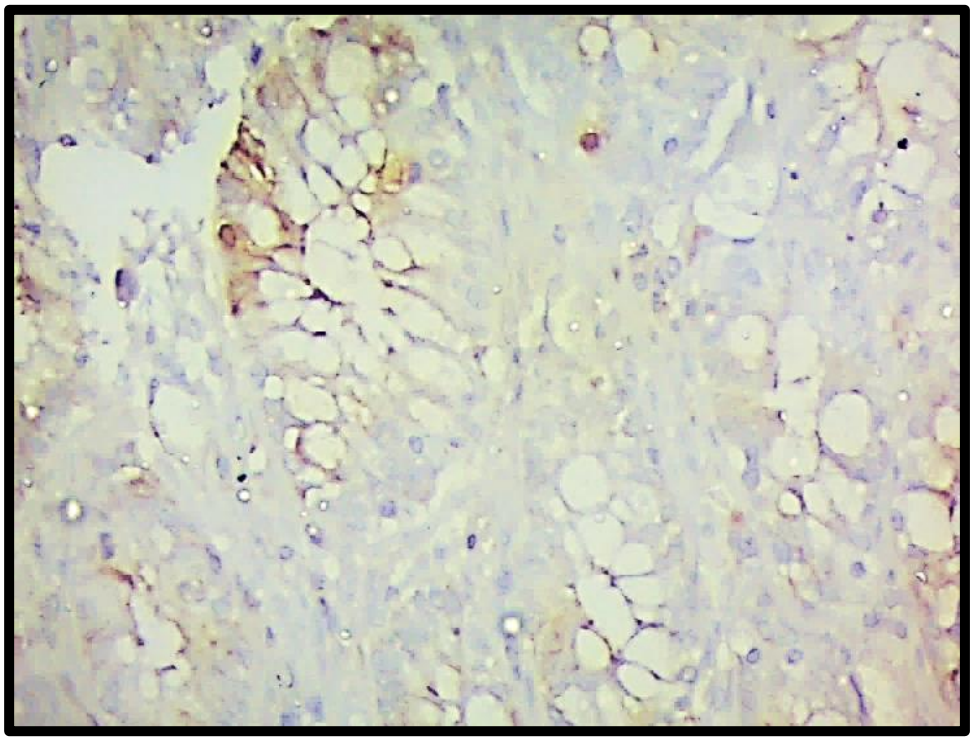


Figure 39: Microphotograph of E Cadherin IHC staining showing low expression with Intensity-2, % Fraction – 1. (original magnification, x400).

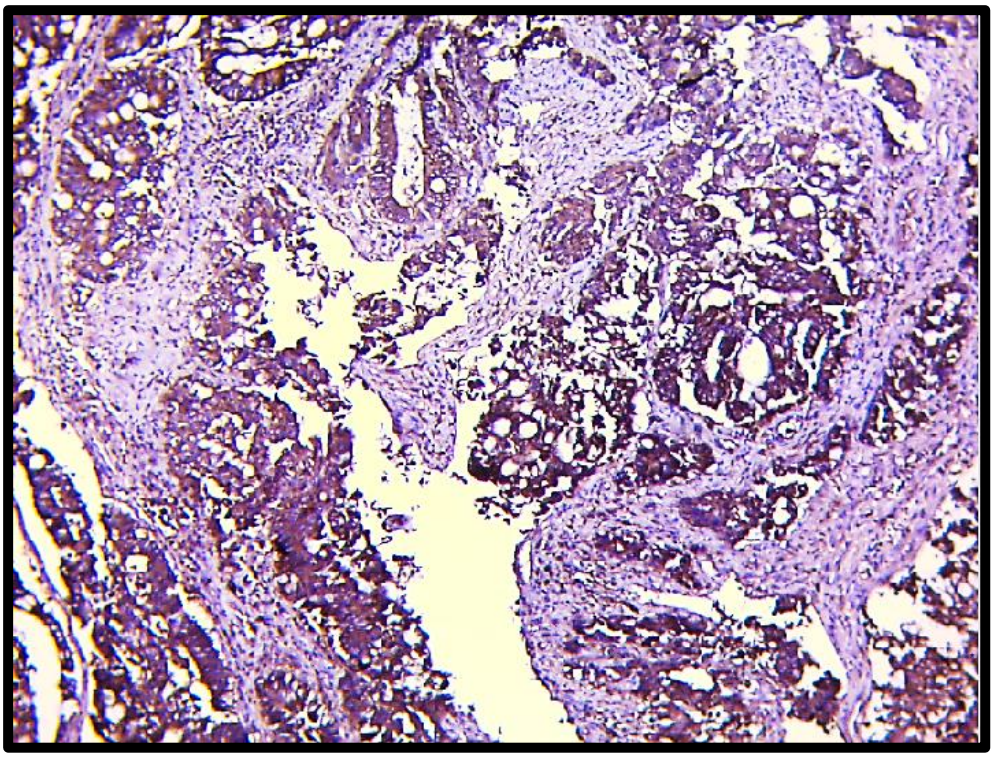


Figure 40: Microphotograph of E Cadherin IHC staining showing high expression with Intensity-3, % Fraction – 3. (original magnification, x100).

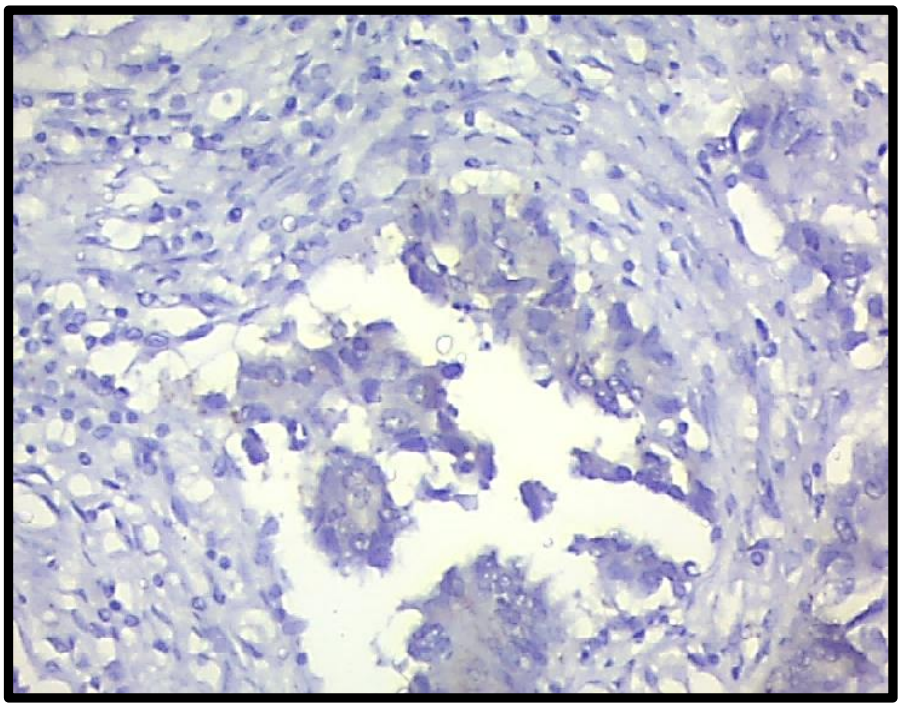


Figure 41: Microphotograph of Beta Catenin IHC staining showing low expression with Intensity-1, % Fraction – 1. (original magnification, x400).

