"A STUDY OF TUMOR ASSOCIATED MACROPHAGES AND THEIR SUBPOPULATION M1 AND M2 BY IMMUNOHISTOCHEMISTRY IN COLORECTAL CANCER"

BY

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DISSERTATION SUBMITTED TO

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IN

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UNDER THE GUIDANCE OF

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LIST OF ABBREVATIONS

CRC	**- Colo-Rectal Carcinoma**
TAM	**- Tumor Associated Macrophage**
CDC	**- Centre for Disease Control and Prevention**
CEA	**- Carcino Embryonic Antigen**
CD 68	**- Cluster of Differentiation 68**
CD 163	**- Cluster of Differentiation 163**
TGF	**- Tumor Growth Factor**
IL	**- Interleukin**
COX-2	**– Cycloxigenase 2**
APR	**- Abdomino-Perineal Resection**
H&E	**- Hematoxylin and Eosin**
FAP	**- Familial adenomatous polyposis**
IHC	**- Immunohistochemistry**

ABSTRACT

BACKGROUND:

Tumors of the colon and rectum are one of the most common malignancies worldwide. However, its incidence was less in India compared to the developed countries. In the recent years, due to westernization, sedentary lifestyle and increased consumption of animal fats with less dietary fibre intake have increased the incidence in India in the past few decades. Family history and Microsatellite instability also predisposes the patient to Colo-rectal carcinoma. Many prognostic factors have been studied in Colo-rectal cancers and have been proved. However newer factors like macrophage infiltration in the tumor microenvironment have been studied. Many theories have been put forth to study these macrophages and their sub population M1 and M2. M1 macrophages are considered to be tumoricidal whereas M2 macrophages are considered to promote tumor growth by releasing growth factors and promoting angiogenesis. Hence, the study of these macrophage subpopulation M1 and M2 can help in assessing the prognosis in patients with Colo-rectal cancers.

AIMS & OBJECTIVES:

1. To determine the expression of CD68 and CD163 in Colorectal Cancer

2. To Correlate the expression of CD68 and CD163 with the histological grade and stage of the tumor

MATERIALS AND METHODS:

All Colorectal carcinoma specimens received in the Department of Pathology from R.L.Jalappa Hospital and Research Center attached to Sri Devaraj Urs Medical College, Tamaka, and Kolar from December 2016 to September 2018 and also the paraffin blocks taken from all cases of Colorectal cancer retrieved from Archives of Department of Pathology from the year January 2008 to November 2016 were included in the study.

Data regarding the clinical details (Age, Sex, Histological grading) were collected. Hand E slides were reviewed for Histopathological types, grade and staging of the tumor. Immunohistochemistry for CD68 and CD163 (Biocare mouse antibody) was performed on all cases of Colorectal Carcinoma using appropriate positive and negative controls by peroxidase and anti peroxidase method.

RESULTS:

A total of 62 cases were studied of which 39 were males and 23 were females. The most common site of tumor was Rectum followed by ascending colon. Majority of the tumors were less than 5 cms.The most common grade was moderately differentiated adenocarcinoma. Maximum number of cases were in Stage III, 23 cases(37.1%) Perineural invasion was seen in 2 cases and lymphovascular invasion was seen in 3 cases. Maximum number of cases (64.5%) were in lymphnode ratio less than <0.111.

Expression of CD 68 was significantly correlating with site of the tumor, Size of the tumor, Grade, and lymphnode ratio. Expression of CD 163 was correlating with T stage, N stage, TNM stage, and Lymphnode ratio.

CONCLUSION:

CD 68 expression was associated with better prognostic factors such as smaller size of tumor, lesser grade and lesser lymphnode ratio(LNR) and CD 163 expression was associated with poorer prognostic factors such as higher T stage, Higher N stage, and higher values of lymphnode ratio(LNR). Hence, CD 69 and CD 163 can serve as a reliable prognostic marker in colo-rectal cancers.

KEY WORDS- Colo-rectal cancer, Immunohistochemistry, Prognosis

TABLE OF CONTENTS

LIST OF TABLES

LIST OF CHARTS

LIST OF FIGURES


Introduction

INTRODUCTION

Tumors of the colon and rectum are the 3 rd most common malignancies in men and second most common malignancy worldwide. [1] They are the 2 nd most common cause of death from cancer.[2] They are included among the most frequently encountered malignancy in the western population and in industrialized countries. The U S SEER database showed that the incidence of colorectal adenocarcinoma was 33.7/100000 and there was an increase of 18% from 1973 to1987.[3] However, in the recent past, there has been a steady increase in the incidence of Colo-rectal cancers in India.

It was estimated that about 875,000 colo-rectal cancer cases were detected in 1996 and constituted to about 8.5% of overall newly detected malignancies.[3]

A variety of environmental and genetic factors play a vital role in the development of these tumors[3]. Tumor microenvironment consisting of leucocytes and fibroblasts are also involved in the progression of colo-rectal cancers.

The concept of macrophages differentiation and activation by classical and alternate pathway in the progression of the disease has been hypothesized and are being studied in the tumors of colon and breast[4].

It has also been studied that the macrophages release cytokines, which favor tumor progression and metastasis[5]

The tumor associated macrophages (TAMs) are broadly classified into two types depending on their mode of activation. The M1 macrophages are activated by classical pathway and M2 macrophages are activated by alternate pathways.

M1 macrophages cause good inflammatory response by releasing pro inflammatory cytokines such as TNF alpha, IL Beta and IL 6 thus fight against the tumor cells and are considered tumoricidal.

The M2 macrophages secrete anti-inflammatory cytokines such as TGF Beta, IL 10 and IL 3 and may help in tumor progression[6].

CD 68 and CD 163 are the proteins expressed by the circulating macrophages, monocyte derived macrophages and tissue macrophages. CD 68 stains cytoplasm of the M1 macrophages that are considered to be tumor suppressive and CD 163 stains the cytoplasm of M2 macrophages that are considered to help in tumor progression.

The patients with Colo-rectal cancer have better prognosis when there is increase density of macrophages at the tumor front which exhibit M1 phenotype, despite the parallel increase of M2 phenotype[7]

On H and E section, it is difficult to differentiate M1 and M2 phenotypes. Hence Immunostaining is used to identify M1 and M2 sub population of macrophages. CD68 is been taken as a marker for M1 macrophage and CD163 is been taken as a marker for M2 macrophage.

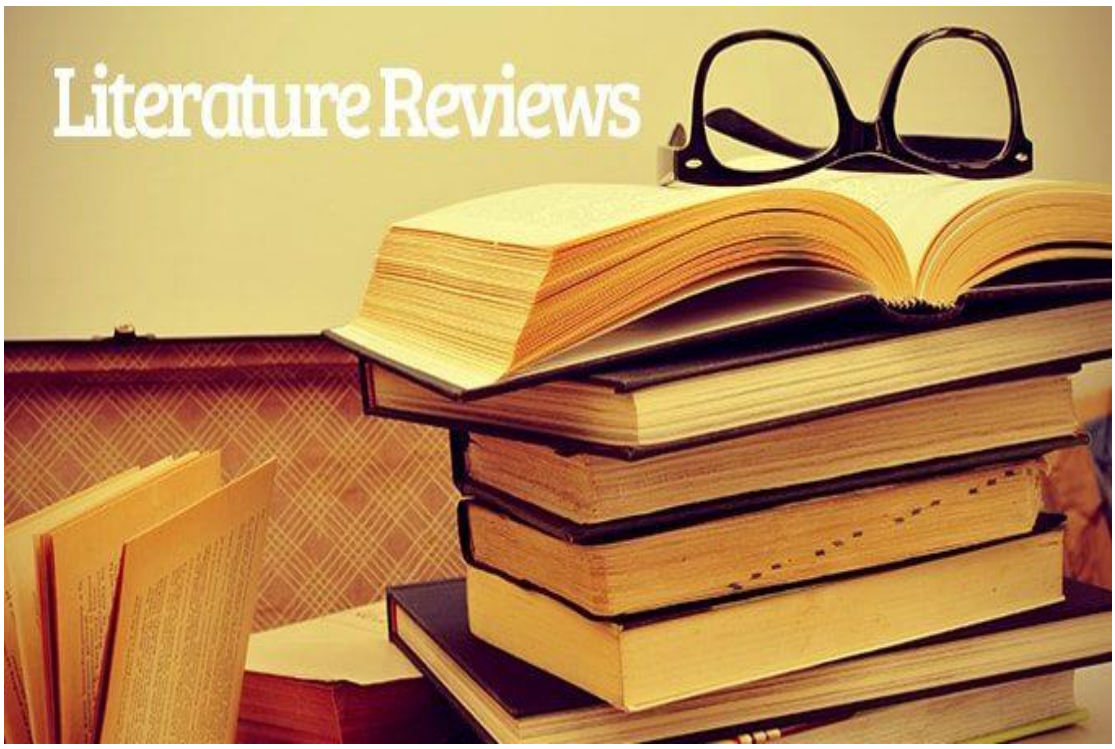
Only few studies determining expression of CD68 and CD163 have been done on Colorectal Cancers and published in Indian Literature so far.

Hence the study is undertaken to determine the expression of CD68 and CD163 in Colorectal Carcinomas.

Aims & Objectives

OBJECTIVES OF THE STUDY

1. To determine the expression of CD68 and CD163 in Colorectal Cancer
2. To Correlate the expression of CD68 and CD163 with the histological grade and stage of the tumor


Literature Reviews

REVIEW OF LITERATURE

EMBRYOLOGY

The caecum, appendix, ascending colon and one half to 2/3rd of transverse colon arises from the hindgut. During development, as early as 6 th week of intrauterine life, the midgut elongates and forms a U- shaped loop called as "Midgut loop" and communicates with the omphaloenteric duct by 10th week.

The midgut loop has a cranial and caudal limb and which is suspended by the mesentry in the abdominal cavity. The cranial loop grows rapidly and forms the intestinal loops.

The caecal swelling is formed by the caudal loop , which appears in the ante mesenteric border of midgut loop which further grows slowly in the apex forming appendix.

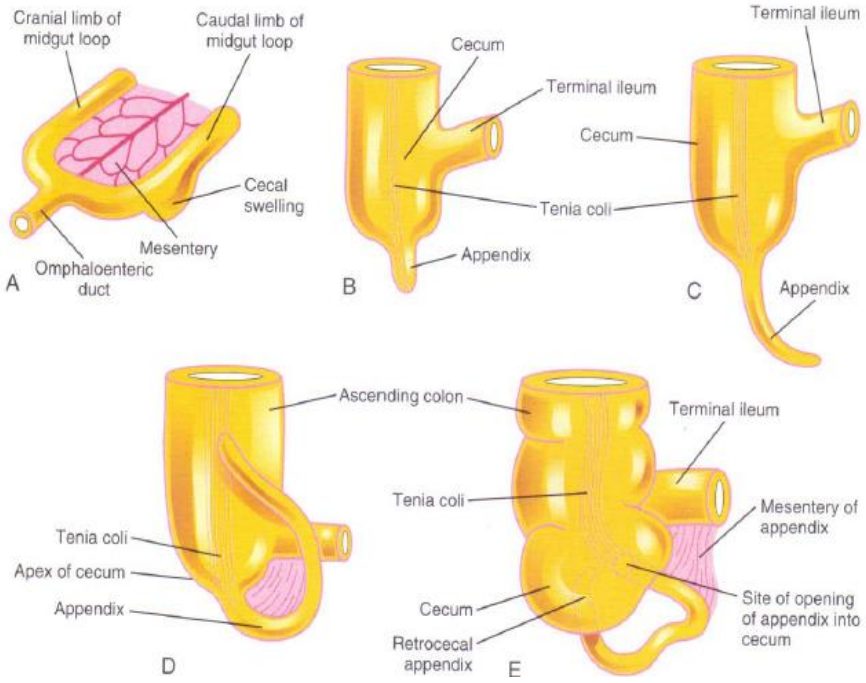


Figure 1 - Showing stages in the developmemt of cecum and appendix. [34]

The Left one third to one half of transverse colon, descending colon, sigmoid colon and part of anal canal is formed by the hindgut. The site of junction 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 Superior mesenteric artery whereas the part of transverse colon derived from the hindgut is supplied by branch of Inferior mesenteric artery.

As the mesentery fuses with parietal peritoneum the descending colon becomes retroperitoneal.

The terminal part of hindgut forms the cloaca and plays an important role in the development of Anal canal and rectum. [34]

ANATOMY

The colon and rectum comprises of distal 1-1.5 metres of the gastro intestinal tract and has been divided into Caecum, ascending colon, transverse colon, descending colon, sigmoid colon and rectum. The rectum is about 8-15 cms and ends with Anal canal but has no peritoneal covering.

The main function of colon or large intestine is the absorption of water and salts and push the solid faeces into the rectum during defecation. [32]

GROSS APPEARANCE AND HISTOLOGY

The identification of large intestine on the external surface is done by examination of caecum and appendix. The other identification points in favour of large intestine are

1. Taeniae coli are the three longitudinal muscle bands of smooth muscles over the surface
2. The contractions in smooth muscles of taeniae coli causes bulges and are known as Haustrations.
3. Accumulations of adipose tissue on the visceral surface are called Epiploic appendages [72].

The large bowel is lined by 4 layers namely

1. Mucosa- The Mucosa is subdivided into Epithelium, Lamina propria and muscularis mucosae. The histology remains the same in the entire length of colon from caecum to rectum. Just above the valves of anal canal, mucosa folds itself longitudinally and are known as “Coloumns of Morgagani”.

It consists of two different types of cells- Goblet cells and Absorptive cells. These cells are arranged in tightly packed crypts which extend below and sits on muscularis mucosae. [32]

2. Sub mucosa- Consists of loose connective tissue, blood vessels and Meissners plexus
3. Muscularis Externa- There are two muscle layers in muscularis externa. 1. Inner circular muscle layer. 2. Outer muscle layer forms three bands that are longitudinal and are known as Taeniae coli. The outer longitudinal layer is thin and seen interspersed between taeniae coli. [33]
4. Serosa- It is the outermost layer and is the site of mesenteric attachment to the colon.[33]

The lymphatics of colon drain to Paracolic group, Mediate nodal groups, Central Lymph nodes and Para aortic group of Lymph nodes[2]

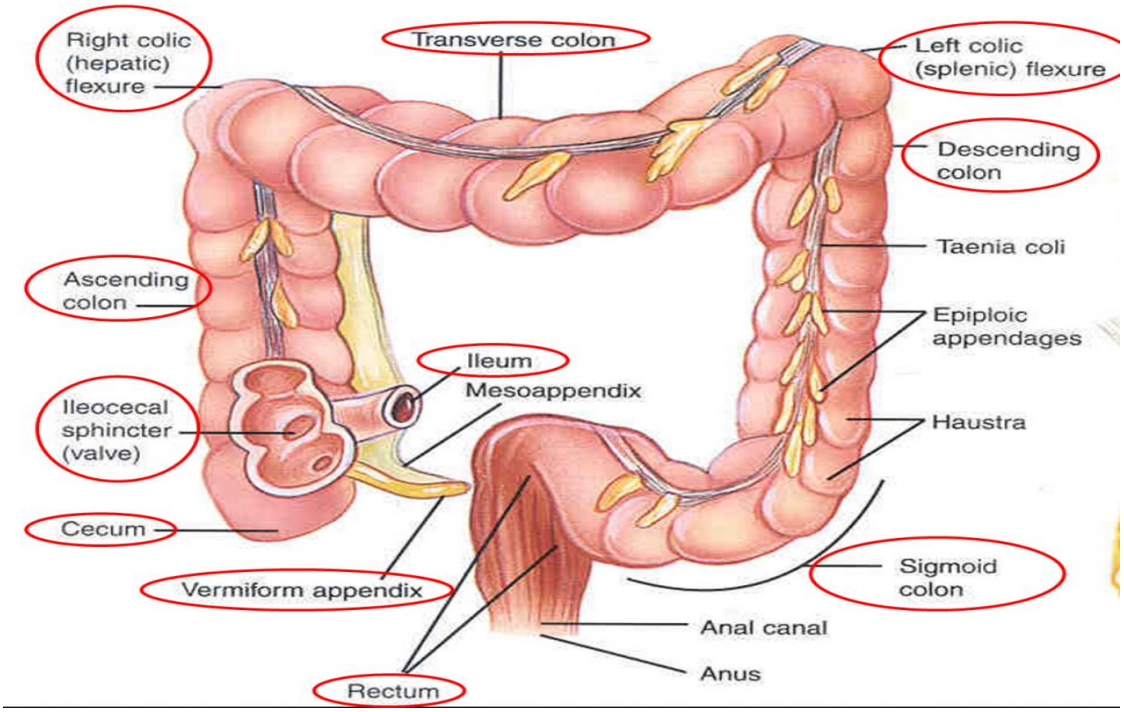


Figure 2 - Anatomy of Colon and Rectum [22]

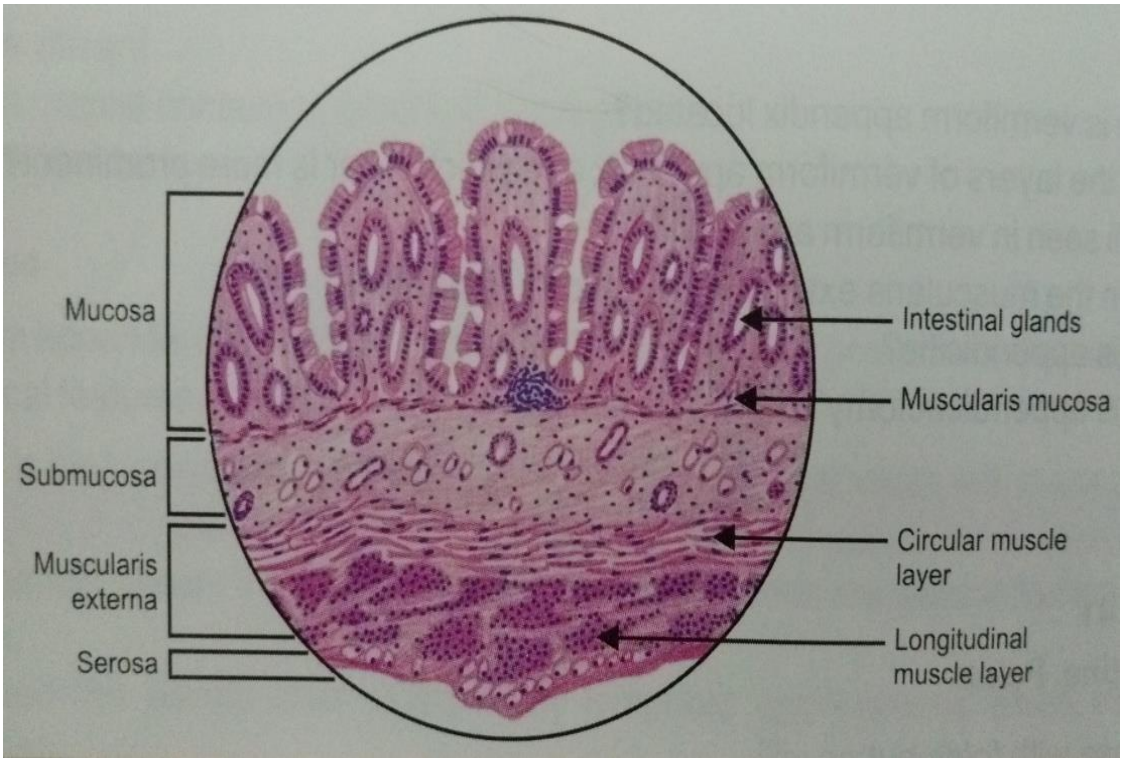


Figure 3 - Histology of Large intestine [23]

INCIDENCE AND EPIDEMIOLOGY:

Colo-rectal cancers are an important cause of morbidity and mortality worldwide and accounts for 9% of all malignancies. There is no sex predeliction but slightly more common in males.

Australia, New Zealand, Canada, the United States, and parts of Europe carry the highest number of cases whereas countries with the lowest risk include China, India, and parts of Africa and South America. It affects about 40/ 100000 population in United States, New Zealand and Australia. [9]

In the year 2016, 134,490 cases of Colo-rectal carcinomas were newly detected in USA and 49,190 cases succumbed to the disease. It is the third most common malignancy in men next to Prostatic and lung cancer in men and in women, it is next to lung cancer and carcinomas of the breast, and cause a heavy burden on health status in United states and worldwide. [25]

Thailand and Japan are burdened with increasing incidence of Colo-rectal malignancies and there has been a steady increase in the past 30 years. Saudi Arabia has been showing the doubling incidence of Colo-rectal carcinoma since 1994 [25].

In United States, It is the second leading cause of death due to cancers. Though the survival in these patients have been influenced by improved diagnostic modalities, its incidence remains unchanged[9]

RISK FACTORS [3,8,9,10,11]

The risk factors for the development of Colo- rectal cancer can be divided into modifiable and non-modifiable risk factors.

NON MODIFIABLE RISK FACTORS

1. Age- More common above the age of 40 years and over 90% of tumors of over 50 years.
2. Adenomatous polyps- A patient with history of adenomas of colon is at increasing risk of developing colo rectal cancer. Over 90% of sporadic tumors arise from these adenomas.
3. Inflammatory bowel disease- A patient with Inflammatory bowel disease is 4-20 times more prone for developing malignancy
4. Family history- A patient with positive family history for colo-rectal cancer or Adenomatous polyps have 20 fold increased risk of developing malignancy
5. Inherited genetic risk- Patients with Familial adenomatous polyposis and Heriditary non polyposis Colo-rectal cancer are at 70-80% higher risk of developing Colo-rectal cancer
6. Ubiquitous somatic mutations- Also known as Microsatellite instability is caused by large number of mutations in the form of insertions or deletions in tumor cells.

MODIFIABLE RISK FACTORS

1. Environmental risk factors- Numerous environmental factors such as lifestyle, urban living, social and cultural practices enhances the risk of developing Colo-rectal cancers.
2. Nutritional practices- Increased animal fat, high meat consumption and reduced dietary fibre intake
3. Physical activity and obesity- Sedentary lifestyle, reduced physical activity and obesity are proved to be a risk factor for colo-rectal cancer
4. Cigarette Smoking- Smoking is an important risk factor for adenomatous polyps and 9 % of colo-rectal cancers are attributed to tobacco smoking.
5. Alcohol Consumption- Heavy alcohol abuse is associated with enhanced risk of colo-rectal cancer.

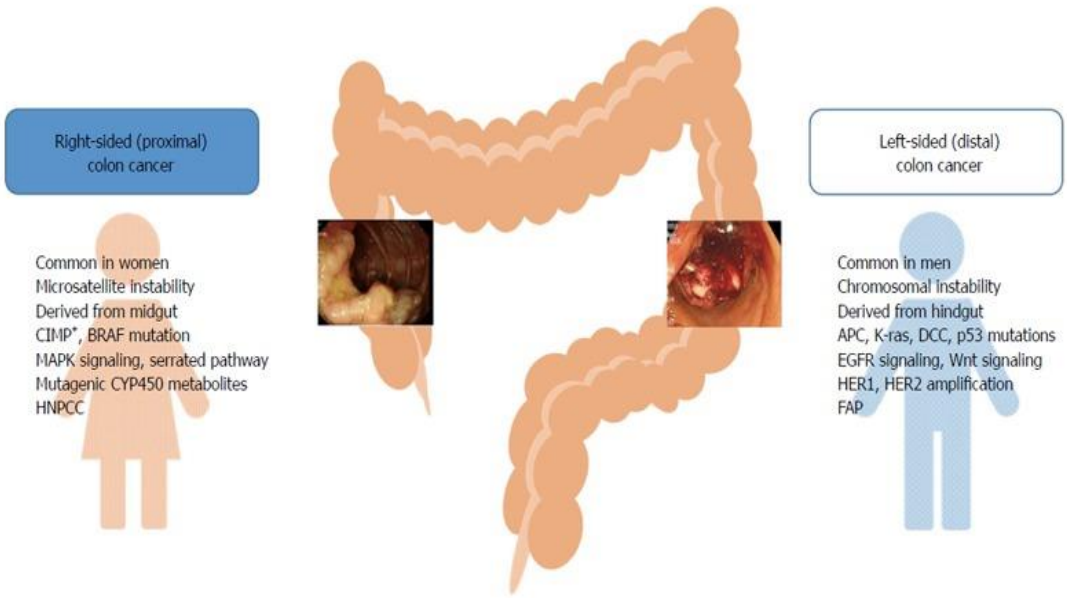


Figure 4- Right and left colon tumors- Clinical and molecular characters[43]

PROTECTIVE FACTORS:

Numerous factors have been proved to be protective for Colo-rectal carcinomas.

1. Consumption of Fish and Fish oils is proved to be a protective factor against colo-rectal carcinoma even in high meat consuming areas. [26]
2. Other factors include the consumption of dietary rich fibres which is considered protective not only for colo-rectal carcinomas but also for other non-infectious bowel disorders. Many of the developed western countries have a low consumption of dietary fibres and may be attributed as cause for the increased incidence. It has been studied that decreased consumption of dietary fibre will induce and increase the carcinogenic changes in intestinal flora leading to increased incidence. [27]

3. High intake of Vitamin D rich food and Calcium supplements have shown to be protective and decrease the incidence of colorectal carcinoma. It has been studied that calcium reduces the proliferation of epithelial lining cells in the colonic mucosa by neutralisation of bile acids, hence reducing the incidence of colon cancer [28]
4. Regular physical exercise has been shown to not only minimize the risk of colonic tumors but also further reduces the morbidity and mortality in detected cases. Exercise has shown to reduce the incidence of colon cancer over 25%[29].
5. Regular use of Aspirin reduces the risk of colon cancer. Studies have shown that the incidence of colo-rectal carcinomas have significantly reduced in patients regularly consuming Aspirin at least twice a week.[30] The hypothesis is that Aspirin inhibits Cycloxigenase- 2 (COX-2) which is the one of the driving parameter in inflammation and progression of colonic tumors. [31]

SCREENING FOR COLO-RECTAL CANCERS:

The process of screening involves the detection of pre-malignant lesions or diagnosis at early stage so as to minimise mortality and morbidity , even earlier to the manifestation of the disease. The advantages of these screening includes-

- Earlier detection
- Lesser economic burden
- Easier to manage
- Less morbidity and mortality [25]

The Centre for Disease Control and Prevention (CDC) and the U.S. Preventive Services Task in USA recommendations are that entire population be screened for Colo-rectal cancers by Sigmoidoscopy, Colonoscopy and Faecal occult blood testing. They recommend regular screening starting from the age of 50 years in normal individuals and even earlier in patients with Inflammatory bowel disease or family history of Familial adenomatous polyposis(FAP) or lynch syndrome [31]

Tumor microenvironment consists of different types of numerous immune cells. Tumor associated macrophages constitute a good number in microenvironment and has a major role to play in progression of tumors. The controversiality of tumor associated macrophages (TAM) in colo-rectal carcinoma has led to numerous studies in the recent years. The TAMs act by changing the tumour cell metabolism, promoting the angiogenesis, remodelling of extra cellular matrix and by altering the other factors in tumor microenvironment. In colo-rectal cancers, TAM s help in the progression of colonic cancers and thus, may further have therapeutic implications [6]

The infiltration of TAMs into the tumor stroma and interaction of these cells with the surrounding microenvironment decides the course of tumor progression. TAM s analyzed using CD163 marker and the results were compared with clinic pathological data. The level of CD 163 expression was more in cases with tumors of high grade and was associated with poorer outcome. It was concluded that TAM infiltrating the tumor stroma is an independent prognostic factor in Colo-rectal carcinomas. [12]

M1 macrophages have microbicidal and tumoricidal activity due to presence of antigen presenting molecules, which is co-stimulatory receptor for lymphocytes and many pro inflammatory cytokines on their surface[13,14].

M2 macrophages are pro- tumorigenic since they produce factors stimulating tumor growth (Eg. Epidermal growth factor, fibroblast growth factor and Transforming growth factor Beta- 1), angiogenesis (Eg. Vascular endothelial growth factor) and tissue Remodelling (Eg. Fibroblast growth factor, fibrin, and matrix metallopeptidases) and also produce immune suppressive cytokines (Eg. IL10 and Transforming growth factor beta).[13,14]

Stroma of the colo-rectal cancers contain a large number of tumor associated macrophages. There is continuous polarisation of anti-tumoral M1 macrophages to M2 macrophages. The inhibition of the EGFR signalling pathway in colon cancer cells alters cytokine secretion and prevents M1 to M2 polarisation thus inhibiting the cancer growth. This can prove to be a great novel therapeutic modality in treatment of colorectal cancers in the coming years. [15]

A study used anti CD-68 antibody for tumor associated macrophages, suggested that TAMs play a significant role in increasing the micro-vessel density and endothelial area and has been postulated that tumor associated macrophages(TAM) have an effect in promoting local tumor growth, invasion and metastasis. Hence agents targeting TAMs can be of help in controlling the tumor growth in these cancers. [16]

CLINICAL FEATURES:

The clinical presentation usually occurs after the disease has been in an advanced stage and most of the CRC patients present with vague abdominal discomfort, altered bowel habits and bleeding per rectum and anemia.

Tumors of descending colon and sigmoid colon may present with obstructive symptoms and these symptoms are less frequently encountered in right colon. A colonoscopic appearance and biopsy from the suspicious site may yield a definitive diagnosis. [17]

WHO HISTOLOGICAL CLASSIFICATION OF TUMOURS OF THE COLON AND RECTUM [3]

Epithelial tumours

Adenoma

Tubular

Villous

Tubulovillous

Serrated

Intraepithelial neoplasia (dysplasia) associated with chronic inflammatory diseases

Low-grade glandular intraepithelial neoplasia

High-grade glandular intraepithelial neoplasia

Carcinoma

Adenocarcinoma

Mucinous adenocarcinoma

Signet-ring cell carcinoma

Small cell carcinoma

Squamous cell carcinoma

Adenosquamous carcinoma

Medullary carcinoma

Undifferentiated carcinoma

Carcinoid (well differentiated endocrine neoplasm)

EC-cell

serotonin-producing neoplasm

L-cell

glucagon-like peptide

PP/PYY producing tumour

Others

Mixed carcinoid-adenocarcinoma

Others

Non-epithelial tumours

Lipoma

Leiomyoma

Gastrointestinal stromal tumour

Leiomyosarcoma

Angiosarcoma

Kaposi sarcoma

Malignant melanoma

Others

Malignant lymphomas

Marginal zone B-cell lymphoma of MALT Type

Mantle cell lymphoma

Diffuse large B-cell lymphoma

Burkitt lymphoma

Burkitt-like /atypical Burkitt-lymphoma

Others

Secondary tumours

Polyps

Hyperplastic (metaplastic)

Peutz-Jeghers

Juvenile

TUBULAR ADENOMAS

Commonly known as Adenomatous polyps and are usually uniformly distributed in all parts of large intestine and less commonly in the rectum. They are usually asymptomatic and may sometimes cause altered bowel habits. Usually less than 1 cms in size and may be sessile or pedunculated. There is Glandular hyperplasia with cellular crowding and may have atypical nuclear features. Immuno expression shows increased positivity for Carcino embryonic antigen (CEA) especially in the atypical areas. [17]

VILLOUS ADENOMA

They are usually solitary and seen in elderly patients. Though Rectum and recto sigmoid areas are the most common site, they can be easily missed even by digital examination as the lesions are very soft. They have a wide base and finger like villi radiate from base. Light microscopy may show crown like pattern with long papillary structures. Treatment depends on the size and extent of the lesion. The risk of progression to malignancy is as high as 29%- 70%. [17]

SERRATED ADENOMAS

They are usually sessile, small measuring not more than 5 mms. They are designated as serrated as the appearance resembles the saw toothed architecture on light microscopy. They have infoldings of the glands into the lumen and are characteristic. Increased mitotic activity may also be seen. [17]

ADENOCARCINOMA

The minimum criteria to be designated as carcinoma is that the tumor cells should completely breech the muscularis mucosae into the sub mucosa. They are usually asyptomatic and most common mode of presentation is change in the bowel habits, haematochezia or anemia for evaluation. Colonoscopy may aid in the early diagnosis. The pattern of growth may be exophytic, with intraluminal growth, diffusely infiltrative/ linitis plastic type with endophytic growth or with complete circumferential involvement. [17]

MUCINOUS CARCINOMA - Maliganant cells with extra cellular mucin pools more than 50% are designated. Usually asoociated with micro satellite instability

SIGNET RING CELL CARCINOMA- The cells should have peripherally placed nucleus with mucin inside the cells and the cells should comprise of more than 50 % population of tumor cells

ADENOSQUAMOUS CARCINOMA- The entity should have mixture of both adenocarcinoma component and squamous cell carcinoma. The foci of squamous cell carcinoma should be convincing and more than one component should be present.

MEDULLARY CARCINOMA- It is a rare tumor with pretty good prognosis and characterised by presence of tumor cells in solid pattern having vesicular nucleus, prominent nucleoli and eosinophilic cytoplasm. [3]

TNM CLASSIFICATION OF TUMOURS OF THE COLON AND RECTUM [3]

T – Primary Tumour

TX Primary tumour cannot be assessed

T0 No evidence of primary tumour

Tis Carcinoma in situ: intraepithelial or invasion of lamina propria3

T1 Tumour invades submucosa

T2 Tumour invades muscularis propria

T3 Tumour invades through muscularis propria into subserosa or into non-peritonealized pericolic or perirectal tissues

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

Lymph nodes

N – Regional Lymph Nodes

NX Regional lymph nodes cannot be assessed

N0 No regional lymph node metastasis

N1 Metastasis in 1 to 3 regional lymph nodes

N2 Metastasis in 4 or more regional lymph nodes

Metastasis

M – Distant Metastasis

MX Distant metastasis cannot be assessed

M0 No distant metastasis

M1 Distant metastasis

Table-1: TNM STAGING OF TUMORS OF COLON AND RECTUM

Stage 0	Tis	N0	M0
Stage 1	T1	N0	M0
	T2	N0	M0
Stage 2	T3	N0	M0
	T4	N0	M0
Stage 3	Any T	N1	M0
	Any T	N2	M0
Stage 4	Any T	N1	M1

DUKES CLASSIFICATION [73]

1. Dukes A: Invasion into but not through the bowel wall
2. Dukes B: Invasion through the bowel wall penetrating the muscle layer but not involving lymph nodes
3. Dukes C: Involvement of lymph nodes
4. Dukes D: Widespread metastases

ASTLER-COLLER CLASSIFICATION [74]

1. Stage A: Limited to mucosa

2. Stage B1: Extending into muscularis propria but not penetrating through it; nodes not involved

3. Stage B2: Penetrating through muscularis propria; nodes not involved

4. Stage C1: Extending into muscularis propria but not penetrating through it. Nodes involved

5. Stage C2: Penetrating through muscularis propria. Nodes involved

6. Stage D: Distant metastatic spread

These two staging systems are no longer used and are completely replaced by TNM staging system.

