"QUANTITATIVE EVALUATION OF LYMPHOID REACTION AND CD8+ T-LYMPHOCYTES AND IT'S ASSOCIATION WITH GRADING AND STAGING OF COLORECTAL CARCINOMA - 5 YEARS STUDY (3 YEARS RETROSPECTIVE AND 2 YEARS PROSPECTIVE STUDY)"

By

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Dissertation submitted to

BLDE (Deemed to be University), Vijayapura, Karnataka

In partial fulfillment of the requirements for the award of the degree of

DOCTOR OF MEDICINE

IN

PATHOLOGY

Under the guidance of

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ABSTRACT

Introduction:

Staging of colorectal carcinoma (CRC) is done on histopathological criteria such as depth of tumor invasion, presence or absence of regional lymph node involvement, distant metastases and on tumor cell differentiation as defined by World Health Organization (WHO). Accurate classification of tumor is necessary for the assessment of prognosis and proper treatment of the patient.

In various studies it was noted that increase in inflammatory cell infiltrate is linked with better survival of the patient and gives prognostic value which is independent of the stage of disease. Quantification of T lymphocytes and inflammatory reaction grading are regarded as important prognostic factors.

Hence the present study was undertaken to assess role of quantitative estimation of lymphoid reaction and CD8+T lymphocyte count and its association with grading and staging in colorectal cancer.

Objectives:

1) Quantitative evaluation of peritumoral lymphoid reaction and CD8+ T lymphocytic infiltration by CD8 IHC marker in colorectal carcinoma.
2) To evaluate the association between lymphoid reaction and CD8+T lymphocytic infiltration with grading and staging of colorectal carcinoma.

Materials and Methods:

H & E stained sections of colorectal carcinoma of resection specimens which were diagnosed as carcinoma in Department of Pathology from May 2015 to May 2020 were evaluated for grading and staging. All cases were evaluated for age, sex, Tumor location, Histological Grade, pT stage, pN stage, and peritumoral LR. Tumour tissue blocks on which quantitative evaluation of peritumoral LR was done, same blocks

were further processed for IHC CD8+ T lymphocytic marker and scores were evaluated. Scores of lymphoid reaction and CD 8+ T lymphocytic infiltration were correlated with grading and staging of colorectal carcinoma.

Results:

Mean age of CRC patient in the present study was 52.8 years with male to female ratio 1:1 and commonest site was proximal colon. Majority of the cases were moderately differentiated adenocarcinoma amounting to 89% and were of stage pT3 amounting to 42.1% followed by stage pT2 and stage pN0 followed by pN1 stage. Peritumoral LR and CD8+ Lymphocytic count was highest in well differentiated adenocarcinoma and pT1 and pT2 stage and in stage pN0

Conclusion:

Peritumoral LR and CD8+T lymphocyte count were high in moderately differentiated adenocarcinoma and in Stage 1 to stage 3 as compared to stage 4b. These findings suggest that there is association of peritumoral LR and grading and staging of CRC and can be considered as prognostic markers for CRC. Hence further extensive evaluation with good number of cases is needed to conclude Peritumoral LR and CD8+T lymphocyte count as prognostic marker of CRC.

Key words: Colorectal carcinoma (CRC), CD8+T lymphocyte, Peritumoral lymphoid reaction (PLR)

LIST OF ABBREVATIONS USED

APC	Adenomatous Polyposis Coli
BRAF	v-raf murine sarcoma viral oncogene homolog B1
CD	Cluster of Differentiation
CEA	Carcinoembryonic Antigen
CIMP	CpG Island Methylator Phenotype
CIN	Chromosomal Instability
CLR	Crohn's like Lymphoid Reaction
CRC	Colorectal Cancer
GPS	Glasgow Prognostic Score
H&E	Hematoxylin and Eosin
LA	Lymphoid Aggregates
LOH	Loss of Heterozygosity
Mod.Diff. Adenoca.	Moderately Differentiated Adenocarcinoma
MSH	MutS Homolog
MSI	Micro Satellite Instability
MSS	Micro Satellite Stability
PLR	Peritumoral Lymphoid Reaction
Poorly. Diff. Adenoca.	Poorly Differentiated Adenocarcinoma
TNM	Tumor, Node, Metastasis

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Approximately 1,200,000 new Colorectal cancer (CRC) cases are reported worldwide which accounts for almost 10% incidence amongst all cancers.[1] CRC was found to be the third most cancer diagnosed. It is one of the commonest causes of cancer-causing death worldwide and is fourth leading cause of cancer related death. At present staging of CRC is done on histopathological criteria such as depth of tumour invasion in the wall of colorectal tissue (T), presence or absence of regional lymph node involvement (N) and distant metastases (M).[2] Accurate categorization of tumour grading and staging is necessary for the assessment of prognosis and proper treatment of the patient.[1]

In various studies it was noted that increase in inflammatory cell infiltrate is associated with better survival of the patient and gives prognostic value which is independent of the stage of disease. Quantification of T lymphocytes and inflammatory reaction grading are considered as important prognostic factors. Immune system has major role not only in pathogenesis of inflammatory and infectious diseases but also in development and progression of cancer.[3,4]

Immune reaction is independent of various other prognostic modalities such as micro-satellite instability, BRAF mutation and LINE-1 hypomethylation. It is anticipated that tumour classification in the future will be based on immune score obtained by calculation of lymphocyte population in the core of tumour and margin of tumour.[2]

Inflammatory and immune reactions occur in response to tumour tissue and presence of lymphoid aggregates around the tumour is termed as Crohn's-like lymphoid reaction (CLR). Prognostic significance of these aggregations is yet not clear.[3]

Data obtained from CRC cohort studies mentioned that identifying number, type and location of tumour immune infiltrates by calculation of lymphocyte population in the core of tumour and peri tumoral lymphoid aggregates can be utilized as an indicator for disease-free and overall survival of patients of CRC.[2]

Hence the present study was undertaken to assess the role of quantitative estimation of lymphoid reaction and CD8+T lymphocyte count and its association with grading and staging in colorectal cancer.

OBJECTIVES OF THE STUDY

1. Quantitative evaluation of peritumoral lymphoid reaction and CD8+ T lymphocytic infiltration by CD8 IHC marker in colorectal carcinoma.
2. To evaluate the association between lymphoid reaction and CD8+T lymphocytic infiltration with grading and staging of colorectal carcinoma.

REVIEW OF LITERATURE

Colorectal cancer epidemiology

Colorectal cancer is one of the most common malignancies and is a major contributor to cancer-related deaths worldwide. Its incidence is increasing in developing countries. It was estimated that in 2018 there will be more than 1.8 million new colorectal cancer cases and 881,000 deaths will occur accounting to one tenth of cancer cases and deaths. Overall, the incidence of colorectal cancer ranks third but mortality rate ranks second. [5]

The incidence of CRC in India is lower than that in western countries and it is the seventh leading cancer in India. Estimated new CRC cases in India in 2018 were 27,605.[5]

Etiopathogenesis of Colorectal cancer

Variations in the incidence of CRC between various countries around the world suggest that dietary and environmental factors account for the illness threat.[6] In the western countries dietary factors such as ingestion of red meat and heavy alcohol consumption may be associated with an increased CRC risk whereas a high-fibre diet may be was associated with a low incidence of CRC in India.[7] Other known risk factors for CRC include smoking, overweight, low physical activity, and inflammatory bowel diseases (IBD). It was mentioned in some studies that the non-steroidal anti-inflammatory drugs (NSAID) intake can decreases the risk of CRC.[8] CRC is widely sporadic. In 5% of CRC patients a characterized germ line mutation is noted. In some studies, it was suggested that risk of CRC due to genetic factors account for up to 35%. The most common genetic factors are hereditary colorectal cancer syndromes such as familial adenomatous polyposis (FAP) with a germline mutation in adenomatous polyposis coli (APC) tumor suppressor gene and Lynch

syndrome with a germline mutation in one of the mismatch repair (MMR) systems of deoxyribonucleic acid (DNA).[9]

Sustained proliferative signalling, evading growth suppressors, activating invasion and metastasis, enabling replicative immortality, inducing angiogenesis, resisting cell death, deregulating cellular energetics, and avoiding immune destruction are the hallmarks of cancer.[10] CRC acquire these traits in the multi-step process of carcinogenesis.[11]

Genetic Model of Colorectal Cancer Progression

Molecular events that lead to colonic adenocarcinoma are heterogeneous and include genetic and epigenetic abnormalities. APC/β-catenin pathway and microsatellite instability pathway are the two genetic pathways which are distinct and plays major role in progression of CRC. Mutations of the APC/β-catenin pathway lead to increased WNT signalling. Mutation involving microsatellite instability pathway leads to defects in DNA mismatch repair.[11]

Progression from Adenoma to Carcinoma

The greater part of CRCs arises from adenomas, either conventional adenomas, sessile serrated adenomas/polyps (SSA/ Ps), or traditional serrated adenomas.[12] There is distinct associations between the histologic type of precursor lesion and the subsequent development of malignancy. There are two comprehensive pathways involved in neoplastic progression of the colorectum, the conventional adenoma pathway and the serrated adenoma/ polyp pathway.[13] In various studies it was mentioned that the conventional adenoma pathway may be responsible approximately in 70% to 80% of CRCs and is much more prevalent in the left colon and rectum than in the right colon. It was also mentioned in some studies that conventional adenomas may subsequently develop malignancy within 15 years in

untreated patients. Hence it was concluded in these studies that endoscopic removal of conventional adenomas may decreases the incidence of subsequent CRC in treated patients. The pathogenic mechanism of serrated pathway has been studied in the past 10 years, and it was mentioned that this mechanism may be responsible roughly in 20% to 30% of all CRC. Most CRC emerging in the serrated pathway develop from SSA/Ps, especially those situated in the right colon.[14] CRC is typically preceded by the development of foci of dysplasia within these polyps.[12]

Colorectal cancer diagnosis

Symptoms associated with CRC include lower GI bleeding, change in bowel habits, abdominal pain, weight loss, change in appetite, weakness, and obstructive symptoms.[15] Physical examination findings in CRC may be a palpable mass, passage of fresh blood per rectum which is usually noted in left-sided colon cancers and rectal cancer. Malena or altered blood may be noted in right-sided colon cancers. In right sided colon cancer lesser degrees of bleeding or hemo-occult-positive stool may be the finding. Lymphadenopathy, hepatomegaly, jaundice or even pulmonary signs may be seen in patients with metastatic disease.[1]

Colorectal cancer screening

Advanced non-invasive technologies such as CT and magnetic resonance colonography are receiving increased attention in clinical studies which demonstrates overall feasibility as well as some advantages over conventional endoscopic screening programs have been less effective at reducing right-sided CRC.[1,12]

"WHO Classification of Colorectal Carcinoma" [16]

Benign Epithelial tumours and precursors

- Serrated dysplasia, low grade
- Serrated dysplasia, high grade
    - Hyperplastic polyp, micro vesicular type
    - Hyperplastic polyp, goblet cell
- Adenomatous polyp, low grade dysplasia
- Adenomatous polyp, high grade dysplasia
    - Tubular adenoma, low grade
    - Tubular adenoma, high grade
    - Villous adenoma, low grade
    - Villous adenoma, high grade
    - Tubulovillous adenoma, high grade
- Glandular intraepithelial neoplasia, low grade
- Glandular intraepithelial neoplasia, high grade

Malignant Epithelial Tumours

- Adenocarcinoma NOS
    - Serrated adenocarcinoma
    - Adenoma like adenocarcinoma
    - Micropapillary adenocarcinoma
    - Mucinous adenocarcinoma
    - Poorly cohesive carcinoma
    - Signet ring cell carcinoma
    - Medullary adenocarcinoma
    - Adenosquamous carcinoma

- Carcinoma undifferentiated, NOS
- Carcinoma with sarcomatoid component

Neuroendocrine tumour NOS

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

Neuroendocrine carcinoma NOS

- Large cell Neuroendocrine carcinoma
- Small cell Neuroendocrine carcinoma

Mixed Neuroendocrine- non- neuroendocrine neoplasm (MiNEN)''[16]

STAGING AND PROGNOSIS OF COLORECTAL CANCER

Staging

CRC staging is done by using the current TNM (tumor, node, metastasis) classification of the American Joint Committee on Cancer (AJCC)/International Union Against Cancer (UICC) staging system.[1]

pT Stage:

Stage pTx refers to the primary tumor that cannot be assessed. In pT0 there is no evidence of primary tumor. pTis refers to the carcinoma in situ showing involvement of lamina propria with no extension through muscularis mucosae. In pT1 tumors invade into muscularis mucosae but not through the submucosa. In pT2 tumor

invades the muscularis propria. In pT3 tumor invades through the muscularis propria into peri colorectal tissues. In pT4 tumor invades the visceral peritoneum or invades or adheres to adjacent organs or structures. In pT4a tumor perforates the visceral peritoneum. In pT4b tumor directly invades into adjacent organs or structures.[1]

pN Stage: Lymph nodes involvement is associated with the prognostic significance of CRC. Minimum 12 lymph nodes should be analysed. Both number of nodes that are positive for tumor and the total number of nodes inspected should be mentioned in the report.[1]

According to TNM classification, in pNx regional lymph nodes cannot be assessed. pN_0 refers to no regional lymph node metastasis. In pN_{1a} one regional lymph node is positive. pN_{1b} is referred when two or three regional lymph nodes are positive. In pN1c no regional lymph nodes are positive, but tumor deposits are seen in the subserosa, mesentery, non peritonealized pericolic, or perirectal or mesorectal tissues. pN_2a indicates metastasis to four to six regional lymph nodes. pN_{2b} indicates metastasis to seven or more regional lymph nodes.[1]

M Stage: If there is no evidence of distant metastases that is referred as $M_{0.}$ Presence of metastasis to one site or organ without peritoneal metastasis is referred as pM_{1a}, whereas metastasis to two or more sites or organs without peritoneal metastasis is denoted as $pM_{1b.}$ pM1c is metastasis to the peritoneal surface alone or with other site or organ metastases.[1]

Prognostic factors

Depth of invasion: Tumors restricted to the submucosa that is those that do not cross the muscularis mucosae have 5-year survival rates in 100% cases while invasion into the submucosa or muscularis propria reduces 5-year survival to 95% and 70% to 90%,

respectively in tumors limited to the primary site. Invasion through the visceral serosal surface or into adjacent organs and tissues reduces survival further.[3]

The presence of lymph node metastases

Most cases with lymph node metastases need radiation or chemotherapy.[11]

Distant metastases to lung, liver, or other sites also limits survival of the patients. Only 15% or fewer of patients with tumors at this stage may survive five years after the diagnosis.[11]

Residual Tumor (R Stage) at Margins of Resection

Proper assessment of margins is also important in colonic carcinomas.[12] Tumors that are completely resected with histologically negative margins are classified as R0. Tumors with a complete gross resection but with microscopically positive margins are classified as R1. Patients who have incomplete resections with grossly positive margins are classified as patients having R2 resection. The R0, R1 and R2 status carry strong prognostic implications.[1]

Histologic Grade

WHO classification categorizes colorectal adenocarcinomas into well, moderate, poorly differentiated adenocarcinomas, and undifferentiated carcinomas based on the percentage of gland formation. In well-differentiated more than 95% gland formation is noted and is also called grade G1. In G2 that is moderately differentiated adenocarcinoma 50–95% gland formation is noted. In poorly differentiated less than 50% gland formation is noted and categorised as G3. When there is no evidence of differentiation then the tumour is graded as undifferentiated carcinoma.[16]

Lymph-Vascular and Perineural Invasion

In small vessel invasion tumor involvement shows thin-walled vessel wall structures lined by endothelium. Smooth muscle layer of an elastic lamina is not identifiable in small vessel tumor invasion. In small vessels invasion small vessels are capillaries, postcapillary venules and lymphatics. Small vessel invasion most of the times is associated with lymph node metastasis. In many studies it is mentioned as independent indicator of adverse outcomes.[1] Perineural invasion is an independent indicator of poor prognosis. Extramural vascular invasion (VI) (invasion of large veins beyond the muscularis propria) is an independent prognostic indicator of disease recurrence and survival in CRC.[17] Intramural VI is of prognostic significance.[12]

Tumor Budding

Presence of small cord and small clusters of the neoplastic epithelium (five or less than five cells) detach from tumor glands and appear to migrate into the adjacent desmoplastic stroma.[12] Presence of high number of tumor budding in tumour tissue is also considered as a significant risk factor for involvement of lymph node metastasis.[18]

Host Immune Response

Immune system and inflammation

Since long ago it was found that lymphocytic reaction is an important indicator of immune response of host towards tumour cells in resected specimens of colorectal malignancies.[19]

In various studies it was noted that there was a significant correlation between increased lymphocytic reaction and favourable prognostic outcome. This correlation was utilized for various changes seen in lymphoid systems such as lymphoid hyperplasia in regional lymph nodes, peritumoral lymphocytic infiltration and

lymphocyte infiltration in the core of tumour. Activation of lymphoid system against tumors can be speculated by the presence of nodular lymphoid aggregates at the periphery of the tumou tissue in carcinoma.[3]

In some studies, it was mentioned that histologic feature of nodular lymphoid aggregates at the periphery of carcinoma also suggests activation of lymphatic system against tumour. Some authors coined the terminology as perivascular lymphocytic cuff to the sheets of small lymphocytes seen around the tumour foci in muscle and pericolic fat. They mentioned that this phenomenon might be host immunologic response to invasive carcinoma.[19]

Graham and Appelman[20] observed that peritumoral lymphoid aggregates in CRC were having morphology as that of the inflammatory component of Crohn disease. Hence, they started referring such lymphoid aggregates as CLR. Eventually the term CLR came into use more frequently even if they were present adjacent to venules. This term became more popular among pathologists as compared to the previous terminology that is perivascular lymphocytic cuffs.[19]

Few studies demonstrated correlation between CLR and favorable prognostic outcome. In those studies, it was found that CLR was associated with mismatch repair status and those authors also mentioned that it acts as a histological marker of microsatellite instability.[19]

Assessment of CLR method, proposed by Graham and Appelman[20] has become very popular and was widely used for assessment of CLR. Based on the number and size of lymphoid aggregate (LA) and number of germinal centers they classified CLR in CRC into 3 grades as follows:[19,20]

Grade 0: Absence of LA or presence of at least single small LA

Grade 1: Presence of small LAs with rare or absent germinal centers

Grade 2: Presence of numerous large LAs with frequent germinal centers

According to Graham's method, Grading of CLR was determined in subjective manner. Hence, few studies were undertaken for evaluation of reproducibility of this method. Also, in few studies attempts were made to formulate optimal criteria for evaluation of CLR status.[19,20]

Ueno et al[19] in their study concluded that CLR can be evaluated semi quantitatively by assessing the maximum diameter of largest lymphoid aggregate within the tumour and they also mentioned that 1-mm rule can be applied to improve reproducibility in evaluation of CLR. They also mentioned that based on CLR status in CRC incidence of recurrence and metastasis in distant organs can be predicted.