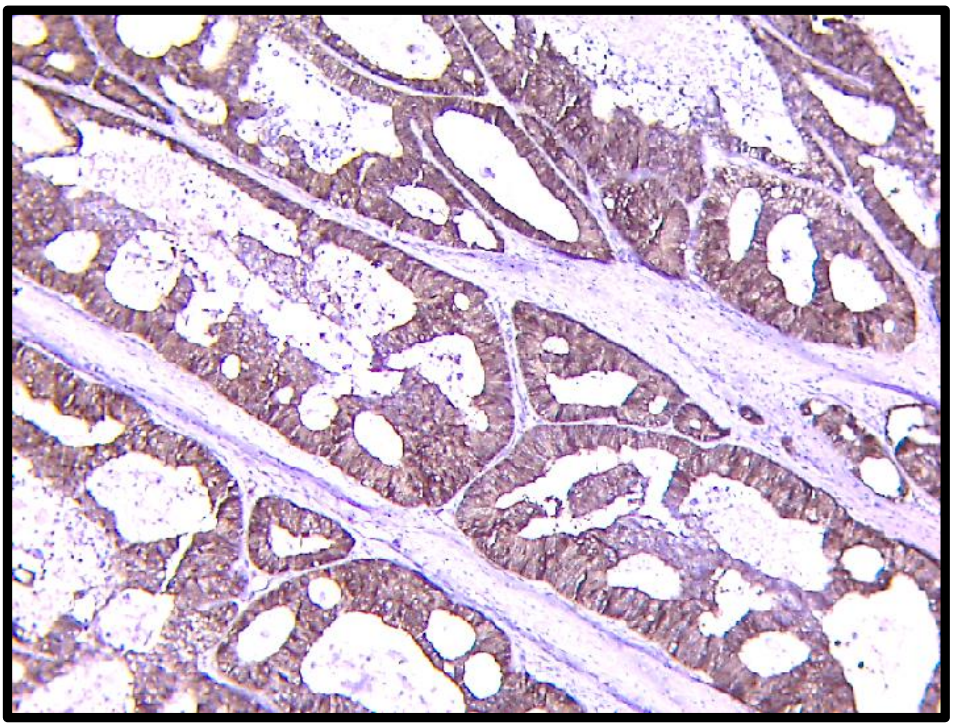


Figure 42: Microphotograph of Beta Catenin IHC staining showing High expression with Intensity-3, % Fraction – 3. (original magnification, x100).

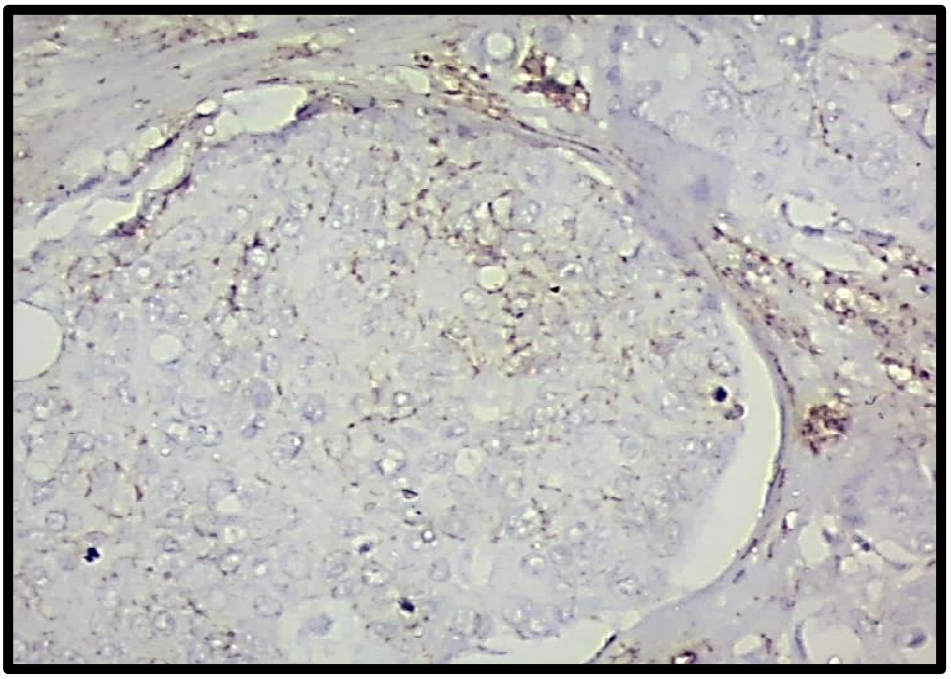


Figure 43: Microphotograph of CD44 IHC staining showing low expression with Intensity-1, % Fraction – 1. (original magnification, x400).

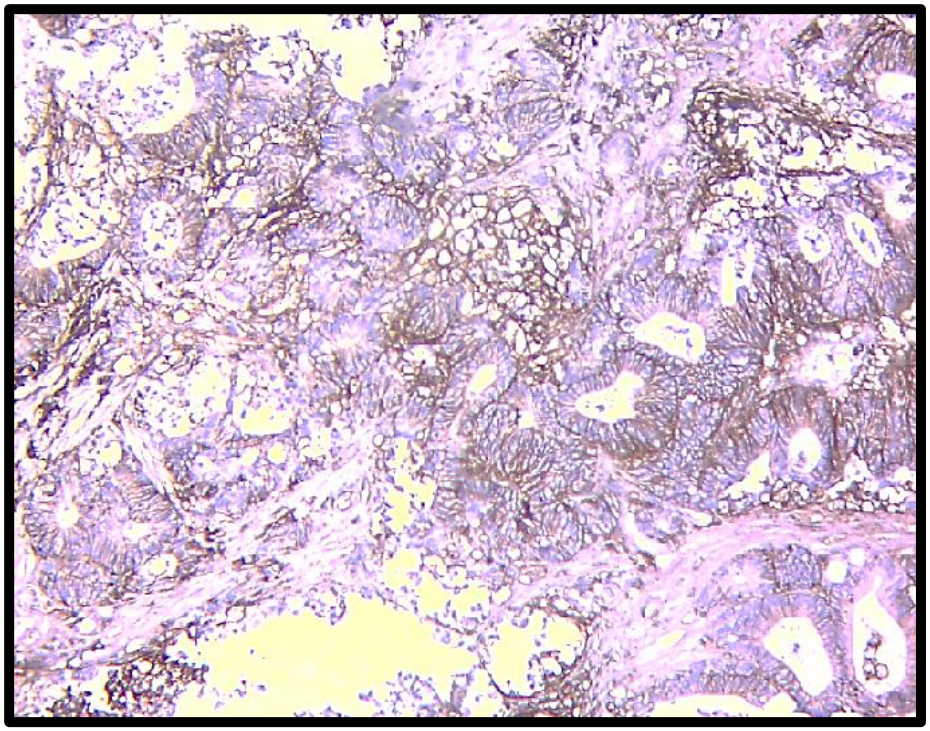


Figure 44: Microphotograph of CD 44 IHC staining showing High expression with Intensity-3, % Fraction – 3. (original magnification, x100).