ANATOMIC STAGE/PROGNOSTIC GROUPS					
Stage	T	N	M	Dukes*	MAC*
0	Tis	N0	M0	–	–
I	T1	N0	M0	A	A
	T2	N0	M0	A	B1
IIA	T3	N0	M0	B	B2
IIB	T4a	N0	M0	B	B2
IIC	T4b	N0	M0	B	B3
IIIA	T1–T2	N1/N1c	M0	C	C1
	T1	N2a	M0	C	C1
IIIB	T3–T4a	N1/N1c	M0	C	C2
	T2–T3	N2a	M0	C	C1/C2
	T1–T2	N2b	M0	C	C1
IIIC	T4a	N2a	M0	C	C2
	T3–T4a	N2b	M0	C	C2
	T4b	N1–N2	M0	C	C3
IVA	Any T	Any N	M1a	–	–
IVB	Any T	Any N	M1b	–	–

Table -2: Comparison TNM, DUKES and Modified Astler-Coller classification system

CD 68 (CLUSTER OF DIFFERENTIATION 68)

Also known as Macrosialin, CD 68 is a 110 kD transmembrane glycoprotein containing 354 amino acids and an important member of scavenger family. It is encoded by CD 68 gene on chromosome 17. Normally it stains cells of macrophage, lineage including Kuffer cells and osteoclasts.

The binding of CD 68 to selectins and organ specific lectins is mediated by Glycosylated extra cellular domain on its surface. Its functions include activation and recruitment of macrophages in a specific site, engulfment of dead cells (Phagocytosis) and foreign bodies.

The lysosomes and late endosomes of the macrophages express CD 68 antigen in the granules thus giving a cytoplasmic staining.

CD 68 positive TAM s in the tumour microenvironment show high serum and stromal levels of VEGF .In this way, by altering the tumor microenvironment, it not only facilitates angiogenesis in the tumour but also reduces the response the tumor to radiotherapy. [18] There is an established fact that increased macrophage index and high vascular grade is negatively associated with reduced relapse free survival and reduced overall survival and is a poor prognostic factor. [19]

Contrary to this, many studies have also shown that M1 macrophages are associated with lesser grade of tumor and better survival in colo-rectal cancers by release of pro-inflammatory cytokines and other inflammatory mediators.

CD 163 (Cluster of differentiation 163)

It is a member of scavenger receptor family and is in a resident tissue macrophage. It acts a receptor for haemoglobin haptoglobin complex and has a pivotal role to perform in body's immune mechanism in response to intravascular and extravascular hemolysis and many bacterial infections. [20]

It has a molecular size of 130 k Da and has 1048 amino acid residues in extracellular domains. A dissolved form of CD 163 is seen in cerebrospinal fluid and is called sCD 163 represents receptor shedding and structural and functional modulation of CD163.

sCD 163 is upregulated in disease likes Diabetes, Gauchers disease, Rheumatoid arthritis and Hodgkin's lymphoma.

Numerous studies have shown than higher expression of CD 163 molecule in Colo-rectal cancers are associated with higher chances of lymph node metastasis, higher grade, increased tumour size and higher chances to tumor recurrence. [21]

PROGNOSTIC FACTORS:

1. Sex- Males have a poorer prognosis compared to females.
2. Age- Extremes of age (Young and elderly) are associated with poorer prognosis.
3. CEA levels- Levels >5 ng/ dl are associated with poor prognosis.
4. Locations- There are studies to prove that tumors involving left side of colon are associated with higher relapse free survival.
5. Inflammation- Tumors having dense inflammation in the tissue tumor interphase are considered as host's response against the tumor cells and are associated with better prognosis.
6. Tumor budding- Presence of isolated tumor cells or cluster of > 5 cells at the invasive front are associate with poor prognosis.
7. Vascular invasion- Invasion of the blood vessel by the tumor cells is a well known prognostic factor and associated with poorer prognosis.
8. Perforation- Large tumors causing perforation are at higher stage and associated with bad prognosis.
9. Lymph node involvement- Involvement of lymph node by the tumor cells increases the stage and associated with bad prognosis.
10. Stage- Higher stage is associated with poor prognosis
11. Grade- Well differentiated tumors (Grade I) are associated with better survival than grade III tumors [17]


MATERIALS &
METHODS

METHODOLOGY

STUDY DESIGN – Observational study.

SOURCE OF DATA : All Colorectal carcinoma specimens received in the Department of Pathology from R.L.Jalappa Hospital and Research Center attached to Sri Devaraj Urs Medical College, Tamaka, and Kolar from December 2016 to September 2018 and also the paraffin blocks taken from all cases of Colorectal cancer retrieved from Archives of Department of Pathology from the year January 2008 to November 2016 were included in the study.

DURATION OF STUDY – Two years

METHOD OF COLLECTION:

All resected Colorectal Carcinoma Specimens confirmed by histopathological examination were included in the study**.**

Data regarding the clinical details (Age, Sex, Histological grading) were collected. Hand E slides were reviewed for Histopathological types, grade and staging of the tumor.

Immunohistochemical staining for CD68 and CD163 (Biocare mouse antibody) was performed on all cases of Colorectal Carcinoma using appropriate positive and negative controls by peroxidase and anti peroxidase method.

PROTOCOL

1) Section Cutting

Sections are cut at approximately 3-4 µ m, floated on to positive charged slides and incubated at 37degree c for one day and further incubated at 58° c overnight.

2) Deparaffinization and Dexylinisation

Xylene –I - 15 mins

Xylene –I I - 15 mins

Ab alcohol – I - 1min

Ab alcohol – II - 1min

90%Alcohol – 1min

70%Alcohol -1min

3) Tap water – 10 min washing

4) Distilled water – 5 min rinsing

5) Antigen Retrieval

Microwave at power 10 for 2 cycles of 6 minutes each in TRIS EDTA BUFFER of PH 9.0. Slides cooled to room temperature.

6) Peroxidase block- 25 min

7) TBS buffer- 3 times wash of 5 min each.

8) Power block- 20 min

9) Drain and cover section with TARGET Ab- 45 min

10) TBS buffer- 3 times wash of 5 min each

11) Probe- 30 min

12) TBS buffer- 3 times wash of 5 min each

13) Super sensitive polyp –HRP- 1 hour 15 min

14) TBS buffer - 3 times wash of 5 min each

15) DAB Color development – 30 min

16) TBS buffer - 3 times wash of 5 min each

17) Hematoxylin Counter stain- 1 min

18) Tap water- 5 min

19) Dehydrate with Xylene

20) Mount with DPX

POSITIVE CONTROL- Tonsil tissue containing macrophages were taken as positive control

TUMOR SIZE

The tumor size was divided two groups. I.e tumors with size less than 5 cms (< 5) and more than 5 cms (>5) according to the study done by Ohnishi K et al [68] on Prognostic role of CD 169 positive macrophages in Colo-rectal cancers.

SELECTION OF HOT SPOTS AND GRADING OF IHC

The CD 68 and CD 163 immuno stained smears were examined under low magnification (10X) and was looked for areas with maximum expression of CD 68 and CD 163 by two observers and were called as" Hot spots" . These hotspots were then viewed under higher magnification (40X) and CD 68 and CD 163 positive cells were counted and the mean was taken. Expression of macrophages antigen CD 68 and CD 163 were graded with the proportion of macrophages staining positive in the tumor stroma. Out of hundred cells counted and the grading is a follows[35, 69]

GRADE+1: Less than 10% cells positive

GRADE+2: More than 10% & less than 50% cells positive

GRADE+3: More than 50% cells positive

LYMPH NODE RATIO(LNR)

Lymph node ratio is the ratio of number of Lymph node with metastasis to the number of Lymph nodes harvested. In the study, LNR was divided into 4 groups according to the study done by Ren JQ et al [70].

LNR 1 - ≤ 0.111

LNR 2 - 0.111 0 to ≤0.200

LNR 3 - 0.200 to ≤0.429

LNR 4 - > 0.429

SAMPLE SIZE

Sample size was estimated by using the proportion of CD163 marker positivity in Colo-rectal cancers in study done by Ivan Shabo et al [12] which was 20% by using the formula

$$\text{Sample size} = \frac{Z_{1-\alpha/2}{}^2 p(1-p)}{d^2}$$

Here

$Z_{1-\alpha/2}$ = Is standard normal variate (at 5% type 1 error ($P<0.05$) it is 1.96 and at 1% type 1 error ($P<0.01$) it is 2.58). As in majority of studies P values are considered significant below 0.05 hence 1.96 is used in formula.
p = Expected proportion in population based on previous studies or pilot studies.
d = Absolute error or precision – Has to be decided by researcher.

P = 20 or 0.20

q = 80 or 0.80

d = 10% or 0.10

Using the above values at 95% Confidence level a sample size of 62 subjects with primary colorectal cancers were included in the study.

STATISTICAL ANALYSIS

Data was entered into Microsoft excel data sheet and was analysed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. **Chi-square test** was used as test of significance for qualitative data.

Continuous data was represented as mean and SD. **ANOVA (Analysis of Variance) or Kruskal Wallis test** was the test of significance to identify the mean difference between more than two groups for quantitative and qualitative data respectively.

Graphical representation of data: MS Excel and MS word was used to obtain various types of graphs such as bar diagram, Pie diagram.

p value (Probability that the result is true) of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

Statistical software: MS Excel**,** SPSS version 22 **(**IBM SPSS Statistics, Somers NY, USA) was used to analyse data.

INCLUSION CRITERIA: All Colorectal Carcinoma Cases.

EXCLUSION CRITERIA:

1. Metastatic tumor to Colo-rectal region.

2. Recurrent lesion.

3. Patient subjected for chemotherapy and Radiotherapy


RESULTS

RESULTS

Table 3: Age distribution of subjects in the study group

		Frequency	Percent(%)
Age	<60 years	42	67.7
	>60 years	20	32.3
	Total	62	100.0

In the study, 67.7%of cases were in the age group <60 years, 32.3% were in the age group >60 years.

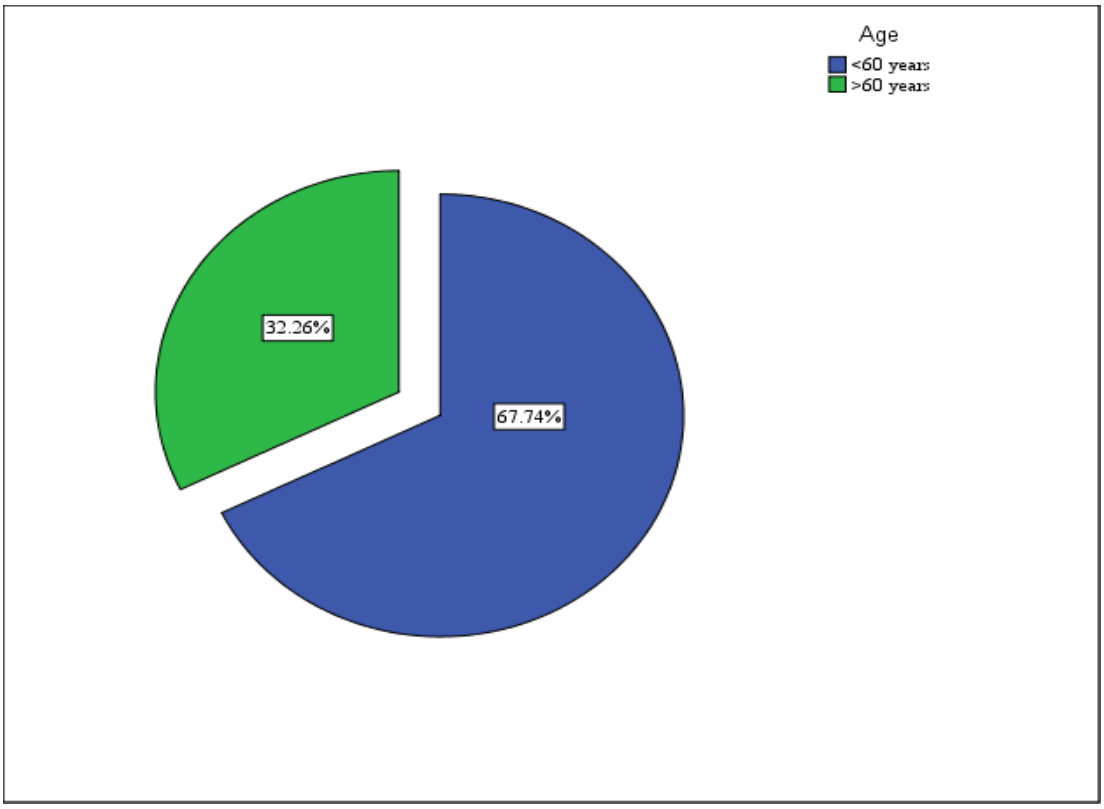


Chart 1: Pie diagram showing Age distribution of subjects in the study

Table 4: Gender distribution of subjects in the study group

		Frequency	Percent(%)
Sex	Male	39	62.9
	Female	23	37.1
	Total	62	100.0

In the study, 62.9% of cases were males and 37.1% were males.

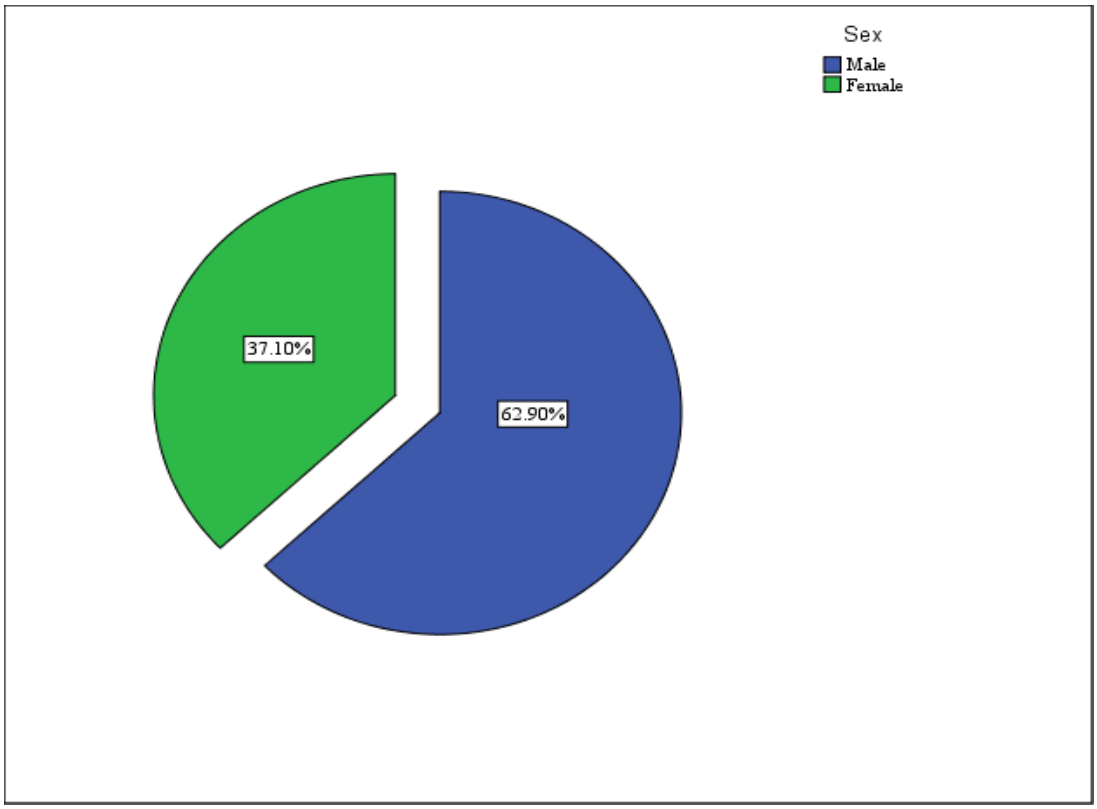


Chart 2: Pie diagram showing Sex distribution of subjects in the study

Table 5: Site of tumor distribution among subjects

		Frequency	Percent(%)
Site	Ascending Colon	13	21.0
	Transverse Colon	6	9.7
	Descending Colon	3	4.8
	Sigmoid Colon	13	21.0
	Rectum	27	43.5
	Total	62	100.0

In the study, the most common site of malignancy was Rectum in 43.5% od cases, followed by ascending colon and sigmoid colon in 21% each , transverse colon in 9.7% and descending colon in 4.8% of cases.

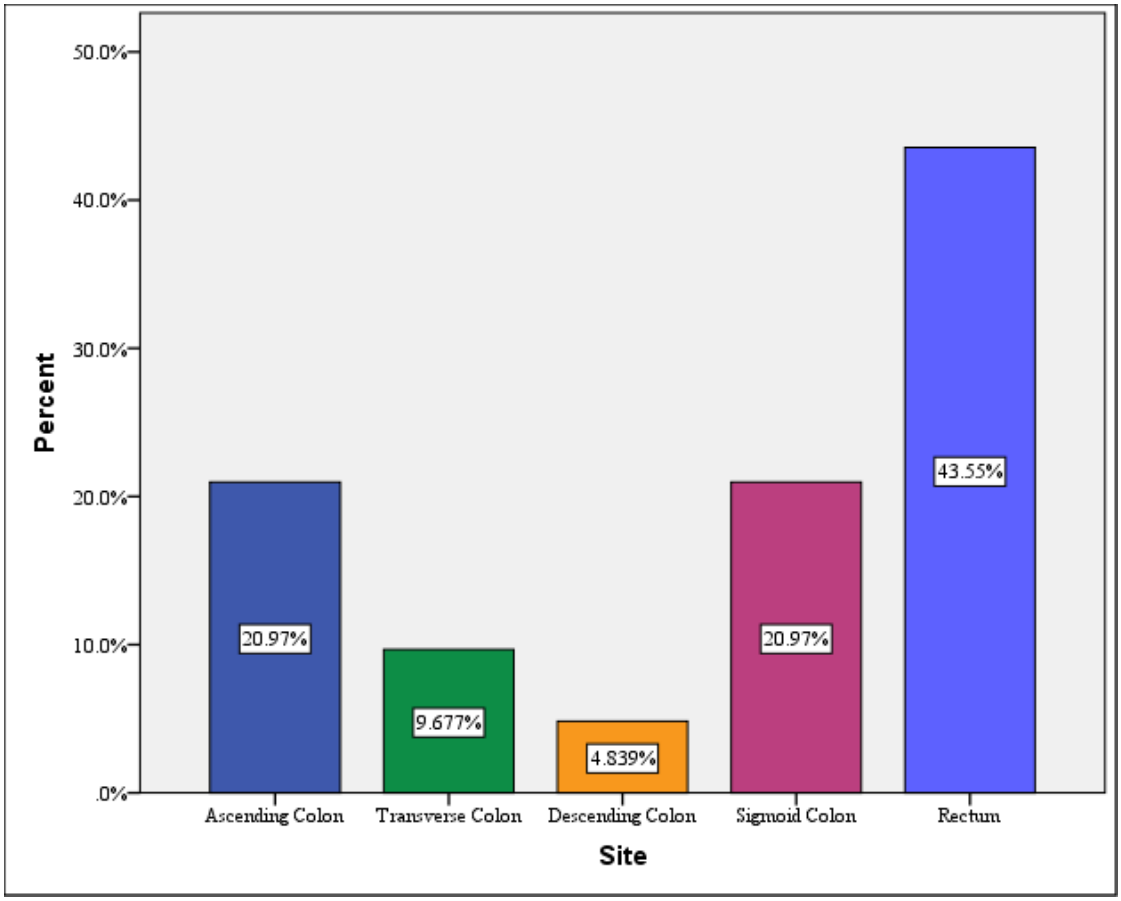


Chart 3 : Bar diagram showing Site of lesion distribution among subjects

Table 6: Type of tumor growth among study subjects

		Frequency	Percent(%)
Growth	Proliferative	8	12.9
	Ulceroproliferative	34	54.8
	Ulcerative/ Infiltrative	20	32.3
	Total	62	100.0

In the study, the most common type of tumor growth was Ulceroproliferative type in 54.8% of subjects followed by Ulcerative/ Infiltrative in 32.3% and proliferative in 12.9% of cases.

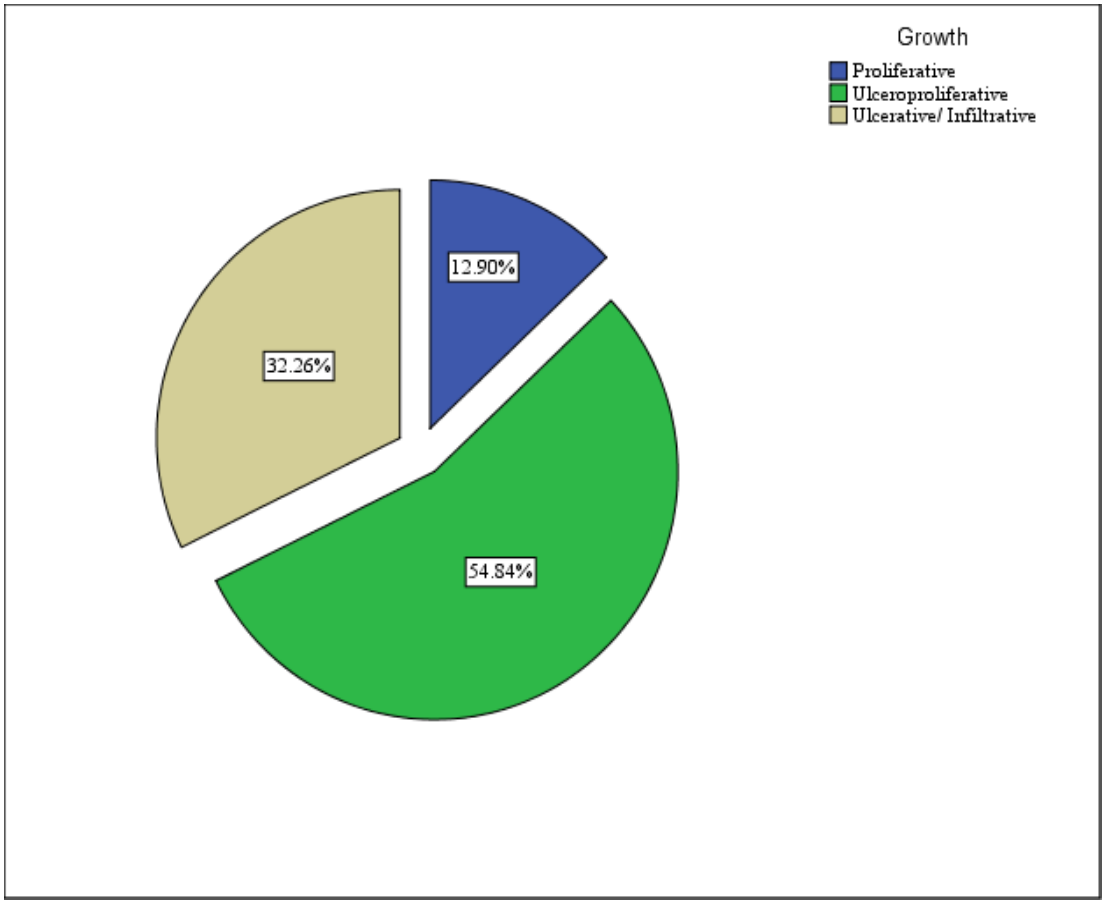


Chart 4 : Pie diagram showing type of Growth distribution among subjects

Table 7: Type of gross specimen distribution among subjects

		Frequency	Percent(%)
Specimen Type	Hemicolectomy	39	62.9
	Abdominoperineal resection(APR)	14	22.6
	Anterior Resection	9	14.5
	Total	62	100.0

In the study, the most common specimen type received was Hemicolectomy in 62.9% of cases followed by APR in 22.6% and Anterior resection in 14.5% of cases.

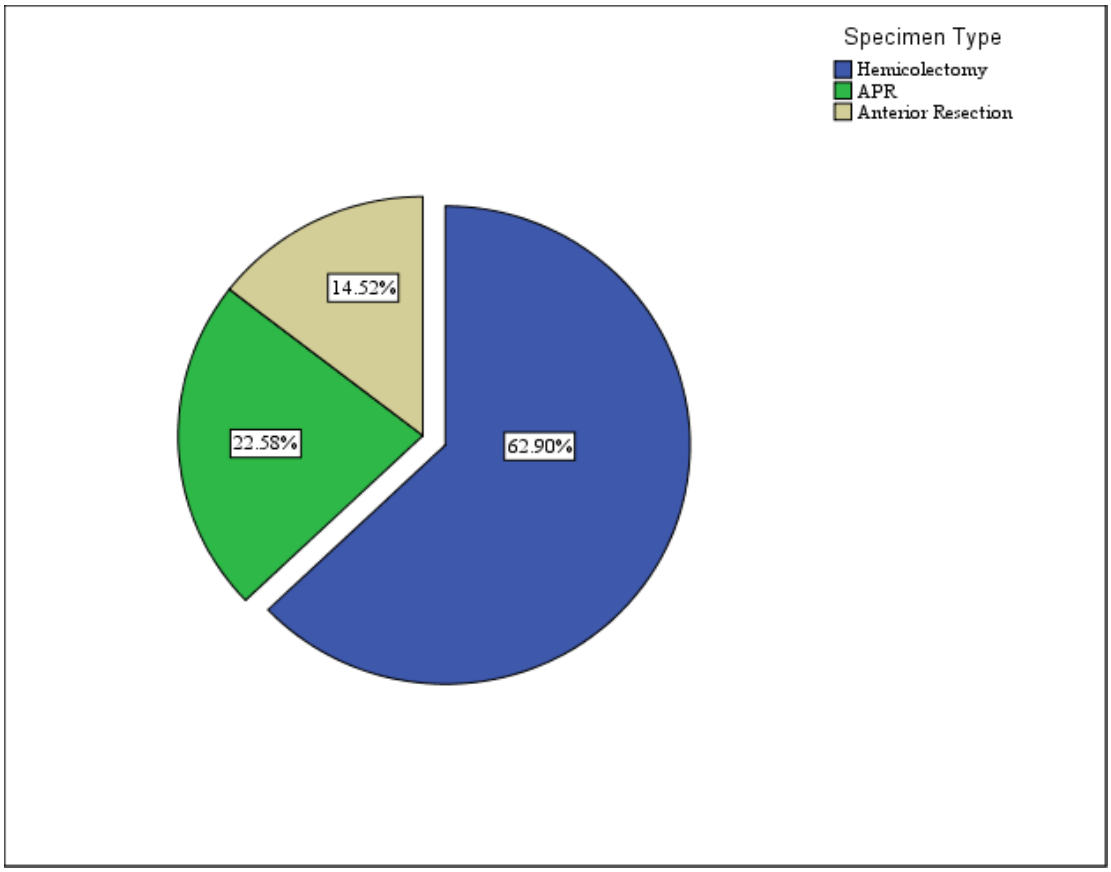


Chart 5: Pie diagram showing Specimen Type distribution among subjects

Table 8: Tumor Size distribution among subjects

		Frequency	Percent(%)
Tumor Size [68]	<50 mms	39	62.9
	>50 mms	23	37.1
	Total	62	100.0

In the study, 62.9% of cases had tumor size <50 mms and 37.1% of cases had tumor size >50 mms.

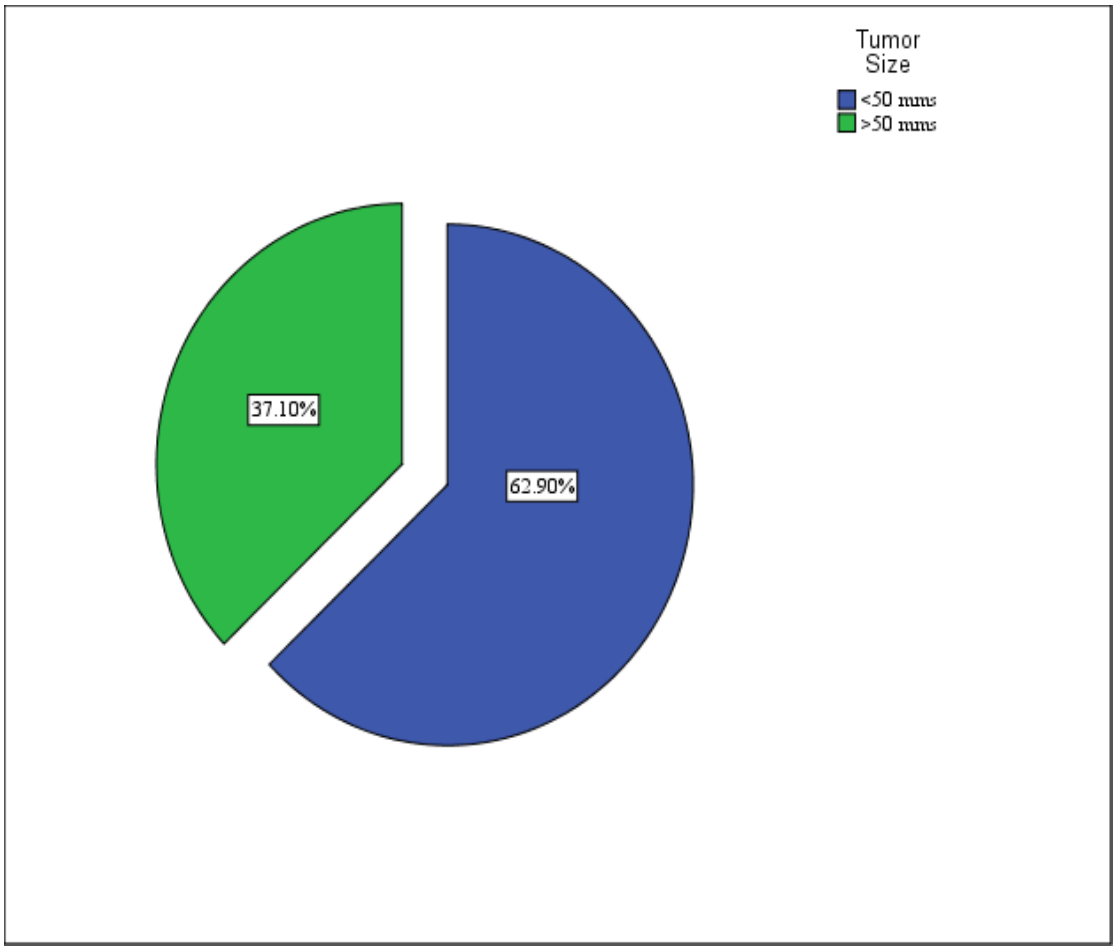


Chart 6: Pie diagram showing Tumor Size distribution among subjects

Table 9: Grading of Malignancy distribution among subjects

		Frequency	Percent(%)
Malignancy Grading	Well Differentiated	20	32.3
	Moderately Differentiated	30	48.4
	Poorly Differentiated	12	19.4
	Total	62	100.0

In the study,32.3% of cases were well differentiated carcinomas, 48.4% were moderately differentiated and 19.4% were poorly differentiated.

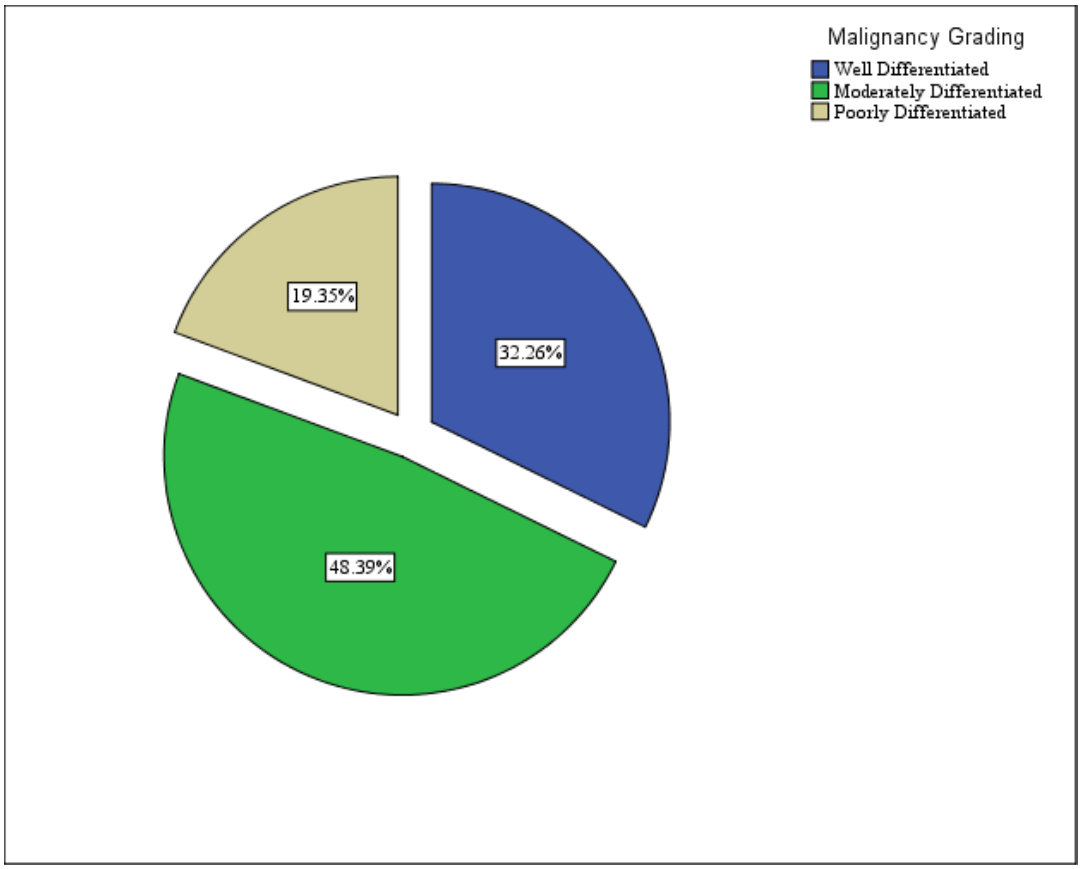


Chart 7 : Pie diagram showing Malignancy grading distribution among subjects

Table 10: T staging distribution among subjects

		Frequency	Percent(%)
T Staging	T1	4	6.5
	T2	16	25.8
	T3	32	51.6
	T4	10	16.1
	Total	62	100.0

In the study, 6.5% of cases were in T1 stage, 25.8% were in T2 stage, 51.6% were in T3 Stage, 16.1% were in T4 Stage.

