HER 2 NEU AND KI67 EXPRESSION AS IMMUNOLOCALISATION IN COLORECTAL CARCINOMA

DISSERTATION

SUBMITTED TO THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY

CHENNAI

In partial fulfillment of
the requirements for the degree of

M.D. (PATHOLOGY)

BRANCH – III

DEPARTMENT OF PATHOLOGY

TIRUNELVELI MEDICAL COLLEGE HOSPITAL

TIRUNELVELI - 627011

MAY-2018

CERTIFICATE

I hereby certify that this dissertation entitled **"HER 2 NEU AND KI67 EXPRESSION AS IMMUNOLOCALISATION IN COLORECTAL CARCINOMA"** is a record of work done by **Dr. BHUVANA. G**, in the Department of Pathology, Tirunelveli Medical College, Tirunelveli, during her postgraduate degree course period from 2015- 2018. This work has not formed the basis for previous award of any degree.

The DEAN
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This is to certify that this Dissertation entitled **"HER 2 NEU AND KI67 EXPRESSION AS IMMUNOLOCALISATION IN COLORECTAL CARCINOMA"** is the bonafide original work of **Dr. BHUVANA. G**, during the period of her Post graduate study from 2015 – 2018, under my guidance and supervision, in the Department of Pathology Tirunelveli Medical College & Hospital, Tirunelveli, in partial fulfillment of the requirement for M.D., (Branch III) in Pathology examination of the Tamilnadu Dr. M.G.R Medical University will be held in MAY 2018.

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DECLARATION

I solemnly declare that this dissertation titled **"HER 2 NEU AND KI67 EXPRESSION AS IMMUNOLOCALISATION IN COLORECTAL CARCINOMA"** submitted by me for the degree of M.D, is the record work carried out by me during the period of 2015-2018 under the guidance of **Prof. Dr. K. SWAMINATHAN, M.D**, Professor of Pathology, Department of Pathology, Tirunelveli Medical College, Tirunelveli. The dissertation is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, towards the partial fulfilment of requirements for the award of M.D. Degree (Branch III) Pathology examination to be held in MAY 2018.

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ACKNOWLEDGEMENT

Though only my name appears on the cover of this dissertation, a great many people have been behind this task and I take this opportunity with immense pleasure to place on record my heartfelt gratitude and respect to all my distinguished resources.

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I shall be failing in my duty, if I do not acknowledge the contributions of my **Patients** who were involved in this study.

Finally, I thank **LORD AND MY PARENTS**, the Creator and the Guardian, without whose will and blessings, this thesis would have never blossomed.

ABBREVIATIONS

1.	Cm	Centimetre
2.	CRC	Colorectal carcinoma
3.	HER2/ neu	Human epidermal growth factor/ neuroblastoma
4.	K-RAS	Kristen- rat sarcoma virus
5.	TP53	Tumor protein 53
6.	APC	Adenomatosis polyposis coli
7.	US	United states
8.	DNA	Deoxy ribo nucleic acid
9.	MMR	Mismatch repair
10.	TGFβRII	Transforming growth factor- beta receptor
11.	MYH	MutY DNA glycosylase
12.	PIK3CA	Phosphatidylinositol -4-5,- bisphosphate 3- kinase catalytic subunit alpha
13.	LOH	Loss of heterozygosity
14.	CIN	Chromosomal instability
15.	CNS	Central nervous system
16.	MSI	Microsatellite instability
17.	MLH1	MutL homolog 1
18.	MSH	MutS protein homolog 2
19.	PMS2	Mismatch repair endonuclease enzyme
20.	CpG	Cytosine- guanine nucleotide
21.	CIMP	CpG island methylator phenotype
22.	MSS	Microsatellite stable
23.	EGFR	Epidermal growth factor receptor

23.	RAS	Rat sarcoma virus
24.	RAF	Rapidly accelerated fibrosarcoma
25.	MAPK	Mitogen activated protein kinase pathway
26.	PI3K/AKT	Phosphotidylinositol 3- kinase / protein kinase B
27.	MEK	Mitogen – activated protein
28.	PTEN	Phosphatase and tensin homolog
29.	PIP3	Phosphotidylinositol triphosphate
30.	MUC1	Mucin 1 cell surface associated
31.	CK20	Cytokeratin
32.	CDX2	Caudal- related homeobox transcription factor
33.	CEA	Carcinoembryonic antigen
34.	AFP	Alpha-feto protein
35.	TNM	Tumor node metastasis
36.	AJCC	American journal cancer committe
37.	PAS	Periodic acid Schiff
38.	TAG-72	Tumor associated glycoprotein
39.	HLA	Human leucocyte antigen
40.	HcG	Human chorionic gonadotropin
41.	PLAP	Placental alkaline phosphatase
42.	IHC	Immunohistochemistry
43.	NET	Neuroendocrine tumors
44.	NOS	Not otherwise specified

CONTENTS

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REF NO:822/PATH/2016

PROTOCOL TITLE: HER 2 NEU AND Ki67 EXPRESSION AS IMMUNOLOCALISATION IN COLORECTAL CARCINOMA

PRINCIPAL INVESTIGATOR: Dr.G. BHUVANA, MBBS.,

DESIGNATION OF PRINCIPAL INVESTIGATOR: POST GRADUATE IN PATHOLOGY
DEPARTMENT & INSTITUTION: TIRUNELVELI MEDICAL COLLEGE, TIRUNELVELI

Dear , Dr. G. Bhuvana,MBBS.,, The Tirunelveli Medical College Institutional Ethics Committee (TIREC) reviewed and discussed your application during the IEC meeting held on 03.08.2016.

THE FOLLOWING DOCUMENTS WERE REVIEWED AND APPROVED

1.	TIREC Application Form	☐
2.	Study Protocol	☐
3.	Department Research Committee Approval	☐
4.	Patient Information Document and Consent Form in English and Vernacular Language	☐
5.	Investigator's Brochure	☐
6.	Proposed Methods for Patient Accrual Proposed	☐
7.	Curriculum Vitae of the Principal Investigator	☐
8.	Insurance /Compensation Policy	☐
9.	Investigator's Agreement with Sponsor	☐
10.	Investigator's Undertaking	☐
11.	DCGI/DGFT approval	☐
12.	Clinical Trial Agreement (CTA)	☐
13.	Memorandum of Understanding (MOU)/Material Transfer Agreement (MTA)	☐
14.	Clinical Trials Registry-India (CTRI) Registration	☐

THE PROTOCOL IS APPROVED IN ITS PRESENTED FORM ON THE FOLLOWING CONDITIONS

1. The approval is valid for a period of 2 year/s or duration of project whichever is later
2. The date of commencement of study should be informed
3. A written request should be submitted 3weeks before for renewal / extension of the validity
4. An annual status report should be submitted.
5. The TIREC will monitor the study
6. At the time of PI's retirement/leaving the institute, the study responsibility should be transferred to a person cleared by HOD
7. The PI should report to TIREC within 7 days of the occurrence of the SAE. If the SAE is Death, the Bioethics Cell should receive the SAE reporting form within 24 hours of the occurrence.
8. In the events of any protocol amendments, TIREC must be informed and the amendments should be highlighted in clear terms as follows:
   a. The exact alteration/amendment should be specified and indicated where the amendment occurred in the original project. (Page no. Clause no. etc.)
   b. The PI must comment how proposed amendment will affect the ongoing trial. Alteration in the budgetary status, staff requirement should be clearly indicated and the revised budget form should be submitted.
   c. If the amendments require a change in the consent form, the copy of revised Consent Form should be submitted to Ethics Committee for approval. If the amendment demands a re look at the toxicity or side effects to patients, the same should be documented.
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   e. Approval for amendment changes must be obtained prior to implementation of changes.
   f. The amendment is unlikely to be approved by the IEC unless all the above information is provided.
   g. Any deviation/violation/waiver in the protocol must be informed.

STANDS APPROVED UNDER SEAL

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CERTIFICATE - II

This is certify that this dissertation work title **"HER 2 NEU AND KI67 EXPRESSION AS IMMUNOLOCALISATION IN COLORECTAL CARCINOMA"** of the candidate **Dr.BHUVANA.G** with registration Number **201513301** for the award of **M.D.** Degree in the branch of **PATHOLOGY (III).** I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion page and result shows **3 percentage** of plagiarism in the dissertation.

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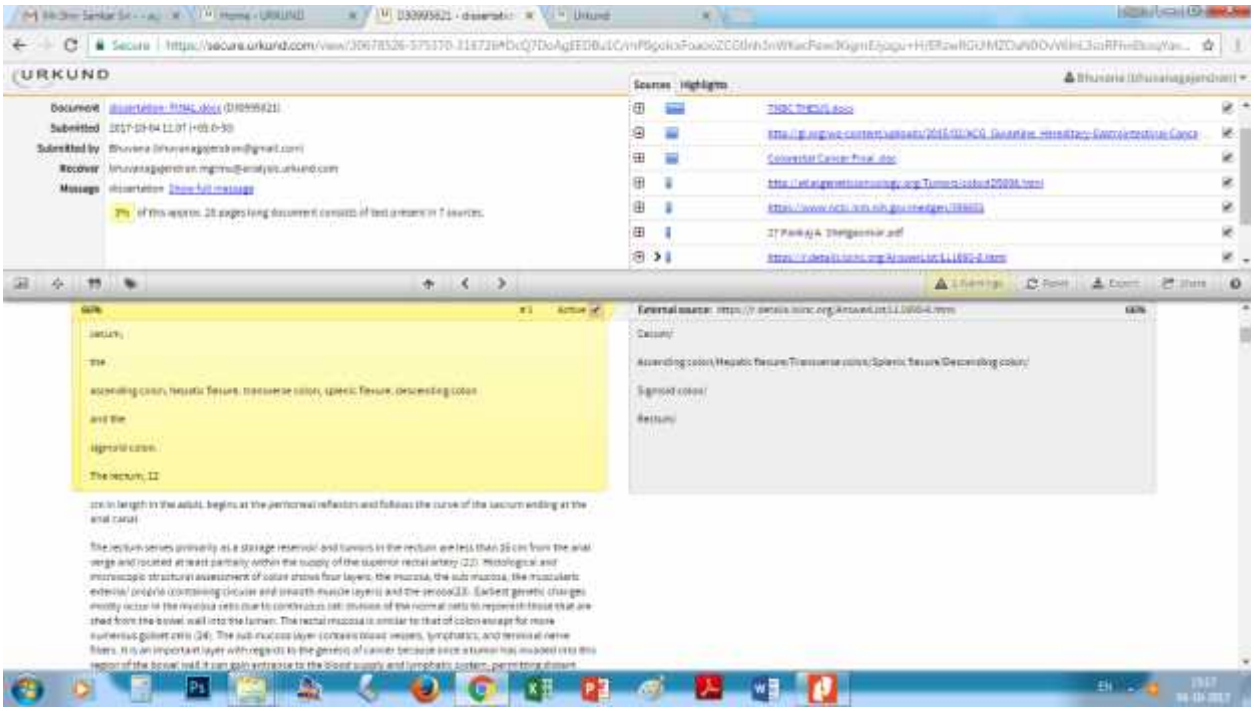
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ascending colon, hepatic flexure, transverse colon, splenic flexure, descending colon
and the
sigmoid colon.
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Ascending colon/Hepatic flexure/Transverse colon/Splenic flexure/Descending colon/
Sigmoid colon/
Rectum/

INTRODUCTION

Cancer of the large bowel (colorectal cancer) includes all cancer originating from the cecum to the anus. Colorectal cancer can be subdivided into colon cancer, which ranges from the cecum to the sigmoid (approximately 15 cm above the anal verge), and rectal cancer, that ranges from the recto-sigmoid to the anus. CRC originates as a noncancerous growth called a polyp that grows on the inner lining of the colon or rectum and propagates slowly, over a period of 10 to 20 years [1, 2].

About one-third to one-half of all individuals will develop one or more adenomas [3, 4]. All adenomas have the potential to become cancerous, fewer than 10% progresses to invasive cancer [5, 6]. The likelihood that an adenoma will become more cancerous as it becomes larger [7].

Cancer originating from the inner lining of the colorectum is called adenocarcinoma and accounts for approximately 96% of all CRCs [8]. Incidence and mortality rates are higher in males than in females [9]. Common symptoms consist of abdominal pain, rectal bleeding, altered bowel habits, and involuntary weight loss.

Globally, the incidence of colorectal cancer differs widely by over 10-fold, with the highest incidence rates in Australia and New Zealand, Europe and North America, and the lowest rates in Africa and Asia [10]. These geographic differences appear to be attributable to differences in dietary and environmental exposures [11]

Several factors have been shown to put individuals at risk to CRC and these include age, the presence of polyps, inflammatory bowel disease, lifestyle, genetic background, and family medical history.

Environmental factors such as obesity, physical inactivity, poor diet, smoking and heavy alcohol consumption account for approximately 80% of all colorectal cancer cases [(12)].

Genetic susceptibility is associated with familial adenomatous polyposis (FAP) and Lynch Syndrome (hereditary non-polyposis colorectal cancer (HNPCC) which accounts for 10% of all colorectal cancer cases. Individuals who have these diseases have an increased lifetime risk of CRC of up to 80% [(12)].

AIMS AND OBJECTIVES

To study the expression pattern of HER2 neu and Ki67 expression of different grades in colorectal carcinomas and to observe the relationship in their staining patterns in various tumor stages.

REVIEW OF LITERATURE

Colonic and rectal cancer is jointly referred to as a single disease called colorectal cancer [13]. It is a disease of western style.

Colorectal cancer is characterized by malignant growth which occurs in the large bowel and occasionally, confined locally for a comparatively long period before metastasis through the bowel wall to lymph nodes and other parts of the body [14, 15].

Colorectal cancer arises due to uncontrolled cell growth in rectum or colon, both part of the large intestine [16, 17]. It evolves either sporadically (85%), or as a part of a hereditary cancer syndrome(<10%) or in the background of inflammatory bowel disease ([18]).

Accumulation of molecular alteration, including the mutation in K-RAS, P53, APC, contribute to colorectal carcinogenesis [19].

EMBRYOLOGY:

The primitive gut forms during the 4th week of gestation when the flat embryonic disc folds in the median and horizontal planes to form a tubular structure that incorporates part of the yolk sac into the embryo. Ventral folding of lateral sides forms the midgut. Ventral folding of cranial and caudal ends form the foregut and the hindgut.

The hindgut gives rise to - distal transverse colon

- descending colon, sigmoid colon, rectum

- proximal anal canal (superior to the pectinate line)

The caudal part of the hindgut known as cloaca is divided by the urorectal septum into the urogenital sinus and the rectum [20]

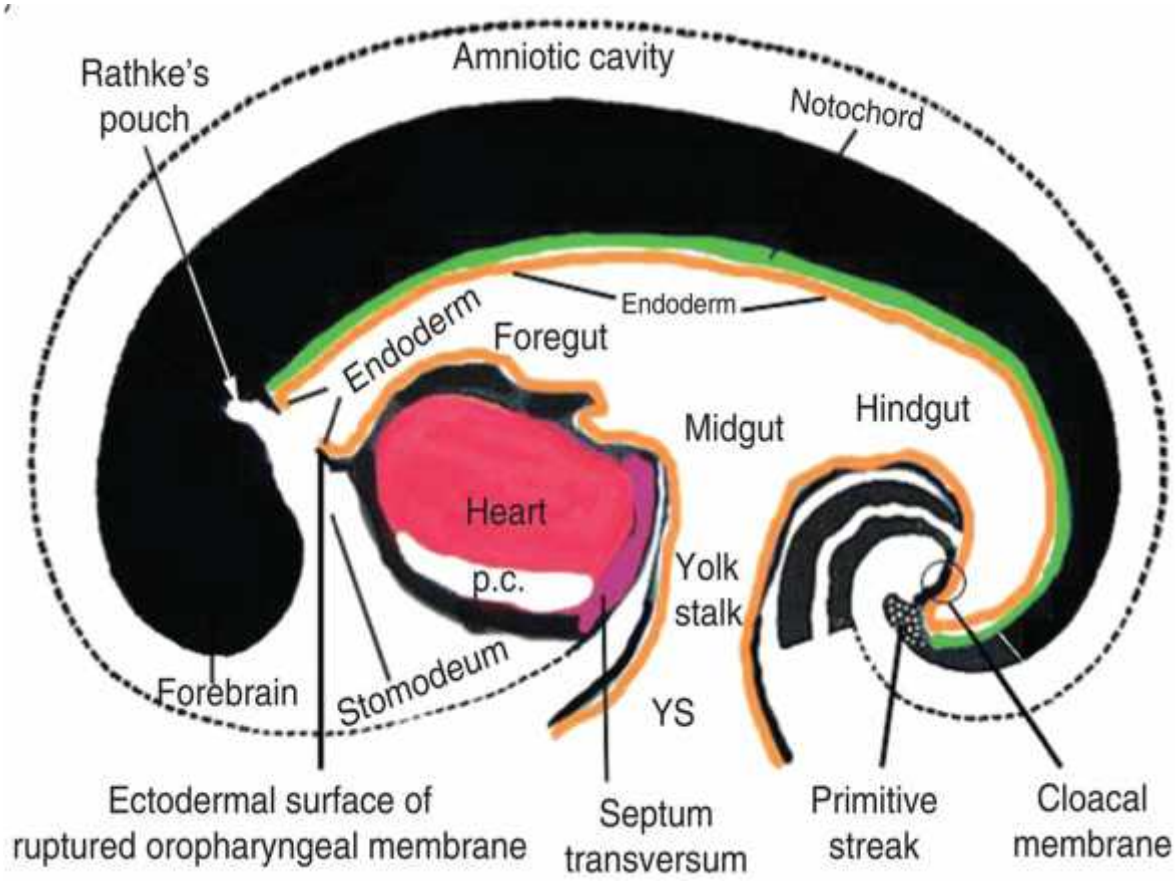


FIGURE 1: FORMATION OF FOREGUT, MIDGUT AND HINDGUT

ANATOMICAL AND MICROSCOPIC FEATURES OF THE LARGE INTESTINE

The large intestine is the final section of the alimentary canal which extends from the terminal ileum to the anal canal [21]. The colon is a tubular structure approximately 1.5 meters long in adults and constitutes of the cecum, the

ascending colon, hepatic flexure, transverse colon, splenic flexure, descending colon and the sigmoid colon. The rectum, 12 cm in length in the adult, begins at the peritoneal reflexion and follows the curve of the sacrum ending at the anal canal.

