"EVALUATION OF CD10 BIOMARKER EXPRESSION IN DIAGNOSIS OF COLORECTAL CARCINOMA - A ONE YEAR CROSS-SECTIONAL STUDY AT TERTIARY CARE CENTRE OF BELAGAVI"

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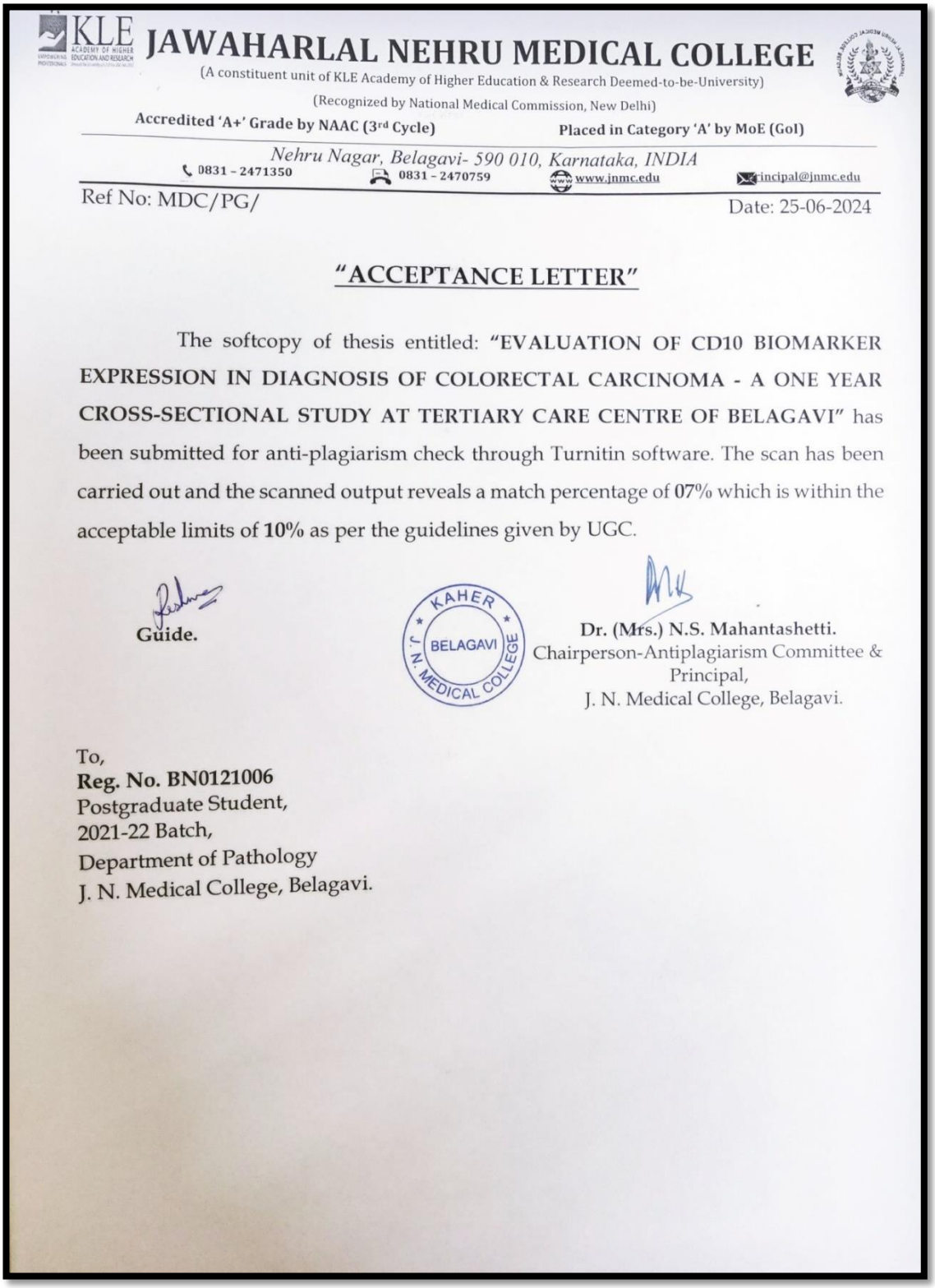

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LIST OF ABBREVIATIONS USED

AJCC	American Joint Committee on Cancer
AKT	Serine-threonine protein kinase / Protein kinase B
APC	Adenomatous polyposis coli
BRAF	B-Rapidly Accelerated Fibrosarcoma
CA 19.9	Carbohydrate antigen 19.9
CEA	Carcinoembryonic antigen
CD10	Cluster of Differentiation 10
CDX2	Caudal-type homeobox 2
CIMP	CpG island methylator phenotype
CIN	Chromo somal instability
CK	Cytokeratin
CRC	Colorectal carcinoma
CT	Computed tomography
DNA	Deoxyribonucleic acid
ERK	Extracellular signal-regulated kinase
FAP	Familial adenomatous polyposis
HNPCC	Hereditary Non-polyposis Colorectal Cancer
IBD	Inflammatory bowel disease
LET	Linear energy transfer
MAPK	Mitogen-activated protein kinase

MENK	Methionine enkephalin
MLH1	MutL protein homolog 1
MMP-9	Matrix Metalloproteinase-9
mRNA	Messenger ribonucleic acid
MSH2	MutS protein homolog 2
MSH6	MutS protein homolog 6
MUC	Mucin
MUTYH	mutY DNA glycosylase
NEP	Neutral endopeptidase
NOS	Not otherwise specified
PMS2	Post meiotic segregation increased, Saccharomyces cerevisiae
PP	Pancreatic polypeptide
PY	Peptide YY / Peptide tyrosine tyrosine
PI3K	Phosphoinositide 3-kinase
RAS	Rat sarcoma
RAF	Rapidly Accelerated Fibrosarcoma
ROS	Reactive oxygen species
TGF-β	Transforming growth factor-β
TNM	Tumor, Node, Metastasis
TPS	Tissue polypeptide specific antigen
Tris-EDTA	Tris-Ethylenediaminetetraacetic acid

UC	Ulcerative colitis
UICC	Union for International Cancer Control
WHO	World Health Organization
WNT	Wingless integrated

ABSTRACT

“Evaluation Of CD10 Biomarker Expression in Diagnosis of Colorectal Carcinoma - a One Year Cross-Sectional Study at Tertiary Care Centre of Belagavi”

Background and Objectives: Colorectal carcinoma (CRC) is the most common malignancy of the gastrointestinal tract. It is the 4th most common cancer in men and 3rd most common cancer in women worldwide and in Indian subcontinent. Few studies have found a role of CD10 marker in cancer progression and prognosis in cases of CRC. The present study aims to evaluate the expression of CD10 as a diagnostic marker in CRC and determine its association with staging and grading of carcinoma.

Methods: The present study was a prospective study which included 40 cases of CRC from January 2023 to December 2023. The clinical details and radiological reports of the patients under study were obtained from the medical records department of the hospital. The specimens were grossed, slides were made and stained for H&E and CD10 marker. Statistical analysis was done. The probability value of ≤0.05 was considered statistically significant.

Results: CD10 was expressed in tumour cells(tCD10), stromal cells(sCD10) and inflammatory cells(iCD10). CD10 expression was positive in 16(40%) cases and negative in 24(60.00%) cases of CRC. CD10 expression was higher in the stromal cells than in the tumour cells of CRC which is statistically significant(p=0.0001). Expression of sCD10 was decreased with increase in grades of carcinoma which was statistically significant (p=0.0072). No correlation between tCD10 expression with grades and stages of CRC was seen.

Conclusion: The findings of this study suggest that CD10 expression in CRC varies significantly among tumor cells, stromal cells and inflammatory cells. There is significant inverse correlation between stromal CD10 expression and tumor grade. These findings underline the complexity of the role of CD10 in the CRC microenvironment and suggest that while CD10 expression varies, it may not be a reliable independent marker for assessing the progression or severity of CRC.

Keywords: Colorectal carcinoma; CD10 marker; Immunohistochemistry.

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INTRODUCTION

Cancer is the leading cause of illness and mortality across the globe. Around 8.2 million deaths and 14 million newly diagnosed cases are directly attributable to cancer.[1] Colorectal carcinoma (CRC) is the most frequent gastrointestinal tract cancer and is a leading cause of morbidity and death globally. It is 4th most common cancer in men and 3rd most common cancer in women worldwide and also in the Indian subcontinent.[2]

Colorectal cancers develop latently, asymptomatically, and may remain undetectable for a long time. Prognosis and treatment of colorectal cancer depend on how advanced the disease is at the time of diagnosis. More than 70% of cases are diagnosed at an early stage so that effective treatment can be given. Unfortunately, approximately 20% of cases presenting late and having metastases have poor prognosis.[3]

Several biomarkers have been used to detect CRC, like carcinoembryonic antigen (CEA), carbohydrate antigen (CA 19.9), and tissue polypeptide specific antigen (TPS). However, these tests do not have diagnostic accuracy.[4] Hence there is a need for additional new biological markers which help in the diagnosis of colorectal carcinoma. One such marker is CD10.[3]

CD10 also known as neprilysin is a membrane-bound metallopeptidase. It is involved in carcinogenesis by the release of bioactive molecules that stimulate invasion, extracellular matrix degradation, promotion of angiogenesis, inhibition of apoptosis, and modulation of immune response.[5] In the past, CD10 was used to distinguish between haematological cancers as it was expressed in acute lymphoblastic leukaemia[5,6] and later studies have shown that it has a significant

impact on the initiation and spread of many other malignancies like endometrial sarcoma, prostate carcinoma[7,8], renal cell carcinoma[7,9], breast carcinoma[7,10], malignant melanoma[11].

In CRC, CD10 expression has been noted in tumor cells, stromal fibroblasts, and also in inflammatory cells.[12] Research in the past has shown the association of this marker with liver metastasis[12,13,14,15,16]. By degrading hepatic MENK, CD10 expression in CRC cells abolishes its anti-tumor impact and promotes liver metastasis.[17]

Studies have also shown that expression of this marker is associated with vascular invasion(veins) and the development of tumors to advanced stages in CRC patients.[18] According to a few studies, CD10 expression could be a valuable biomarker for determining the biological characteristics of early CRC.[19]

OBJECTIVES

1) To determine the potential of CD10 marker for the diagnosis of colorectal carcinoma
2) To determine the association of CD10 expression with the staging of CRC
3) To determine the association of CD10 expression with histological grading of carcinoma

REVIEW OF LITERATURE

LARGE INTESTINE AND RECTUM:

Embryology:

The caudal region of the midgut gives rise to the large intestine.[20]

The caecum, the appendix as well as the ascending colon, and the proximal two-thirds part of the transverse colon develop from the distal mid-gut tube.

The left side of the colon starting from the transverse colon to the proximal part of the anal canal develop from hindgut. Toldt fascia connects the mesentery to the peritoneum on the posterior wall of body cavity in areas of the ascending colon and descending colon.

The proximal part of the anal canal develops from the endoderm of hindgut and the distal part develops from the ectoderm near the proctodaeum. The anal pit is formed by the proliferation and invagination of the ectoderm in the proctodaeum area on surface of a portion of the cloaca. The pectinate line represents a line of junction between the endoderm and ectoderm slightly below the columns of Morgagni (Anal columns).[20,21]

ANATOMY:

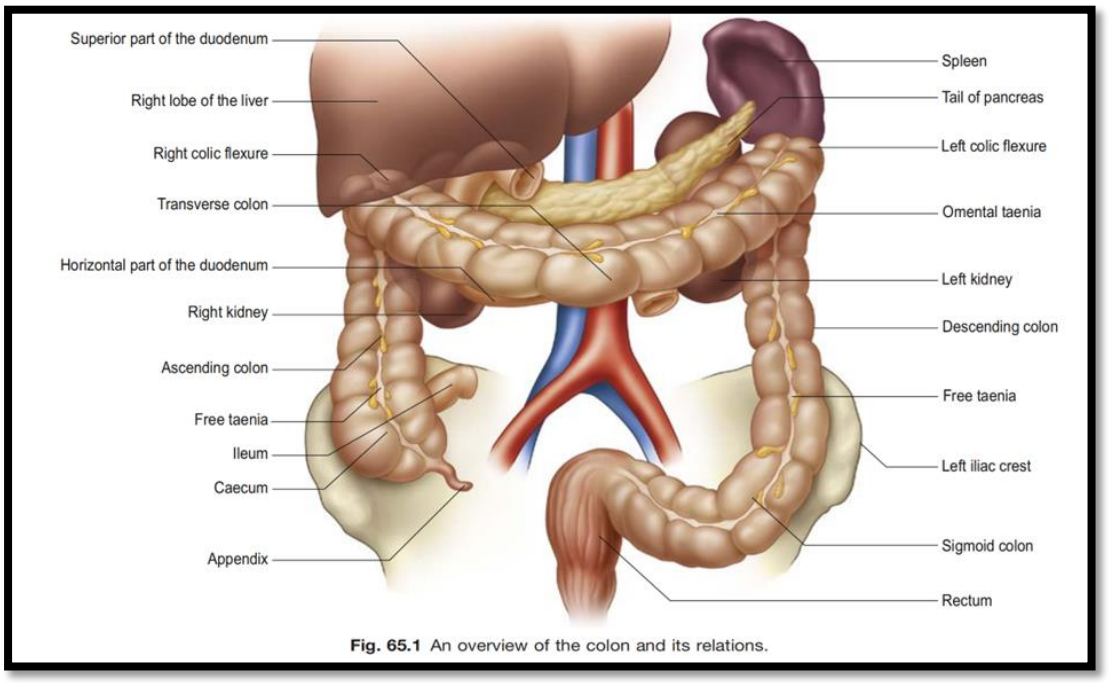


Fig1: Gray's Anatomy, the Anatomical Basis of Clinical Practice.42nd edition. 2021 Page no. 1186.[22]

The large intestine or colon is about 1.5 m long and it extends from the ileocecal junction to the anus.[23] It is a large tube that encircles the small intestine loops.[24]

Cecum

It is the sacculated portion of the large intestine that extends downward below the entrance of the ileum as an approximately 6 to 8-cm blind pouch. It is located in the right iliac fossa, above the lateral half part of the inguinal ligament and is almost entirely, invested with the peritoneum.

Appendix

The appendix is an extended diverticulum situated approximately 3 cm below the ileocecal junction, that arises from the posteromedial portion of the cecum.

The length of the appendix ranges from 2 to 20 cm, and its diameter is roughly 5 mm.

Ascending colon

The ascending colon is roughly 15 cm long. From the ileocecal junction, it starts and ascends to the hepatic flexure. It is surrounded by the peritoneum on the anterior side as well as on both sides.[24]

Transverse colon

The transverse colon is the longest segment of the large bowel and is approximately 45 cm long. It is suspended by a part that is 10 to 15cm wide which gives varying mobility to the colon but in between it is comparatively fixed at places of each flexure. It is entirely covered by peritoneum.[24]

Descending colon

This part of the colon starts from the splenic flexure and courses downward to the brim of the true pelvis. It is approximately 25 cm long and is invested by the peritoneum on its anterior and lateral parts only.[24]

Sigmoid colon

It measures approximately 35 to 40 cm in length. It is a movable, omega-shaped loop and is entirely covered by the peritoneum. The mesosigmoid is attached to the pelvic walls and is resting in a recess known as the intersigmoid fossa.

Rectum

The rectum is approximately 12–15 cm long with three lateral curves. The middle curve is convex to the left and the upper and lower curves are convex to the right.

The upper third of the rectum is covered by the peritoneum anteriorly and laterally, the middle third is covered by the peritoneum only anteriorly and the lower third of the rectum is entirely extraperitoneal.[24,25]

Blood supply:

The superior mesenteric artery supplies the right-sided colon (The caecum, the appendix, the ascending colon, and the right two-thirds part of the transverse colon). The inferior mesenteric artery supplies structures developing from the hindgut.

The rectum receives blood supply from the superior rectal artery, the middle rectal artery, and the inferior rectal artery which are branches of the inferior mesenteric artery, the internal iliac artery, and the internal pudendal artery respectively. The internal pudendal arteries give rise to the inferior rectal branches that supply the anal canal.

Venous drainage of the colon and rectum follows the arterial supply of it.[20,23]

Lymphatic drainage:

The colon drains into the epicolic lymph nodes, paracolic lymph nodes, and also in intermediate lymph nodes, and terminal lymph nodes (superior mesenteric and inferior mesenteric nodes). The part of the rectum and anal canal above the dentate line void into the internal iliac group and pararectal lymph nodes and below the dentate line void into the superficial inguinal lymph nodes.[20,23,25]

Histology of colon and rectum

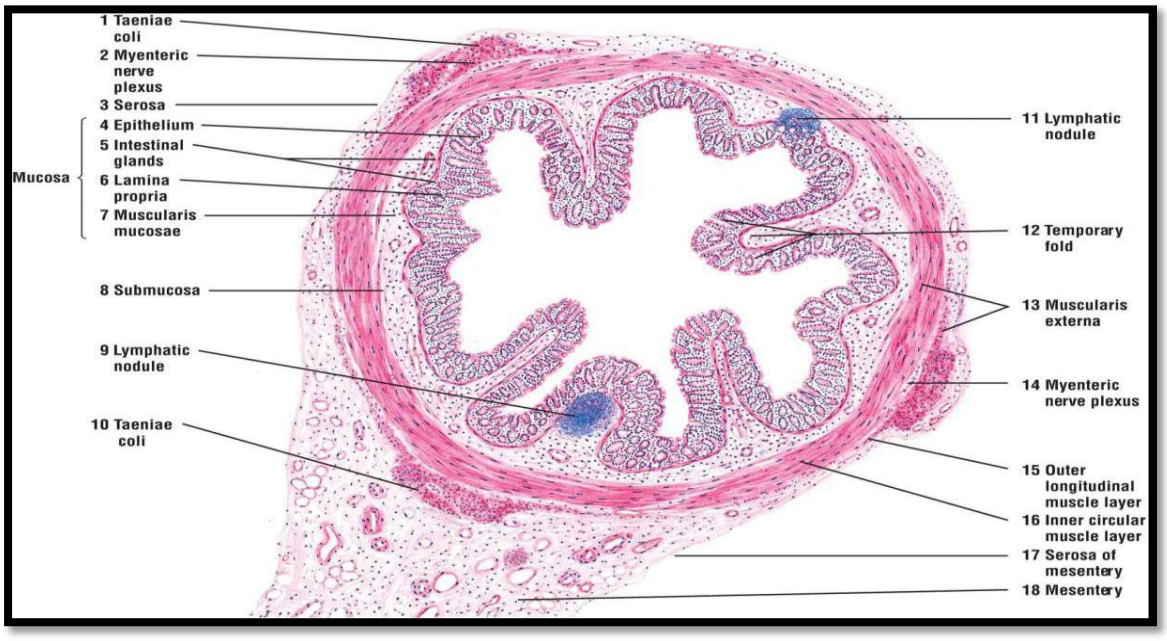


Fig 2: Large intestine: Transverse section of the colon with mesentery (H and E stain, Low magnification view).[26]

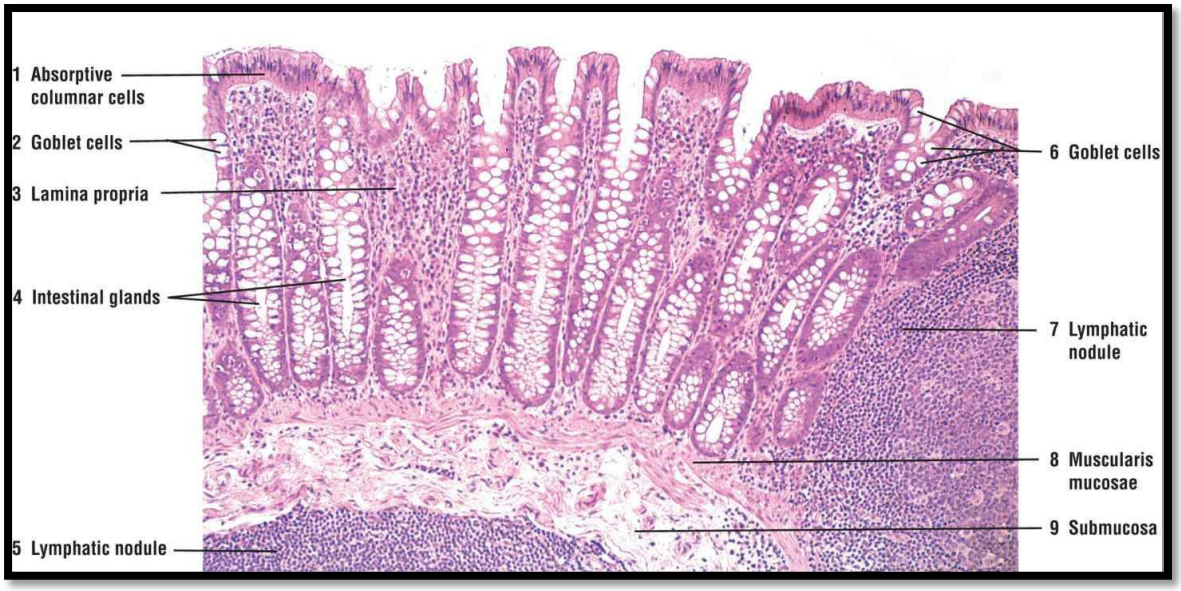


Fig 3: Large intestine: Wall of the colon. (H and E stain,30x).[26]

Colon

The wall of the colon has four layers as mucosa, submucosa, muscularis propria and serosa. The mucosa comprises of columnar epithelium, intestinal glands, lamina propria, and muscularis mucosae. The submucosa consists of blood vessels, connective tissue fibres, and nerves. The muscularis propria (externa) is made up of an inner circular smooth muscle layer and an outer longitudinal smooth muscle layer. Between the muscle layers of the muscularis externa is where the parasympathetic ganglia of the myenteric nerve plexus are found. The serosa covers the transverse colon and sigmoid colon.[26,27]