In a study done on association between the CD8+ T lymphocytic infiltration in colorectal cancer concluded that there is possible influence of viral infection for the clinical outcome and CD8+ T lymphocytic infiltration in colorectal carcinoma.[2]

Various types of immune cells infiltrate the CRC like other solid malignancies. The immune system is classified into the innate and adaptive immune systems. Neutrophils, mast cells, natural killer (NK) cells, dendritic cells (DC), and tumor-associated macrophages are cells of the innate immune system and can be easily detected in these tumors.[21]Moreover, advanced tumors recruit specific myeloid subsets that represent phenotypically heterogeneous but a functionally similar population of CD11bGr1 cells, called myeloid-derived suppressor cells.[22] These cells share some characteristic with monocytes, macrophages, neutrophils, and DC and help suppress antitumor immune responses and tumor angiogenesis. Cells of the adaptive immune system are also recruited into colorectal and colitis-associated tumors, where they have either pro- or antitumorigenic roles. T cells, for instance, are required for inflammation, cancer development, and tumor progression, as well as for

anticancer immunity. A well-defined balance between immunosurveillance (by CD8+T cells and CD4+ T cells_) and tumor promoting inflammation (by innate immune cells and various subtypes of T cells) was observed in sporadic CRC.[23]

Tumors require blood vessels to attain nutrients and oxygen. Vascular endothelial growth factor (VEGF) plays a major role in angiogenesis regulation and overexpresses in CRC. High VEGF expression and high blood vessel density have been accompanied by worse prognosis in CRC, indicating that the tumors with effective angiogenesis show aggressive behavior.[24] In addition to tumor cells, the inflammatory cells in tumor stroma are significant sources of angiogenesis.[25]

The immune response to CRC has profound molecular, biological, and clinical implications. In the tumor microenvironment (TME) immune cell populations are associated with distinct molecular events (e.g., mismatch repair deficiency), histopathological features, and overall and cancer-specific survival.[26]

Since long ago it was found that lymphocytic reaction is an important indicator of the immune response of the host towards tumor cells in resected specimens of colorectal malignancies.[19]

In various studies, it was noted that there was a significant correlation between increased lymphocytic reaction and favourable prognostic outcome. This correlation was utilized for various changes seen in lymphoid systems such as lymphoid hyperplasia in regional lymph nodes, peritumoral lymphocytic infiltration, and lymphocyte infiltration in the core of the tumor. Activation of the lymphoid system against tumors can be speculated by the presence of nodular lymphoid aggregates at the periphery of the tumor tissue in carcinoma.[3]

In some studies, it was mentioned that the histologic feature of nodular lymphoid aggregates at the periphery of carcinoma also suggests activation of the

lymphatic system against tumors. Some authors coined the terminology as a perivascular lymphocytic cuff to the sheets of small lymphocytes seen around the tumor foci in muscle and pericolic fat. They mentioned that this phenomenon might be a host immunologic response to invasive carcinoma.[19]

Cytotoxic T lymphocytes were established as important players in anti-tumor immunity in CRC. Cytotoxic T lymphocytes can kill target cells upon being exposed to a tumor cell antigen/HLA1 complex for which their T-cell receptor is specific.[12]

Usually, most of the CRC tumors are immunogenic and infiltrated by T lymphocytes. An increased amount of T lymphocytes infiltrating the tumor in CRC has repeatedly proven to be associated with a better prognosis.[4]

Crohn's-like lymphoid reaction

In CRCs, an inflammatory reaction pattern that consists of numerous transmural lymphoid aggregates referred to as Crohn's like lymphoid reaction (CLR). Graham and Appelman[20] observed that peritumoral lymphoid aggregates in CRC were having morphology as that of the inflammatory component of Crohn disease. Hence, they started referring such lymphoid aggregates as CLR. Eventually, the term CLR came into use more frequently even if they were present adjacent to venules. This term became more popular among pathologists as compared to the previous terminology that is perivascular lymphocytic cuffs.[19]

Few studies demonstrated a correlation between CLR and favourable prognostic outcome. In those studies, it was found that CLR was associated with mismatch repair status and those authors also mentioned that it acts as a histological marker of microsatellite instability.[19]

Presence of tumor-infiltrating lymphocytes (TILs), and specifically CD8+ cytotoxic T lymphocytes (CTLs) were correlated with the immune status in a study

done by recent researchers and their research study indicated an interrelationship between the host-inflammatory response and carcinogenesis. Inflammatory reaction induced by host tissue against tumor tissue has a crucial role in determining the occurrence, progression, and dissemination of some cancers. [27–29] In various studies it was observed that the cytotoxic T lymphocytes (CTLs) intensity is a favorable biomarker to determine the prognosis of many cancers, including CRC. In these studies, it was mentioned that an increase in inflammatory cell infiltrate is associated with better survival of the patient and gives a prognostic value that is independent of the stage of the disease. [27-29]

Genetic prognostic and predictive markers

Microsatellite Instability

MSI-H tumors are more frequently right-sided, high grade, and mucinous histology but a good prognosis.[30] Majority of MSI tumors are sporadic because of epigenetic inactivation of the M LH J gene. MSI-H is important because it not only is a good prognostic factor, but it also predicts a poor response to 5-FU chemotherapy.[4]

BRAF

BRAF mutation, present in 8% to 10% of CRCs, is linked to a subset of MSI-H tumors.MSI status and BRAF mutation are prognostic factors that interact significantly. Although BRAF mutation is associated with poor prognosis, the presence of MSI may attenuate its adverse impact. MSI without BRAF is a good prognostic factor, whereas MSI-H with BRA F mutation portends slightly worse survival.[30]

CpG Island Methylator Phenotype

CpG island methylator phenotype (CIMP), is an aberrant silencing tumor suppressor gene. Half of CRCs shows loss of allelic LOH that involves chromosome

18q. However, other genes in this region, such as SMAD2 and SMAD4, may also be relevant to CRC development. DCC expression is greatly reduced or absent in many colorectal carcinomas, and loss of DCC is associated with metastasis and an adverse prognosis.[1]

SMAD4

SMAD4, which is localized to band 18q21, is a common downstream regulator and tumor suppressor gene in the TFG-β pathway. Sporadic mutations are present in 2.1% to 20% of CRCs, with limited data suggesting a poor prognostic impact.[1]

Blood and serum prognostic markers

Hematological parameters and systemic protein markers have been associated with prognostic significance in CRC.[31] CEA levels also may be used as a response marker for the treatment of Stage IV disease and is a significant molecule for metastasis.[30] Systemic inflammatory markers have also shown promise in CRC prognostication.4 Especially, the Glasgow prognostic score (GPS) comprised of serum levels of C reactive protein (CRP) and albumin and blood neutrophil/lymphocyte ratio have been found to have strong prognostic value.[32]

Colorectal cancer treatment

Surgery is the primary modality of treatment for CRC. Resection is the only therapy required for early-stage CRC. Adjuvant chemotherapy, radiotherapy (RT) or chemoradiotherapy (CRT) reduces the mortality in surgically treated patients having high risk of recurrence. Neoadjuvant RT or CRT improves the results of the treatment of locally advanced rectal cancer. In addition to the traditional cytotoxic drugs, the last two decades have led to the introduction of monoclonal antibodies in CRC treatment.[1]

Surgical treatment

In the surgical treatment of CRC, the target is to remove the tumor along with the associated lymphatics en bloc with 5–10 cm of normal bowel on either side of the primary tumor. Usually the remaining parts of the bowel are anastomosed together. However, in the abdominoperineal resection of the rectum, also the anus is removed and the end of the remaining sigmoid colon is brought to the surface of the abdomen as a colostomy.[33] In addition to the resection of the primary tumor, the resection of colorectal liver metastases in selected patients has been slowly adopted as the standard of care during the last 20 years.[34]

Neoadjuvant treatment for rectal cancer

Neoadjuvant therapy for locally advanced rectal cancer may reduce the risk of local relapse, improve resectability, help to preserve the sphincter function and help to avoid stoma.[35] Combined modality preoperative therapy (radiation and 5-FU plus leucovorin) may lead to down staging and improved local control of rectal cancer after surgical resection. Neoadjuvant therapy has become the treatment of choice for T3, T4 and node-positive rectal cancers. Neoadjuvant therapy is associated with a relative risk reduction of local recurrence of 50%. The role of the pathologist in examining specimens from patients who have had preoperative chemoradiation therapy for rectal cancer is to carefully evaluate the presence of a residual primary tumor and the presence or absence of lymph node metastases.[12]

MATERIALS AND METHODS

Source of data

- Resected specimens of colorectal carcinoma sent to the Histopathology section of the Department of Pathology, B.L.D.E. (Deemed to be University) Shri B M Patil Medical College Hospital & Research Centre, Vijayapura.
- Study period: 1stMay, 2015 – 30th May, 2020 (5 years study 3 years retrospective and 2 years prospective)

Methods of collection of data:

Resected specimens of colorectal carcinomas, which were diagnosed as carcinoma on histopathology were evaluated for grading and staging. H & E stained sections of Tumor tissue sections were evaluated for lymphoid reaction as per the method proposed by Graham and Appelman.[19,20] Number of tumour sections examined per case was two slides of tumour tissue.

Each case was evaluated under the following headings –

1. Age of the patient
2. Sex
3. Tumour location
4. Histological grading
5. pT stage -AJCC (7th edition) Classification of CRC
6. pN stage -AJCC (7th edition) Classification of CRC
7. Peritumoral Lymphoid reaction - Lymphoid reaction in peritumoral area was classified into 3 grades based on the number and size of LAs and number of germinal centres as mentioned below -

Grade 0: No LA or single small LA in both tumour sections

Grade 1: Small LAs with rare or absent germinal centers

Grade 2: Numerous large LAs with frequent germinal centers[3]

Tumour tissue blocks on which quantitative evaluation of lymphoid reaction was done, those were further be processed for Immuno-histochemistry CD8+ marker.

Scores of CD8+ T lymphocytic infiltration were evaluated as per the study done by Kleist *et al*.[2] as mentioned below

Negative – CD8 + T lymphocytic infiltration $<1/mm^2$,

Low - CD 8+ T lymphocytic infiltration $1\text{-}59/mm^2$,

Moderate - CD 8+ T lymphocytic infiltration $60\text{-}119/mm^2$,

High – CD8 + T lymphocytic infiltration $\geq 120/mm^2$

Scores of lymphoid reaction and CD 8+ T lymphocytic infiltration were correlated with grading and staging of colorectal carcinoma as mentioned in Table1 and Table 2

Table 1: Association between Peritumoral lymphoid reaction with grading and staging of colorectal carcinoma[19]

Clinical parameters	**Grade 0 = No LA or at most single small LA** (n – number of samples)	**Grade 1= Small LAs with rare or absent germinal centers** (n – number of samples)	**Grade 2 = Numerous large LAs with frequent germinal centers.** (n-number of samples)
Age(years)			
Mean(range)			
Gender			
Male			
Female			
Tumour site			
Proximal colon			
Distal colon			
Rectum			
Histological grade			
Well			
Moderate			
Poor			
pT stage			
1(Tumour invasion up to sub mucosa)			

≤2 (Tumour invasion up to muscularis propria)			
3 (Tumour invasion through muscularis propria into peri colorectal tissues)			
4 (Tumour directly invades or is adherent to other organs or structures)			
pN stage			
pNx (Regional lymph nodes cannot be assessed			
pN0 (No regional lymph nodes)			
pN1 (1-3 regional lymph nodes)			
pN2 (≥ 4 regional lymph nodes)			

Table 2: Association between CD8+ T lymphocytic count (IHC) with grading and staging of colorectal carcinoma[2]

Clinical parameters	**Negative<1/mm^2** n– number of samples	**Low 1-59/mm^2** n – number of samples	**Moderate 60 - 119/mm^2** n – number of samples	**High>120/mm^2** n – number of samples
Age (years)				
Mean (range)				
Gender				
Male				
Female				
Tumour site				
Proximal colon				
Distal colon				
Rectum				
Histological grade				
Well				
Moderate				
Poor				
pT stage				
1(Tumour invasion up to sub mucosa)				
<2 (Tumour invasion up to muscularis propria				
3 (Tumour invasion through the muscularis				

propria into peri colorectal tissues)				
4 (Tumour directly invades or is adherent to other organs or structures)				
pN stage				
pNx (Regional lymph nodes cannot be assessed				
p0 (No regional lymph nodes)				
pN1 (1-3 regional lymph nodes)				
pN2 (≥ 4 regional lymph nodes)				

Inclusion criteria:

- Resected specimens of colon and rectum which were histologically diagnosed as colorectal carcinoma were included.

Exclusion criteria:

- Resected specimen of colorectal carcinoma with extensive autolysis.

Scores of lymphoid reaction and CD 8+ T lymphocytic infiltration were correlated with grading and staging of colorectal carcinoma

Sample Size

With anticipated correlation coefficient between CD8+T Lymphocyte count with IHC and clinical outcome r=0.500 as mentioned in study done by Vayrynen et al[3]. At 95% confidence level and 10% power by using statistical formula,

The standard normal deviate for $\alpha = Z_\alpha = 1.96$

The standard normal deviate for $\beta = Z_\beta = 1.282$

$C = 0.5 * \ln[(1+r)/(1-r)] = 0.299$

- Total sample size was -

$$N = [(Z_\alpha + Z_\beta)/C]^2 + 3$$

$$= 38$$

Statistical analysis

All characteristics were summarized descriptively. For continuous variables, the summary statistics of mean± standard deviation (SD) were used. For categorical data, the number and percentage were used in the data summaries and diagrammatic presentation. Chi-square (χ^2) test was used for association between two categorical variables.

The formula for the chi-square statistic used in the chi square test is:

$$\chi_c^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

The subscript "c" are the degrees of freedom. "O" is observed value and E is expected value. C= (number of rows-1) * (number of columns-1)

The difference of the means of analysis variables between two independent groups was tested by unpaired t test.

The statistics to test whether the means are different were calculated as follows:

$$t = \frac{(\overline{x_1} - \overline{x_2}) - (\mu_1 - \mu_2)}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

where $\bar{x}_1$ = mean of sample 1

$\bar{x}_2$ = mean of sample 2

n_1 = number of subjects in sample 1

n_2 = number of subjects in sample 2

s_1^2 = variance of sample 1 = $\frac{\Sigma(x_1 - \bar{x}_1)^2}{n_1}$

s_2^2 = variance of sample 2 = $\frac{\Sigma(x_2 - \bar{x}_2)^2}{n_2}$

If the p-value was < 0.05, then the results were considered to be statistically significant otherwise it was considered as not statistically significant. Data were analyzed using SPSS software v.23 (IBM Statistics, Chicago, USA)and Microsoft office 2007.