DISCUSSION

DISCUSSION

Colorectal carcinoma is one of the most frequent malignant tumors globally, ranking third in incidence and fourth in mortality.[53] Approximately it accounts 10 % of the global cancer burden.[54]

Age >60 years, polyps, adenomas, diet (red meat, animal fat, alcohol), obesity, sedentary lifestyle, and positive family history are risk factors for colorectal cancer. Between 61 and 70 years of age, the incidence rises.

Conventional prognostic parameters for colorectal cancer include TNM staging, tumor grade, lymphatic, perineural invasion, venous invasion, and tumor border architecture.[12,13,14,17]

The activation of EMT during cancer growth enables cancer cells to develop characteristics including migratory, invasive, and stem-like traits.[3]

In the present study, we used E Cadherin, Beta-catenin for the evaluation of Epithelial-Mesenchymal Transition and CD44 in the assessment of stem cells based on studies done by Choi et al., Ryu Hs et al. and Briede et al.[7,55,56]

We studied association of the IHC expression of E Cadherin, Beta-catenin, and CD44 with clinicopathological parameters.

Comparison of Age Distribution with other studies:

Table 24: Comparison of Age Distribution with other studies:

STUDY	MEAN AGE
Yasuhito Iseki et al., (2017) (n=49)	59
Wafaey Gomaa et al., (2019) (n=196)	62
Ji Eun Choi et al., (2017) (n=286)	65
Melincovici et al. (2020) (n=31)	70
Present Study (n= 50)	**68**

In the current study, the major part of the patients were in the age group of 61 to 70 years (36%), followed by 51 to 60 years (34%), 41 to 50 years (16%), 70 years (10%) and the least in ≤ 30 years and 31-40 years (2%). In the present study mean age was 68 years, which was comparable to similar studies (Yasuhito Iseki et al.,[55] Wafaey Gomaa et al.,[56] Ji Eun Choi et al.,[57] and Melincovici et al.,[58]).

Comparison of Gender Distribution with other studies:

Table 25: Comparison of Gender Distribution with other studies

Study	Males	Females	M:F
Yasuhito Iseki et al., (2017) (n=49)	57.1%	42.8%	1.3:1
Wafaey Gomaa et al., (2019) (n=196)	56.6%	43.4%	1.3:1
Ji Eun Choi et al., (2017) (n=286)	39.1%	60.8%	0.65:1
Melincovici et al., (2020) (n=31)	51.6%	48.3%	1.06:1
Present Study	**48%**	**52%**	**0.9:1**

In the current study,52% were of the female gender, and 48% were male. Studies by Yasuhito Iseki et al.,[55] Wafaey Gomaa et al.,[56] and Melincovici et al.,[56] had a slight male predominance. In the study done by Choi et al.,[57] Female predominances were noted similar to the present study.

Comparison of the Location of the tumor with other studies :

Table 26: Comparison of the Location of the tumor with other studies

Studies		Rajkumar et al.,[59] (2022) (n=100)	Yasuhito Iseki et al., (2017) (n=49)	Archilla et al.,[60] (2021) (n=342)	**Present Study**
Sites	**Rectum**	38%	46.7%	48%	**46.8%**
	Colon	62%	53.3%	46%	**38%**
	Rectosigmoid	Not specified	Not specified	6%	**15.2%**

In the current study, the Rectum is affected in 48% of the participants, followed by the colon (46%) and rectosigmoid (6%). Archilla et al.,[60] reported the Rectum as a more common site than Colon and Rectosigmoid locations, which was relevant to our study. Whereas other studies by Rajkumar et al.[59] and Yasuhito Iseki et al. [55] have noted the colon has a higher incidence of colorectal carcinoma.

Comparison of Histological Grading with other studies:

Table 27: Comparison of Histological Grading with other studies

Studies		Rajkumar et al. (2022) (n=100)	Madalina Palaghia et al. (2016) (n=65)	Yasuhito Iseki et al. (2017) (n=49)	**Present Study (n=50)**
Malignancy Grading	**Well Differentiated**	13	8	44	**18**
	Moderately Differentiated	74	48	44	**24**
	Poorly Differentiated	13	9	5	**5**
	Mucinous Adenocarcinoma	Not specified	Not specified	5	**5**

In the current study, 48% of the patients had Moderately differentiated Adenocarcinoma, followed by Well-differentiated Adenocarcinoma (36%), Poorly differentiated Adenocarcinoma (10%), and Mucinous Adenocarcinoma (6%). This is similar to studies by Rajkumar et al.,[59] and Madalina Palaghia et al.,.[61] where predominant tumors were graded as Moderately differentiated.

In Yasuhito Iseki et al.[55] studies, both well-differentiated Adenocarcinoma and Moderately differentiated Adenocarcinoma had equal numbers.

Comparison of Pathological T Staging Distribution with other studies:

Table 28: Comparison of Pathological T Staging Distribution with other studies

		Gomma et al. (2021) (n=196)	Melincovici et al. (2020) (n=31)	Yasuhito Iseki et al (2017) (n=49)	**Present study (n=50)**
T Staging	**T1**	1.5%	6.4%	48% (T1&T3)	**0%**
	T2	18.4%	29.03%		**26%**
	T3	70.4%	54.08%	52% (T4)	**62%**
	T4	9.7%	9.67%		**12%**

In the present study, 62% of cases were categorized in the T3 Stage. Comparable findings were noted by Gomma et al.,[56] and Melincovici et al.,[58] While the T4 Stage was the predominant category in the study done by Yasuhito Iseki et al.,[5]

Comparison of Pathological TNM Staging of Tumor with other studies :

Table 29: Comparison of Pathological Staging of Tumor with other studies

		Ji Eun Choi Et Al. (2017) (n=286)	Banias L et al, (2019) (n=112)	Melincovici et al. (2020) (n=31)	**Present study (n=50)**
TNM Stage of Tumor	**I**	49.7% (I+II)	7.1%	6.45%	**24%**
	II		53.6%	29.03%	**40%**
	III	71.4% (III+IV)	39.3%	54.8%	**34%**
	IV		0%	9.67%	**2%**

In our study, most cases were Stage II cancers (40%), similar to the study by Banias et al.[62] On the contrary, studies by Melincovici et al.,[58,] and Ji Eun Choi et al.,[57,] had a higher number of Stage III and Stage IV cancers.