The rectum serves primarily as a storage reservoir and tumors in the rectum are less than 16 cm from the anal verge and located at least partially within the supply of the superior rectal artery [22].

Histological and microscopic structural assessment of colon shows four layers; the mucosa, the sub mucosa, the muscularis externa/ propria (containing circular and smooth muscle layers) and the serosa[23].

Earliest genetic changes mostly occur in the mucosa cells due to continuous cell division of the normal cells to replenish those that are shed from the bowel wall into the lumen. The rectal mucosa is similar to that of colon except for more numerous goblet cells [24].

The sub mucosa layer contains blood vessels, lymphatics, and terminal nerve fibers. It is an important layer with regards to the genesis of cancer because once a tumor has invaded into this region of the bowel wall it can gain entrance to the blood supply and lymphatic system, permitting distant spread throughout the body[23].

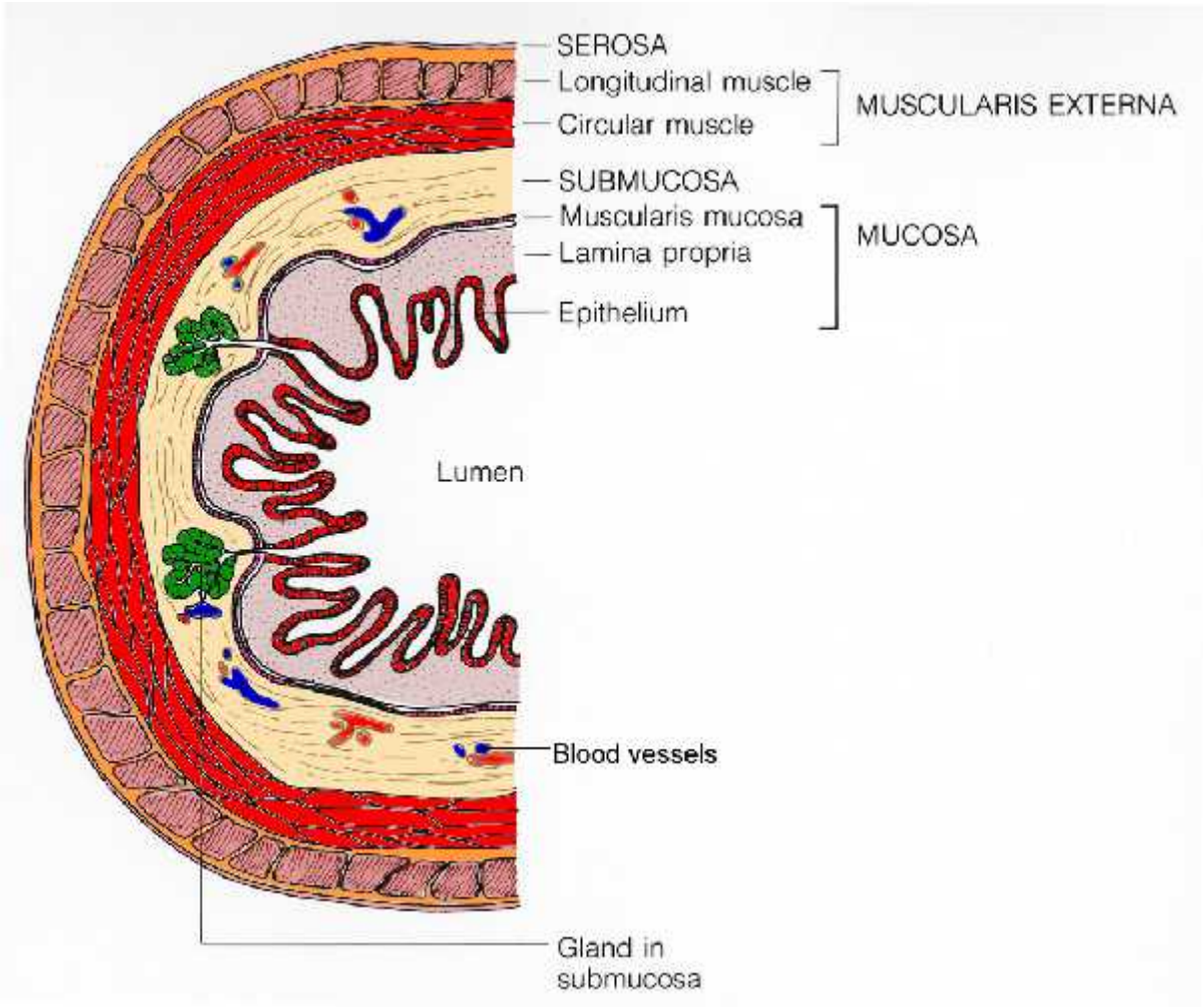


FIGURE 2: LAYERS OF THE COLON

EPIDEMIOLOGY

In 2017, there will be an approximately 95,520 new cases of colon cancer and 39,910 cases of rectal cancer diagnosed in the US [25]. Approximately 4.6% of men (1 in 22) and 4.2% of women (1 in 24) will be diagnosed colorectal cancer in their lifetime.[25]

RELATIONSHIP BETWEEN ANATOMICAL SITE AND EPIDEMIOLOGY

Cancers of rectum and colon share similar precancerous lesions and conditions as well as histopathological appearances and mode of spread. On the other hand, important sex differences exist between colon and rectum.

Up to the age of 55 years, the colon cancer is more common in women and thereafter becomes slightly more common in men [26]. In contrast, rectal cancer is found with equal frequency in both sexes up to the age of 55, but then becomes more common in males [26, 27]

Proximal cancers are more likely to be diploid and show DNA microsatellite instability and other genetic differences [28, 29, 30]. Five circumstances have been recommended in which the risk of cancer of the right colon appears to be increased selectively: female sex, previous cholecystectomy – notably in women, low blood cholesterol prior to surgery, nulliparity and following hormone therapy for prostatic cancer [31]. Selenium deficiency also explain the increased incidence of right-sided cancers [32]

AGE AND SEX

Cancer of the colon and rectum usually present in the seventh decade and worldwide incidence rates show the disease to be more common in males. In low-risk (developing) areas the mean age at diagnosis is about 50 years [33].

Cancers within low-risk areas or low-risk populations show an unusually high incidence of high-grade and mucinous types [34, 35]

ENVIRONMENTAL FACTORS

It includes obesity, sedentary behavior, and a high-meat, high-calorie, fat-rich, fiber-deficient diet, and has been linked to increased colorectal cancer risk [36, 37].

Alcohol consumption and tobacco smoking further increase the risk for colorectal cancer. Alcohol entering the colon is microbially metabolized into acetaldehyde, which degrades folate in vivo [37, 38].

Because folate is required for DNA synthesis and repair, folate deficiency can lead to chromosome breakage, uracil misappropriation, and other DNA precursor imbalances, all of which can contribute to carcinogenesis [39]

Cigarette smoking has been associated with a twofold to threefold increase in the risk of developing colorectal adenoma[40,41] This is due to the ability of the gastrointestinal tract and circulatory system to spread cigarette carcinogens to colorectal mucosa, elevating the risk of inflammation, mutagenesis, and carcinogenesis[37]

In addition to the above environmental risk factors, high blood levels of insulin, gastrointestinal inflammation, and certain meat-cooking methods may also increase the risk of colorectal carcinogenesis [37]. Hyperinsulinemia increases the

risk of colorectal cancer through promotion of colon cell proliferation and reduction of apoptosis [42].

Evidence suggests that about 1% of all colorectal cancer cases are due to the chronic inflammation associated with ulcerative colitis, and the risk for developing cancer directly correlates to the amount of time a patient has endured the inflammatory condition [43].

Certain modes of cooking meat may also increase the risk of developing colorectal cancer. Studies have demonstrated that with frying, boiling, charcoal broiling, or other modes in which meat is cooked at extremely high temperatures, mutagenic heterocyclic amines, and polycyclic aromatic hydrocarbons can form, leading to the production of N-nitroso, a known human carcinogen in the colon [37, 44]. Other compounds such as quinoxaline and pyridine can also increase risk of colorectal cancer, particularly distal adenomas [45]

PROTECTIVE FACTORS

One such protective factor is the ingestion of fish and fish oil, a high intake of dietary fiber, a high intake of vitamin D, a high calcium intake, habitual exercise, and routine use of aspirin.

GENETIC FACTORS

The term ‘genetic’ does not naturally imply that cancer is hereditary. Indeed, most of the genetic variations are acquired at the somatic level and are not constitutional or germline.

An important example would be the inheritance of a mutation in the APC gene, the tumor suppressor gene implicated in the autosomal dominant disorder familial adenomatous polyposis. A second example would be the inheritance of a mutated DNA mismatch repair (MMR) gene responsible for the autosomal dominant disorder hereditary non-polyposis colorectal cancer (HNPCC) or Lynch syndrome [46].

Polyposis syndromes other than FAP are linked with an increased risk of colorectal cancer. These include juvenile polyposis and hyperplastic polyposis [47] and mixed polyposis [48]. A locus on chromosome 15q has been linked to a syndrome of hereditary colorectal adenoma and carcinoma in which small numbers of adenomas and serrated polyps occur [49].

The APCI1307K mutation occurring in Ashkenazi Jews results in a short mononucleotide repeat that in turn is susceptible to truncating mutation. The resulting phenotype is an increased frequency of adenoma and carcinoma in affected families [50]. Germline mutation of TGFβRII and MYH have also been described as rare causes of familial colorectal cancer [51, 52].

Genetic factors need not necessarily operate exclusively at the somatic level within the tumor genome. They could also influence intermediary metabolism, either through failing to detoxify potential mutagens or through an enhanced synthesis of initiating or promoting compounds.

Given the fact that acetylation is involved in the metabolism of arylamine carcinogens, interest has been generated in the observation that fast acetylators are at increased risk of developing colorectal cancer[53,54]. The glutathione S-transferases detoxify carcinogens including the epoxides of polycyclic aromatic hydrocarbons [55].

The glutathione S-transferase null genotype has been associated with an increased risk of colorectal cancer [56, 57, 58]. Hypomethylation of DNA occurs as an early step in colorectal carcinogenesis and may be a factor in chromosomal instability [59].

Apolipoprotein E (ApoE) regulates cholesterol metabolism. Individuals with the £4 allele absorb a greater percentage of their luminal cholesterol. This allele was less common in individuals with proximal colorectal cancers [60].

The cytochromes P450 (CYP1A1 and CYP2D6) are implicated in the conversion of polycyclic aromatic hydrocarbons to their DNA binding carcinogenic forms [61]. Homozygosity for the MspI mutant genotype of CYP1A1 has been associated with colorectal cancer [62].

HISTOGENESIS OF COLORECTAL CANCER:

Adenoma carcinoma sequence describes the stepwise progression from normal to dysplastic epithelium to carcinoma associated with accumulation of multiple clonally selected genetic alterations.

It is accompanied by series of molecular alterations that include mutational activation of oncogenes and the inactivation of tumor-suppressor genes [63]

ADENOMA CARCINOMA SEQUENCE:

A number of genetic and epigenetic changes affecting genes controlling cell proliferation and/or cell death trigger the development of carcinoma [64].Most CRCs arise sporadically from adenomatous polyps.

The evolvement of carcinomas through different histopathological steps was suggested in 1974[65], and some years later Vogelstein et al [66] described genetic alterations in several genes accompanying this stepwise progression from benign adenoma to a malignant carcinoma.

This has been named the adenoma-carcinoma sequence and includes mutations in the Adenomatous polyposis coli (APC), Kirsten-ras (K-ras) and TP53 genes amongst others [67].

Mutations in the APC gene, which cause FAP if mutations are inherited, is one of the most frequently mutated genes in CRC and an early event in the development of CRC[68].

K-ras mutations are also observed as early events [69, 70] whereas mutations in the tumor suppressor gene TP53 is considered to be a relatively late event in colorectal carcinogenesis [69, 71].

Each cell with a malignant subclone will have accumulated multiple mutations. The generation of an adenoma is followed by the spatial reorganization of the proliferative compartment.

Instead of sequestered within crypt base, proliferative cells accumulate superficially where they are exposed directly to luminal carcinogens and promoting influences. Expansion of the neoplastic clone through growth further raises the size of the target population. The accumulation of genetic damage may occur at the level of DNA or at a chromosomal level through disruption of cell cycle checkpoint mechanisms [72]

The final step of conversion of adenoma to adenocarcinoma is accompanied by multiplicity of phenotypic changes implicating enzymes in metabolic pathways [73], increased telomerase activity[74] , growth factors promoting stromal proliferation and angiogenesis[75,76], proteolytic enzymes facilitating local invasion [77,78], numerous changes to secretory and membrane-associated glycoproteins[79], alterations in cell adhesion molecules[80] as well as development of aneuploidy.

In colorectal carcinomas, three distinct pathways of genomic instability have been recognized.

1) Chromosomal instability
2) Microsatellite instability
3) CpG island methylator phenotype pathways

Recently mutations involving other genes have been described – TGFβR and PIK3CA.

CHROMOSOMAL INSTABILITY PATHWAY

The expression of a mutator phenotype in human cancers as an early step in tumor progression has been described [81, 82]. This is known as the chromosomal instable phenotype, and these cells typically display numerous chromosomal aberrations and it includes activation of proto-oncogenes(KRAS) and inactivation of at least 3 tumor suppressor genes – Loss of APC, loss of p53, LOH18q and also TGFβR, PIK3CA.

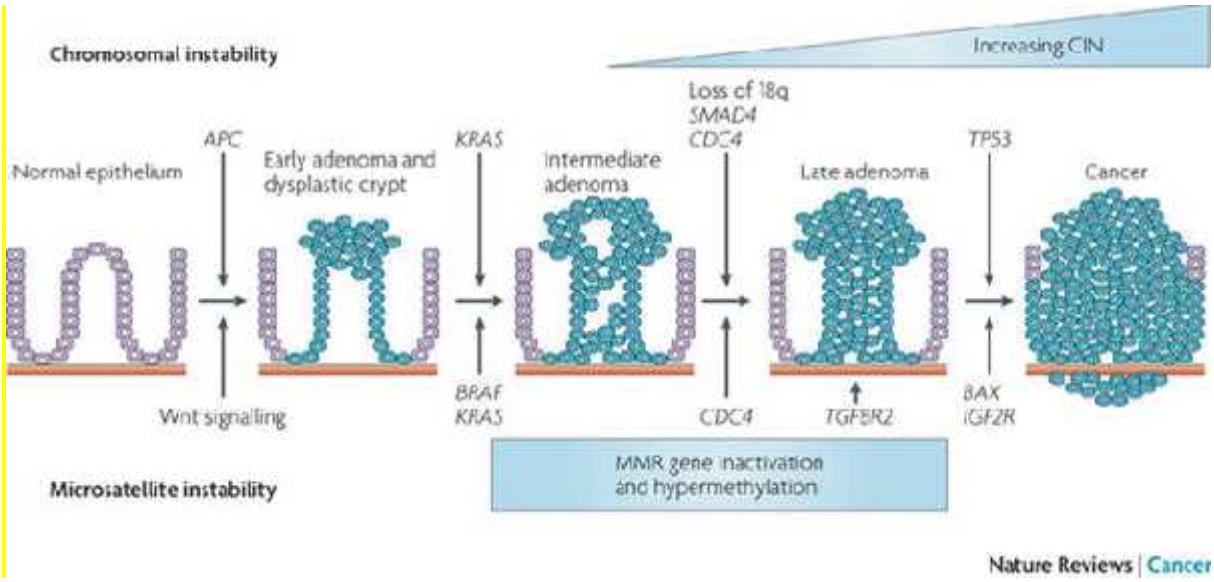


FIGURE 4 : CHROMOSOMAL INSTABILITY PATHWAY

These events lead to the transformation of the normal colonic epithelium to colon adenocarcinoma. A larger part of MSS tumors follows the CIN pathway. Characteristically for CIN tumors are that they are often aneuploid or polypoid,

highly differentiated, rarely mucinous, have no lymphocytic infiltration, poor prognosis, and no specific tumor site predominance [83].