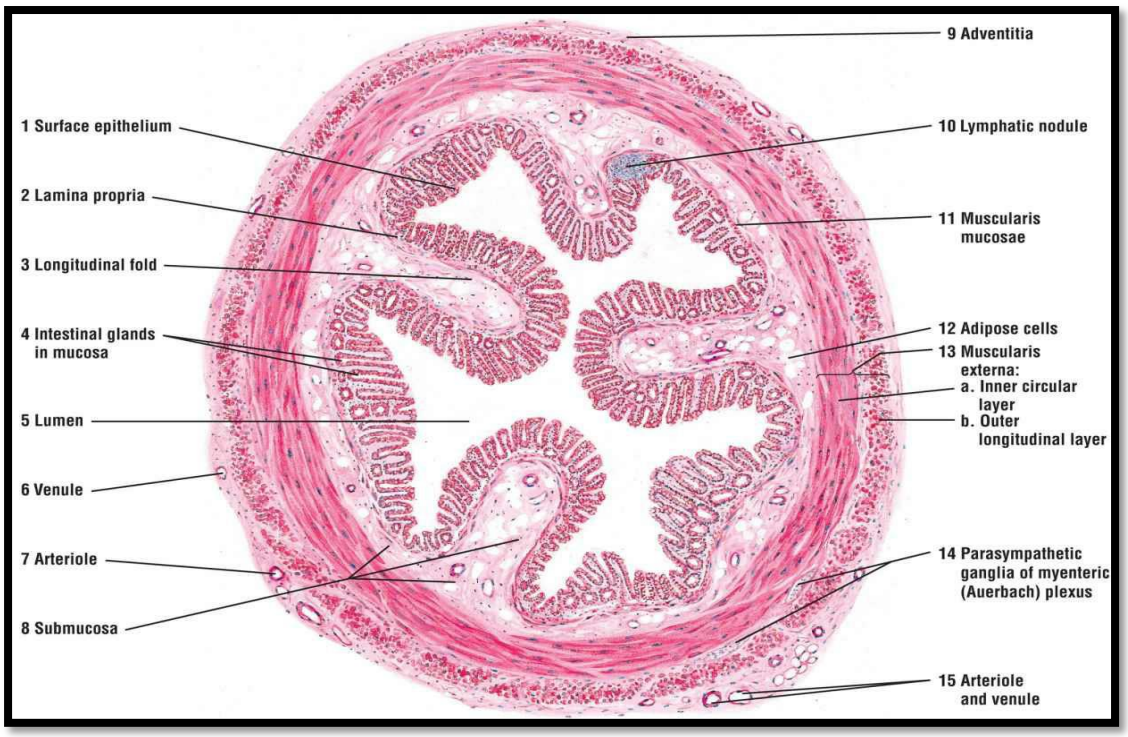


Fig 4: Transverse section of rectum. (H and E stain, low magnification view).[26]

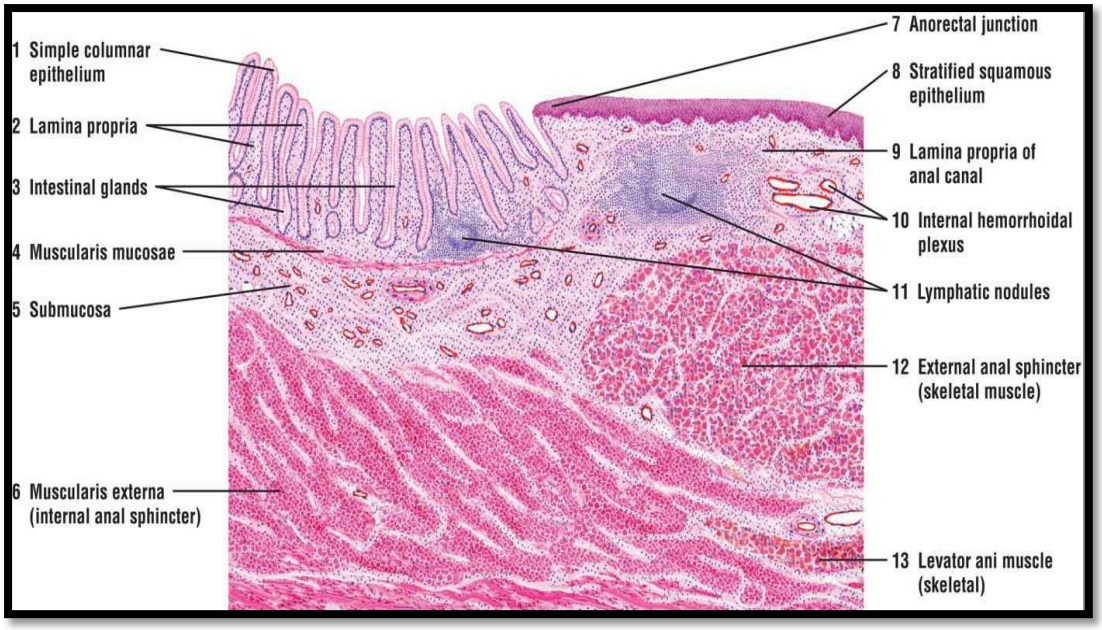


Fig 5: Longitudinal section of Anorectal junction (H and E stain, Low magnification view).[26]

Rectum

The colon and the upper rectum have similar histological features. The lowest portion of the rectum is corresponding to the area of the anal canal situated above the anorectal junction. The columnar epithelium changes to the stratified squamous epithelium of the skin in the area of the anal canal below the anorectal junction.[26,27]

The function of the large intestine are absorption of water and electrolytes like sodium and potassium. It also plays a role in the production and absorption of vitamins like vitamin K, and B vitamins including biotin. The large intestine helps in processing indigestible food material or chyme after absorption of most nutrients in the small intestine.[28]

The rectum acts as a temporary reservoir for the storage of feces. It also plays a role in maintaining continence and controlling defecation.[29]

COLORECTAL EPITHELIAL TUMORS:

Colorectal epithelial tumors are classified in different categories by the WHO (2019)[30] (Annexure I)

EPIDEMIOLOGY OF CRC:

About 9% of all malignancies are colorectal carcinomas. It ranks as the fourth most frequent global cause of cancer death.[1] It is more prevalent in affluent nations like North America, Australia, and Europe though it is less common in developing nations like Africa and Asia [1,2,31] emphasizing the significance of environmental, genetic, and nutritional factors.

In India, the annual incidence rates for colon carcinoma and rectal carcinoma in men are 5.36 and 5.17/100,000, respectively. 4.3/100000 women are diagnosed with colon cancer each year. Men were comparably at higher risk than women.[32] The incidence of CRC appears to be increasing in young patients (≤50 years of age).[33]

The majority of CRCs originate from adenomas, which serve as their precursor lesions. This phenomenon is termed as adenoma-carcinoma sequence.[34,35]

Colorectal adenoma develops from the normal epithelium of the colon and rectum. Adenomas later develop into more advanced adenomas. When significant mutations arise, advanced adenoma becomes early CRC.[35]

Adenoma to carcinoma transformation

Although not all adenomas develop into carcinomas, it is believed that most carcinomas have adenomas as their precursors. Tubular, Tubulovillous, Villous, and Serrated adenomas are the different types of adenomas.[36]

The tubular adenoma progresses to adenocarcinoma over a period of more than 10 -15 years and is accompanied by alterations first in the WNT signalling pathway, followed by the RAS-RAF-MAPK, PI3K-AKT and TGF-β pathways.[37,38]

Approximately 25% adenomas are of the tubular type, whereas over 75% are villous. Higher risk of cancer is associated with villous adenomas.[39,40,41]

Carcinoma will inevitably progress in those with inherited colon cancer syndromes and adenomas if surgery is not performed. As there is an almost inevitable adenoma-carcinoma sequence in FAP patients, they must be treated with surgery which has a positive impact on life expectancy of the patient. In FAP, polyps develop in early teenage and they will have an almost 100% risk of developing CRC by the age 40 if these patients are not treated by prophylactic colectomy.[42,43,44,45,46,47]

ADENOMA CARCINOMA SEQUENCE:

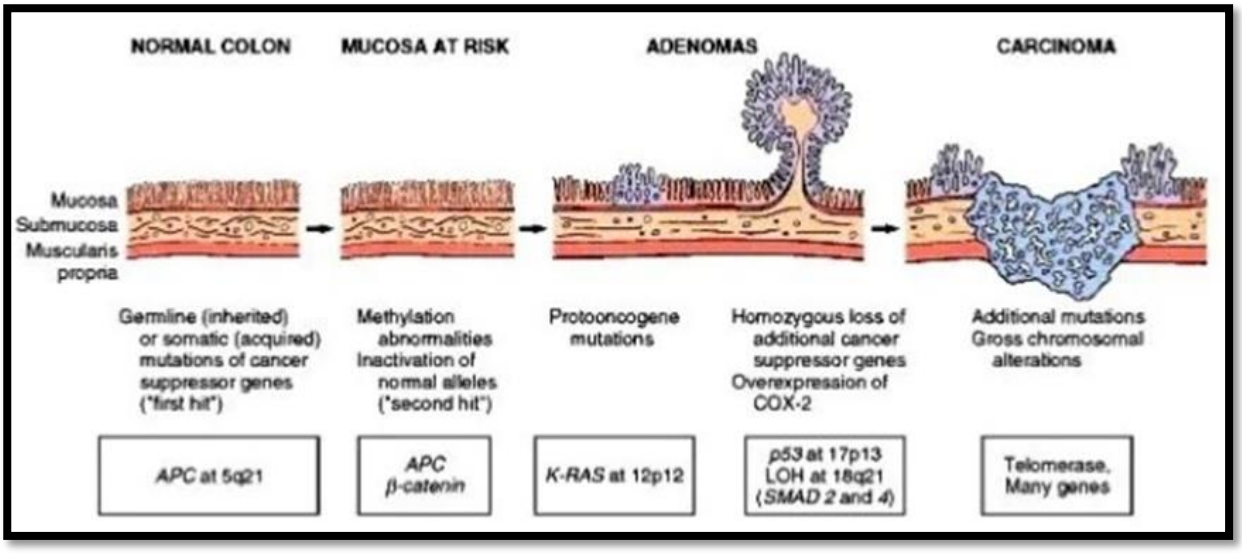


Fig 6: Adenoma carcinoma sequence[81]

The "adenoma-carcinoma sequence" is a multi-step process of colorectal cancer development that describes the progression from normal to dysplastic epithelium to carcinoma associated with the accumulation of multiple clonally selected genetic mutations. The concept of adenoma-carcinoma sequence provides an excellent model to study the genesis of invasive carcinoma and affords means of preventing CRC by removal of precursor lesions endoscopically.[48]

COLORECTAL ADENOCARCINOMAS:

ETIOLOGY AND RISK FACTORS:

The aetiology of colorectal adenocarcinomas is multifactorial like environmental, constitutional etc.

1) Dietary factors:

Research has indicated that consuming a diet high in fat and animal proteins, especially red meat and processed meat increases the risk of CRC.[49,50] The presence of growth-promoting dietary components including heme and arginine, an increased mutagenic intestinal environment, and an intestinal inflammatory response are the primary factors linked to the carcinogenic effects of red and processed meat. Reactive oxygen species (ROS) produced by heme iron promote the oxidation of proteins, lipids, and DNA, which increases colonic damage.[51]

Research has also indicated that a high dietary fiber intake can lower the chance of developing CRC by as much as 50%. A high fiber diet reduces the transit time of stool throughout the colon, which reduces the amount of contact between potentially carcinogenic substances and colonic epithelium and increases the water content of faeces, which dilutes carcinogens and procarcinogens present in faeces. It

is also thought that it stimulates the growth of beneficial gut microbiota, which in turn ferments fiber and produces short-chain fatty acids, which are substances that are suggested to exert tumor-suppressive effects.

Intake of fruits and vegetables has been linked to low risk of CRC development. Vegetables and fruits are rich in vitamins, minerals, folic acid and plant sterols, which have strong anti-inflammatory and antioxidant qualities and may prevent cellular and DNA damage.[52,53,54]

2) Cigarette Smoking:

Smoking is associated with a considerable rise in the risk of both sporadic colorectal adenomas and colorectal carcinomas.[55,56] Early age of initial use and pack years are associated with a considerable increase in cancer risk. Apart from being associated with BRAF mutations in colon malignancies, CpG Island Methylator Phenotype (CIMP), and microsatellite instability, smoking may also function as a tumor initiator.[57]

3) Alcohol consumption:

Due to aberrant DNA methylation, alcohol consumption also raises the risk of adenomas and malignancies. Increased consumption of alcohol is associated with considerable rise in the risk of colorectal carcinoma.[58,59,60]

4) Environmental risk factors:

Research on migrating populations and their progeny provides some indication of environmental risk. The incidence of colorectal cancer tends to rise near the population-average levels in the host nation among migrants moving from low-risk to high-risk countries. Other than migration, a few other geographic factors affect

variations in colon cancer incidence. Living in an urban area is among them. Urban dwellers consistently experience a higher incidence.[61]

5) Physical Activity and Obesity:

One further factor linked to colorectal cancer is the rising prevalence of obesity in both men and women, which can be linked to a lack of physical activity in everyday routines. Physical activity level and frequency have an inverse relationship with the risk of colorectal cancer.[53,62,63] Regular exercise and a balanced diet can help lower the risk of colorectal cancer, the evidence being more for colonic than for rectal cancer.[61,64]

6) Radiation exposure

Research conducted on radiation facility personnel and survivors of atomic bombs has shown that low-LET radiation exposure is a risk factor for colorectal carcinogenesis. Furthermore, research on radiation therapy for paediatric cancer and prostate cancer[65] patients revealed a markedly elevated risk of colon cancer recurrence, supporting the idea that therapeutic radiation is a risk factor for colorectal cancer.[66]

Additionally, it has been projected that the incidence of colon cancer from diagnostic radiation exposures is higher than that of the population that has not been exposed, particularly from procedures like CT that include many lengthy exposures.[66,67,68]

7) Diverticulitis:

The risk for CRC is high in patients with complicated diverticulitis as compared to patients with uncomplicated diverticulitis.[69]

8) Inflammatory bowel disease:

Long-standing Ulcerative colitis and Crohn's colitis have an approximate 2–3 times increased risk of CRC. About 3.2% of all UC patients will develop CRC, with cumulative risks of 1%, 3%, and 7% after 10, 20, and 30 years of illness, respectively.[70,71]

CRC developing in IBD is considered a model of inflammation-induced carcinogenesis. Prolonged inflammation causes DNA damage due to oxidative stress, which triggers the activation of tumor promoter genes and the deactivation of genes that prevent tumor growth.[72] The sequence of inflammation, dysplasia and cancer is characterized by a steady increase in markers of oxidative damage and double-strand breaks in DNA.[73]

Compared to the general population, patients with IBD develop colorectal cancer at a younger age and have a slightly lower 5-year survival rate.[74] Risk factors for the development of colorectal cancer in IBD patients are duration of disease, extent of disease, younger age at the time of diagnosis, and severity of inflammation. In cases of IBD cancer most commonly developed on the left side of the colon, particularly in the rectum and anal canal.[75,76]

9) Schistosomiasis:

Individuals with Schistosoma japonicum infection have a higher risk of developing CRC. Infection with schistosomiasis frequently results in intestinal involvement.[77,78,79] There is egg deposition and associated granulomatous inflammation in the intestine which leads to chronic colitis and is associated with polyp formation.[77,80] It is hypothesized that people may be more susceptible to colorectal cancer as a result of this persistent chronic inflammation.[77]

10) Genetic factors:

A) Familial adenomatous polyposis (FAP) -

FAP is an autosomal dominant condition in which numerous colorectal adenomas are developed in patients during adolescence. It occurs due to somatic mutations in the adenomatous polyposis coli (APC) gene.[81,82,83] This gene is an important negative regulator of the WNT signalling pathway. A diagnosis of classic FAP requires the presence of at least 100 polyps, though thousands may be present. FAP-associated polyps are morphologically identical to sporadic adenomas, except for their numbers.[81] FAP is accountable for around 0.5 to 1% of all cases of colorectal cancer and affects men and women equally.[83,84,85]

If a preventive colectomy is not performed, 75% of Familial adenomatous polyposis patients will have colon carcinoma by the time they are 30 years old, and by the time they are 50 years old, this number rises to 100%. Prophylactic colectomy is therefore accepted course of therapy. Colectomy protects against CRC, although patients still have a chance of developing carcinoma elsewhere. Among FAP patients the ampulla of Vater, and the stomach are frequent extracolonic locations of adenomas.

Some polyposis patients have autosomal recessive disorder termed MUTYH-associated polyposis or MAP. Compared to FAP, this has less than 100 polyps, develops at a later age, and has a delayed onset of colon carcinoma.[81]

B) Hereditary Non-polyposis Colorectal Cancer (HNPCC)-

HNPCC is caused by mutations in mismatch repair genes, which encode proteins that detect, eliminate, and repair mistakes during DNA replication. It is also

called Lynch syndrome and the original description of Lynch syndrome was based on a familial clustering of malignancies at multiple sites, including the brain, small bowel, hepatobiliary system, pancreas, ovary, ureters, stomach, colorectum, and skin.[81,86]

The most prevalent syndromic form of colon carcinoma is HNPCC which is estimated to represent 2% to 4% of all colorectal malignancies. Colon cancers in people with HNPCC frequently arise in the right colon and usually manifest at a younger age than sporadic colon cancers. Although there are at least five MMR genes, MSH2 or MLH1 mutations account for the majority in the patients with HNPCC.[81]

Pathways of Colorectal Carcinogenesis

Adenoma–Carcinoma Sequence

The multistep process of cancer development known as the "adenoma-carcinoma sequence" provides a comprehensive explanation for the development of colorectal adenocarcinomas. The majority of colorectal carcinomas develop from pre-cancerous polyps, which are generally divided into two groups: serrated polyps and conventional tubular adenomas. Adenomas arise when regular systems that control DNA repair and cell division are disrupted. Adenomatous polyps progressively grow larger, acquire more dysplastic characteristics, and ultimately obtain the ability to invade. The change from a normally proliferating epithelium to one that is hyperproliferative is marked by sequential changes in important growth regulating genes. Additional genetic changes identify the transition from early to intermediate neoplasia, advanced polyps with high-grade dysplasia, and ultimately invasive malignancies. Depending on a number of circumstances, the transition from adenoma to carcinoma can take anywhere between 10 and 40 years.[87]

CIN pathway

The CIN pathway is seen in about 65%–70% of sporadic colorectal tumors.[87,88,89] It is characterized by chromosomal changes such as somatic copy number alterations which are caused by aneuploidy, insertions, deletions, amplifications or loss of heterozygosity. Tumors which develop through this pathway are non-hypermutated tumors. Almost all CIN tumors have activated WNT pathways, and 80% of these tumors have been found to have APC mutations.[87,90]

MSI pathway

The MSI pathway is the primary mechanism for hypermutable phenotype of colorectal tumors. Mutations in DNA mismatch repair (MMR) genes (MLH1, MSH2, MSH6, PMS2) cause instability within microsatellite regions. MSI is seen in nearly 15% of sporadic colorectal carcinomas and almost all colorectal tumors in people with Lynch syndrome which is the most common hereditary colon cancer syndrome and is linked to germline mutations in DNA MMR genes.[87,91]

Serrated Neoplasia Pathway

Nearly 15% of CRCs are thought to develop from serrated polyps through the serrated neoplasia pathway. It is thought that BRAF mutations arise early in the serrated pathway, constitutively activating the MAPK—ERK pathway and inducing uncontrollably massive cell divisions leading to carcinoma.[87,92]

Clinical features:

Symptoms of CRC are nonspecific and insidious. Up to 35% of patients of CRC present in an emergency. Individuals with colon carcinoma are completely asymptomatic in one-third of cases, despite the fact that the disease typically

manifests in an emergency setting with prior symptoms including abdominal discomfort, weight loss, and diarrhoea.[93]

The clinical presentation in CRC depends upon the location of the tumor in the colon and also on the stage of the tumor i.e., early or advanced. Patients with right-sided colon carcinoma mainly present with symptoms of anemia like fatigue, and weakness. Left-sided CRC patients may present with occult bleeding, bowel habit changes, cramps, and pain in the lower quadrant of the left side. Patients may also present with abdominal pain and obstruction.[81]

In addition to these, additional intestinal and extraintestinal symptoms unique to the disease process are present in CRC that arise in specific conditions of IBD, polyposis syndromes, and HNPCC [81]

Staging of CRC:

In the past many years, pathologists have used the classic Duke's staging provided in 1932 which was later modified (Annexure II), and the Astler-Coller staging, which was provided in 1954(Annexure III). However, the AJCC and the Union for International Cancer Control (UICC) recommended TNM system[97] (Annexure IV) quickly replace these two classifications as part of a drive for a more universal and uniform method of reporting.[94]

Pathologic features:

Gross:

The majority of CRCs are either ulcerative/infiltrating or polypoid. The ulcerative/infiltrating type is centrally ulcerated and has a less elevated surface. The polypoid CRC appears as a bulky mass with clearly defined rolled borders and a distinct border that separates it from the normal intestine.

There is another variant called flat or depressed carcinoma which is thought to arise de novo.[98]

In the proximal colon tumors frequently develop as polypoidal, exophytic masses which grow in the wall of the ascending colon and cecum and they rarely cause obstruction. On the other hand, carcinomas in the distal part of the colon typically form annular lesions which form "napkin-ring" constrictions and cause narrowing of the lumen.[81]

Rarely (0.3%), CRC can resemble linitis plastica of the stomach grossly.[94]

Microscopy: The most common type (90%) of all CRCs is Adenocarcinoma.

1) Adenocarcinoma, NOS

The common CRC is a well-to-moderately differentiated adenocarcinoma secreting variable amounts of mucin. The tumor cells consist of a combination of goblet and columnar cells, with occasional neuroendocrine cells and Paneth cells.

The carcinoma elicits an inflammatory and desmoplastic reaction, which is prominent at the edge of the tumor. The inflammatory reaction consists of lymphocytes, plasma cells and histiocytes. Villous or papillary patterns may be present on the tumor surface. The tumor cells may be seen invading all the layers of the colon and extending into the pericolic fat, invading perineurial spaces and lymphovascular spaces.[98]

2) Mucinous adenocarcinoma:

Mucinous adenocarcinoma accounts for 10 % of all CRCs. It is a special type of CRC where large lakes of extracellular mucin are formed, and mixed with tumor cells.