RESULTS AND ANALYSIS

Total 38 cases of resected specimens of CRC received in the Department of Pathology from 2015 to 2020 were evaluated for age and sex of the patient, tumor location, histological grading, pT and pN staging. Tumor sections were evaluated for LR in peritumoral area. Tumor tissue blocks of same sections were processed for CD8+ T lymphocytic marker and CD8+ T lymphocytic marker scores were evaluated.

Table 3: Distribution of cases according to age in patients with CRC

Age (Years)	No. of patients	Percentage
30-40	9	23.6%
41 - 50	7	18.4%
51 - 60	11	28.9%
61 - 70	8	21.2%
71-80	3	7.9%
Total	38	100.0%

Age of the youngest CRC patient in the present study was 30 years and oldest patient was 80 years with a mean age of 52.8 years. Most of the cases were in the age group of 51 - 60 years.

Table 4: Distribution of the cases of CRC according to Sex

Gender	No. of patients	Percentage
Female	19	50.0%
Male	19	50.0%
Total	38	100.0%

Male to female ratio in the present study was 1:1.

Table 5: Distribution of the cases of CRC according to tumor site involvement

Tumor site	No. of patients	Percentage
DISTAL COLON	13	34.21%
PROXIMAL COLON	14	36.8%
RECTUM	11	28.9%
Total	38	100%

Commonest site for CRC observed in the present study was proximal colon followed by distal colon

Table 6: Distribution of cases of CRC according to Histopathological grade

Histopathological grades	No. of patients	Percentage
Poorly differentiated adenocarcinoma	2	5.3%
Moderately differentiated adenocarcinoma	34	89.5%
Well differentiated adenocarcinoma	2	5.3%
Total	38	100.0%

Majority of the cases of CRC in the present study were moderately differentiated adenocarcinoma amounting to 89%. Two cases each of well-differentiated adenocarcinoma and poorly differentiated adenocarcinoma were noted.

Table 7: Distribution of cases of CRC according to pT stage

$_{P}$T stage	No. of patients	Percentage
pT stage 1(Tumour invasion up to sub mucosa)	3	7.9%
pT stage ≤2 (Tumour invasion up to muscularis propria)	14	36.8%
pT stage 3 (Tumour invasion through muscularis propria into peri colorectal tissues)	16	42.1%
pT stage 4 (Tumour directly invades or is adherent to other organs or structures)	5	13.2%
Total	38	100.0%

Majority of the cases were of stage pT3 amounting to 42.1% followed by stage pT2 and stage pT4.

Table 8: Distribution of cases of CRC according to pN stage

pN stage	No. of patients	Percentage
pNx (regional lymph nodes cannot be assessed)	4	10.5%
pN0 (No regional lymph nodes)	18	47.4%
pN1 (1-3 regional lymph nodes)	11	28.9%
pN2 (≥ 4 regional lymph nodes)	5	13.2%
Total	38	100.0%

Majority of the cases amounting to 47.4% were of stage pN0 followed by pN1 stage amounting to 28.9%.

Table 9: Distribution of cases of CRC according to peritumoral lymphoid reaction grading

Grading of peritumoral lymphoid reaction	No. of patients	Percentage
Grade 0	1	2.6%
Grade 1	21	55.3%
Grade 2	16	42.1%
Total	38	100.0%

Grade 0 Peritumoral lymphoid reaction was observed in single case of poorly differentiated adenocarcinoma.

Table 10: Distribution of cases of CRC according to CD8+T(IHC)lymphocytic count

CD8+T(IHC)lymphocytic count	No. of patients	Percentage
Low	3	7.9%
Moderate	15	39.5%
High	20	52.6%
Total	38	100.0%

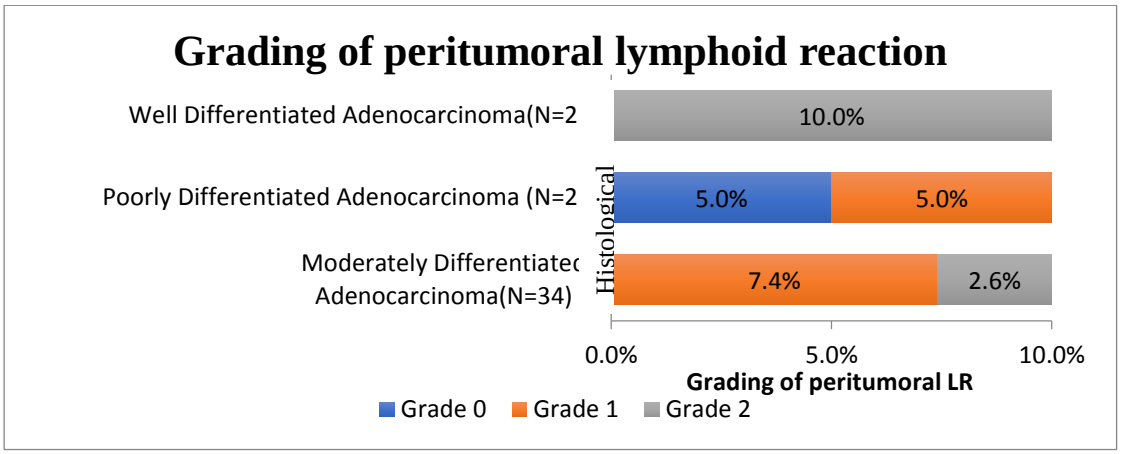
Out of 38 cases of CRC, 20 (52.6%) cases were showing high CD8+ Lymphocytic count (52.6%) and 15 cases were showing moderate CD8+ Lymphocytic counts.

Table 11: **Association between peritumoral LR and histological grading of the CRC**

Histological grade	Grading of peritumoral lymphoid reaction						
	Grade 0		Grade 1		Grade 2		p value
	N	%	N		N	%	
Moderately differentiated adenocarcinoma(N=34)	0	0.0%	25	74%	9	26%	0.001*
Poorly differentiated adenocarcinoma (N=2)	1	50.0%	1	50.0%	0	0%	
Well differentiated adenocarcinoma(N=2)	0	0.0%	0	0.0%	2	100%	

Note: * significant at 5% level of significance ($p<0.05$)

Figure 1: Association between peritumoral LR and histological grading of the CRC



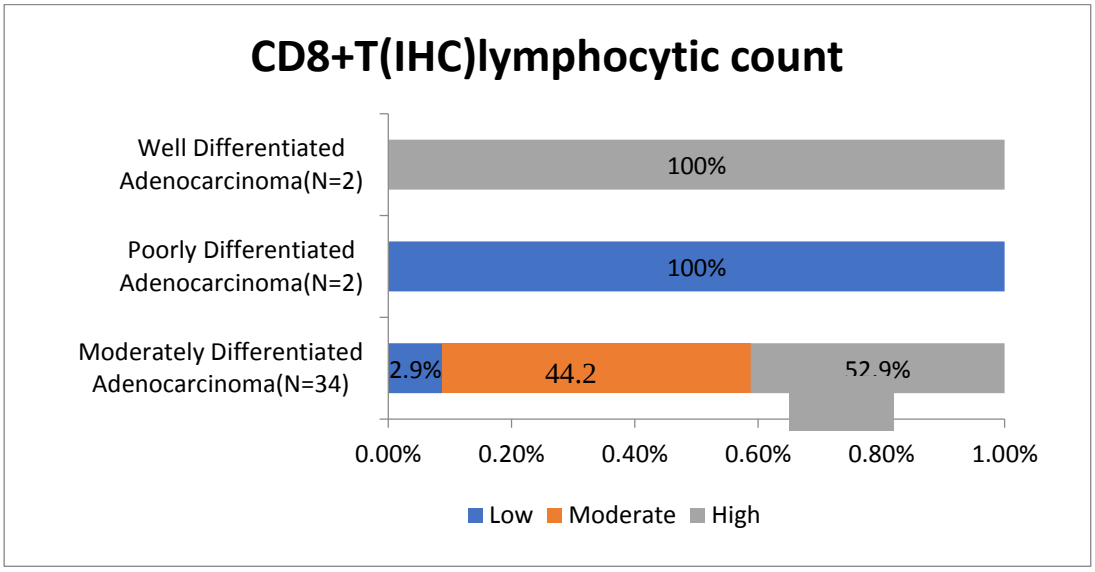
Out of 34 cases of moderately differentiated adenocarcinoma in 25 cases Grade 1 peritumoral LR was observed. Peritumoral LR was highest in well differentiated adenocarcinoma and moderate in moderately differentiated adenocarcinoma. One case of poorly differentiated adenocarcinoma had grade zero peritumoral LR and one case had grade one peritumoral LR. Statistically significant difference was noted in grading of peritumoral LR between well, moderate and poorly differentiated adenocarcinoma having p value 0.001.

Table 12: Association betweenCD8+T(IHC)lymphocytic count and Histological grade

Histological grade	CD8+T(IHC)lymphocytic count						p value
	Low		Moderate		High		
	N	%	N	%	N	%	
Moderately differentiated adenocarcinoma(N=34)	1	2.9%	15	44.2%	18	52.9%	0.032 *
Poorly differentiated adenocarcinoma(N=2)	2	100%	0	0.0%	0	0.0%	
Well differentiated adenocarcinoma(N=2)	0	0.0%	0	0.0%	2	100%	

Note: * significant at 5% level of significance (p<0.05)

Figure 2: Distribution of Histological grade according to CD8+T(IHC)lymphocytic count



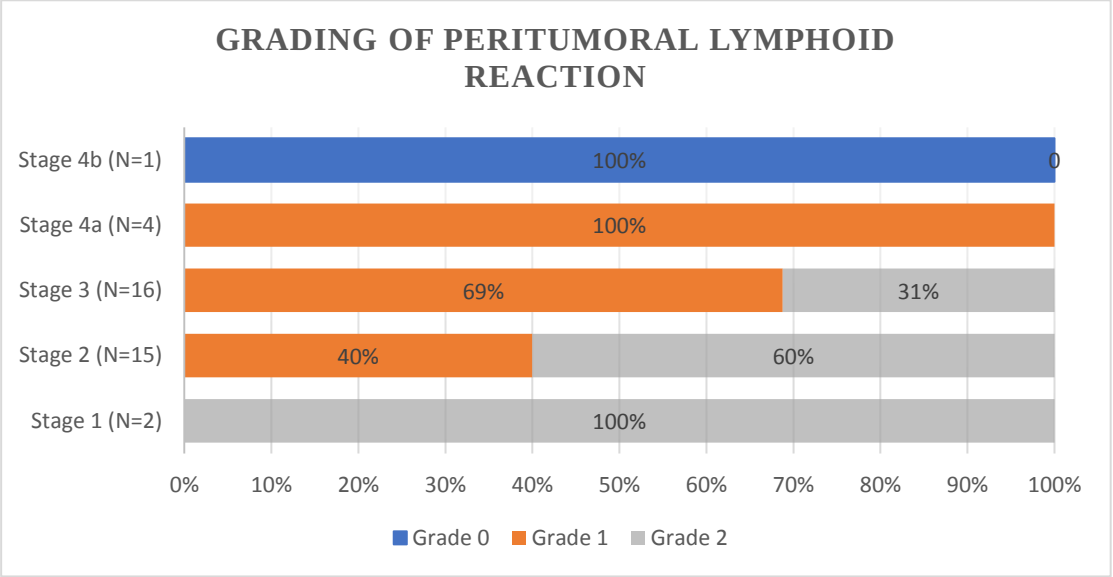
In moderately differentiated adenocarcinoma out of 34 cases in 15 cases moderate CD8+ T lymphocytic count and 18 cases high CD8+ T lymphocytic count was

noted. In all cases of well differentiated adenocarcinoma CD8+ T lymphocytic count was high. In all cases of poorly differentiated adenocarcinoma CD8+ T lymphocytic count was low. There was statistically significant difference in CD8+ T lymphocytic count of moderately differentiated adenocarcinoma, poorly differentiated adenocarcinoma and well differentiated adenocarcinoma having p value 0.032.

Table 13: Association between stage according to Grading of peritumoral lymphoid reaction

pT Stage	**Grading of peritumoral lymphoid reaction**						**p value**
	Grade 0		**Grade 1**		**Grade 2**		
	N	**%**	**N**	**%**	**N**	**%**	
pTStage1 (N=2)	0	0.0%	0	0.0%	2	100%	0.293
pT Stage 2(N=15)	0	0.0%	6	40%	9	60%	
pT Stage 3(N=16)	0	0.0%	11	69 %	5	31%	
pT Stage 4a(N=4)	0	0.0%	4	100%	0	0.0%	
pT Stage 4b(N=1)	1	100.0%	0	0.0%	0	0.0%	

Figure 3: Distribution of pT stage according to Grading of peritumoral lymphoid reaction

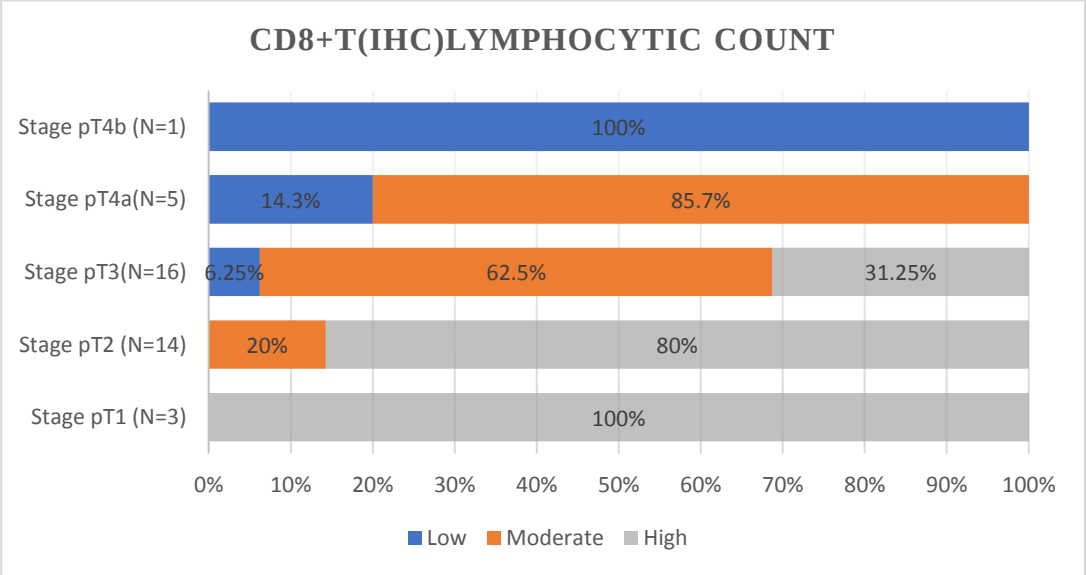


In stage pT1 and pT2 majority of the cases were showing Grade 2 peritumoral LR amounting to 100% and 60% respectively. In 100% of the stage pT4a grade 1 peritumoral LR was noted. Peritumoral LR is higher in pT stage 1 and lowest in pT stage 4b. However, the difference was statistically not significant.

Table 14: Association between stage according to CD8+T(IHC)lymphocytic count

pT Stage	CD8+T(IHC)lymphocytic count						p value
	Low		Moderate		High		
	N	%	N	%	N	%	
pT Stage 1(N=3)	0	0.0%	0	0.0%	3	100%	0.410
pT Stage 2(N=14)	0	0.0%	2	14.3%	12	85.7%	
pT Stage 3(N=16)	1	6.25%	10	62.5%	5	31.25%	
pT Stage 4a(N=5)	1	20%	4	80%	0	0.0%	
pT Stage 4b(N=1)	1	100%	0	0.0%	0	0.0%	

Figure 4: Distribution of pT stage according to CD8+T(IHC)lymphocytic count

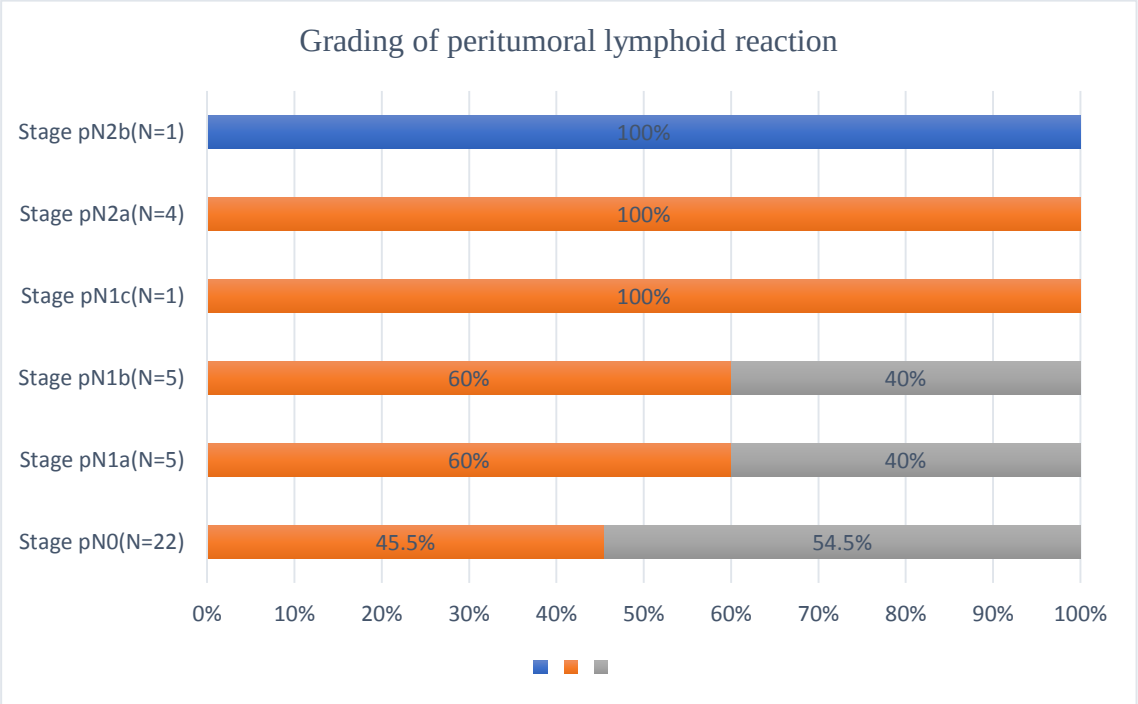


In stage pT1 and pT2 majority of the cases were showing high CD8+ T lymphocytic count. In pT stage pT4a and pT4b majority of the cases were showing moderate and low CD8+ T lymphocytic count respectively. However, the difference was statistically not significant.