APC (ADENOMATOSIS POLYPOSIS COLI) GENE AND Wnt SIGNALING PATHWAY

APC- associated polyposis includes familial adenomatous polyposis (FAP), attenuated FAP, Gardner syndrome, and turcot syndrome.

FAP is a CRC- predisposition syndrome in which hundreds to thousands of precancerous polyp develops. It accounts for <1% of colon cancers. Patients with attenuated FAP have lesser polyps, the site of polyps is in more proximal part of colon and cancer develops at a later age.

Gardner syndrome is a subtype of FAP-associated with osteomas and soft tissue tumors. Turcot syndrome manifests as colonic polyps along with CNS tumors.

The APC/Wnt/ β- catenin pathway plays a major role in both sporadic and hereditary colorectal carcinoma. The majority (98%) of APC mutations are either frameshift or nonsense mutations leading to the synthesis of a truncated protein. This mutation is found in approximately 30%-70% 0f sporadic adenomas and sporadic colorectal carcinomas [84, 85, 86]

TP53 MUTATION

The TP53 gene is involved in the control of the cell cycle and apoptosis and is commonly mutated in colorectal carcinoma [87, 88]. The p53 protein induces G1 cell-cycle arrest and facilitates DNA repair prior to a cell committing to the process of DNA replication.

If DNA repair is unsuccessful, p53 induces cell death (apoptosis). TP53 mutation is generally believed to occur at the time of transition from adenoma to cancer. The incidence of TP53 mutations is highest in the distal colon [89, 90, 91]

Inherited or germline mutations in TP53 are the cause of Li-Fraumeni syndrome, a cancer predisposition syndrome associated with a variety of neoplasms, including soft tissue sarcoma, osteosarcoma, premenopausal breast cancer, brain tumors, and adrenocortical carcinoma.

18q LOSS OF HETEROZYGOSITY

LOH in the region of 18q21 is frequently seen in advanced colorectal cancer. The DCC (Deleted in Colorectal Carcinoma) gene is located on the long arm of chromosome 18. It encodes the transmembrane protein DCC. DCC is a "conditional tumor suppressor gene"

In contrary to the common types of transmembrane receptors, DCC blocks cell growth in the absence of its ligand, netrin-1.Approximately 70% of colorectal carcinomas show LOH in the DCC gene region.

Netrin-1 is produced deep in the crypts of colorectal mucosa. As epithelial cells differentiate and move toward the surface, the concentration of netrin-1 decreases. When DCC gene is mutated, netrin-1 will not bind to DCC transmembrane protein, resulting in abnormal cell survival.

MICROSATELLITE INSTABILITY (MSI) AND MISMATCH REPAIR PATHWAYS

During cell replication, DNA polymerase “reads” an intact DNA strand as a template and uses it to synthesize an identical copy. The mismatch repair (MMR) system checks and repairs defects that were overlooked by DNA polymerase.

MSI is the hallmark of Lynch syndrome. It is responsible for only 15%-20% of cases. Lynch syndrome accounts for 3%-5% of all colorectal cancers. It is an autosomal dominant disorder caused by germline mutations in one of the several MMR genes.

Lynch syndrome can be diagnosed when a mutation is found in one of the four mismatch repair genes. The genes are MLH1, MSH2, MSH6, PMS2. MSI serves as a marker for the loss of DNA MMR activity. Inactivation of the MMR enzymes can arise either through aberrant methylation of promoter CpG islands of MLH1 gene or via point mutations in a member of the MMR family.”

Microsatellite high” (MSI-H) is defined as the presence of instability in >30% of the markers. “ Microsatellite – low “(MSI-L) is defined as the presence of instability in 10%-29% of markers, “microsatellite –stable “(MSS) is defined as no unstable markers[92].The majority of MMR defects in sporadic colorectal

carcinoma are due to epigenetic silencing of MLH1 gene expression by promoter hypermethylation [93,94,95]

EPIGENETIC INSTABILITY AND CpG METHYLATION

Epigenetic changes are chemical alterations of DNA resulting in changes in gene expression.

The main epigenetic modifier is methylation of cytosine located within the dinucleotide CpG[96,97], and CpG sites are widely and unsymmetrically distributed in the genome. When CpG islands are positioned in the promoter region of a gene it will result in transcriptional silencing of the gene expression.

The methylation of multiple CpG islands, defined as CpG island methylator phenotype (CIMP), seems to be an event in about half of all sporadic CRCs[98], and MSI tumors are often caused by epigenetic silencing of the DNA mismatch repair gene MLH1[99].

Aberrant methylation of MLH1 occurs in 80% of sporadic MSI colorectal cancers. CpG island methylator phenotype (CIMP) is a subclass of colorectal cancers with a high percentage of the gene being hypermethylated. These subclasses of tumors commonly have BRAFV600E mutations [100]

Endothelial growth factor binds to the extracellular domain of EGFR leading to receptor dimerization. Following dimerization, the intracellular domain of EGFR

is autophosphorylated and stimulates multiple downstream proteins of the RAS/RAF/MAPK and PI3K/AKT pathways.

KRAS and BRAF are components of the MAP kinase (MAPK) pathway. The RAS/RAF/MAPK pathway controls cell proliferation, differentiation, senescence, and apoptosis.

The RAS oncogenes include HRAS, NRAS, KRAS. KRAS is the most commonly mutated RAS family member in colorectal carcinoma and is mutated in 40% of sporadic colorectal carcinomas.

The mitogen-activated protein kinase pathway (MAPK) is the primary cell proliferation signal transduction pathway from the cell surface to the nucleus. This activation uses a sequence of intermediate proteins including RAS, RAF, and MEK.

BRAF is a member of RAF family of serine/threonine kinases and arbitrates cellular responses to growth signals through the RAS-RAF-MAP kinase pathway.

PI3K/AKT PATHWAY, PTEN AND TGFβ RECEPTOR

The phosphoinositide 3- kinases (PI3K)/AKT/mammalian target of rapamycin(mTOR) is an alternative EGFR- mediated signaling pathway.[101]

PTEN is a phosphatase that negatively regulates the PI3K/AKT signaling pathway by dephosphorylating PIP3 to inhibit activation of AKT via hyperactivation of PI3K signaling.[102]

TGFβ is a group of multifunctional proteins that regulate many cellular processes through binding to TGFβ receptors. Three types of TGFβ receptors (type I, type II, type III) are identified in most cells.[103]. TGFβ receptor type II is mutated in up to 90% of colon cancers with MSI.

TGFβRII appears to function in two ways during tumorigenesis. In early stages of tumorigenesis, TGFβRII negotiates tumor- suppressive effects, but in late stages, it increases tumor progression by inhibiting tumor cell death and immune repression. It also induces epithelial to mesenchymal transformation (EMT) known to induce tumor progression, invasion, and metastasis [104]

Mutation of TGFβRII will inhibit with EMT and shorten the invasiveness and metastatic capability of the tumors. EMT has been shown to be defective in MSI colon cancer cells.

Tumors with MSI but without TGFβRII mutations can undergo EMT in vitro in response to TGFβRII, which suggests that TGFβRII and not MSI status may be the key determinant of invasiveness and metastasis and prognosis [105,106]

PATHOGENESIS

The cause and pathogenesis of colorectal carcinoma are related to both environmental and genetic factors.

The environmental factors are largely dietary, particularly in terms of fat and animal protein. The genetic factors include familial adenomatous polyposis, and other forms of polyposis, hereditary nonpolyposis colorectal cancer syndrome (Lynch syndrome) and related nonpolyposis associated hereditary conditions [107].

EREDITARY COLORECTAL CANCER SYNDROME AND OTHER FORMS OF FAMILIAL CANCER

The most common of these syndromes is the Lynch Syndrome, also known as Hereditary Non- Polyposis Colon Cancer (HNPCC), which is an autosomal dominant disorder and accounts for 1-6% of all malignancies of the colon [108].

This syndrome is characterized by early onset of CRC (mean age < 45 years), with tumors predominantly located in the right colon (70%) [108], and often associated with synchronous (18%) or metachronous (24%) tumors.

The carcinomas most commonly affect the proximal colon. They are also at increased risk for metastasis at other sites like liver, uterine corpus, urinary bladder.

Hereditary nonpolyposis colorectal cancer syndrome is due to germline mutations in any one of the genes responsible for the repair of DNA mismatches, most commonly MLH1, MSH2, MSH6, PMS2 producing high levels of microsatellite instability.

TABLE1: CRITERIA FOR IDENTIFYING PATIENTS WITH LYNCH SYNDROME

AMSTERDAM CRITERIA [109]	REVISED BETHESDA GUIDELINES[110]
1. Atleast 3 relatives with CRC or a lynch syndrome – associated cancer (endometrium, small bowel, ureter or renal pelvis)	Tumors from individuals should be tested for MSI in the following situations.
2. One first- degree relative of the other two, and	1. CRC diagnosed in a patient <50 years of age.
3. At least two affected generations, and	2. Presence of synchronous, metachronous CRC or other lynch syndrome associated tumors, regardless of age.
4. One cancer diagnosed before the age of 50 years, and	3. CRC with MSI-H histology diagnosed in a patient who is <60 years of age.
5. FAP excluded in the CRC case	4. CRC diagnosed in one or more first degree relatives with a lynch syndrome-related tumor, with one of the cancers being diagnosed under age 50 years.
All criteria must be fulfilled for further analyses.	5. CRC diagnosed in two or more first- or second-degree relatives with lynch syndrome-related tumors, regardless of age

The second most frequent inherited syndrome, the Familial Adenomatous Polyposis syndrome (FAP), accounts for less than 1% of all CRC [111]. From early adolescence and onwards, patients with this condition develop hundreds to thousands of polyps in the colon and rectum.

There are several clinical variations of FAP, such as Gardner's syndrome [112], Turcot's syndrome [113] and the attenuated form of FAP [114].

FAP is caused by germline mutations in the tumor suppressor gene APC (adenomatous polyposis coli), also found to be frequently mutated in sporadic colorectal cancers.

Genetic testing is routinely used for detection of FAP. Flexible sigmoidoscopy at the age of 10-12 years old is recommended for screening for polyps in APC gene mutation carriers [115].

Once polyps are detected, annual colonoscopy for polyp screening is recommended and when the polyp burden increases, prophylactic colectomy is offered.

Other inherited CRC syndromes are rare and include the Peutz-Jegher's syndrome with mucocutaneous pigmentation and gastrointestinal hamartomas, the juvenile polyposis syndrome with multiple hamartomatous polyps spread throughout the gastrointestinal tract, and Cowden's disease with multiple hamartomatous polyps, neurologic and dermatologic symptoms [108].

COLORECTAL POLYPS

The two main histological types of polyps in the colorectal mucosa are hyperplastic and adenomatous polyps. Carcinogenesis starting in hyperplastic polyps develops through serrated adenomas and is suggested to be caused by microsatellite instability [116,117]. This link to serrated adenomas may represent a carcinogenetic pathway largely independent of the adenoma-carcinoma sequence [116].

Risk factors are polyp size (>1 cm), multiple polyps (>20), family history of hyperplastic polyposis or CRC [118]

Most colon carcinomas develop from adenomas, which are separated into three histologically different types: tubular (75%), villous (10%) and tubulovillous (15%).

Increased size, grade of dysplasia and villous structure is associated with increased risk of malignancy.

Removal of adenomas in the colon and rectum decreases this risk of developing CRC [119,120]. The transformation of adenomas to invasive cancer involves a wide specter of genetic events including alterations of oncogenes, tumor suppressor- and mismatch repair genes [121,122,123].

CLINICAL FEATURES:

The appearance of colorectal cancer is dependent on the site of a tumor and extent of disease. Patients with early cancers are mostly asymptomatic and diagnosis is mostly made through population screening.

Carcinomas of the large bowel may present with rectal bleeding, change in bowel habits, anemia resulting from chronic blood loss, and vague abdominal pain.

Proximal cancers rarely cause gross rectal bleeding because the blood tends to mix with the stool and degrade during colonic transit. This occult blood loss means such patients often present with iron deficiency anemia [124].

In contrast, distal rectal tumors may present with fresh rectal bleeding, pelvic pain or tenesmus[125].

Symptoms of tumors confined to the recto sigmoid portion are most often false and/or painful urge to defecate (tenesmus), narrow stool and hematochezia[126]. In a few cases, patients without recent symptoms present as an emergency with intestinal obstruction, fistulation or perforation [127].

Intestinal obstruction is common when the tumor is located in the left colon, and rare for tumors in the caecum or ascending colon. Perforation may occur rarely [128]

WHO CLASSIFICATION OF HISTOPATHOLOGIC TYPES OF COLORECTAL CARCINOMA

Adenocarcinoma, not otherwise specified

Mucinous adenocarcinoma

Signet ring carcinoma

Small cell carcinoma

Micropapillary adenocarcinoma

Serrated adenocarcinoma

Cribriform comedo-type adenocarcinoma

Adenosquamous carcinoma

Squamous cell carcinoma

Medullary carcinoma

Undifferentiated carcinoma

GROSS

Up to 40% of all large bowel cancers occur in the rectum and rectosigmoid area. The sigmoid colon accounts for a further 25%. Of the remaining bowel, the ascending colon is a site of predilection.

Most cancers of the colon and rectum are ulcerating tumors with raised everted edges. Ulcerating tumors may involve the bowel circumference to produce stenosis and obstruction.

Some growths may be circumferential yet show little evidence of ulceration. Such annular growths have been called ‘string carcinoma’.

String carcinomas occur with the greatest frequency in the transverse and descending colon. About 10% of colorectal cancers will show a mucoid appearance on the cut surface of the tumor due to the secretion of abundant mucus by the tumor cells. Most cancers of the colon and rectum remain approximately small and well circumscribed related to gastric carcinoma.

Most colorectal cancers are one of two the polypoid or the ulcerative – infiltrating type. The polypoid cancers present as a bulky mass with well-defined and rolled margins and a sharp dividing line with the normal bowel. The ulcerative – infiltrating cancers have less elevated surface and are centrally ulcerated [(128)] Polypoid cancers have a better prognosis than ulcerative lesions.

MICROSCOPY

Adenocarcinoma can be divided into three grades based on the arrangement of cells with regard to the degree of tubular (acinar) formation.

GRADE 1 CANCERS (low –grade or well – differentiated tumors) accounts for 15-20% of colorectal adenocarcinomas, and are composed mainly of simple tubules, in which nuclear polarity is easily ascertained and the nuclei are of uniform size.

GRADE 2 CANCERS (average- grade or moderately differentiated tumors) accounts for 60-70% of colorectal adenocarcinomas, composed of tubules that may be simple, complex, or slightly irregular, the nuclear polarity is barely discernible or lost.

GRADE 3 TUMOURS (poorly differentiated tumors) accounts for 15-20 % of colorectal adenocarcinomas, characterized by a predominance of the absence of glandular differentiation (solid- like pattern) as well as loss of nuclear polarity (107)

IMMUNOHISTOCHEMISTRY

The adenocarcinomas of the large bowel show positivity for MUC1 and MUC3. Expression of MUC13 in poorly differentiated tumors. They are also positive for CK20 and negative for CK7. Other markers are villin, cathepsin B, neuropilin -1, SRCA-2, cadherin -17.

MUCINOUS ADENOCARCINOMA

It accounts for approximately 10% of colorectal cancers. The definition of mucinous carcinoma is that at least 50% of the lesion must be mucinous.

Mucinous carcinomas usually present at a more advanced stage, more extensive perirectal spread, greater incidence of lymph node involvement, and an overall poorer prognosis.

Mucinous carcinomas show a high rate of microsatellite instability, CIMP, young adults and children, villous adenomas, cancers arising secondary to therapeutic irradiation, ulcerative colitis, and colorectal cancers in low – incidence developing countries .[107]

IMMUNOHISTOCHEMISTRY

They show positivity for MUC2.

CRIBRIFORM COMEDO-TYPE ADENOCARCINOMA

This type of adenocarcinoma has extensive areas of cribriform glands with central necrosis as seen in cribriform and comedo in situ duct carcinoma of the breast.

SIGNET RING CARCINOMA

This is a rare form of colorectal carcinoma, affecting young patients. Grossly presents as diffuse infiltration of the wall. Microscopically, the tumor arises in a diffuse fashion, with little if any glandular formation. The intracellular

accumulation of mucin results in displacement of the nucleus and a typical signet ring configuration of the cells. [128]

IMMUNOHISTOCHEMISTRY

These tumors express MUC2, MUC5AC, CDX2. They also show positivity for CK20 and negative for CK7.

SMALL CELL CANCER

A rare variant is small cell cancer, which comprises less than 1% of colorectal cancers.

Histologically, these cancers are indistinguishable from small cell carcinoma of the lung (oat cell type and intermediate type). They have an extremely poor prognosis and have lymph node and liver metastasis.

IMMUNOHISTOCHEMISTRY

These neoplasms express neuron-specific enolase (84%), Leu-7 (18%), synaptophysin (50 %), chromogranin (37 %)

UNDIFFERENTIATED CANCER

These are uncommon cancers, representing less than 1% of colorectal cancers.