As per the definition given by WHO, 50% mucinous foci should be present to consider it a mucinous adenocarcinoma. They are associated with tumors with MSI and have a worse prognosis than nonmucinous colorectal carcinomas.[98]

3) Signet ring carcinoma:

It is a rare (1%) type of CRC. Grossly, it presents as widespread invasion of the colon wall or may be seen arising in an adenomatous polyp. By definition, the tumor should comprise more than 50% of signet ring cells, regardless of the quantity of extracellular mucin. Microscopically, there is diffusely arranged tumor cells with very less formation of glands. The intracellular accumulation of mucin results in displacement of the nucleus and a typical signet ring configuration of the cells.[98]

4) Medullary adenocarcinoma:

It is characterized by sheets of malignant cells with vesicular nuclei, prominent nucleoli, and abundant cytoplasm and shows infiltration by lymphocytes and neutrophils. It is frequently associated with MSI in combination with BRAF mutations and has a good prognosis.[30]

5) Serrated adenocarcinoma:

This type shows morphological similarities with serrated polyps. It shows glandular serration admixed with mucinous areas. Tumor cells have a low nuclear-to-cytoplasmic ratio. 10 to 15% of CRCs are classified as serrated adenocarcinoma.[30]

6)Micropapillary adenocarcinoma:

It accounts for 5 to 20% of CRCs. It shows tumor cells arranged in small clusters within the stroma and >5% of tumor should show this characteristic. This

type has poor prognostic factors like a lymphovascular invasion, and perineural invasion and has an increased risk for metastasis to lymph nodes.[30]

7)Adenoma like adenocarcinoma:

This is an invasive adenocarcinoma in which more than 50 % of invasive areas have adenoma-like features with villous structures. Mild desmoplastic reaction and pushing growth pattern are seen. It has a high KRAS mutation rate. It shows a favorable prognosis.[30]

8)Adenosquamous carcinoma:

It is a rare type and accounts for <0.1% of CRCs and shows features of adenocarcinoma and squamous cell carcinoma both.[30]

9)Undifferentiated carcinoma:

This type lacks morphological, immunohistochemical, and molecular evidence of differentiation. They are infrequently associated with MSI.[30]

10) Carcinomas with sarcomatoid components:

This type is characterized by partly undifferentiated histology and sarcomatoid features like rhabdoid or spindle cell components**.** The tumor cells are dyscohesive, large and show rhabdoid cells with abundant eosinophilic rhabdoid bodies in the cytoplasm, within myxoid matrix. Areas of glandular differentiation and pleomorphic spindle cells are also seen.[30]

Histological grading of colorectal adenocarcinoma:

Based mostly on the arrangement of cells concerning the degree of tubule (acini) formation, adenocarcinomas can be categorized into three grades. The grade is determined by the percentage of tumors showing well-formed glands.[94]

1) Grade 1 or well-differentiated adenocarcinoma shows glands in > 95% of the tumor.
2) Grade 2 or moderately differentiated adenocarcinoma shows 50% to 95% of glands in the tumor.
3) Grade 3 or poorly differentiated adenocarcinoma shows 5% to 49% of glands in the tumor.
4) Grade 4 or undifferentiated carcinoma shows less than 5% of glands in the tumor.

Signet ring carcinoma and mucinous adenocarcinoma are considered grade 3 carcinomas.[99]

Prognostic factors:

Several clinical and pathological characteristics are associated with the prognosis of CRCs. The most significant predictor of the clinical behaviour of CRC is the anatomic extent of the tumor or pathologic stage.[98,100] Serosal involvement and regional lymph node involvement are also a critical prognostic parameter. Tumor subtype has a significant impact on prognosis because signet ring carcinoma, mucinous carcinoma, and undifferentiated carcinomas are more clinically aggressive tumors. Also, high-grade carcinomas are aggressive as compared to low-grade carcinomas. Tumors with an infiltrative margin have a more aggressive clinical course than tumors with an expanding border and smooth rounded contours. Other factors

like extremes of age (very young and very old), male gender, perforation of the bowel wall, vascular invasion, perineural invasion, and prominent tumor angiogenesis have adverse prognoses.[53] In colorectal carcinoma, lymphovascular invasion is a stage-independent indicator of a poor outcome.[101]

Histochemical and immunohistochemical characteristics of CRC:

Mucin stains are positive in most of the CRCs. MUC1 and MUC3 immunostains are the primary mucin protein cores expressed by conventional CRCs. There is also expression of MUC13, particularly in poorly differentiated carcinomas. Recently, it has been discovered that elevated expression of MUC2, MUC5AC, and MUC6 is linked to the serrated pathway of colon carcinoma.

All colorectal adenocarcinomas are positive for cytokeratin (CK); the most prevalent pattern is positivity for CK20 and negativity for CK7. However, CK7 positivity can be seen in some poorly differentiated CRCs. CDX2 is found by immunohistochemistry in the overwhelming majority of CRCs. CEA is also used in the screening of CRCs.

Tumor-associated glycoprotein 72 (TAG-72) is detected in almost all invasive colon carcinomas, most adenomatous polyps, and even in normal colon mucosa; however, the expression pattern and frequency vary depending on the condition.

Other markers expressed by CRCs, regardless of differentiation are villin, neuropilin-1, and cathepsin B. Also, human chorionic gonadotropin (HCG) immunoreactivity is seen in a significant proportion of colorectal carcinomas and this appears to be more frequent in mucinous and poorly differentiated carcinomas.[98]

In the past few years, few studies have used CD10 marker in the diagnosis of CRC.

CD10 BIOMARKER:

Cluster of differentiation 10 (CD10), is a type II integral membrane protein of the M13 family that ranges in size from 90 to 110 kDa. It is also known as neprilysin or neutral endopeptidase (NEP). The NEP protein consists of 3 domains: a transmembrane hydrophobic domain, an extracellular domain with catalytic activity, and a cytoplasmic N terminal domain.[5,102,103]

Earlier it was known as the common acute lymphoblastic leukemia antigen (CALLA) since it was shown to be present in the cells of acute lymphoblastic leukemia but later discovered to be expressed in the lung, kidney, adrenal glands, intestine, endometrium, and prostate. As CD10 was found in different cells it indicates that it has a diverse biological role that is not limited to hematological malignancies.[5]

Structural features and molecular features of CD10:

CD10 is located on chromosome 3 at the 3q25.2 cytogenetic region and is a single copy that is larger than 45 kb. CD10 exhibits alternative splicing in the 5' untranslated region, resulting in four distinct mRNA transcripts though the coding region is unaltered.

A 3-dimensional secondary structural model of the enzyme CD10 reveals an active site with 400 residues, situated in the center of the pocket.[5,104,105] This site has two histidine-active sites that are responsible for the binding of cofactor zinc and one glutamate-active site that is responsible for catalysis. The activity of CD10 is

inhibited by phosphoramidon which is a metabolite isolated from Streptomyces, by binding to its active enzymatic site.[5]

Few studies have found the role of CD10 biomarker in cancer development and prognosis in cases of CRC. The present study aims to evaluate the expression of CD10 as a diagnostic marker in colorectal carcinoma and determine its association with staging and grading of carcinoma.

METHODOLOGY

Study design: Cross-sectional study

Source of Data: Specimens of colorectal carcinoma received at the Histopathology laboratory at KLE'S DR. PRABHAKAR KORE HOSPITAL and MEDICAL RESEARCH CENTER, BELAGAVI.

Study Period: One year- 1st January 2023 to 31st December 2023

Sample Size: 40

Sampling technique: Universal sampling.

On an average, number of specimens of Colorectal carcinoma received in histopathology department per year was 40. So, 40 specimens of CRC were studied.

Inclusion Criteria: All the specimens diagnosed histopathologically as CRC.

Exclusion Criteria: Benign lesions and biopsy.

Ethical clearance: The ethical clearance was acquired from the Institutional Ethics Committee, JNMC, Belagavi before the commencement of study.

Data collection procedure: A total 40 cases of colorectal carcinoma received at the histopathology laboratory were studied. The specimens were kept in 10% formalin overnight for fixation. The specimens were taken for grossing on the next day and representative tissue bits were given. The tissue bits were taken for processing in a tissue processor.

Paraffin embedded blocks were prepared. Sections measuring 3-4 µ thick were cut using microtome and taken on to the slides and then stained using haematoxylin and eosin stain for histopathological evaluation.

Histological evaluation:

The slides were evaluated by a pathologist and grading was done as per World Health Organization criteria and reporting was done.

Grading:1) Well-differentiated (Grade1)

2) Moderately differentiated (Grade2)

3) Poorly differentiated (Grade3)

Immunohistochemical analysis:

CD10 staining was performed manually using an anti CD10 antibody (Vitro Master diagnostica, Clone56C6) using peroxidase-antiperoxidase method and H2O2-diaminobenzidine as substrate-chromogen. Antigenic unmasking was done on the sample before it was incubated with the primary antibody. This was done by heating the sample in Tris-EDTA buffer at pH 8 in a pressure cooker at 60 degrees Celsius.

Immunohistochemical staining evaluation:

Brown color in the membranous area or cytoplasm of the cell is considered as positive immunoexpression. The samples with less than 10% of stained cells are considered negative and samples with more than 10% of stained cells are considered positive for immunoexpression of CD10 .[5]

The percentage of tumor cells stained with biomarker is used as an indicator of the degree of biomarker expression.[5]

Score 0 – percentage of stained cells less than 10%

Score 1 – percentage of stained cells is 11- 25%

Score 2 – percentage of stained cells is 26 -50 %

Score 3 – percentage of stained cells more than 50 %

The percentage of stained stromal cells was assessed and scoring was given.[2]

Score 0 – percentage of stained cells is 0-5 %

Score 1 – percentage of stained cells is 5-30 %

Score 2 – percentage of stained cells is 30-60 %

Score 3 – percentage of stained cell more than 60 %

Statistical Analysis: The data was analyzed using The SPSS Software version 23 and Microsoft Word and Excel were used to generate Graphs and tables.

Descriptive statistics such as Mean & Percentages were calculated. The difference in the number of cases with the expression of CD10 in tumor cells and stromal cells was analyzed by chi-square test. The relationship between the expression of markers and grading and staging of carcinoma was analyzed using Spearman's rank correlation coefficient method.

The probability (P) value of < 0.05 was considered statistically significant.

RESULTS

Data was collected for the 40 colorectal carcinoma cases.

The data obtained from this study was compiled and tabulated. It was then statistically analyzed.

The age group of patients ranged from years ≤ 60 years to ≥71 years (Mean = 62.30 years) (Table 1 and Fig 7)

Table 1: Distribution of cases according to age

Age groups	No of cases	% of cases
<=60yrs	15	37.50
61-70yrs	13	32.50
>=71yrs	12	30.00
Total	40	100.00
Mean	62.30	
SD	12.97	

Fig 7: Distribution of cases according to age

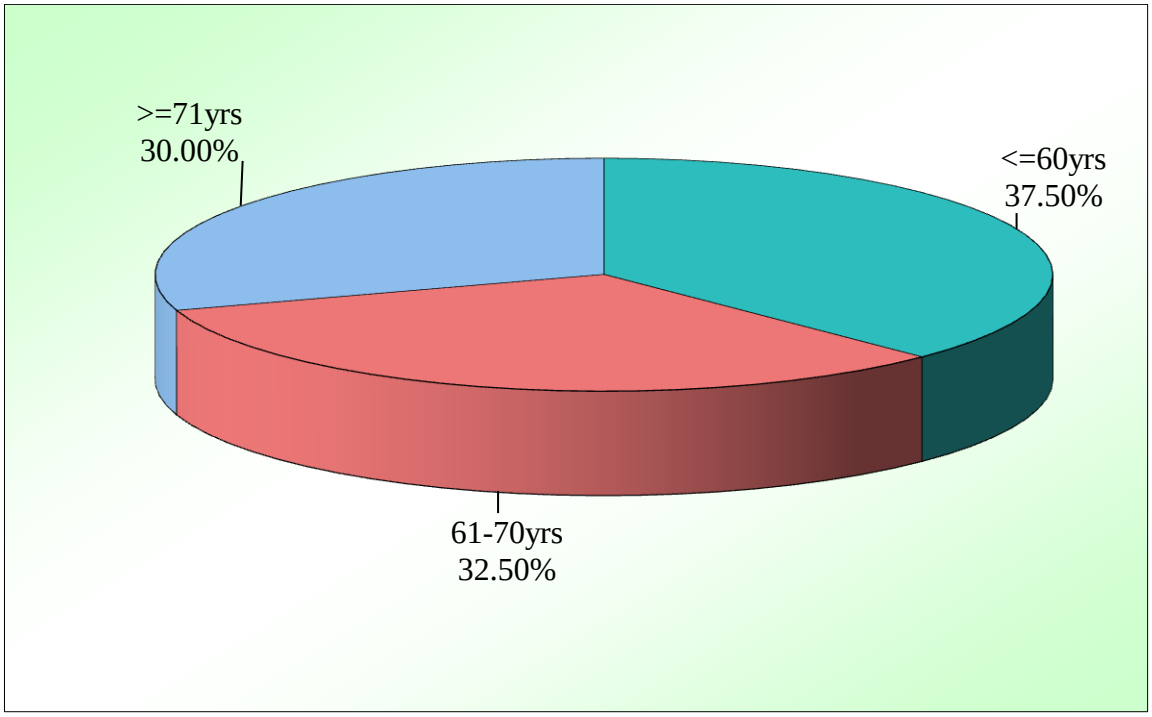
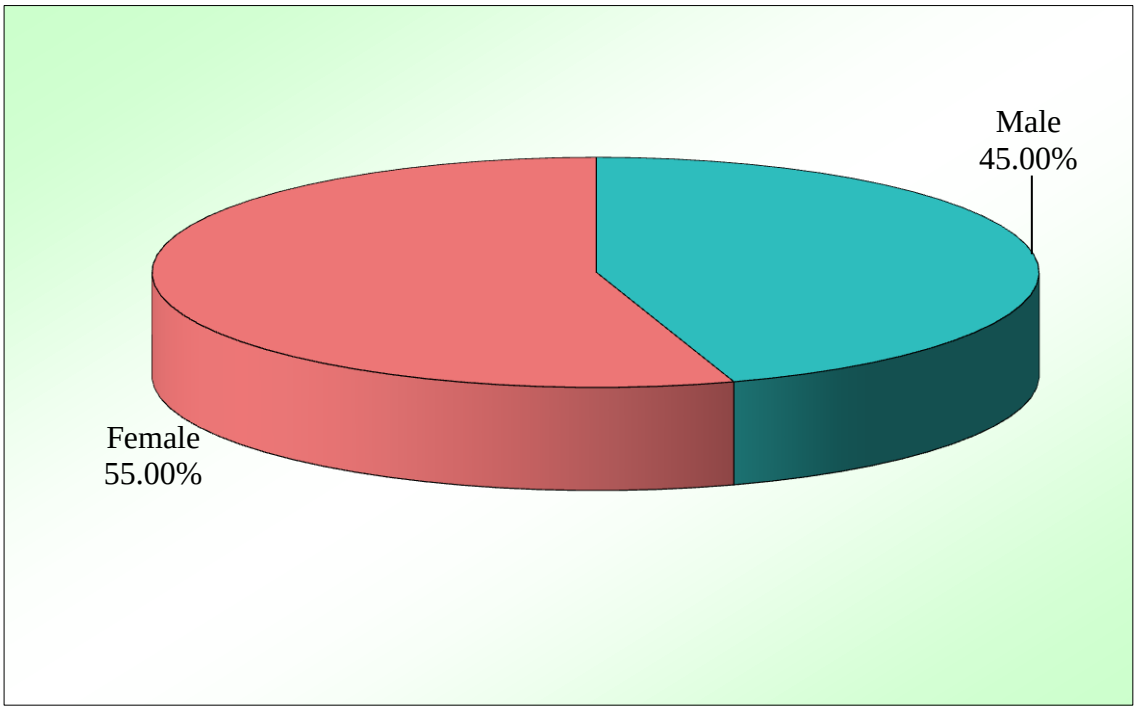


Table 2: Distribution of cases according to gender

Gender	No of cases	% of cases
Male	18	45.00
Female	22	55.00
Total	40	100.00

Fig 8: Distribution of cases according to gender

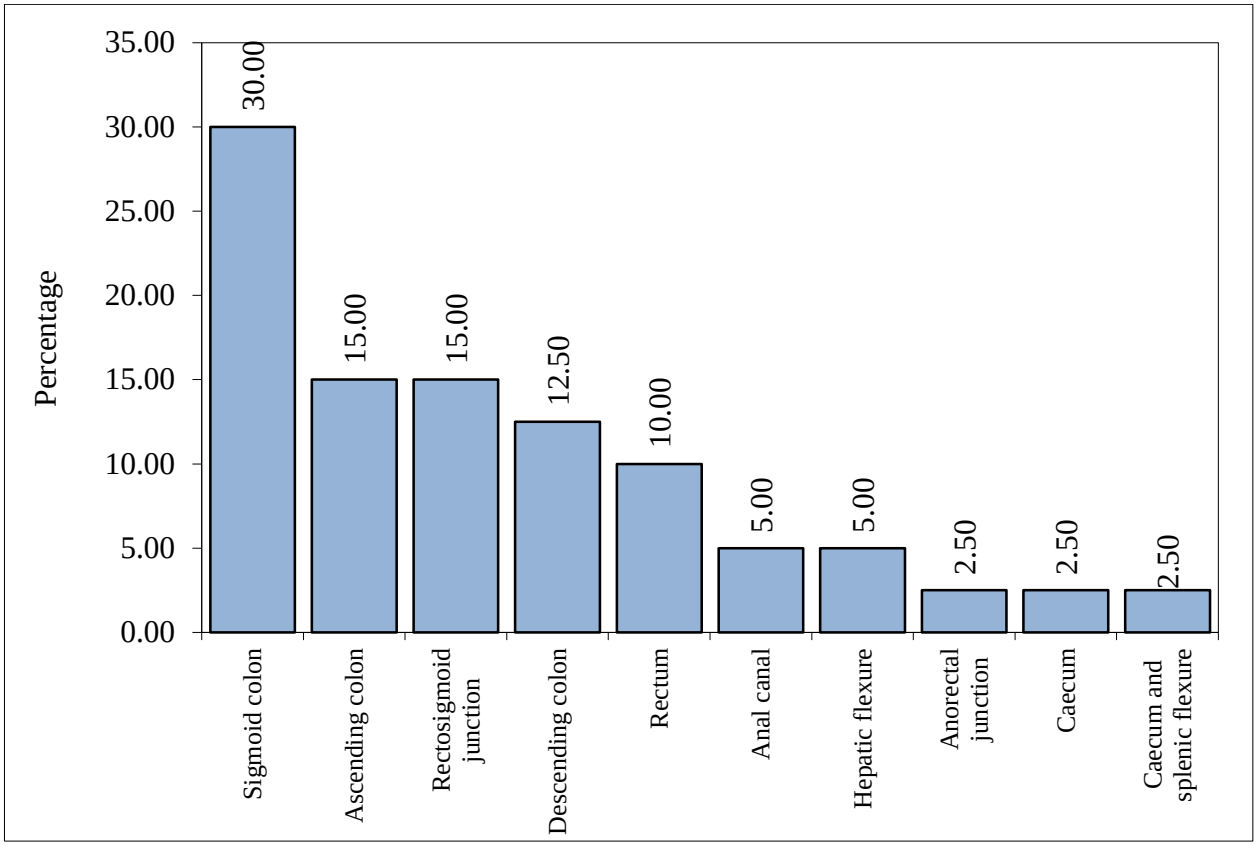


In 40 CRC cases, 18(45%) were male patients and 22(55%) were female patients. (Table 2 and Fig 8)

Table 3: Cases distribution according to the site of lesion

Site of lesion	No of cases	% of cases
Ascending colon	6	15.00
Anal canal	2	5.00
Anorectal junction	1	2.50
Rectosigmoid junction	6	15.00
Caecum	1	2.50
Caecum and splenic flexure	1	2.50
Descending colon	5	12.50
Hepatic flexure	2	5.00
Sigmoid colon	12	30.00
Rectum	4	10.00
Total	40	100.00

Fig 9: Cases distribution according to the site of lesion

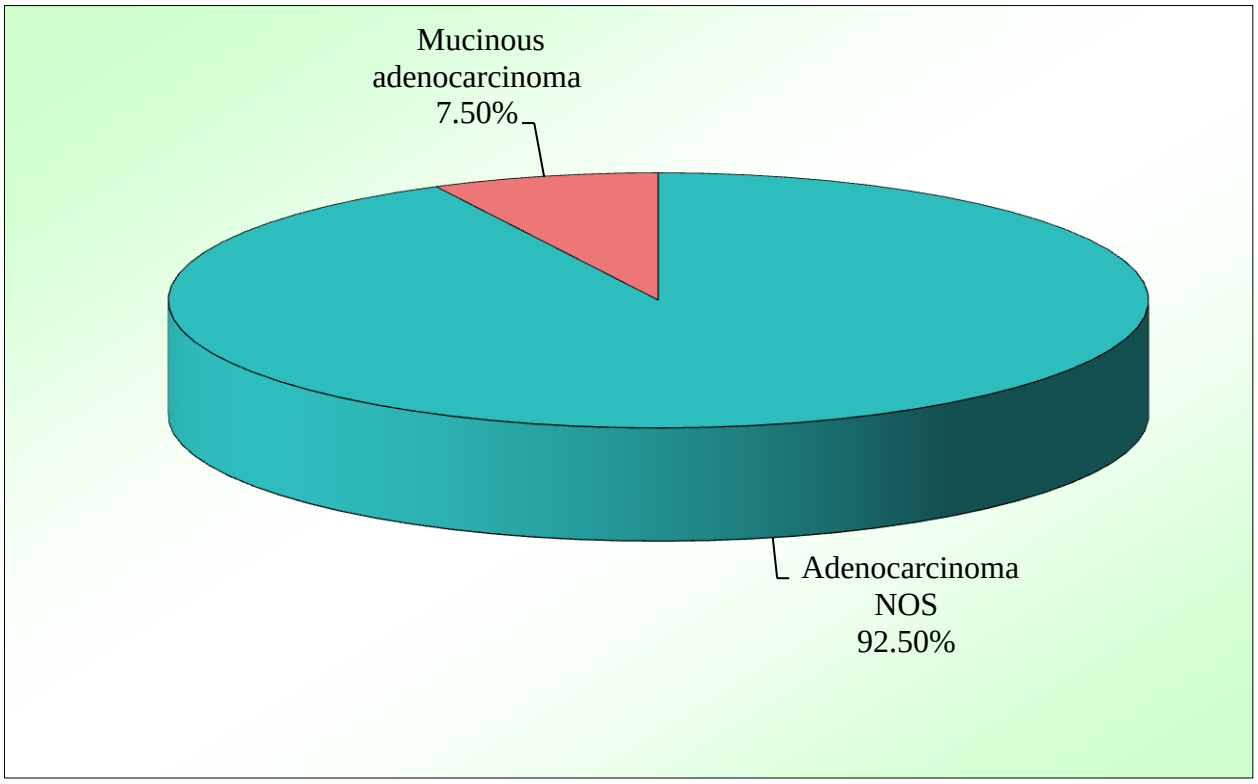


Among 40 CRC cases site wise, 12 cases (30.00%) in sigmoid colon, 6 cases (15.00%) in rectosigmoid junction and ascending colon, 5 cases (12.50%) in descending colon, 4 cases (10.00%) in rectum, 2 cases (5.00%) in anal canal and hepatic flexure, 1 case (2.50%) each in anorectal junction, caecum, splenic flexure were seen. (Table 3 and Fig 9)

Table 4: Distribution of cases according to diagnosis

Diagnosis	No of cases	% of cases
Adenocarcinoma NOS	37	92.50
Mucinous adenocarcinoma	3	7.50
Total	40	100.00