Comparison of perineural & lymphovascular Invasion with other studies:

Table 30: Comparison of perineural & lymphovascular Invasion with other studies

		Gomma et al. (2021) (n=196)	Yasuhito Iseki et al (2017) (n=49)	Melincovici et al. (2020) (n=31)	**Present study(n=50)**
Perineural Invasion	**Absent**	Not Specified	Not Specified	77.41%	**92%**
	Present			22.58%	**8%**
Lymphovascular Invasion	**Absent**	87.8%	91%	78.3%	**94%**
	Present	12.2%	9%	21.7%	**6%**

Perineural and Lymphovascular invasion is the critical prognostic factors in colorectal carcinoma. In the present study, 8% of cases had perineural invasion, and 6% showed Lymphovascular invasion. Similar findings were noted by Gomma et al.,[56] Yasuhito Iseki et al.,[55] and Melincovici et al.,[56]

Statistical analysis of Loss of E-cadherin expression to various Histopathological parameters with other studies:

Table 31: Statistical analysis of Loss of E-cadherin expression to various Histopathological parameters with other studies

Loss of E Cadherin	Gomma et al.(2021) (n=196)	Yasuhito Iseki et al (2017) (n=49)	Ji Eun Choi Et Al. (2017) (n=286)	Melincovici et al. (2020) (n=31)	**Present Study (n=50)**
	p value	*p* value	*p* value	*p* value	***p* value**
Grading	0.46	0.81	0.00	0.018	**0.336**
T staging	0.7	0.44	0.04	Not Specified	**0.033**
LN Mets	0.8	0.24	0.001	0.277	**0.006**
TNM Staging	Not Specified	Not Specified	0.001	0.81	**0.04**
Lymphovascular invasion	0.12	0.3	0.009	0.39	**0.30**

Tumor invasion, migration, and metastasis are thought to be aided by cell-cell adhesion molecules. Malignant cells must separate from adjacent cells in order for a carcinoma to spread. This mechanism necessitates a breakdown of the E-cadherin/catenin complex, that seems to be totally vital in the genesis and progress of human carcinomas.[56] E-cadherin was found in high concentrations in membranes of non-cancerous colorectal epithelial cells. CRC cells exhibited a loss of membrane expression.[57] Loss of E-cadherin function decreases cell to cell contacts and impairs intercellular signaling but does not lead to direct tumor transformation. The subcellular distribution of E-cadherin is influenced by cell to cell contact in conjunction with membranous localization.[56]

In the current study, most participants had low (60%) E-cadherin expression, and 40% had high Expression of E-cadherin. Ji Eun Choi et al.,[57] and Melincovici et al.,[58] found a substantial association between loss of Expression of E-cadherin as well as cancer grading; such an association was not found in our study. T stage, Lymph nodal metastasis, and TNM staging showed a significant association with loss of Expression of E cadherin in our study, which was relatable to the study done by Ji Eun Choi et al.,[57] Lymphovascular invasion and Perineural invasion didn't show any association significantly with the loss of E cadherin expression. According to Yun J et al.,[63] low E-cadherin expression is correlated to poor survival outcomes. Gomma et al.,[56] found that some prognostic parameters are associated with decreased E-cadherin expression in CRC , its also associated with disease relapse in primary CRC and independent predictor for relapse of disease. For colorectal cancer development, these results could indicate that the Wnt/b-catenin pathway is crucial.

Statistical analysis of Accumulation of Beta-catenin expression to various Histopathological parameters with other studies:

Table 32: Statistical analysis of Accumulation of Beta-catenin expression to various Histopathological parameters with other studies

High Expression of Beta Catenin	Gomma et al. (2021) (n=196)	Melincovici et al. (2020) (n=31)	Ji Eun Choi Et al. (2017) (n=286)	**Present Study (n=50)**
	p value	*p* value	*p* value	***p* value**
Grading	0.71	0.23	0.001	**1**
T staging	0.39	Not Specified	0.59	**0.000**
LN Mets	0.95	0.046	0.012	**0.122**
TNM Staging	Not Specified	0.93	0.034	**0.005**
Lymphovascular invasion	0.41	0.7	0.007	**0.80**

Beta-catenin is important for epithelial integrity and carry double role in tumour growth reliant on its cellular localization.[5] Membrane Beta-catenin interact with intracellular region of E-cadherin to generate a complex that holds cell-cell adhesion. Nearly 60-80% of CRC are caused by an aberrant stimulation of the Wnt signalling system via its central molecule, Beta-catenin. It may occur in three biological places inside a cancer epithelial cell: the membrane, the nucleus, and the cytoplasm.[56]

In the nonappearance of Wnt signals, Beta-catenin is phosphorylated and inactivated protein complex, inhibiting phosphorylation and stabilising nuclear translocation, permitting cytoplasmic accumulation and Beta-catenin.[5]

In the current study, most participants had excessive cytoplasmic and nuclear expression (60%) of Beta-catenin levels, and 40 % had low Expression of beta cadherin. Increased Expression of Beta-catenin showed a strong association with TNM staging, which was also

seen in the Study by Ji Eun Choi et al.,[57] The rest of the parameters did not show significant p-value as noted in other studies such as Gomma et al.,[56] and Melincovici et al.,[58]

In study done by Bhattacharya et al.,[5] and Wong et al.,[64], a statistically major positive correlation was achieved between beta-catenin subcellular localization and their corresponding membranous, cytoplasmic, and nuclear score related to the AJCC-TNM Stage ($r = 0.512$; $p < 0.001$) of colorectal Adenocarcinoma.

However, the study done by Gomma et al. [56] stated that loss of Beta-catenin expression was significantly associated with aggressive behavior, high Stage, and distant metastases, and aggressive colorectal cancer is connected with a decreased expression of the Beta-catenin. In a study done by Wu et al.,[69] observed that high nuclear β-catenin expression seemed to be a poor prognostic factor and is related with liver metastasis and invasion.

Statistical analysis of CD 44 expression to various Histopathological parameters with other studies:

Table 33: Statistical analysis of CD 44 expression to various Histopathological parameters with other studies:

High Expression of CD44	Yasuhito Iseki et al(2017) (n=49)	Wang et al. (2022) (48 studies meta-analysis)	Ji Eun Choi Et al. (2017) (n=286)	**Present Study (n=50)**
	p value	*p* value	*p* value	***p* value**
Grading	0.55	0.25	0.36	**0.7**
T staging	0.07	0.46	0.02	**0.068**
LN Mets	0.71	0.04	0.005	**0.018**
TNM Staging	Not Specified	Not Specified	Not Specified	**0.064**
Lymphovascular invasion	0.18	0.153	0.51	**0.25**

CD44 was not expressed in non-neoplastic colorectal epithelial cells, but it was shown to be membranous in CRC cells.[57] CD44, a transmembrane glycoprotein of class 1, is essential for lymphocyte homing, angiogenesis, inflammation, cell proliferation, and motility and in conjunction with HA and glycosaminoglycans, it plays a key role in cell-to-ECM adhesion.[55]

According to the results of current meta-analysis, CD44 overexpression in colorectal cancer is an adverse prognostic marker that predicts a high grade and metastases in lymph nodes and distant regions.[7]

In the present study, the high Expression of CD44 indicated a significant association with Lymph nodal metastasis; related results were also noted in the study by Wang et al.,[64] and Ji Eun Choi et al.,[57] Rest of the parameters were not showing any statistical significance.