They are malignant epithelial tumors that have no glandular structures or other features suggesting definite differentiation. The absence of intracytoplasmic mucin helps to differentiate these tumors from poorly differentiated adenocarcinoma [128]

MEDULLARY CARCINOMA

These tumors are located in the proximal colon and have a female predominance. Grossly, these tumors are large heavy masses with an expansile growth pattern. Microscopically, the tumor cells have eosinophilic or amphophilic cytoplasm with rounded nuclei with prominent nucleoli. The tumor cells comprise of undifferentiated small to medium rounded tumor cells arranged in closely packed, trabecular or solid patterns.

Medullary carcinomas are well circumscribed and have a prominent Crohn- like reaction, focal gland formation, and mucinous features [128]

IMMUNOHISTOCHEMISTRY

These tumors are immunoreactive for MUC1, MUC1, and TF3. They also show consistent loss of staining for MLH1 and CDX2 [107]

SQUAMOUS AND ADENOSQUAMOUS CANCERS

These are extremely rare tumors, and they have been associated with ulcerative colitis, schistosomiasis, and pelvic irradiation.

To make a diagnosis of primary colorectal squamous cancer, the following criteria must be met: There must be no other sites of squamous cancer in the body and no involvement of cloacogenic or squamous – lined mucosa.

SERRATED ADENOCARCINOMA

This is a newly described entity that encompasses 7.5% of all colorectal carcinomas and 17.5 % of proximal carcinomas. It is more common in females (9.3%) than males (5.8%). They belong to the category of CIMP-H tumors, the majority being MSI-L with TSAs as their precursor lesions. A lesser percentage has MSI-H with SSA and mixed polyps being the precursor lesions.

MICROSCOPY: A serrated pattern of epithelium with eosinophilic cytoplasm with well-preserved nuclear polarity. The nuclei can be bland, or vesicular with prominent nuclei, but the nuclei are not hyperchromatic, overlapping or stratified as seen in adenocarcinoma

40% of serrated adenocarcinomas show mucinous differentiation, and 17.6% are mucinous adenocarcinoma with a well-preserved serrated pattern. They have eosinophilic papillary rods and eosinophilic cell balls floating in mucin.

Poorly differentiated serrated adenocarcinoma cells grow in a trabecular pattern. The tumor cells show abundant eosinophilic cytoplasm with vesicular nuclei and prominent nucleoli.

MICROPAPILLARY ADENOCARCINOMA

Micropapillary adenocarcinoma is an uncommon histologic variant with an aggressive behavior described in breast and urinary bladder.

Microscopically it consists of balls/ clusters of neoplastic cells with eosinophilic cytoplasm and pleomorphic nuclei surrounded by cleftlike spaces. A minimum of 5 % of a tumor with micropapillary features is required for the diagnosis.

These tumors have a greater incidence of lymph node metastasis, greater number of positive lymph nodes, more an advanced stage, greater incidence of distant metastasis, regardless of the percentage of the micropapillary component.

UNUSUAL FORMS OF COLORECTAL CANCER

CARCINOSARCOMA

It is an extremely rare tumor, occurring in both large and small intestine. These cancers have areas of typical adenocarcinoma merging with sarcoma (spindle cells and sarcoma with osseous and cartilaginous differentiation)

IHC

Cytokeratin reactivity of the sarcomatous element is helpful in making diagnosis

CHORIOCARCINOMA

In this type, adenocarcinoma with tumor cells producing human chorionic gonadotropin.

CLEAR CELL TYPE

This pattern resembles renal clear cell adenocarcinoma. Primary clear cell colonic cancers produce glycogen and negative for mucin production. They also

express carcinoembryonic antigen (CEA), which is helpful in differentiating these lesions from metastatic renal cell carcinoma.

HEPATOID ADENOCARCINOMA

These tumors have combined features of classic adenocarcinoma and tumor indistinguishable from hepatocellular carcinoma. Polyclonal carcinoembryonic antigen (CEA) shows cytoplasmic staining for the glandular component and canalicular staining for the hepatoid component. The serum alpha - fetoprotein (AFP) is markedly elevated.

RECTAL NEUROENDOCRINE TUMOUR

These tumors are larger than 2 cm and display invasion of muscularis propria and/or that have 2 or more mitosis per 10 high power field. These tumors can histologically mimic prostate cancer.

IHC: IHC staining for prostate - specific antigen, prostate –specific acid phosphatase (80%), chromogranin, synaptophysin, CD57, CDX2 may be helpful [(107)]. Rectal neuroendocrine tumors show weak expression of CDX2.

LARGE CELL NEUROENDOCRINE CARCINOMA

This is an uncommon neoplasm representing less than 1% of colorectal neoplasms. Histologically, they are indistinguishable to that seen in the lung.

IHC: Synaptophysin, neuron-specific enolase, chromogranin, CD57, CD56, CDX2. 44% tumors express c-kit (CD117) with variable immunoreactivity.

WHO CLASSIFICATION OF MALIGNANT LYMPHOMAS OF THE COLON AND RECTUM

1. Marginal zone lymphoma of MALT type
2. Mantle cell lymphoma
3. Diffuse large B-cell lymphoma
4. Burkitt lymphoma
5. B –cell lymphoma unclassifiable

TABLE2: THE TNM STAGING SYSTEM OF THE AJCC FOR COLORECTAL CANCER

PRIMARY TUMOR (T)	REGIONAL LYMPH NODES (N)	DISTANT METASTASIS (M)
TX- primary tumor cannot be assessed	NX- lymph nodes cannot be assessed	MX- distant metastasis cannot be assessed
T0- No evidence of primary tumor	N0- No regional lymph node metastasis	M0- No distant metastasis
Tis- Carcinoma in situ, intraepithelial or invasion of lamina propria	N1- Metastasis in 1-3 regional lymph nodes	M1- Distant metastasis
T1- Tumor invades submucosa	N1a- Metastasis in one regional lymph node	M1a- Metastasis confined to one organ or site
T2- Tumor invades muscularis propria	N1b- Metastasis in 2-3 regional lymph node	M1b- Metastasis in more than one organ/site or peritoneum
T3- Tumor invades through the muscularis propria into pericolorectal tissues	N1c- Tumor deposits in subserosa, mesentry or nonperitonealized pericolic or perirectal tissues without regional nodal metastasis	
T4a- Tumor penetrates the visceral peritoneum	N2- Metastasis in 4 or more regional lymph nodes	
T4b- Tumor invades or is adherent to other organs or structures	N2a-Metastasis in4-6 regional lymph nodes	
	N2b- Metastasis in 7 or more regional lymph nodes	

PREDICTIVE FACTORS:

Factors which have an impact on the patient feedback to a certain therapy are called predictive factors. Some bio-molecular factors that predict the response to cytotoxic therapy have been identified, and are therefore important when allocating patients to different therapeutic regimens.

The predictive value of K-ras mutation status in therapy with antibodies to epidermal growth factor receptor (EGFR) is well documented [129,130,131] and in regular clinical use.

Human cancer cells lines with disruption of TP53 have shown a reduced therapeutic response to fluorouracil in experimental studies[132], and reduced effect of FU-based chemotherapy has been reported in TP53mutated tumors has been reported[133,134].

PROGNOSTIC FACTORS

The prognosis of CRC range widely among patients and depends on a number of factors. Currently, the gold standard of prognostication is the clinico-pathological staging based on the TNM classification system.

Stage of the disease at presentation has a subtle effect on the prognosis. However, prognosis also differs between patients with the same TNM stage, and many clinical, histopathological and biomolecular markers have a potential impact on the outcome.

1. AGE: Tumour occurring in very young and very the old patients are associated with poor prognosis
2. SEX: The prognosis is better for females than for males
3. CEA serum levels: CEA belongs to the immunoglobulin superfamily and is attached to the cell membrane by glycosylphosphatidylinositol anchor and released in soluble form by phospholipase C or D [135,136] and is recommended for determining prognosis, surveillance followed after curative resection, and as a monitoring therapy in advanced CRC [137,138]. Elevated serum CEA levels more than 5.0 ng /ml shown to have an adverse effect on prognosis
4. Tumour location: This factor remains controversial
5. Tumour multiplicity: The survival rates for synchronous or metachronous malignancies of the large bowel is similar to those patients of solitary colorectal carcinomas.
6. Local extent: The prognosis is excellent if focal microscopic carcinoma is discovered incidentally in a polyp, and if a tumor is restricted to mucosa and submucosa
7. Tumour size: Although a correlation exists between size of the tumor and prognosis, there are too many exceptions for this to be a reliable prognostic indicator
8. Tumour edge: Advanced colorectal carcinomas with a nonpolypoid edge seem to have a worse prognosis than polypoid tumors

9. Tumor differentiation grade: low differentiated grade is associated with poor outcomes[107]
10. Obstruction: This feature has been found to be an indicator of a worsened prognosis independent of duke's staging
11. Perforation: Perforation resulting from extensive tumor invasion of the bowel wall is linked to poor prognosis
12. Tumour margins and inflammatory reaction: Carcinomas having pushing margins and an inflammatory infiltrate at the interphase between tumor and the neighboring tissue have a better prognosis
13. Tumor budding: It is defined as the presence of isolated tumor cells or clusters of more than 5 cells at the invasive tumor front, as a strong and independent prognostic marker of poor outcome.
14. Pericolonic tumor deposits: It indicates a poor prognostic sign.
15. Perineural invasion: It is a sign of advanced disease, and is an unfavorable prognostic indicator.
16. Lymphovascular invasion: It is regarded as a step in the pathway of spread to regional lymph nodes and increases the risk of metastases[139], and is associated with poor outcome.[140]
17. Surgical margins: Tumour involvement of the radial margin (defined by adventitial soft tissue margin by non peritonealised surface) is a single most critical factor in predicting local recurrence in rectal carcinoma.

18. Tumor thickness. Measurement of the tumor thickness in the ‘central depressed area’ of the tumor is said to correlate with the incidence of lymph node and liver metastases and with prognosis [141].
19. Microscopic tumor type. Mucinous carcinoma, signet ring carcinoma, and anaplastic carcinoma have a worse prognosis than the ordinary type of adenocarcinoma, whereas medullary carcinoma is said to be associated with an improved outcome (AJC category IIB).
20. Mucin-related antigens. Colorectal carcinomas that express the mucin-associated antigens sialyl-Tn and sialyl-Lewis(x) antigen have been said to run a more aggressive clinical course [142,143].
21. Cell proliferation. Determination of S-phase fraction has shown a relation between the survival rate in some studies [144].
22. Oncogenes and tumor suppressor gene expression. KRAS mutation at certain codon sites has been found to be much more common in patients with recurrent disease [145] (AJC category IIB). P53 over-expression was found to be an independent predictor of survival [146,147,148] (AJC category IIB). Expression of the CMYC oncogene has been found to correlate with the degree of differentiation of the tumor[149]. Over-expression of thymidylate synthase mRNA or protein is associated with a poor prognosis and resistance to chemotherapy [150]. Absence of P27 expression (a cell cycle inhibitor with a potential tumor suppressor function) is associated with a poor prognosis [151] (AJC category IIB).

23. Lymph node involvement. Once the tumor has spread to the lymph nodes, the 5-year survival rate drops sharply (AJC category I, as part of the staging). The location and duration of lymph node involvement are also significant. The greater the number of lymph nodes involved, the poor the prognosis.
24. Microscopic grade. There is a definite relationship between the microscopic grade of the tumor and its prognosis [152,153,154,155] especially if the tumors are stratified into two rather than three or five categories (low-grade and high-grade) (AJC category IIA).

IMMUNOHISTOCHEMICAL MARKERS

Histochemically, the large number of CRC are positive for mucin stains. The most well-documented carbohydrates are the polysaccharide chains constituting glycoproteins and glycolipids (glycoconjugates). The malignant behavior is likely to be explained by structural defects in cell membrane glycoconjugates.

The main mucin protein cores expressed by conventional adenocarcinoma of large bowel are MUC1 and MUC3 (as opposed to MUC2 in mucinous carcinoma) [156,157].

MUC1 is widely distributed in glandular epithelia and most strongly expressed in crypt base of the colonic mucosa [158]. MUC2 is the main secretory mucin in the colorectum and specific to goblet cells.[159,160] A high proportion of colorectal cancer shows marked up-regulation for

MUC1.[161,162]. There is also an expression of MUC13, in poorly differentiated tumors[163].

Sialic acid associated with MUC2 loses O- acetyl groups in colorectal cancer and is strongly PAS-positive [164].Colorectal cancers showing high levels of DNA microsatellite instability (MSI-H) are more likely to be mucinous. This is reflected in their frequent up-regulation not only of MUC2 but also of gastric mucin (M1 or MUC5AC) [165]

Colorectal adenocarcinomas are invariably positive for cytokeratin[166]. The most common pattern is represented by positivity for CK20 and negativity for CK7. This is of great significance in differential diagnosis between colorectal adenocarcinomas and adenocarcinomas of other sites, such as lung and ovary [167,168,169]. However aberrant patterns of immunoreactivity (such as positivity for CK7) can be found in poorly differentiated adenocarcinomas[170,171]

Colorectal adenocarcinoma also shows positivity for CEA. A variety of monoclonal antibodies to different epitopes of CEA molecule are available. Of these, the epitopes of group 1or 2 have been found to have the greatest degree of sensitivity and specificity [172]. However, no evidence has yet been offered for the existence of site-specific CEA species [173]

The CDX2 is normally expressed throughout embryonic and postnatal life within nuclei of intestinal epithelial cells from the proximal duodenum to the distal rectum [174,175].

CDX2 is a caudal- type homeobox gene which encodes a transcription factor that plays an essential role in the proliferation and differentiation of intestinal epithelial cells. It is found in majority of colorectal adenocarcinomas [176]

Tumor-associated glycoprotein (TAG-72), recognized by monoclonal antibody B72.3 also present in invasive colorectal carcinomas, but also in hyperplastic and adenomatous polyps and even in normal mucosa.

Carcinoma of the large bowel often shows loss of blood group isoantigens and of HLA A, B, C expression, particularly if poorly differentiated.[177,178]. These tumors also acquire reactivity for blood group substance H [179,180]. Immunoreactivity for the secretory component of immunoglobulin is strong in well-differentiated tumors [181]

Normal goblet cells express the blood group substances A, B, H, and Le^b according to blood group and secretor status of the individual. The classification of blood group carbohydrate sequences is determined by the structure of the polysaccharide backbone [182].

The type 1 chain family includes H1, Le^b, Le^a, SLe^a. The type 2 chain counterparts are H2, Le^y, Le^x, SLe^x. Monoclonal antibodies against these blood group antigens [183-189] against closely related di- and trifucosylated variants [161] and against the core sugar sequences have demonstrated a bewildering range of changes within cancer mucin.

Upregulation of MUC1 could underlie the increased expression of type 2 blood group substances Le^x and Le^y [161,162]

Other markers expressed by colorectal carcinoma regardless of differentiation are villin (a cytoskeletal protein associated with the axial microfilament bundles of brush border microvilli)[190], cathepsin B (a lysosomal cysteine proteinase)[191], neuropilin-1 (a molecule present in the developing nervous system)[192], SRCA2(an ATPase crucial to many cell functions)[193] and cadherin-17 (also known as liver- intestine-cadherin)[194]

Calretinin can be expressed in a minority of colorectal carcinomas. It also shows reactivity for HCG[195,196], particularly common in mucinous and poorly differentiated tumors.

PLAP has been detected in approximately 10% of all colorectal carcinomas [197]. Estrogen and progesterone receptors are usually absent or present in a small minority of tumors[198,199].

Racemase, a marker for prostatic adenocarcinoma, also expressed in over half of the colorectal adenocarcinomas.[200]

IMMUNOHISTOCHEMISTRY

Immunohistochemistry is one of the powerful ancillary methods used in pathology today which has revolutionized the study of disease and its prognosis. The most useful aspect of IHC is that it is a powerful and cost-effective tool applicable in light microscopy.

The morphologic observations made by pathologists are validated by the use of IHC. Immunohistochemistry (IHC), or immunocytochemistry, is a method

for localizing specific antigens in tissues or cells placed on antigen-antibody recognition.

The main advantages of IHC which makes it a good companion to pathologist are, it can be done in regular laboratories under the light microscope without specialized devices.

Standard fixation techniques can be used and it is permanent which can be done on archival material with an additional benefit of good sensitivity and specificity.

USES OF IHC:

- Classifying undifferentiated tumors, lymphomas, neuroendocrine and soft tissue tumors.
- Detection and accurate assay of tumor biologic factors of prognostic and predictive values.
- Detection of metastatic cells in bone marrow, lymph nodes and serous fluids when the cell groups are too less or confusing.

Clearly, the validity of immunohistochemistry in diagnostic histopathology depends in great measure on the quality of immunostains. In addition to antibody quality, three other factors have a major impact on immunohistochemistry.

- Tissue fixation and processing
- Unmasking of epitopes

- Sensitivity of detection system

Among the variously available fixatives, formaldehyde is the most popular because of its low cost, ease of preparation and because it preserves morphologic details with few artifacts. However, formaldehyde fixation results in a variable loss of immunoreactivity by its masking or damaging some antibody binding sites.

IHC is performed in formalin fixed paraffin embedded tissue blocks. The results were interpreted

HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 2 (HER-2 / neu)

HER-2 is also known as proto-oncogene Neu. The oncogene neu is so-named because it was derived from a rodent glioblastoma cell line, which is a type of a neural tumor. HER 2 protein is known to form clusters in cell membranes that might play a role in tumorigenesis.