Fig 10: Distribution of cases according to diagnosis

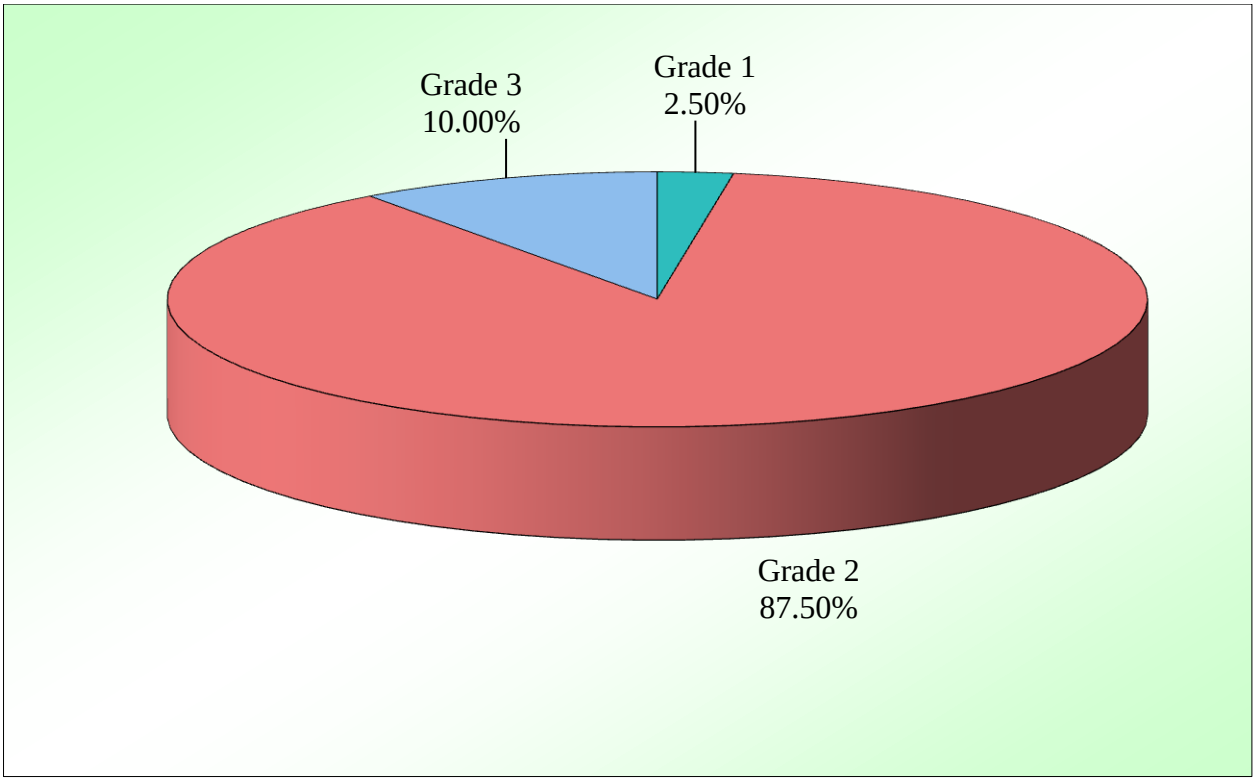


Among 40 cases, 37 (92.50%) cases were Adenocarcinoma NOS and 3 (7.50%) cases were Mucinous adenocarcinoma. (Table 4 and Fig 10)

Table 5: Distribution of cases according to grades of carcinoma

Grades of carcinoma	No of cases	% of cases
Grade 1	1	2.50
Grade 2	35	87.50
Grade 3	4	10.00
Total	40	100.00

Fig 11: Distribution of cases according to grades of carcinoma

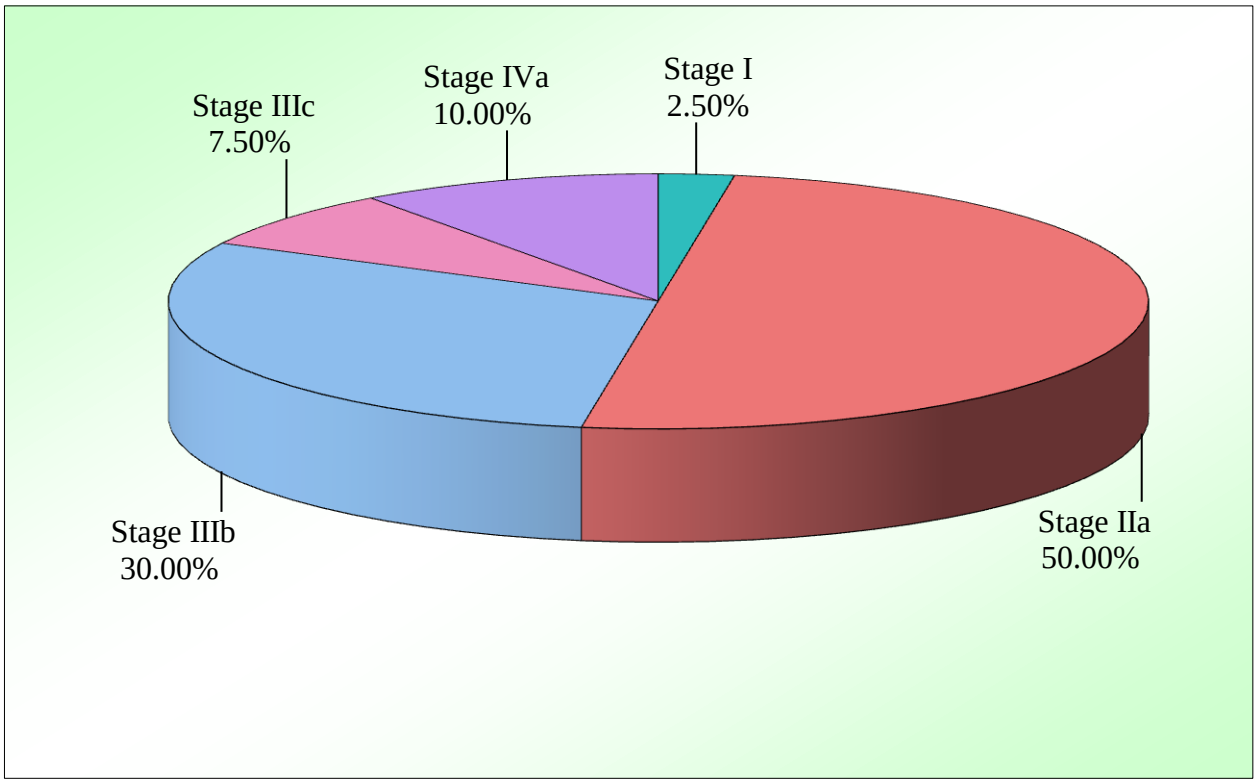


Among 40 cases, 35 (87.50%) cases were of Grade 2 (Moderately differentiated adenocarcinoma), 4 (10.00%) cases were of Grade 3 (Poorly differentiated adenocarcinoma) and 1(2.50%) case was of Grade 1 (Well differentiated adenocarcinoma). (Table 5 and Fig 11)

Table 6: Distribution of cases according to stages of carcinoma

Stages of carcinoma	No of cases	% of cases
Stage I	1	2.50
Stage IIa	20	50.00
Stage IIIb	12	30.00
Stage IIIc	3	7.50
Stage IVa	4	10.00
Total	40	100.00

Fig 12: Distribution of cases according to stages of carcinoma

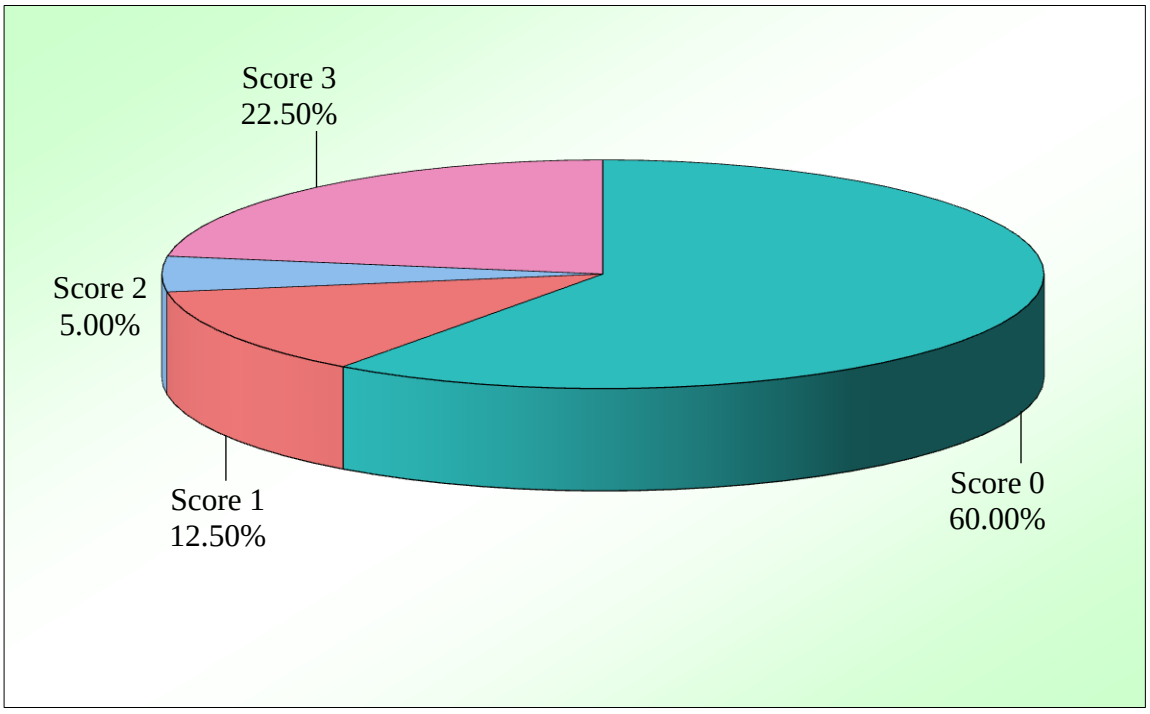


As per TNM Staging (AJCC) in 40 cases of CRC, 20(50.00%) cases of stage IIa, 12(30.00%) cases of stage IIIb, 4 (10.00%) cases of stage IVa, 3 (7.50%) cases of stage IIIc and 1(2.50%) case of stage I were seen. (Table 6 and Fig 12)

Table 7: Distribution of cases according to score of CD10 expression in tumor cells (tCD10)

Score of tCD10 expression	No of cases	% of cases
Score 0	24	60.00
Score 1	5	12.50
Score 2	2	5.00
Score 3	9	22.50
Total	40	100.00

Fig 13: Distribution of cases according to score of CD10 expression in tumor cells (tCD10)



CD 10 expression in tumor cells-

Score 0(less than 10% cells) - seen in 24 (60.00%) cases

Score 1(11%- 25% cells) - seen in 5 (12.50%) cases

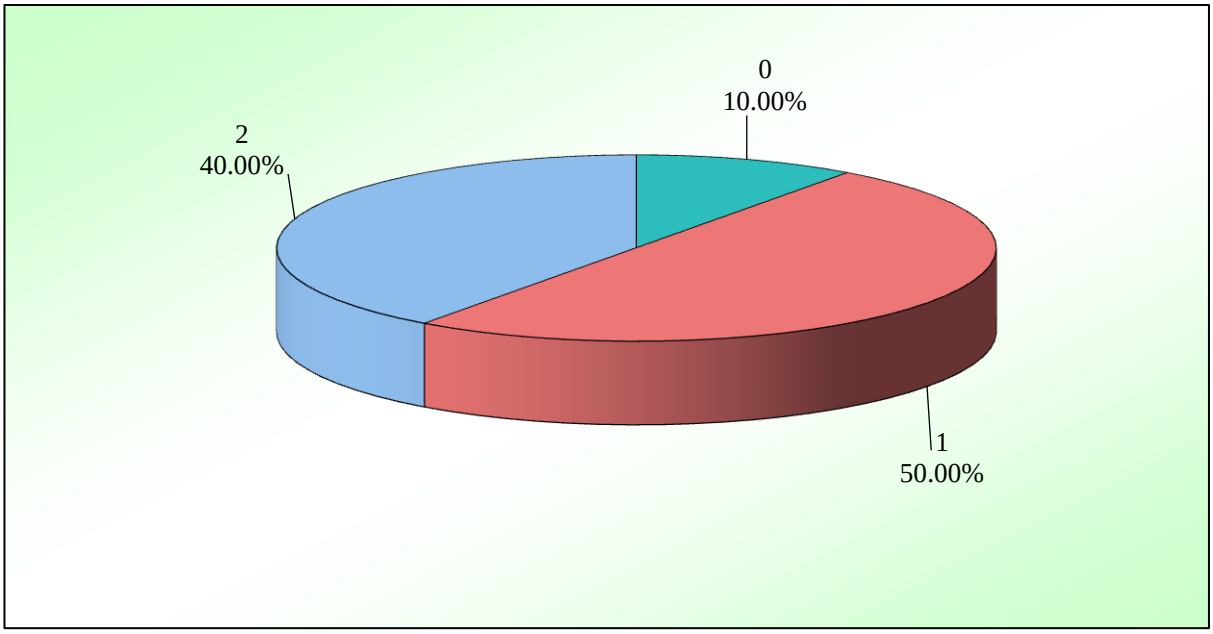
Score 2(26%-50% cells) - seen in 2 (5.00%) cases

Score 3(more than 50% cells) - seen in 9(22.50%) cases. (Table 7 and Fig 13)

Table 8: Distribution of cases according to score of CD10 expression in stromal cells (sCD10)

Score of sCD10 expression	No of cases	% of cases
Score 0	4	10.00
Score 1	20	50.00
Score 2	16	40.00
Total	40	100.00

Fig 14: Distribution of cases according to score of CD10 expression in stromal cells (sCD10)



CD10 expression in stromal cells-

Score 0- seen in 4 (10%) cases

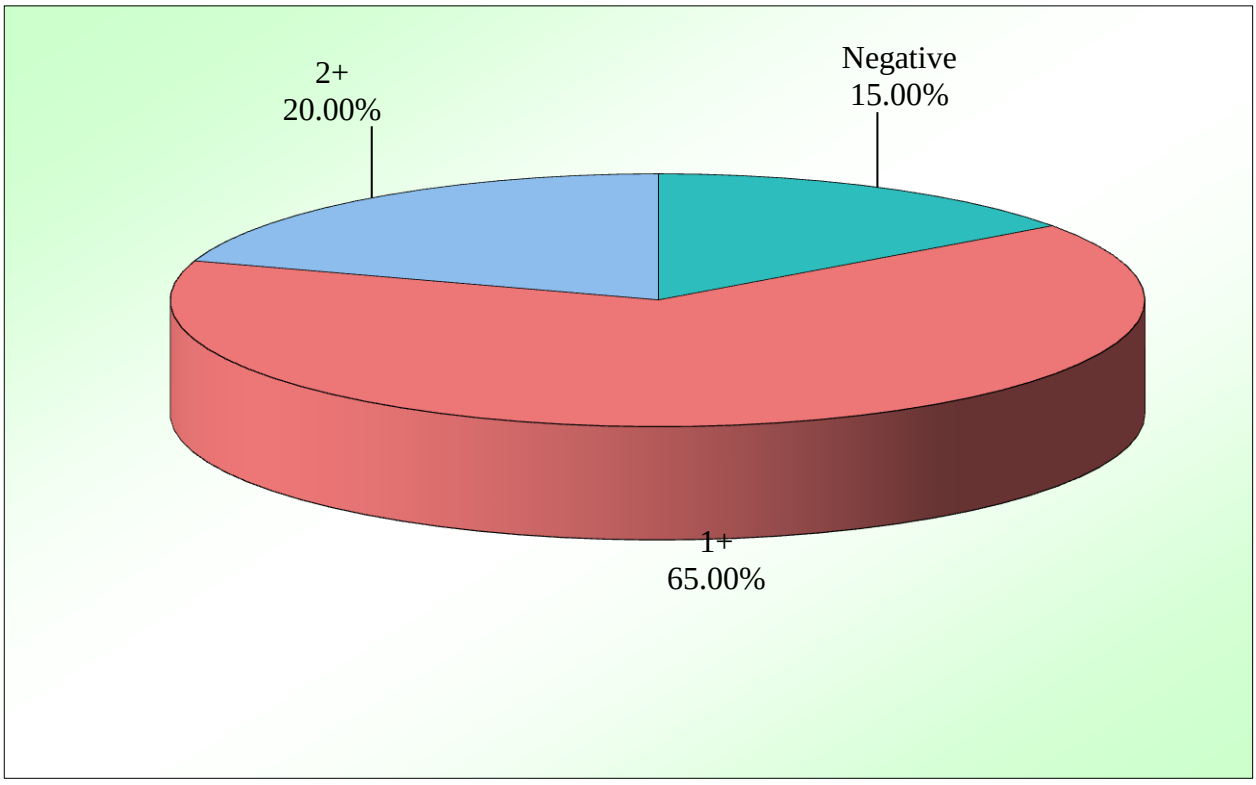
Score1- seen in 20(50%) cases

Score 2- seen in 16(40%) cases (Table 8 and Fig 14)

Table 9: Distribution of cases according to score of CD10 expression in inflammatory cells (iCD10)

iCD10 expression	No of cases	% of cases
Negative	6	15.00
1+	26	65.00
2+	8	20.00
Total	40	100.00

Fig 15: Distribution of cases according to score of CD10 expression in inflammatory cells (iCD10)



CD10 expression in inflammatory cells-

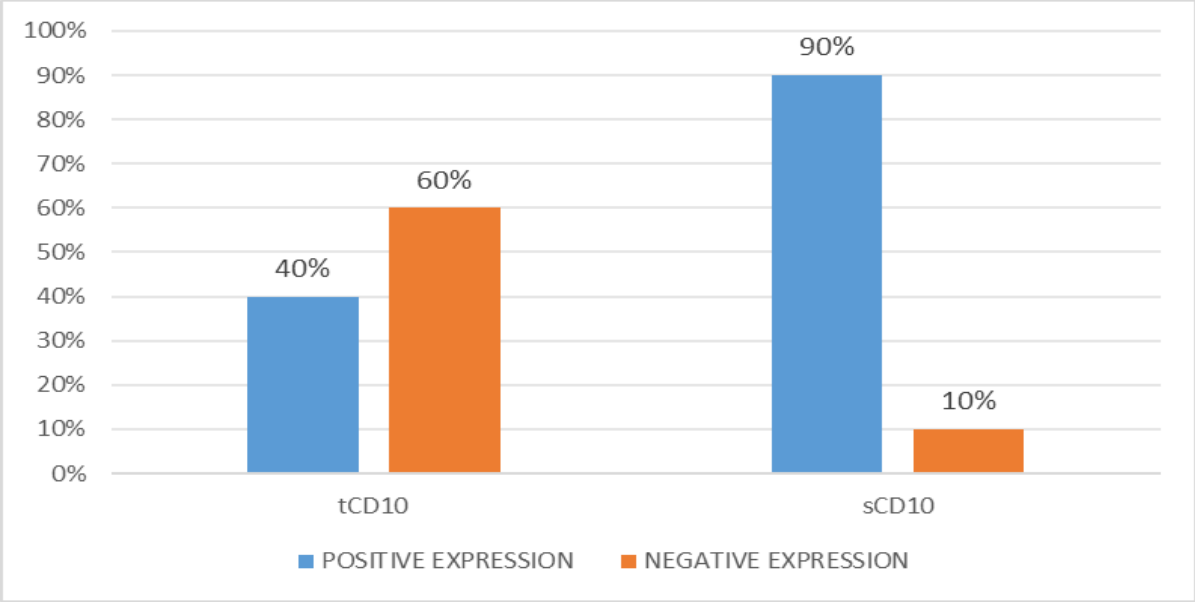
6 (15.00%) cases were negative, 26(65.00%) cases showed 1+ score and 8(20%) cases showed 2+ score. (Table 9 and Fig 15)

Table 10: Comparison of CD10 expression in tumor cells (tCD10) and stromal cells (sCD10) in CRC

CRC cases	tCD10	%	sCD10	%
Positive expression	16	40.00	36	90%
Negative expression	24	60.00	04	10%
Total	40	100.00	40	100.00
Chi square test= 21.9780, p=0.0001				

*p<0.05

Fig 16: Comparison of CD10 expression in tumor cells (tCD10) and stromal cells (sCD10) in CRC

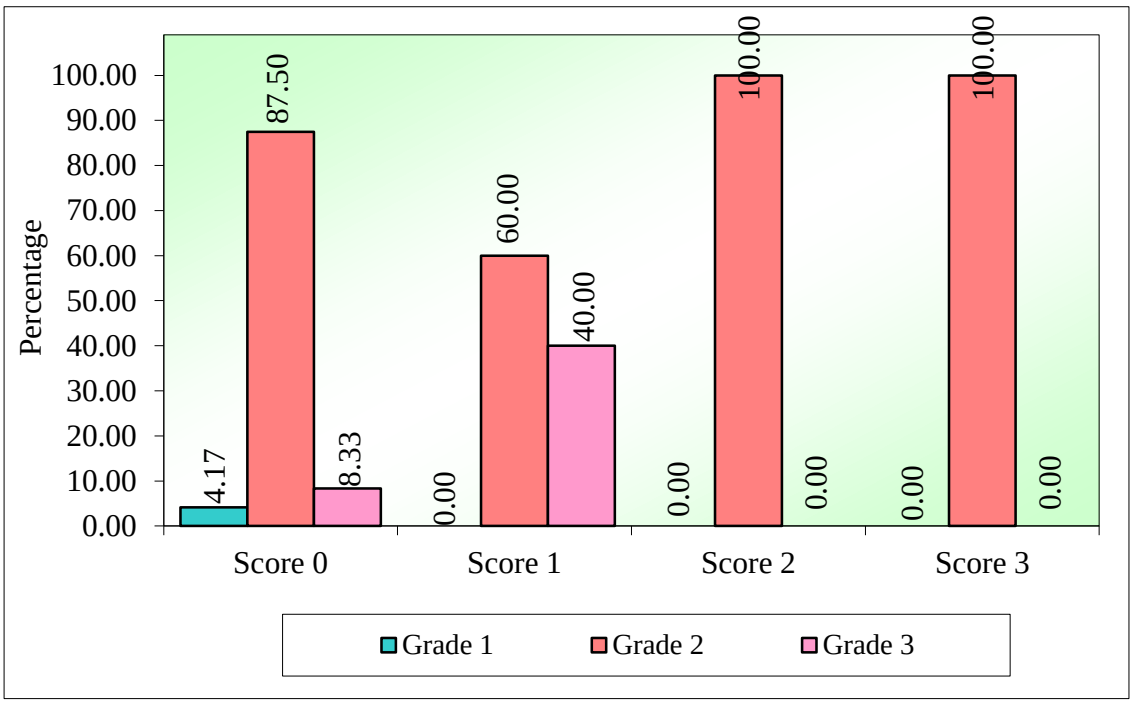


Out of 40 cases, 16(40%) cases showed positive tCD10 expression and 24(60%) cases showed negative tCD10 expression. Whereas 36(90%) cases showed positive sCD10 expression and 4(10%) cases were negative for sCD10 expression. The difference in the CD10 expression in tumor cells and CD10 expression in stromal cells in CRC cases was statistically significant (Chi-square test= 21.9780, p=0.0001). (Table 10 and Fig 16)