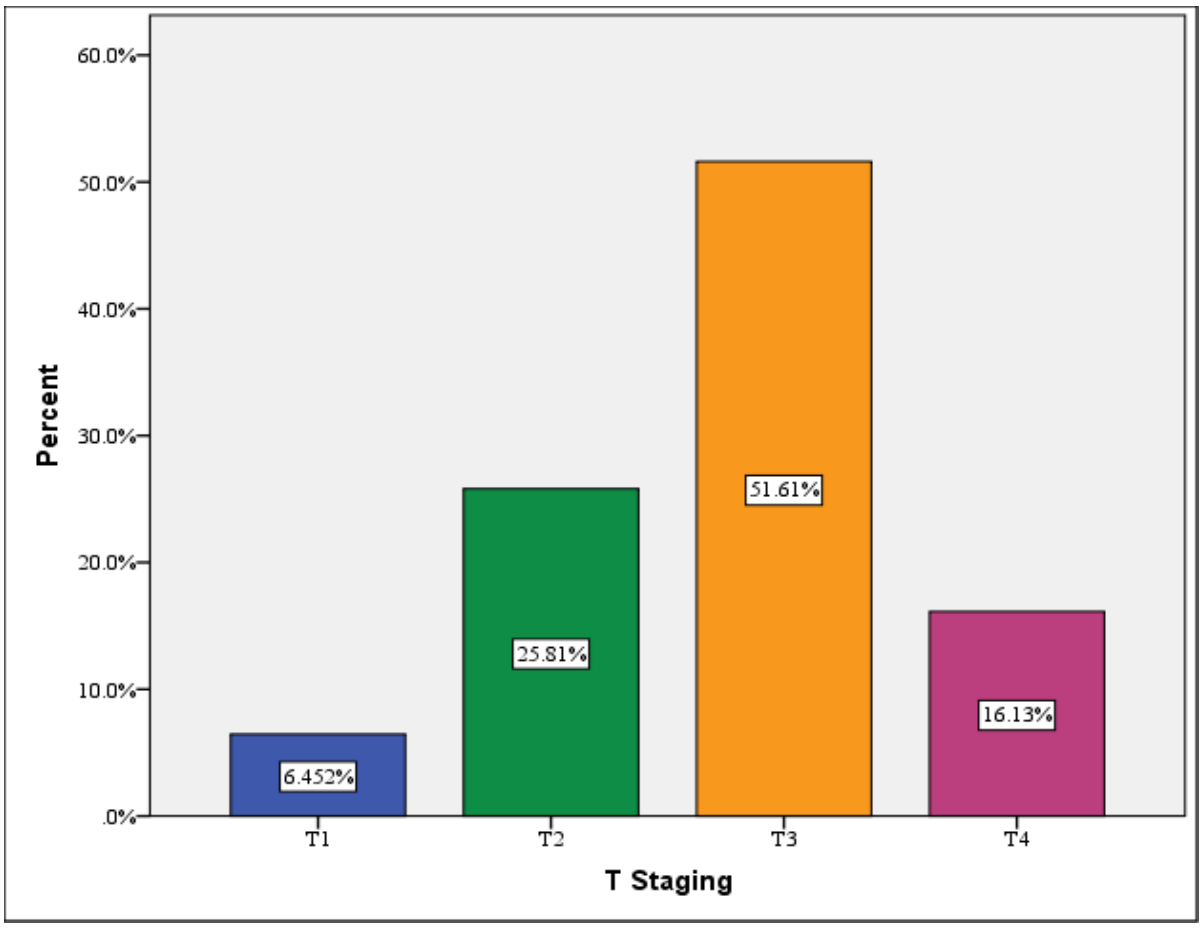


Chart 8 : Bar diagram showing T staging distribution among subjects

Table 11: N staging distribution among subjects

		Frequency	Percent(%)
N Staging	N0	39	62.9
	N1	21	33.9
	N2	2	3.2
	Total	62	100.0

In the stud, 62.9% of cases were in N0 stage, 33.9% were in N1 stage and 3.2% were in N2 stage.

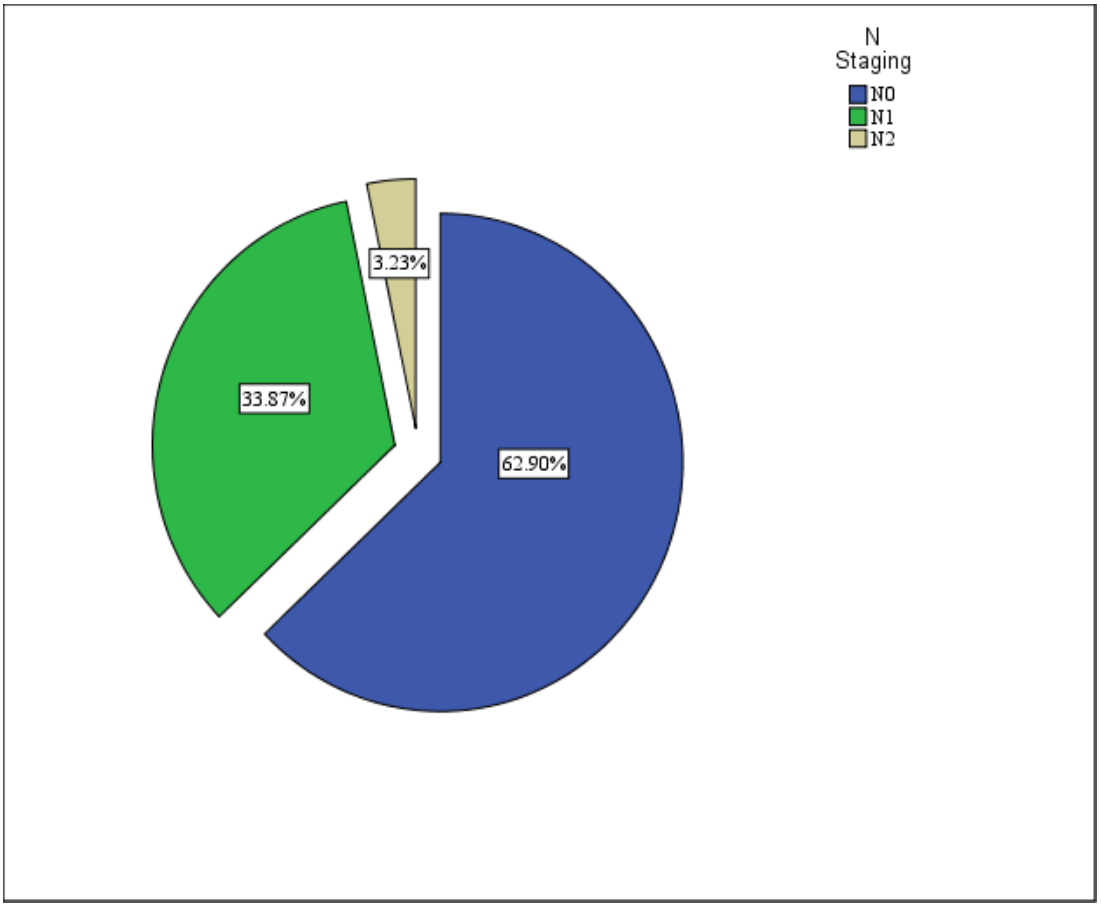


Chart 9: Pie diagram showing N staging distribution among subjects

Table 12 : Stage of Tumor distribution among subjects

		Frequency	Percent(%)
Stage of Tumor	I	17	27.4
	II	22	35.5
	III	23	37.1
	Total	62	100.0

In the study 27.4% were in Stage I, 35.5% were in Stage II and 37.1% were in Stage III.

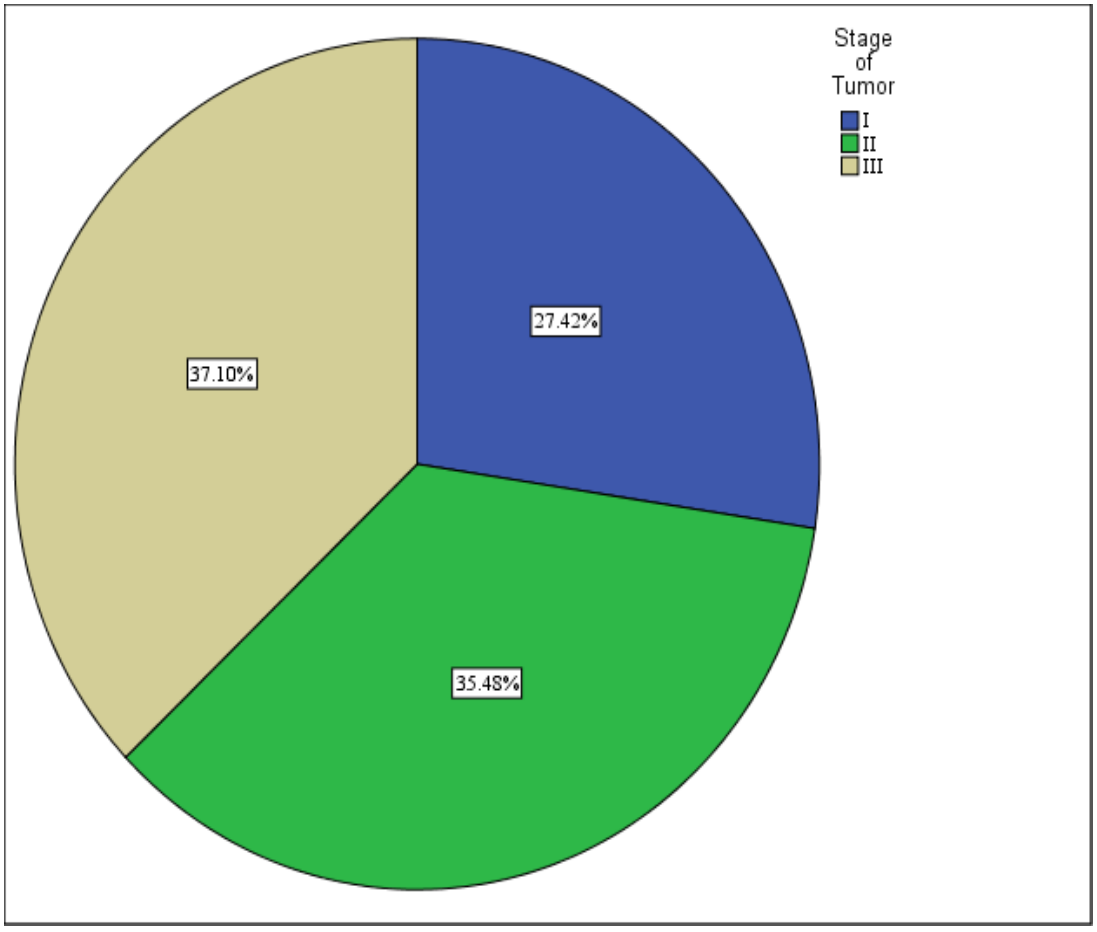


Chart 10 : Pie diagram showing Stage of Tumor distribution among subjects

Table 13: Other parameters in the study subjects

		Frequency	Percent(%)
Perineural Invasion	Absent	60	96.8
	Present	2	3.2
Lymphovascular Invasion	Absent	59	95.2
	Present	3	4.8

In the study 3.2% of cases had Perineural Invasion, 4.8% of cases had Lymphovascular Invasion and 4.8% of cases had Perforation.

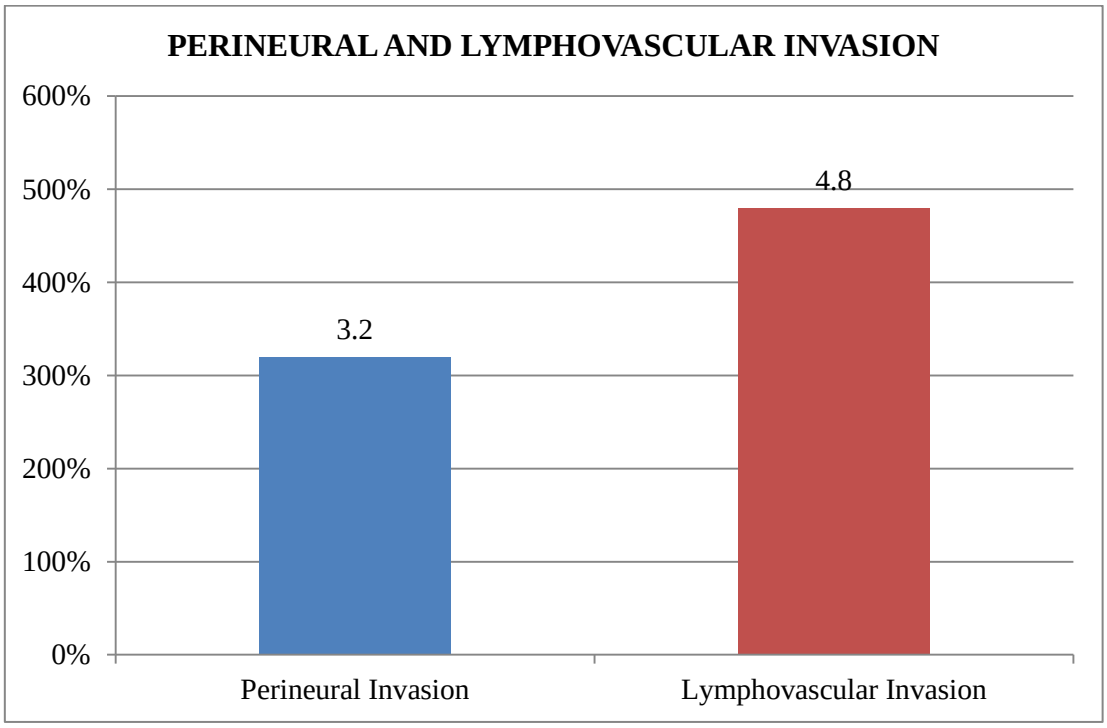


Chart 11 : Bar diagram showing Perineural Invasion and Lymphovascular Invasion in the study subjects

Table 14: Lymph node ratio (LNR) distribution among subjects

		Frequency	Percent(%)
LNR	<0.111	40	64.5
	0.111 to 0.200	3	4.8
	0.200 to 0.429	5	8.1
	>0.429	14	22.6
	Total	62	100.0

In the study, 64.5% of cases had LNR <0.111, 4.8% had LNR 0.111 to 0.200, 8.1% had LNR 0.200 to 0.429 and 22.6% of cases had LNR >0.429.

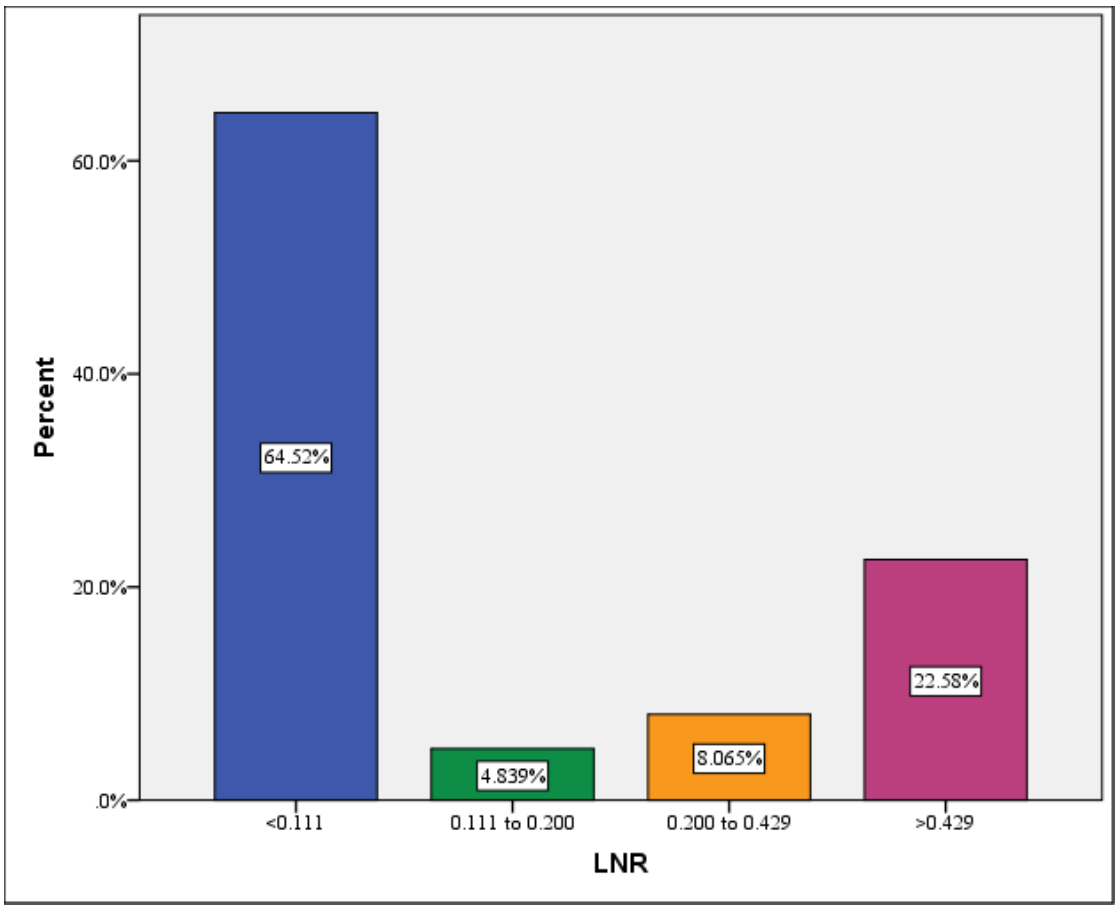


Chart 12 : Bar diagram showing LNR distribution among subjects

Table 15: CD 68 distribution among subjects

		Count	Percent (%)
CD 68	<10% of Cells	24	38.7%
	>10% to <50% of Cells Positive	24	38.7%
	>50% of Cells Positive	14	22.6%

In the study, 38.7% of cases expressed CD 68 in <10% of cells, 38.7% of cases expressed CD 68 in >10% to <50% of Cells and 22.6% of cases expressed CD 68 in >50% of Cells.

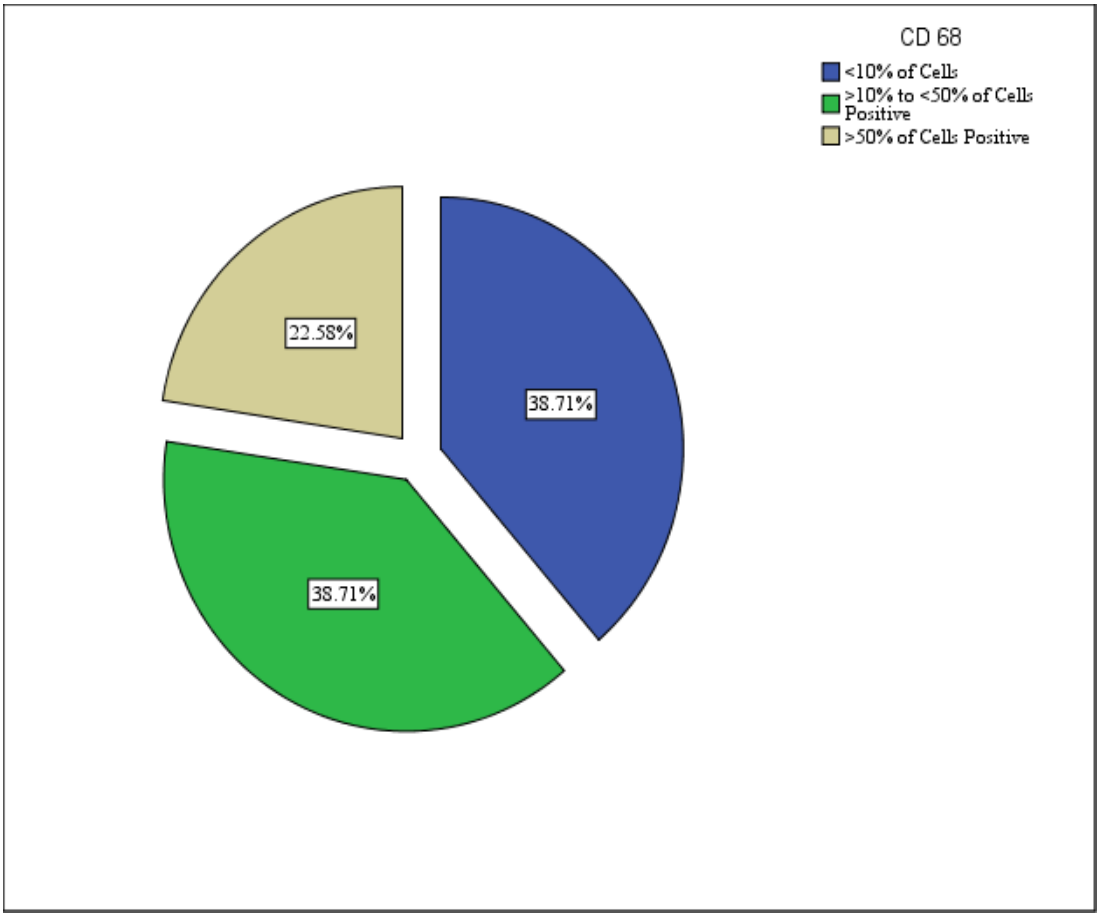


Chart 13 : Pie diagram showing distribution of CD 68 expression among subjects

Table 16 : CD 163 distribution among subjects

		Count	Percent (%)
CD 163	<10% of Cells	6	9.7%
	>10% to <50% of Cells Positive	36	58.1%
	>50% of Cells Positive	20	32.3%

In the study, 9.7% of cases expressed CD 163 in <10% of Cells, 58.1% of cases expressed CD 163 in >10% to <50% of Cells and 32.3% of cases expressed CD 163 in >50% of Cells.

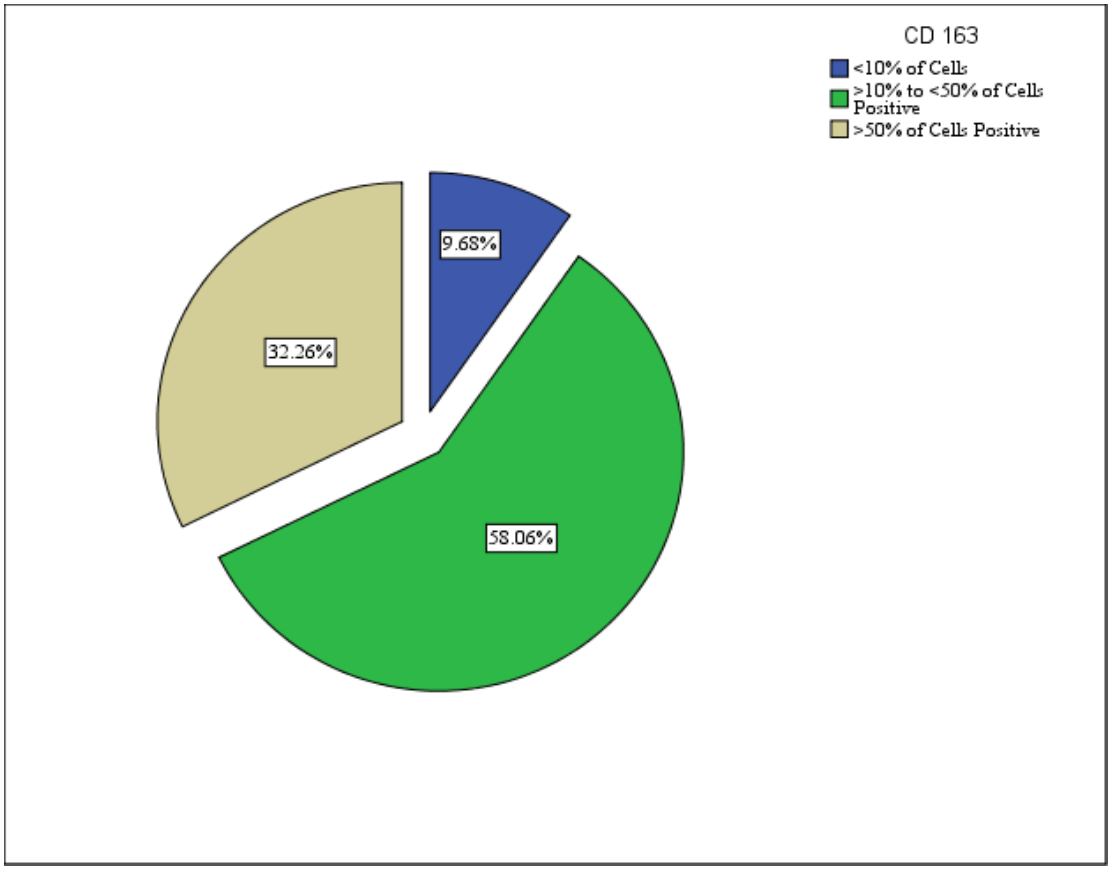


Chart 14: Pie diagram showing distribution of CD 163 expression among subjects

COMPARISION OF DIFFERENT PROGNOSTIC MARKERS WITH CD 68

Table 17: Association between Age and CD 68 expression

			CD 68			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
Age	<60 years	Count	17	15	10	42
		%	40.5%	35.7%	23.8%	100.0%
	>60 years	Count	7	9	4	20
		%	35.0%	45.0%	20.0%	100.0%
Total		Count	24	24	14	62
		%	38.7%	38.7%	22.6%	100.0%

$\chi 2 = 0.494$, df =2, p = 0.781

In the study, there was no significant association between Age and CD 68 expression.

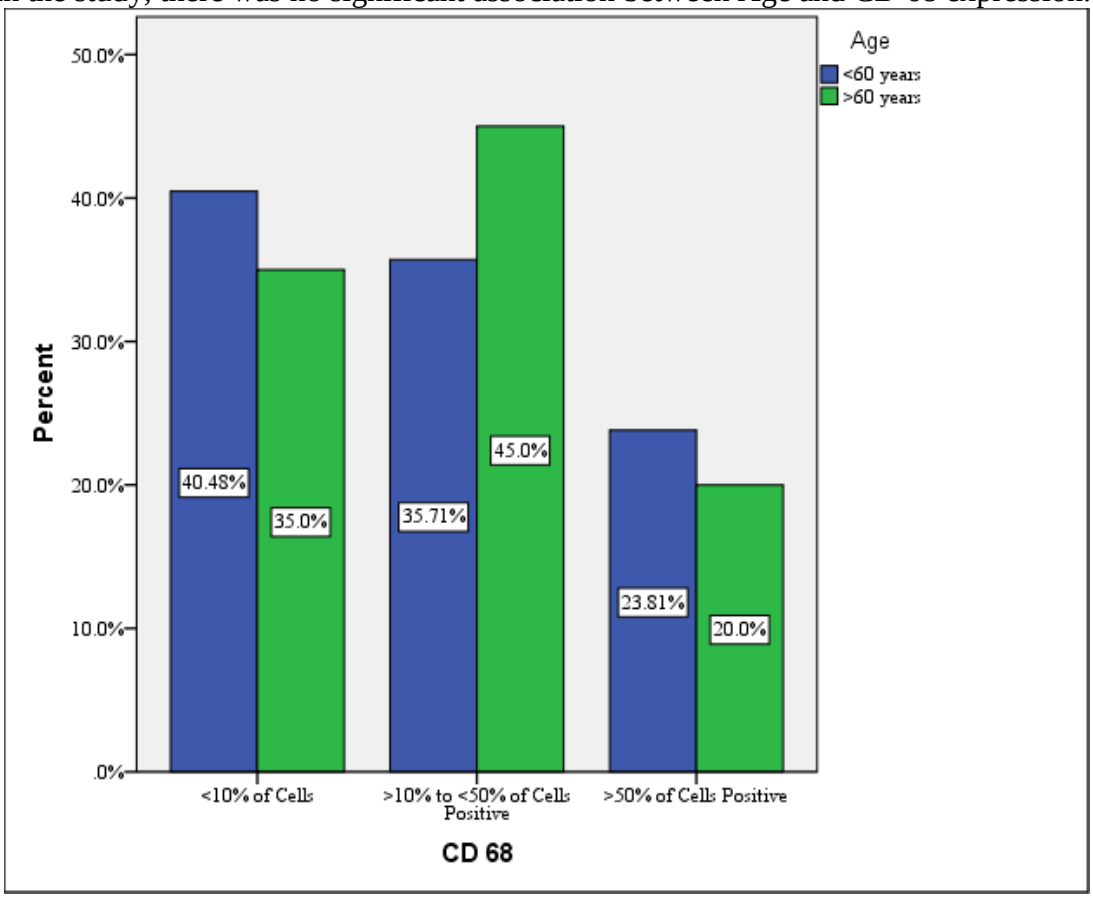


Chart 15: Bar diagram showing Association between Age and CD 68 expression.

Table 18: Association between Sex and CD 68 expression

			CD 68			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
Sex	Male	Count	13	18	8	39
		%	33.3%	46.2%	20.5%	100.0%
	Female	Count	11	6	6	23
		%	47.8%	26.1%	26.1%	100.0%
Total		Count	24	24	14	62
		%	38.7%	38.7%	22.6%	100.0%

$\chi 2 = 2.489$, df =2, p = 0.288

In the study, there was no significant association between Sex and CD 68 expression.

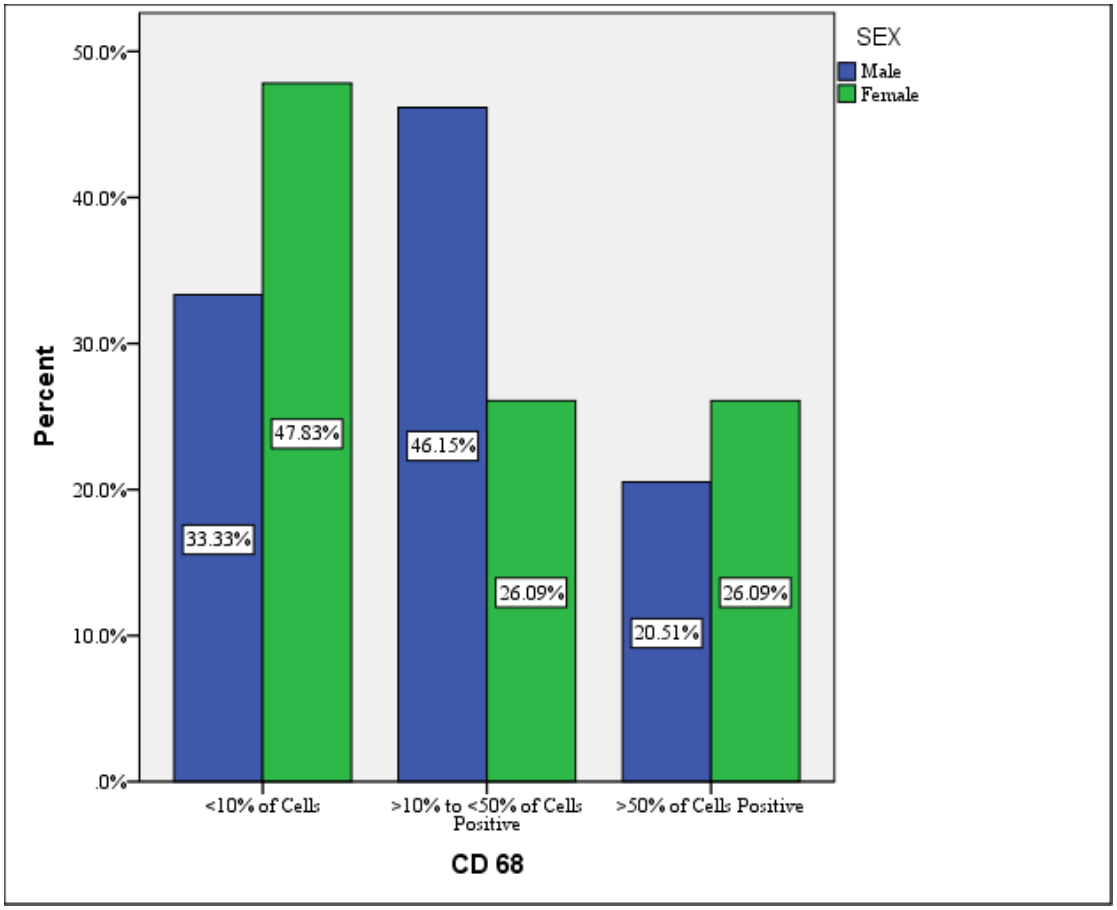


Chart 16: Bar diagram showing Association between Sex and CD 68 expression.

Table 19: Association between Site and CD 68 expression

			CD 68			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
Site	Ascending Colon	Count	4	7	2	13
		%	30.8%	53.8%	15.4%	100.0%
	Transverse Colon	Count	2	0	4	6
		%	33.3%	0.0%	66.7%	100.0%
	Descending Colon	Count	2	1	0	3
		%	66.7%	33.3%	0.0%	100.0%
	Sigmoid Colon	Count	2	6	5	13
		%	15.4%	46.2%	38.5%	100.0%
	Rectum	Count	14	10	3	27
		%	51.9%	37.0%	11.1%	100.0%
Total		Count	24	24	14	62
		%	38.7%	38.7%	22.6%	100.0%

$\chi 2 = 16.37$, df =8, p = 0.037*

In the study, there was statistically significant association between Site of tumor and CD 68 expression.