The proto-oncogene HER-2/neu is localized to chromosome 17q and it encodes a 185kD transmembrane protein that lacks a natural ligand. HER-2 activation initiates signal cascades including the MAPK (Mitogen-activated protein kinase) and PI3K/AKT (3-kinase) pathways that are essential for cell proliferation and differentiation [(201)].

In normal cells, activation of this receptor controls normal cell growth, differentiation, and motility [(202)]. In cancer cells dysregulation of these pathways and increased expression of HER-2/neu promotes tumor cell

growth and migration [203,204]. Over-expression of Her2/neu has been suggested as a factor of poor prognosis, decreased survival and increased metastasis in various malignant tumors [205].

HER2/neu is important as a target of the monoclonal antibody trastuzumab (marketed as Herceptin). Trastuzumab is useful only in cancers where the HER2/neu receptor is overexpressed. One of the mechanisms of how trastuzumab works that it binds to HER2/neu is by increasing p27, a protein that halts cell proliferation [206].

TABLE3: GRADING OF IMMUNOHISTOCHEMICAL STAINING FOR HER2/NEU EXPRESSION

SCORE	STAINING PATTERN	INTERPRETATION
0	No staining at all or very slight partial membrane staining in less than 10% of tumor cells	Negative
1+	Faint barely perceptible membrane staining in more than 10% of tumour cells. Cells stained in only part of the membrane.	Negative
2+	Weak to moderate complete membrane staining observed in more than 10% of tumour cells	Weakly positive
3+	Strong complete membrane staining in more than 30% of tumour cells	Strongly positive

The Ki-67 gene is present on the long arm of the human chromosome 10 (10q25).The half-life of Ki-67 protein has been estimated about 60-90minutes. The Ki-67 antigen is detected in G1, S, G2 and M phases of the cell cycle but not in G0 phase.

The Ki-67 is a protein phosphorylated via serine and threonine with a critical role in cell division. Cellular proliferation is fundamental to support tissue homeostasis and is essential in oncogenesis.

Assessment of tumor cell proliferation may predict tumor behavior[207]. An increasing number of studies have proposed that Ki67 may be a significant factor in cancer grading and prognostic evaluation. It has been shown that Ki67 immunohistochemical (IHC) staining is an effective method of assessing the prognosis in a number of tumor types [208,209].

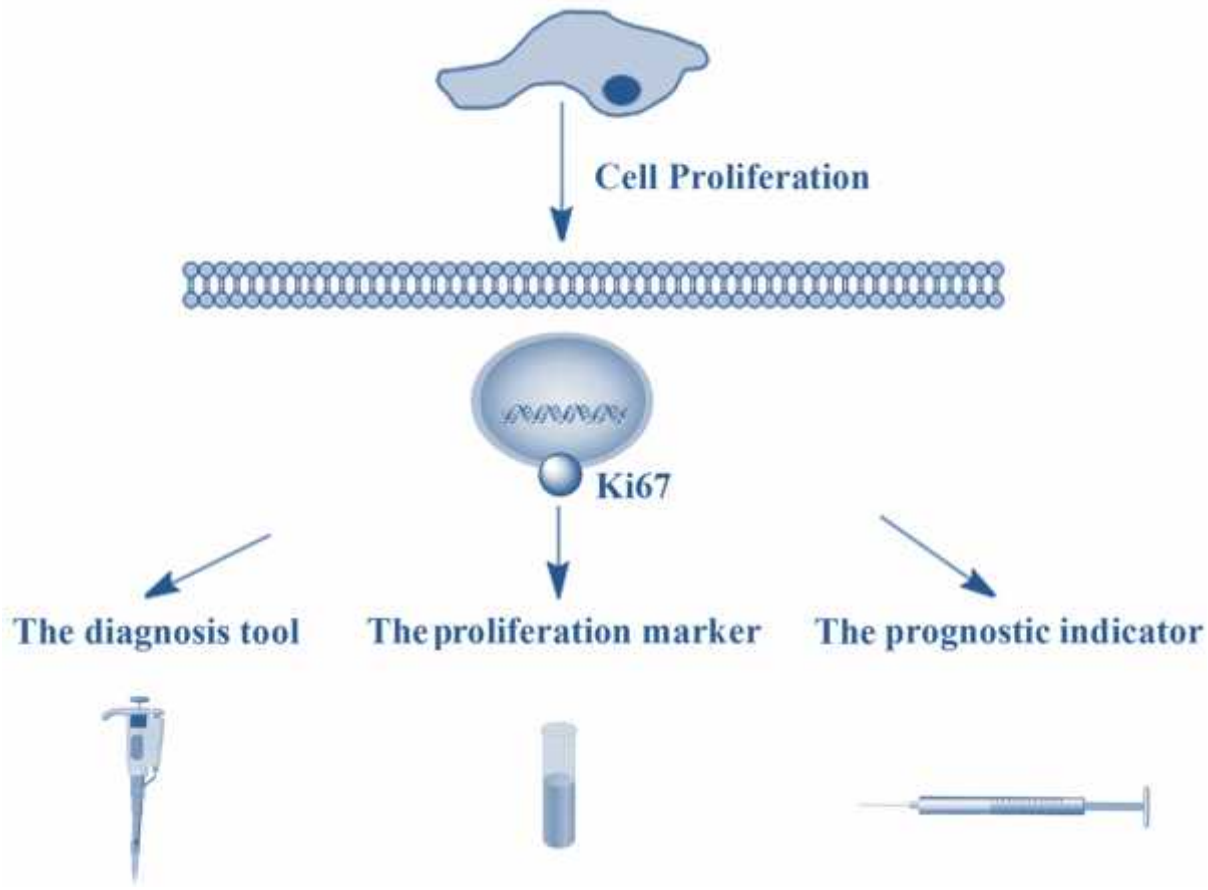


FIGURE 3: SCHEMATIC DIAGRAM OF Ki67 AS A MOLECULAR TARGET IN DIAGNOSIS OF CANCER

The Ki-67 expression is predicted as the percentage of the tumor cells positively stained by the antibody, with nuclear staining being the most frequent criterion of positivity.

MIB-1 is a monoclonal antibody it recognizes the Ki-67 nuclear antigen in the formalin fixed paraffin embedded tissue sections and its reactivity is not altered if there is a delay in fixation [(210)].

For statistical analyses, the staining results were categorized into three groups (weak, moderate, high) according to the percentage of Ki67-positive tumor cells as follows: low Ki67, 0%–10%; moderate Ki67, more than 10% and up to 25%; high Ki67, 25% and more.

This Ki67 labeling system is also currently being applied in grading breast cancer [(211)]. In well-differentiated neuroendocrine tumors(NETs), Ki67 staining of core biopsies of the primary usually provides a reliable method of proliferation assessment for prognosis of metastatic NETs to the liver [(212)]. However, only a few studies exist on the prognostic role of Ki67 in CRC and have partially shown contradictory results [(213-216)]

Ki-67 is a nonhistone nuclear protein closely associated with proliferating cells [(217)]. In patients with CRC, a high Ki-67 expression is usually associated with a higher histological grade of the tumor, lymph node involvement and shorter disease-free interval [(217,218)].

MATERIALS AND METHODS

The study was conducted after obtaining from Institutional Ethical Committee of Tirunelveli Medical College, Tirunelveli. The prospective study was carried out in the Department of Pathology, Tirunelveli Medical College, and Hospital, Tirunelveli from 2015-2017

SOURCE

Formalin-fixed, paraffin-embedded tissue blocks from 43 surgically resected colorectal tissues which were diagnosed as colorectal carcinoma variants by histopathological examination were retrieved along with their hematoxylin and eosin stained slides and they were examined

SAMPLE SIZE

A total of 43 cases were included in this study. Clinical data like patient age, sex, and other relevant details were noted from the pathology records.

INCLUSION CRITERIA

- Adenocarcinoma , NOS
- Mucinous adenocarcinoma
- Signet ring carcinoma
- Micropapillary carcinoma
- Squamous cell carcinoma
- Adenosquamous carcinoma
- Medullary carcinoma

- Undifferentiated carcinoma

EXCLUSION CRITERIA

- Benign lesions of colon and rectum

MATERIALS REQUIRED

1. Donor blocks which contain formalin fixed paraffin embedded tissue obtained from all the cases of colorectal adenocarcinoma
2. Hematoxylin and eosin stained tissue sections made from the donor blocks.
3. Black glass marking pen for marking area of interest.
4. Microtome and incubator for obtaining tissue sections and to dewax the sections
5. Positively charged slides for holding tissue sections for IHC
6. Chemicals for preparing antigen retrieval solutions and for wash buffers.
7. Pressure cookers for antigen retrieval
8. Kit for performing immunohistochemistry which includes primary antibody

   (HER 2 neu & ki-67) and universal kit . Microscope, used for interpretation and grading of IHC

METHODOLOGY

The method of performing immunohistochemistry over the paraffin tissue includes the following steps.

1. Collection of the donor blocks
2. Preparation of the recipient paraffin blocks
3. Immunohistochemistry and analysis

COLLECTION OF DONOR BLOCKS

The hematoxylin and eosin stained sections which were prepared from formalin fixed paraffin embedded blocks of all the cases of colorectal adenocarcinoma in the department of pathology during the study period were retrieved.

The corresponding formalin fixed paraffin embedded tissues were also obtained which constituted the donor block. Then the hematoxylin and eosin stained slides which contained full sections were examined and the area of interest was marked by using black glass marking pen. The area of interest is nothing but the area of tumor containing well preserved and well stained malignant cells. Then these marked areas on the slides were matched with the donor blocks and the corresponding areas over the donor blocks were also marked with the help of black glass marking pen. This area was used as the site for obtaining cores for the recipient block.

PREPARATION OF THE RECIPIENT PARAFFIN BLOCKS

The empty paraffin recipient blocks with minimum size of 25mm x 25mm were first prepared by freshly poured molten wax in the metal moulds. Then it was allowed to cool.

IMMUNOHISTOCHEMISTRY

SECTION CUTTING

Sections were taken at 5 microns thickness on the surface of the APES (3-aminopropyltriethoxysilane) coated slides. This was followed by incubation of slides at 58-60^{0}c for one hour.

ANTIGEN RETRIEVAL

In our institution we followed antigen retrieval by using pressure cooker as it produces even heating with lesser disadvantages as compared to other methods.

PROCEDURE FOR IMMUNOHISTOCHEMISTRY AS GIVEN BY MANUFACTURER (PATH N SITU)

1. Cut 3mm sections on charged slides and incubate at 60-70 ^{0}C for 1 hour.
2. Deparafinize by 2 changes of xylene 15 minutes each.
3. Hydrate through descending grades of alcohol as follows:
    - Absolute alcohol – two changes ,5 minutes each
    - 90% alcohol – 5 minutes
    - 70% alcohol – 5 minutes
    - Wash in distilled water , two changes , 2 minutes each
4. Antigen retrieval for 15 -20 minutes in MERS. pH of retrieval buffer may be either 6,8 or 9.5 according to the marker.
5. Wash in distilled water , two changes, 2 minutes each
6. Wash in PBS /TBS for 2 minutes

7. Do endogenous peroxidise blocking by adding H2O2 on the section, keep for 5 minutes. Wash in the wash buffer for 2 minutes , twice
8. Add primary antibody and keep for 30 minutes in a moist chamber. Then wash in wash buffer 2 times , 2 minutes each
9. Add Polyexcel Target binder reagent and keep for 12 minutes. Wash in two changes of buffer, 2 minutes each.
10. Add Polyexcel HRP and incubate for 12 minutes. Wash with buffer, 2 minutes two changes.
11. Add working DAB chromogen (1mlDAB Buffer + 1 drop DAB Chromogen, mix well) and keep for 2-5 minutes, then wash in distilled water.
12. Counterstain with Hematoxylin for 30 seconds, wash with water
13. Dehydrate (70%, 90%, and absolute), clear (xylene) and mount as usual.

IMMUNOHISTOCHEMICAL EVALUATION

Immunohistochemical analysis of HER 2 neu, KI-67 were donne in paraffin embedded tissue samples using polymer HRP system based on non-biotin polymeric technology.

5 µ thick sections from formalin fixed paraffin embedded tissue samples were transferred onto APES coated slides. Heat induced antigen retrieval was done.

The antigen was bound was bound with rabbit monoclonal antibody against HER2 neu , ki-67 and then detected by the addition of secondary antibody

conjugated with horse radish peroxidise- polymer and diaminobenzidine substrate.

BIOMARKERS USED IN IHC

Antigen	**Species(clone)**	**Dilution**	**Control slide**
HER 2neu	Rabbit monoclonal	Ready to use	Breast
KI-67	Rabbit monoclonal	Ready to use	Breast

OBSERVATION AND ANALYSIS

STATISTICAL ANALYSIS

The data collected were analyzed and the evaluated the relationship of colorectal carcinoma to clinicopathologic features was done. Chi –square test was used. Determining the probability factor (p-value) assessed the significance of results. When p-value were found to be less than 0.05 or less than 0.01, the results were considered statistically significant.

TABLE 3: AGE DISTRIBUTION OF PATIENTS

Of the 43 patients included in the study majority of the patients were more than 50 years, who constituted 68% of the group. Patient's age range from 17-76 years with a mean age of 53 years.

AGE	NO.OF PATIENTS (n-43)	PERCENTAGE(%)
<30	3	7
31-50	12	28
>50	28	65

CHART 1: AGE DISTRIBUTION OF PATIENTS

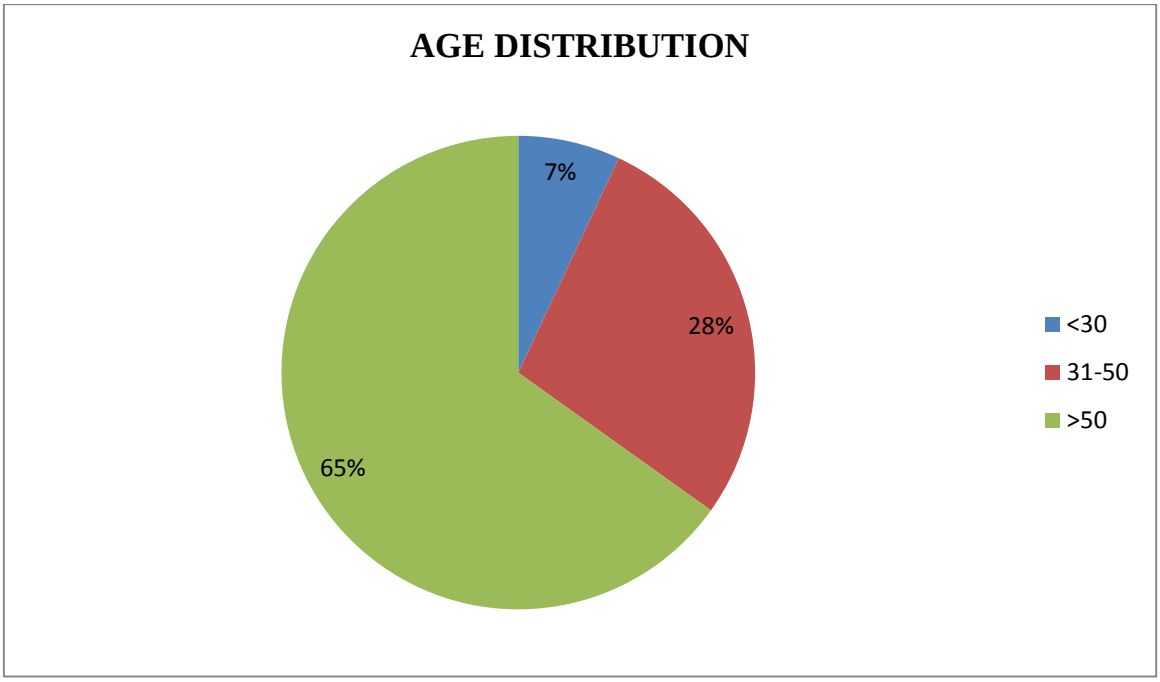
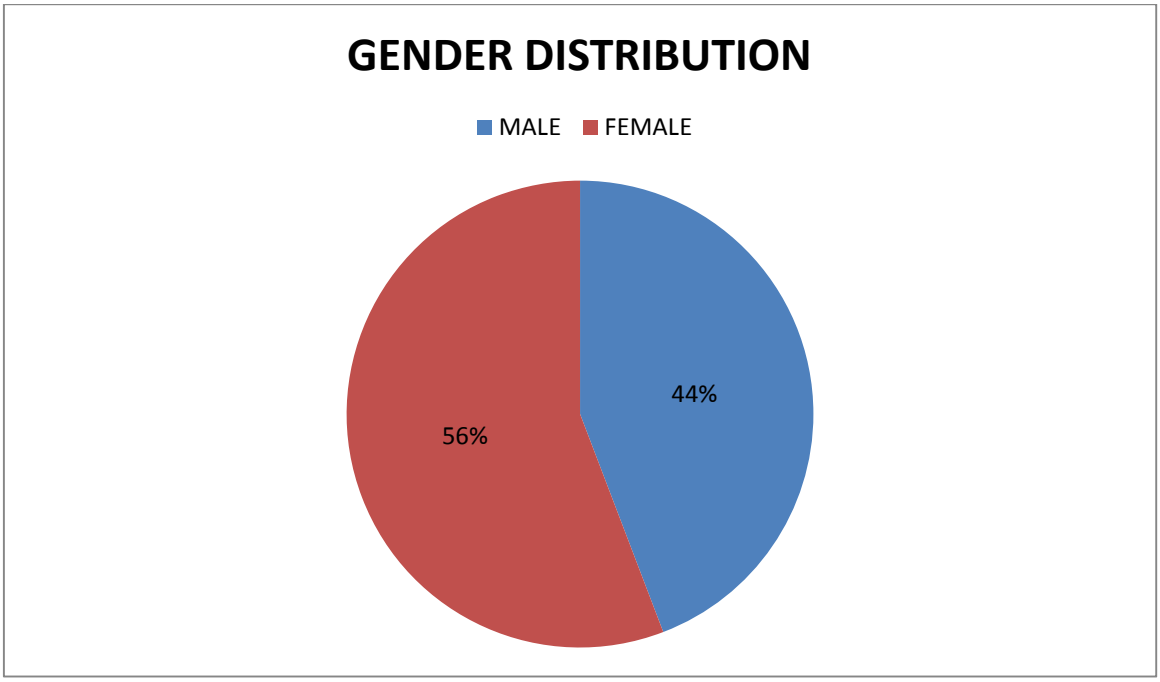


TABLE 4 : GENDER DISTRIBUTION OF PATIENTS

Out of 43 casse, 24 (56%) were females and 19(44%) were males.