Table 11: Association between CD expression in tumor cells (tCD10) with grades of carcinoma

Score of tCD10 expression	Grades of carcinoma							
	Grade 1	%	Grade 2	%	Grade 3	%	Total	%
Score 0	1	4.17	21	87.50	2	8.33	24	60.00
Score 1	0	0.00	3	60.00	2	40.00	5	12.50
Score 2	0	0.00	2	100.00	0	0.00	2	5.00
Score 3	0	0.00	9	100.00	0	0.00	9	22.50
Total	1	2.50	35	87.50	4	10.00	40	100.00
Spearman's rank correlation, Sp. R=0.0318, p=0.8454								

Fig 17: Association between CD10 expression in tumor cells (tCD10) with grades of carcinoma

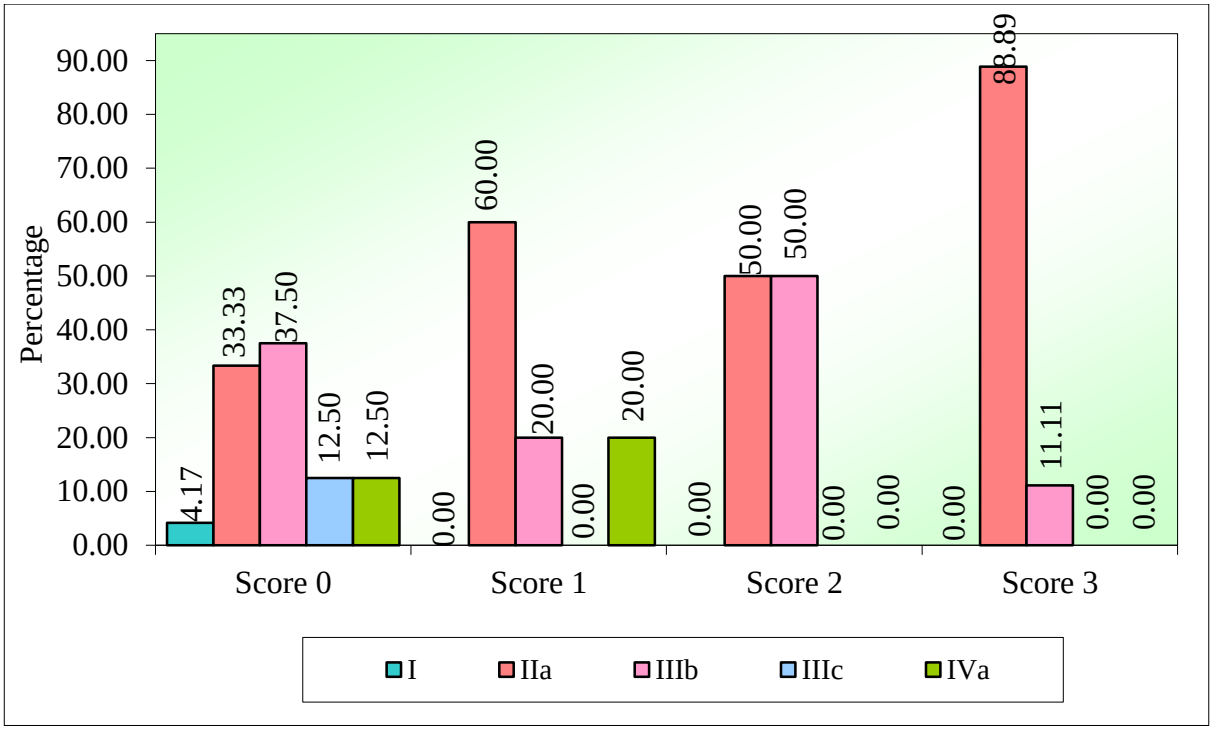


CD10 expression in tumor cells was compared with grades of carcinoma. There was no statistically significant correlation found between CD10 expression and grades of carcinoma. (Spearman's rank correlation (Sp. R) = 0.0318 and p-value = 0.8454) (Table 11 and Fig 17)

Table 12: Association between CD10 expression in tumor cells (tCD10) with stages of carcinoma

Score of tCD10 expression	Stages of carcinoma									
	I	%	IIa	%	IIIb	%	IIIc	%	IVa	%
Score 0	1	4.17	8	33.33	9	37.50	3	12.50	3	12.50
Score 1	0	0.00	3	60.00	1	20.00	0	0.00	1	20.00
Score 2	0	0.00	1	50.00	1	50.00	0	0.00	0	0.00
Score 3	0	0.00	8	88.89	1	11.11	0	0.00	0	0.00
Total	1	2.50	20	50.00	12	30.00	3	7.50	4	10.00
Spearman's rank correlation, Sp. R= -0.1492, p=0.3583										

Fig 18: Association between CD10 expression in tumor cells (tCD10) with stages of carcinoma



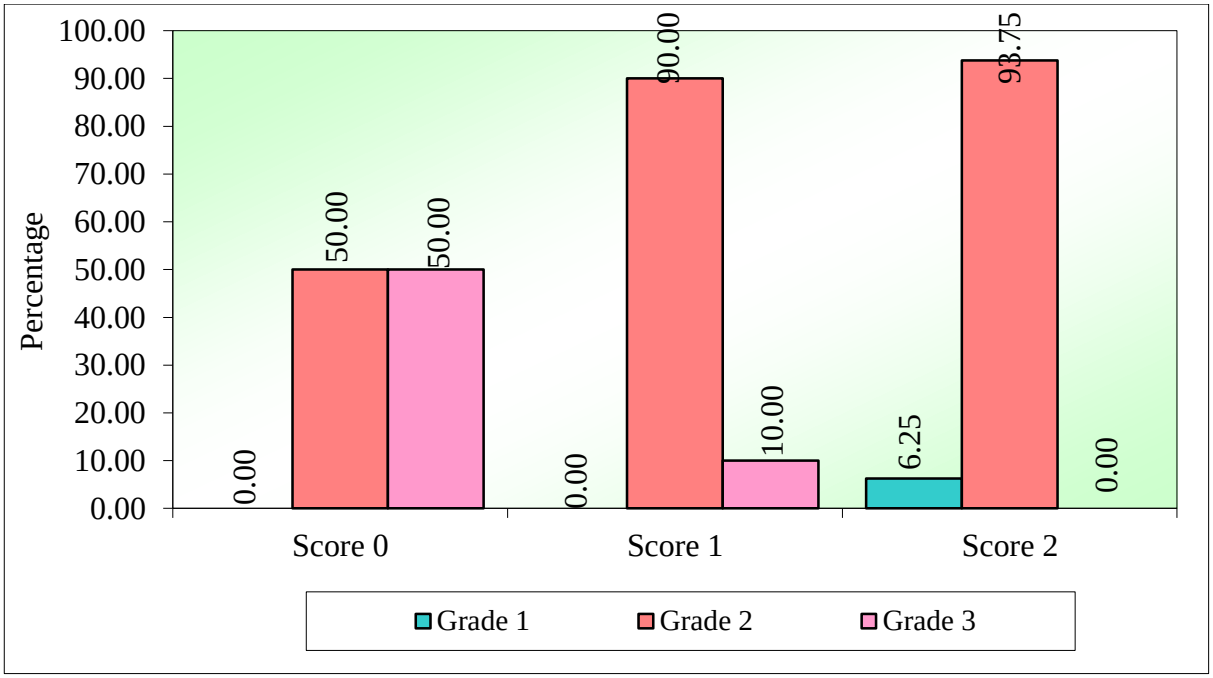
CD10 expression was compared with stages of carcinoma. No statistically significant correlation was found (Spearman's rank correlation (SP. R) = -0.1492 and p value= 0.3583) (Table 12 and Fig 18)

Table 13: Association between CD10 expression in stromal cells (sCD10) with grades of carcinoma

Score of sCD10 expression	Grades of carcinoma							
	Grade 1	%	Grade 2	%	Grade 3	%	Total	%
Score 0	0	0.00	2	50.00	2	50.00	4	10.00
Score 1	0	0.00	18	90.00	2	10.00	20	50.00
Score 2	1	6.25	15	93.75	0	0.00	16	40.00
Total	1	2.50	35	87.50	4	10.00	40	100.00
Spearman's rank correlation, Sp. R= -0.4184, p=0.0072*								

*p<0.05

Fig 19: Association between CD10 expression in stromal cells (sCD10) with grades of carcinoma

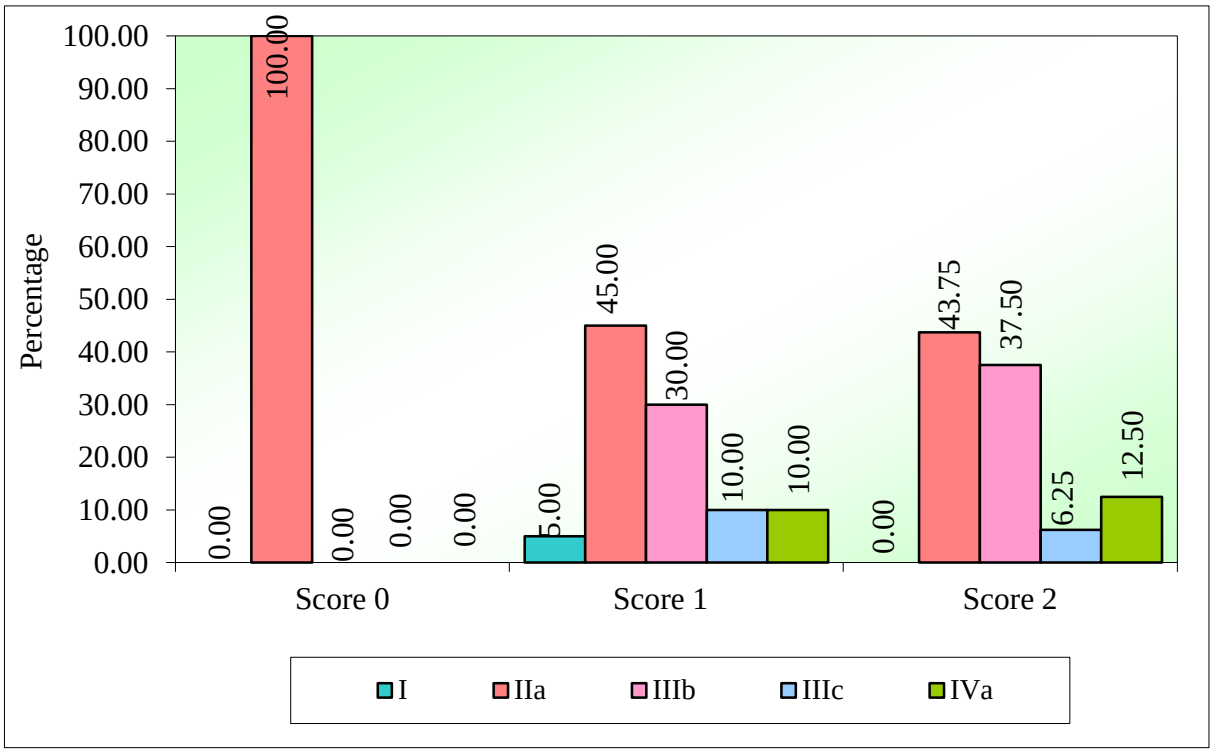


CD10 expression in stromal cells was compared with grades of carcinoma. A slight negative correlation was seen which was statistically significant. CD10 expression was decreased with an increase in the grade of carcinoma. (Spearman's rank correlation (SP. R) = -0.4184 and p value= 0.0072) (Table 13 and Fig 19)

Table 14: Association between CD10 expression in stromal cells (sCD10) with stages of carcinoma

Score of sCD10 expression	Stages of carcinoma									
	I	%	IIa	%	IIIb	%	IIIc	%	IVa	%
Score 0	0	0.00	4	100.0	0	0.00	0	0.00	0	0.00
Score 1	1	5.00	9	45.00	6	30.00	2	10.00	2	10.00
Score 2	0	0.00	7	43.75	6	37.50	1	6.25	2	12.50
Total	1	2.50	20	50.00	12	30.00	3	7.50	4	10.00
Spearman's rank correlation, Sp. R=-0.1168, p=0.4728										

Fig 20: Association between CD10 expression in stromal cells (sCD10) with stages of carcinoma

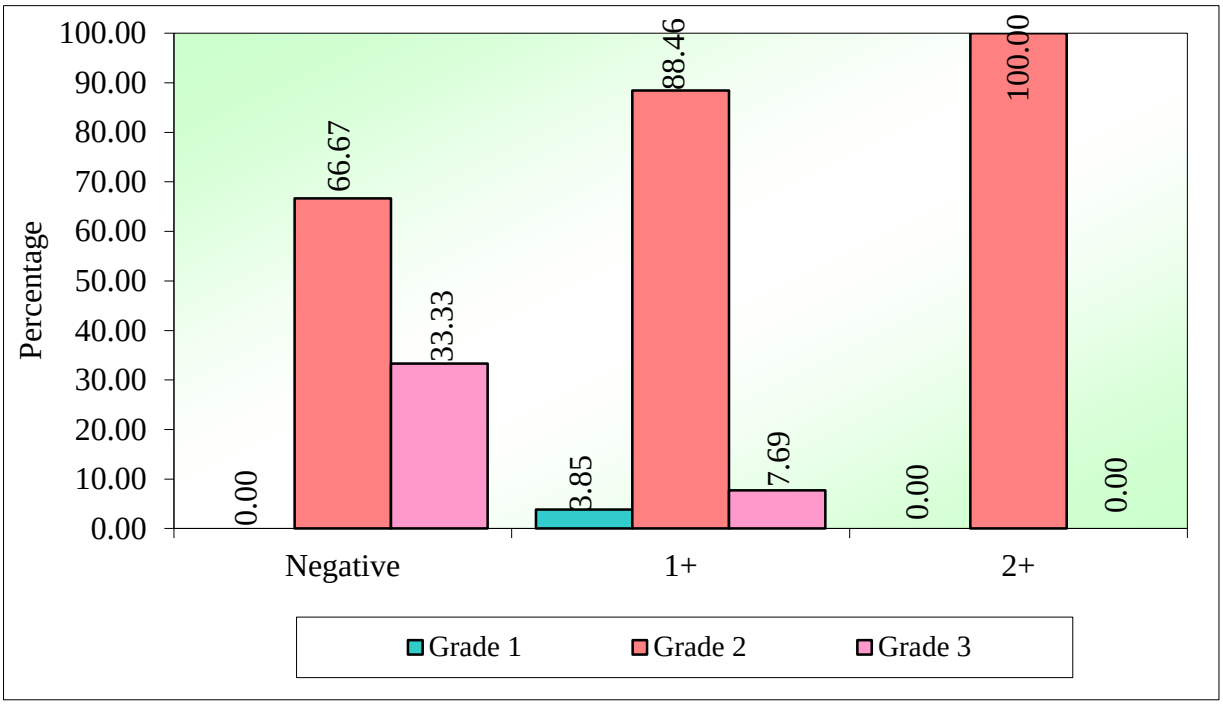


The association of CD10 expression in stromal cells with the stage of carcinoma was studied. No statistically significant correlation was seen. (Spearman's rank correlation, Sp. R=-0.1168, p=0.4728) (Table 14 and Fig 20)

Table 15: Association between CD10 expression in Inflammatory cells (iCD10) with grades of carcinoma

iCD10 expression	Grades of carcinoma							
	Grade 1	%	Grade 2	%	Grade 3	%	Total	%
Negative	0	0.00	4	66.67	2	33.33	6	15.00
1+	1	3.85	23	88.46	2	7.69	26	65.00
2+	0	0.00	8	100.00	0	0.00	8	20.00
Total	1	2.50	35	87.50	4	10.00	40	100.00
Spearman's rank correlation, Sp. R=-0.2646, p=0.0990								

Fig 21: Association between CD10 expression in Inflammatory cells (iCD10) with grades of carcinoma

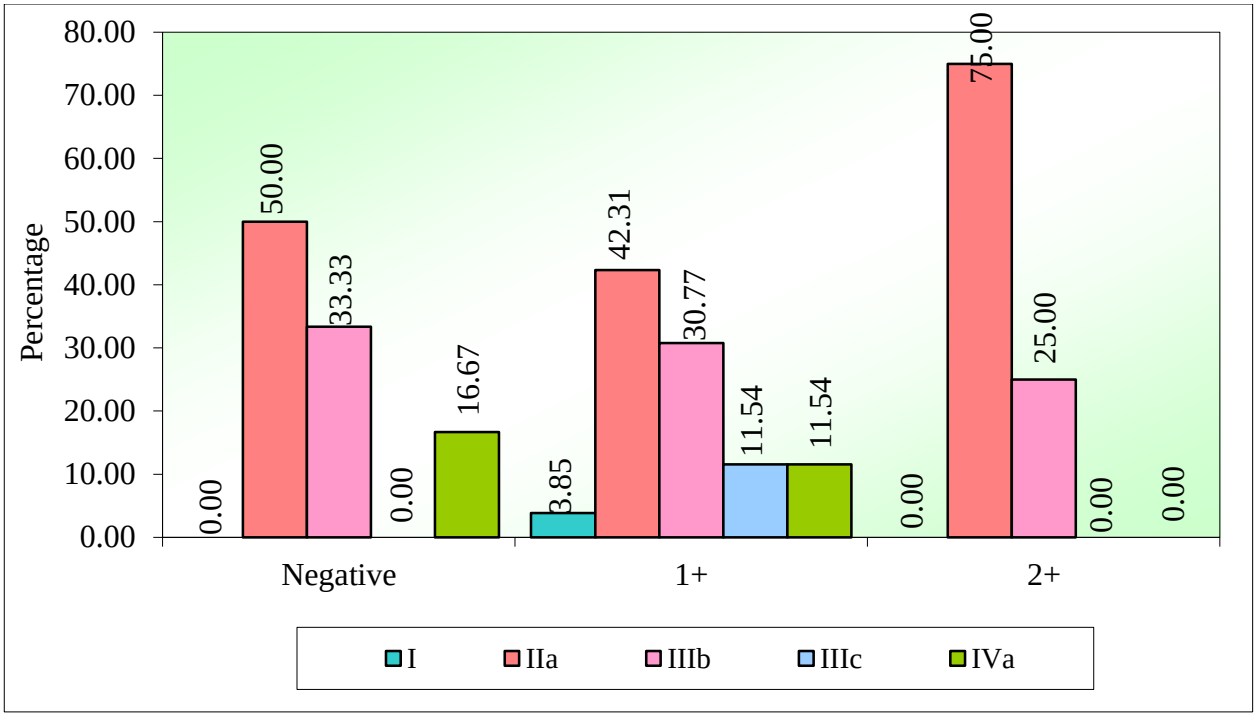


CD10 expression in inflammatory cells was compared with grades of CRC. No statistically significant correlation was seen. (Spearman's rank correlation, Sp. R=-0.2646, p=0.0990) (Table 15 and Fig 21)

Table 16: Association between CD10 expression in Inflammatory cells (iCD10) with stages of carcinoma

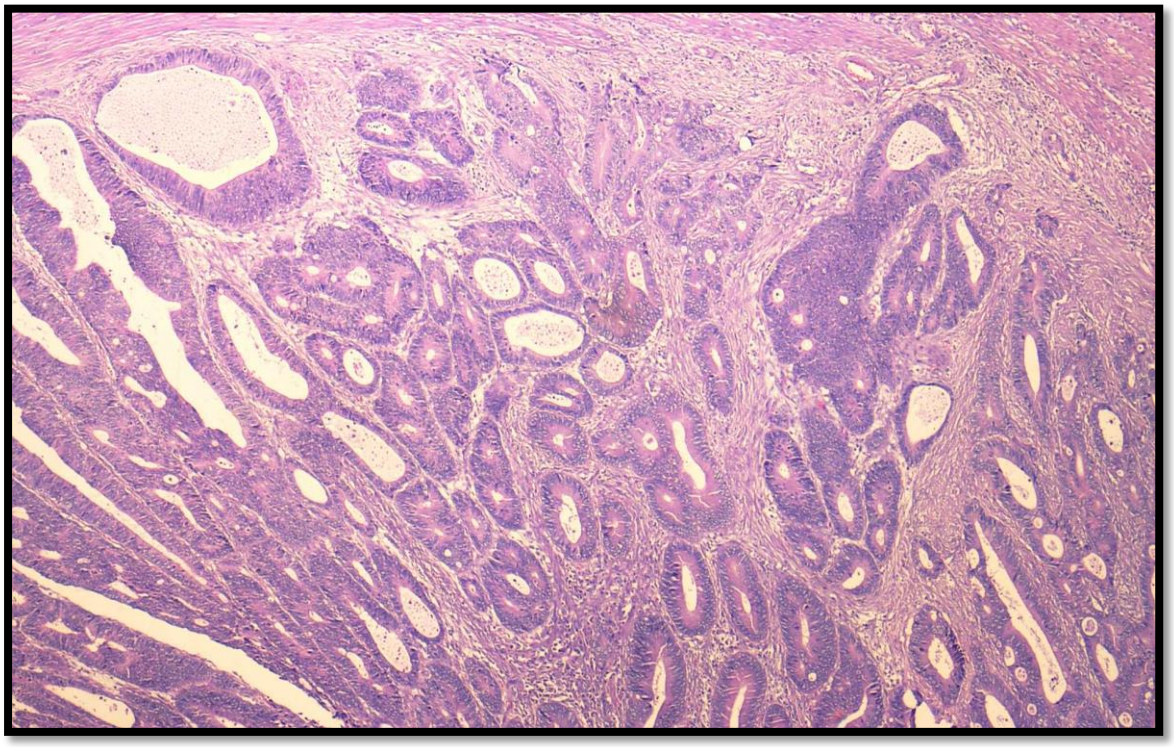
iCD10 expression	Stages of carcinoma									
	I	%	IIa	%	IIIb	%	IIIc	%	IVa	%
Negative	0	0.00	3	50.00	2	33.33	0	0.00	1	16.67
1+	1	3.85	11	42.31	8	30.77	3	11.54	3	11.54
2+	0	0.00	6	75.00	2	25.00	0	0.00	0	0.00
Total	1	2.50	20	50.00	12	30.00	3	7.50	4	10.00
Spearman's rank correlation, Sp. R=-0.2128, p=0.1873										

Fig 22: Association between CD10 expression in Inflammatory cells (iCD10) with stages of carcinoma