The meta-analysis done by Wang et al.,[65] concluded that poor differentiation, lymph node metastases, distant metastasis, and poor overall survival were linked to high CD44 Expression. Hong et al.[66] noted that among 162 colorectal carcinoma cases were not statistically significant association between pT ($p = 0.578$), pTNM stage ($p = 0.711$), and Grade ($p = 0.144$). Nonetheless, they discovered a greater expression in primary cancer than in paired lymph node metastases, as shown by p 0.001.

Yasuhito Iseki et al.,[55] reported that CD44 Expression, which is a poor prognosis indicator for patients undergoing curative surgery, was not shown to be a predictive predictor for patients with unresectable metastatic CRC.

Statistical Analysis of altered protein expression of EMT with CD 44:

In our study altered protein expression of EMT (Ecadherin and Beta Catenin) did not show any significant association with CD 44 . Similar findings were noted in the study done by Choi et al.,[57]and Ngan CY et al.,[67]

There is a strong relationship between CD44 and EMT in colorectal cancer, which is defined by the loss of epithelial markers such as E-cadherin and the acquisition of mesenchymal features in tumour cells. EMT results in the improvement of CD44-positive cancer stem cells.[7] In certain studies, EMT has been found to suppress the growth of stem cell-like features (Celia-Terrassa et al.,[68] 2012; Korpal et al.,[69] 2011), which opposes the idea of EMT-induced stemness. Similar results were seen in our study, where EMT showed no statistical significance with CD 44 expression. Another study found that Twist1 is required to develop CSC features; however, cancer stemness is individual of EMT or tumor invasion, showing that EMT and stemness are controlled independently (Beck et al., [70] 2015).

Statistical Analysis of EMT with other parameters :

Table 34: Statistical analysis of altered protein expression of EMT to various Histopathological parameters with other studies:

Altered protein expression of EMT markers	Ji Eun Choi Et al. (2017) (n=286)	Ngan CY et al., (2017) (n=140)	**Present Study (n=50)**
	p value	*p* value	***p* value**
Grading	0.007	0.643	**0.57**
T staging	0.7	0.54	**0.032**
LN Mets	0.026	0.03	**0.04**
TNM Staging	0.05	-	**0.016**

Cells undergo epithelial-mesenchymal transition (EMT) to become mesenchymal. Epithelial cells lose cell-to-cell contact and cell polarity during EMT, increasing mobility and invasiveness. EMT involves downregulation of epithelial markers and abnormal overexpression of mesenchymal markers.

In our Study, EMT showed a statistical significance with T stage(p=0.032), TNM staging (p=0.016), and Lymph nodal metastasis(p=0.04). Our results were similar to the studies done by Choi et al,[57] and Ngan Cy et al.[67]

According to the study by Choi et al, EMT-related protein expression, excluding snail, was strongly correlated with adverse clinicopathological characteristics. Previous studies observed that EMT-related marker expression is linked to tumour size, differentiation, growth patterns, metastasis, and poor prognosis.[57] Ribatti et al, concluded that EMT controls tumour development, progression, and metastasis in the tumour microenvironment.[3] Hypoxia, oxidative stress, food restriction, inflammation, and EMT transcription factors initiate and promote the mesenchymal phenotype and targeted therapeutics against EMT signalling regulators may assist cancer patients by targeting EMT-undergoing cells.[71]

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 2020 - 2022.
- A total of 50 cases of Colorectal carcinoma who underwent surgical resection were studied. H & E Slides of these cases were reviewed and performed immunohistochemistry against EMT markers ("E cadherin and Beta-Catenin") and stem cell marker -CD44.
- The altered protein expression of EMT ("E cadherin and Beta-Catenin" was evaluated and correlated with clinicopathological data of cases such as histological grading, lymph-node status, staging and lympho-vascular invasion. Later EMT and CD44 were also correlated.
- Peak incidence was in the 61-70 years age group (36%). Most frequent site of the tumor was rectum (48%) and most common side was Left (56%).
- Majority of cases were showed Moderately differentiated Adenocarcinoma (48%) and majority of the patients were belonging to T3 stage of tumor (62%)
- Most of the patients belong to TNM Stage II (40%) followed by TNM Stage III (34%).
- IHC expression of "E Cadherin, Beta Catenin and CD 44" was categorized into Low expression (0 or 1) or High expression (2 or 3).
- 60% cases showed low expression for E cadherin, 60% cases showed high expression for Beta-Catenin and 58% showed high expression for CD44.
- Low expression of E cadherin was found to be significantly associated with higher T stage($p=0.03$) , TNM Staging ($p=0.04$) and the presence of lymph node metastasis ($p=0.006$).

- High Beta Catenin expression was noticed to have significant correlation with higher T stage (p=0.006) and TNM staging (p=0.005).
- High CD 44 expression was found to be significantly associated with Lymph node metastasis (p=0.01).
- Altered expression of EMT related proteins ("E Cadherin and Beta-Catenin") showed significant correlation with higher T stage(p=0.03), TNM staging(p=0.016) and Lymph-node metastasis(0.04).
- These EMT markers ("E Cadherin and Beta-Catenin") and Cancer stem cell marker (CD -44) can be used as prognostic markers for predicting tumour growth and lymphnode metastasis.

CONCLUSION

CONCLUSION

In this study, altered expressions of EMT-related proteins (E Cadherin and Beta-catenin) showed significant association with the TNM staging and Lymphnode metastasis in Colorectal Carcinoma.

High expression of CD 44 was significantly associated Lymphnode metastasis.

EMT & Cancer Stem Cell Immunohistochemistry markers are possible biomarkers for aggressive tumor behaviour and lymphnode metastasis. Colorectal carcinoma expressing EMT and cancer stem cell Immunohistochemistry markers may offer prospective candidates for molecular targeted therapeutics for colorectal carcinoma in the future.

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ANNEXURES

ANNEXURE-I

INFORMED CONSENT FORM

STUDY TITLE: ASSOCIATION BETWEEN THE IMMUNOHISTOCHEMISTRY EXPRESSION OF E-CADHERIN, BETA-CATENIN AND CD-44 IN COLORECTAL CARCINOMA

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 Date:

(subject) Place

Name and signature / thumb impression Date:

Place:

(Witness/Parent/ Guardian/ Husband)

ANNEXURE-II
PATIENT INFORMATION SHEET

STUDY TITLE: ASSOCIATION BETWEEN THE IMMUNOHISTOCHEMISTRY EXPRESSION OF E-CADHERIN, BETA-CATENIN AND CD-44 IN COLORECTAL ADENOCARCINOMA

PLACE OF STUDY: Sri Devaraj Urs Medical College affiliated to R.L Jalappa Hospital and Research Centre ,Tamaka, Kolar.