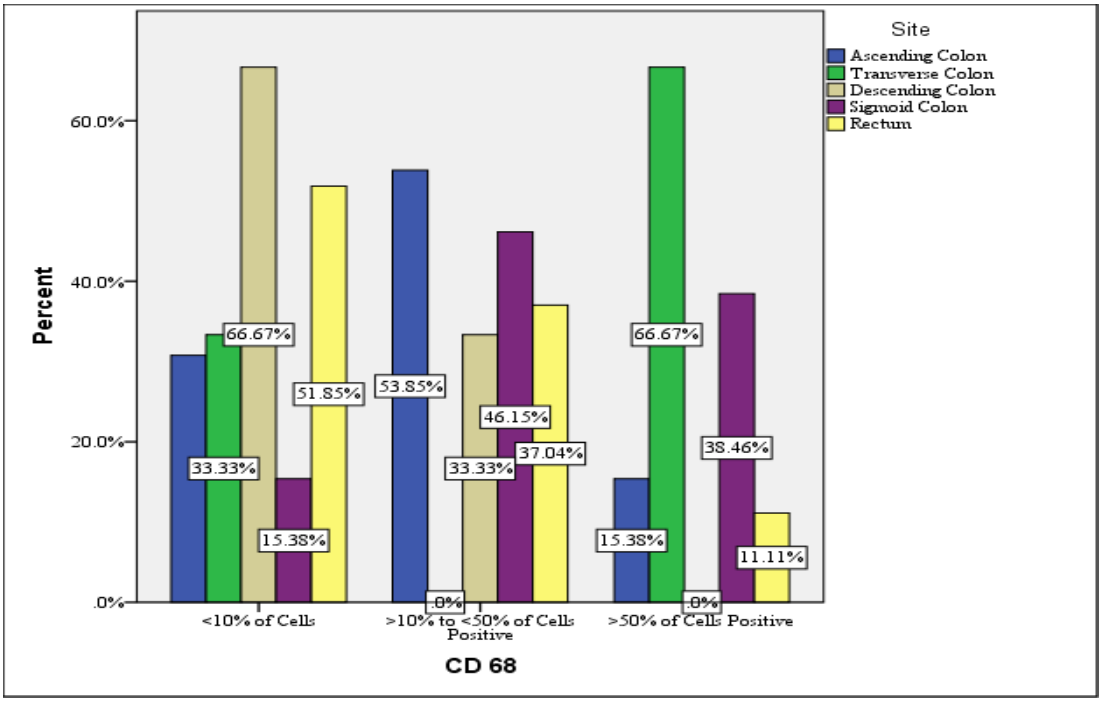


Chart 17: Bar diagram showing Association between Site of tumor and CD 68 expression.

Table 20: Association between type of Tumor growth and CD 68 expression

			CD 68			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
Growth	Proliferative	Count	5	2	1	8
		%	62.5%	25.0%	12.5%	100.0%
	Ulceroproliferative	Count	11	16	7	34
		%	32.4%	47.1%	20.6%	100.0%
	Ulcerative/ Infiltrative	Count	8	6	6	20
		%	40.0%	30.0%	30.0%	100.0%
Total		Count	24	24	14	62
		%	38.7%	38.7%	22.6%	100.0%

$\chi 2 = 3.833$, df =4, p = 0.429

In the study, there was no significant association between Growth and CD 68 expression.

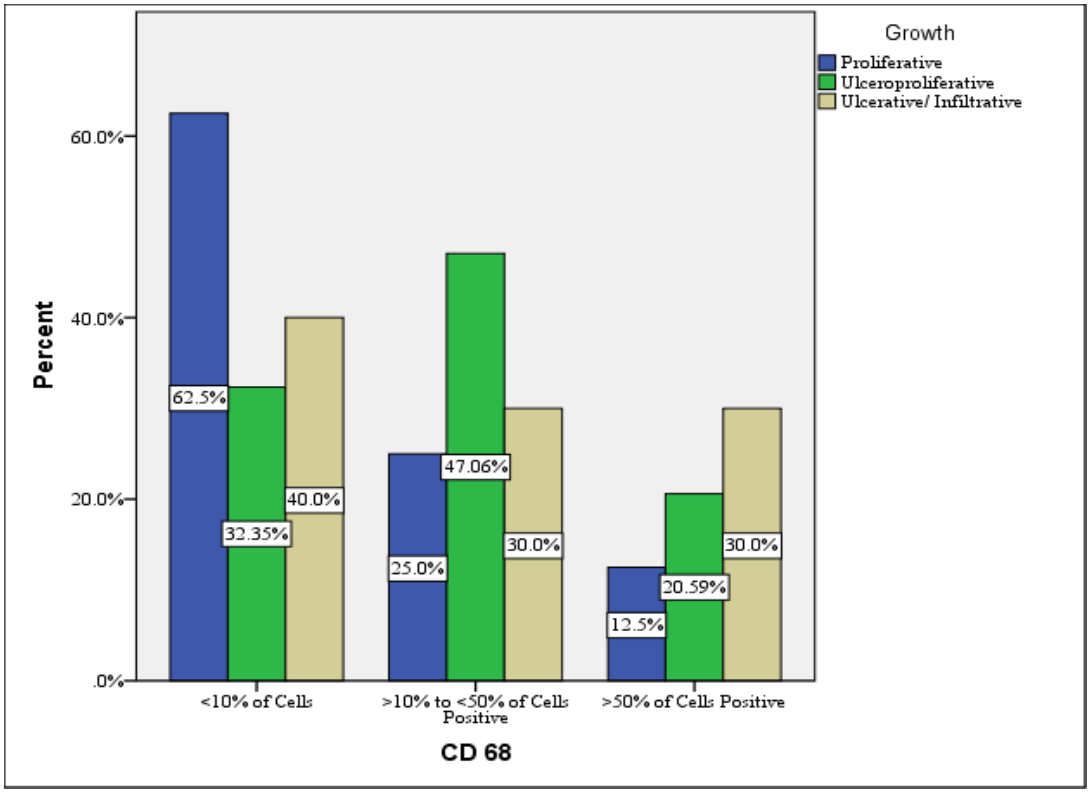


Chart 18 : Bar diagram showing Association between type of tumor growth and CD 68 expression.

Table 21 : Association between Specimen type and CD 68 expression.

			CD 68			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
Specimen Type	Hemicolectomy	Count	12	16	11	39
		%	30.8%	41.0%	28.2%	100.0%
	APR	Count	9	4	1	14
		%	64.3%	28.6%	7.1%	100.0%
	Anterior Resection	Count	3	4	2	9
		%	33.3%	44.4%	22.2%	100.0%
Total		Count	24	24	14	62
		%	38.7%	38.7%	22.6%	100.0%

χ 2 =5.595, df =4, p = 0.232

In the study, there was no significant association between Specimen Type and CD 68 expression.

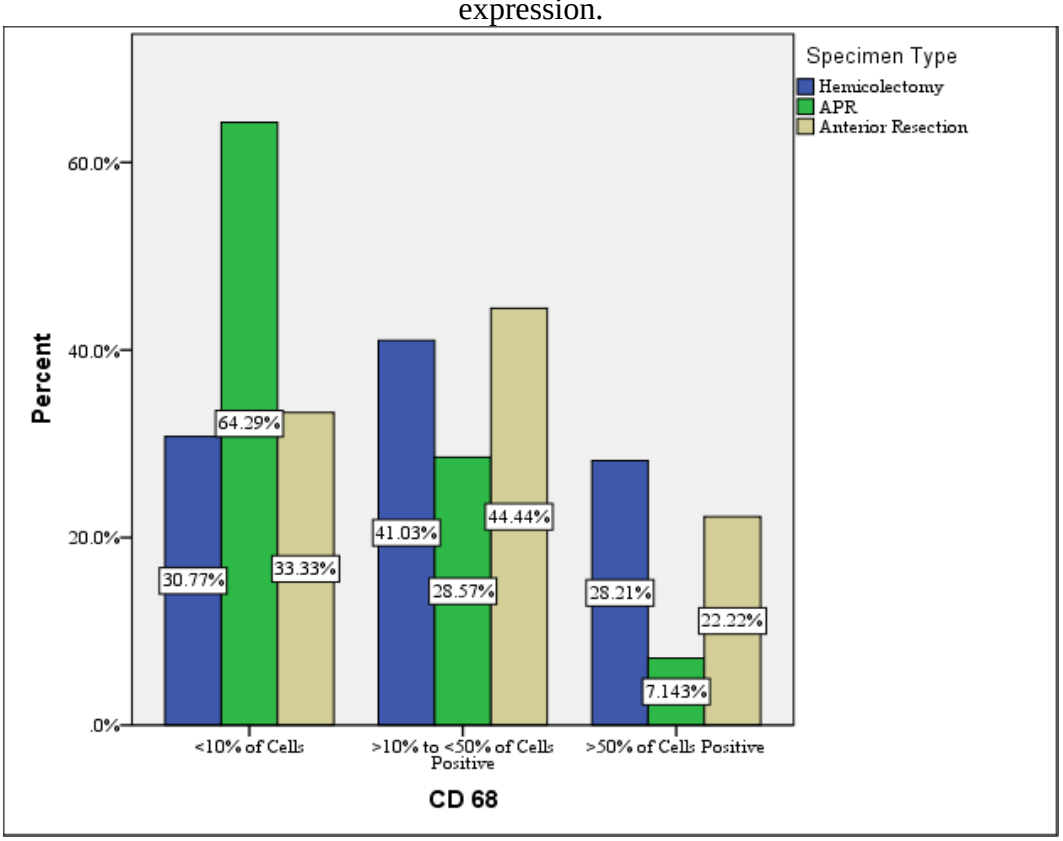


Chart 19 : Bar diagram showing Association between Specimen type and CD 68 expression.

Table 22 : Association between Tumor Size and CD 68 expression.

			CD 68			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
Tumor Size	<50 mms	Count	16	11	12	39
		%	41.0%	28.2%	30.8%	100.0%
	>50 mms	Count	8	13	2	23
		%	34.8%	56.5%	8.7%	100.0%
Total		Count	24	24	14	62
		%	38.7%	38.7%	22.6%	100.0%

$\chi 2 = 6.264$, df =2, p = 0.044*

In the study, there was a statistically significant correlation between Tumor Size and CD 68 expression.

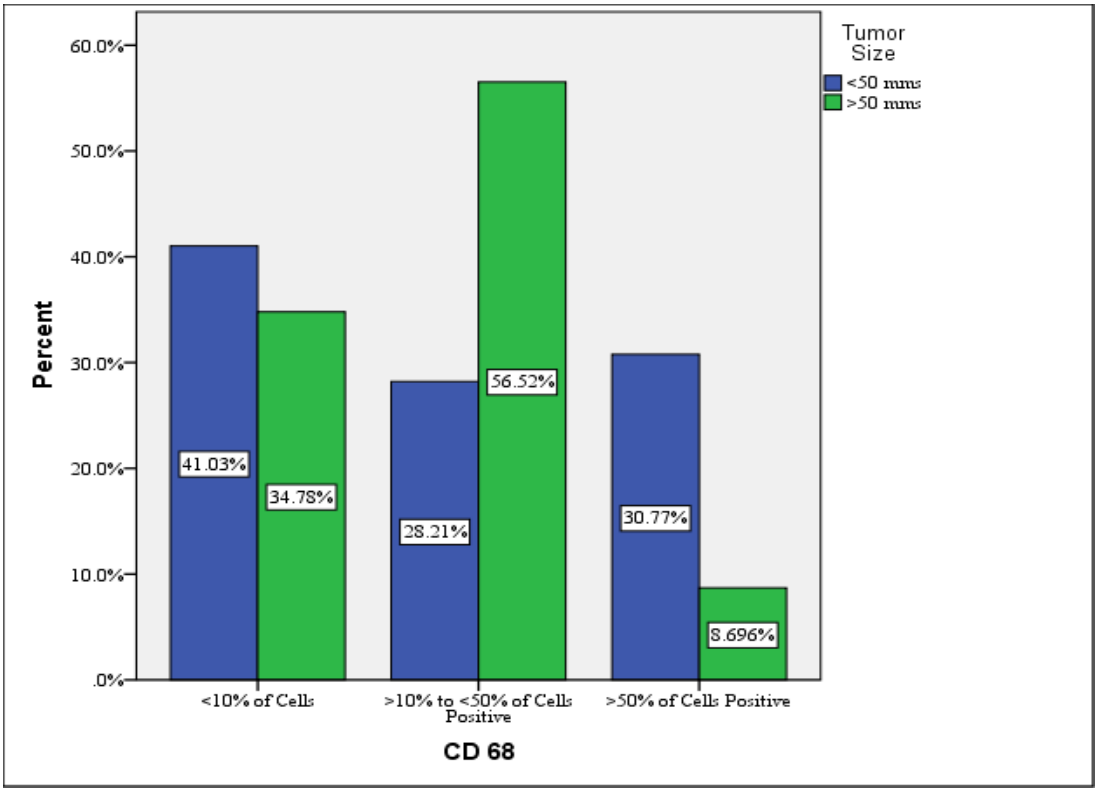


Chart 20: Bar diagram showing Association between Tumor Size and CD 68 expression.

Table 23 : Association between Malignancy Grade and CD 68 expression.

			CD 68			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
Malignancy Grading	Well Differentiated	Count	4	7	9	20
		%	20.0%	35.0%	45.0%	100.0%
	Moderately Differentiated	Count	15	11	4	30
		%	50.0%	36.7%	13.3%	100.0%
	Poorly Differentiated	Count	5	6	1	12
		%	41.7%	50.0%	8.3%	100.0%
Total		Count	24	24	14	62
		%	38.7%	38.7%	22.6%	100.0%

$\chi 2 = 9.989$, df =4, p = 0.041*

In the study, there was a statistically significant association between Malignancy Grade and CD 68 expression.

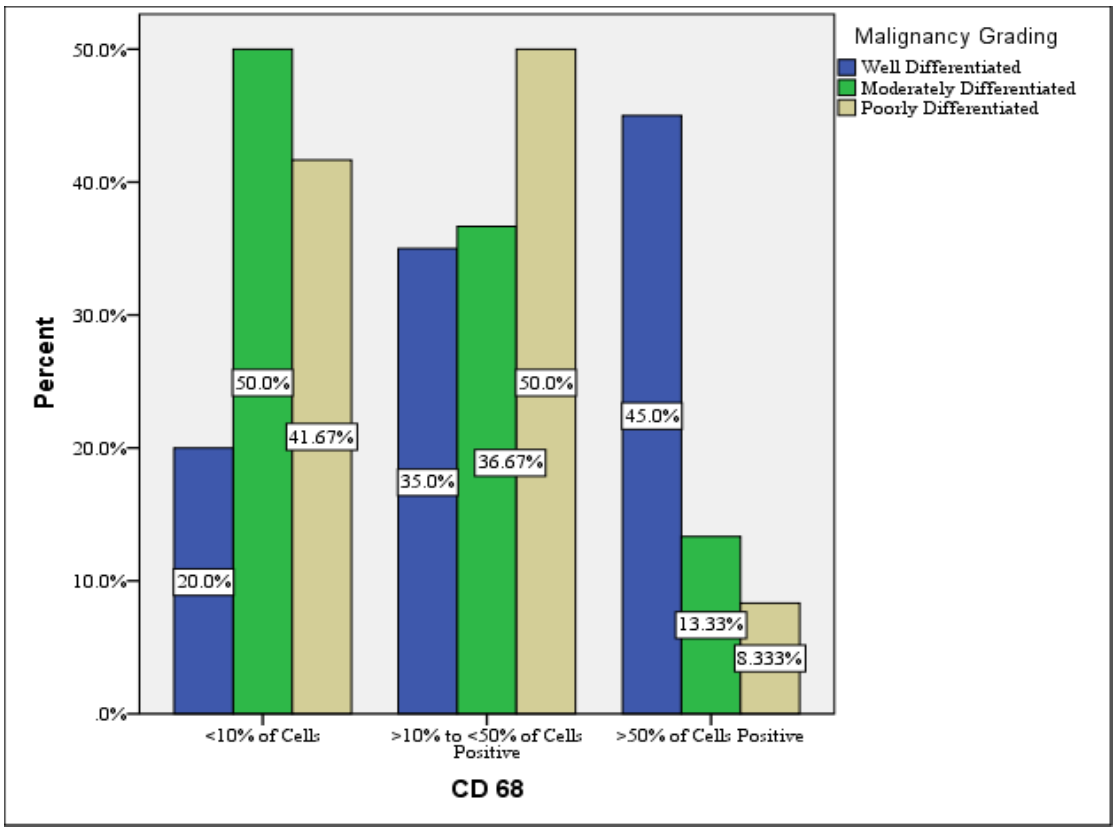


Chart 21: Bar diagram showing Association between Malignancy Grading and CD 68 expression.

Table 24 : Association between T Stage and CD 68 expression.

			CD 68			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
T Stage	T1	Count	1	1	2	4
		%	25.0%	25.0%	50.0%	100.0%
	T2	Count	5	6	5	16
		%	31.2%	37.5%	31.2%	100.0%
	T3	Count	14	12	6	32
		%	43.8%	37.5%	18.8%	100.0%
	T4	Count	4	5	1	10
		%	40.0%	50.0%	10.0%	100.0%
Total		Count	24	24	14	62
		%	38.7%	38.7%	22.6%	100.0%

$\chi 2 = 3.953$, df =6, p = 0.683

In the study, there was no significant association between T Stage and CD 68 expression.

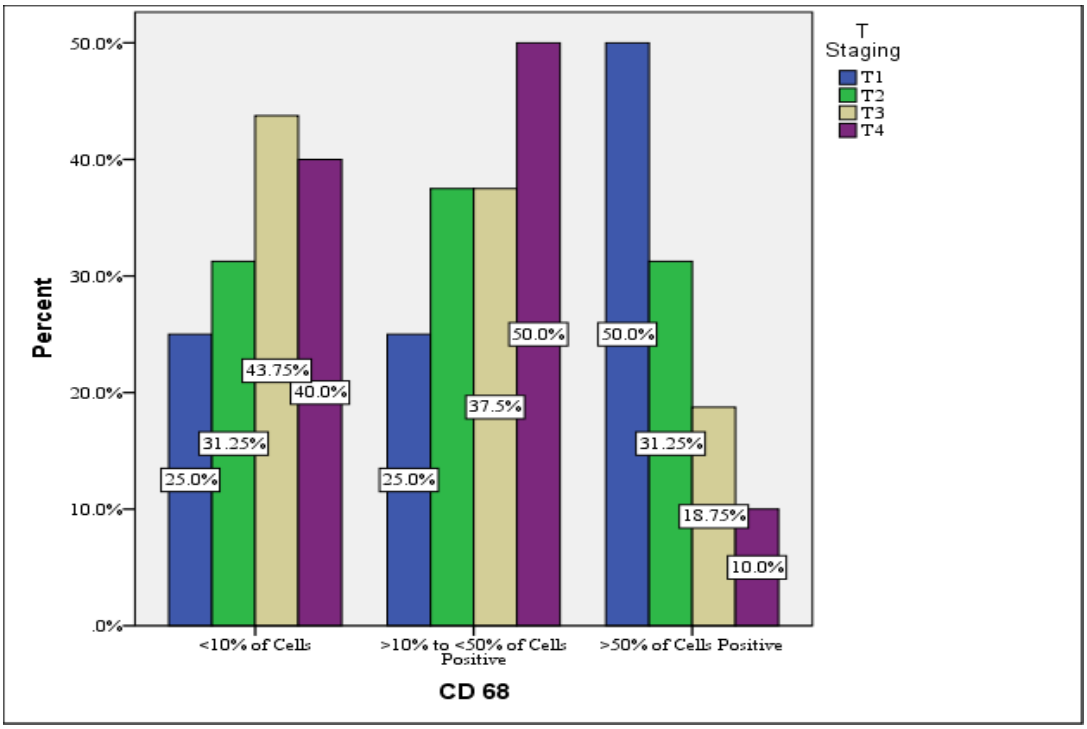


Chart 22 : Bar diagram showing Association between T Stage and CD 68 expression.

Table 25 : Association between N Stage and CD 68 expression.

			CD 68			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
N Staging	N0	Count	13	13	13	39
		%	33.3%	33.3%	33.3%	100.0%
	N1	Count	10	10	1	21
		%	47.6%	47.6%	4.8%	100.0%
	N2	Count	1	1	0	2
		%	50.0%	50.0%	0.0%	100.0%
Total		Count	24	24	14	62
		%	38.7%	38.7%	22.6%	100.0%

$\chi 2 = 6.977$, df =4, p = 0.137

In the study, there was no significant association between N Stage and CD 68 expression.

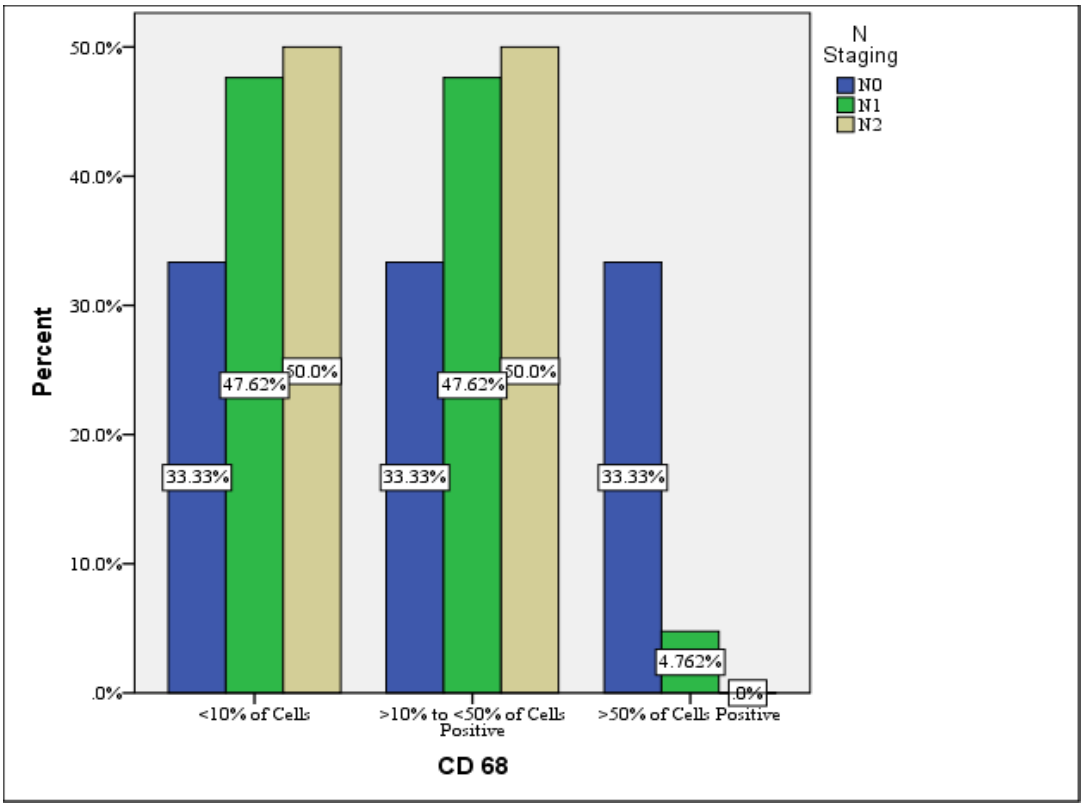


Chart 23: Bar diagram showing Association between N Stage and CD 68

Table 26 :Association between Tumor Stage and CD 68

			CD 68			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
Tumor Stage	I	Count	5	5	7	17
		%	29.4%	29.4%	41.2%	100.0%
	II	Count	8	8	6	22
		%	36.4%	36.4%	27.3%	100.0%
	III	Count	11	11	1	23
		%	47.8%	47.8%	4.3%	100.0%
Total		Count	24	24	14	62
		%	38.7%	38.7%	22.6%	100.0%

χ 2 =8.014, df =4, p = 0.091

In the study, there was no significant association between Tumor Stage and CD 68 expression.

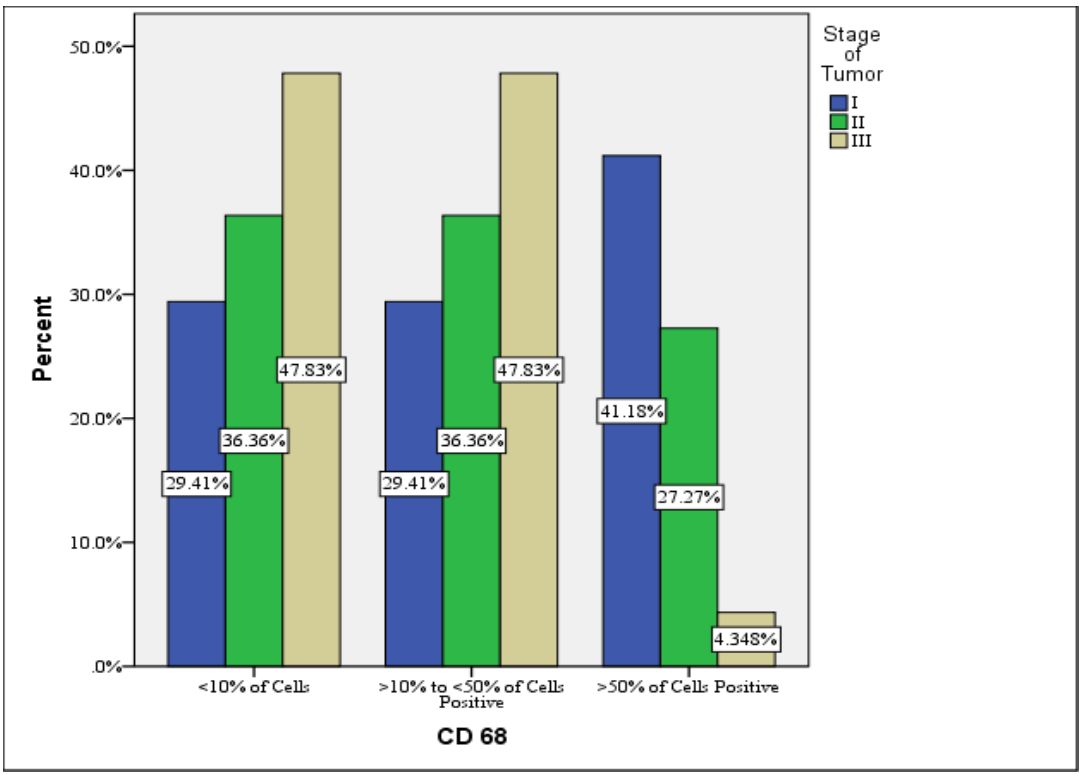


Chart 24: Bar diagram showing Association between Tumor Stage and CD 68 expression.

Table 27 : Association between Perineural invasion and CD 68

			CD 68			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
Perineural invasion	Absent	Count	23	24	13	60
		%	38.3%	40.0%	21.7%	100.0%
	Present	Count	1	0	1	2
		%	50.0%	0.0%	50.0%	100.0%
Total		Count	24	24	14	62
		%	38.7%	38.7%	22.6%	100.0%

$\chi 2 = 1.556$, df =2, p = 0.459

In the study, there was no significant association between Perineural invasion and CD 68 expression.

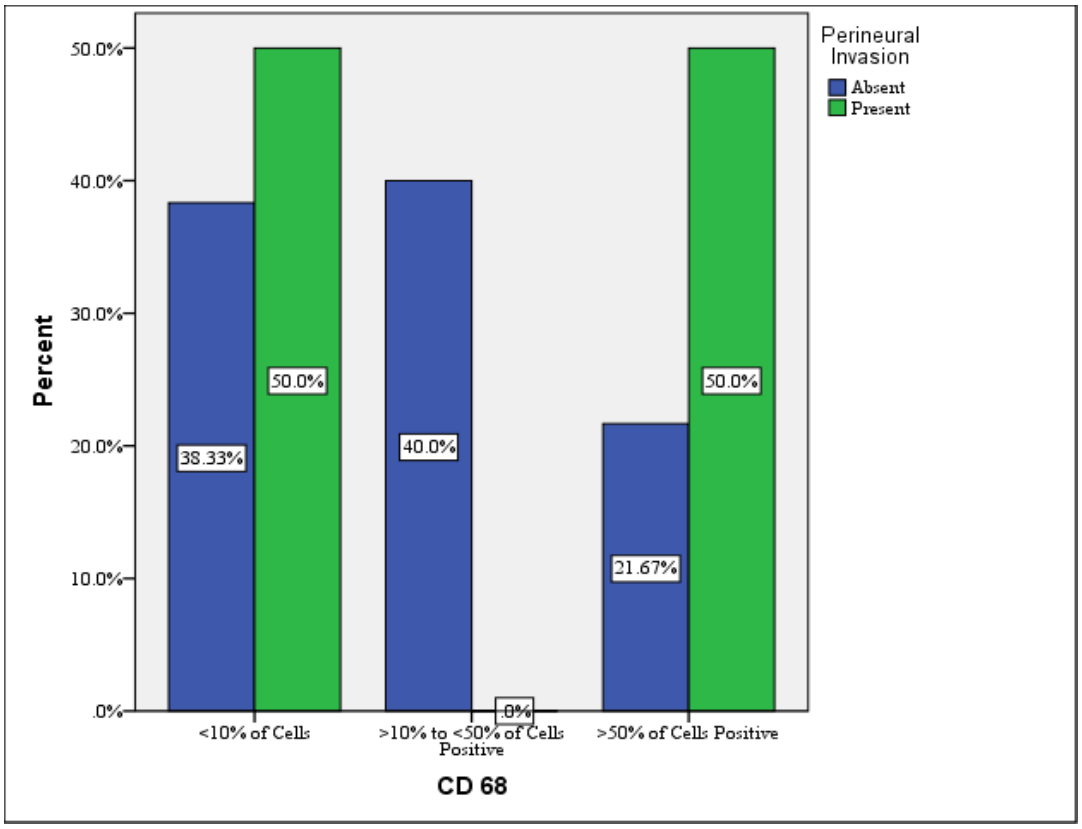


Chart 25: Bar diagram showing Association between Perineural invasion and CD 68 expression.

Table 28: Association between Lymphovascular Invasion and CD 68 expression.

			CD 68			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
Lymphovascular Invasion	Absent	Count	23	23	13	59
		%	39.0%	39.0%	22.0%	100.0%
	Present	Count	1	1	1	3
		%	33.3%	33.3%	33.3%	100.0%
Total		Count	24	24	14	62
		%	38.7%	38.7%	22.6%	100.0%

χ 2 =0.209, df =2, p = 0.901

In the study, there was no significant association between Lymphovascular Invasion and CD 68 expression.

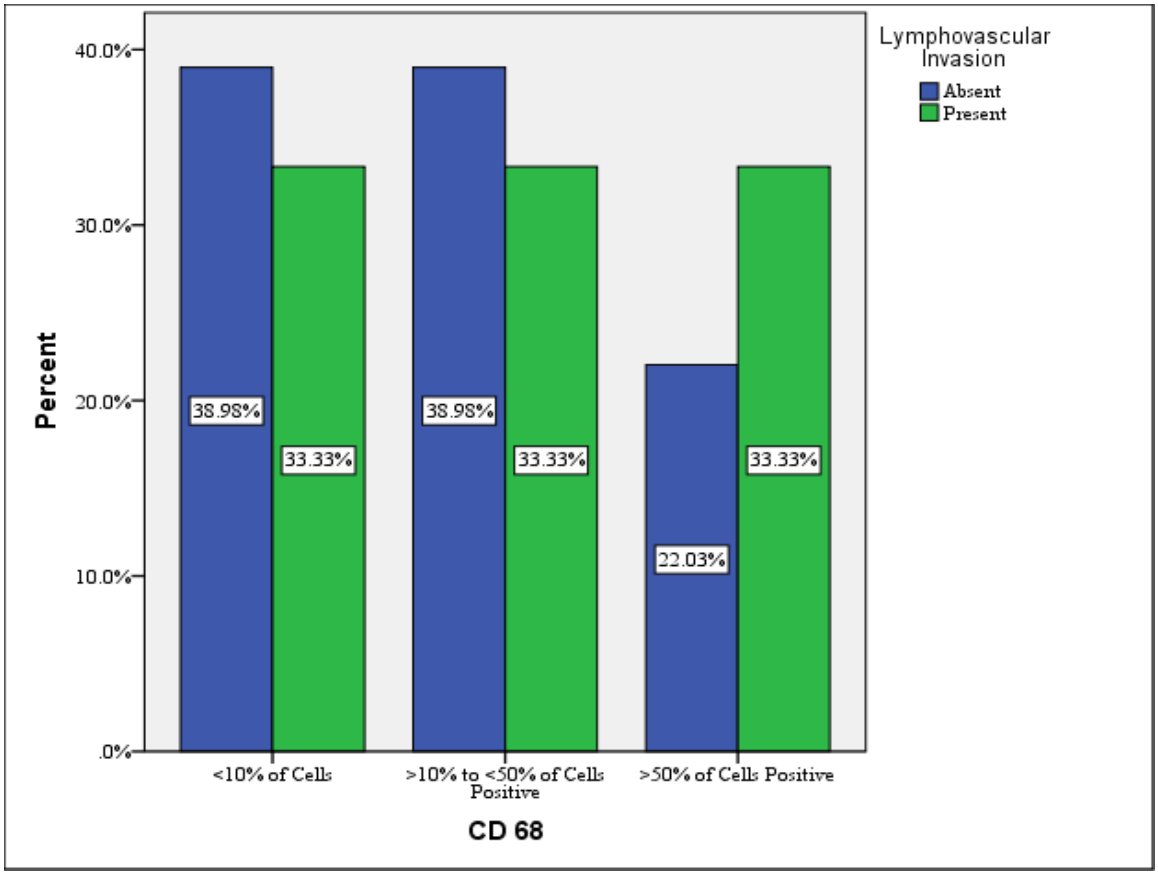


Chart 26 : Bar diagram showing Association between Lymphovascular Invasion and CD 68 expression.

Table 29:Association between No of Positive Lymph nodes and CD 68 expression.

			CD 68			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
No of Positive Lymph nodes	0	Count	12	13	13	38
		%	31.6%	34.2%	34.2%	100.0%
	1	Count	3	4	0	7
		%	42.9%	57.1%	0.0%	100.0%
	2	Count	6	3	0	9
		%	66.7%	33.3%	0.0%	100.0%
	3	Count	2	3	1	6
		%	33.3%	50.0%	16.7%	100.0%
	4	Count	0	1	0	1
		%	0.0%	100.0%	0.0%	100.0%
	6	Count	1	0	0	1
		%	100.0%	0.0%	0.0%	100.0%
Total		Count	24	24	14	62
		%	38.7%	38.7%	22.6%	100.0%

$\chi 2 = 12.619$, df =10, p = 0.246

In the study, there was no significant association between No of Positive Lymph nodes and CD 68 expression.