GENDER	NO.OF CASES (n-43)	PERCENTAGE(%)
Male	19	44
Female	24	56

CHART2 : GENDER DISTRIBUTION OF PATIENTS



DISTRIBUTION OF SAMPLES BASED ON HISTOPATHOLOGICAL TYPE

Of the 43 patients analysed 49% were moderately differentiated adenocarcinoma,33% were well differentiated adenocarcinoma and Mucinous carcinoma constitutes 12% of the cases.

TABLE 5 : DISTRIBUTION OF SAMPLES BASED ON HISTOLOGIC TYPE

HPE DIAGNOSIS	NO.OF PATIENTS (n-43)	PERCENTAGE (%)
Well differentiated adenocarcinoma	14	33
Moderately differentiated adenocarcinoma	21	49
Poorly differentiated adenocarcinoma	1	2
Mucinous carcinoma	5	12
Signet ring cell carcinoma	1	2
Invasive squamous cell carcinoma	1	2

CHART 3: DISTRIBUTION OF SAMPLES BASED ON HISTOLOGIC TYPE

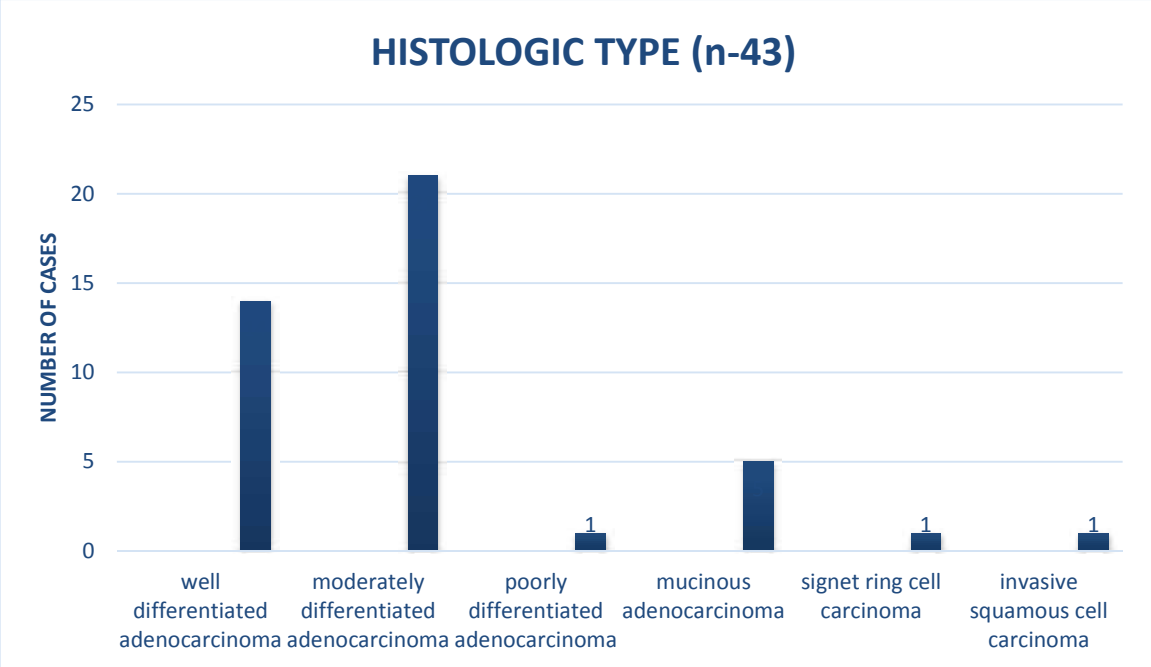
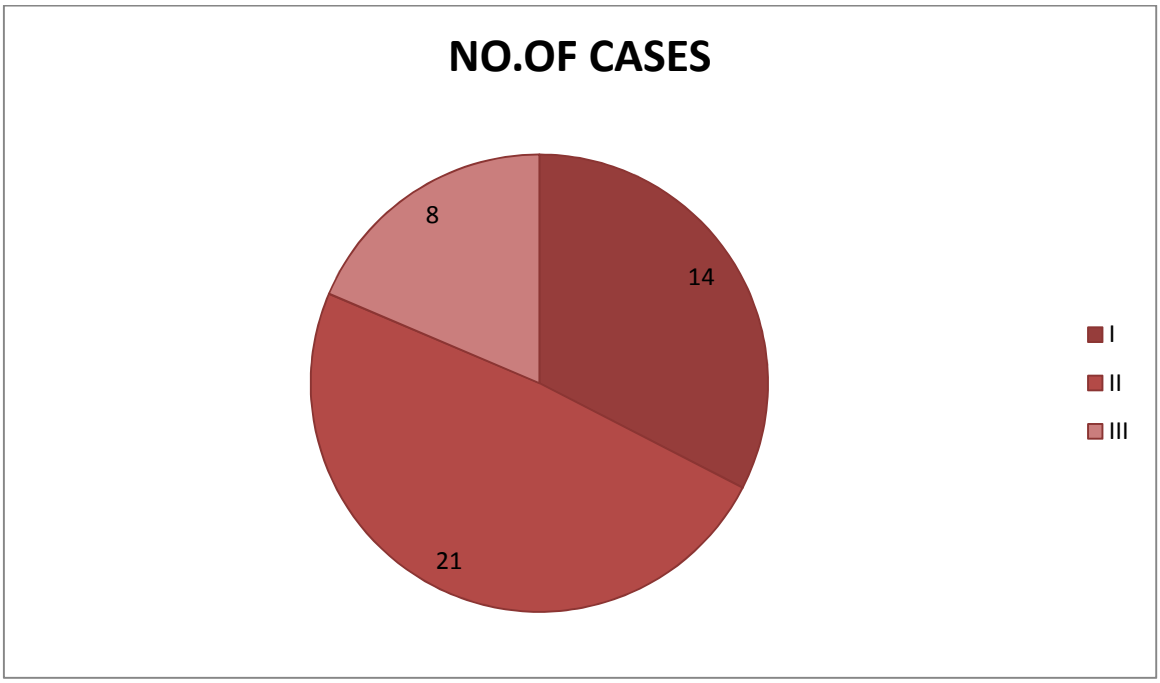


TABLE 6 : DISTRIBUTION OF SAMPLES BASED ON HISTOLOGIC GRADE

Of the 43 cases, 21 cases (49%) were grade II, followed by 14 cases (33%) were grade I, and 8 cases (19%) were under grade III.

HISTOLOGIC GRADE	NO. OF PATIENTS(n-43)	PERCENTAGE(%)
I	14	33
II	21	49
III	8	19

CHART 3: DISTRIBUTION OF SAMPLES BASED ON HISTOLOGIC GRADE



DISTRIBUTION OF HER2neu & KI-67

Out of 43 cases, 38 cases (88%) were HER2 positive, 5 cases(12%) were negative & ki-67 out of 43 cases, 14 cases(33%)shows high proliferative index, 28 cases (65%) shows low proliferative index for the above tumors.

TABLE 7 : HER2 neu & KI-67

STATUS	**NO.OF PATIENTS(n-43)**	**PERCENTAGE**
HER2		
Positive	38	88
Negative	5	12
KI-67		
High	14	33
Intermediate	1	2
Low	28	65

CHART4 : GRAPH REPRESENTING HER2 RECEPTOR STATUS

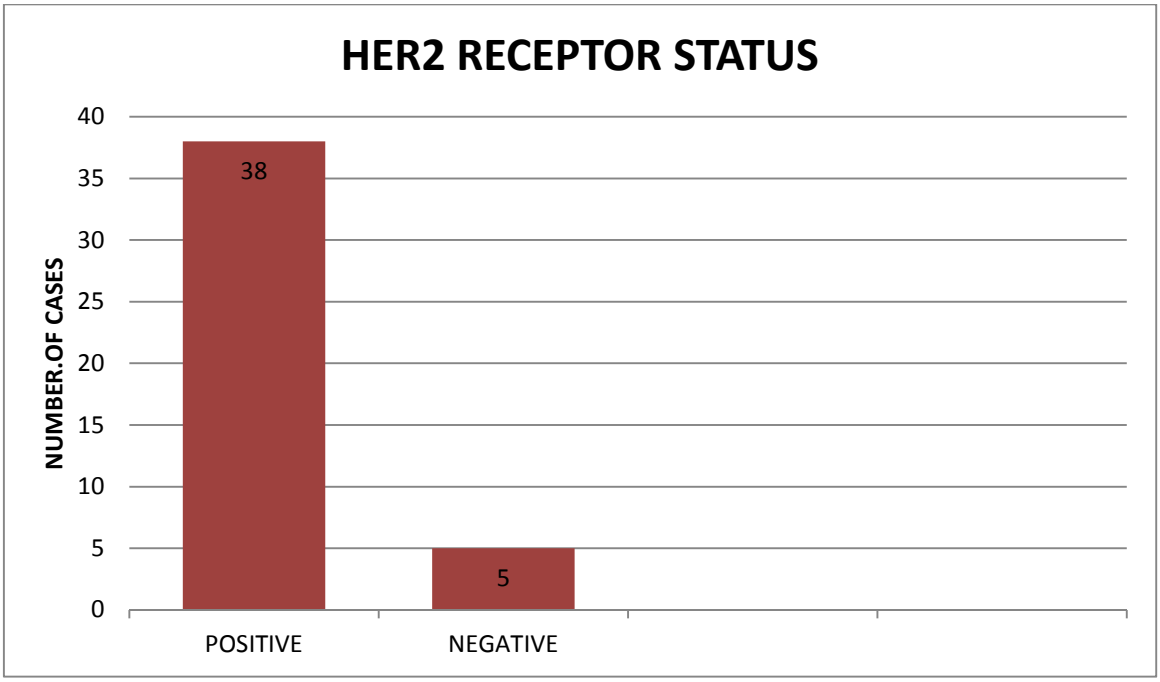
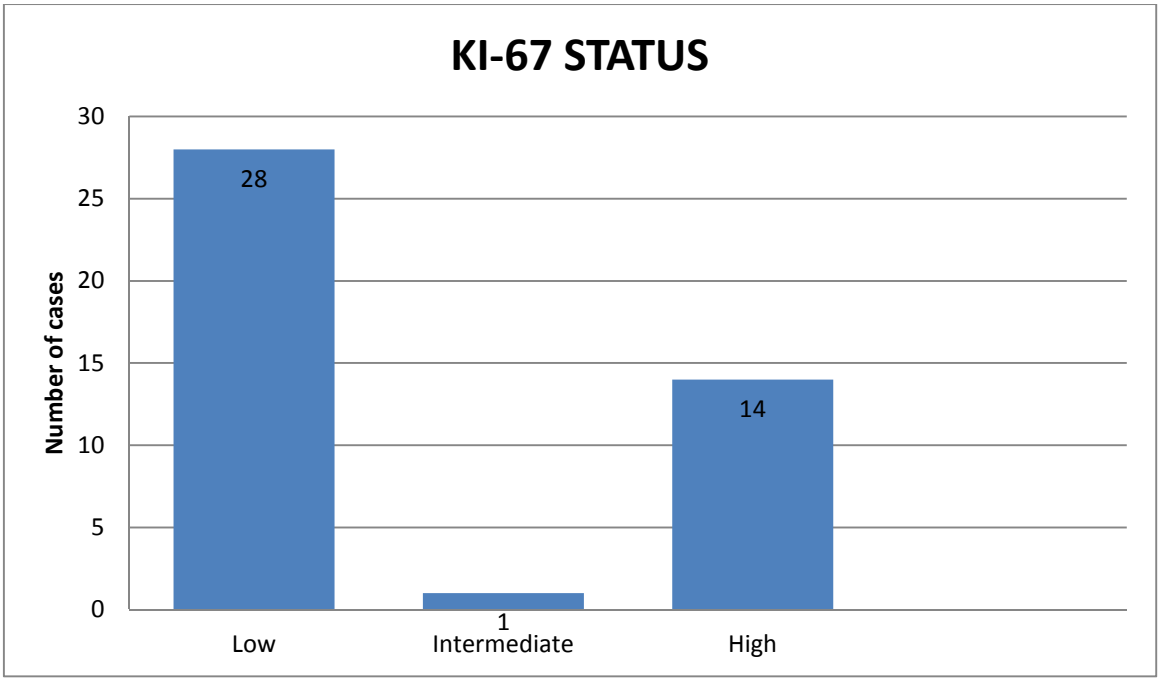


CHART5 : GRAPH REPRESENTING KI-67 STATUS



CORRELATION BETWEEN HISTOLOGIC TYPE AND HER2 neu

In the present study, 3 cases of moderately differentiated adenocarcinoma were HER2 positive and 18 cases were HER2 negative. 2 cases of well differentiated adenocarcinoma were HER2 positive and 12 cases were HER2 negative. 5 cases of mucinous adenocarcinoma were HER2 negative. The relationship between HER2 neu and histologic type were insignificant (p value – 0.992) when assessed by chi – square test

TABLE 8 : CORRELATION BETWEEN HISTOLOGIC TYPE AND HER2 neu

HISTOLOGIC TYPE	HER2 (+)	HER2(-)	P value Chi-square test
Well differentiated carcinoma	2	12	
Moderately differentiated carcinoma	3	18	
Poorly differentiated carcinoma	0	1	
Mucinous carcinoma	0	5	
Signet ring cell carcinoma	0	1	0.992
Invasive squamous cell carcinoma	0	1	

CORRELATION BETWEEN HISTOLOGIC TYPE AND KI-67

In our study,7 cases of moderately differentiated adenocarcinoma shows high proliferative activity, 13 cases shows low proliferative activity. In well differentiated adenocarcinoma, 6 cases shows high proliferative activity and 8 cases shows low proliferative activity. In mucinous carcinoma , 5 cases shows low proliferative activity. The relationship between histologic type and KI-67 was statistically insignificant (p value – 0.693) when assessed by chi-square chart.

TABLE 9 : CORRELATION BETWEEN HISTOLOGIC TYPE AND KI-67

Histologic type	**Low**	**Intermediate**	**High**	**P value Chi-square test**
Well differentiated adenocarcinoma	8	0	6	0.693
moderately differentiated adenocarcinoma	13	1	7	
Poorly differentiated adenocarcinoma	0	0	1	
Mucinous carcinoma	5	0	0	
Signet ring cell carcinoma	1	0	0	
Invasive squamous cell carcinoma	1	0	0	

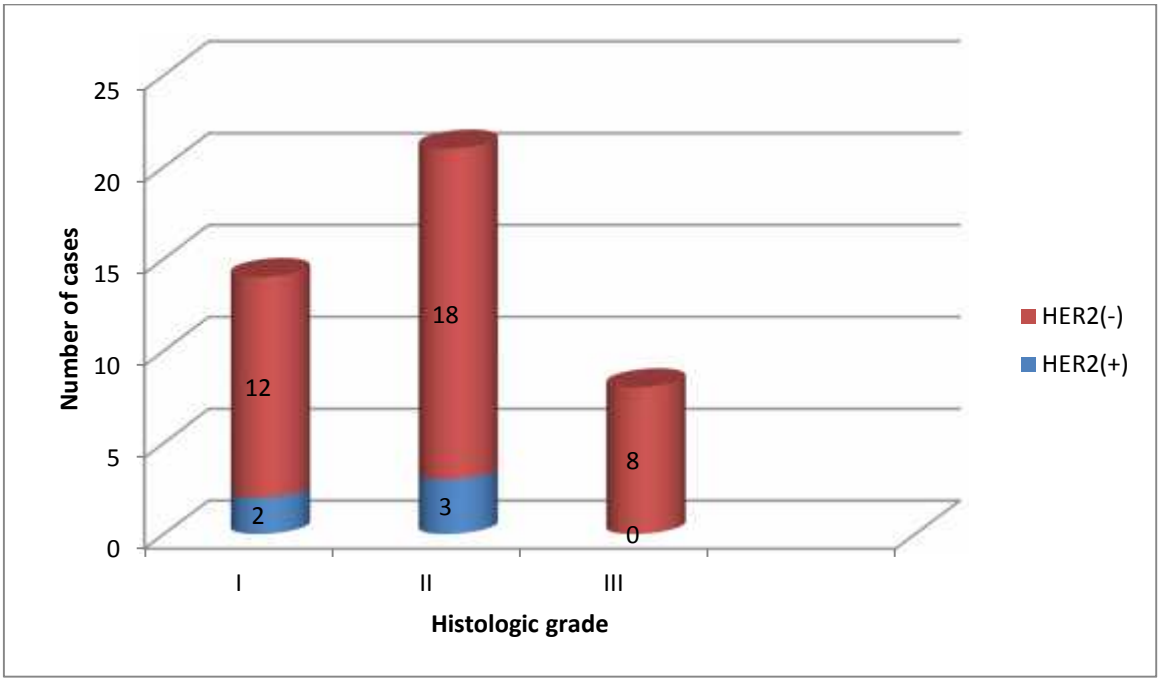
CORRELATION BETWEEN HISTOLOGIC GRADE AND HER2 neu

In our study, grade I tumors shows 2 cases of HER2 positive and 12 cases were HER2 negative. Grade II tumors shows 3 cases of HER2 positive and 18 cases were HER2 negative. Grade III tumors shows 8 cases of HER2 negative. The relationship between histologic grade and HER2neu were statistically insignificant (p value- 0.656) when assessed by chi-square test.