The main aim of the study is to determine the of IHC expression of E cadherin, beta catenin, CD44 in colorectal Adenocarcinoma and to find the Correlation of the IHC expression of E cadherin, beta catenin and CD44 with Histopathological grade and stage of colorectal adenocarcinoma

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 breast carcinoma specimens of the patients. The specimens will be collected from the department of pathology, SDUMC,Kolar.

This study will be 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. This study does not change the treatment protocol . Immunohistochemistry will be self financed.

For any clarification you are free to contact the investigator.

PRINCIPAL INVESTIGATOR : Dr. AMRUTHA.T

PHONE: 9703009116

ANNEXURE III

STUDY PROFORMA

NAME: AGE: SEX:

HOSPITAL NO: BIOPSY NO: CASE NO:

NATURE OF SPECIMEN:

DIAGNOSIS:

GROSS:

Specimen size:

Site

Specimen type:

Tumor size:

HISTOLOGY:

GRADING:

TNM STAGE OF DISEASE:

TREATMENT DETAILS:

IHC EXPRESSION &SCORING

E CADHERIN -
LOW/HIGH

BETA CATENIN -LOW/HIGH

CD 44 -LOW/HIGH

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 = LOCATION OF TUMOR

POSITIVE LN= LYMPHNODE METASTASIS

TNM=TUMOUR NODE METASTASIS

LVI= LYMPHOVASCULAR INVASION

PNI= PERINEURAL INVASION

E CADHERIN (I)= ECADHERIN INTENSITY SCORE

ECADHERIN (P)= FRACTION PERCENTAGE OF POSITIVE EXPRESSION SCORE

E CADHERIN (T)= TOTAL E-CADHERIN EXPRESSION SCORE

BETA-CATENIN (I)= BETA CATENIN INTENSITY SCORE

BETA CATENIN (P)= FRACTION PERCENTAGE OF POSITIVE EXPRESSION SCORE

BETA CATENIN (T)= TOTAL BETA CATENIN EXPRESSION SCORE

CD 44 (I)= CD 44 INTENSITY SCORE

CD 444 (P)= FRACTION PERCENTAGE OF POSITIVE EXPRESSION SCORE

CD 44 (T)= TOTAL CD 44 EXPRESSION SCORE

HIGH= HIGH EXPRESSION

LOW= LOW EXPRESSION

SI NO.	AGE	SEX	YEAR	HOSPITAL NO.	BIOPSY NO.	SIDE	SITE	HISTOPATHOLOGICAL DIAGNOSIS	GRADE	SIZE OF TUMOR	LN RETRIEVED	positive LN	POSITIVE LN	LVI	PNI	T staging	pTNM staging	STAGE	E CADHERIN			BETA CATENIN			CD 44		
																			E CADHERIN (I)	E CADHERIN (P)	E CADHERIN (T)	BETA CATENIN(I)	BETA CATENIN(P)	BETA CATENIN(T)	CD 44(I)	CD 44(p)	CD 44(T)
1	60	F	2018	359750	B-50-18	RIGHT	COLON	MODERATELY DIFFERENTIATED ADENOCARCINOMA	LOW	3X5.5X0.5CM	5	ABSENT	0	ABSENT	ABSENT	2	PT2N0MX	I	2	2	HIGH	2	1	LOW	3	2	HIGH
2	60	M	2018	413664	B-429-18	LEFT	COLON	WELL DIFFERENTIATED ADENOCARCINOMA	LOW	4.8X3.5X2.5CM	0	ABSENT	0	ABSENT	ABSENT	4	PT4N0MX	II	1	1	LOW	3	3	HIGH	3	2	HIGH
3	51	F	2012	835745	B-1866-12	LEFT	RECTUM	MODERATELY DIFFERENTIATED ADENOCARCINOMA	LOW	6.8X5.4X2CM	6	PRESENT	1	ABSENT	ABSENT	3	PT3N1MX	III	2	1	LOW	3	3	HIGH	3	2	HIGH
4	73	F	2012	837910	B-1790-12	RIGHT	RECTUM	MODERATELY DIFFERENTIATED ADENOCARCINOMA	LOW	5.4X3.8X1.6CM	11	PRESENT	2	ABSENT	ABSENT	3	PT3N1MX	III	1	2	LOW	2	3	HIGH	2	2	LOW
5	85	F	2012	841155	B-2213-12	RIGHT	COLON	WELL DIFFERENTIATED ADENOCARCINOMA	LOW	8x4.5x2.2cm	9	PRESENT	1	ABSENT	ABSENT	4	PT4N1MX	III	3	2	HIGH	2	1	LOW	2	3	HIGH
6	45	F	2012	836409	B-2480-12	LEFT	RECTUM	POORLY DIFFERENTIATED ADENOCARCINOMA	HIGH	7.8X3.4X1.6CM	8	PRESENT	2	ABSENT	ABSENT	3	PT3N1MX	III	1	2	LOW	3	2	HIGH	3	3	HIGH
7	67	M	2012	816143	B-1358-12	RIGHT	RECTUM	WELL DIFFERENTIATED ADENOCARCINOMA	LOW	3.5X2X0.3 CM	0	ABSENT	0	ABSENT	ABSENT	2	PT2NXMX	I	3	2	HIGH	2	1	LOW	1	1	LOW
8	51	F	2012	835745	B-1899-12	RIGHT	RECTUM	MODERATELY DIFFERENTIATED ADENOCARCINOMA	LOW	6.6X4.5X1.5CM	8	PRESENT	1	ABSENT	ABSENT	3	PT3N1MX	III	2	1	LOW	3	3	HIGH	3	2	HIGH
9	55	F	2013	882182	B-298-13	LEFT	COLON	MODERATELY DIFFERENTIATED ADENOCARCINOMA	LOW	5X3.5X0.4 CM	7	PRESENT	2	ABSENT	ABSENT	2	PT2N1MX	III	1	1	LOW	2	2	LOW	2	1	LOW