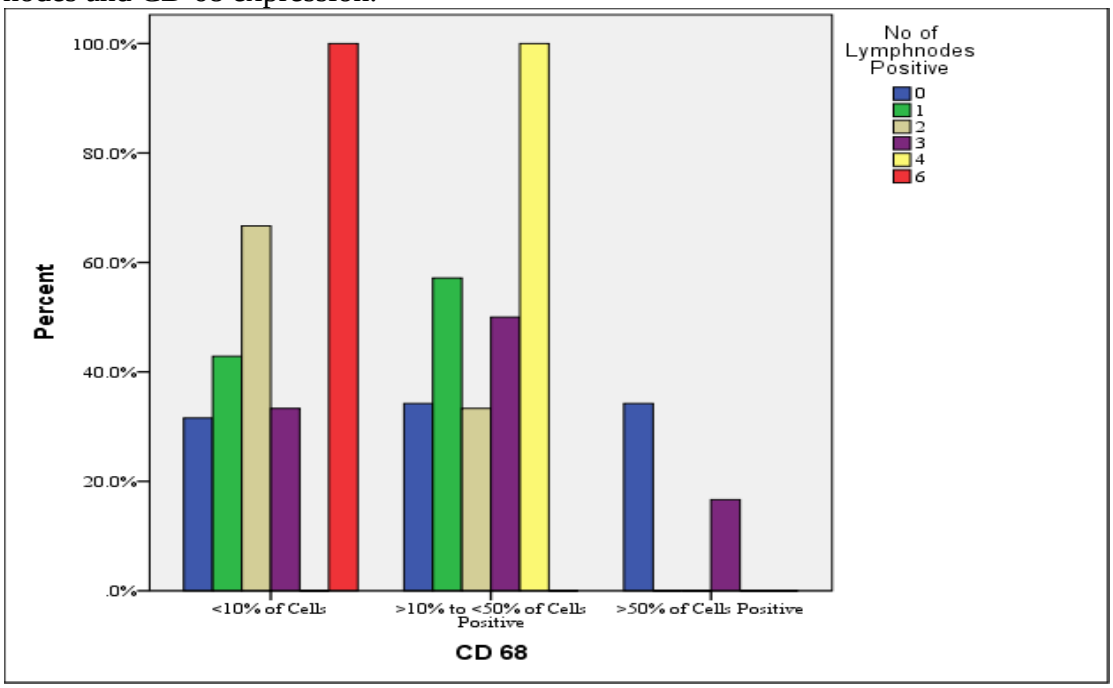


Chart 27 : Bar diagram showing Association between No of Positive Lymph nodes and CD 68 expression.

Table 30: Association between LNR Positive and CD 68 expression

			CD 68			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
LNR	<0.111	Count	12	15	13	40
		%	30.0%	37.5%	32.5%	100.0%
	0.111 to 0.200	Count	2	1	0	3
		%	66.7%	33.3%	0.0%	100.0%
	0.200 to 0.429	Count	5	0	0	5
		%	100.0%	0.0%	0.0%	100.0%
	>0.429	Count	5	8	1	14
		%	35.7%	57.1%	7.1%	100.0%
Total		Count	24	24	14	62
		%	38.7%	38.7%	22.6%	100.0%

$\chi 2 = 14.503$, df =6, p = 0.024*

In the study, there was significant association between Lymph node ratio and CD 68 expression. Among those with LNR <0.111, 30% had CD 68 in <10% of Cells, 37.5% in >10% to <50% of Cells Positive and 32.5% in >50% of Cells Positive. Among those with LNR 0.111 to 0.200, 30% had CD 68 <10% of Cells, 33.3% had >10% to <50% of Cells Positive.

Among those with LNR 0.2 to 0.429, 100% of cases had <10% of cells.

Among those with LNR >0.429, 35.7% had <10% of Cells, 57.1% had >10% to <50% of Cells Positive and 7.1% had >50% of Cells Positive.

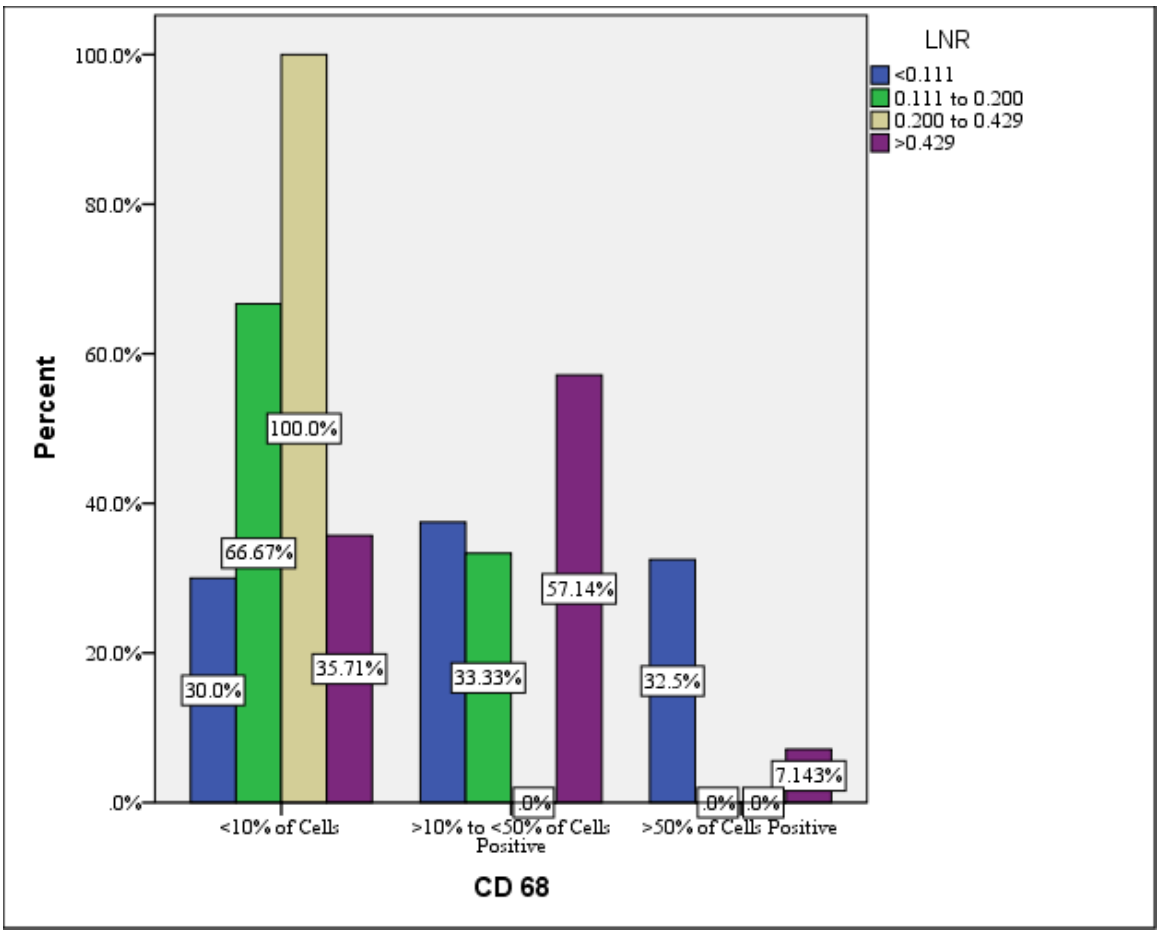


Chart 28 : Bar diagram showing Association between LNR and CD 68 expression.

ASSOCIATION OF CD 163 WITH DIFFERENT HISTOLOGICAL PARAMETERS

Table 31: Association between Age and CD 163 expression.

			CD 163			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
Age	<60 years	Count	4	26	12	42
		%	9.5%	61.9%	28.6%	100.0%
	>60 years	Count	2	10	8	20
		%	10.0%	50.0%	40.0%	100.0%
Total		Count	6	36	20	62
		%	9.7%	58.1%	32.3%	100.0%

$\chi 2 = 0.882$, df =2, p = 0.643

In the study, there was no significant association between Age and CD 163 expression.

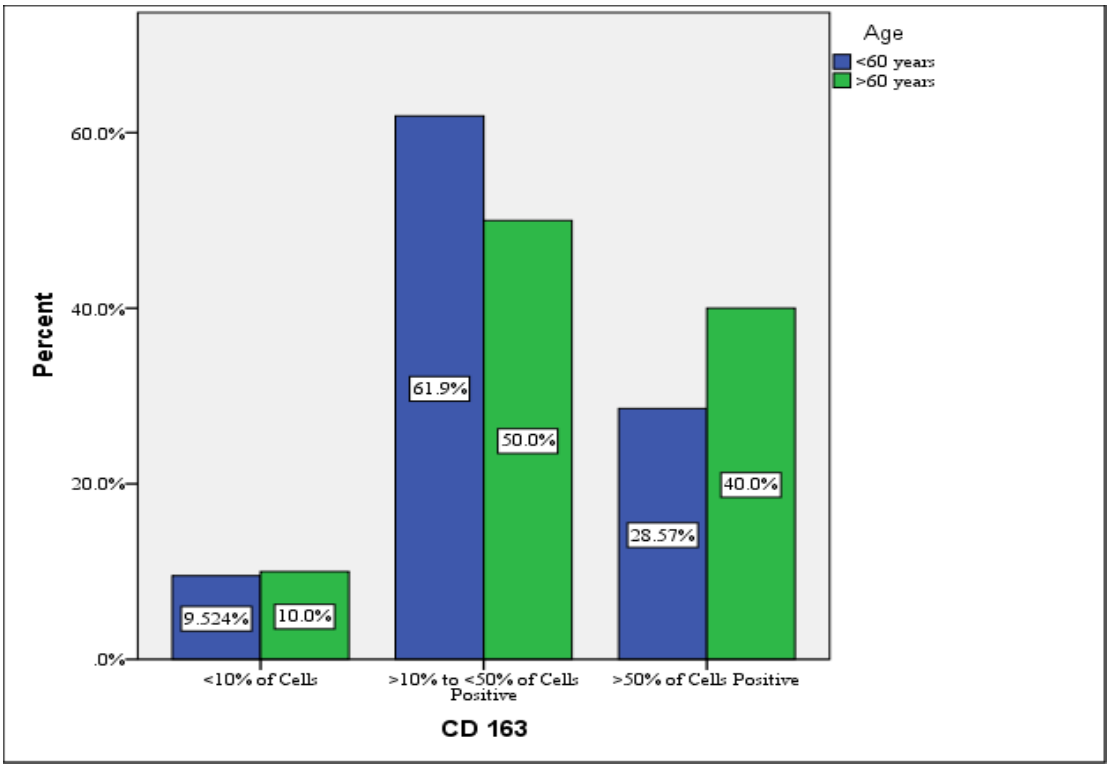


Chart 29 : Bar diagram showing Association between Age and CD 163 expression.

Table 32 : Association between Sex and CD 163 expression.

			CD 163			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
Sex	Male	Count	3	23	13	39
		%	7.7%	59.0%	33.3%	100.0%
	Female	Count	3	13	7	23
		%	13.0%	56.5%	30.4%	100.0%
Total		Count	6	36	20	62
		%	9.7%	58.1%	32.3%	100.0%

$\chi 2 = 0.481$, df =2, p = 0.786

In the study there was no significant association between Sex and CD 163 expression.

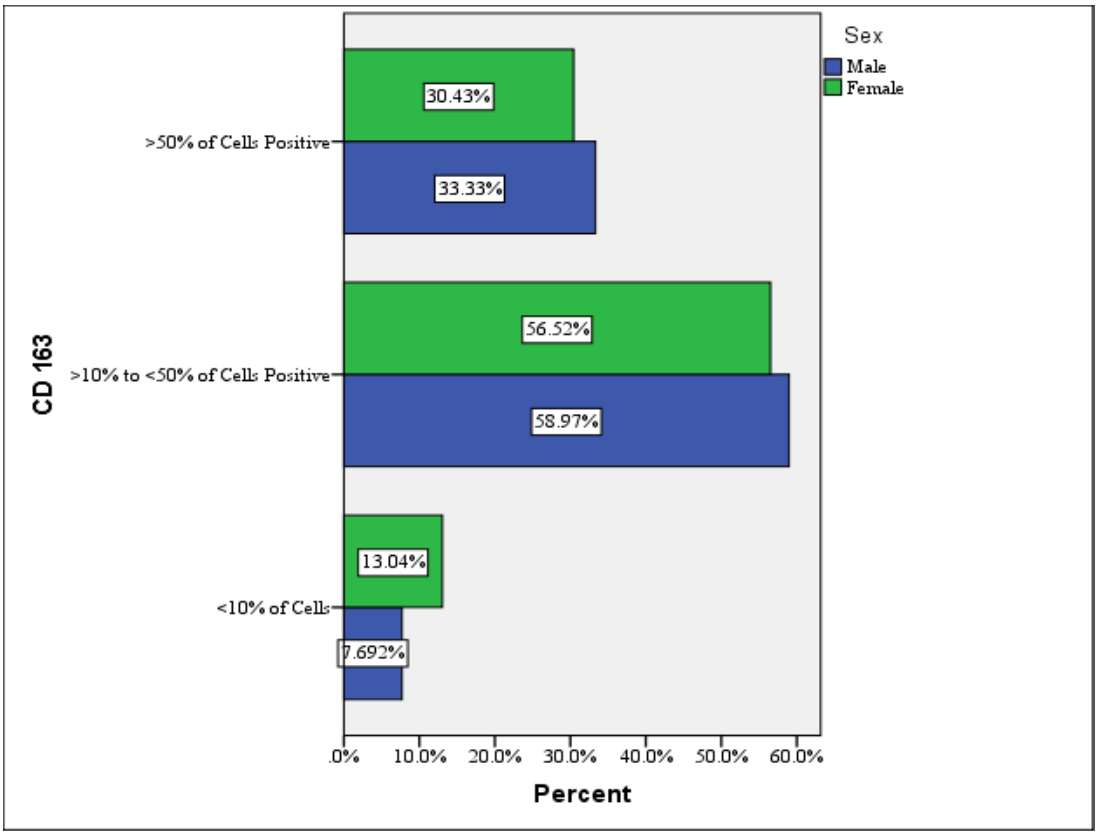


Chart 30 : Bar diagram showing Association between Sex and CD 163 expression.

Table 33: Association between Site and CD 163 expression.

			CD 163			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
Site	Ascending Colon	Count	1	4	8	13
		%	7.7%	30.8%	61.5%	100.0%
	Transverse Colon	Count	1	5	0	6
		%	16.7%	83.3%	0.0%	100.0%
	Descending Colon	Count	0	2	1	3
		%	0.0%	66.7%	33.3%	100.0%
	Sigmoid Colon	Count	1	8	4	13
		%	7.7%	61.5%	30.8%	100.0%
	Rectum	Count	3	17	7	27
		%	11.1%	63.0%	25.9%	100.0%
Total		Count	6	36	20	62
		%	9.7%	58.1%	32.3%	100.0%

$\chi 2 = 8.997$, df =8, p = 0.343

In the study, there was no significant association between Site and CD 163 expression.

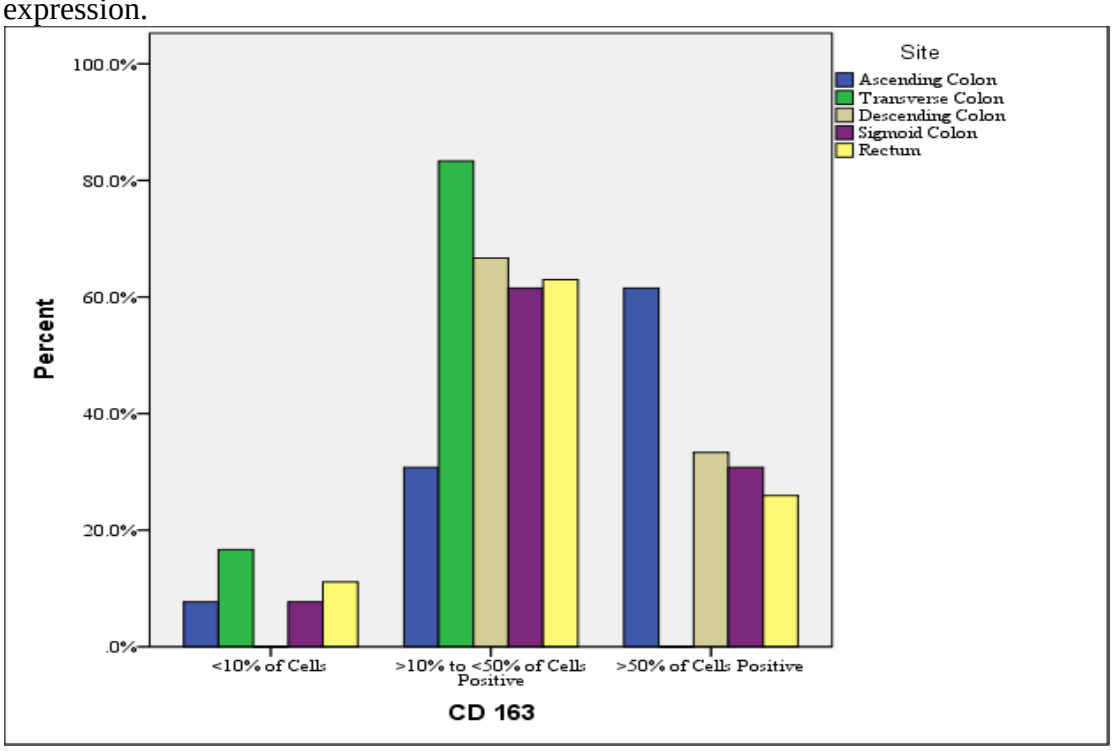


Chart 31 : Bar diagram showing Association between Site and CD 163 expression

Table 34: Association between type of tumor growth and CD 163 expression.

			CD 163			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
Growth	Proliferative	Count	1	3	4	8
		%	12.5%	37.5%	50.0%	100.0%
	Ulceroproliferative	Count	4	20	10	34
		%	11.8%	58.8%	29.4%	100.0%
	Ulcerative/ Infiltrative	Count	1	13	6	20
		%	5.0%	65.0%	30.0%	100.0%
Total		Count	6	36	20	62
		%	9.7%	58.1%	32.3%	100.0%

χ 2 =2.32, df =4, p = 0.677

In the study, there was no significant association between type of tumor growth and CD 163 expression.

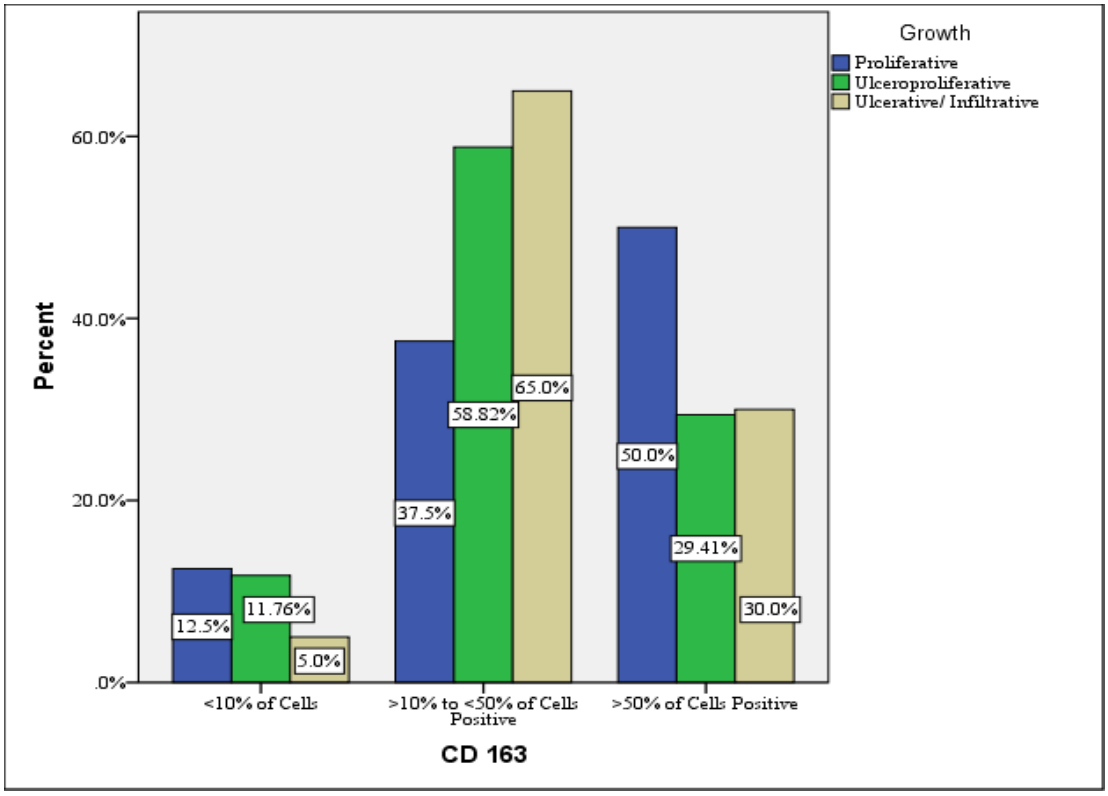


Chart 32 : Bar diagram showing Association between type of tumor growth and CD 163 expression.

Table 35: Association between Specimen Type and CD 163 expression.

			CD 163			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
Specimen Type	Hemicolectomy	Count	4	22	13	39
		%	10.3%	56.4%	33.3%	100.0%
	APR	Count	1	9	4	14
		%	7.1%	64.3%	28.6%	100.0%
	Anterior Resection	Count	1	5	3	9
		%	11.1%	55.6%	33.3%	100.0%
Total		Count	6	36	20	62
		%	9.7%	58.1%	32.3%	100.0%

$\chi 2 = 323$, df =4, p = 0.988

In the study, there was no significant association between Specimen Type and CD 163 expression.

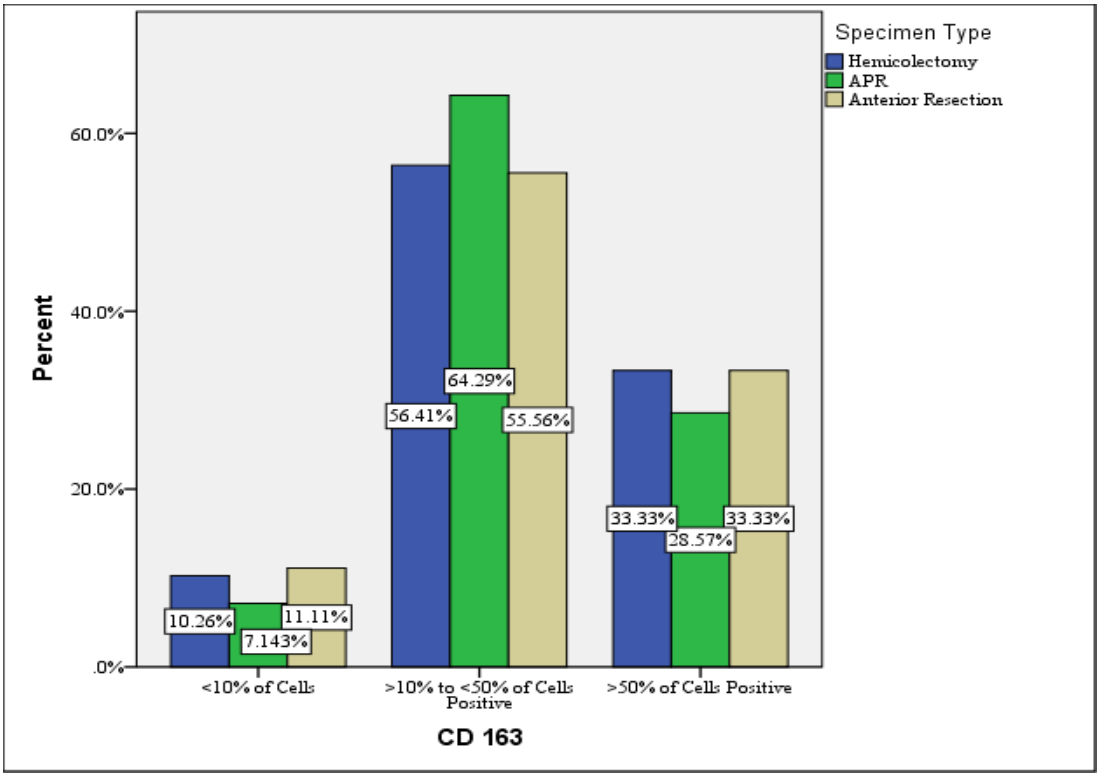


Chart 33 : Bar diagram showing Association between Specimen Type and CD 163 expression

Table 36: Association between Tumor Size and CD 163 expression.

			CD 163			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
Tumor Size	<50 mms	Count	5	24	10	39
		%	12.8%	61.5%	25.6%	100.0%
	>50 mms	Count	1	12	10	23
		%	4.3%	52.2%	43.5%	100.0%
Total		Count	6	36	20	62
		%	9.7%	58.1%	32.3%	100.0%

$\chi 2 = 2.719$, df =2, p = 0.257

In the study,there was no significant association between Tumor Size and CD 163 expression.

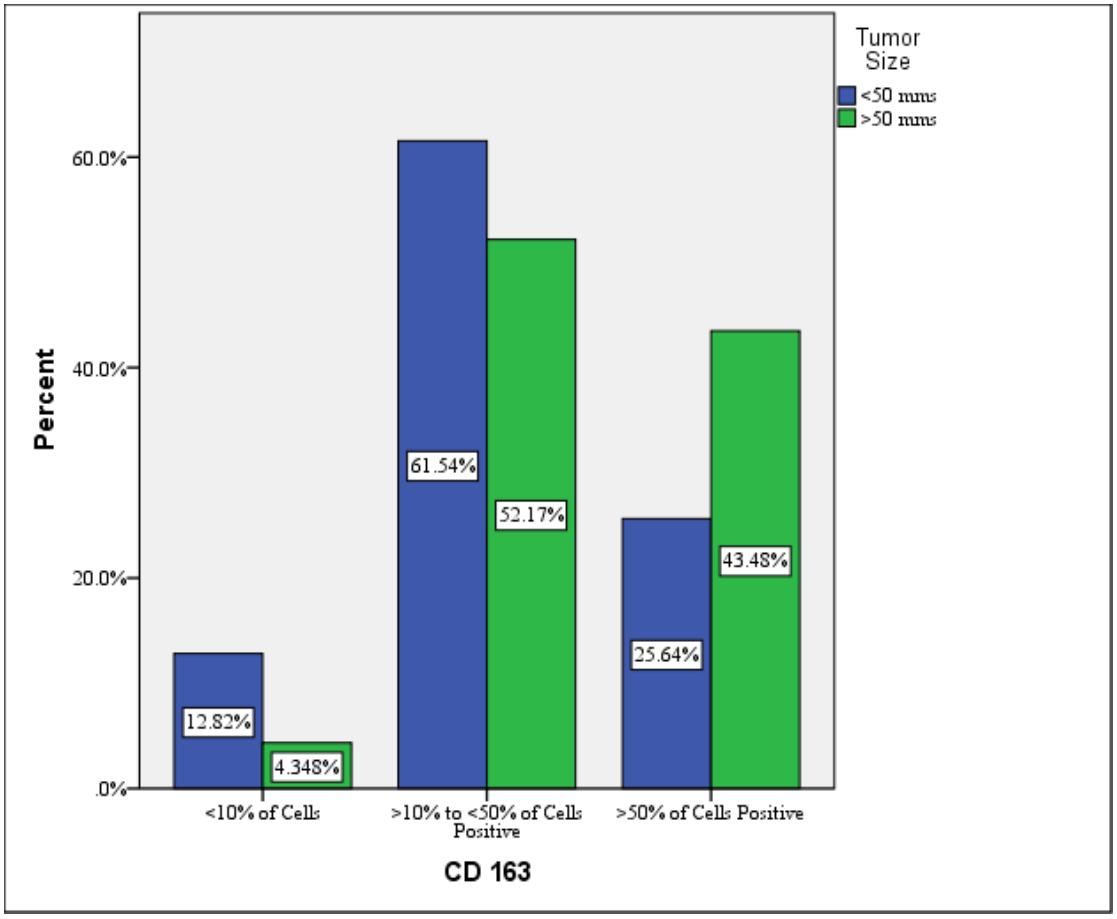


Chart 34: Bar diagram showing Association between Tumor Size and CD 163 expression

Table 37: Association between Malignancy Grade and CD 163 expression.

			CD 163			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
Malignancy Grading	Well Differentiated	Count	4	13	3	20
		%	20.0%	65.0%	15.0%	100.0%
	Moderately Differentiated	Count	2	18	10	30
		%	6.7%	60.0%	33.3%	100.0%
	Poorly Differentiated	Count	0	5	7	12
		%	0.0%	41.7%	58.3%	100.0%
Total		Count	6	36	20	62
		%	9.7%	58.1%	32.3%	100.0%

$\chi 2 = 8.722$, df =4, p = 0.067

In the study, there was no significant association between Malignancy Grade and CD 163 expression.

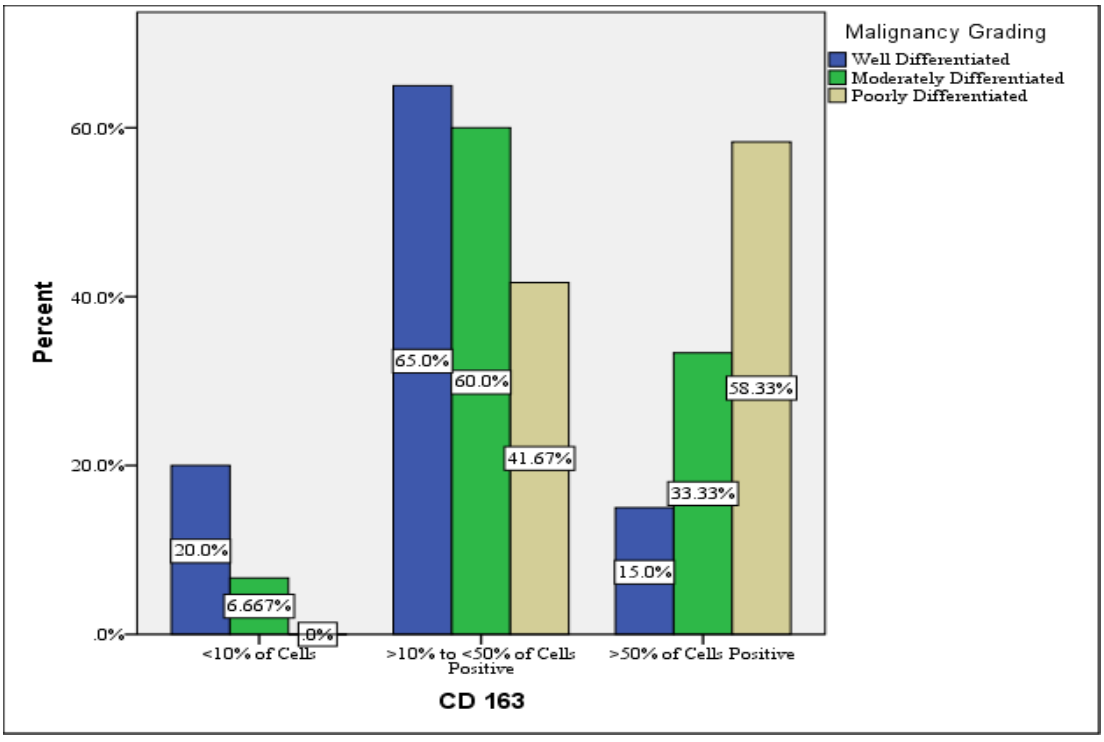


Chart 35 : Bar diagram showing Association between Malignancy Grade and CD 163 expression.

Table 38: Association between T Stage and CD 163 expression.

			CD 163			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
T Staging	T1	Count	3	1	0	4
		%	75.0%	25.0%	0.0%	100.0%
	T2	Count	2	9	5	16
		%	12.5%	56.2%	31.2%	100.0%
	T3	Count	0	21	11	32
		%	0.0%	65.6%	34.4%	100.0%
	T4	Count	1	5	4	10
		%	10.0%	50.0%	40.0%	100.0%
Total		Count	6	36	20	62
		%	9.7%	58.1%	32.3%	100.0%

$\chi 2 = 23.58$, df =6, p = 0.0001*

In the study, there was significant association between T Staging and CD 163 expression. Among those with T1, 75% had CD 163 <10% of Cells, 25% had >10% to <50% of Cells Positive. Among those with T2 stage, 12.5% had <10% of Cells, 56.2% had >10% to <50% of Cells Positive and 31.2% had >50% of Cells Positive.