Table 15: Association between stage according to Grading of peritumoral lymphoid reaction

pN stage	Grading of peritumoral lymphoid reaction						p value
	Grade 0		Grade 1		Grade 2		
	N	%	N	%	N	%	
Stage pN0(N=22)	0	00%	10	45.5%	12	54.5%	0.818
Stage pN1a(N=5)	0	0.0%	3	60%	2	40%	
Stage pN1b(N=5)	0	0.0%	3	60%	2	40%	
Stage pN1c(N=1)	0	0.0%	1	100%	0	0.0%	
Stage pN2a(N=4)	0	0.0%	4	100%	0	0.0%	
Stage pN2b(N=1)	1	100%	0	0.0%	0	0.0%	

Figure 5: Distribution of pN stage according to Grading of peritumoral lymphoid reaction

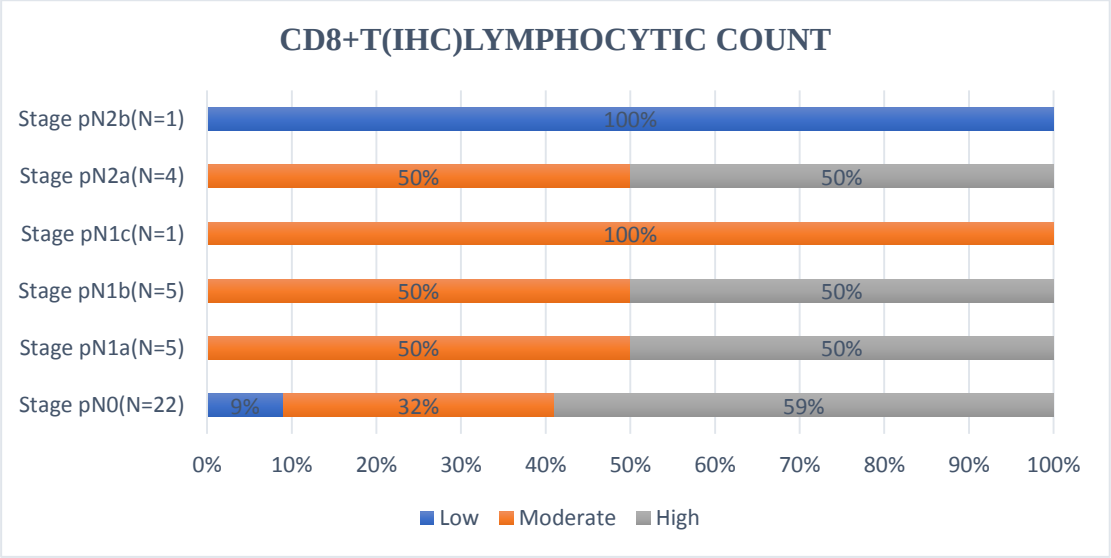


In stage pN0 majority of the cases were showing Grade 2 peritumoral LR. Only one case of stage pN2b was noted in the present study showing Grade 0 peritumoral LR.

Table 16: Association between stage according to CD8+T(IHC)lymphocytic count

pN stage	CD8+T(IHC)lymphocytic count					
	Low		Moderate		High	
	N	%	N	%	N	%
Stage pN0(N=22)	2	9%	7	32%	13	59%
Stage pN1a(N=6)	0	00%	3	50%	3	50%
Stage pN1b(N=4)	0	0.0%	2	50%	2	50%
Stage pN1c(N=1)	0	0.0%	1	100%	0	0.0%
Stage pN2a(N=4)	0	0.0%	2	50%	2	50%
Stage pN2b(N=1)	1	100%	0	0.0%	0	0.0%

Figure 6: Distribution of pN stage according to CD8+T(IHC)lymphocytic count



In pN0 stage most of the cases were showing High CD8+ T lymphocytic count. In the present study one case of stage pN2b was notedshowing low CD8+ T lymphocytic count. However, the difference was statistically not significant.

Table 17: Association between Peritumoral lymphoid reaction with grading and staging of colorectal carcinoma

Parameters	Grading of peritumoral lymphoid reaction					
	Grade 0		Grade 1		Grade 2	
	N	%	N	%	N	%
Age						
31-40	1	11%	8	89%	0	0%
41-50	0	0%	4	57%	3	43%
51-60	0	0%	7	64%	4	36%
61-70	0	0%	4	50%	4	50%
71-80	0	0%	3	100%	0	0%
Age (Mean±SD)	60±0		51.1±13.9		54.8±11.7	
Sex distribution						
Male	1	5%	14	74%	4	21%
Female	0	0%	12	63%	7	37%
Tumour site						
Distal colon	0	0%	8	62%	5	38%
Proximal colon	1	7%	9	64%	4	29%
Rectum	0	0%	9	82%	2	18%
Histological grade						
Moderately Differentiated Adenocarcinoma	0	0%	25	74%	9	26%
Poorly Differentiated Adenocarcinoma	1	50%	1	50%	0	0%
Well Differentiated Adenocarcinoma	0	0%	0	0%	2	100%
pT stage						
1	0	0%	0	0%	2	100%
2	0	0%	6	40%	9	60%
3	0	0%	11	69%	5	31%
4a	0	0%	4	100%	0	0%
4b	1	100%	0	0%	0	0%
pN stage						

pN0	0	0%	10	45.5%	12	54.5%
pN1a	0	0%	3	60%	2	40%
pN1b	0	0%	3	60%	2	40%
pN1c	0	0%	1	100%	0	0%
pN2a	0	0%	4	100%	0	0%
pN2b	1	100%	0	0%	0	0%

There was no significant association of peritumoral LR when association was compared with age and sex of the patients. When association of peritumoral LR was studied for tumour site peritumoral lymphoid reaction was high in rectum as compared to proximal colon.

Peritumoral lymphoid reaction was high in well differentiated adenocarcinoma, pT stage 1 and stage pN0.

Table 18: Association of CD8+ T lymphocytic count (IHC) with grading and staging of colorectal carcinoma.

Parameters	CD8+T(IHC)lymphocytic count					
	Low		Moderate		High	
	N	%	N	%	N	%
Age						
31-40	1	11%	2	22%	6	67%
41-50	0	0%	3	43%	4	57%
51-60	2	18%	5	45%	4	36%
61-70	1	13%	3	38%	4	50%
71-80	1	33%	1	33%	1	33%
Age (Mean±SD)	58.8±15.1		54.9±11.2		49.8±13.1	
Sex distribution						
Male	2	11%	8	42%	9	47%
Female	3	16%	6	32%	10	53%
Tumour site						
Distal colon	0	0%	4	31%	9	69%
Proximal colon	3	21%	5	36%	6	43%
Rectum	2	18%	5	45%	4	36%
Histological grade						
Moderately differentiated adenocarcinoma	3	9%	17	50%	14	41%
Poorly differentiated adenocarcinoma	2	100%	0	0%	0	0%
Well differentiated adenocarcinoma	0	0%	0	0%	2	100%
pT stage						
1	0	0%	0	0%	3	100%
2	0	0%	2	14.3%	12	85.7 %
3	1	6.25%	10	62.5%	5	31.25 %
4a	1	20%	4	80%	0	0%
4b	1	100%	0	0%	0	0%

pN stage						
pN0	2	9%	7	32%	13	59%
pN1a	0	0%	3	50%	3	50%
pN1b	0	0%	2	50%	2	50%
pN1c	0	0%	1	100%	0	0%
pN2a	0	0%	2	50%	2	50%
pN2b	1	100%	0	0%	1	20%

CD8+ T lymphocytic count was high in 31 to 40 years of age group and was slightly higher in males as compared to females. When association of CD8+ T lymphocytic count was studied for tumour site CD8+ T lymphocytic count was high in distal colon. CD8+ T lymphocytic count was high in well differentiated adenocarcinoma, pT stage 1 and stage pN0.

GROSS IMAGES AND PHOTOMICROGRAPH

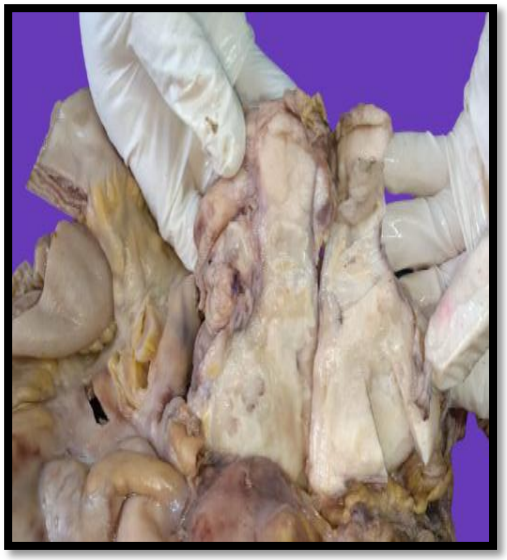


Fig.7 Gross morphology of resected colorectal specimen

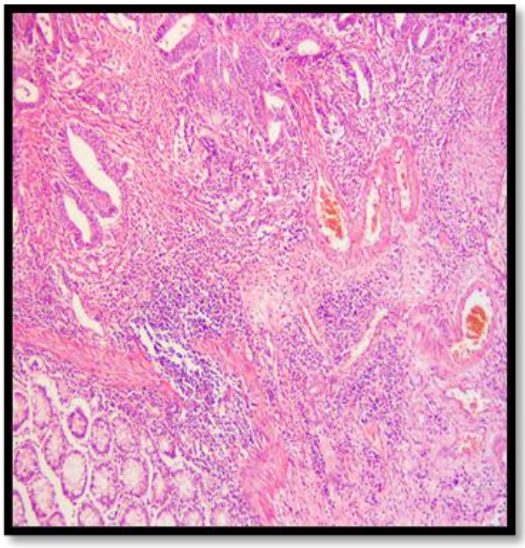


Fig.8 Photomicrograph of Mod. Diff. Adenocarcinoma showing grade 2 Peritumoral LR (H & E stain,100X)

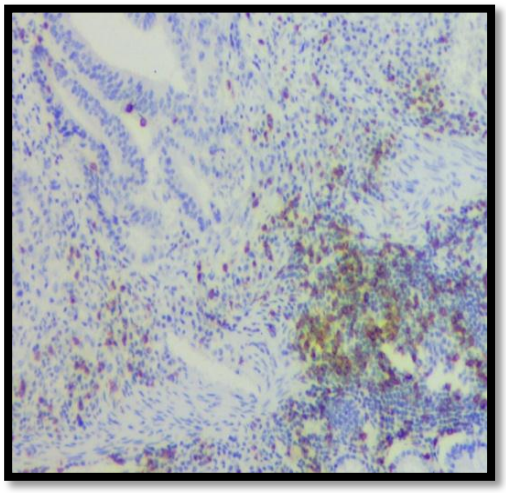


Fig.9 Photomicrograph of Mod. Diff. Adenoca. showing high CD8+T lymphocytic count (IHC marker,100X)

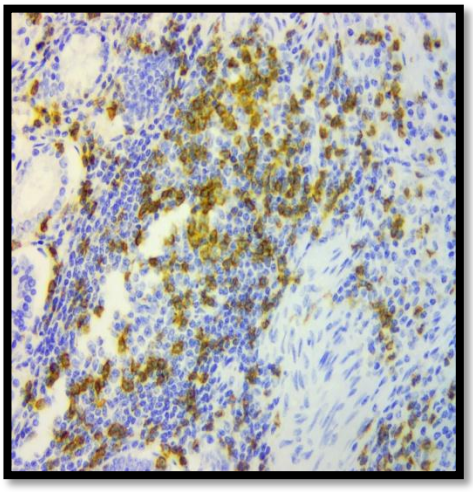


Fig.10 Photomicrograph of Mod. Diff. Adenoca. Showing high CD8+ T lymphocytic count (IHC marker,400X)

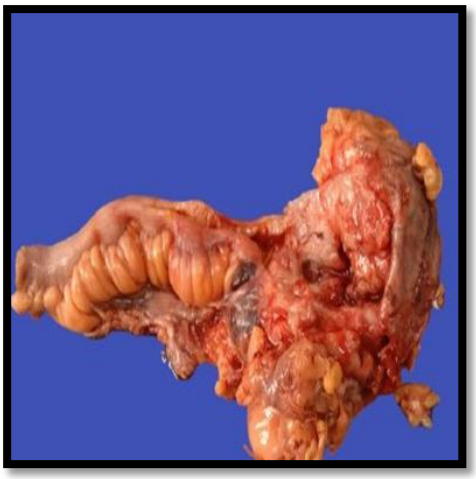


Fig.11 Gross morphology of resected colorectal specimen

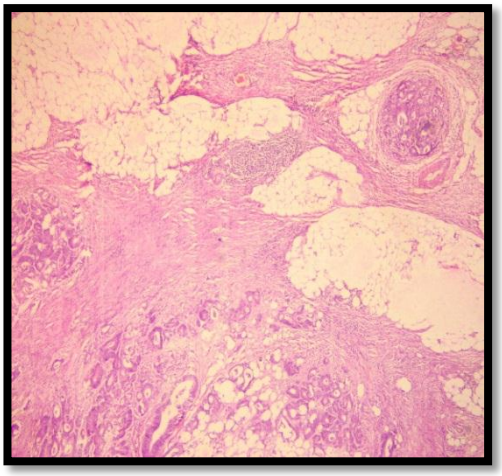


Fig.12 Photomicrograph of Mod. Diff. Adenoca.showing grade 1 Peritumoral LR (H & E stain,100X)

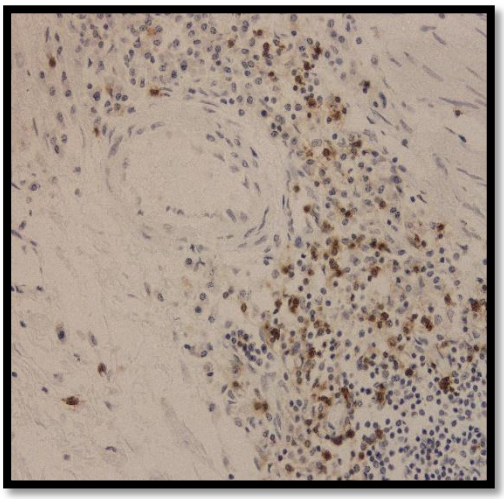


Fig.13 - Photomicrograph of Mod. Diff. Adenoca. showing high CD8+ T lymphocytic count (IHC marker,400X)

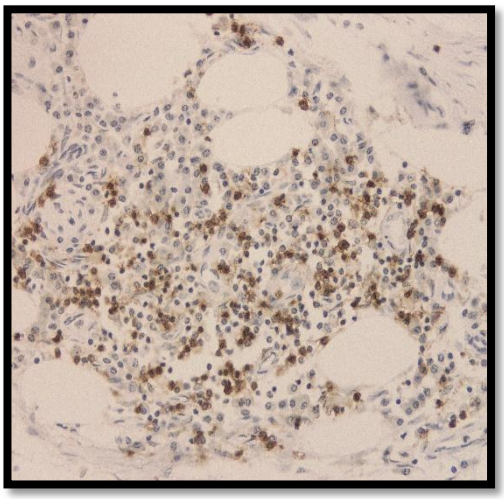


Fig.14 Photomicrograph of Mod. Diff. Adenoca. showing high CD8+ T lymphocytic count (IHC marker,400X)