10	60	M	2013	878863	B-331-13	LEFT	RECTUM	MODERATELY DIFFERENTIATED ADENOCARCINOMA	LOW	6.4X4.8X2.1CM	6	PRESENT	1	ABSENT	ABSENT	3	PT3N1MX	III	2	1	LOW	2	3	HIGH	3	2	HIGH
11	67	M	2013	883102	B-427-13	RIGHT	RECTUM	WELL DIFFERENTIATED ADENOCARCINOMA	LOW	3.8X4X1.2CM	3	ABSENT	0	ABSENT	ABSENT	2	PT2NXMX	I	1	2	LOW	2	3	HIGH	1	1	LOW
12	46	M	2013	879624	B-443-13	RIGHT	COLON	WELL DIFFERENTIATED ADENOCARCINOMA	LOW	5.6X4.8X1.5CM	5	ABSENT	0	ABSENT	ABSENT	3	PT3N0MX	II	2	2	LOW	3	2	HIGH	2	2	LOW
13	60	M	2013	903057	B-851-13	LEFT	RECTUM	MODERATELY DIFFERENTIATED ADENOCARCINOMA	LOW	7.5X3.8X2.1CM	9	PRESENT	1	ABSENT	ABSENT	3	PT3N1MX	III	2	1	LOW	2	1	LOW	2	3	HIGH
14	65	M	2013	958439	B-2098-13	RIGHT	COLON	POORLY DIFFERENTIATED ADENOCARCINOMA	HIGH	6.5X5X2.3CM	12	PRESENT	4	ABSENT	ABSENT	3	PT3N2MX	III	1	1	LOW	2	3	HIGH	3	3	HIGH
15	65	F	2013	940986	B-1844-13	LEFT	RECTUM	WELL DIFFERENTIATED ADENOCARCINOMA	LOW	5.8X4.6X2CM	8	ABSENT	0	ABSENT	ABSENT	3	PT3N0MX	II	2	3	HIGH	2	3	HIGH	3	2	HIGH
16	65	F	2013	928495	B-1652-13	RIGHT	RECTUM	MODERATELY DIFFERENTIATED ADENOCARCINOMA	LOW	4.2X5X1CM	9	ABSENT	0	ABSENT	ABSENT	2	PT2N0MX	I	3	3	HIGH	2	1	LOW	2	0	LOW
17	70	F	2013	854002	B-1714-13	LEFT	RECTUM	POORLY DIFFERENTIATED ADENOCARCINOMA	HIGH	5.2X4X0.8CM	2	ABSENT	0	ABSENT	ABSENT	2	PT2N0MX	I	2	3	HIGH	2	2	LOW	2	0	LOW
18	60	M	2014	981042	B-223-14	RIGHT	COLON	POORLY DIFFERENTIATED ADENOCARCINOMA	HIGH	3.3X3X1.5CM	10	PRESENT	1	ABSENT	ABSENT	3	PT3N1MX	III	2	2	LOW	2	2	LOW	3	3	HIGH
19	50	F	2014	32776	B-1999-14	RIGHT	RECTUM	MODERATELY DIFFERENTIATED ADENOCARCINOMA	LOW	6 X 4 X 1.6CM	9	ABSENT	0	ABSENT	ABSENT	3	PT3N0MX	II	2	3	HIGH	1	2	LOW	2	1	LOW
20	60	M	2015	155065	B-1593-15	RIGHT	COLON	MODERATELY DIFFERENTIATED ADENOCARCINOMA	LOW	4.5X3.8X 2.5CM	7	PRESENT	3	PRESENT	ABSENT	3	pT3N1Mx	III	2	1	LOW	2	3	HIGH	2	3	HIGH
21	70	M	2015	208706	B-3187-15	RIGHT	RECTUM	WELL DIFFERENTIATED ADENOCARCINOMA	LOW	8.5X5X0.5CM	0	ABSENT	0	ABSENT	ABSENT	4	PT4NXMX	IIB	2	2	LOW	3	3	HIGH	3	2	HIGH
22	55	F	2016	305665	B-2067-16	LEFT	COLON	MODERATELY DIFFERENTIATED ADENOCARCINOMA	LOW	7.4 X 6 X 1.5 CM	2	ABSENT	0	ABSENT	ABSENT	2	pT2 N0 Mx	I	2	3	HIGH	2	2	LOW	2	3	HIGH
23	76	F	2016	304816	B-2001-16	LEFT	COLON	WELL DIFFERENTIATED ADENOCARCINOMA	LOW	6 X 4 X 1 CM	7	ABSENT	0	ABSENT	ABSENT	2	T2 N0 Mx	I	1	2	LOW	3	3	HIGH	2	2	LOW
24	45	F	2016	254597	B-1885-16	RIGHT	RECTUM	MODERATELY DIFFERENTIATED ADENOCARCINOMA	LOW	1X1X2.4 CM	0	ABSENT	0	ABSENT	ABSENT	3	T3N0MX	IIA	2	2	LOW	2	3	HIGH	1	3	HIGH
25	60	F	2016	289663	B-2936-16	LEFT	COLON	POORLY DIFFERENTIATED ADENOCARCINOMA	HIGH	4.5X3.8X 2.5CM	9	PRESENT	3	ABSENT	ABSENT	4	T4aN1cMx	IIIB	1	2	LOW	2	3	HIGH	2	2	LOW
26	60	F	2016	239287	B-213-16	RIGHT	RECTUM	WELL DIFFERENTIATED ADENOCARCINOMA	LOW	4 X 2 X 2.5 CM	3	ABSENT	0	ABSENT	ABSENT	2	PT2N0MX	I	3	2	HIGH	2	1	LOW	2	2	LOW
27	68	M	2016	283041	B-48-16	LEFT	RECTUM	MODERATELY DIFFERENTIATED ADENOCARCINOMA	LOW	5 X 5 X 1 CM	1	ABSENT	0	ABSENT	ABSENT	2	T2 N0 Mx	I	3	3	HIGH	2	1	LOW	2	0	LOW
28	45	F	2017	402459	B-474-17	RIGHT	RECTUM	MODERATELY DIFFERENTIATED ADENOCARCINOMA	LOW	1.2 X 2 X 1.5 CM	6	PRESENT	3	PRESENT	PRESENT	3	T3 N1 Mx	IIIB	1	1	LOW	3	3	HIGH	3	2	HIGH
29	45	M	2017	428218	B-1207-17	LEFT	RECTUM	MODERATELY DIFFERENTIATED ADENOCARCINOMA	LOW	5 X 4 X 3 CM	25	PRESENT	6	PRESENT	PRESENT	3	pT3 N0 Mx	IIA	2	2	HIGH	2	1	LOW	2	3	HIGH
30	84	M	2017	502643	B-2504-17	LEFT	COLON	MODERATELY DIFFERENTIATED ADENOCARCINOMA	LOW	4 X 3.5 X 1 CM	0	ABSENT	0	ABSENT	ABSENT	3	T3 N0 Mx	IIA	1	3	HIGH	2	3	HIGH	2	3	HIGH
31	63	F	2018	308796	B-382-17	RIGHT	COLON	WELL DIFFERENTIATED ADENOCARCINOMA	LOW	12 X 9 X 5 CM	8	ABSENT	0	ABSENT	ABSENT	3	pT3 N0 Mx	IIA	2	2	HIGH	2	1	LOW	2	2	LOW