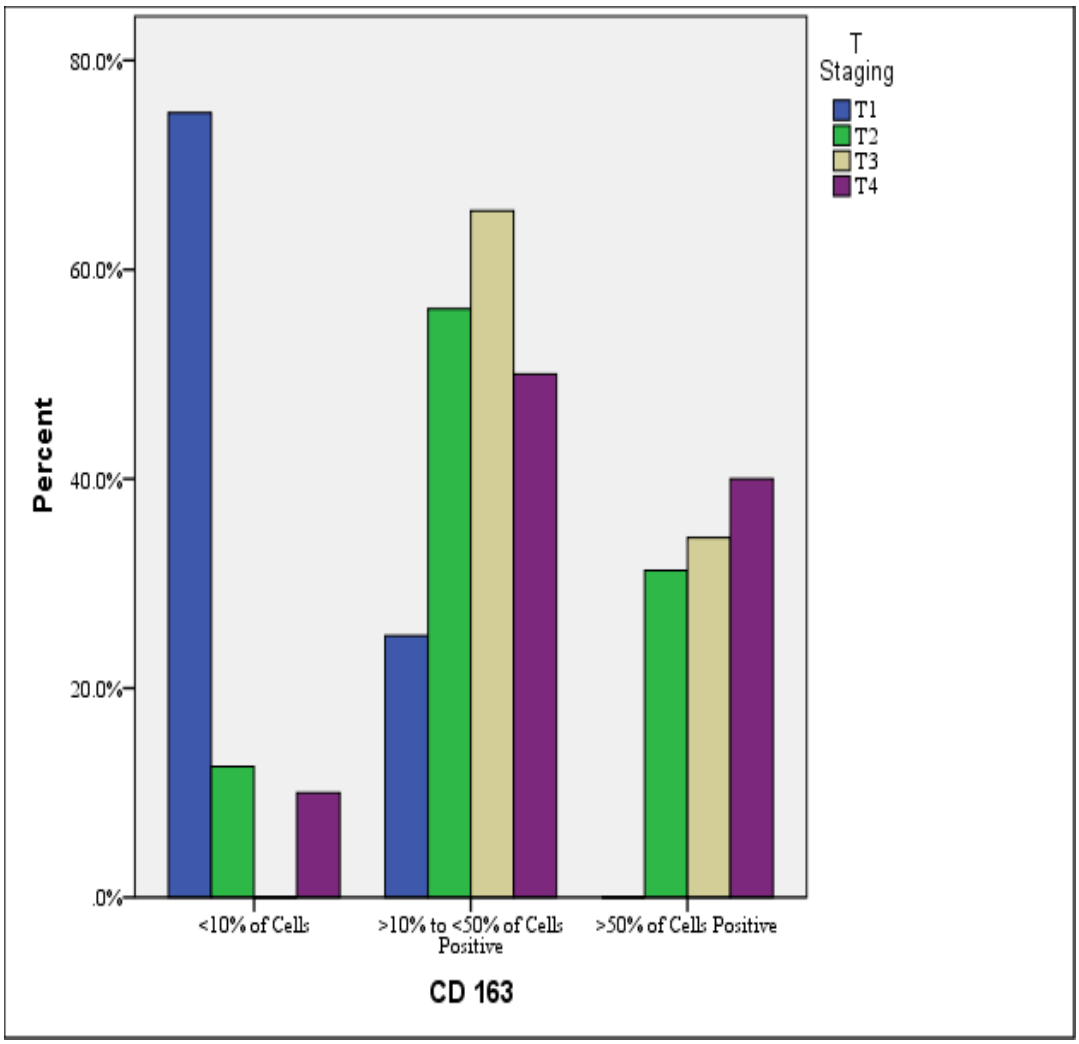


Chart 36 : Bar diagram showing Association between T Stage and CD 163 expression.

Table 39: Association between N Stage and CD 163 expression.

			CD 163			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
N Staging	N0	Count	6	27	6	39
		%	15.4%	69.2%	15.4%	100.0%
	N1	Count	0	8	13	21
		%	0.0%	38.1%	61.9%	100.0%
	N2	Count	0	1	1	2
		%	0.0%	50.0%	50.0%	100.0%
Total		Count	6	36	20	62
		%	9.7%	58.1%	32.3%	100.0%

$\chi 2 = 15.2$, df =4, p = 0.004*

In the study, there was significant association between N Staging and CD 163 expression. Among those with N0, 15.4% had <10% of Cells expressing CD 163, 69.2% had >10% to <50% of Cells Positive and 15.4% had >50% of Cells Positive. Among those in N1 Stage, 38.1% had >10% to <50% of Cells Positive and 61.9% had >50% of Cells Positive. Among those with N2 Stage, 50% had >10% to <50% of Cells Positive and 50% had >50% of Cells Positive.

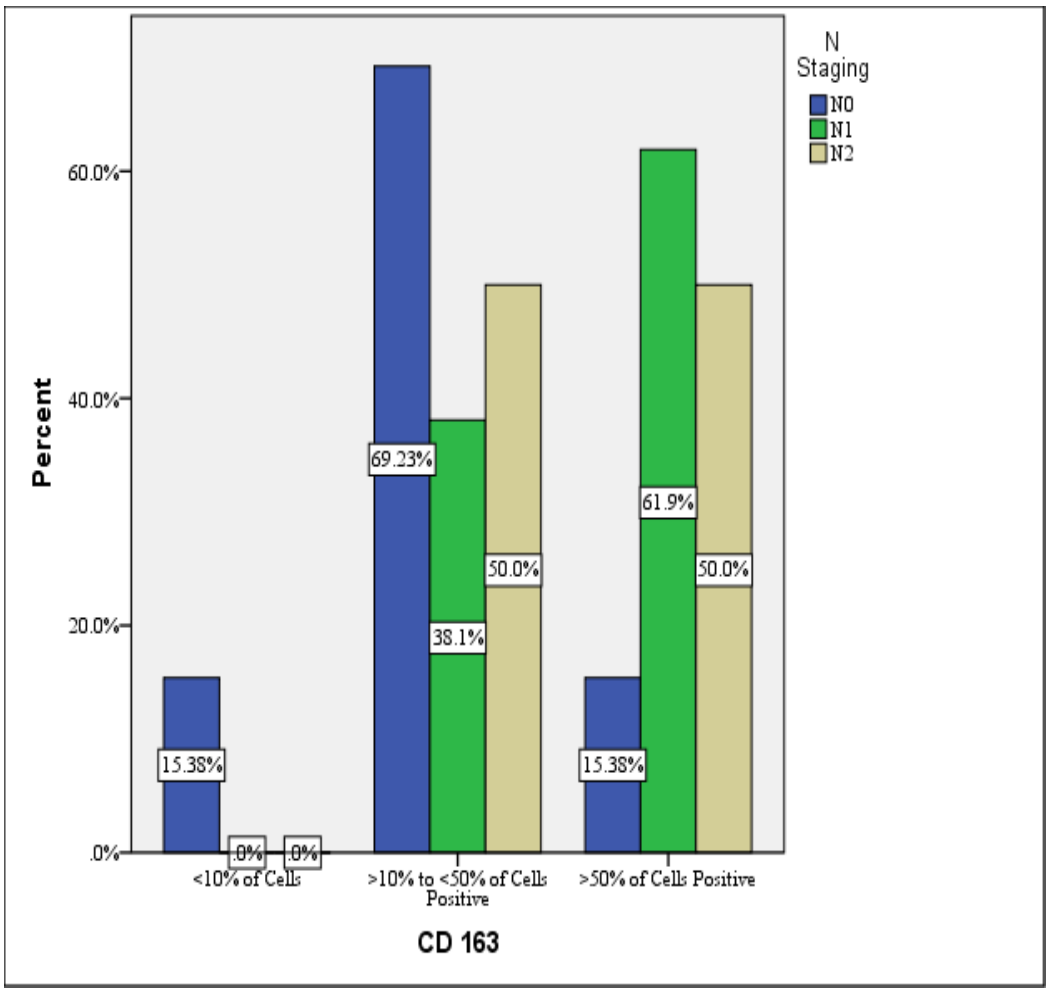


Chart 37 : Bar diagram showing Association between N Stage and CD 163 expression

Table 40 : Association between Tumor Stage and CD 163 expression

			CD 163			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
Tumor Staging	I	Count	5	9	3	17
		%	29.4%	52.9%	17.6%	100.0%
	II	Count	1	18	3	22
		%	4.5%	81.8%	13.6%	100.0%
	III	Count	0	9	14	23
		%	0.0%	39.1%	60.9%	100.0%
Total		Count	6	36	20	62
		%	9.7%	58.1%	32.3%	100.0%

$\chi 2 = 22.62$, df =4, p <0.001*

In the study, there was significant association between Tumor Staging and CD 163 expression. Among those in I stage, 29.4% had CD 163 expression <10% of Cells, 52.9% had >10% to <50% of Cells Positive and 17.6% had >50% of Cells Positive. Those in II stage, 4.5% had <10% of Cells, 81.8% had >10% to <50% of Cells Positive and 13.6% had >50% of Cells Positive. Those in III stage, 39.1% had >10% to <50% of Cells Positive and 60.9% had >50% of Cells Positive.

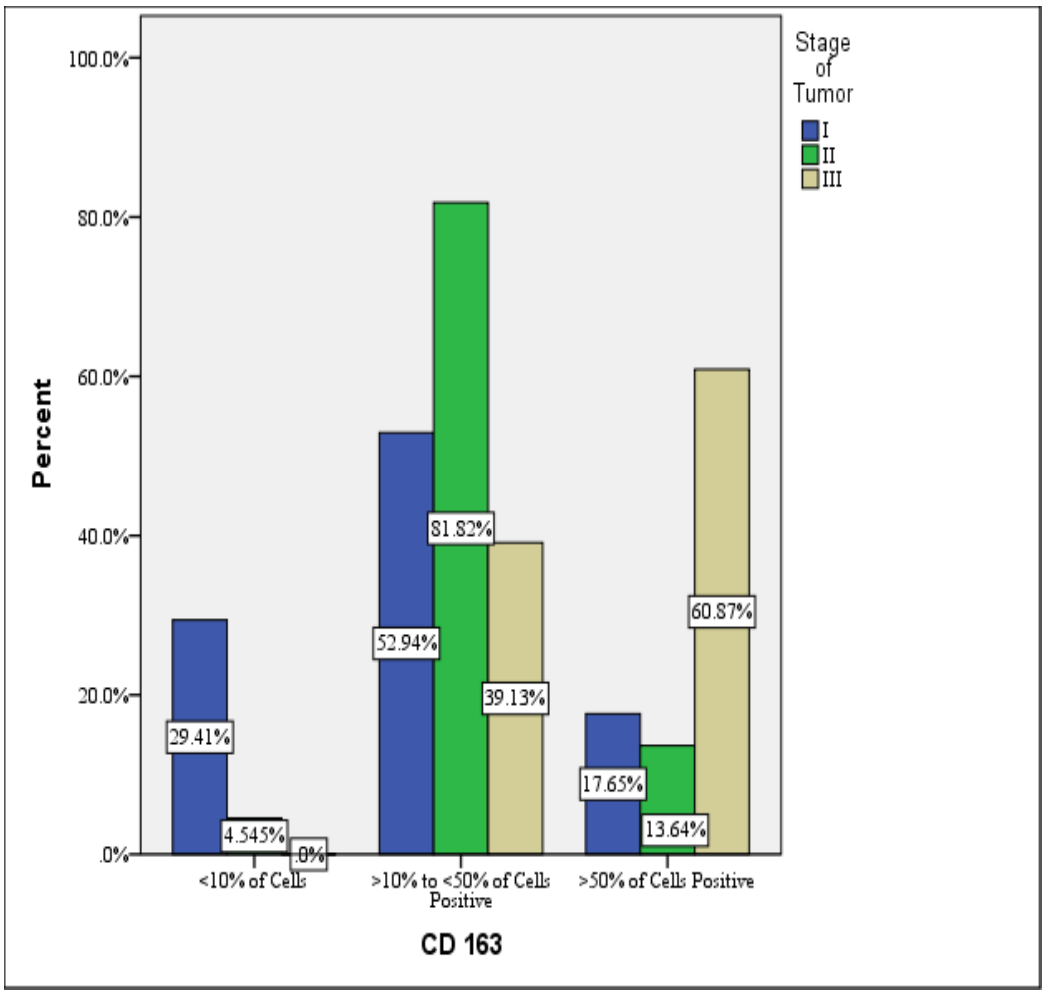


Chart 38: Bar diagram showing Association between Tumor Stage and CD 163

Table 41: Association between Perineural invasion and CD 163 expression.

			CD 163			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
Perineural invasion	Absent	Count	6	35	19	60
		%	10.0%	58.3%	31.7%	100.0%
	Present	Count	0	1	1	2
		%	0.0%	50.0%	50.0%	100.0%
Total		Count	6	36	20	62
		%	9.7%	58.1%	32.3%	100.0%

χ 2 =0.425, df =2, p = 0.809

In the study, there was no significant association between Perineural invasion and CD 163 expression.

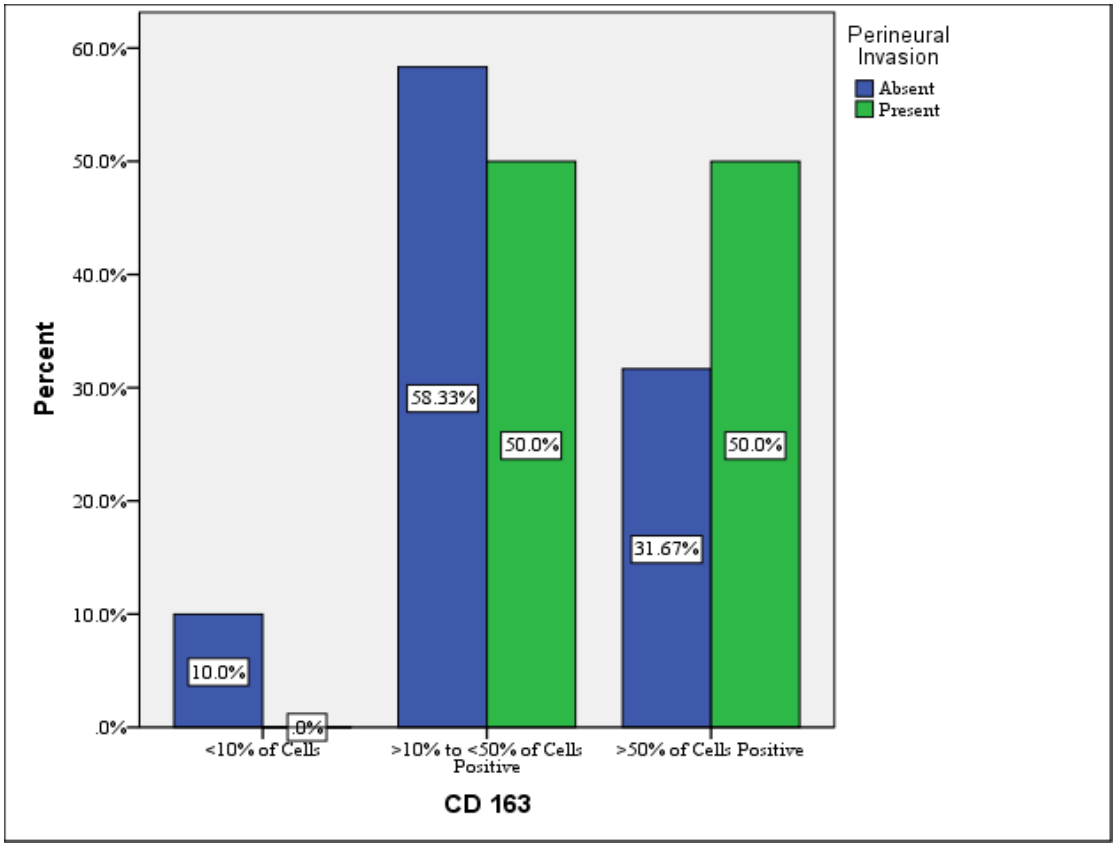


Chart 39 : Bar diagram showing Association between Specimen Type and CD 163 expression

Table 42: Association between Lymphovascular Invasion and CD 163 expression.

			CD 163			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
Lymphovascular Invasion	Absent	Count	6	34	19	59
		%	10.2%	57.6%	32.2%	100.0%
	Present	Count	0	2	1	3
		%	0.0%	66.7%	33.3%	100.0%
Total		Count	6	36	20	62
		%	9.7%	58.1%	32.3%	100.0%

χ 2 =0.346, df =2, p = 0.841

In the study, there was no significant association between Lymphovascular Invasion and CD 163 expression.

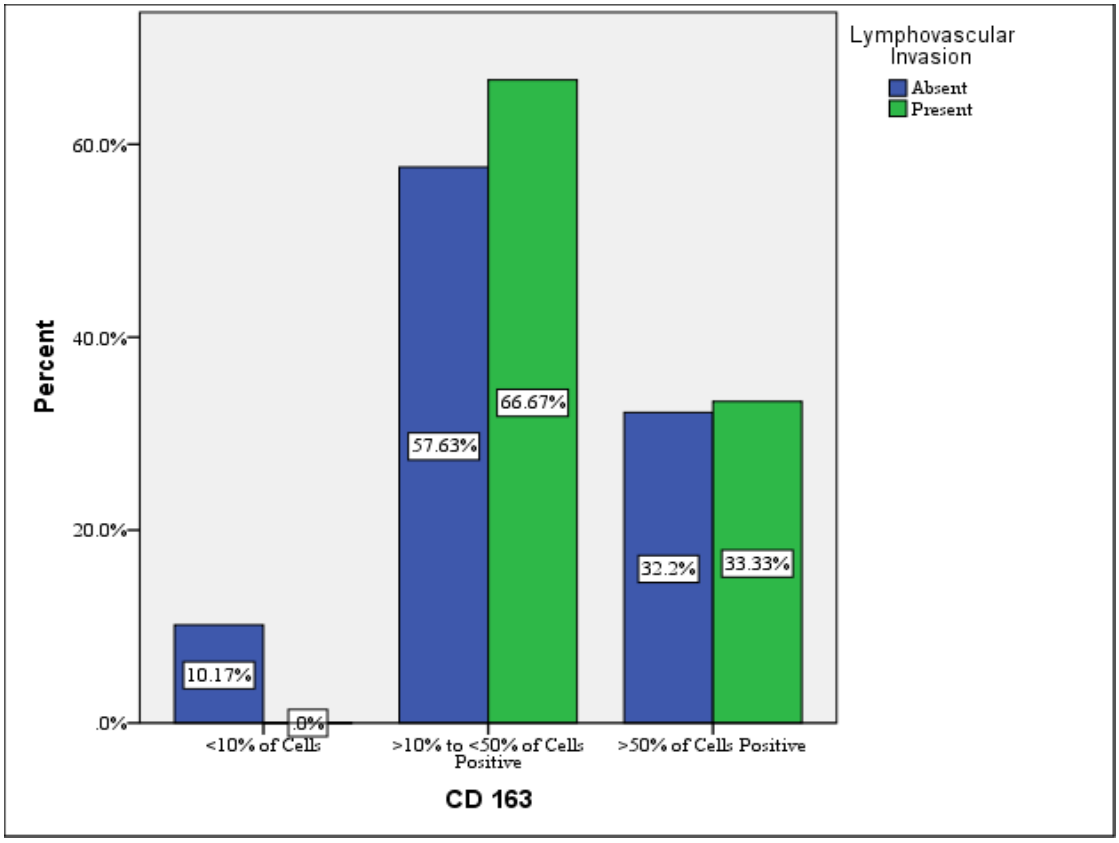


Chart 40: Bar diagram showing Association between Lymphovascular Invasion and CD 163 expression.

Table 43: Association between number of positive LN and CD 163 expression.

			CD 163			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
Positive LN	0	Count	6	27	5	38
		%	15.8%	71.1%	13.2%	100.0%
	1	Count	0	4	3	7
		%	0.0%	57.1%	42.9%	100.0%
	2	Count	0	3	6	9
		%	0.0%	33.3%	66.7%	100.0%
	3	Count	0	1	5	6
		%	0.0%	16.7%	83.3%	100.0%
	4	Count	0	0	1	1
		%	0.0%	0.0%	100.0%	100.0%
	6	Count	0	1	0	1
		%	0.0%	100.0%	0.0%	100.0%
Total		Count	6	36	20	62
		%	9.7%	58.1%	32.3%	100.0%

$\chi 2 = 22.93$, df =10, p = 0.011*

In the study, there was significant statistical association between CD 163 expression and number of positive Lymph nodes. Among those with no Lymph nodes metastasis, 71.1% had CD 163 expression in >10% to <50% of Cells, 13.2% had >50% of Cells Positive and 15.8% of cases had <10% of Cells positive. Among those with 1 lymph node, 57.1% had >10% to <50% of Cells Positive and 42.9% had >50% of Cells Positive. Among those with 2 lymph nodes, 33.3% had >10% to <50% of Cells Positive and 66.7% had >50% of Cells Positive. Among those with 3 lymph nodes positive 16.7% had >10% to <50% of Cells Positive and 83.3% had >50% of Cells Positive.

Among those with 4 lymph nodes positive, 100% had >50% of Cells Positive. Among those with 6 lymph nodes positive, 100% had >10% to <50% of Cells Positive.

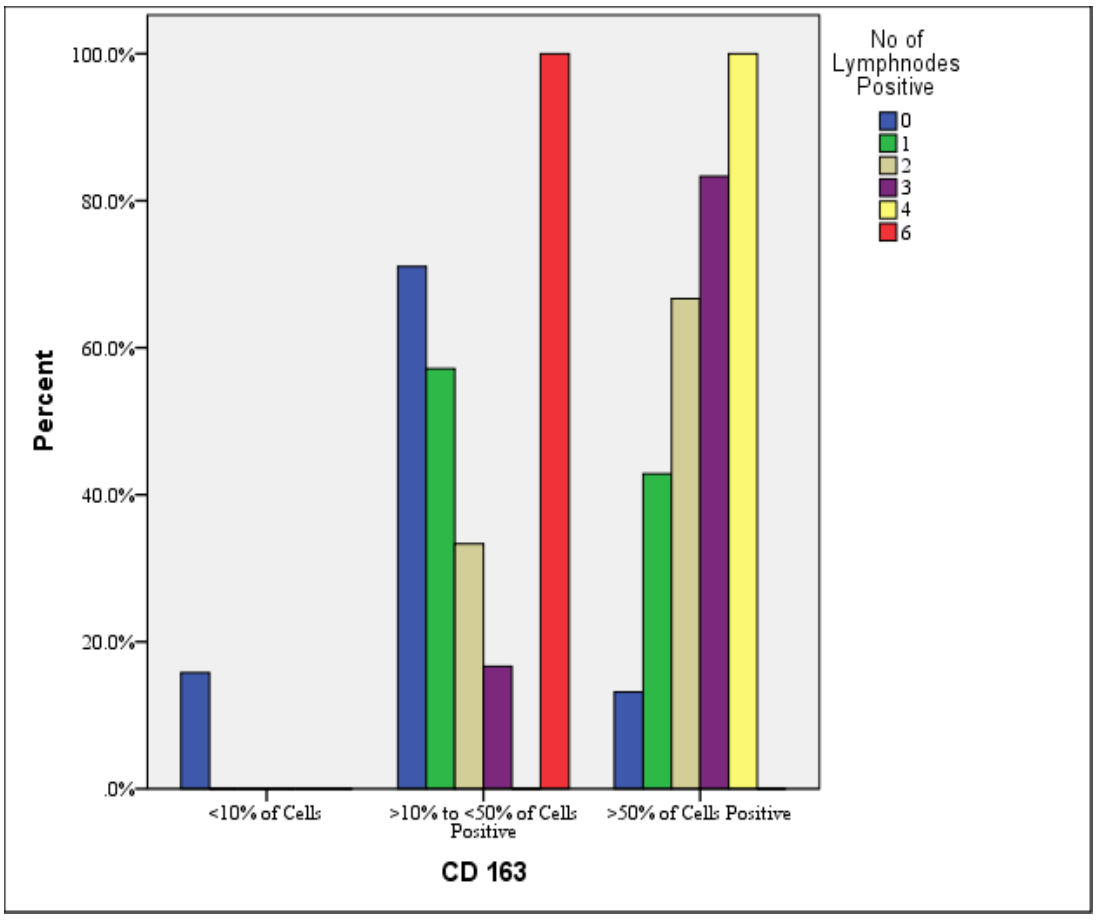


Chart 41 : Bar diagram showing Association between number of positive Lymph nodes and CD 163 expression.

Table 44 : Association between Lymph node ratio (LNR) and CD 163 expression.

			CD 163			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
LNR	<0.111	Count	6	29	5	40
		%	15.0%	72.5%	12.5%	100.0%
	0.111 to 0.200	Count	0	2	1	3
		%	0.0%	66.7%	33.3%	100.0%
	0.200 to 0.429	Count	0	2	3	5
		%	0.0%	40.0%	60.0%	100.0%
	>0.429	Count	0	3	11	14
		%	0.0%	21.4%	78.6%	100.0%
Total		Count	6	36	20	62
		%	9.7%	58.1%	32.3%	100.0%

$\chi 2 = 23.63$, df =6, p = 0.001*

In the study, there was significant association between Lymph node ratio(LNR) and CD 163 expression. Among those with LNR <0.111, 15% of cases had <10% CD 163 expression, 72.5% had >10% to <50% of Cells Positive and 12.5% had >50% of Cells Positive. Among those with LNR 0.111 to 0.200, 66.7% had >10% to <50% of Cells Positive, 33.3% had >50% of Cells Positive. Among those with LNR 0.200 to 0.429, 40.0% had >10% to <50% of Cells Positive, 60% had >50% of Cells Positive. Among those with >0.429, 21.4% had >10% to <50% of Cells Positive and 78.6% had >50% of Cells Positive.

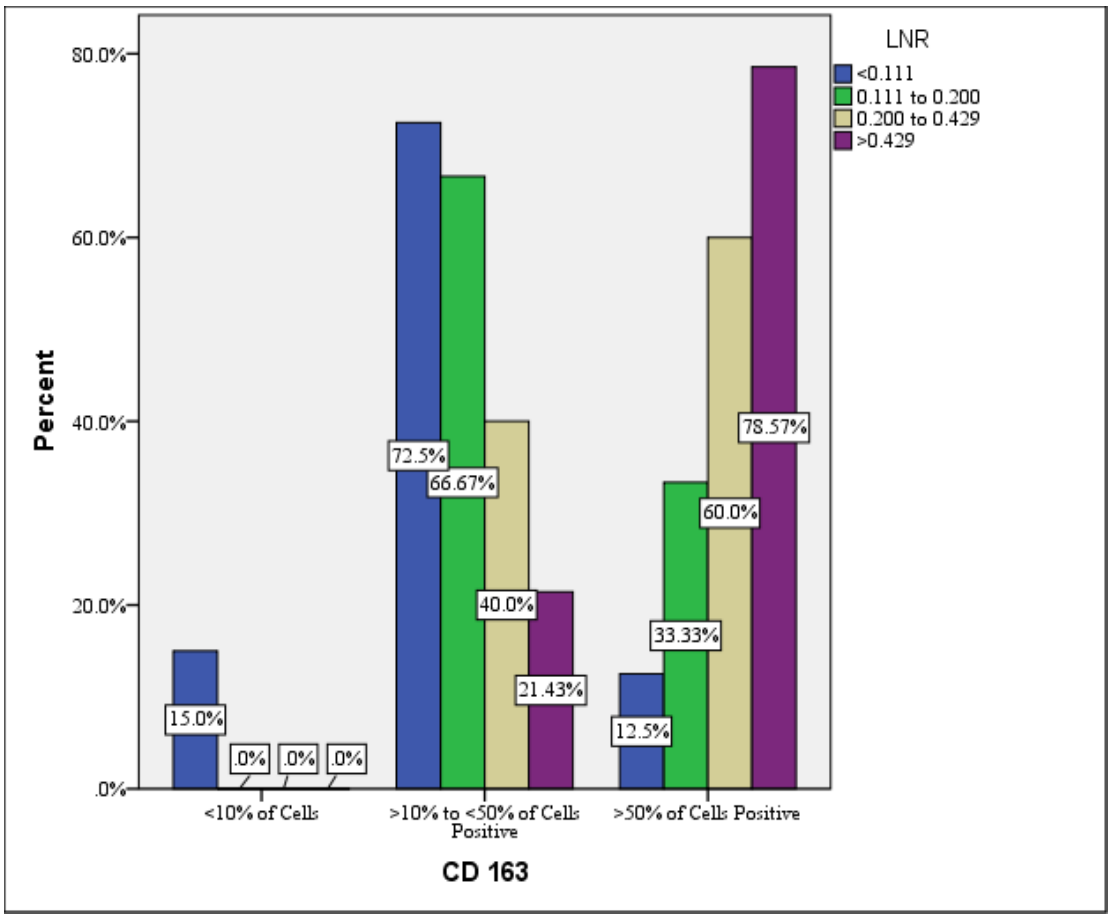


Chart 42 : Bar diagram showing Association between LNR and CD 163 expression.

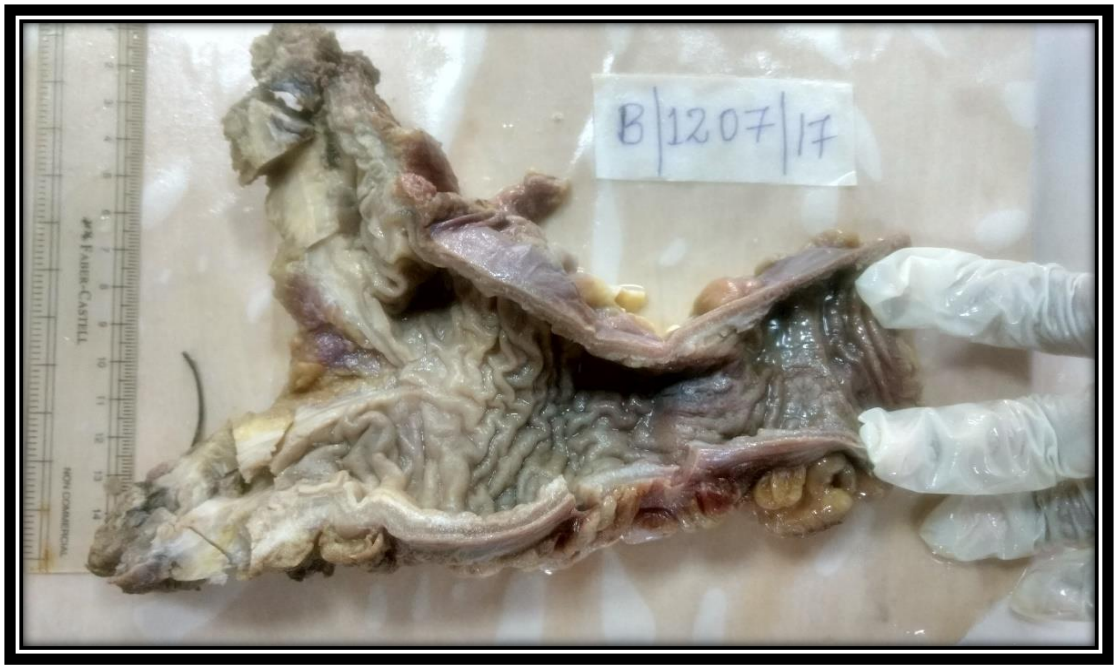


Figure 5 - Cut section of the colon showing Grey white tumor area

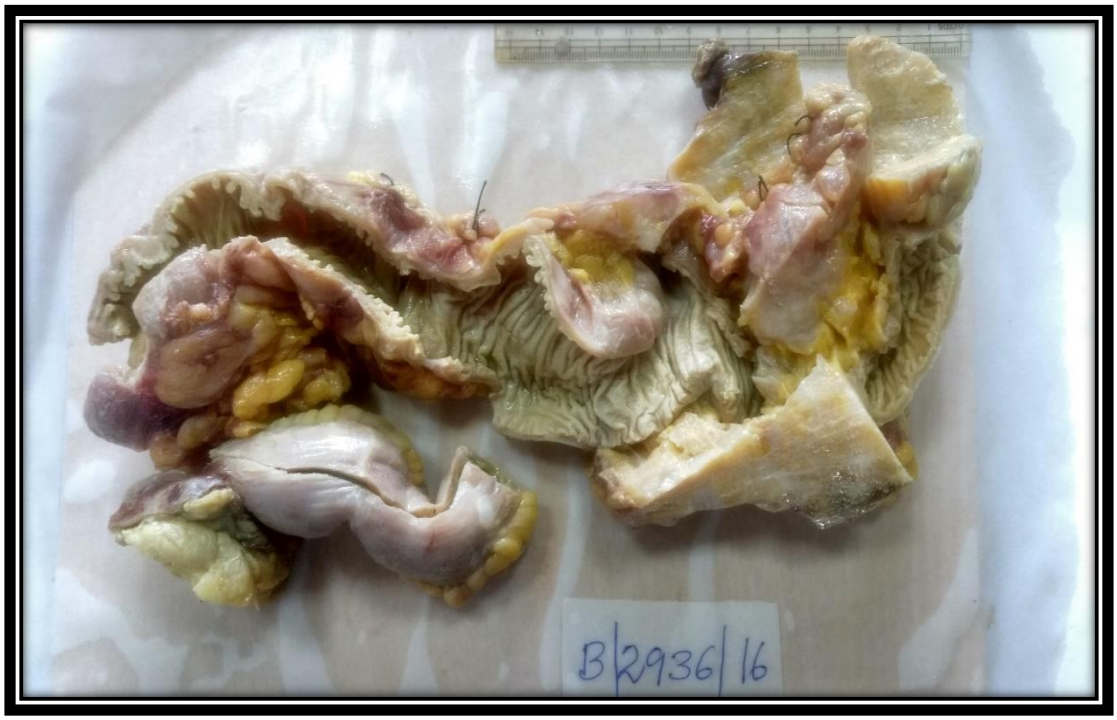


Figure 6 – Cut section showing Grey white tumor measuring 4.5x3x2cms. Serosa was involved by the tumor in this case

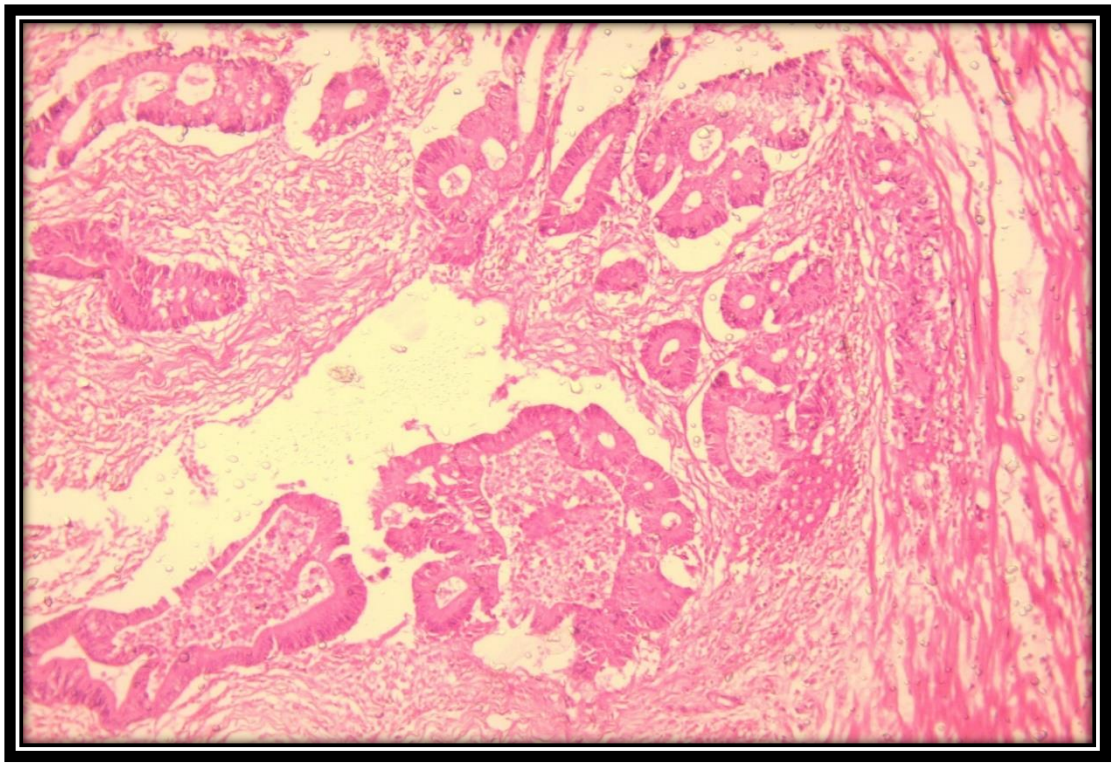


Figure 7 – H&E Sections showing Well differentiated adenocarcinoma of colon (x 10X)