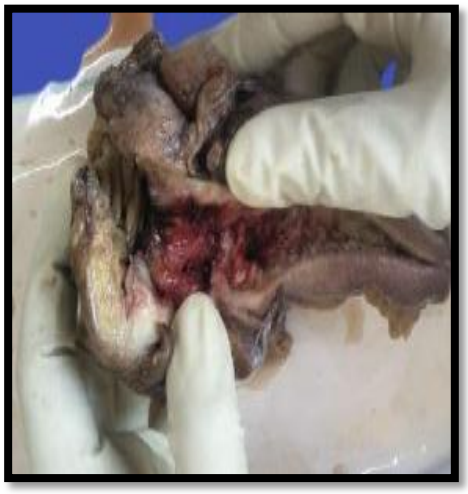


Fig.15 Gross morphology of resected colorectal specimen

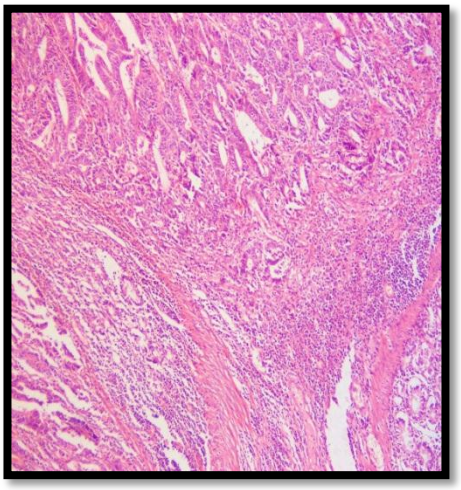


Fig.16 Photomicrograph of Mod. Diff. Adenoca. showing grade 1 Peritumoral LR (H & E stain,100X)

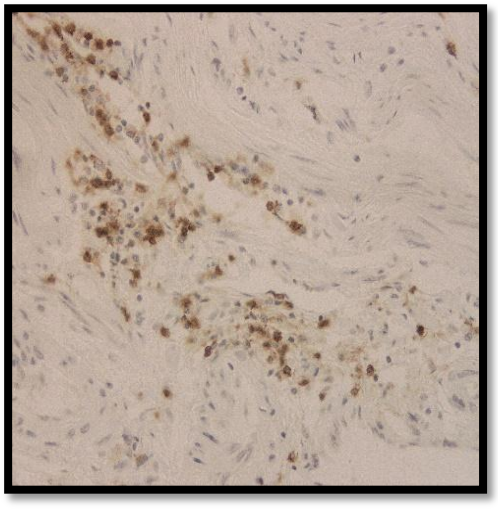


Fig.17 Photomicrograph of Mod. Diff. Adenoca. showing low CD8+T lymphocytic count (IHC marker,40X)

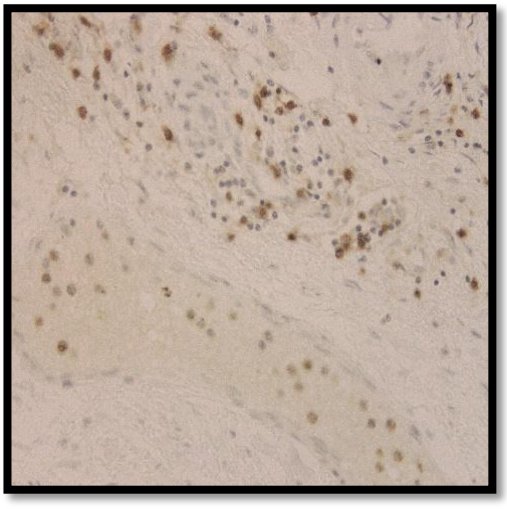


Fig.18- Photomicrograph of Mod. Diff. Adenoca. showing low CD8+ T lymphocytic count (IHC marker,400X)

Fig.19 Gross morphology of resected colorectal specimen

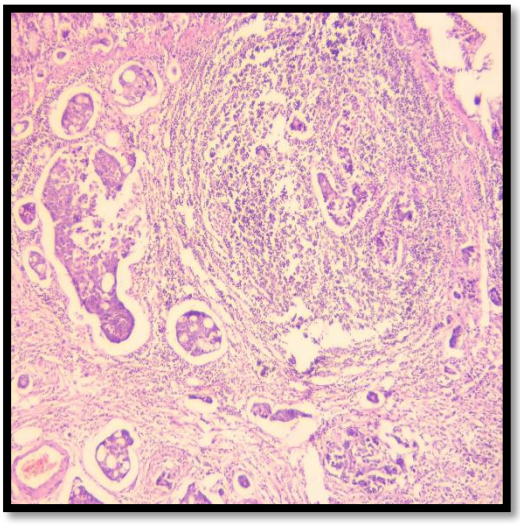


Fig.20 Photomicrograph of Mod. Diff. Adenoca. showing grade 2 Peritumoral LR (H & E stain,100X)

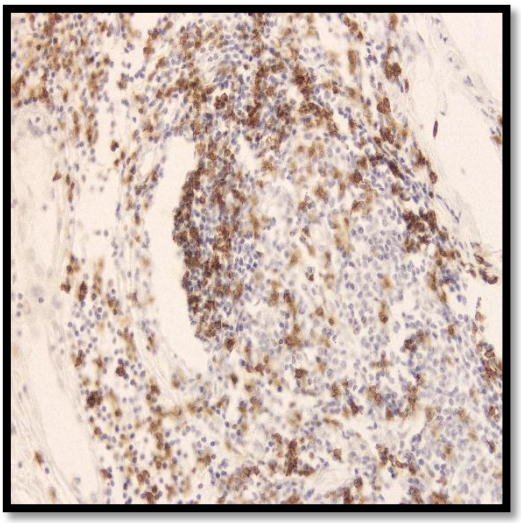


Fig.21 Photomicrograph of Mod. Diff. Adenoca. showing high CD8+ T lymphocytic count (IHC marker,400X)

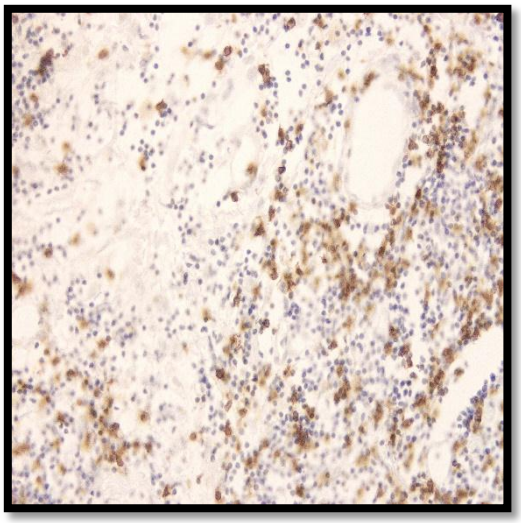


Fig.22 - Photomicrograph of Mod. Diff. Adenoca. showing high CD8+ T lymphocytic count (IHC marker,400X)

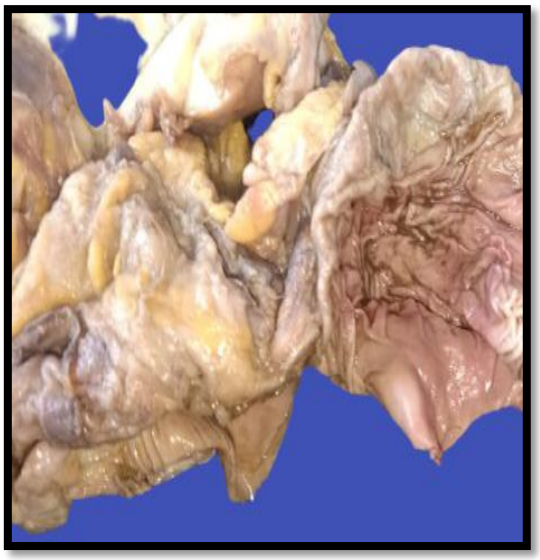


Fig.23 Gross morphology of resected colorectal specimen

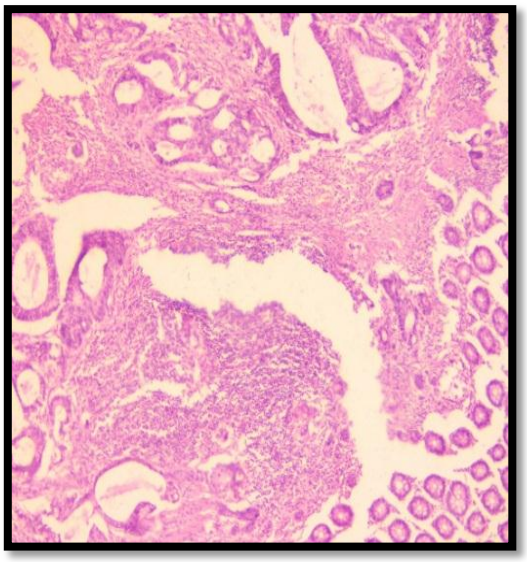


Fig.24 Photomicrograph of Mod. Diff. Adenoca. showing grade 1 Peritumoral LR (H & E stain,100X)

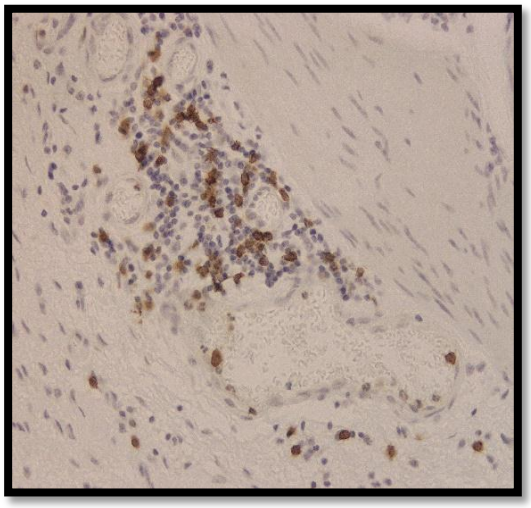


Fig.25 Photomicrograph of Mod. Diff. Adenoca. showing moderate CD8+ T lymphocytic count (IHC marker,400X)

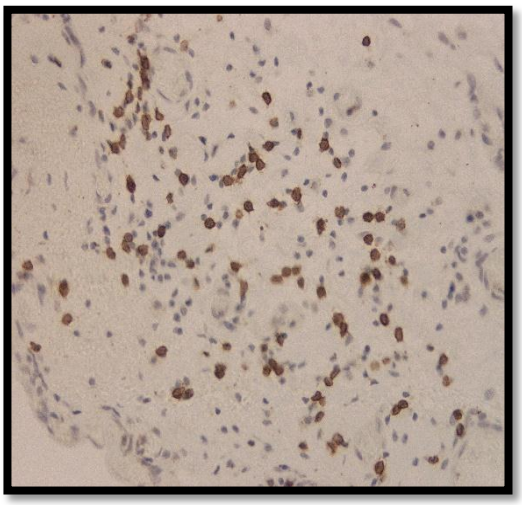


Fig.26 Photomicrograph of Mod. Diff. Adenoca. showing moderate CD8+ T lymphocytic count (IHC marker,400X)

Fig.27 Gross morphology of resected colorectal specimen

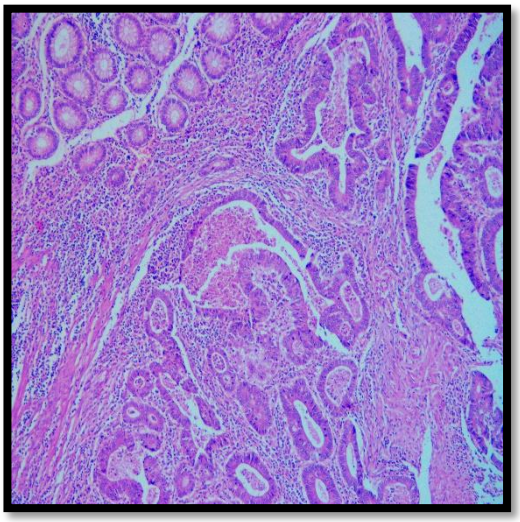


Fig.28 Photomicrograph of Well Diff. Adenoca. showing grade 2 Peritumoral LR (H & E stain,100X)

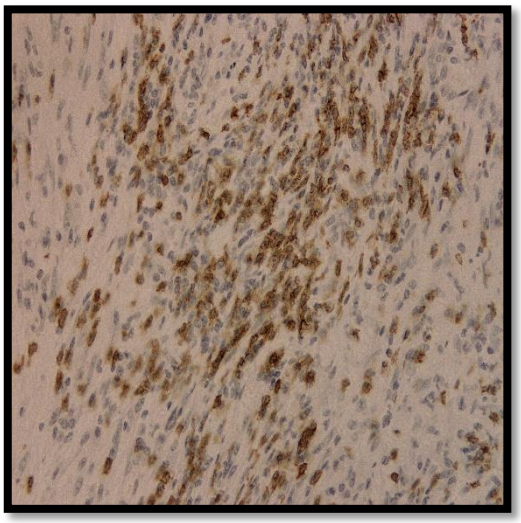


Fig.29 Photomicrograph of Well Diff. Adenoca. showing high CD8+ T lymphocytic count (IHC marker,400X)

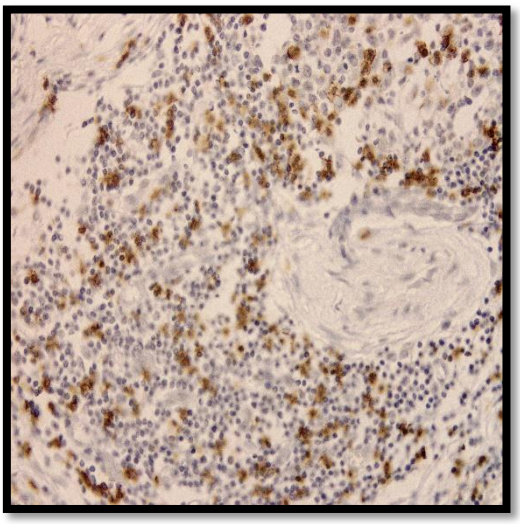


Fig.30 Photomicrograph of Well Diff. Adenoca. showing high CD8+ T lymphocytic count (IHC marker,400X)

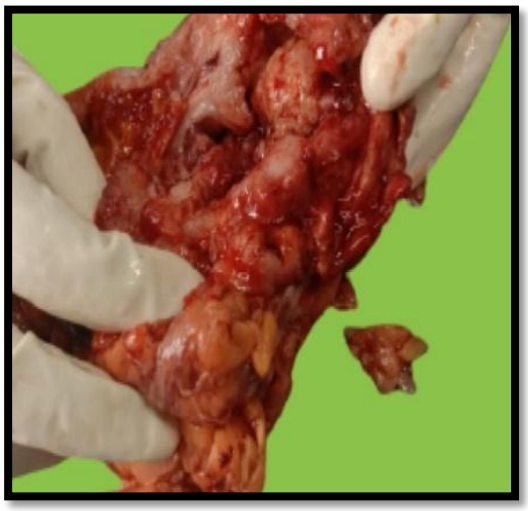


Fig.31 Gross morphology of resected colorectal specimen

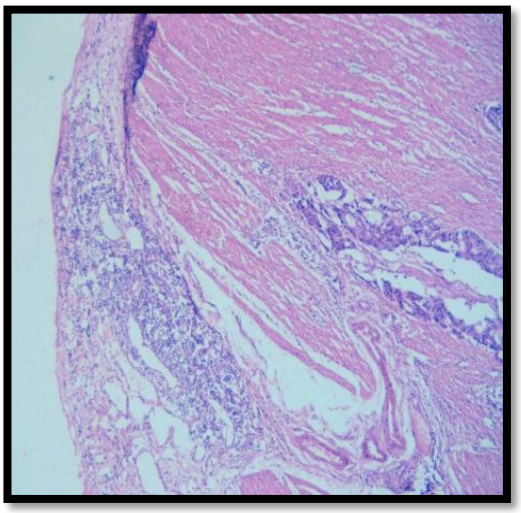


Fig.32Photomicrograph of Well Diff. Adenoca. showing grade 2 Peritumoral LR (H & E stain,100X)

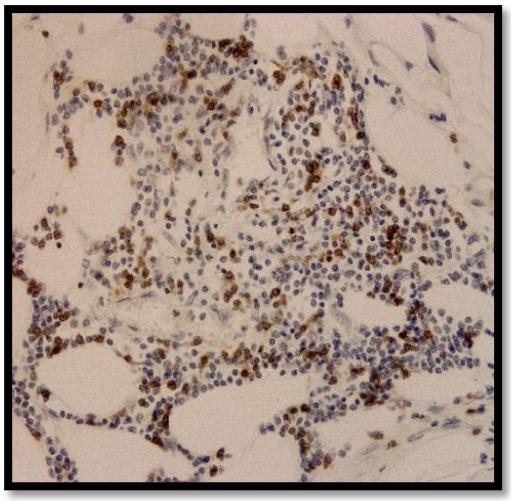


Fig.33 Photomicrograph of Well Diff. Adenoca. showing high CD8+ T lymphocytic count (IHC marker,400X)