SI NO.	AGE	SEX	YEAR	HOSPITAL NO.	BIOPSY NO.	SIDE	SITE	HISTOPATHOLOGICAL DIAGNOSIS	GRADE	SIZE OF TUMOR	LN RETRIEVED	positive LN	POSITIVE LN	LVI	PNI	T staging	pTNM staging	STAGE	E CADHERIN			BETA CATENIN			CD 44			
																			E CADHERIN (I)	E CADHERIN (P)	E CADHERIN (T)	BETA CATENIN(I)	BETA CATENIN(P)	BETA CATENIN(T)	CD 44(I)	CD 44(p)	CD 44(T)	
32	70	M	2018	548316	B-613-18	LEFT	RECTUM	MODERATELY DIFFERENTIATED ADENOCARCINOMA	LOW	4.8 X 1.8 X 2 CM	3	ABSENT	0	ABSENT	PRESENT	3	T3 N0 Mx	IIA	2	2	HIGH	3	3	HIGH	2	1	LOW	
33	56	M	2018	550703	B-782-18	LEFT	RECTOSIG MOID	MUCINOUS ADENOCARCINOMA	LOW	3.5 X 2.8 X 0.8 CM	1	ABSENT	0	ABSENT	ABSENT	3	PT3N0MX	II	3	1	LOW	3	2	HIGH	3	3	HIGH	
34	30	M	2018	553372	B-1515-18	LEFT	RECTUM	MUCINOUS ADENOCARCINOMA	LOW	3.5 X 1.4 X 0.1 CM	6	PRESENT	2	ABSENT	ABSENT	3	T3 N1 Mx	IIIB	1	1	LOW	2	2	HIGH	2	2	HIGH	
35	62	M	2018	598766	B-429-18	RIGHT	COLON	WELL DIFFERENTIATED ADENOCARCINOMA	LOW	7.3X4.5X1.2CM	3	ABSENT	0	ABSENT	ABSENT	4	T4N0MX	II	1	1	LOW	3	3	HIGH	3	2	HIGH	
36	51	F	2018	615361	B-2030-18	RIGHT	COLON	MODERATELY DIFFERENTIATED ADENOCARCINOMA	LOW	2.3 X 1.4 X 1.4 CM	0	ABSENT	0	ABSENT	ABSENT	3	pT3 Nx Mx	IIA	2	1	LOW	2	2	LOW	2	3	HIGH	
37	72	M	2019	700297	B-728-19	RIGHT	COLON	WELL DIFFERENTIATED ADENOCARCINOMA	LOW	5.5X3X1CM	10	PRESENT	2	ABSENT	ABSENT	3	PT3N1MX	III	3	2	HIGH	3	3	HIGH	3	1	LOW	
38	42	F	2019	716626	B-2627-19	RIGHT	RECTUM	MUCINOUS ADENOCARCINOMA	LOW	4 X 2.5 X 1 CM	0	ABSENT	0	ABSENT	ABSENT	3	pT3 Nx Mx	IIA	2	1	LOW	2	2	LOW	2	3	HIGH	
39	61	F	2019	728096	B-1592-19	LEFT	COLON	WELL DIFFERENTIATED ADENOCARCINOMA	LOW	3.5 X 3 X 0.4 CM	0	ABSENT	0	ABSENT	ABSENT	2	pT2 Nx Mx	I	2	2	LOW	2	2	LOW	2	3	LOW	
40	53	F	2019	683154	B-1467-19	LEFT	RECTUM	WELL DIFFERENTIATED ADENOCARCINOMA	LOW	4.5 X 3 X 2.5 CM	3	ABSENT	0	ABSENT	ABSENT	3	pT3 N0 Mx	IIA	3	3	HIGH	1	1	LOW	1	1	LOW	
41	65	F	2019	655446	B-253-19	LEFT	COLON	MODERATELY DIFFERENTIATED ADENOCARCINOMA	LOW	3.5 X 2.5 X 1 CM	7	PRESENT	3	ABSENT	ABSENT	3	pT3 N1b Mx	IIIB	1	1	LOW	2	3	HIGH	2	3	HIGH	
42	35	M	2020	843200	B-1209-20	LEFT	RECTUM	MODERATELY DIFFERENTIATED ADENOCARCINOMA	LOW	2 X 2 X 0.5 CM	11	ABSENT	0	ABSENT	ABSENT	3	pT3 N0 Mx	IIA	2	3	HIGH	3	3	HIGH	3	2	HIGH	
43	75	F	2020	844349	B-838-20	LEFT	RECTOSIG MOID	WELL DIFFERENTIATED ADENOCARCINOMA	LOW	2 X 1.5 X 0.7 CM	1	ABSENT	0	ABSENT	ABSENT	3	pT3 N0 Mx	IIA	2	1	LOW	2	2	HIGH	2	1	LOW	
44	65	M	2020	875828	B-1732-20	LEFT	COLON	WELL DIFFERENTIATED ADENOCARCINOMA	LOW	2.5 X2 X 1.2 CM	20	ABSENT	0	ABSENT	ABSENT	4	pT4a N0 Mx	IIB	2	1	LOW	2	3	HIGH	3	1	LOW	
45	65	M	2020	866322	B-1590-20	LEFT	COLON	WELL DIFFERENTIATED ADENOCARCINOMA	LOW	8X7 CM	43	ABSENT	0	ABSENT	ABSENT	3	pT3 N0 Mx	IIA	3	2	HIGH	3	2	HIGH	2	2	HIGH	
46	60	F	2020	867528	B-1466-20	LEFT	COLON	WELL DIFFERENTIATED ADENOCARCINOMA	LOW	2.5 X 1.5 X 0.5 CM	1	PRESENT	1	ABSENT	PRESENT	3	pT3 N1a Mx	IIIB	2	2	LOW	2	3	HIGH	3	3	HIGH	
47	45	F	2020	846813	B-1265-20	RIGHT	RECTUM	MODERATELY DIFFERENTIATED ADENOCARCINOMA	LOW	3 X 2.5 X 0.5 CM	15	ABSENT	0	ABSENT	ABSENT	2	pT2 N0 Mx	I	2	2	HIGH	2	1	LOW	3	2	HIGH	
48	60	F	2020	841770	B-707-20	LEFT	COLON	MODERATELY DIFFERENTIATED ADENOCARCINOMA	LOW	3.5 X 3 X 1.5 CM	4	ABSENT	0	ABSENT	ABSENT	2	pT2 N0 Mx	I	2	3	HIGH	2	1	LOW	2	1	LOW	
49	68	M	2021	891338	B-172-21	LEFT	COLON	MODERATELY DIFFERENTIATED ADENOCARCINOMA	LOW	2.5 X 2.5 X 1.5 CM	0	ABSENT	0	ABSENT	ABSENT	3	pT3 Nx Mx	IIA	2	1	LOW	2	3	HIGH	2	2	LOW	
50	65	M	2021	903275	B-578-21	LEFT	RECTOSIG MOID	MODERATELY DIFFERENTIATED ADENOCARCINOMA	LOW	5X3.5X2.6 CM	22	PRESENT	9	ABSENT	ABSENT	3	pT3N2bM1	IVC	2	0	LOW	3	3	HIGH	2	3	HIGH	
51	68	M	2021	943507	B-1550-21	LEFT	COLON	WELL DIFFERENTIATED ADENOCARCINOMA	LOW	8.5X6X4 CM	1	ABSENT	0	ABSENT	ABSENT	3	PT3N0MX	IIA	1	2	LOW	2	2	LOW	2	2	LOW	