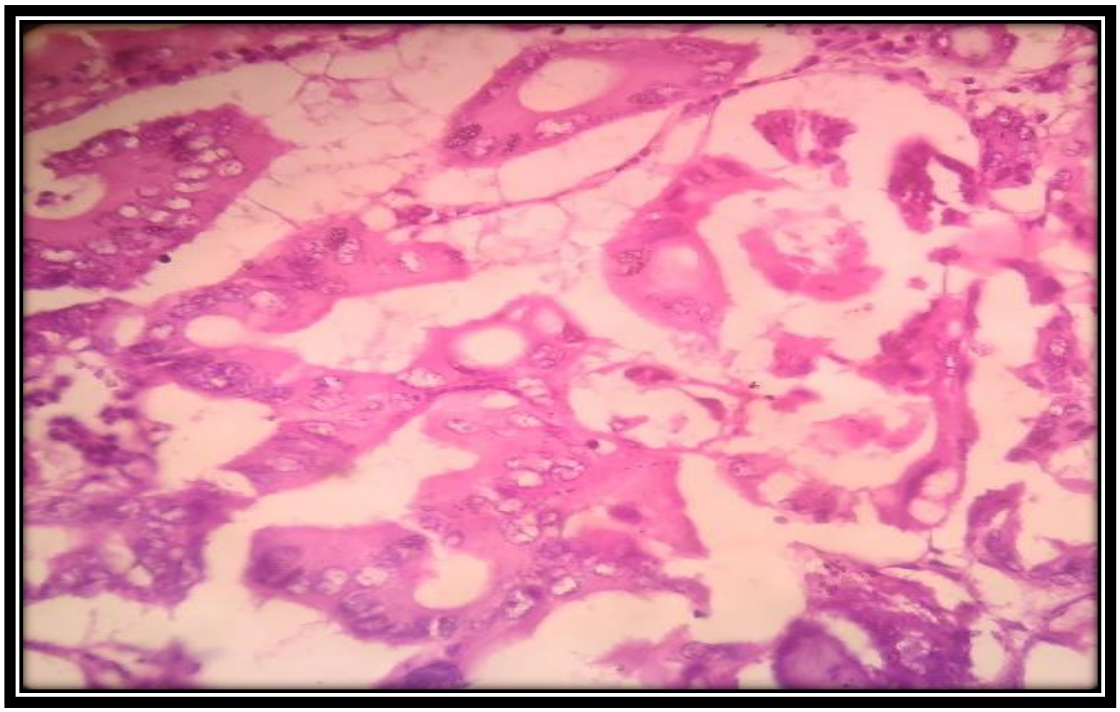


Figure 8 - H & E Sections showing Moderately differentiated adenocarcinoma of colon (x 40X)

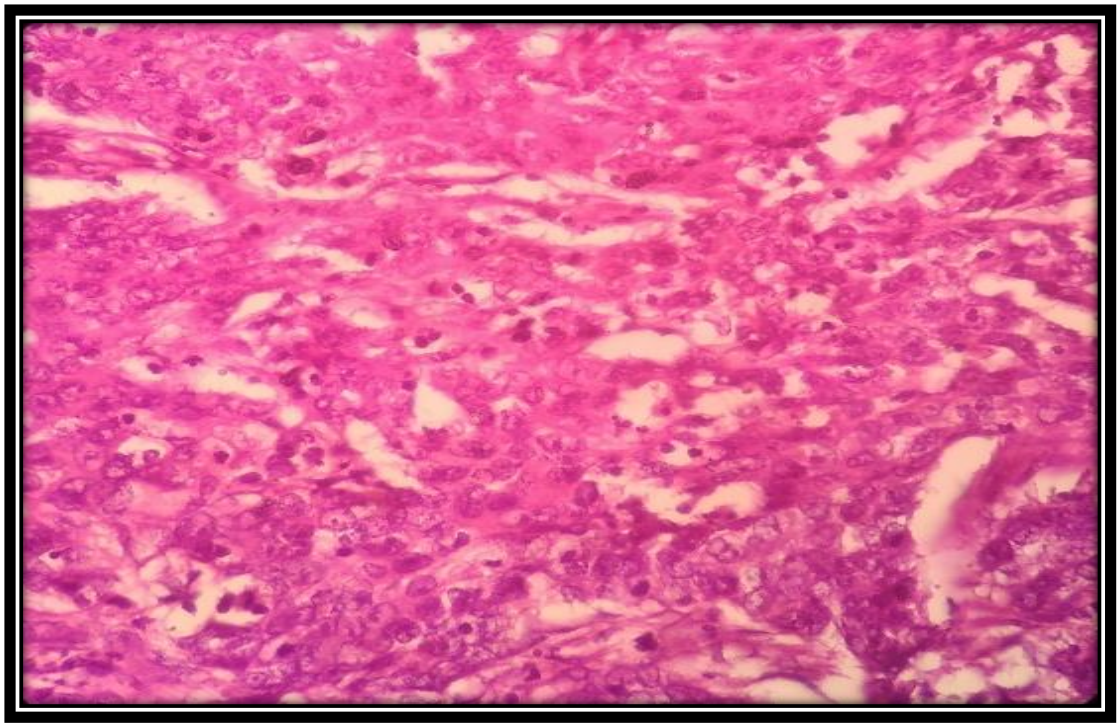


Figure 9 - H & E Section showing Poorly differentiated adenocarcinoma of Colon (x 40X)

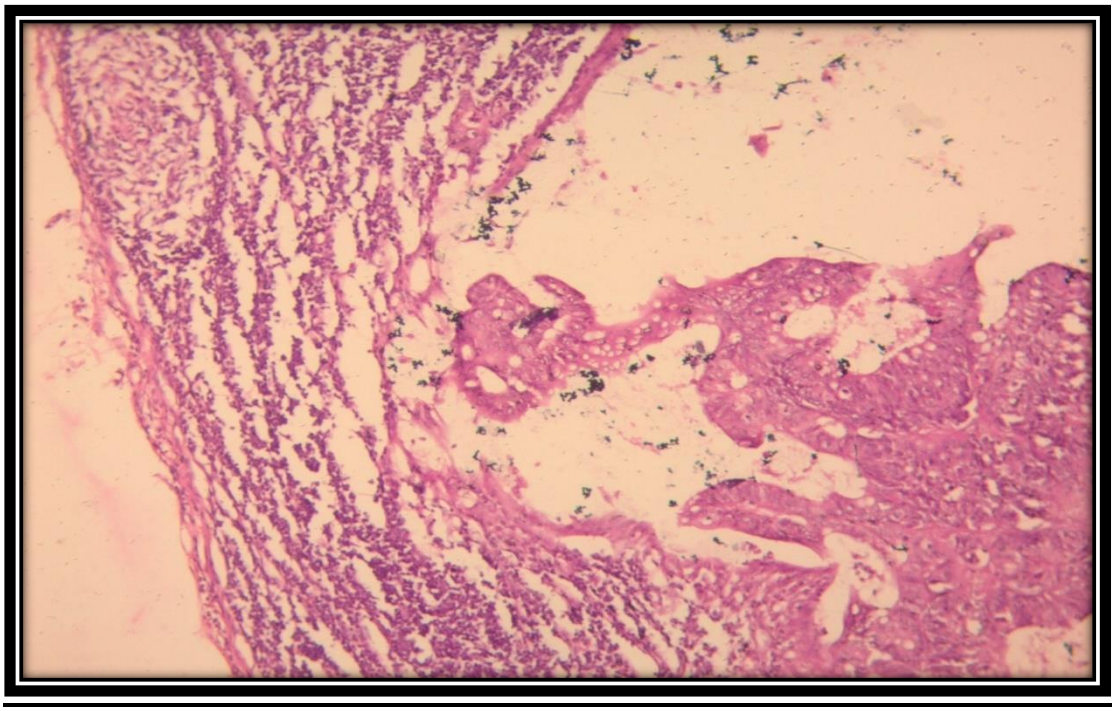


Figure 10 - H&E stained section showing adenocarcinoma metastases in Lymph node (x 40X)

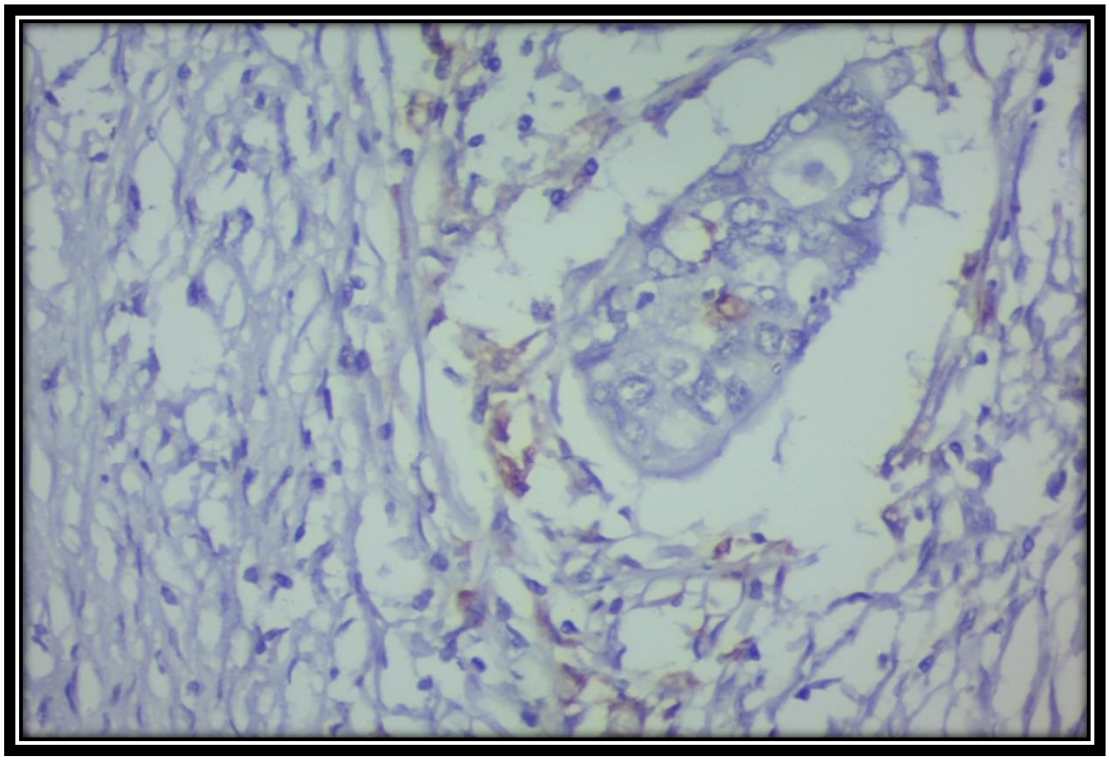


Figure 11–IHC staining with CD 68 showing Less than 10% cells positive (x 40X)

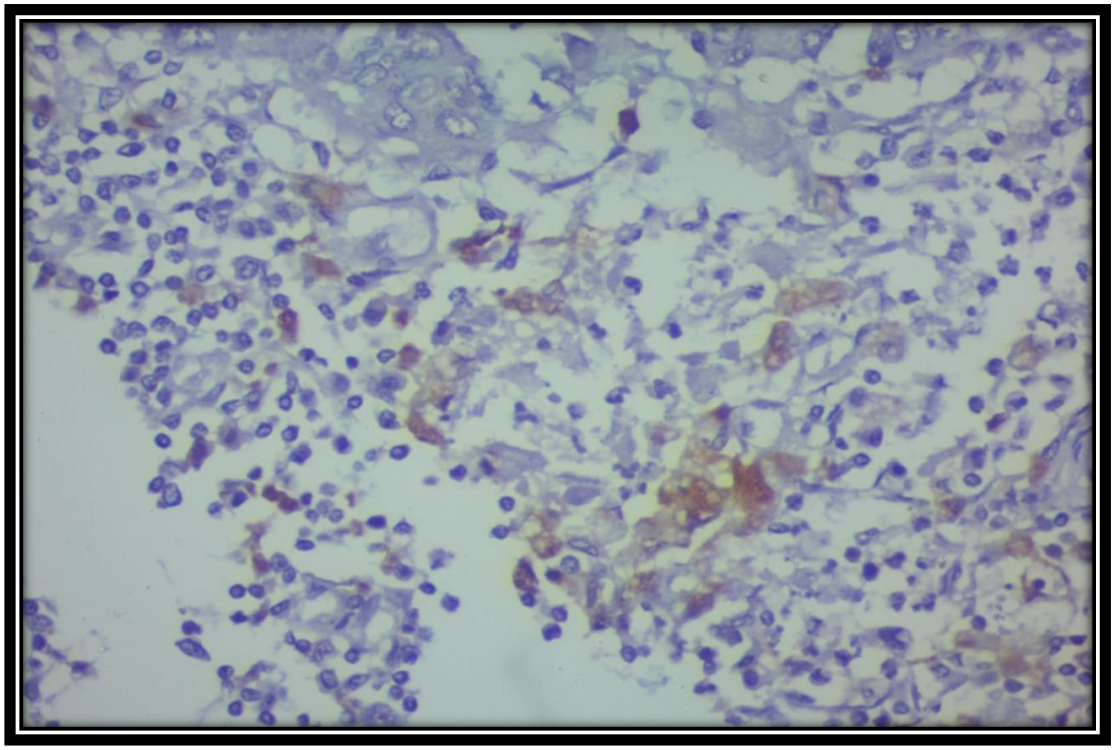


Figure 12 - IHC staining with CD 68 showing More than 10% & less than 50% cells positive (x 40X)

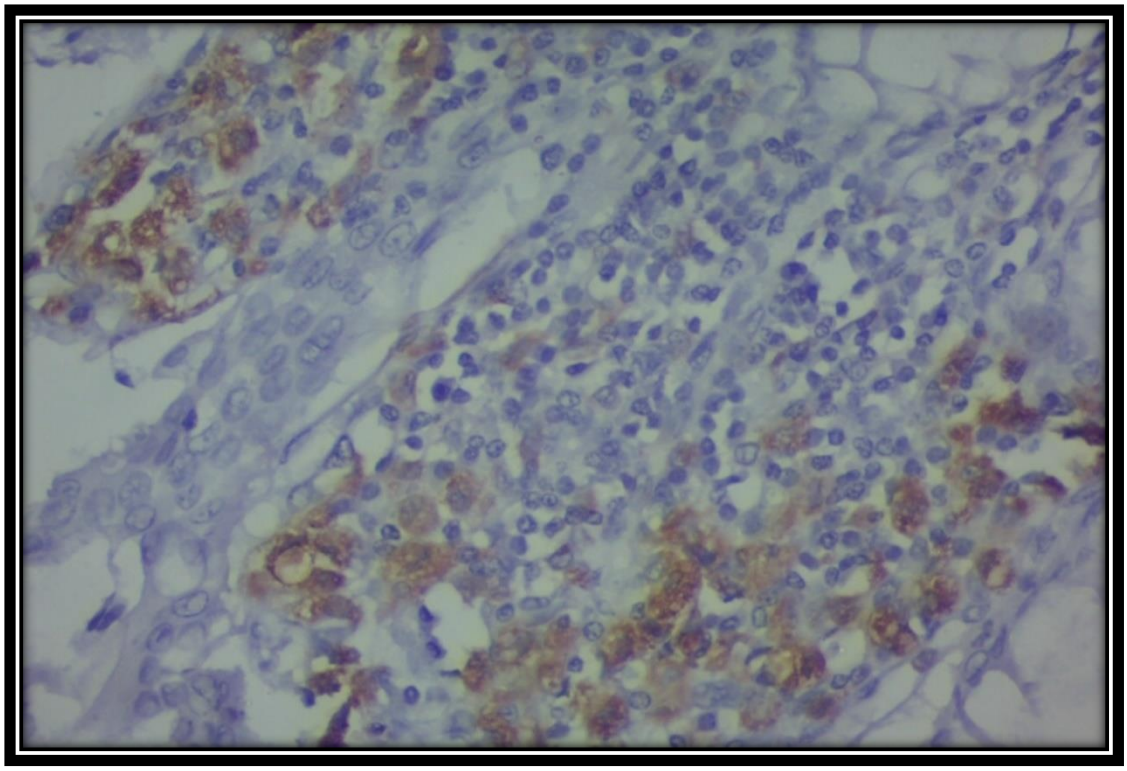


Figure 13- IHC staining with CD 68 showing More than 50% cells positive (x 40X)

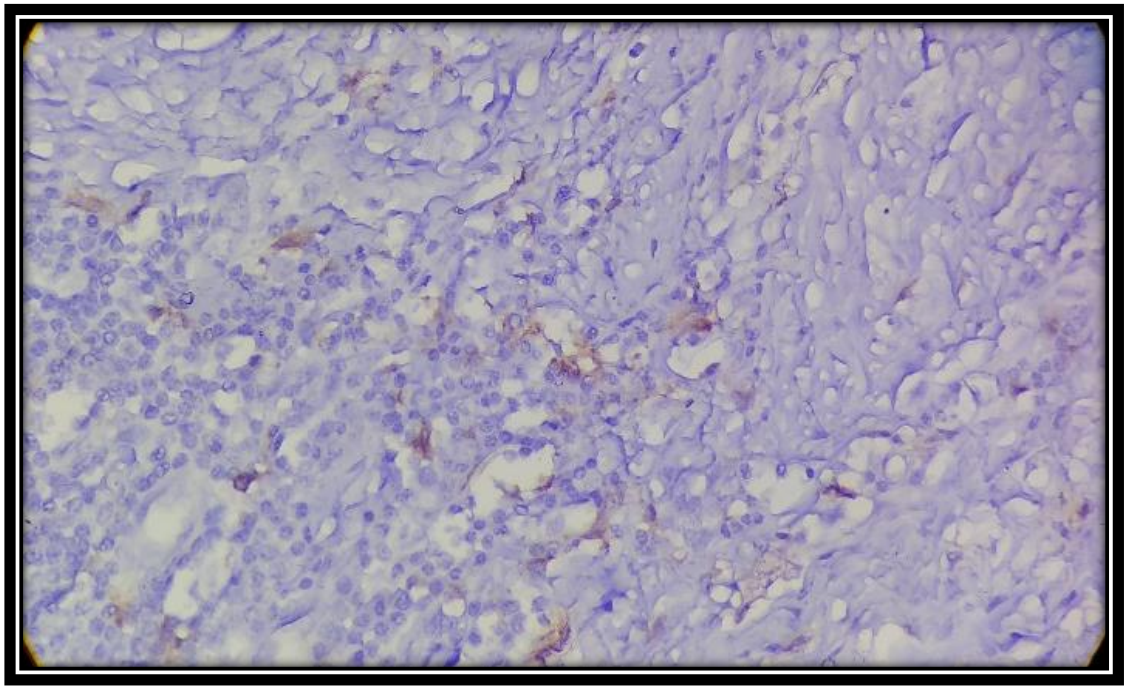


Figure 14- IHC staining with CD 163 showing Less than 10% cells positive (x 40X)

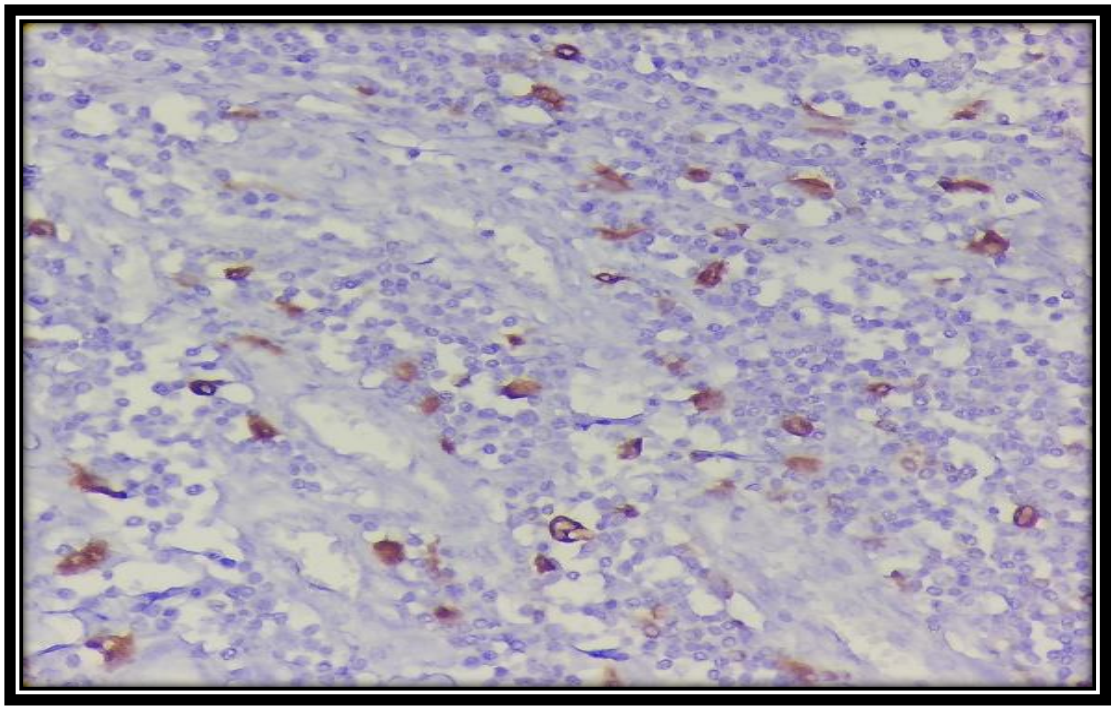


Figure 15- IHC staining with CD 163 showing More than 10% & less than 50% cells positive (x 40X)

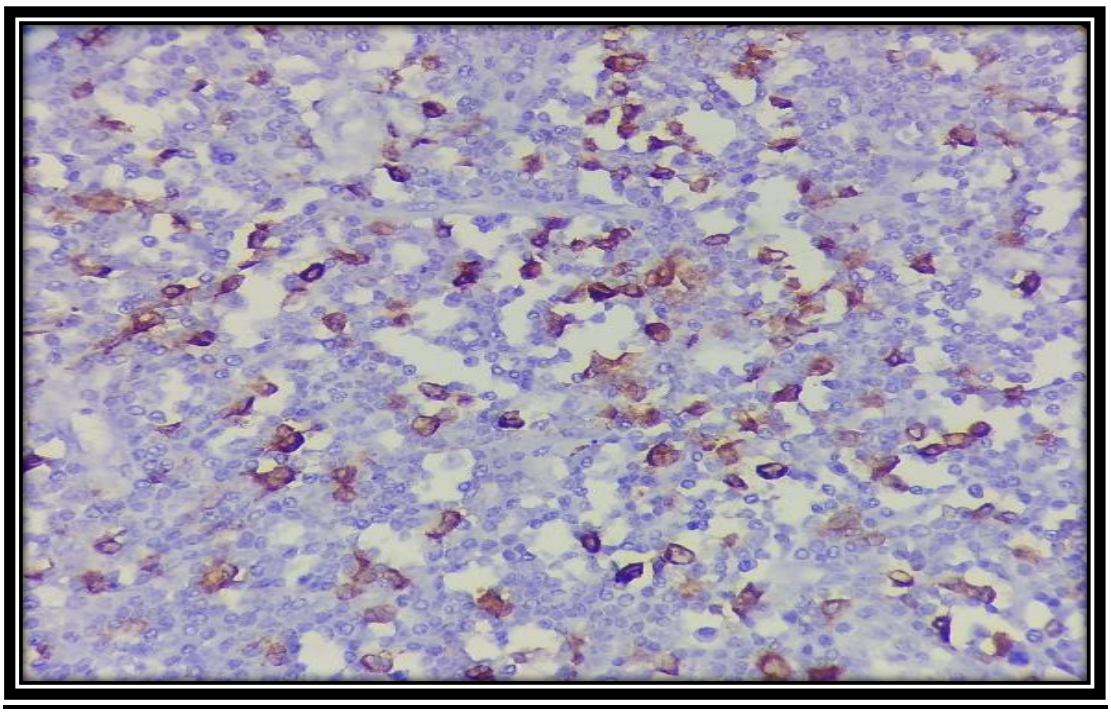


Figure 16 - IHC with CD 163 showing More than 50% cells positive(x 40X)


DISCUSSION

DISCUSSION

Table 45 - Comparison of age distribution with other studies

Age	Gulubova M et al [40] (2013)	Forssell et al [41] (2007)	Present Study (2018)
≤ 60 years	66(32.5%)	90 (19.3%)	42 (67.7%)
> 60 years	137(67.5%)	137(67.5%)	20 (32.3%)

In the present study, the majority of the cases, n=42(67.7%) were less than 60 years of age and 20 (32.3%) cases were over the age of 60 years. Other studies done by Gulubova M et al [40] and Forssell et al [41] observed that majority of the cases, I.e 137(67.5%) and 137(67.5%) were above the age of 60 years respectively.

In the study, there was no significant association between age distribution and CD 68 and CD 163 expression. I.e the expression of CD 68 and CD 163 antigens are independent of the age of the patient in colo-rectal cancers.

Table 46 - Comparison of sex distribution with other studies

	Gulubova M et al [40] (2013)	Majek O et al [42] (2013)	Present study (2018)
Males	128 (61.0%)	86704 (52.5%)	39(62.9%)
Females	82 (39.0%)	78292 (47.5%)	23(37.1%)

The present study showed that maximum number of cases of colo-rectal cancers were in male population. This was similar to the observations made by Gulubova M et al [40] and Majek O et al [42] showing that incidence of colo-rectal cancers are more common in men than in women.

Though macrophage infiltration into the tumor stroma is considered as a prognostic factor, its prognostic association with the gender of the patient is not proved. In the present study involving 36 male cases and 23 female cases, there was no statistically significant correlation between the gender of the patient and expression of CD 68 and CD 163 showing that macrophages infiltrating the tumor stroma is independent of patient's gender.

Table 47 - Comparison of distribution of site of tumor with other studies

Site	Wei Q at al [44] (2016)	Pirzada MT et al [45] (2017)	Patra T et al [46] (2017)	Present study (2018)
Right colon	25(41.0%)	46 (9.6%)	87(23.2%)	16(25.8%)
Left colon	13(21.3%)	14(30.2%)	94(25%)	19(30.6%)
Rectum	23(37.7%)	287 (60.2%)	194(51.7%)	27(43.5%)

In the study, the tumors were broadly classified into right colon that includes ileocecal junction, cecum, ascending colon, hepatic flexure of transverse colon. The left-sided colon consisted of splenic flexure, descending colon and sigmoid colon and rectum. It was further subdivided into ascending colon, transverse colon, descending colon, sigmoid colon and rectum for further statistical analysis.

The maximum number of cases were rectal carcinomas which was similar to the study done by Pirzada MT et al [45] and Patra T et al [46] whereas maximum colo-rectal tumors were seen in right colon as per the observations made by Wei Q at al [44]

There was a statistically significant correlation between the site of tumor and CD 68 expression ($p = 0.037$) but not for CD 163.

It has been an established data that left sided colon cancers are associated with better prognosis than right sided colonic cancers[71]. In the present study, maximum number of tumors were located in left colon and significant correlation between the CD 68 antigen supports the concept that infiltration by M1 macrophages is a good a good prognostic factor.

In the present study, 39 (62.9%) cases were hemicolectomy specimens, 14(22.5%) cases underwent abdominal perineal resections and 9(14.5%) cases underwent anterior resection.

In the study, 8(12.9%) cases had proliferative growth pattern on gross, ulceroproliferative in 34(54.8%) cases and ulcerative or infiltrative growth pattern in 20(32.2%) cases.

There was no statistically significant correlation between the different growth patterns of tumor with CD 68 and CD 163 expression. This could be attributed to the concept that, though infiltrative growth patterns are considered to be associated with bad prognosis due to higher T stage, the infiltration of the macrophages into the tumor stroma does not depend on the type of tumor growth.

Table 48 - Comparision of size of tumor with other studies

Tumor size	Chen HC et al [47] (2017)	Present study (2018)
≤ 5 cms	143(64.7%)	39(62.9%)
>5 cms	78(35.3%)	23(37.09%)

In the study, majority of the cases were less than 5 cms I.e 39(62.9%) which is similar to the observations made by Chen HC et al [47]

There was a statistically significant correlation between the tumor size and CD 68 expression ($p = 0.044$) but no significance was seen with expression of CD 163.

Tumor size is a well proven prognostic factor in Colo-rectal cancers as smaller tumours are associated with better prognosis. In the study, as there was a significant correlation between CD 68 and size of the tumor, it supports the hypothesis that CD 68 positive M1 macrophages in the tumor stroma are a good prognostic markers and is associated with lesser T stage and better survival.

Table 49 - Comparison of Tumor grade with other studies

Tumor grade	Telfah A et al [48] (2015)	Resch A [49] (2015)	Present study (2018)
Well differentiated	37(7.4%)	27(18.6%)	20(32.2%)
Moderately differentiated	280(56%)	72(49.7%)	30(48.4%)
Poorly differentiated	138(36.6%)	46(31.7%)	12(19.3%)

Telfah A et al [48] and Resch A [49] studied the grading of colo-rectal carcinomas and noted that moderately differentiated adenocarcinomas were the most common grade followed by poorly differentiated type. Similar observations were made in the present study that moderately differentiated carcinomas were the most common grade encountered and accounted to 48.4% of cases.

There was a statistically significant association between Grade of the tumor and CD 68 expression but not with CD163.

The moderately differentiated adenocarcinomas and well differentiated adenocarcinomas which are the majority in the present study group are associated with better prognosis compared to poorly differentiated adenocarcinomas and M1 macrophages posses antigen presenting molecules, which is co-stimulatory receptor for lymphocytes and many pro inflammatory cytokines on their surface. This immune mechanism is considered as body's response against the tumor cells and helps in tumor differentiation and further supports the hypothesis that CD 68 expression is a good prognostic marker in colo-rectal cancers.

Table 50 - Comparision of TNM staging with other studies

TNM staging	Vlad C et al [50] (2015)	Zhao M et al [51] (2017)	Present study (2018)
I	56(17.6%)	78,070(26.5%)	4(6.4%)
II	75(23.6%)	79,906(27.2%)	16(25.8%)
III	178(56.3%)	80,865(27.5%)	32(51.6%)
IV	8(2.5%)	54,775(18.6%)	10(16.1%)

In the present study, maximum number of cases were in stage III category (51.6%) which is similar to the observations done by Vlad C et al [50] and Zhao M et al [51] in their studies. Stage III was followed by stage II(25.8%), Stage IV(16.1%) and stage I(6.4%).

The stage of the tumor was statistically significant only with CD 163 expression and not with CD 68.

In the present study, stage III and stage IV tumors constituted the majority and are designated as advance disease which is associated with poorer outcomes. The significant association of CD 163 with the stage in the study adds on to the theory put forth by Eden S et al[13,14] that CD 163 which is a marker for M2 macrophages is pro-tumorogenic and associated with bad prognosis.

Table 51 - Comparison of T staging with other studies

T stage	Kim SM et al [52] (2017)	Ladeira KM et al [53] (2016)	Present study (2018)
T1	1(0.97%)	63 (5.9%)	4(6.4%)
T2	3(2.91%)	148 (13.9%)	16(25.8%)
T3	50(48.5%)	758 (71.2%)	32(51.6%)
T4	49(47.5%)	63 (5.9%)	10(16.1%)

In concordance with the observations made in the studies conducted by Kim SM et al [52] and Ladeira KM [53],

The maximum number of cases were in the T3 stage which means the maximum number of cases invaded through muscularis propria into subserosa or into non-peritonealized pericolic or perirectal tissues which is associated with higher stage. It is similar to the observations made in the present study with 51.6% (n=32) of cases in T3 stage.

There was a statistically significant association between expression of CD 163 with the T stage whereas CD 68 was not showing any significant correlation.

The expression of CD 163 by M2 macrophages is considered to be a bad prognostic factor as they are hypothesized to promote tumor growth by releasing growth factors and promoting angiogenesis [12]. The significant association between Cd 163 expression and higher T stage supports this hypothesis.

Table 52 - Comparison of N staging with other studies

N stage	Liu Q et al [54] (2018)	Soylu L et al [55] (2017)	Moug SJ et al [56] (2009)	Present study (2018)
N0	28,935(66.98%)	112(58.9%)	120(61.5%)	39(62.9%)
N1	9936(23. %)	45(23.7%)	49(25.1%)	21(33.8%)
N2	4325(10.01%)	33(17.4%)	26(13.3%)	2(3.22%)

According to the observations made by Liu Q et al [54] , Soylu L et al [55] and Moug SJ et al [56], maximum number of cases were in N0 category with 66.98% , 58.9% and 61.5% respectively and the least number of cases were seen on N2 category with 10.01%, 17.4% and 13.3% respectively. Similar trend was seen in the present study with maximum number of cases in N0 category (62.9%) and least number of cases in N2 category (3.22%).

There was a statistically significant correlation between N stage and CD 163 expression whereas CD 68 did not show any correlation.

Shabo I et al [12] studied the expression of CD 163 in colo-rectal cancers and concluded that its expression is a bad prognostic factor, but the N stage was not included in his study. In the present study, majority of cases were in N0 stage and the observation in the N stage showed that CD 163 is a good prognostic factor which is in contrast to the study done by Shabo I et al [12]. This could be due to the more number of cases in N0 category and a larger sample size could provide a better understanding in this regard.