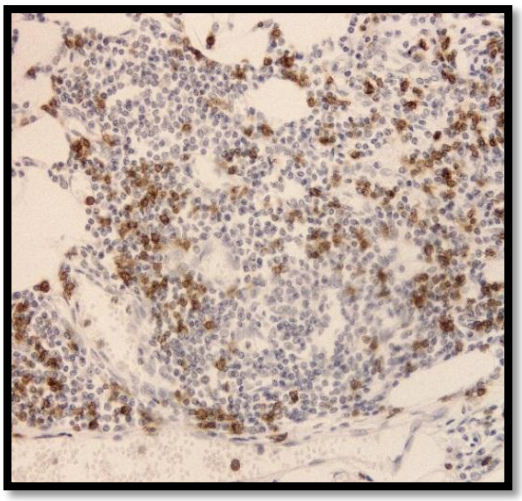


Fig.34 Photomicrograph of Well Diff. Adenoca. showing highCD8+ T lymphocytic count (IHC marker,400X)

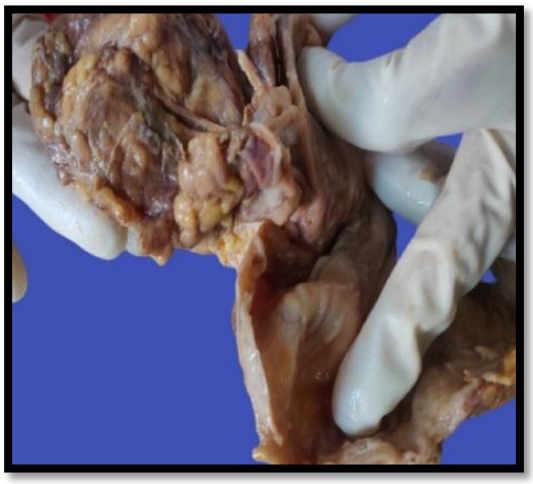


Fig.35 Gross morphology of resected colorectal specimen

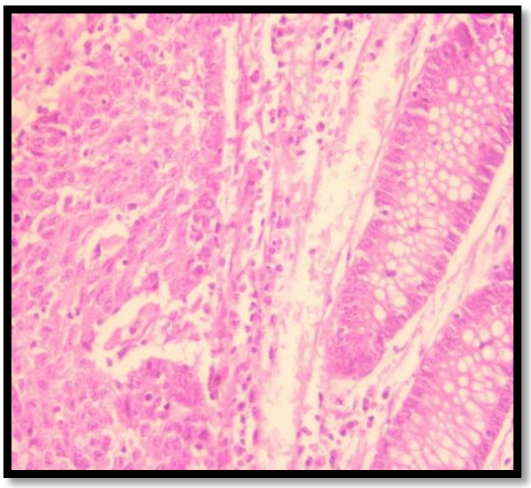


Fig36 Photomicrograph of Poorly. Diff. Adenoca. showing grade 1 Peritumoral LR (H & E stain,100X)

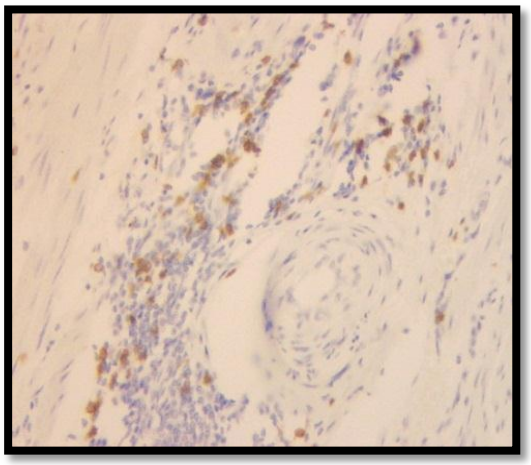


Fig.37 Photomicrograph of Poorly Diff. Adenoca. showing low CD8+ T lymphocytic count (IHC marker,400X)

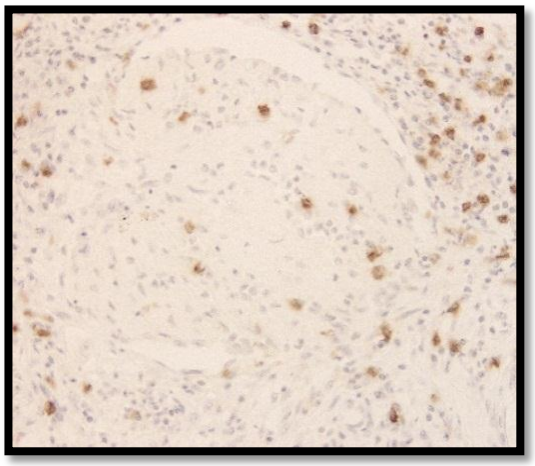


Fig.38 Photomicrograph of Poorly Diff. Adenoca. showing low CD8+ T lymphocytic count (IHC marker,400X)

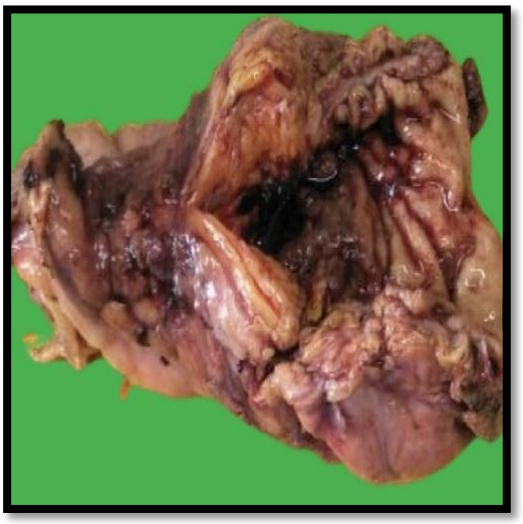


Fig.39 Gross morphology of resected colorectal specimen

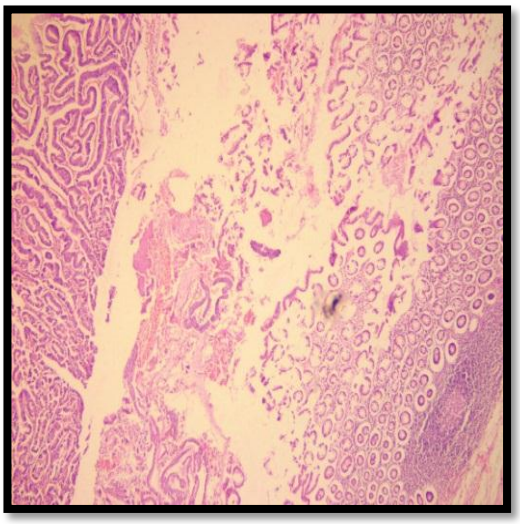


Fig.40 Photomicrograph of Mod. Diff. Adenoca. showing grade 2 Peritumoral LR (H & E stain,100X)

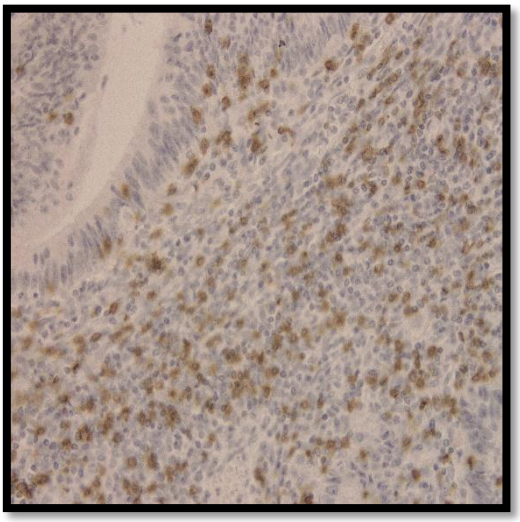


Fig.41 Photomicrograph of Mod. Diff. Adenoca. showing high CD8+ T lymphocytic count (IHC marker,400X)

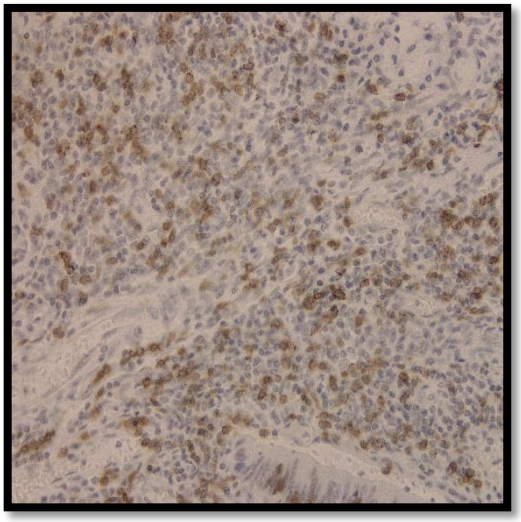


Fig.42 Photomicrograph of Mod. Diff. Adenoca. showing high CD8+ T lymphocytic count (IHC marker,400X)

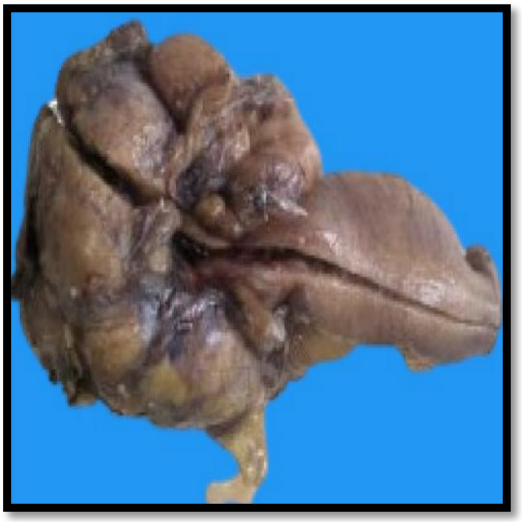


Fig.43 Gross morphology of resected colorectal specimen

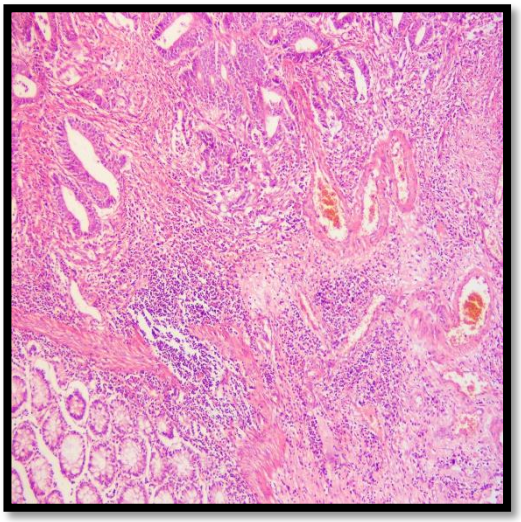


Fig.44 Photomicrograph of Mod. Diff. Adenoca. showing grade 2 Peritumoral LR (H & E stain.100X)

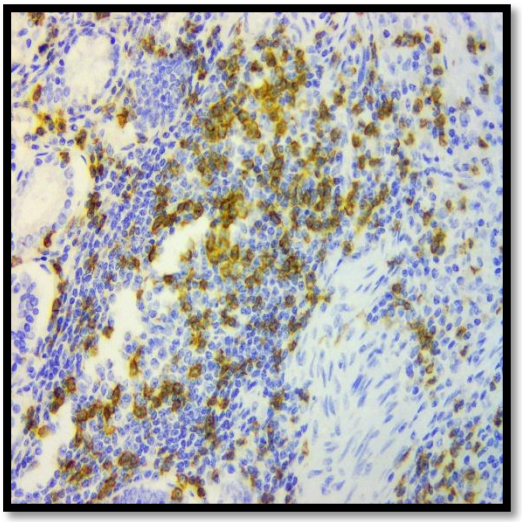


Fig.45 Photomicrograph of Mod. Diff. Adenoca. showing moderate CD8+ T lymphocytic count (IHC marker,400X)

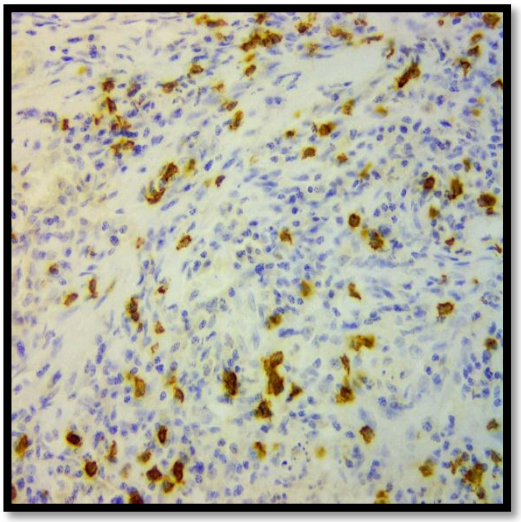


Fig.46 Photomicrograph of Mod. Diff. Adenoca. showing moderate CD8+ T lymphocytic count (IHC marker,400X)

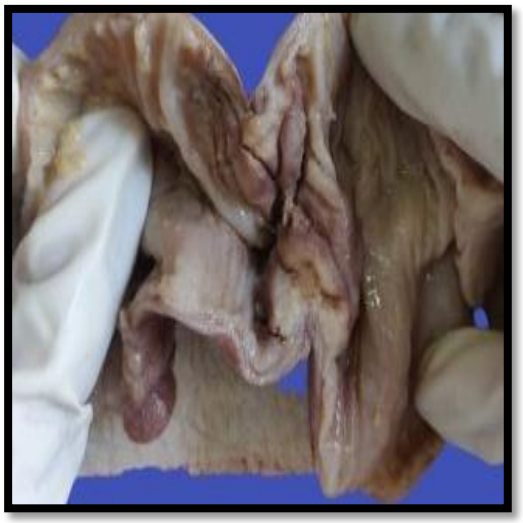


Fig.47 Gross morphology of resected colorectal specimen

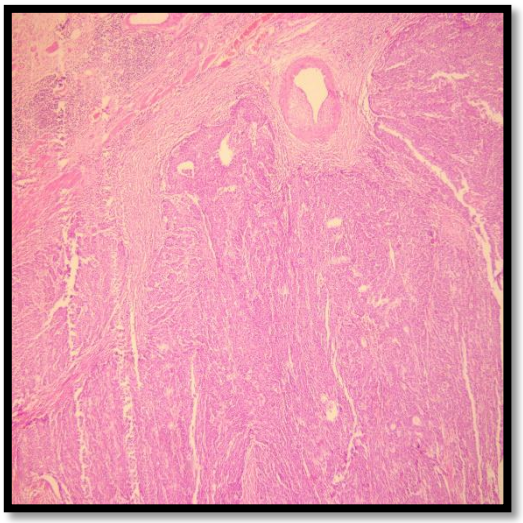


Fig.48 Photomicrograph of Poorly Diff. Adenoca. showing grade 0 Peritumoral LR (H & E stain,100X)

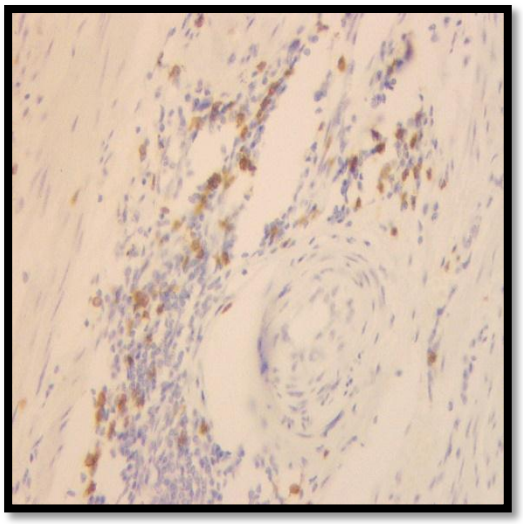


Fig.49 Photomicrograph of Poorly Diff. Adenoca. showing low CD8+ T lymphocytic count (IHC marker,400X)

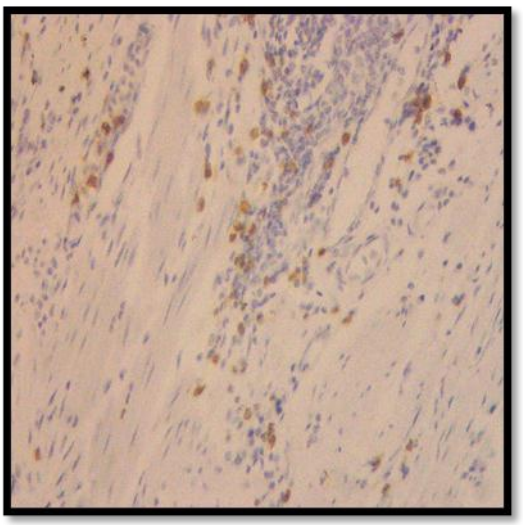


Fig.50 Photomicrograph of Poorly Diff. Adenoca. showing low CD8+ T lymphocytic count (IHC marker,400X)