TABLE 10 : CORRELATION BETWEEN HISTOLOGIC GRADE AND HER2 neu

Histologic grade	**HER2 (+)**	**HER2 (-)**	**P value Chi square test**
I	2	12	0.656
II	3	18	
III	0	8	

CHART6 : CORRELATION BETWEEN HISTOLOGIC GRADE AND HER2 neu



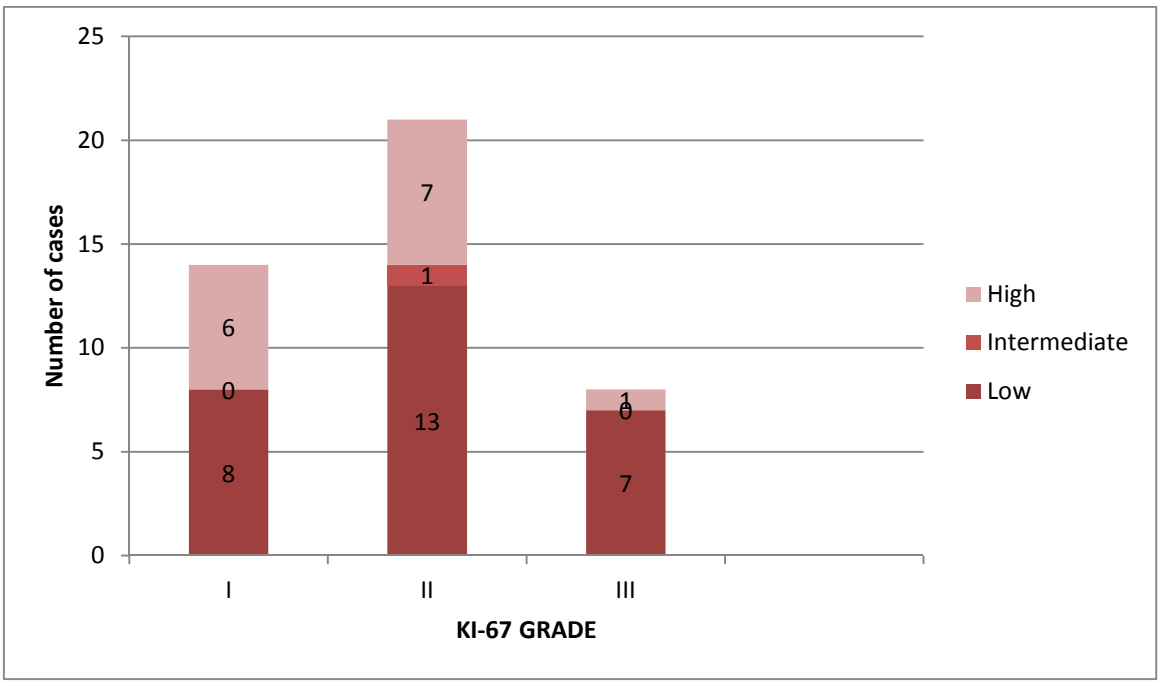
CORRELATION BETWEEN HISTOLOGIC GRADE AND KI-67

In our study , grade I tumors shows 6 cases of high proliferative index and 8 cases of low proliferative index. Grade II tumors shows 7 cases of high proliferative index and 13 cases of low proliferative index, 1 case shows intermediate proliferative index. Grade III tumors shows 1 case of high proliferative index and 7 cases of low proliferative index. The relationship between histologic grade and KI-67 was statistically insignificant (p value – 0.512) when asssessed by chi-square test.

TABLE 11 : CORRELATION BETWEEN HISTOLOGIC GRADE AND KI-67

GRADE	LOW	INTERMEDIATE	HIGH	P value Chi-square test
I	8		6	
II	13	1	7	0.512
III	7		1	

CHART 7 : CORRELATION BETWEEN HISTOLOGIC GRADE AND KI-67



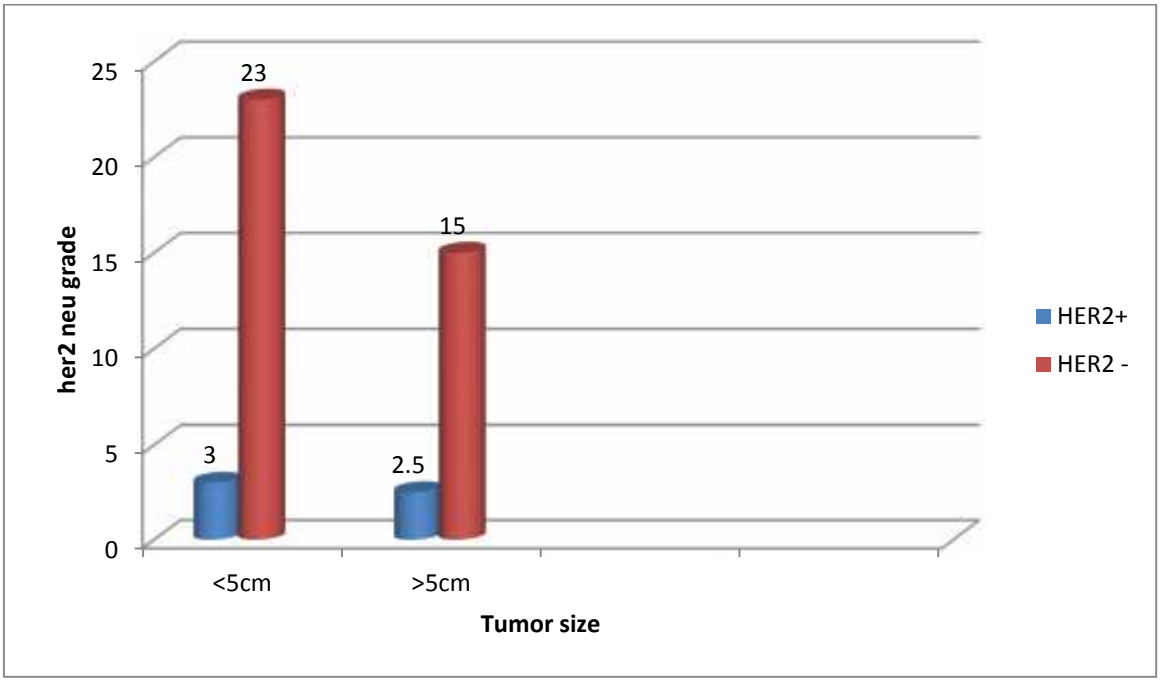
CORRELATION BETWEEN TUMOR SIZE AND HER2 neu

In our study, out of 43 cases tumor size of less than 5cm, shows 3 cases of HER2 neu positive and 23 cases of HER2 neu negative. Tumor size of more than 5 cm shows 2 cases of HER2 neu positive and 15 cases were HER2 neu negative. The relationship between tumor size and her2 neu expression was statistically insignificant (p value -0.715) when assessed by chi-square test.

TABLE 12 : CORRELATION BETWEEN TUMOR SIZE AND HER2 neu

Tumor size	**Her2 (+)**	**Her 2 (-)**	**P value** **Chi –square test**
<5cm	3	23	
>5cm	2	15	0.715

CHART8 : CORRELATION BETWEEN TUMOR SIZE AND HER2 neu



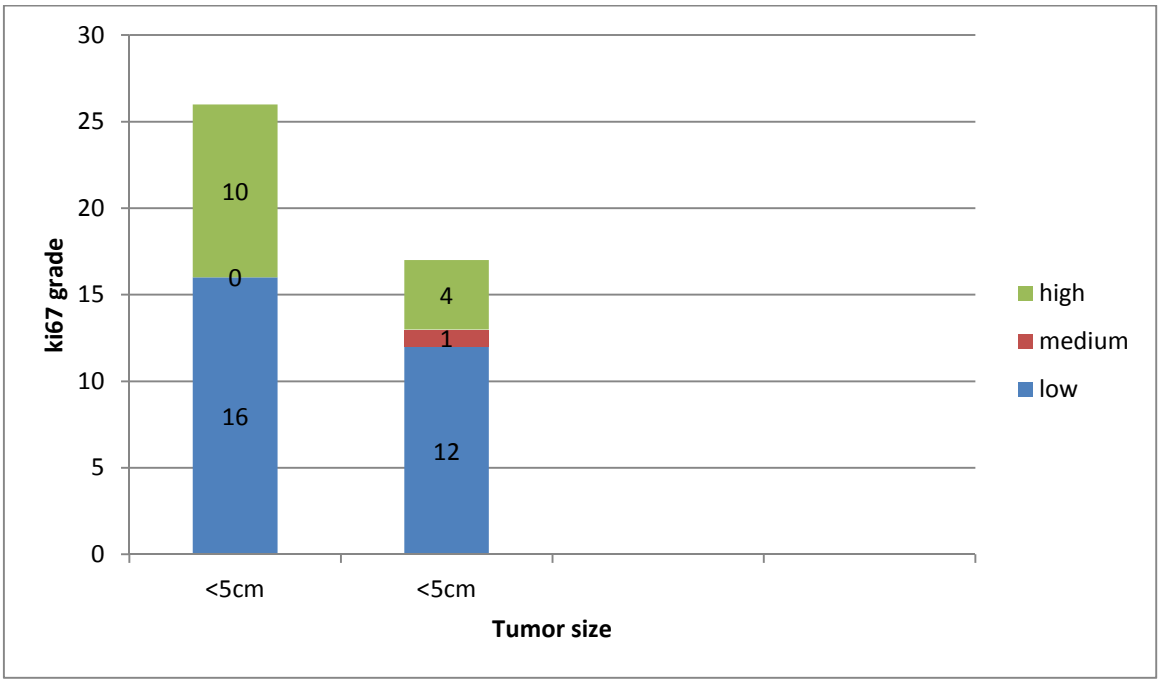
CORRELATION BETWEEN TUMOR SIZE AND KI-67

In our study, out of 43 cases, tumor size of less than 5cm shows 10 cases with high proliferative index and 16 cases with low proliferative index. Tumor size of more than 5 cm shows 4 cases with high proliferative index and 12 cases with low proliferative index and 1 cases with intermediate proliferative index.

TABLE 13 : CORRELATION BETWEEN TUMOR SIZE AND KI-67

TUMOR SIZE	LOW	INTERMEDIATE	HIGH	P value Chi square test
<5cm	16	0	10	
>5cm	12	1	4	0.307

CHART9 : CORRELATION BETWEEN TUMOR SIZE AND KI67



RELATIONSHIP BETWEEN HER2 neu AND CLINICOPATHOLOGICAL VARIABLES

In our study , her 2 neu expression was correlated with age, gender, tumor location, lymph node status. Compared with age, more than 50 years shows 4 cases of her2 positive and 11 cases shows her2 negative. The relationship between her2 neu expression and age was statistically insignificant (p value- 0.857) when assessed by chi-square test.

Her2 neu expression was correlated with gender of which 3 cases were her2 neu positive and 21 cases were her2 neu negative in females. 2 cases were her2 neu positive and 17 cases were her2 neu negative in males. This was staistically insignificant (p value – 0.646) when assessed by chi-square test.

Her 2 neu expression was correlated with tumor location of which 28% was in right hemicolon , 26% was in left hemicolon, 40% in rectum and 7% in recto-sigmoid. The relationship between tumor location and her2 neu expression was statistically insignificant (p value – 0.791) when assessed by chi-square test.

Her 2 neu expression was correlated with lymph node status, of which not involved by tumor was 35% with 3 cases of her2 positive and 13 cases of her2 negative. Metastatic nodes was 37% of which 1 cases were her2 positive , 15 cases were her2 negative. Nodes couldnt be assessed was 28% of which 2 cases were her2 positive and 10 cases were her2 negative. The relationship between lymph node status and her2 was statstically insignificant (p value – 0.601) when assessed by chi-square test.

TABLE 14 : RELATIONSHIP BETWEEN HER2 neu AND CLINICOPATHOLOGICAL VARIABLES

PARAMETER	NUMBER	HER2(+)	HER2(-)	P value Chi square test
AGE				
<30	3	0	3	
31-50	12	1	11	0.857
>50	28	4	24	
GENDER				
Male	19	2	17	
Female	24	3	21	0.646
SITE				
Right colon	12	2	10	
Left colon	11	2	9	0.791
Rectum	17	1	16	
Recto-sigmoid	3	0	3	
Lymph node status				
Not involved by tumor	15	2	13	
Metastatic	16	1	15	0.601
Couldn't be assessed	12	2	10	

CORRELATION BETWEEN KI-67 AND CLINICOPATHOLOGICAL VARIABLES

In our study , ki-67 was correlated with age, gender, tumor location, lymph node status. Compared with age, patients with more than 50 years show high proliferative index. The relationship between age and ki-67 expression was statistically insignificant (p value – 0.308) when assessed by chi-square test.

Compared with gender, female patients show high proliferative index. The relationship between gender and ki-67 expression was statistically insignificant (p value – 0.22) when assessed by chi-square test.

Compared with tumor location, rectum shows high proliferative index. The relationship between tumor location and ki-67 expression was statistically insignificant (p value – 0.821)

Compared with lymph node status, 13 cases of metastatic nodes show low proliferative index. The relationship between lymph node status and ki-67 expression was statistically significant (p value – 0.004) when assessed by chi-square test.

TABLE 15: CORRELATION BETWEEN KI-67 AND CLINICOPATHOLOGICAL VARIABLES

PARAMETER	NUMBER	LOW	INTERMEDIATE	HIGH	P value Chi square test
AGE					
<30	3	3	0	0	0.308
31-50	12	8	1	3	
>50	28	17	0	11	
GENDER					
Male	19	14	1	4	
Female	24	14	0	10	0.22
SITE					
Right colon	12	7	1	4	
Left colon	11	8	0	3	
Rectum	17	11	0	6	0.821
Recto-sigmoid	3	2	0	1	
Lymph node status					
Not involved by tumor	15	12		3	
Metastatic	16	13	1	2	0.004
Couldn't be assessed	12	3		9	

DISCUSSION

Colorectal cancer (CRC) is the fourth most common malignant disease with over one million novel cases and over 5,00,000 deaths each year worldwide[219]. Early diagnosis of CRC, successful surgical treatment, better knowledge of its clinico-pathological prognostic factors and response to adjuvant therapy have contributed to improved outcome in affected patients.

Immunohistochemistry refers to the process of localizing proteins in the cells of a tissue section, thus exploiting the principle of antibodies binding specifically to antigens in biological tissues.

Immunohistochemistry is relatively inexpensive, widely available, easy to preserve and less time consuming and it requires a routine microscope.

Although the tumor is diagnosed histopathologically on light microscopy, various immunological markers are expressed by colorectal carcinomas and depending on them, the treatment and the prognosis differ.

Her2 neu is a useful marker, to predict the outcome of colorectal cancers. Its over-expression correlates with poor prognosis. It is used to predict the patient response to adjuvant chemotherapy and endocrine therapy and to select patients for immunotherapy with a targeted monoclonal antibody therapy. The patients who overexpress Her2 neu should respond to transtuzumab (Herceptin) therapy, independent of the tissue origin of the cancer.

COMPARITIVE STUDY OF AGE AND SEX DISTRIBUTION IN CRC

In our study it includes 43 patients of colorectal adenocarcinoma. Mean age of the patients recruited in the study was 53 years (range 17-76 years).

El- Bolkainy et al [220] observed the mean age was 51 years, which was similar to our present study. Rajesh Singh et al [221] observed the mean age was 60-69 years.

In our study, 19(44%) were male patients and 24(56%) were female patients.

In Manmeet kaur gill et al, 24(60%) were male patients and 16(40%) were female patients. Ghaffarzadegan , schuell also found the same sex distribution in their studies.[222,223] Cressy et al [224] reported that higher incidence was detected in females representing (63%) of cases, which was similar to our present study.