Table 53 - Comparison of Perineural invasion with other studies

Perineural invasion	Huh JW et al [57] (2010)	Liebig C et al [58] (2009)	Sukhni ES et al [59] (2017)	Present study (2018)
Present	57 (16.7%)	55 (22.1%)	15,734 (11.1%)	2 (3.33%)
Absent	284 (83.28%)	194 (77.9%)	1,26,300(88.9%)	60 (96.7%)

Perineural invasion is one of the important prognostic factor in colo-rectal cancers. In the present study, only two cases had perineural invasion accounting to 3.33%. Higher number of cases with perineural invasion was seen in study by Liebig C et al [58] with 22.1% followed by Huh JW et al [57] and Sukhni ES et al [59] with 16.7% and 11.1% respectively.

Perineural invasion is one of the proved bad prognostic factor in Colo-rectal cancer. However, in the present study, there was no statistically significant association between CD 68 and CD 163 expression with perineural invasion. This suggests that perineural invasion is an independent prognostic variable irrespective of CD 68 and CD 163 expression.

Table 54 - Comparision of Lymphovascular invasion with other studies

Lympho vascular invasion	Lim SB et al [60] (2010)	Chang CS et al [61] (2012)	Lopes CR et al [62] (2012)	Present study (2018)
Present	610 (25.2%)	16 (5.5%)	112(44.6%)	3(4.8%)
Absent	1807(74.76%)	276 (90.9%)	139(55.37%)	59(95.2%)

Among the observations made in different studies, a study by Lopes CR et al [62] showed higher number of cases (44.6%) showing lymphovascular invasion followed by 25.2% of cases as observed by Lim SB et al [60].

The present study had only 4.8% of cases with lymphovascular invasion which is similar to the observations made by Chang CS et al [61](5.5%).

There was no statistically significant correlation between expression of CD 68 and CD 163 with lymphovascular invasion.

The loss of significance of CD 68 and CD 163 with lymphovascular invasion may be due to less number of cases with lymphovascular invasion cases(4.8%) encountered in the study. Further studies with more number of lymphovascular invasion positive cases can provide better reliable data in this regard.

Table 55 - Comparision of Lymph node ratio (LNR) with other studies

	LNR	Ren QJ et al [70] (2012)	Present study (2018)
LNR 1	<0.111	36 (24.8%)	40 (64.5%)
LNR 2	0.111 to 0.200	37 (25.5%)	3 (4.8%)
LNR 3	0.200 to 0.429	37 (25.5%)	5 (8%)
LNR 4	>0.429	35 (24.1%)	14(22.5%)

Lymph node ratio is one of the important, newer factors in determining the prognosis of Colo rectal cancers. It has also been studied in tumors of stomach, pancreas, bladder and breast [65]. Lymph node ratio is the proportion of the number of Lymph node with tumor deposits to the number of Lymph nodes examined[63]. It has also been studied that increased harvesting of Lymph nodes during surgery in colorectal cancers is associated with better outcomes [64]. Different cut-off values have been studied by various authors for determining Lymph node ratio. In general, higher Lymph node ratio is associated with poor 3 year relapse free survival, higher tumor stage, perineural invasion and overall survival [66,67].

Ren QJ et al [70] studies LNR in colo rectal cancer patients with Stage III disease and observed that maximum number cases were in LNR 2 and LNR 3 which is associated with poor prognosis. In the present study, maximum number of cases were in LNR1 category. This may be attributed to the concept that Ren QJ et al [70] used only stage III tumors that had higher chance of Lymph node metatstasis.

There was a statistically significant relationship between Lymph node ratio and CD 163 expression but not with expression of CD 68.

LNR has been proved as a bad prognostic marker in breast cancer. Numerous studies are being done in colo-rectal cancers to assess its prognostic significance. M2 macrophages are hypothesized to be tumor promotive and are associated with bad prognosis. The significant correlation in the present study adds on to CD163 as a bad prognostic marker.

Table 56 - Correlation of Expression of CD 68 and CD 163

			CD 68			Total
			<10% of Cells	>10% to <50% of Cells Positive	>50% of Cells Positive	
CD 163	<10% of Cells	Count	1	3	2	6
	>10% to <50% of Cells Positive	Count	16	10	10	36
	>50% of Cells Positive	Count	7	11	2	20
Total		Count	24	24	14	62

There was no statistically significant correlation between expression of CD 163 with CD 68 expression.

CD 68 stains M1 macrophages that are considered to be anti-tumorigenic whereas CD 163 stains M2 macrophages that are pro-tumorigenic and both these macrophages have contrary effects on tumor progression. This is supported by no statistical significance between these two entities in the present study.


Conclusion

CONCLUSION

Majority of the patients were in the golden age group of less than 60 years.

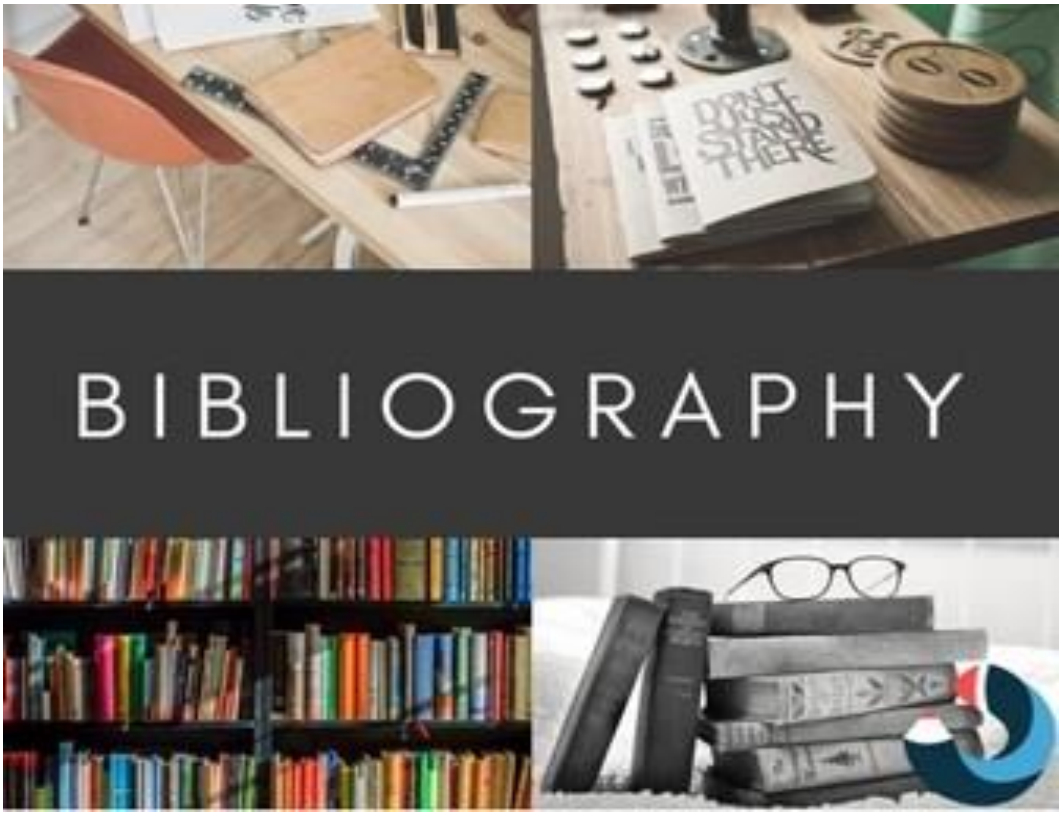
CD 68 expression was associated with better prognostic factors such as smaller size of tumor, lesser grade and lesser Lymph node ratio(LNR).

CD 163 expression was associated with poorer prognostic factors such as higher T stage, Higher N stage, and higher values of Lymph node ratio(LNR).

Hence, CD 68 and CD 163 can be used as novel biomarkers in assessing the prognosis in Colo-rectal cancer patients. Further studies may help in improving the therapeutic modalities by targeted therapies.

SUMMARY

1. The present study was conducted in the Department of Pathology , Sri Devaraj Urs Medical College, Tamaka, Kolar from December 2016 to September 2018. Also,Retrospective cases from January 2008 to November 2016 were included in the study.
2. A total of 62 cases were studied of which 39 were males and 23 were females
3. Majority of the subjects were less than 60 years of age 42 cases(67.7%)
4. The most common site of tumor was Rectum 27 cases,(43.5%) followed by ascending colon 13 cases (21%).
5. The most common growth was Ulceroproliferative type, 34 cases(54.8%)
6. Majority of the tumors were less than 5 cms, 39 cases(62.9%)
7. The most common grade was moderately differentiated adenocarcinoma, 30 cases (48.4%)
8. The most common T stage was T3, 32 cases(51.6%) and most common N stage was N0, 39 cases, (62.9%)
9. Maximum number of cases were in Stage III, 23 cases(37.1%)
10. Perineural invasion was seen in only 2 cases (3.2%) and lymphovascular invasion was seen in 3 cases(4.8%)
11. Maximum number of cases (64.5%) were in Lymph node ratio less than <0.111.
12. CD 68 expression was associated with smaller size of tumor, lesser grade and lesser Lymph node ratio(LNR).
13. CD 163 expression was associated with higher T stage, Higher N stage, and higher values of Lymph node ratio(LNR).


BIBLIOGRAPHY

BIBILIOGRAPHY

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCON estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424.

2. Rosai J, Ackerman's. Gastrointestinal tract. In: Rosai, Ackerman, editors. Surgical Pathology, Vol 2 (10^{th} edition). New Delhi: Mosby;2011.p.585-855.

3. 3.Hamilton SR, Aaltonen LA. (Eds):World Health Organisation Classification of Tumours. Pathology and Genetics of Tumors of the Digestive System. IARC Press:Lyon 2000.

4. Yang L, Zhang Y. Tumor-associated macrophages: from basic research to clinical application. J Hematol Oncol 2017; 10: 1-12.

5. Noy R, Pollard JW. Tumor-associated macrophages: from mechanisms to therapy. J Immunity 2014; 41: 49-61.

6. Zhong X, Chen B, Yang Z. The Role of Tumor-Associated Macrophages in Colorectal Carcinoma Progression. Cell Physiol Biochem 2018; 45: 356-65.

7. Edin S, Wikberg M L, Dahlin AM, Rutegard J, Oberg K , Oldenborg PA et al. The Distribution of Macrophages with a M1 or M2 Phenotype in Relation to Prognosis and the Molecular Characteristics of Colorectal Cancer. Plos One 2012; 7: 1-12.

8. Boyle P, Langman JS. ABC of colorectal cancer: Epidemiology. BMJ 2000; 321: 805-8.

9. Haggar FA, Boushey RP. Colorectal Cancer Epidemiology: Incidence, Mortality, Survival, and Risk Factors. Clin Colon Rectal Surg 2009; 22: 191-7.

10. Janaout V, Kollaraova H. Epidemiology of colorectal cancer. CECOG 2000; 4: 1-10.

11. Jeter JM, Kohlmann W, Gruber SB. Genetics of colorectal cancer. Oncology 2006; 20: 269–76.

12. Shabo I, Olsson H, Elkarim R, Sun x, Svanvik J. Macrophage infiltration in tumor stroma is related to tumor cell expression of CD163 in colorectal cancer. Cancer microenviron 2014; 7: 61-9.

13. Edin S, Wikberg ML, Rutegard J, Oldenborg PA, Palmqvist R. Phenotyping Skewing of Macrophages in vitro by secreted factors from colorectal cancer cells. PLoS One 2013; 8: 1-10.

14. Edin S, Wikberg ML, Dahlin AM, Rutegård J, Öberg A, Oldenborg PA et al. The Distribution of macrophages with a M1 or M2 phenotype in relation to prognosis and the molecular characteristics of colorectal cancer. PLoS One 2012; 7: 1-12.

15. Zhang W, Chen L, Ma Kai, Zhao Y, Liu X, Wang Y et al. Polarisation of macrophages in the tumor microenvironment is influenced EGFR signalling within colon cancer cells. Oncotarget 2016; 7: 75366-76.

16. Marech I, Ammendola M, Sacco R, Samarco G, Zuccala V, Zizzo N. Tumor associated macrophages correlate with microvascular bed extension in colorectal cancer patients. J Cell Mol Med 2016; 20: 1373-60.

17. Rosai J, Ackerman's. Gastrointestinal tract. In: Rosai, Ackerman, editors. Surgical Pathology, Vol 2 (10th edition). New Delhi: Mosby;2011.p.585-855.

18. Alpha F, Bazan NG, Belayev L, Eighth N. Microglia are the primary innate immune effector cells of the CNS and they represent a unique myeloid: Handbook of Clinical Neurology,2016 Related terms: Neuroinflammation Apoptosis in Nervous System Injury Role of Microglia in Neuronal and Oligodendrocyte. 2018;1-5.

19. Leek RD, Lewis CE, Whitehouse R, Greenal M, Clarke J, Harris L A. Association of Macrophage Infiltration with Angiogenesis and Prognosis in Invasive Breast Carcinoma. Cancer Res 1996; 56: 4625-9.

20. Fabriek BO, Bruggen RV, Deng DM, Ligtenberg AJM, Nazmi K, Schornagel K et. al. The macrophage scavenger receptor CD163 functions as an innate immune sensor for bacteria. Blood 2009; 113: 887-92.

21. Schaer DJ, Schaer CA, Buehler PW, Boykins RA, Schoedon, G, Alayash AI et al. CD163 is the macrophage scavenger receptor for native and chemically modified hemoglobins in the absence of haptoglobin. Blood 2006; 107: 373-80.

22. Krishna G. BD Chaurasia's Human anatomy Regional and applied Dissection and Clinical. 5th ed. India: CBC publishers and distributors;2015.

23. In L, way AS. Learn In A Simple Way Manage Your Time 1996 Histology of Large Intestine (COLON).2018;1-5.

24. Shabo I, Olsson H, Sun XF, Svanvik J. Expression of the macrophage antigen CD163 in rectal cancer cells is associated with early local recurrence and reduced survival time. Int J Cancer 2009; 125: 1826-31.

25. Marley AR, Nan H. Epidemiology of colorectal cancer. Int J Mol Epidemiol Genet 2016;7:105-114.

26. Caygill CP, Hill MJ. Fish, n-3 fatty acids and human colorectal and breast cancer mortality. Eur J Cancer Prev 1995; 4: 329-32.

27. Burkitt DP. Epidemiology of cancer of the colon and rectum. Cancer 1971; 28: 3-13.

28. Gorham ED, Garland CF, Garland FC, Grant WB, Mohr SB, Lipkin M et. al. Vitamin D and prevention of colorectal cancer. J Steroid Biochem Mol Biol 2005; 97: 179-94.

29. Baena R, Salinas P. Diet and colorectal cancer. Maturitas 2015; 80: 258-64.

30. Chan AT, Ogino S, Fuchs CS. Aspirin and the risk of colorectal cancer in relation to the expression of COX-2. N Engl J Med 2007; 356: 2131-42.

31. CDC - Colorectal Cancer Screening Tests [Internet]. Cdc.gov; 2014 [21 November 2018]. Available from: http://www.cdc.gov/cancer/colorectal/basic_info/screening/tests.htm.

32. Young B, Woodfard P, O'Dowd G. Gastrointestinal tract. Wheater's functional Histology. A text and colour atlas. 6th ed. USA: Churchill Livingston Elservier; 2014.p.251-275.

33. Eroschenko VP. Digestive system:Small and Large Intestines. diFiore's Atlas of Histology with Functional Correlations. 11th ed. Philadelphia: Wolters Kluwer;2008.p.291-312.

34. Moore KL, Persaud TVN, Torchia MG. The developing human:clinically oriented embryology. 9th ed. Philadelphia:Elsevier;2013.

35. Bagul N, Roy S, Ganjre A, Kathariya R, Meher A, Singh P. Quantitative assessment of tumor associated macrophages in head and neck squamous cell carcinoma using CD68 marker: An Immunohistochemical study. J Clin Diagn Res 2016; 10: 81-4.

36. 36.Gaddis GM, Gaddis ML. Introduction to biostatistics: Part 4, Statistical inference techniques in hypothesis testing. *Ann Emerg Med*. 1990; 19: 820-5.

37. Pratap P. Sample size in clinical research, the number we need. Int J Med Sci Public Health. 2012; 1: 5–9.

38. Sundar Rao PSS, Richard J. Introduction to Biostatistics and research methods. 5th ed. New Delhi: Prentice hall of India Private Limited; 2012. 86-160.

39. 39.Elenbaas, RM, Elenbaas, JK, Cuddy PG. Evaluating the medical literature, part II: Statistical analysis. Ann Emerg Med 1983; 12: 610-20.

40. Guluboav M, Ananiev J, Yovchev Y, Julianov A, Karashmalakov A, Vlaykova T. The density of macrophages in colorectal cancer is inversely correlated to TGF-β1 expression and patients' survival. J Mol Hist 2013; 44: 679–92.

41. Forssell J, Oberg A, Henriksson ML, Stenling R, Jung R, Palmqvist R. High macrophage infiltration along the tumor front correlates with improved survival in colon cancer. Clin Cancer Res 2007; 13: 1472-9.

42. Majek O. Gondos A, Jansen L, Emrich K, Holleczek B, Katalinic A et. al. Sex differences in colorectal cancer survival: Population based analysis of 164,996 colorectal cancer patients in Germany. PLoS One 2013; 8: 1-7.

43. Kim SE, Paik HY, Yoon H, Lee JE, Kim N, Sung MK. Sex and gender specific disparities in colorectal cancer risk. World J Gastroenterol 2015; 21: 5167-75.

44. Wei Q, Wang X, Gao J, Li J, Li J, QI c et. al. Clinicopathologic and molecular features of colorectal adenocarcinoma with signet ring cell component. PLos One 2016; 11: 1-12.

45. Pirzada MT, Ahmed MJ, Muzzafar A, Nasir IUI, Shah MF, Khattak S, Syed AA. Rectal carcinoma: demographics and clinicopathological features from Pakistani population perspective. Cureus 2017; 9:e1375. doi: 10.7759/cureus.1375.

46. Patra T, Mandal S, Alam N, Murmu N. Clinicopathological trends of colorectal carcinoma patients in a tertiary cancer centre in Eastern India. Clin Epidemiol Glob Health 2018; 6: 39-43.

47. Chen CH, Hsieh MC, Hsiao PK, Lin EK, Lu YJ, Wu SY. A critical reappraisal for the value of tumor size as a prognostic variable in rectal adenocarcinoma. J Cancer 2017; 8: 1927-34.

48. Telfah A, Obeidat M, Kamar AA, Bawa neh A, Arabeiat A, Al-Kafaween H Fayyad L. Clinicopathological characteristics of colorectal cancers at KHMC. Appl Med Res 2015; 1: 22-5.

49. Resch A, Langer C. Prognostic value of tumor grading in colorectal cancer: Systematic analysis of primary and metastatic tumor tissue. Graz;2015

50. Vlad C, Kunelac P, Vlad D, Irimie A, Cadariu PA. Evaluation of clinical, morphopathological and therapeutic prognostic factors in rectal cancer. Experience of a tertiary oncology center. J BUON 2015; 20: 92-9.

51. Zhao M, Liu H, Tang Y, Meng X, Yu J, Wang Q et. al. Clinicopathological features and prognostic factors for patients with colorectal cancer who are 75 years and older. Oncotarget 2017; 8: 80002-11.

52. Kim MS, Park EJ, Kang J, Min BS, Lee KY, Kim NK, Baik SH. Prognostic factors predicting survival in incurable stage IV colorectal cancer patients who inderwent palliative primary tumor resection. Retrospective cohort study. Int J Surg 2018; 49: 10-5.

53. Ladeira KM, Martins SFF. Prognostic impact of the number of resected Lymph node on survival in colorectal cancer. J Coloproctol 2016; 36: 130-8.

54. Liu Q, Luo D, Cai S, li Q, Li X. P-TNM staging sytem for colon cancer: combination of P-stage and AJCC TNM staging system for improving prognostic prediction and clinical management. Cancer Manag Res 2018; 10: 2303-14.

55. Soylu L, Aydin OU, Cekmen N, Atalay F. Lymph node evaluation and survival after resection of colorectal cancer. Med Science 2017; 6: 182-8.

56. Moug SJ, Saldanha JD, McGregor JR, Balsitis M, Diament RH. Positive Lymph node retrieval ratio optimises patient staging in colorectal cancer. Br J Cancer 2009; 100: 1530-3.

57. Huh JW, Kim HR, Kim YJ. Prognostic value of perineural invasion in patients with stage II colorectal cancer. Ann Surg Oncol 2010; 17: 2066-72.

58. Liebig C, Ayala G, Wilks J, Verstovsek G, Liu H, Agarwal N et. al. Perineural invasion is an independent predictor of outcome in colorectal cancer. J Clin Oncol 2009; 27: 5131-7.

59. AL-Sukhni E, Attwood K, Gabriel EM, LeVea CM, Kanehira K, Nurkin SJ. Lymphovascular and perineural invasion are associated with poor prognostic features and outcomes in colorectal cancer: A retrospective cohort study. Int J Surg 2017: 37: 42-9.

60. Lim SB, Yu CS, Jang SJ, Kim TW, Kim JH, Kim JC. Prognostic significance of lymphovascular invasion in sporadic colorectal cancer. Dis Colon Rectum 2010; 53: 377-84.

61. Chang SC, Lin CC, Wang HS, Yang SH, Jiang JK, Lan YT et. al. Lymphovascular invasion determines the outcome of stage I colorectal cancer patients. Formosan J Surg 2012; 45: 141-5.

62. Lopes RC, Silveira S Jr, Koch KS. Incidence of angiolymphatic invasion in colorectal cancer. J Coloproctol 2012; 32: 240-5.

63. Kobayashi H, Enomoto M, Higuchi T, Uetake H, Iida S, Ishikawa T et.al. Clinical significance of Lymph node ratio and location of nodal involvement in patients with right colon cancer. Dig Surg 2011; 28: 190-7.

64. Amri R, Klos CL, Bordeianou L, Berger DL. The prognostic value of Lymph node ratio in colon cancer is independent of resection length. Am J Surg 2016; 212: 251-7.

65. Mirzaei AZ, Abdorrazaghi F, Lotfi M, Nejad BK, Shayanfar N. prognostic value of Lymph node ratio in comparison to Lymph node metastases in stage III colon cancer. Iranian J Pathol 2015; 10: 127-35.

66. Deng Y, Peng J, Zhao Y, Sui Q, Zhao R, Lu Z et. al. Lymph node ratio as a valuable prognostic factor for patients with colorectal liver-only metastasis undergoing curative resection. Cancer Manag Res 2018; 10: 2083-94.

67. Ghahramani L, Pourahmad S, Mohammadianpanah M. P0018 prognostic value of total number of lymph nodes identified and ratio of lymph nodes in resected colorectal cancer. Eur J Cancer 2014; 50: e14. https://doi.org/10.1016/j.ejca.2014.03.062.

68. Ohnishi K, Komohara Y, Saito Y, Miyamoto Y, Watanabe M, Baba H, Takeya M. CD 169-positive macrophages in regional Lymph nodes are associated with a favourable prognosis in patients with colorectal carcinoma. Cancer Sci 2013; 104: 1237-44.

69. Harris JA, Jain S, Ren Q, Zarineh A, Liu C, Ibrahim S. CD163 and CD 68 in tumor associated macrophages of classical Hodgkin lymphoma. Diag Pathol 2012; 7: 1-6.

70. Ren JQ, Liu JW, Chen ZT, Liu Sj, Huang SJ, Huang Y, Hong JS. Prognostic value of the Lymph node ratio in stage III colorectal cancer. Chin J Cancer 2012; 31: 241-7.

71. Colorectal cancer survival linked to primary tumor location-National Cancer Institute. 2016 May 27.[Accessed on 22 November 2018] Available from: https://www.cancer.gov/news-events/cancer-currents-blog/2016/colorectal-survival-location.

72. Faiz O, Blackburn S, Moffat D. Anatomy at a Glance.3rd ed. Delhi:Wiley;2011.p.1-176.

73. Patten D.K, Layfield D, Arya S, Leff DR, Paraskeva PA. Single Best Answers in Surgery.Boca Raton:CRC Press;2015.p.107.

74. Astler VB, Coller FA: The prognostic significance of direct extension of carcinoma of the colon and rectum. Ann Surg1954;139:846-59.

ANNEXURES

PROFORMA

Name:

Case no:

Age/sex:

Hospital no:

Biopsy no:

Clinical history:

Pain abdomen ☐

Altered bowel habits ☐

Mass per abdomen ☐

Bleed per rectum ☐

Histopathological diagnosis:

GROSS:

Specimen size

Site- Specimen type-

Growth- Tumor size-

MICROSCOPY:

Grade-

Pathological T- N- M-

Stage-

No. of Lymph nodes retrieved-

No. of Positive Lymph nodes-

Extracapsular Extension- Yes / No

Lymph node ratio –

Perineural Invasion- Yes / No

Lymphovascular invasion- Yes / No

IMMUNOHISTOCHEMICAL FINDINGS:

CD68:

CD163:

FINAL IMPRESSION:

SIGNATURE

KEYS TO MASTER CHART

SEX	1- MALE 2- FEMALE
AGE	1- ≤60 YEARS 2- > 60 YEARS
SPECIMEN TYPE	1- HEMICOLECTOMY 2- APR 3- ANTERIOR RESECTION
SITE	1- ASCENDING COLON 2- TRANSVERSE COLON 3- DECENDING COLON 4- SIGMOID COLON 5- RECTUM
HISTOPATHOLOGY DIAGNOSIS	1- ADENOCARCINOMA
MALIGNANCY GRADING	1- WELL DIFFERENTIATED 2- MODERATELY DIFFERENTIATED 3- POORLY DIFFERENTIATED
GROWTH	1- PROLIFERATIVE 2- ULCEROPROLIFERATIVE 3- ULCERATIVE/ INFILTRATIVE
PATHOLOGICAL T	1- T1 2- T2

	3- T3 4- T4
PATHOLOGICAL N	0- N0 1- N1 2- N2
STAGE	1- I 2- II 3- III
TUMOR SIZE	1- ≤ 50 MMS 2- > 50 MMS
PERINEURAL INVASION	0- ABSENT 1- PRESENT
LYMPHOVASCULAR INVASION	0- ABSENT 1- PRESENT
LYMPH NODES EXAMINED	NUMBER OF LYMPH NODES
POSITIVE LYMPH NODES	NUMBER OF POSITIVE LYMPH NODES
LYMPH NODE RATIO	1- ≤0.111 2- 0.111- ≤0.200

	3- 0.200- ≤0.429 4- >0.429
EXTRACAPSULAR EXTENSION	0- ABSENT 1- PRESENT
CD 68	1- < 10% OF CELLS 2- >10% TO < 50% OF CELLS POSITIVE 3- > 50% OF CELLS POSITIVE
CD 163	1- < 10% OF CELLS 2- >10% TO < 50% OF CELLS POSITIVE 3- > 50% OF CELLS POSITIVE

H.NO	YEAR	B.NO	SEX	AGE	SITE	HISTOPATHDIAGNOSIS	MALIGNANCYGRADING	GROWTH	PATHOLOGICALT	PATHOLOGICALN	PATHOLOGICALM	PATHOLOGICALTNMSTAGE	TUMORSIZE	SPECIMENTYPE	PERINEURALINVASION	LYMPHOVASCULARINVASION	LYMPHNODESEXAMINED	POSITIVELN	LNR	EXTRACAPSULAREXTENSION	CD 68	CD 163
762580	2012	8	2	2	1	1	2	1	3	1	x	III	2	1	0	0	2	2	4	0	2	3
816143	2012	1358	1	2	5	1	1	2	1	0	x	I	1	2	0	0	0	0	1	0	2	1
837910	2012	1790	2	2	5	1	2	2	3	1	x	III	2	2	0	0	2	2	4	0	1	2
840354	2012	1814	1	1	5	1	2	3	3	0	x	II	1	3	0	0	5	0	1	0	1	2
835745	2012	1866	2	1	5	1	2	3	3	1	x	III	2	3	0	0	2	1	4	0	2	3
841155	2012	2213	1	2	4	1	1	3	4	1	x	III	2	1	0	0	4	1	3	0	1	2
836409	2012	2480	2	1	5	1	3	2	3	0	x	II	1	2	0	0	4	2	4		1	3
882182	2013	298	2	1	1	1	2	2	2	1	x	III	2	1	0	0	20	2	1	0	2	2
878863	2013	331	1	1	5	1	2	3	3	1	x	III	2	3	0	0	2	1	4	0	2	3
88312	2013	427	1	2	5	1	1	1	2	0	x	I	1	2	0	0	0	0	1	0	1	2
879624	2013	443	1	1	4	1	1	2	3	0	x	II	1	1	0	0	2	0	1	0	2	2
903057	2013	851	1	1	5	1	2	2	3	1	x	III	2	2	0	0	7	1	2	0	1	2
928495	2013	1652	2	2	5	1	2	2	2	0	x	I	1	3	0	0	8	0	1	0	1	3
854002	2013	1714	2	2	5	1	3	2	2	0	x	I	1	2	0	0	8	0	1	0	1	3
940986	2013	1844	2	2	5	1	1	1	3	0	x	II	1	3	0	0	13	0	1	0	3	2
958439	2013	2098	1	2	1	1	3	2	3	2	x	III	2	1	0	0	4	4	4	0	2	3
981047	2014	223	1	1	1	1	3	2	3	1	x	III	2	1	0	0	5	1	2	0	2	3
1018817	2014	1999	2	1	5	1	2	2	3	0	x	II	1	2	0	0	6	0	1	0	1	2
119999	2015	588	1	1	1	1	3	1	3	1	x	III	2	1	0	0	8	2	3	0	1	3
155065	2015	1593	1	1	1	1	2	3	3	1	x	III	2	1	0	1	9	1	1	0	2	2
208706	2015	3187	1	2	5	1	1	2	4	0	x	II	2	1	0	0	0	0	1	0	2	2
213721	2015	3378	1	1	2	1	2	1	3	0	x	II	1	1	0	0	9	0	1	0	1	2
28304	2016	48	1	2	5	1	2	2	2	0	x	I	1	2	0	0	1	0	1	0	2	3
239287	2016	213	2	1	5	1	1	1	1	0	x	I	1	1	0	0	3	0	1	0	1	1
304816	2016	2001	2	2	4	1	1	2	2	0	x	I	1	1	0	0	7	0	1	0	2	2
305665	2016	2067	2	1	3	1	2	2	2	0	x	I	2	1	0	0	2	0	1	0	1	2
289663	2016	2936	2	1	1	1	3	1	4	1	x	III	1	1	0	0	4	3	4	0	1	3
254597	2016	1885	2	1	5	1	2	3	3	0	x	II	1	1	0	0	0	0	1	0	1	2
391947	2017	254	2	1	5	1	2	3	3	1	x	III	2	1	0	0	6	3	4	0	1	2
402459	2017	474	2	1	5	1	2	3	3	1	x	III	1	2	1	1	6	3	4	0	3	3
428218	2017	1207	1	1	5	1	2	3	3	2	x	III	1	2	1	1	25	6	3	0	1	2
502643	2017	2504	1	2	4	1	2	3	3	0	x	II	1	1	0	0	0	0	1	0	3	3

615361	2018	2030	2	1	3	1	2	3	3	0	x	II	1	1	0	0	0	0	1	0	1	2
541581	2018	382	2	2	4	1	1	2	3	0	x	II	1	1	0	0	8	0	1	0	3	2
548316	2018	613	1	2	1	1	2	2	3	0	x	II	1	3	0	0	3	0	1	0	1	2
550703	2018	782	1	1	5	1	2	2	3	0	x	II	1	3	0	0	1	0	1	0	2	2
566919	2018	941	1	1	1	1	1	2	3	1	x	III	2	1	0	0	6	3	4	0	2	3
553372	2018	1515	1	1	5	1	3	3	4	1	x	III	1	2	0	0	2	2	4	0	1	2
	2013	33	1	1	2	1	2	2	3	1	x	III	1	1	0	0	5	1	2	0	1	2
	2012	146	1	2	1	1	2	2	3	1	x	III	2	1	0	0	8	2	3	0	1	3
690751	2011	782	2	1	5	1	3	2	3	0	x	II	2	2	0	0	0	0	1	0	2	2
638644	2011	1736	1	2	4	1	2	2	2	1	x	III	2	1	0	0	5	3	4	0	2	3
733193	2011	1804	1	1	4	1	3	3	3	0	x	II	2	1	0	0	0	0	1	0	2	2
735191	2011	1994	2	1	2	1	1	3	2	0	x	I	1	1	0	0	0	0	1	0	3	2
579655	2010	1504	1	1	1	1	3	1	4	1	x	III	1	1	0	0	4	2	4	0	2	3
	2009	1023	1	1	2	1	1	2	2	0	x	I	1	1	0	0	0	0	1	0	3	2
359750	2008	50	2	1	4	1	2	2	2	0	x	I	2	1	0	0	0	0	1	0	3	2
384675	2008	77	1	1	4	1	1	2	4	1	x	III	1	1	0	0	4	3	4	0	2	3
402577	2008	208	2	1	4	1	1	2	2	0	x	I	1	1	0	0	0	0	1	0	2	1
407387	2008	265	1	1	4	1	1	3	3	0	x	II	1	1	0	0	4	0	1	0	3	2
413664	2008	429	1	1	4	1	1	2	4	0	x	II	1	1	0	0	6	0	1	0	3	2
425636	2008	661	1	1	3	1	2	3	4	0	x	II	1	1	0	0	2	0	1	0	2	3
438421	2008	1002	1	1	5	1	2	2	3	0	x	II	1	2	0	0	11	0	1	0	2	2
432848	2008	796	1	1	1	1	1	2	2	0	x	I	1	1	0	0	6	0	1	0	3	2
413277	2008	406	1	1	5	1	2	2	4	0	x	II	1	2	0	0	0	0	1	0	1	2
458197	2008	1510	1	1	4	1	1	3	2	1	x	III	2	1	0	0	8	2	3	0	1	3
354832	2008	1264	1	2	5	1	2	3	4	0	x	II	2	1	0	0	0	0	1	0	2	1
453464	2008	1344	1	2	5	1	3	2	2	0	x	I	1	3	0	0	2	0	1	0	2	2
459520	2008	1743	2	1	1	1	2	2	1	0	x	I	1	3	0	0	6	0	1	0	3	1
470873	2008	1789	1	1	5	1	3	3	1	0	x	I	2	1	0	0	2	0	1	0	3	2
470610	2008	1792	1	1	2	1	1	2	2	0	x	I	1	1	0	0	1	0	1	0	3	1
456410	2008	1373	1	2	2	1	1	3	3	0	x	II	1	1	0	0	0	0	1	0	3	2