Fig.51 Gross morphology of resected colorectal specimen

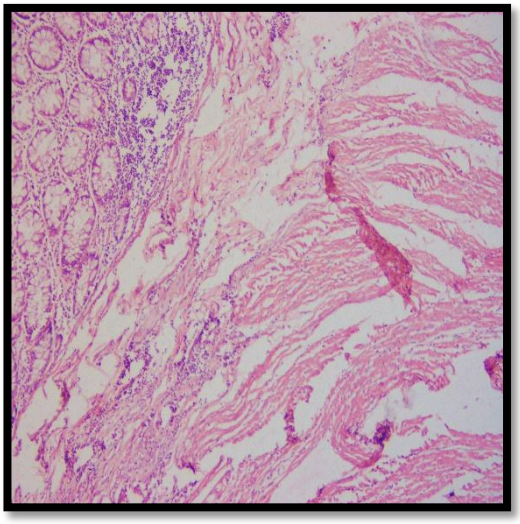


Fig.52 Photomicrograph of Mod. Diff. Adenoca. showing grade 1 Peritumoral LR (H & E stain,100X)

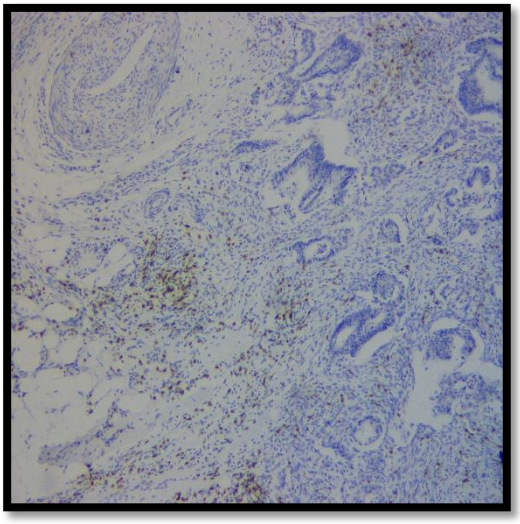


Fig.53 Photomicrograph of Mod. Diff. Adenoca. showing high CD8+ T lymphocytic count (IHC marker,100X)

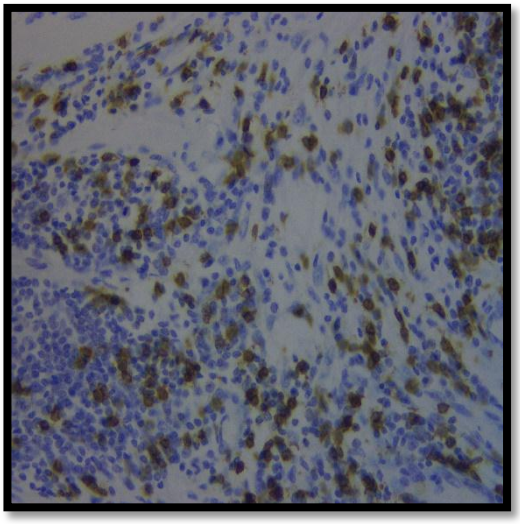


Fig.54 Photomicrograph of Mod. Diff. Adenoca. showing high CD8+ T lymphocytic count (IHC marker,400X)

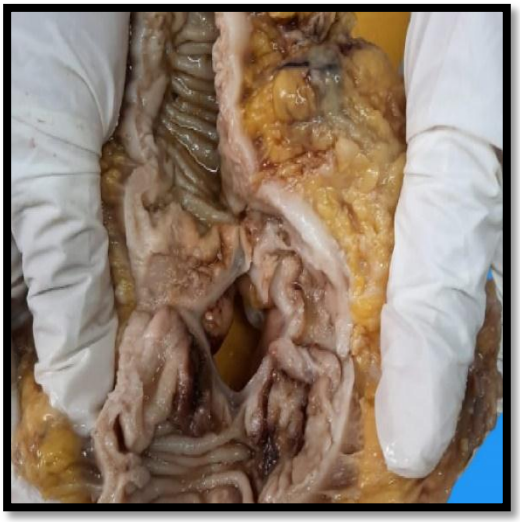


Fig.55 Gross morphology of resected colorectal specimen

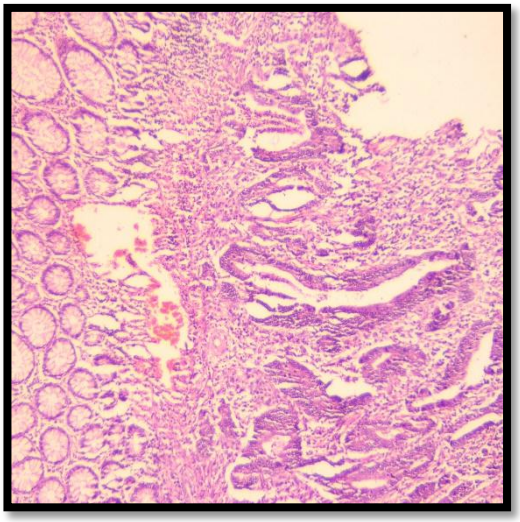


Fig.56 Photomicrograph of Mod. Diff. Adenoca. showing grade 1 Peritumoral LR (H & E stain,100X)

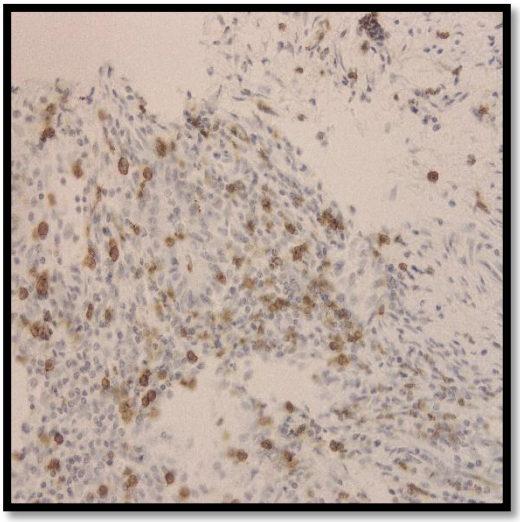


Fig.57 Photomicrograph of Mod. Diff. Adenoca. showing moderate CD8+ T lymphocytic count (IHC marker,400X)

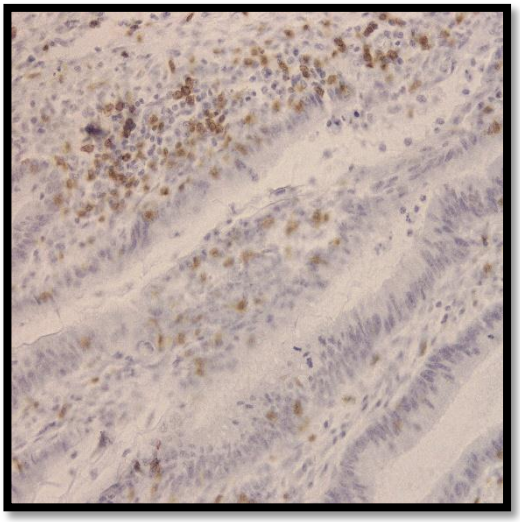


Fig.58 Photomicrograph of Mod. Diff. Adenoca. showing high CD8+ T lymphocytic count (IHC marker,400X)

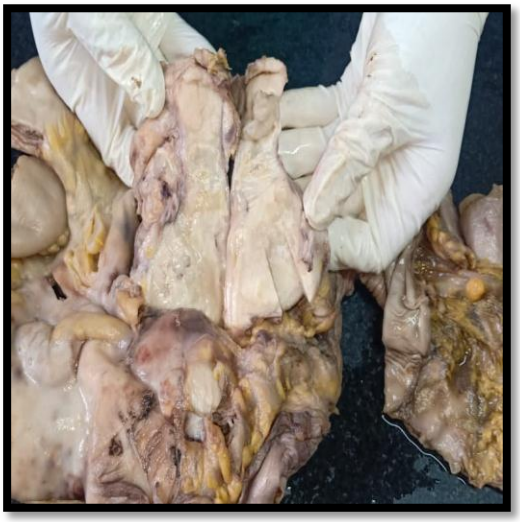


Fig.59 Gross morphology of resected colorectal specimen

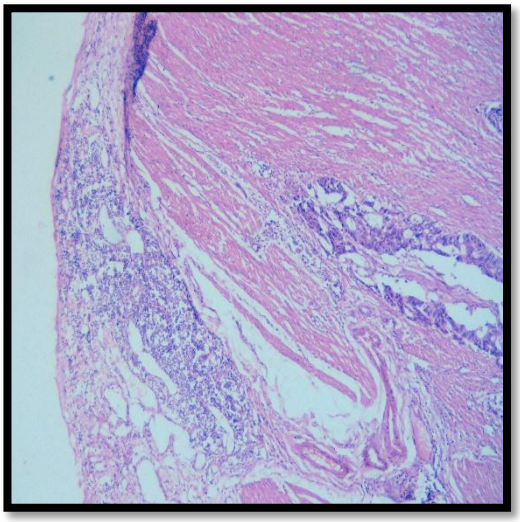


Fig.60 Photomicrograph of Mod. Diff. Adenoca. showing grade 2 Peritumoral LR (H & E stain,100X)

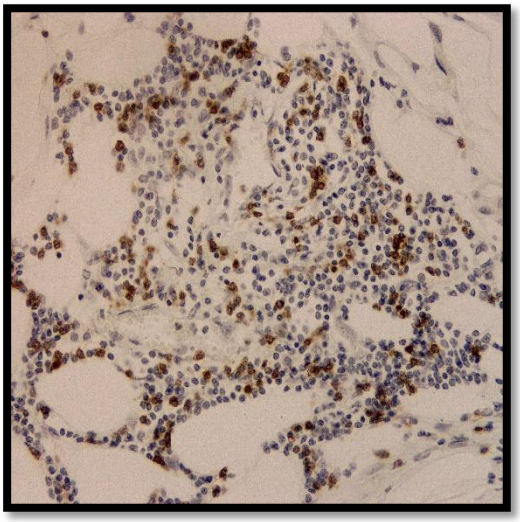


Fig.61 Photomicrograph of Mod. Diff. Adenoca. showing high CD8+ T lymphocytic count (IHC marker,400X)

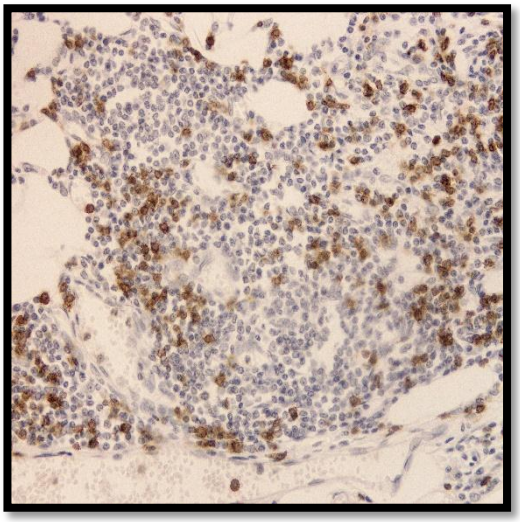


Fig.62 Photomicrograph of Mod. Diff. Adenoca. showing high CD8+ T lymphocytic count (IHC marker,400X)

DISCUSSION

Lymphocytic reaction in the resected specimens of CRC has long been recognized as an indicator of host immune responses to tumor cells. Present study focuses on Peritumoral LR, CD8 +T lymphocytic count and its association with Grading and staging in resected specimens of CRC.[19]

Age group of patients in this study varied from 30 to 80 years with a mean age of 52.8 years. Most of the cases were in the age group of 50-59 years (28.9%). In study done by Vayrynen JP *et al*[3], Ueno et al[19], Rozek LS *et al*[36], Kleist *et al*[2], Hu X *et al*[37] mean age was 67,61.8,70.1,65 and 57.6 respectively. In the study done by these authors age range was 22 to100 years.

In present study, male to female ratio was 1:1. In other authors study incidence of CRC was slightly higher in females as compared to males.[3,38] In the present study high number of cases were noted in proximal colon. Similar finding was noted in various studies of CRC.[2,3,39]

Table 19: Comparison of Histological grade of CRC in present study with other authors studies

Authors	Well Differentiated Adenocarcinoma	Moderately Differentiated Adenocarcinoma	Poorly Differentiated Adenocarcinoma
Vayrynen JP *et al*[3]	101(24.2%)	246(58.9%)	71(17.0%)
Rozek LS *et al*[36]	175(28.2%)	346(55.7%)	97(15.6%)
Present study	2(5.3%)	34(89.5%)	2(5.3%)

In the present study maximum number of cases were moderately differentiated adenocarcinoma amounting to 89.5%. followed by well differentiated

adenocarcinoma (5.3%) and poorly differentiated adenocarcinoma (5.3%). Similar observations were noted in studies done by other authors, however in their studies percentage of moderately differentiated adenocarcinoma varied from 55 to 58%.[3,36]

In the present study grading of peritumoral LR was done as Grade 0, Grade1 and Grade 2 and it was observed that in all cases of well differentiated adenocarcinoma Grade 2 PLR was observed. Vayrynen JP *et al*[3] in their study after grading of PLR they did further evaluation of LR as CLR density median. In their study CLR density median was 0.41 in well differentiated adenocarcinoma. Uneno et al[19] in their study PLR was categorized into two groups, active CLR and inactive CLR. In active CLR size of LA was 1mm or more than 1mm and in inactive CLR size of LA was less than 1mm. However, in their study they have done correlation of LA with prognosis and survival of the patient. In the present study moderately differentiated adenocarcinoma in 74% of the cases Grade 1 PLR was noted and in poorly differentiated adenocarcinoma Grade 0 was noted in 50% of the cases and grade 1 in 50% of the cases. In study done by Vayrynen JP *et al*[3] CLR density median was 0.43 in moderately differentiated adenocarcinoma and 0.37 in poorly differentiated adenocarcinoma. In study done by Vayrynen JP *et al*[3] high density CLR (≥0.38 LAs/mm) was associated with well differentiated adenocarcinoma whereas low density CLR associated with poorly differentiated adenocarcinoma. Similar observations were noted in the present study.

In study done by Vayrynen JP et al[3] CLR density was highest in stage 1 amounting to 0.49 and in stage 2 and 3 CLR density medians was 0.45 and 0.40 respectively. In stage 4 in their study CLR density median was lowest amounting to 0.31. In the present study PLR was highest in stage 1 followed by stage 2 amounting to 100% and 60% respectively. In stage 3, Grade 2 PLR was noted in 31% of the

cases and in stage 4a, PLR was grade 1 in 100% of the cases, in stage 4b PLR was grade 0 in all cases. However, in stage 4b, grade 0 was observed in 100% of cases indicating that PLR is significantly associated with pT staging of the tumor.

In study done by Vayrynen JP *et al*[3] CLR density was highest in pN0 amounting to 0.45 and in stage pN1a CLR density median was 0.39. In stage pN2a CLR density median was lowest amounting to 0.31. In the present study PLR was highest in stage pN0 followed by stage pN1a and stage pN1b. In stage pN2a, Grade 1 PLR was noted in 100% of cases. In stage pN2b PLR grade 0 was observed in all cases. However, in stage pN2b, grade 0 was observed in 100% of cases indicating that PLR is associated with pN staging of the tumor.

In present study scoring of CD8+ T lymphocytic count was categorized into low, moderate and high. It was observed that all cases of well differentiated adenocarcinoma showed high and all cases of poorly differentiated adenocarcinoma showed low CD8+ T lymphocytic count. In moderately differentiated adenocarcinoma 52.9% of cases were showing high count followed by moderate and low CD8+ T lymphocytic count. In study done by Kleist *et al*[2] well and moderately differentiated adenocarcinoma were showing high (94.1%) CD8+T lymphocytic count. Whereas poorly differentiated adenocarcinoma was showing CD8+T lymphocytic count negative in 33.3% and low in 20% cases. Similar observations were noted in the present study. Calik I *et al*[40] in their study mentioned that CD8+ T lymphocytic density score low when the mean CD8+ T lymphocytic count was less than 200 and high when it was more than 200. In their study in poorly differentiated adenocarcinoma CD8+ T lymphocytic density was lowest in 69.6%. Present study findings were correlating with these findings.

In study done by Kleist *et al*[2] CD8+ T lymphocytic count was high in pT 2 and pT3 stages amounting to 82.7% and 82.4% respectively and low CD8+ T lymphocytic count was noted in pT4 stage. In study done by Calik I et al[40] it was observed that CD8+ T lymphocytic density was 53.8% and 46.9% in stage pT1 and stage pT2 respectively. In stage pT3 CD8+ T lymphocytic count was low in 73.1%. In present study highest CD8+ T lymphocytic count was in stage pT1 followed by stage pT2 amounting to 100% and 85.7% respectively, indicating that CD8+ T lymphocytic count is significantly associated with pT staging of the tumor.