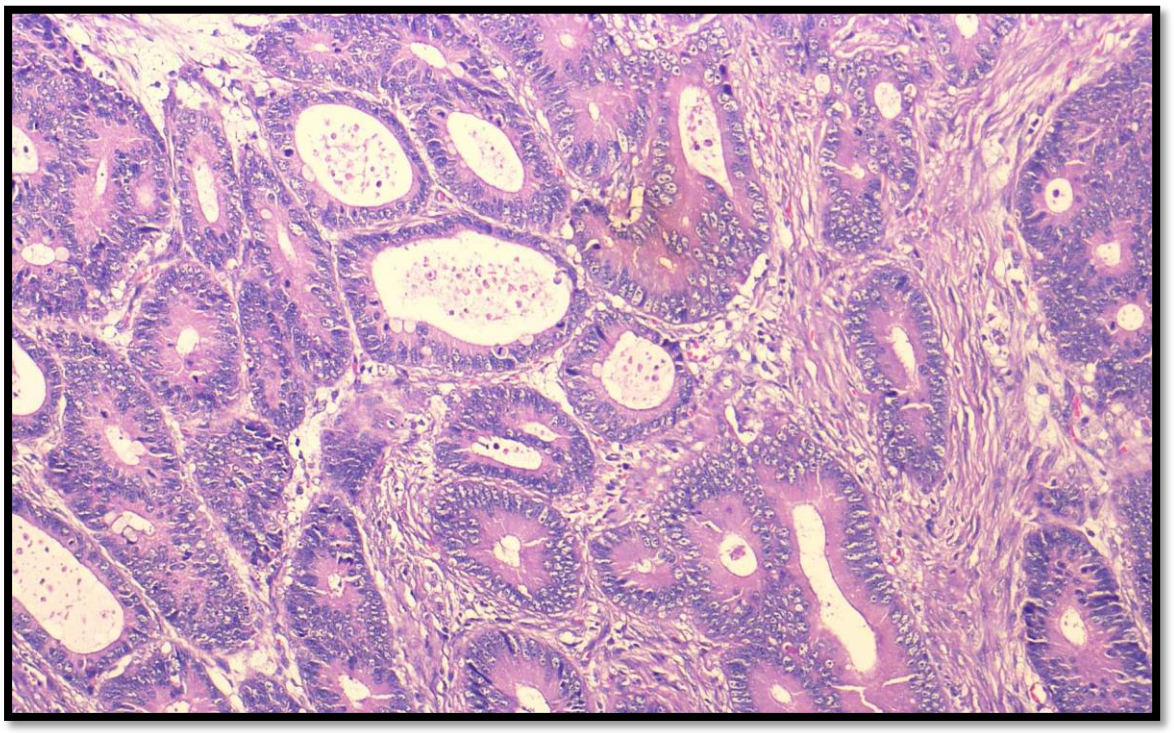


CD10 expression in inflammatory cells was compared with stages of carcinoma. No statistically significant correlation was seen. (Spearman's rank correlation, Sp. R=-0.2128, p=0.1873)(Table 16 and Fig 22)

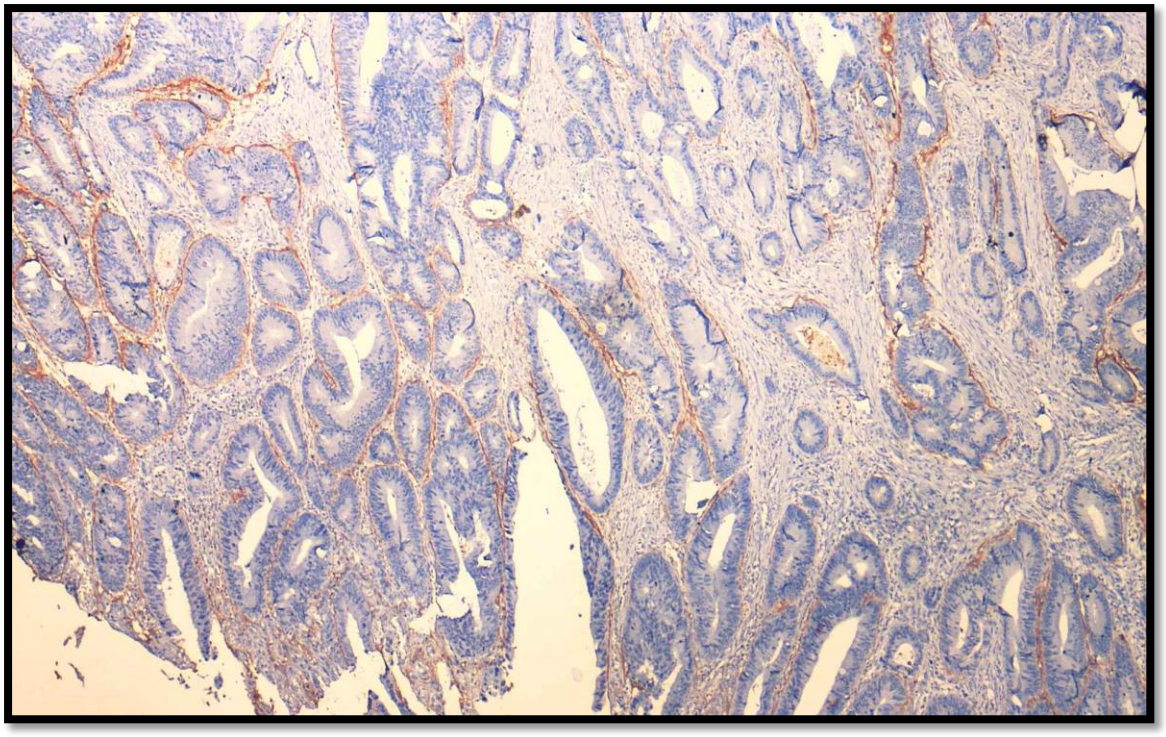
PHOTOMICROGRAPHS



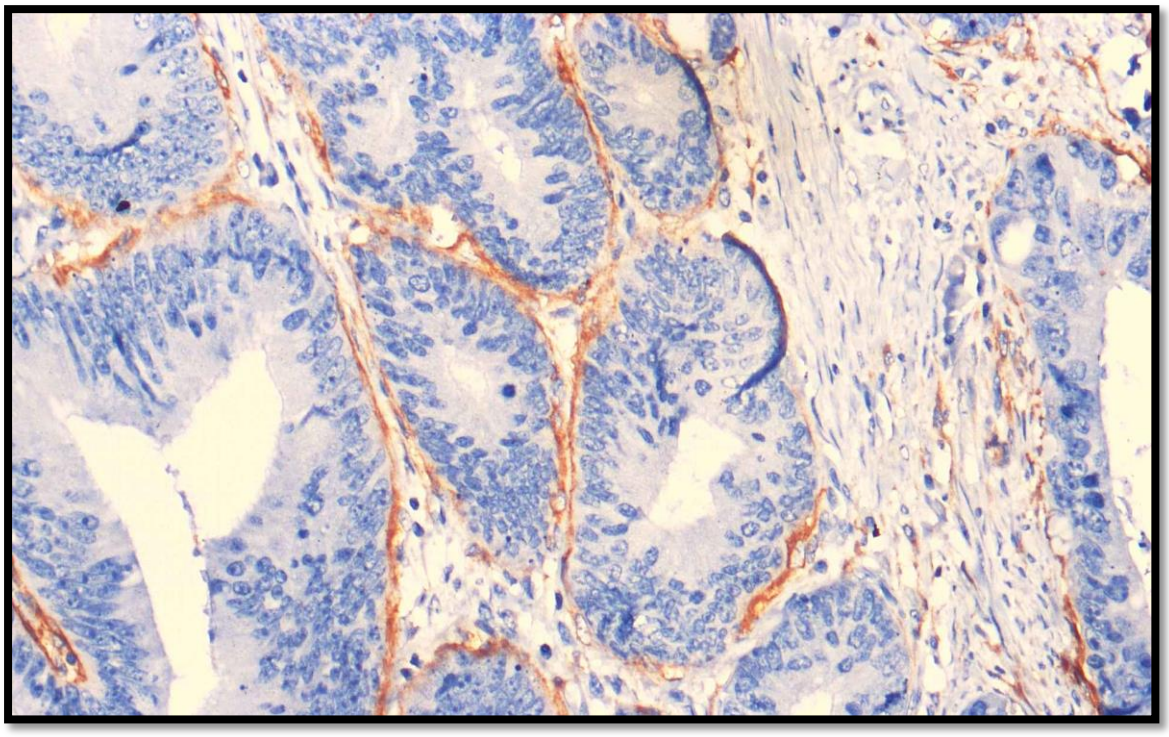
Photomicrograph 1: Well-differentiated Adenocarcinoma NOS (H & E 40X)



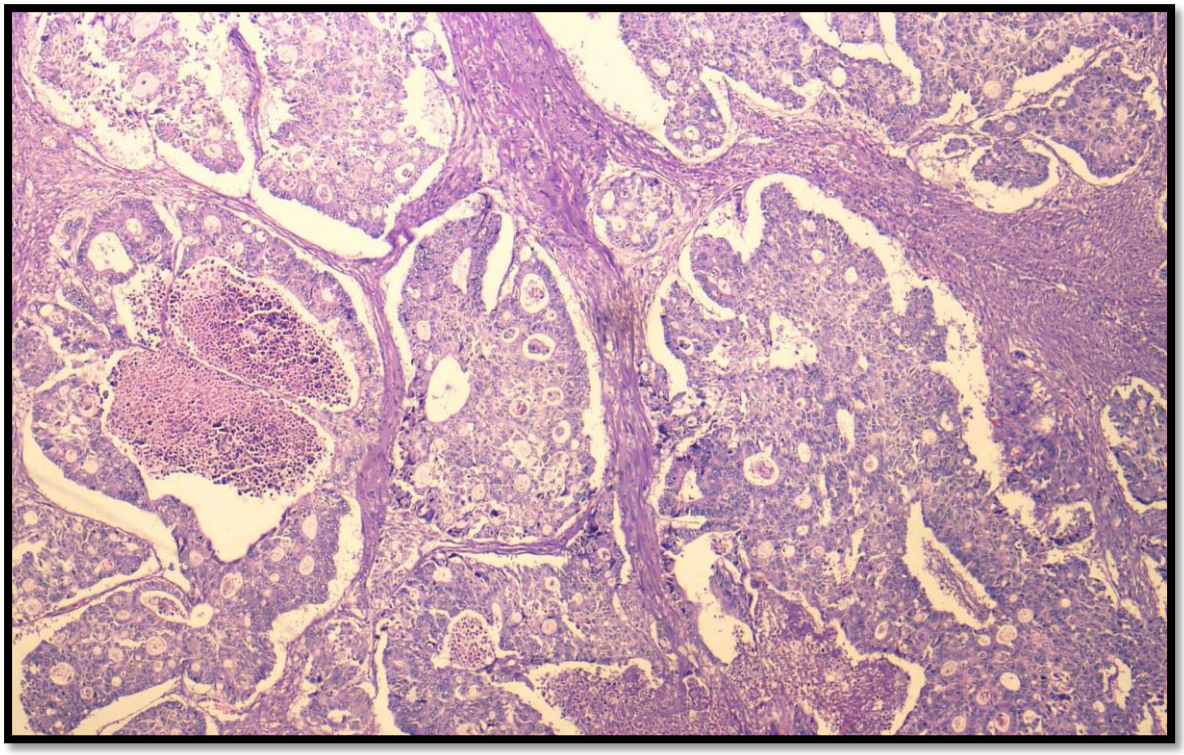
Photomicrograph 2: Well-differentiated Adenocarcinoma NOS (H & E 100X)



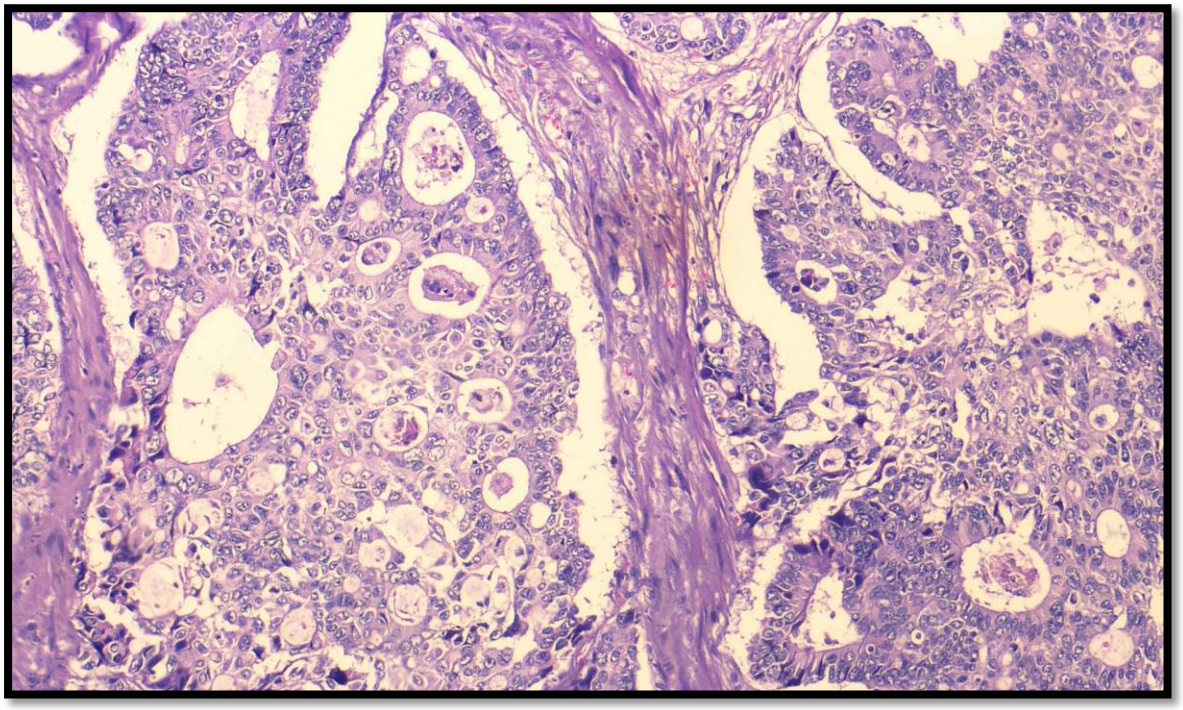
Photomicrograph 3: CD10 negativity in tumor cells and positivity in stromal cells in Well-Differentiated Adenocarcinoma NOS (IHC 40X)



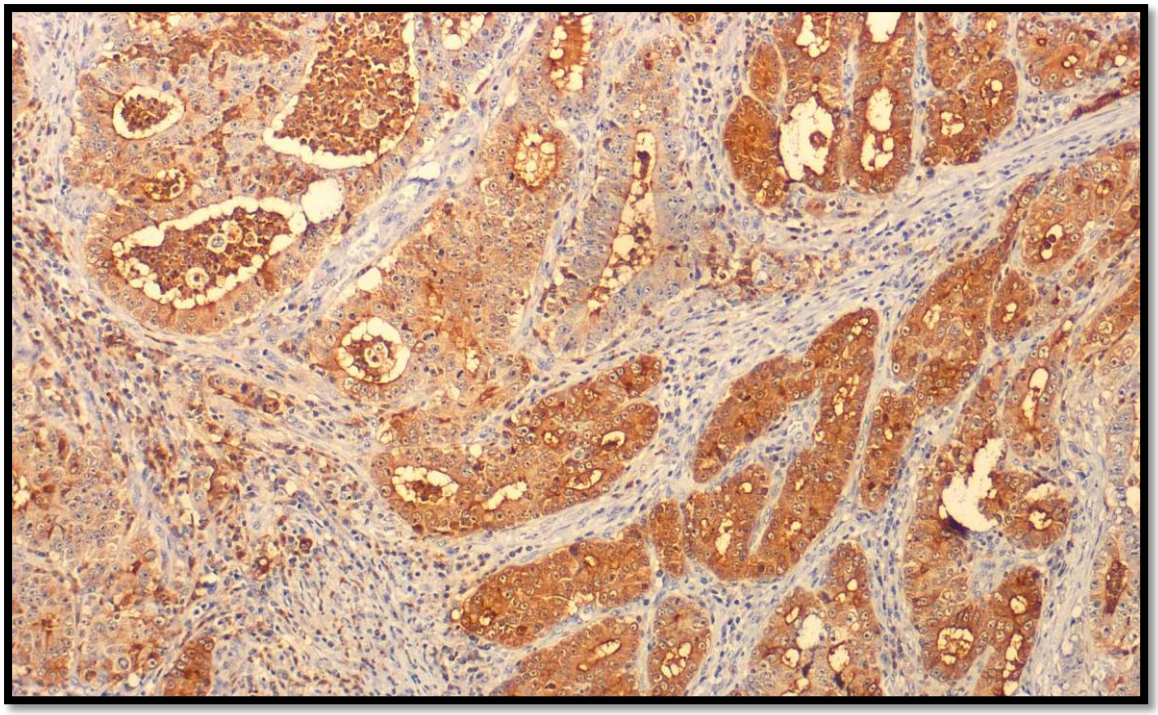
Photomicrograph 4: CD10 negativity in tumor cells and positivity in stromal cells in Well- Differentiated Adenocarcinoma NOS (IHC 200X)



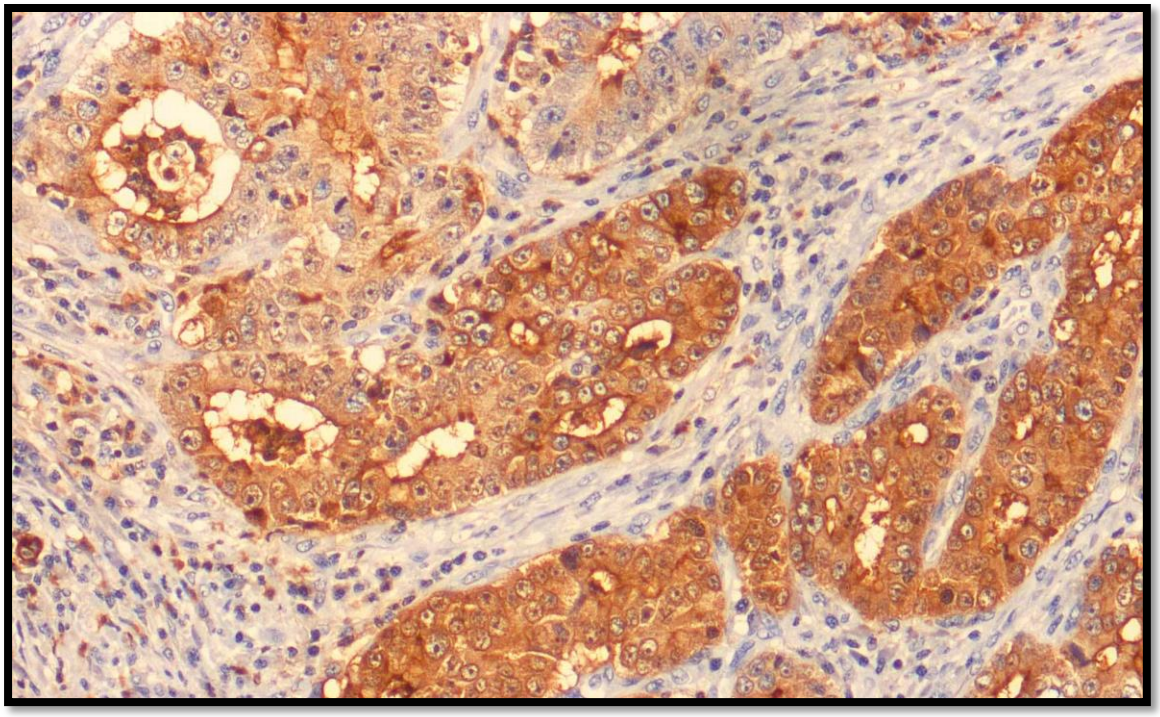
Photomicrograph 5: Moderately differentiated adenocarcinoma NOS (H & E 40X)



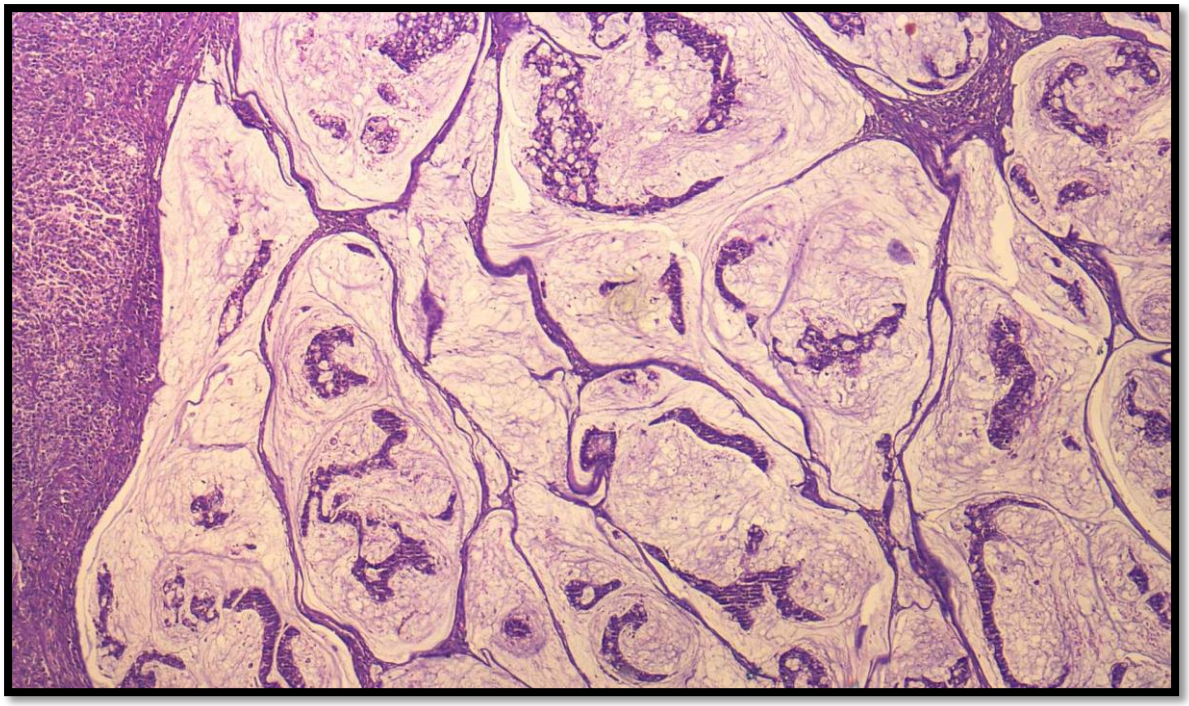
Photomicrograph 6: Moderately differentiated adenocarcinoma NOS (H & E 100X)