In the present study highest CD8+ T lymphocytic count observed in stage pN0 followed by stage pN1a and stage pN1b. In study done by Calik I *et al*[40] high CD8+ T lymphocytic density was noted in pN0 amounting to 53.8%. These findings are correlating with the present study findings.

SUMMARY

A retrospective and prospective observational study of quantitative evaluation of lymphoid reaction and CD8+ T-lymphocytes and its association with grading and staging of colorectal carcinoma received in the Department of Pathology from 2015 to 2020 were evaluated for age and sex of the patient, tumor location, histological grading, pT and pN staging. Total 38 cases of resected specimens of CRC were studied. Tumor sections were evaluated for LR in peritumoral area. Tumor tissue blocks of same sections were processed for CD8+ T lymphocytic marker and CD8+ T lymphocytic marker score was evaluated.

Age of the youngest CRC patient in the present study was 30 years and oldest patient was 80 years with a mean age of 52.8 years. Male to female ratio in the present study was 1:1 and commonest site of CRC was proximal colon followed by distal colon

Majority of the cases of CRC in the present study were Moderately Differentiated Adenocarcinoma amounting to 89%. Majority of the cases were of stage pT3 amounting to 42.1% followed by stage pT2 and stage pT4. Majority of the cases amounting were of stage pN0followed by pN1 stage.

Out of 38 cases of CRC, 20 (52.6%) cases were showing high CD8+ Lymphocytic count. Peritumoral LR was highest in well differentiated adenocarcinoma. In 25 cases of moderately differentiated adenocarcinoma Grade 1 peritumoral LR was observed. Statistically significant difference was noted in grading of peritumoral LR between well, moderate and poorly differentiated adenocarcinoma having p value 0.001.

In moderately differentiated adenocarcinoma out of 34 cases in 17 cases moderate CD8+ T lymphocytic count and 14 cases high CD8+ T lymphocytic count

was noted. In all cases of well differentiated adenocarcinoma CD8+ T lymphocytic count was high. In all cases of poorly differentiated adenocarcinoma CD8+ T lymphocytic count was low. There was statistically significant difference in CD8+ T lymphocytic count having p value 0.032.

In stage pT1 and pT2 majority of the cases were showing Grade 2 peritumoral LR amounting to 100% and 60% respectively. Peritumoral LR was higher in pT stage 1 and lowest in pT stage 4b. However, the difference was statistically not significant. In stage pT1 and pT2 majority of the cases were showing high CD8+ T lymphocytic count. In pT stage pT4a and pT4b majority of the cases are showing low CD8+ T lymphocytic count.

In stage pN0 majority of the cases were showing Grade 2 peritumoral LR.In pN0 stage most of the cases were showing High CD8+ T lymphocytic count.

CONCLUSION

Peritumoral LR and CD8+T lymphocyte count were high in moderately differentiated adenocarcinoma as compared to poorly differentiated adenocarcinoma. Peritumoral LR was high in Stage 1 to stage 3 as compared to stage 4b. Also, CD8+ T lymphocytic count was high in stage 1 as compared to stage 4. These findings suggest that there is association of peritumoral LR and grading and staging of CRC and can be considered as prognostic markers for CRC. However, sample size of well and poorly differentiated adenocarcinoma are very less in number and further extensive evaluation with good number of cases is needed to conclude Peritumoral LR and CD8+T lymphocyte count as prognostic marker of CRC.

Limitation:

Low sample size and follow up was not possible in many cases hence cannot predict prognosis.

Recommendations:

Extensive evaluation with a greater number of cases and standardization of the methods used for quantitative evaluation of peritumoral lymphoid reaction and CD8+T lymphocyte count.

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ANNEXURE-I

ETHICAL CLEARANCE

B.L.D.E (Deemed to be University)
SHRI.B.M.PATIL MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE
VIJAYAPUR – 586103

IEC/NO: 286/2018
17-11-2018

INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 13-11-2018 at 03-15 PM scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has accorded Ethical Clearance.

Title : Quantitative evaluation of lymphoid reaction and CD8+T lymphocytes and It's association with grading and staging of colorectal carcinoma-5 years study (3 years retrospective and 2 years prospective study)

Name of P.G. Student : Dr Getha Rani Lingutla.
Department of Pathology.

Name of Guide/Co-investigator: Dr.Surekha.U.Arakeri, Professor of Pathology.

DR RAGHAVENDRA KULKARNI
CHAIRMAN
Institutional Ethical Committee
Medical College Hospital & Research Centre
Vijayapur-586103.

Following documents were placed before E.C. for Scrutinization:

1) Copy of Synopsis/Research Project
2) Copy of informed consent form.
3) Any other relevant documents.

ANNEXURE-II

BLDE (DEEMED TO BE UNIVERSITY),

SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTER, VIJAYAPURAA-586103

INFORMED CONSENT FOR PARTICIPATION

INDISSERTATION/RESEARCH

I, the undersigned,___, S/O D/O W/O_____, aged_years, ordinarily resident of____do hereby state/declare that Dr_____of _____Hospital has examined me thoroughly on ___at_____(place) and it has been explained to me in my own language that I am suffering from __ disease (condition) and this disease/condition mimic following diseases. Further Doctor informed me that he/she is conducting dissertation/research titled under the guidance of Dr _____ requesting my participation in the study. Apart from routine treatment procedure, the pre-operative, operative, post-operative and follow-up observations will be utilized for the study as reference data.

Doctor has also informed me that during conduct of this procedure adverse results may be encountered. Among the above complications most of them are treatable but are not anticipated hence there is chance of aggravation of my condition and in rare circumstances it may prove fatal in spite of anticipated diagnosis and best treatment made available. Further Doctor has informed me that my participation in this study will help in evaluation of the results of the study which is useful reference to treatment of other similar cases in near future, and also, I may be benefited in getting relieved of suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept

secret and not assessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on information given by me, I can ask any clarification during the course of treatment / study related to diagnosis, procedure of treatment, result of treatment or prognosis. At the same time I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of treatment, I the undersigned Shri/Smt____under my full conscious state of mind agree to participate in the said research/dissertation.

Signature of patient: Signature of doctor:

Witness: 1.

2.

Date:

Place

ANNEXURE III

PROFORMA FOR STUDY:

NAME : OP/IP No.:

AGE :

SEX : D.O. A:

RELIGION : D.O. D:

OCCUPATION :

RESIDENCE :

Presenting Complaints :

Past history :

Personal history :

Family history :

General physical examination:

Pallor present/absent

Icterus present/absent

Clubbing present/absent

Lymphadenopathy present/absent

Oedema present/absent

Built poor/average/well

VITALS: PR: RR:

BP: TEMPERATURE:

WEIGHT:

SYSTEMIC EXAMINATION:

CLINICAL DIAGNOSIS:

OPERATIVE FINDINGS:

GROSS EXAMINATION FINDINGS OF RESECTED SPECIMEN:

HISTOPATHOLOGICAL EXAMINATION OF RESECTED SPECIMENS:

Tumor Site -

Histopathology Diagnosis -

Peritumoral Lymphoid reaction –

Grade 0: No LA or single small LA in both tumour section.

Grade 1: Small LAs with rare or absent germinal centres.

Grade 2: Numerous large LAs with frequent germinal centers.[3]

CD8+ T (IHC Marker) lymphocytic count –

Negative – CD8 + T lymphocytic infiltration $<1/mm^2$,

Low - CD 8+ T lymphocytic infiltration $1\text{-}59/mm^2$,

Moderate - CD 8+ T lymphocytic infiltration $60\text{-}119/mm^2$,

High – CD8 + T lymphocytic infiltration $\geq 120/mm^2$

Grading: a) Well differentiated Adenocarcinoma

b) Moderately differentiated Adenocarcinoma

c) Poorly differentiated Adenocarcinoma

Staging (AJCC 8^{th} edition): a) pT stage

b) pN stage

Table 1: Association between Peritumoral lymphoid reaction with grading and staging of colorectal carcinoma[19]

Clinical parameters	**Grade 0 = No LA or at most single small LA** (n – number of samples)	**Grade 1= Small LAs with rare or absent germinal centres** (n – number of samples)	**Grade 2 = Numerous large LAs with frequent germinal centres.** (n-number of samples)
Age(years)			
Mean(range)			
Gender			
Male			
Female			
Tumour site			
Proximal colon			
Distal colon			
Rectum			
Histological grade			
Well			
Moderate			
Poor			
pT stage			
≤2 (Tumour invasion up to muscularis propria)			
3 (Tumour invasion through muscularis propria into peri colorectal tissues)			

4 (Tumour directly invades or is adherent to other organs or structures)			
pN stage			
p0 (No regional lymph nodes)			
pN1 (1-3 regional lymph nodes)			
pN2 (≥ 4 regional lymph nodes)			

Table 2: Association between CD8+ T lymphocytic count (IHC) with grading and staging of colorectal carcinoma[2]

Clinical parameters	**Negative<1/mm^2** n– number of samples	**Low 1-59/mm^2** n – number of samples	**Moderate 60 -119/mm^2** n – number of samples	**High>120/mm^2** n – number of samples
Age (years)				
Mean (range)				
Gender				
Male				
Female				
Tumour site				
Proximal colon				
Distal colon				
Rectum				
Histological grade				
Well				
Moderate				
Poor				
pT stage				
<2 (Tumour invasion up to muscularis propria				
3 (Tumour invasion through the muscularis propria into peri colorectal tissues)				

4 (Tumour directly invades or is adherent to other organs or structures)				
pN stage				
p0 (No regional lymph nodes)				
pN1 (1-3 regional lymph nodes)				
pN2 (≥ 4 regional lymph nodes)				

KEY TO MASTERCHART

SL NO.	-	Serial Number
OP/IP No	-	Outpatient/ Inpatient Number
LABNO.	-	Laboratory Number
HPR NO.	-	Histopathology report number
Yrs	-	Years
Clinical Dx	-	Clinical Diagnosis
HPR Dx	-	Histopathology Diagnosis
IHC	-	Immuno Histo Chemistry
pT, N, M	-	Pathological Tumor size, Node, Metastasis Staging

MASTERCHART

S.No.	OP/IP No.	Pt name	Age	Sex	HPR no.	Clinical Dx	HPR Dx	Stage	Grading of peritumoral lymphoid reaction	CD8+T(IHC)lymphocytic count/ mm^2
1	17583	Sangappa	72	Male	3543/15	Rectum	Moderately Differentiated Adenocarcinoma	pT3N1b	Grade 1	Moderate (92 /mm^2)
2	82116	Mahadevi	40	Female	1417/16	Distal colon	Moderately Differentiated Adenocarcinoma	pT3N0	Grade 1	Moderate(117 /mm^2)
3	222531	Ningappa	55	Male	3829/16	Rectum	Moderately Differentiated Adenocarcinoma	pT3N0	Grade 1	Moderate (69 /mm^2)
4	21173	Malenenea	65	male	3935/16	Distal colon	Moderately Differentiated Adenocarcinoma	pT3N1	Grade 1	Moderate (110/mm^2)
5	272835	Anand	47	Male	4681/16	Rectum	Well Differentiated Adenocarcinoma	pT2N1a	Grade 2	High (221 /mm^2)
6	6852	Shankarappa	55	Male	62/17	Distal colon	Moderately Differentiated Adenocarcinoma	pT3Nx	Grade 2	High (192 /mm^2)
7	20833	Siddappa	61	Male	4225/17	Distal colon	Moderately Differentiated Adenocarcinoma	pT3N1	Grade 1	Moderate (96/mm^2)
8	22042	Basappa	58	Male	4474/17	Distal colon	Moderately Differentiated Adenocarcinoma	pT3N0	Grade 1	Moderate (118 /mm^2)
9	403429	Mulimani	30	Male	7626/17	Distal colon	Moderately Differentiated Adenocarcinoma	pT3N0	Grade 1	Moderate (86 /mm^2)
10	312180	Kamala Bai	62	Female	6093/17	Rectum	Moderately Differentiated Adenocarcinoma	pT2Nx	Grade 2	High (195/mm^2)
11	6106	Laxmi Bai	69	Female	1131/18	Rectum	Moderately Differentiated Adenocarcinoma	pT3N0	Grade 2	High (216 /mm^2)
12	8166	Malajan	58	Female	1481/18	Distal colon	Moderately Differentiated Adenocarcinoma	pT2N1a	Grade 2	High (150 /mm^2)
13	9657	Managala	54	Female	1846/18	Proximal colon	Moderately Differentiated Adenocarcinoma	pT2N0	Grade 1	Moderate (118 /mm^2)
14	142190	Chandrappa	75	Male	306/16	Proximal colon	Moderately Differentiated Adenocarcinoma	pT3N1c	Grade 1	Moderate (96 /mm^2)
15	2155	Ratnabai	80	Female	669/19	Rectum	Moderately Differentiated Adenocarcinoma	pT3N0	Grade 1	Moderate e (112 /mm^2)
16	33681	Shamimbanu	65	Female	1146/19	Distal colon	Moderately Differentiated Adenocarcinoma	pT3N0	Grade 2	High (138 /mm^2)
17	13364	Mohammadin	40	Male	4772/19	Proximal colon	Poorly Differentiated Adenocarcinoma	pT4bN2b	Grade 0	Low (45 /mm^2)
18	18264	Kulsumu	45	Female	4091/19	Proximal colon	Moderately Differentiated Adenocarcinoma	pT4aN1aM1a	Grade 1	Moderate (86 /mm^2)
19	22789	Sushilabai	30	Female	5158/19	Proximal colon	Moderately Differentiated Adenocarcinoma	pT1N0	Grade 2	High (179 /mm^2)
20	40037	Mumtaz	55	Female	676/17	Rectum	Moderately Differentiated Adenocarcinoma	pT3N0	Grade 2	High (122 /mm^2)
21	42944	mahadevappa	65	Male	209/20	Rectum	Moderately Differentiated Adenocarcinoma	pT3N1b	Grade 1	Moderate (79/mm^2)
22	27996	parashuram	38	Male	428/20	Distal colon	Moderately Differentiated Adenocarcinoma	pT2N0	Grade 2	High (217 /mm^2)
23	74431	Ayyana patar	45	Male	1203/20	Proximal colon	Moderately Differentiated Adenocarcinoma	pT2N1a	Grade 2	High (226 /mm^2)
24	93150	sayabanna nabar	60	Male	1491/20	Rectum	Moderately Differentiated Adenocarcinoma	pT2Nx	Grade 2	High (126 /mm^2)
25	6645	pooja	30	Female	1271/20	Distal colon	Moderately Differentiated Adenocarcinoma	pT1Nx	Grade 2	High (150 /mm^2)
26	24751	shakira	56	Female	404/20	Proximal colon	Moderately Differentiated Adenocarcinoma	pT2N0	Grade 2	High (134 /mm^2)
27	5939	tangevva madar	50	Female	1176/20	Proximal colon	Moderately Differentiated Adenocarcinoma	pT4aN0	Grade 1	Moderate (102 /mm^2)

28	13859	gunasagari	55	Female	2286/20	Distal colon	Moderately Differentiated Adenocarcinoma	pT2N2a	Grade 1	Moderate (98/mm^2)
29	13628	bangarewwa	60	Female	2517/20	Rectum	Moderately Differentiated Adenocarcinoma	pT2N2a	Grade 1	High (124/mm^2)
30	30517	Irrappa chandram	38	Male	7290/19	Proximal colon	Moderately Differentiated Adenocarcinoma	pT4aN0	Grade 1	Low (57 /mm^2)
31	13364	Gurappa	43	Male	3197/19	Rectum	Moderately Differentiated Adenocarcinoma	pT2N0	Grade 1	High (196 /mm^2)
32	142190	Baby nuchi	38	Female	2738/18	Proximal colon	Moderately Differentiated Adenocarcinoma	pT3N1b	Grade 2	High (122 /mm^2)
33	25850	Hanumanth	62	Male	5254/17	Proximal colon	Poorly Differentiated Adenocarcinoma	pT3N0	Grade 1	Low (58 /mm^2)
34	139818	Irrappa	58	Male	2712/17	Distal colon	Moderately Differentiated Adenocarcinoma	pT2N0	Grade 2	High (132 /mm^2)
35	231759	Shekila	38	Female	4561/17	Proximal colon	Moderately Differentiated Adenocarcinoma	pT2N2a	Grade 1	High (214 /mm^2)
36	25039	Shiddamma	50	Female	1733/15	Proximal colon	Moderately Differentiated Adenocarcinoma	pT2N1b	Grade 2	High (201 /mm^2)
37	396080	Abdul Rahman	65	Male	7432/19	Distal colon	Well Differentiated Adenocarcinoma	pT1N0	Grade 2	High (178/mm^2)
38	8831	Tangemma	42	Female	1663/20	Proximal colon	Moderately Differentiated Adenocarcinoma	pT4N2M1	Grade 1	Moderate (82 /mm^2)