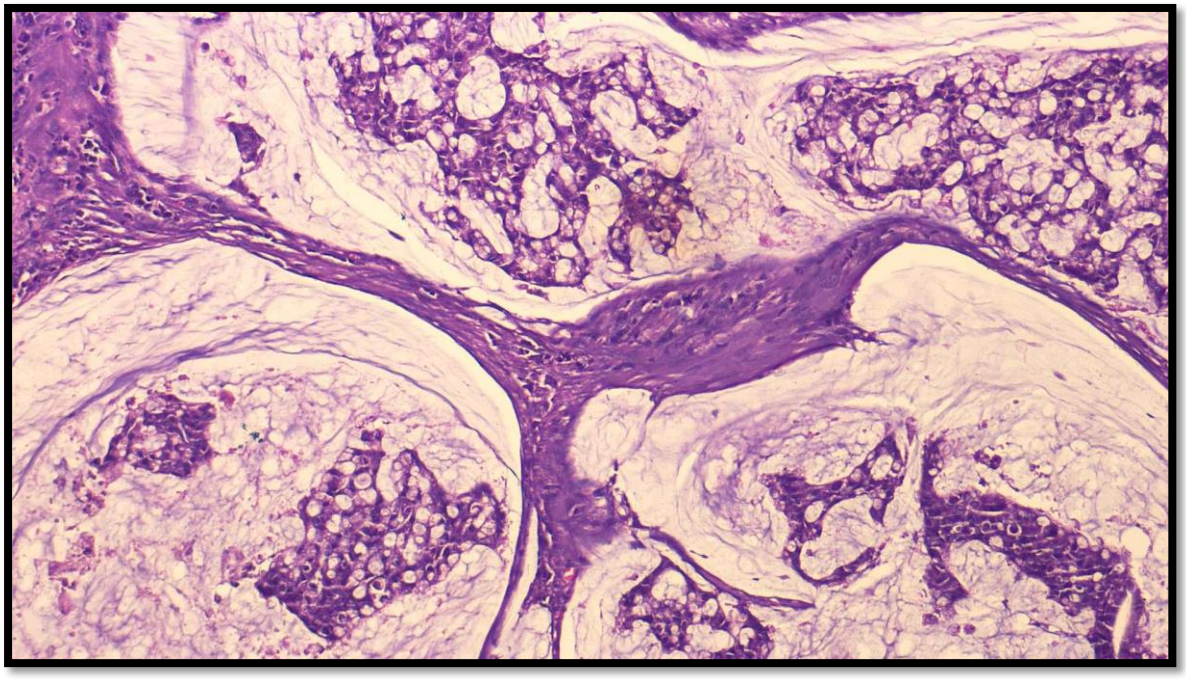
Photomicrograph 7: CD10 strong positivity in tumor cells and stromal cells in Moderately differentiated adenocarcinoma NOS (IHC 100X)



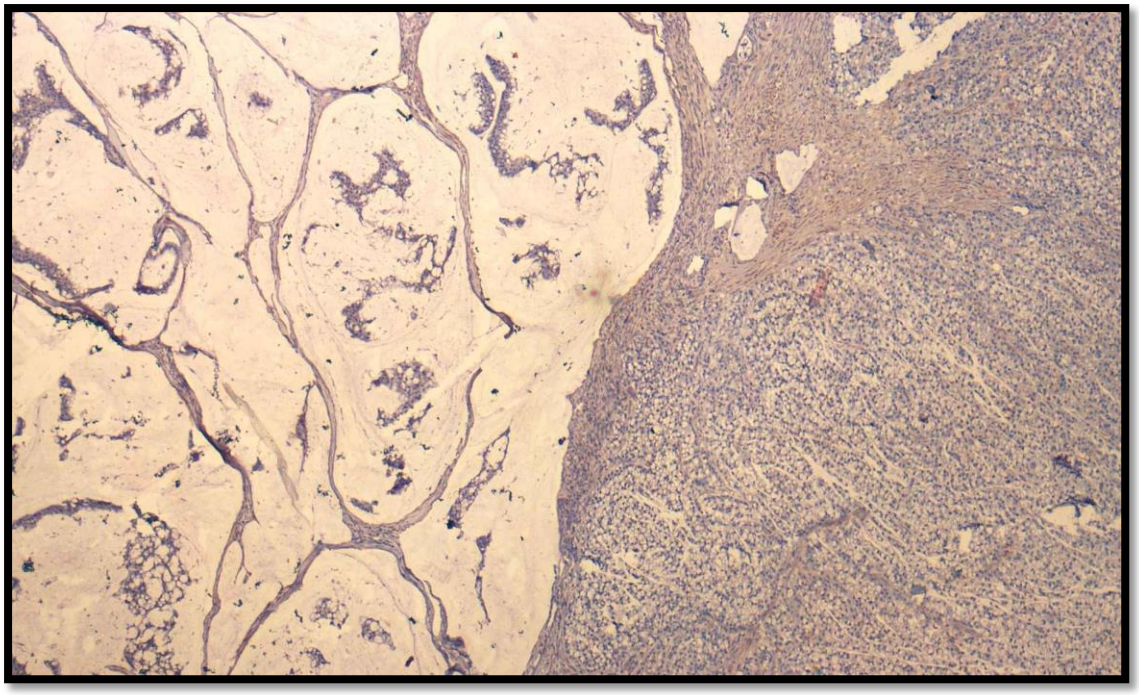
Photomicrograph 8: CD10 strong positivity in tumor cells and stromal cells in Moderately differentiated adenocarcinoma NOS (IHC 200X)



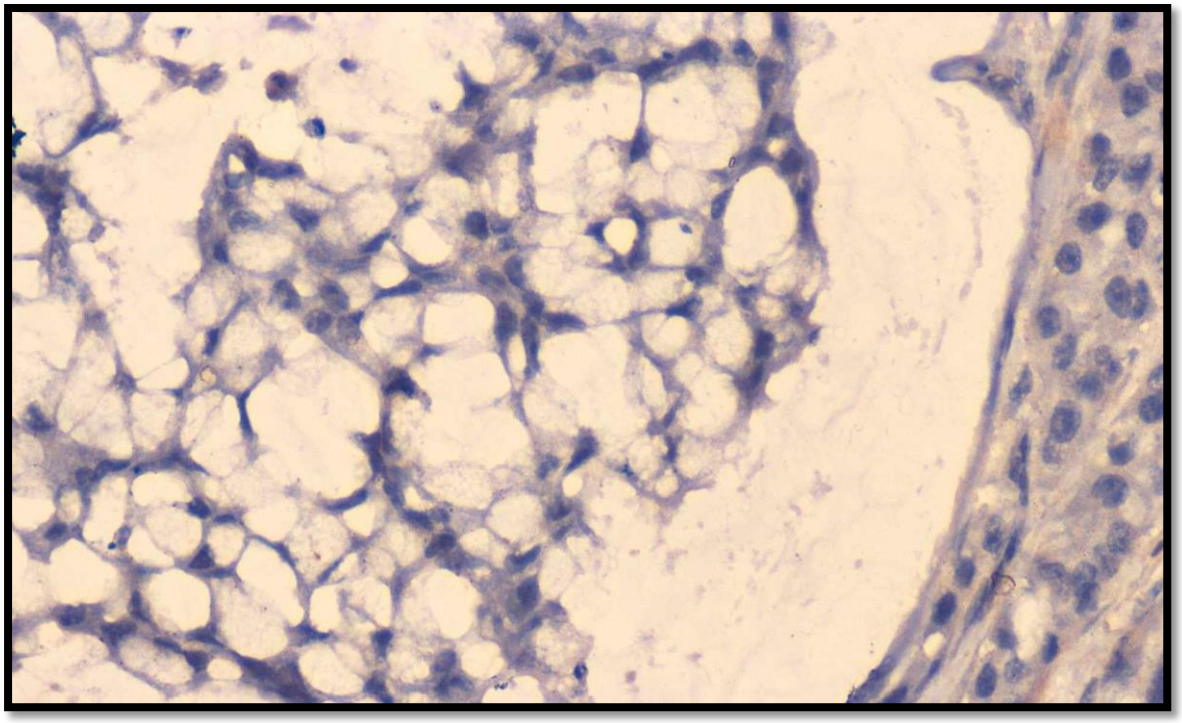
Photomicrograph 9: Mucinous adenocarcinoma (H & E 40X)



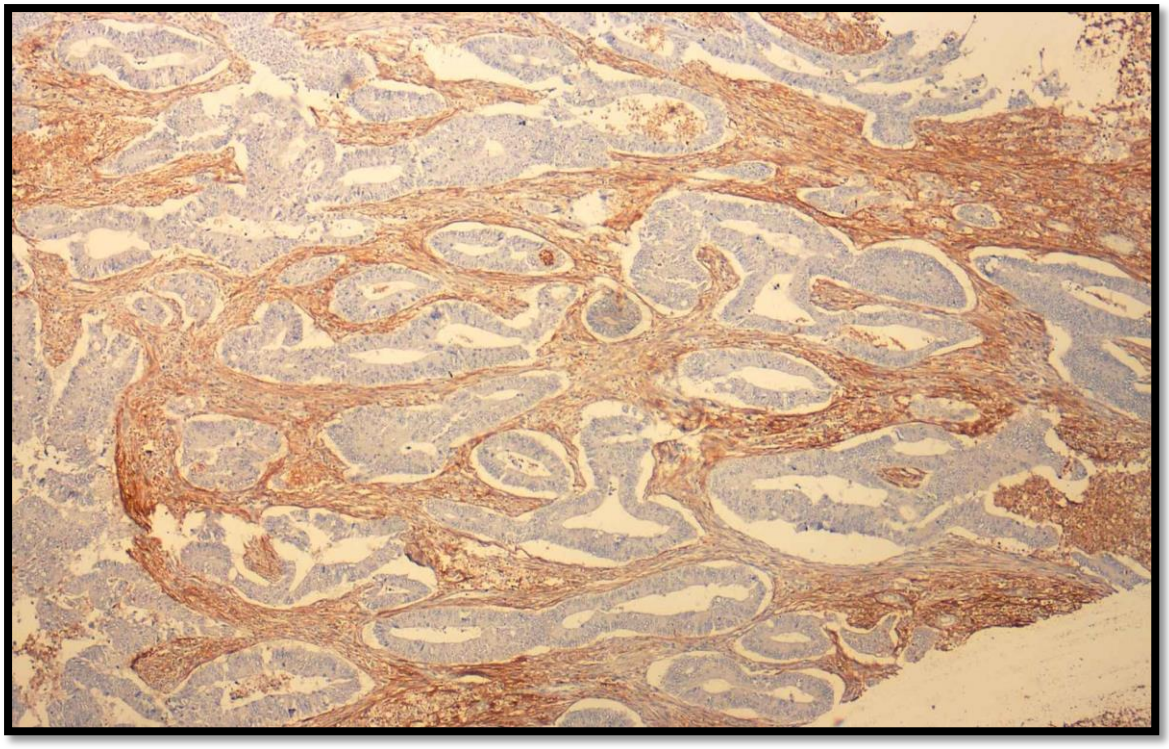
Photomicrograph 10: Mucinous adenocarcinoma (H & E 100X)



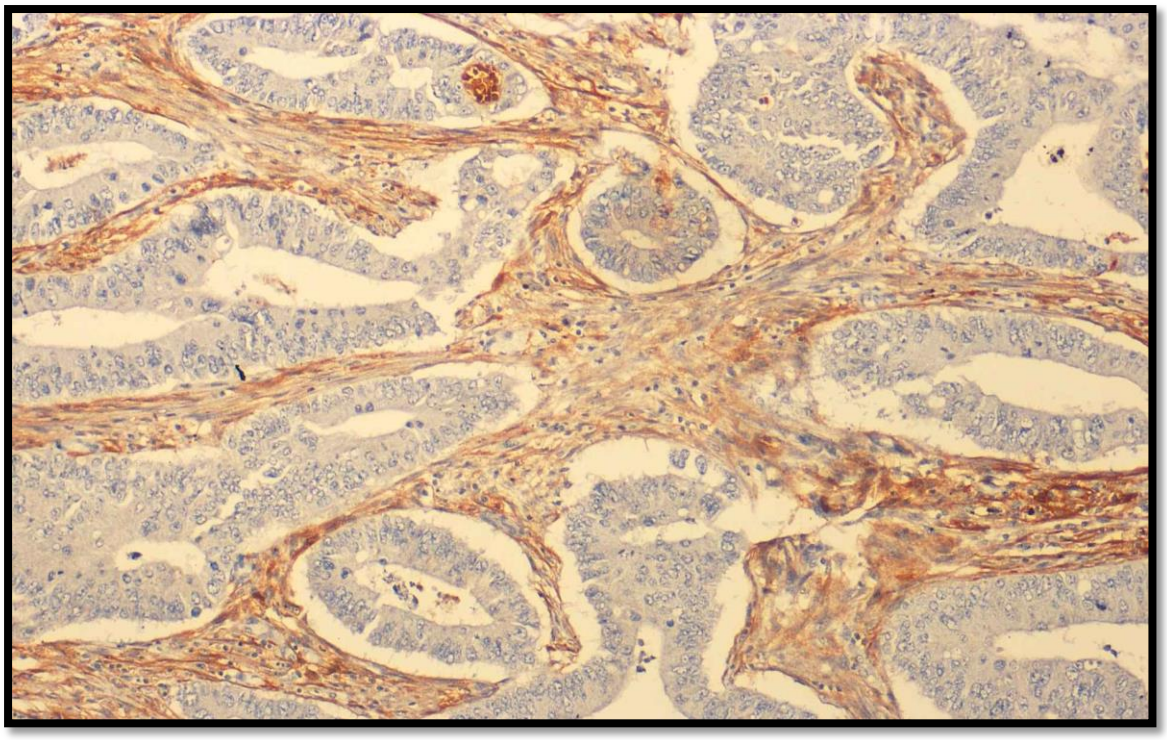
Photomicrograph 11: CD10 negativity in tumor cells of Mucinous adenocarcinoma (IHC 40X)



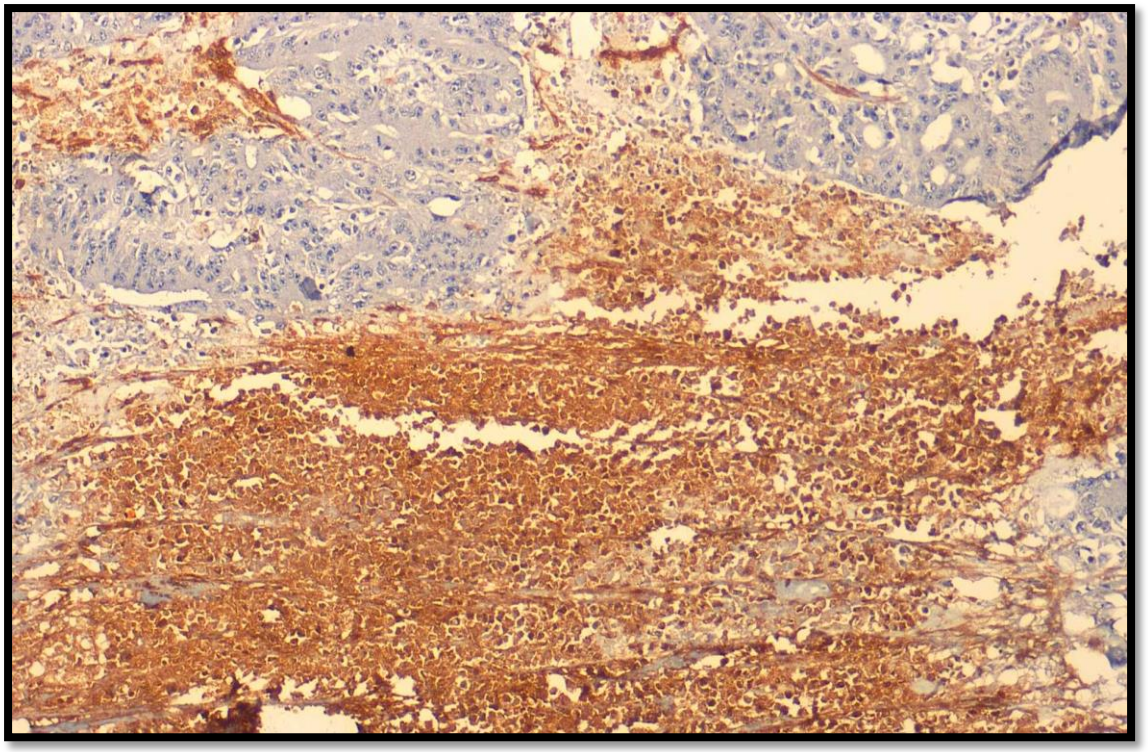
Photomicrograph 12: CD10 negativity in tumor cells of Mucinous adenocarcinoma (IHC 400X)



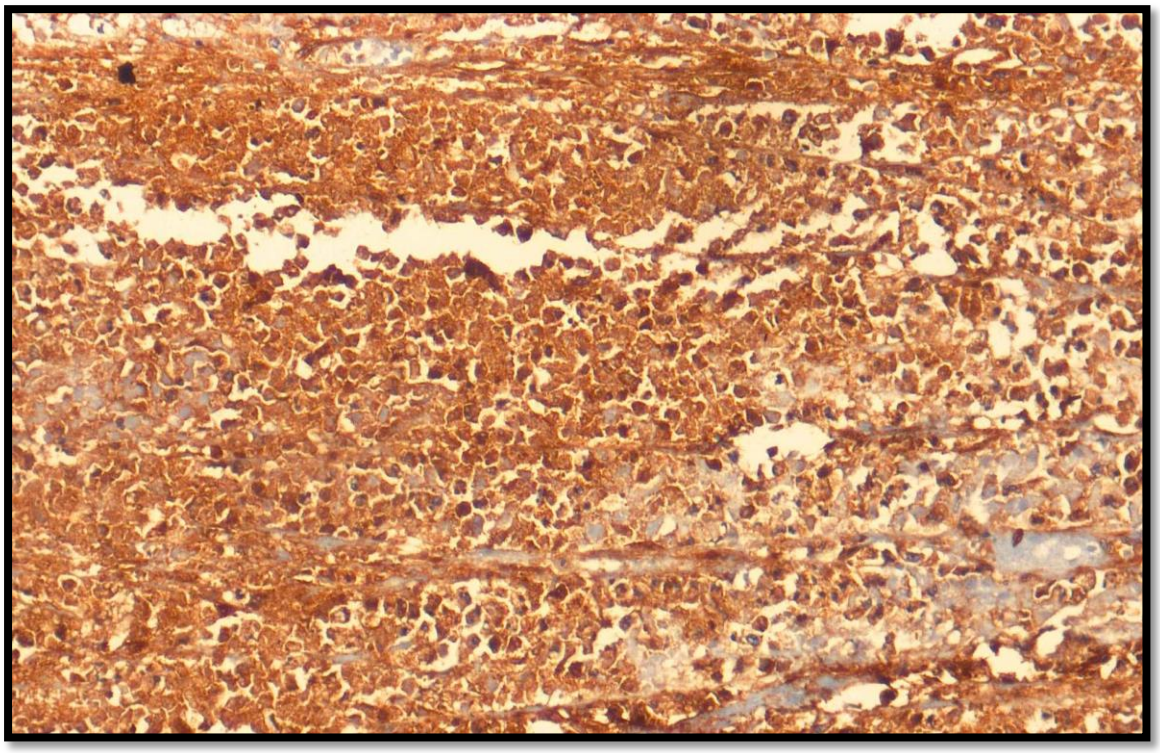
Photomicrograph 13: CD10 positivity in stromal cells in Moderately differentiated adenocarcinoma (IHC 40X)



Photomicrograph 14: CD10 positivity in stromal cells in Moderately differentiated adenocarcinoma (IHC100X)



Photomicrograph 15: CD10 positivity in inflammatory cells (IHC 40X)



Photomicrograph 16: CD10 positivity in inflammatory cells (IHC 100X)

DISCUSSION

Colorectal carcinoma (CRC) is the most common cause of cancer-related deaths in the USA. Globally, CRC affects over a million people each year. Around 20% of patients have metastatic (stage IV) CRC at the time of diagnosis.[7]

As genetic changes take place at an early stage of tumor growth, the potential for metastasis of a primary tumor may be predicted through molecular profiling of particular tumor markers. This suggests that adjuvant therapy may also be beneficial for patients with aggressive tumors.[113] One such marker is CD10.

Studies have observed deranged CD10 expression during the development of many cancers. In lung adenocarcinoma, upregulated expression of CD10 in tumor stroma is considered unfavorable for prognosis. An increase in stromal CD10 expression is associated with increasing grades of tumor in breast carcinoma.[5] CD10 is often expressed in mature neutrophils, lymphoid progenitor cells, and healthy tissues of the kidney, small intestine, and liver but not in healthy colorectal tissue. It has also been detected in malignant melanoma, colorectal, and prostate cancers, etc.[113] The role of CD10 in CRC is still under research.[2] Few studies have found the role of CD10 biomarker in cancer development and prognosis in cases of CRC.

Our study was done to evaluate the use of CD10 marker in the diagnosis of CRC and to correlate its expression with the staging and grading of carcinoma. The study evaluated total 40 cases of CRC.

In the present study, the mean age for CRC was 62.30 years as compared to the study done by Magadhi Malvika et al [2] and Zurawski J. et al[3] where the mean age group was 46.50 years and 59.02±10.16 years respectively. In the study done by Khairy Rasha A et al [5] mean age was 54.87 years, with ages ranging from 29 to 78

years. Levindo Alves de Oliveria et al [16] studied 130 patients with a mean age of 64.20±9.6 years.

In the study done by Zurawski J. et al.[3], 60.2% were females and 39.8% were males. Whereas equal gender distribution (50% females and 50% males) was seen in a study done by Levindo Alves de Oliveria et al. [16] In the present study, out of 40 cases, 22 cases (55%) were females and 18 cases (45%) were males. Similar gender distribution was found in a study done by Khairy Rasha A. et al.[5]

In the study done by Khairy Rasha A. et al.[5] most common tumor site was the right-sided colon (36.7% cases) and in 26.7% cases the site was a rectosigmoid junction. Levindo Alves de Oliveria et al.[16] found tumor in the colon in 60% cases and in the rectum in 40% cases. In the study done by B R, Ashwini et al.[106], 53.65% cases showed tumor in the colon and 46.34% cases showed the tumor in the rectum. In our study, the tumor was seen most commonly in the sigmoid colon (30% cases), followed by the ascending colon and rectosigmoid junction (15% cases).

B R, Ashwini et al.[106] included 37(90.24%) cases of Adenocarcinoma, 2(4.87%) cases of Mucinous adenocarcinoma and Signet ring cell adenocarcinoma each. The present study comprised of 92.50% cases of Adenocarcinoma NOS and 7.50% cases of Mucinous adenocarcinoma.

Khairy Rasha A. et al.[5] studied CD10 expression in tumor cells and stromal cells of CRC and premalignant lesions. They found 80% cases with positive tCD10 expression and 20% cases with negative tCD10 expression in invasive CRC. Positive sCD10 expression was seen in 57% cases and negative sCD10 expression was seen in 43% cases in their study. Magadhi Malvika et al.[2] studied stromal CD10 expression in CRC and compared it with lymph node metastasis. We studied immunohistochemical

expression of CD10 in tumor cells(tCD10), stromal cells (sCD10), and inflammatory cells (iCD10) and compared it with grades and stages of CRC. Out of 40 cases, 40% cases showed positive tCD10 expression and 60% cases showed negative tCD10 expression. Whereas 90% cases showed positive sCD10 expression and 10% cases were negative for sCD10 expression. We also studied CD10 expression in inflammatory cells which showed 85% cases were positive for iCD10 and 15% cases were negative for iCD10.

In a study by Khairy Rasha A. et al[5]., they did not find a significant association between the CD10 expression in tumor cells and CD10 expression in stromal cells in colorectal cancer (CRC). However, in our study, we found a statistically significant difference in the expression of CD10 in tumor cells and stromal cells in CRC cases (Chi-square test= 21.9780, p=0.0001).

The study done by Levindo Alves de Oliveria et al[16] observed similar CD10 expression in different grades of CRC. Also, Oshima et al.[108] found no difference in tCD10 expression in various grades of adenocarcinoma. Khairy Rasha A. et al.[5] observed no significant correlation between histological grades of CRC and tCD10 expression. Whereas a study done by Sato et al.[107] found higher expression of tCD10 in grade 1 and grade 2 CRC and lower expression in grade 3 CRC. Fujimoto Y et al.[13] identified that tCD10 expression was higher in grade 1 adenocarcinoma. In our study, we found that 100% (only 1 case) was of well-differentiated adenocarcinoma (Grade1), 60% (21/35) cases of moderately differentiated adenocarcinoma (Grade 2) and 50% (2/4) cases of Poorly differentiated adenocarcinoma (Grade 3) showed negative expression of CD 10 in tumor cells. 40% (14/35) cases of Grade 2 and 50% (2/4) cases of Grade 3 adenocarcinoma showed positive tCD10 expression. There was no difference in tCD10 expression in different grades of CRC. So, we did not find a

significant correlation between CD10 expression in tumor cells with grading of carcinoma (p=0.8454).

The research studies that are currently available suggest that CD10 cleaves the amino-terminal peptide bonds of hydrophobic amino acids to inactivate a wide variety of neuropeptides that are physiologically active and consequently, it removes these active neuropeptides from the microenvironment that are needed for cell signaling. It is anticipated that a decrease in CD10 expression could be the cause of tumor progression due to the availability of increased peptide concentrations in the tumor environment, which will enhance cell signaling and promote tumor growth.[5]

Khairy Rasha A. et al.[5] studied CD10 expression with depth of tumor invasion and they found inverse correlation between tCD10 expression and depth of invasion which was statistically significant. These results imply that tCD10 may play a role in the invasiveness of early CRC.[5] Decreased CD10 expression may be linked to the development of tumors by allowing peptide refurbishments that enhance cancer cell signaling and promote tumor growth. Fujita S, et al.[109] and Khairy Rasha A. et al.[5] did not find any correlation between tCD10 expression and stage of carcinoma we also found similar results with these studies (p=0.3583).

Zurawski J. et al.[3], Jang TJ et al.[110]and Magadhi Malvika et al.[2] observed that CD10 was expressed in stromal cells in CRC. A previous study proposed that the local inflammatory process in malignant tumors could be the cause of sCD10 expression.[110,114] According to recent research, the majority of CD10-positive immune cells are neutrophil granulocytes that are derived from myeloid cells.[13,110] Neutrophils control the invasion at several levels. Neutrophils can promote the invasion of tumors by releasing MMP-9, which degrades extracellular matrix (ECM).[13] We also found that CD10 was expressed in stromal cells (sCD10) and inflammatory cells(iCD10).

Studies done by Magadhi Malvika et al.[2] and Khanh D.T. et al.[13] did not find a correlation between CD10 expression in stromal cells and grades of carcinoma. However, few studies by Khairy Rasha A. et al.[5], Jang TJ et al.[110,] and Bernescu I, et al.[111] found an increase in sCD10 expression with an increase in grades of carcinoma though no statistical significance was found by most of them. According to Khanh D.T. et al.[13], stromal CD10 expression may be used as a potential biomarker in CRC along the adenoma-carcinoma sequence. B R, Ashwini, et al.[106] observed a decrease in the sCD10 expression in conjunction with loss of differentiation in the CRC. We found that sCD10 expression was decreased with an increase in grades of carcinoma which was statistically significant (p=0.0072) and it was in concordance with a study done by B R, Ashwini et al.[106]

Magadhi Malvika et al.[2] found increased sCD10 expression with an increase in depth of invasion of a tumor but no correlation with lymph node status. The present study found no correlation between sCD10 expression and the stage of CRC which was similar with the findings of Khairy Rasha A. et al.[5]

Jang TJ et al.[110] found CD10 expression in inflammatory cells and compared it with depth of tumor and lymph node metastasis but did not find a correlation between them. In the present study, we did not find a statistically significant association between iCD10 expression with grade and stage of CRC.

The findings from our study suggest that while sCD10 expression may play a role in tumor differentiation, the variable expression of CD10 in tumor cells, stromal cells and inflammatory cells indicates that it may be used along with other markers for assessing the progression or severity of CRC.

SUMMARY

This study examined 40 cases of colorectal carcinoma (CRC) to investigate demographic, pathological characteristics, and CD10 expression in tumor cells, stromal cells, and inflammatory cells. The results revealed the following key findings:

- The mean age of the patients was 62.30 years, with 37.50% of patients being 60 years or less than 60 years. Females represented a slightly higher proportion (55%) compared to males (45%).
- The sigmoid colon was the most common site of lesions, accounting for 30.00% of cases, followed by the rectosigmoid junction and ascending colon, each with 15.00% of cases.
- On histopathological examination most of the cases were of Adenocarcinoma NOS, constituting 92.50% of the cases, with the majority being Grade 2 (87.50%), and only a few cases were diagnosed with Mucinous adenocarcinoma (7.50%).
- 50.00% of the cases were of Stage IIa, followed by Stage IIIb in 30.00% cases.
- Most cases (60.00%) exhibited a score of 0 in tumor cells (less than 10% of cells expressing CD10). There was no statistically significant correlation between CD10 expression and the grades ($p=0.8454$) or stages ($p=0.3583$) of carcinoma.
- CD10 expression in stromal cells showed a slight negative correlation with grades of carcinoma (Spearman's rank correlation = -0.4184, $p=0.0072$), indicating decreased CD10 expression with higher carcinoma grades. However, no significant correlation was found with stages of carcinoma ($p=0.4728$).
- CD10 expression in inflammatory cells did not show any statistically significant correlation with grades ($p=0.0990$) or stages ($p=0.1873$) of carcinoma

LIMITATIONS OF THE STUDY

- The relatively small sample size of 40 cases and a limited number of well-differentiated and poorly differentiated adenocarcinoma cases at our center may have limited our ability to detect a statistically significant correlation between the expression of CD10 and the grading and staging of carcinoma. Future research with a larger sample size is needed to validate these findings and provide more strong conclusions regarding the role of CD10 in CRC.
- Longitudinal studies that track patient outcomes over time are essential to fully understand the prognostic significance of CD10 expression in CRC.

CONCLUSION

The findings of the present study suggest that while there is variability in the expression of CD10 in tumor cells among different CRC cases, this variability does not correlate significantly with either tumor grade or stage. Despite the lack of significant associations, the observed trends provide a foundation for further research. It is possible that CD10 expression in tumor cells could interact with other molecular pathways or be influenced by factors not accounted for in this study.

CD10 expression in stromal cells is higher than in tumor cells which is statistically significant. There is a significant inverse correlation between stromal CD10 expression and tumor grade.

CD10 expression in inflammatory cells does not show a significant correlation with grades and stages. This suggests a limited role for CD10 expression in inflammatory cells in CRC.

These findings underline the complexity of the role of CD10 in the CRC microenvironment and suggest that while CD10 expression varies, it may not be a reliable independent marker for assessing the progression or severity of CRC.

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ANNEXURES

ANNEXURE I

WHO Classification of Colorectal Epithelial Tumors (2019)[30]

Benign epithelial tumors and precursors

1) Serrated dysplasia, low grade
2) Serrated dysplasia, high grade
   - Hyperplastic polyp-micro vesicular type
   - Hyperplastic polyp- goblet cell
3) Adenomatous polyp, low grade dysplasia
4) 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
5) Glandular intraepithelial neoplasia, low grade
6) Glandular intraepithelial neoplasia, high grade

Malignant epithelial tumors

1) Adenocarcinoma, NOS
   - Adenoma-like adenocarcinoma
   - Micropapillary adenocarcinoma
   - Mucinous adenocarcinoma
   - Poorly cohesive carcinoma
   - Signet ring cell carcinoma
   - Medullary adenocarcinoma
   - Adenosquamous carcinoma
   - Carcinoma, undifferentiated, NOS
   - Carcinoma with sarcomatous component
2) Neuroendocrine tumor, NOS
   - Neuroendocrine tumor grade 1
   - Neuroendocrine tumor grade 2
   - Neuroendocrine tumor grade 3
   - L cell tumor
   - Glucagon-like peptide-producing tumor
   - PP / PYY-producing tumor
   - Enterochromaffin cell carcinoid
   - Serotonin-producing carcinoid
3) Neuroendocrine carcinoma, NOS
   - Large cell neuroendocrine carcinoma
   - Small cell neuroendocrine carcinoma
4) Mixed neuroendocrine – nonneuroendocrine neoplasm (MiNEN)

ANNEXURE II

Modified Duke's Staging[95]:

A: Tumor confined to mucosa.

B1: Tumor confined to the submucosa with no lymph node metastasis.

B2: Tumor confined to the muscle layer, with no lymph node metastasis.

C1: The tumor did not invade the bowel wall with lymph node metastasis.

C2: Tumor invaded the intestinal wall and lymph node metastasis.

D: Distant metastasis.

ANNEXURE III

Astler-Coller staging[96]:

A: Tumor confined to mucosa

B1: Tumor invasion up to muscularis layer. No lymph node metastasis.

B2: Tumor invasion up to serosa. No lymph node metastasis.

B3: Tumor invasion up to adjacent organs. No lymph node involvement.

C1: Tumor invasion up to muscularis layer and lymph node involvement.

C2: Tumor invasion up to serosa and lymph node involvement.

C3: Tumor invasion up to adjacent organs with lymph node involvement

D: Metastasis to distant sites.

ANNEXURE IV

The following is the AJCC (8th edition) TNM classification of CRC[97]:

Primary tumor:

TX – Primary tumor cannot be assessed.

T0 – No evidence of primary tumor.

Tis – Carcinoma in situ: intraepithelial or invasion of lamina propria.

T1 – Tumor invades submucosa.

T2 – Tumor invades muscularis propria.

T3 – Tumor invades through the muscularis propria into peri colorectal tissues.

T4a – Tumor penetrates to the surface of the visceral peritoneum.

T4b – Tumor directly invades or is adherent to other organs or structures

Regional lymph nodes:

NX – Regional lymph nodes cannot be assessed.

N0 – No regional lymph node metastasis.

N1 – Metastasis in 1- 3 regional lymph node(s).

N1a – Metastasis in 1 regional lymph node.

N1b – Metastasis in 2 - 3 regional lymph nodes.

N1c – Tumor deposit(s) in the subserosa, mesentery, or non-peritonealised

pericolic or perirectal tissues without regional nodal metastasis.

N2 – Metastasis in 4 or more regional lymph nodes.

N2a – Metastasis in 4 – 6 regional lymph nodes.

N2b – Metastasis in 7 or more regional lymph nodes

Metastasis:

M0 – No distant metastasis.

M1 – Distant metastasis.

M1a –Metastasis confined to one organ or site (e.g., liver, lung, ovary, nonregional node).

M1b – Metastasis in more than one organ/site

M1c– Metastasis to peritoneum.

ANNEXURE V - INFORMED CONSENT FORM

"Evaluation of CD10 biomarker expression in diagnosis of colorectal carcinoma- a one-year cross-sectional study at tertiary care centre of Belagavi"

Name of Student/Principal Investigator: REG NO: BN0121006

Name of Guide/Co-Investigators: -

Objectives: To study the potential of CD 10 marker expression in the diagnosis of colorectal carcinoma and its association with staging and histological grading of CRC.

Explanation of procedure: During this study, you will be asked questions regarding history and background and you are supposed to answer to the best of your knowledge. If you agree to enroll yourself in this study, you will be interviewed regarding your present, past and family history and your clinical manifestations.

Withdrawal from participation in the study: Participation in this study is voluntary. You will be free to decide whether to participate in this study or continue participation once enrolled. In case you decide to withdraw your participation, you are free to do so. However, please convey the decision to the principal investigator.

Possible benefits from participating in the study: You will/ will not have nor get any benefits by participating in this study. The data gathered will help the population at large.

Possible risks from participating in the study: There are no risks involved in participating in this study.

Privacy and confidentiality: The information collected from you will be coded, to prevent any person from identifying you. Your identity will never be revealed. The

data collected from you will be kept confidential and only processed or aggregated data will be used for publication.

Financial incentives: You will not receive any payment for participating in this study.

Authorization for publication of aggregated data: Results obtained after processing of the aggregated data will be published for scientific purposes and or presented to scientific groups. However, your identity will never be revealed.

Questions: In case of any questions with regard to this study, you are free to contact:

1. Principal Investigator, Department of Pathology, J.N. Medical College.

2. Guide, Professor, Department of Pathology, J.N. Medical College.

If you have any question or complaints with regard to your right as study participant you may contact the Chairperson, Ethical Committee of JNMC, 0831-2473777 Extension 4052.

Legal rights: By signing this consent form, we are not waving any of your legal rights.

ANNEXURE VI - CONSENT STATEMENT

I am making a voluntary decision to participate in the study **"Evaluation of CD10 biomarker expression in diagnosis of colorectal carcinoma- a one-year cross sectional study at tertiary care center of Belagavi''**. My signature below indicates that I have decided to participate and I have read the information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the participant:

Signature or left thumb impression of the participant:

Name of the witness:

Signature or left thumb impression of the witness:

Name of the investigator:

Signature of the investigator:

Date:

ANNEXURE VII

STEPS OF TISSUE PROCESSING

Automated tissue processor Leica TP 1020 was used for processing the specimens and includes the following steps:

1. Two changes of 10% formalin for 3 hours in one followed by 1 hour in the second container.
2. Dehydration with graded concentrations of alcohol (50%, 70%, 80%, 95%) for one hour each followed by two changes of absolute alcohol for 30 minutes each.
3. Clearing by xylene- two changes for 1 hour each.
4. Paraffin wax impregnation – two changes for 2 hours each.
5. Embedding in paraffin wax followed by making a block.
6. Sections were cut using a semi-automated microtome at a setting of 3-5 microns.
7. Sections were floated in a water bath at 600 C.
8. Mounting of sections done on egg albumin-coated slides for hematoxylin and eosin staining and mounted on poly-l-lysine-coated slides for IHC.

ANNEXURE VIII

IMMUNOHISTOCHEMICAL STAINING PROCEDURE FOR CD10:

1. Cut the tissue sections on microtome with the thickness of 3µ and collect them on coated slides
2. Bake the sections at 37 degrees Celsius for overnight.
3. Before the test bake it at 60 degrees Celsius for 1 hour.

Deparaffinize step:

4. Xylene I for 10 minutes
5. Xylene II for 10 minutes
6. Absolute alcohol I for 10 minutes
7. Absolute alcohol II for 10 minutes
8. Rinse in water – 5 minutes
9. Rinse in distilled water- 1 minute
10. Antigen retrieval (Tris Buffer+ EDTA)
11. Prepare the required amount of buffer and cook the slides in a pressure cooker for 3 whistles
12. Allow it to cool to room temperature for 1 hour
13. Wash it with wash buffer 3 times with a gap of 2 minutes each.
14. Apply 3% Hydrogen peroxide- 8 to 10 minutes
15. Wash with wash buffer 3 times with a gap of 2 minutes each
16. Primary antibody CD10 (56C6) incubated for 45 to 60 minutes in a closed chamber at room temperature.
17. Wash with wash buffer 3 times with a gap of 2 minutes each.

18. Apply the primary antibody amplifier master for 15 minutes in a closed chamber.
19. Wash with wash buffer 3 times with a gap of 2 minutes each.
20. Apply polymer HRP for 25- 30 minutes in a closed chamber.
21. Wash with wash buffer 3 times with a gap of 2 minutes each.
22. Apply DAB chromogen for 10 minutes.
23. Wash slowly under running tap water from the backside.
24. Counterstain with Haematoxylin for 2 minutes.
25. Blueing in warm water for 1 minute.
26. Clear in xylene and mount with DPX.

The lymph node was used as a positive control

ANNEXURE IX – MASTERCHART

Serial no	IP No	Age	Gender	Type of surgery	Site	Diagnosis	Grade	TNM stage	AJCC Stage	CD10 score- Tumour cells	CD10- stromal cells	CD10 - Inflammatory cells
1	1176378	37	F	Right hemicolectomy	Hepatic flexure	Adenocarcinoma NOS	Grade 2	pT3N0M0	IIa	0	2+	2+
2	1167551	51	F	Right hemicolectomy	Ascending colon	Adenocarcinoma NOS	Grade 2	pT3N0M0	IIa	0	1+	Negative
3	1176781	72	M	Anterior resection	Sigmoid colon	Adenocarcinoma NOS	Grade 2	pT3N2aM0	IIIc	0	3+	1+
4	6918242	36	M	Right hemicolectomy	Ascending colon	Adenocarcinoma NOS	Grade 2	pT3N1bM0	IIIb	0	1+	2+
5	1174123	75	M	Left hemicolectomy	Sigmoid colon	Adenocarcinoma NOS	Grade 2	pT3N0M0	IIa	3	1+	2+
6	1167692	62	F	Right hemicolectomy	Ascending colon	Adenocarcinoma NOS	Grade 2	pT3N0M0	IIa	0	1+	2+
7	1100527	66	M	Abdominoperineal Resection(APR)	Rectosigmoid junction	Adenocarcinoma NOS	Grade 1	pT2N0M0	I	0	1+	1+
8	19391/3029839	51	M	Anterior resection	Sigmoid colon	Adenocarcinoma NOS	Grade 2	pT3N1aM0	IIIb	3	1+	Negative
9	1101478	61	M	Left hemicolectomy	Descending colon	Adenocarcinoma NOS	Grade 1	pT2N0M0	I	0	2+	1+
10	1126348	64	M	APR	Anal canal	Adenocarcinoma NOS	Grade 2	pT2N0M0	I	1	1+	1+
11	1192614	75	F	Left hemicolectomy	Sigmoid colon	Adenocarcinoma NOS	Grade 2	pT3N1bM0	IIIb	0	1+	1+
12	1187244	60	F	Anterior resection	Sigmoid colon	Adenocarcinoma NOS	Grade 2	pT3N2bM1a	IVa	0	2+	1+
13	1186214	30	F	Anterior resection	Rectosigmoid junction	Adenocarcinoma NOS	Grade 2	pT3N0M0	IIa	3	1+	1+
14	20483/3033211	68	F	Anterior resection	Rectosigmoid junction	Adenocarcinoma NOS	Grade 2	pT3N1cM0	IIIb	2	2+	1+
15	1198567	74	M	Anterior resection	Sigmoid colon	Adenocarcinoma NOS	Grade 2	pT3N0M0	IIa	3	1+	1+
16	1197980	74	M	Anterior resection	Sigmoid colon	Adenocarcinoma NOS	Grade 2	pT3N0M0	IIa	3	2+	1+
17	1195978	73	F	Anterior resection	Sigmoid colon	Adenocarcinoma NOS	Grade 2	pT3N0M0	IIa	3	1+	Negative
18	1107187	76	M	Anterior resection	Sigmoid colon	Adenocarcinoma NOS	Grade 1	pT2N0M0	I	0	2+	1+
19	1106641	67	F	APR	Anorectal junction	Adenocarcinoma NOS	Grade 2	pT3N0M0	IIa	3	2+	1+
20	1153352	62	F	Right hemicolectomy	Ascending colon	Adenocarcinoma NOS	Grade 2	pT2N0M0	I	0	2+	2+
21	10007437	62	F	Anterior resection	Rectosigmoid junction	Adenocarcinoma NOS	Grade 2	pT3N0M0	IIa	3	1+	2+
22	6380983	60	F	Right hemicolectomy	Ascending colon	Adenocarcinoma NOS	Grade 2	pT3N1aM0	IIIb	2	1+	1+
23	111007	55	F	Anterior resection	Sigmoid colon	Adenocarcinoma NOS	Grade1	pT3N1cM0	IIIb	3	2+	1+
24	1098462	65	F	Low anterior resection	Rectum	Adenocarcinoma NOS	Grade 2	pT3N0M0	IIa	1	3+	2+
25	1107834	65	M	Anterior resection	Rectum	Adenocarcinoma NOS	Grade 2	pT3N0M0	IIa	3	1+	1+
26	10001704	56	F	Left hemicolectomy	Descending colon	Adenocarcinoma NOS	Grade 1	pT3N1aM0	IIIb	0	2+	1+
27	10000698	87	F	Right hemicolectomy	Ascending colon	Adenocarcinoma NOS	Grade 2	pT3N1bM0	IIIb	1	2+	1+
28	1208394	81	M	Low anterior resection	Rectum	Adenocarcinoma NOS	Grade 2	pT3N2bM1a	IVa	3	1+	Negative
29	1116853	76	M	Anterior resection	Descending colon	Adenocarcinoma NOS	Grade 2	pT3N2aM0	IIIc	2	1+	1+
30	10017967	53	M	Right hemicolectomy	Caecum	Adenocarcinoma NOS	Grade 2	pT2N0M0	I	0	1+	1+
31	10012582	40	M	Right extended hemicolectomy	Caecum and splenic flexure	Adenocarcinoma NOS	Grade 2	pT3N1cM0	IIIb	2	2+	2+
32	1169139	48	F	APR	Anal canal	Adenocarcinoma NOS	Grade3	pT3N0M0	IIa	1	1+	Negative
33	11168902	68	F	Anterior resection	Descending colon	Adenocarcinoma NOS	Grade 2	pT3N1aM0	IIIb	2	1+	1+
34	10027887	63	M	Anterior resection	Sigmoid colon	Adenocarcinoma NOS	Grade 2	pT3N1bM1a	IVa	3	2+	1+
35	3035556/21251	70	F	Anterior resection	Rectosigmoid junction	Adenocarcinoma NOS	Grade 2	pT2N0M0	I	0	1+	1+
36	1199911	76	F	Right extended hemicolectomy	Hepatic flexure	Mucinous adenocarcinoma	Grade3	pT3N1bM0	IIIb	2	3+	Negative
37	3029447/6802392	55	F	APR	Rectum	Mucinous adenocarcinoma	Grade3	pT3N2bM1a	IVa	1	2	1+
38	1098628	74	M	Low anterior resection	Sigmoid colon	Mucinous adenocarcinoma	Grade3	pT3N0M0	IIa	2	2+	1+
39	1199430	60	M	Anterior resection	Descending colon	Adenocarcinoma NOS	Grade2	pT3N1aM0	IIIb	1	3+	1+
40	3023099	44	F	Anterior resection	Rectosigmoid junction	Adenocarcinoma NOS	Grade2	pT3N0M0	IIa	2	1+	1+