COMPARITIVE STYDY OF HISTOLOGIC TYPES IN COLORECTAL CARCINOMAS

In our study, histologic types of colorectal carcinoma, 84% cases were adenocarcinoma, 12% were mucinous adenocarcinoma. Lanza et al, [225] reported 85% were adenocarcinomas, 10-15% were mucinous adenocarcinomas.

Usual type adenocarcinoma was the most common histologic type reported by Bhagyalakshmi et al and also in other studies [226,227,228]. In one study [229]mucinous carcinomas accounted for 11.6% cases and signet ring for 4% of cases.

Sen et al observed moderately differentiated adenocarcinoma (69.1%) constitutes the most common type, followed by well differentiated and poorly differentiated adenocarcinomas (11.8%)

COMPARITIVE STUDY OF HISTOLOGIC GRADE IN COLORECTAL CARCINOMA

In our study, majority was grade II tumors(49%) , followed by grade I tumors(33%). Bhagyalakshmi et al reported majority were grade I tumors.

In several other studies [230,231,232,233,234], CRCs were mostly of grade II tumors (moderately differentiated).

Dalal A. Elwy et al reported 72% cases of grade II tumors (moderately differentiated) in accordance with the results obtained by Triest et al [235]. Sharifi et al [236] reported majority of the cases were grade I tumors (well differentiated).

COMPARITIVE STUDY BETWEEN HER2 neu AND KI-67 EXPRESSION IN CRC

Regarding the immunohistochemical staining for HER2 neu, in our study 88% cases were HER2 neu positive. Mckay et al [237] studied the HER2 neu expression in large cohort of colorectal tumors, HER2 neu was expressed in 81.8% cases.

Tavangar et al reported 12 cases (21.8%) oh HER2neu positivity and it shows a significant correlation (p-0.005) between a more advanced stage of the disease and the prevalence of Her 2 neu over-expression.

Kunio et al observed no Her 2 neu positivity in colorectal carcinomas, because the number of Her 2 positive patients with colon cancer was small compared to those with breast and stomach cancers.

In our study, 28 cases (65%) shows low proliferative index and 14 cases (33%) shows high proliferative index. Uzma et al observed 62% of cases show high proliferative index and 38% cases shows low proliferative index, indicating a variation in proliferative activity. Worldwide, CRC showed a wide- ranged variation of Ki67, ranging from 13-90% [238-242] indicating a variation in proliferative activity.

COMPARITIVE STUDY BETWEEN HISTOLOGIC TYPE AND HER2 NEU AND KI67 EXPRESSION IN CRC

In our study, no statistically significant relationship was detected between HER2 neu expression and histologic types. The same was reported by Kavanagh et al [243].

Ki67 expression with histologic types shows no statistically significant relationship between them.

Ahmed et al observed the proliferative activity was higher in non mucinous tumors than the mucinous ans signet ring carcinoma. Lanza et al observed higher levels of Ki67 reactivity in mucinous tumors than non-mucinoid adenocarcinomas.

Uzma et al observed Ki-67 proliferative index was high in non mucinous tumors than in mucinous or signet ring cell carcinoma.

COMPARITIVE STUDY BETWEEN HISTOLOGIC GRADE ANF HER2 NEU AND KI67 EXPRESSION IN CRC

In our study, no statistically significant relationship was observed between histologic grades and HER2 neu expression. This result was consistent with Goldstein and Armin and Gruenberger et al. In another study a significant correlation was observed between histologic grades and HER2 neu expression done by Steel et al, Mckay et al, Ghaffarzadegan et al and Deng et al.

In our study, Ki67 expression with histologic grade was not statistically insignificant. A study conducted in Japan concluded Ki67 positivity was lower in poorly differentiated and mucinous carcinoma compared with well differentiated and moderately differentiated adenocarcinoma , suggesting that proliferative activity is lower in cancers with poor differentiation.

COMPARITIVE STUDY BETWEEN OTHER CLINICOPATHOLOGIC VARIABLES IN CRC

In our study, no statistically significant relationship was reported between tumour site and HER2 neu expression. The same finding was reported by Mohammadi et al [(244)] and Koenders et al [(243)].

In contrast a decreasing frequency of HER2 neu positive tumors from colon to rectum was reported by Gruenberger et al [(244)] and Koretz et al [(245)].

Li et al [246] observed that correlation was expressed between her2 neu expression and tumor size and distant metastasis (both $p<0.05$), but not correlated with the other clinicopathologic parameters.

In our study, no statistically significant relation was detected between patient's age and sex among studied colorectal cases and HER2 neu expression. This agrees with other studies performed by Ghaffardegan et al [247], Gruenberger et al [244] and Mohammadi et al [242]. In contrast Kountourakis et al [248] demonstrated a statistically significant expression of HER2 neu in the old age group.

In our study, there is statistically significant relationship with lymph node status and Ki-67 expression.

Other studies also observed that there is no correlation between Ki67 and clinicopathologic parameters as age, gender, tumor location, nodal status. This lack of relation is due to considerable heterogenecity in CRCs [238, 249].

In our study out of 43 cases, 40% cases were located in the rectum, 28% in right hemicolon, 26% in left hemicolon. In dalal A. Elwy et al, 36% in right colon, 20% in left colon. The rectum represents 32% cases.

Tumors of right colon outnumber those of the left colon. This agrees with Smyrk [250]who detected a shift toward right-sided cancers occurig during 20th century and Fenoglio-Preiser et al[251] who stated that in low risk carcinomas, carcinomas of the cecum and ascending colon occur more frequently than carcinomas of the

left colon, whereas in high-risk countries, colorectal carcinomas more commonly arise in rectosigmoid region. Tavangar et al reported 45.6% with involvement of right colon and 54.4% cases with involvement of the left colorectal region [252]

In B Ingold Heppner et al, although statistically not significant, HER2 positive tumors tend to be more frequent in sigmoid colon / rectum (p value- 0.063)

Bhagyalakshmi et al, rectum was the most commonly affected (45.1%) followed by right and left colon. In one study [230] colon was more commonly affected site than rectum. In another study [221] rectum was the most common site affected. In two other studies [226,253] rectosigmoid was found to be the most common site affected by CRC.

In our study no statistically significant link was observed between HER2 neu expression and lymph node status. This result was consistent with Mckay et al. In another study, Park et al found over expression of HER2 neu in 12.5% of cases. Tumors which showed positive Her2 neu showed higher rate of nodal metastasis.

SUMMARY

This study was conducted in Department of Pathology, Tirunelveli Medical College from 43 cases of colorectal carcinomas. Paraffin embedded tissue blocks were retrieved.

Among 43 cases, 21 cases (49%) were moderately differentiated adenocarcinoma, 14 cases (33%) were well differentiated adenocarcinoma, 5 cases (12%) were mucinous carcinoma, 1 cases (2%) were poorly differentiated, signet ring cell and invasive squamous cell carcinoma.

Among 43 cases, 38 cases (88%) show Her2 neu positive and Ki67 shows 14 cases (33%) showing high proliferative index and 65 % cases showing low proliferative index.

Among 43 cases, 14 cases (33%) were grade 1 tumors, 21 cases (49%) were grade II tumors, 8 cases (19%) were grade III tumors.

Among 43 cases, only 5 cases show Her 2 neu positivity, in Ki-67 only 14 cases shows high proliferative index, 21 cases shows low proliferative index.

There is statistically significant relationship between Ki-67 and lymph node status.

In our study, there is no statistically significant relationship between clinico - pathological variables and Her 2 / neu expression.

CONCLUSION

Thus it was concluded that colorectal carcinomas which expresses HER2 neu could carry a poor prognosis and therefore require a different therapeutic approach, as these cases could respond to transtuzumab (Herceptin) therapy as well as other new modalities.

Immunohistochemical technique for detection of Ki-67 proliferative index is simple and applicable to surgical specimens.

However, it is not enough to monitor Ki-67 proliferation index alone for prognosis in colorectal cancer as it was not significantly related to variable clinicopathologic parameters.

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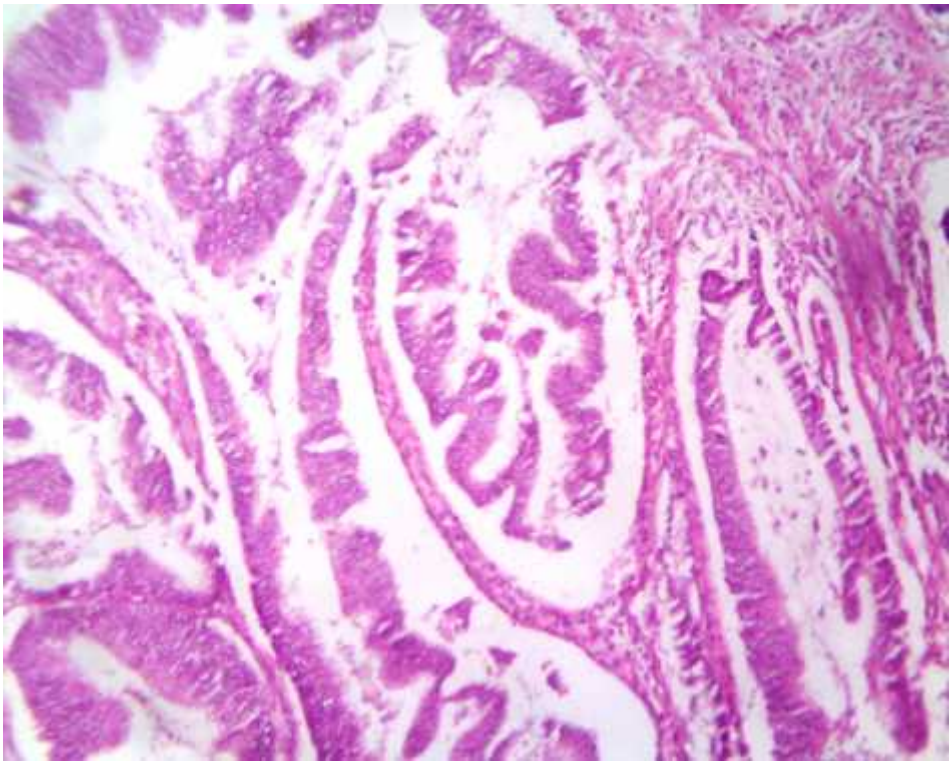

FIGURE 5: Well differentiated adenocarcinoma-GRADE I (H&E X 400)

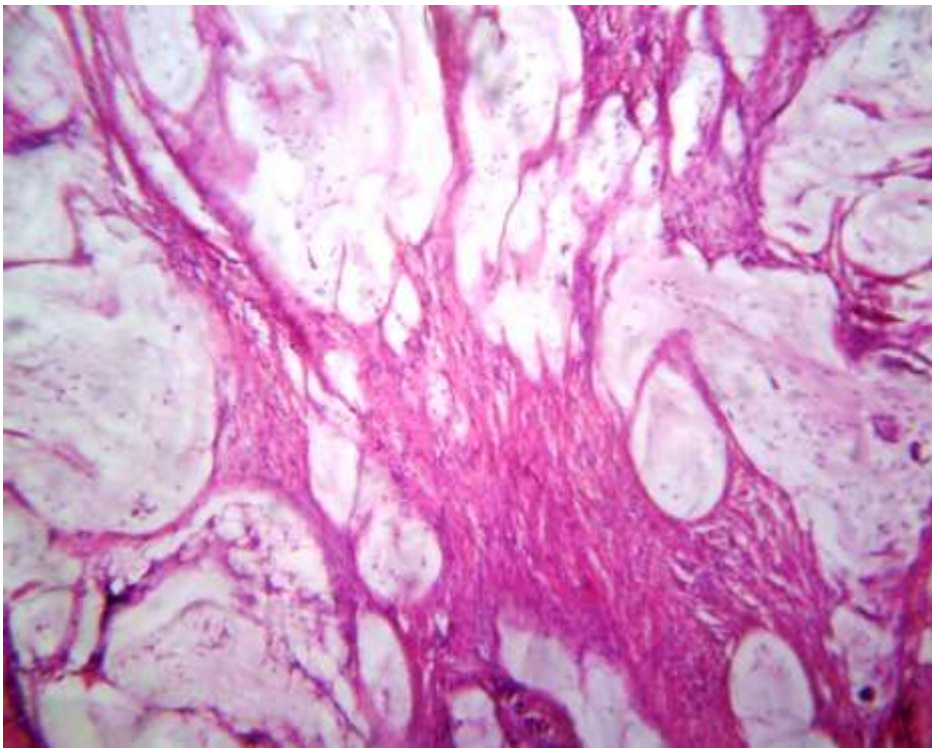

FIGURE 6: Mucinous carcinoma (H&E X 100)

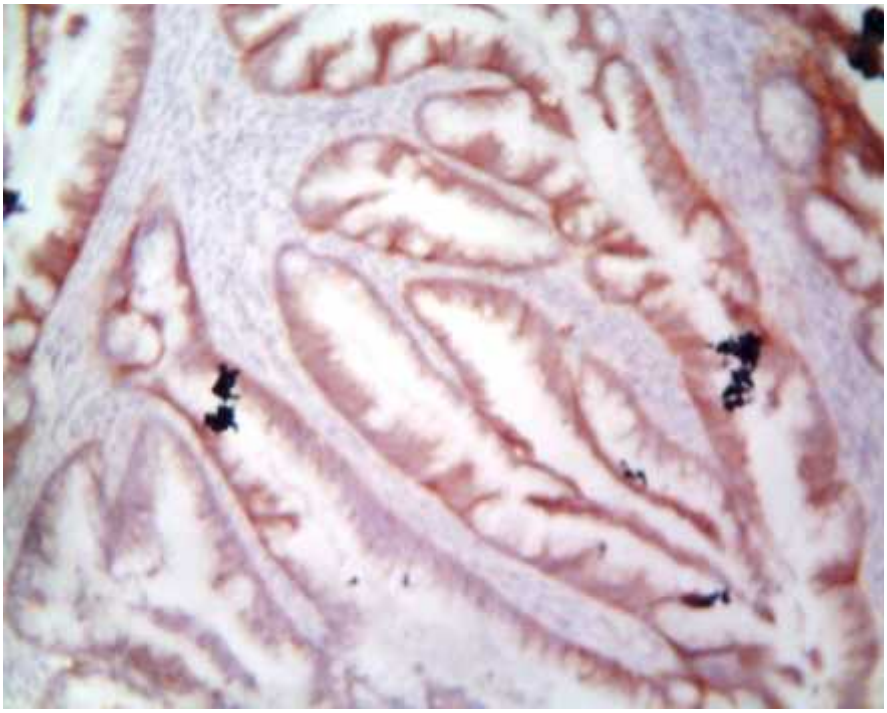

FIGURE 7: Well differentiated adenocarcinoma showing strong HER2 (+) of score 3 in the above tumor (IHC,X 100)

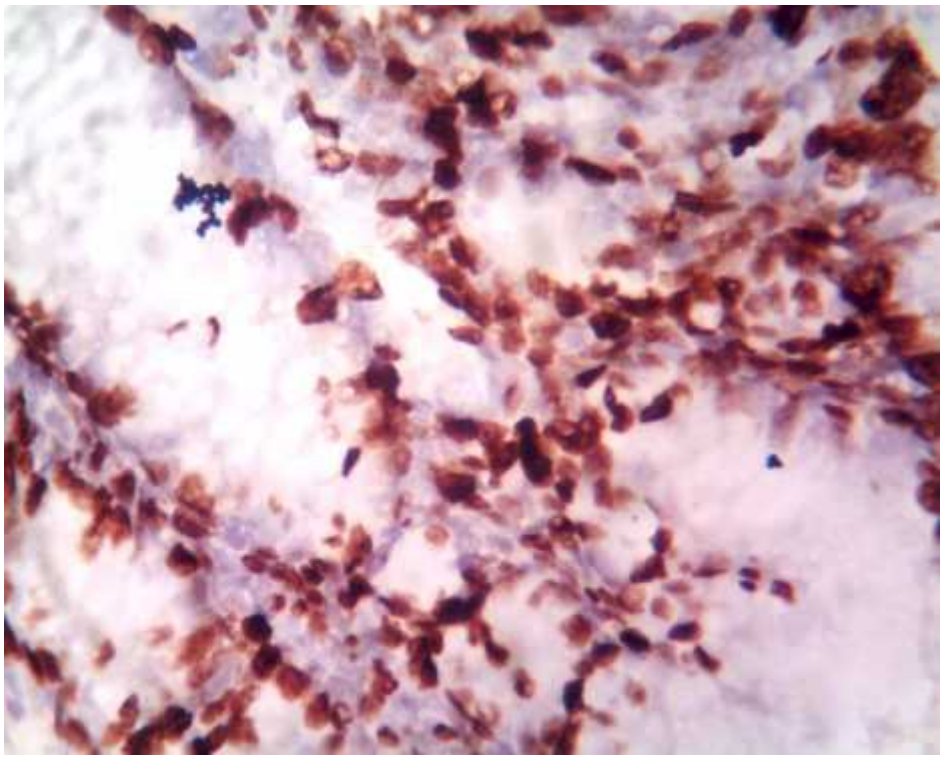

FIGURE 8: Well differentiated adenocarcinoma showing strong nuclear positivity of KI-67 in the above tumor (IHC, X400)

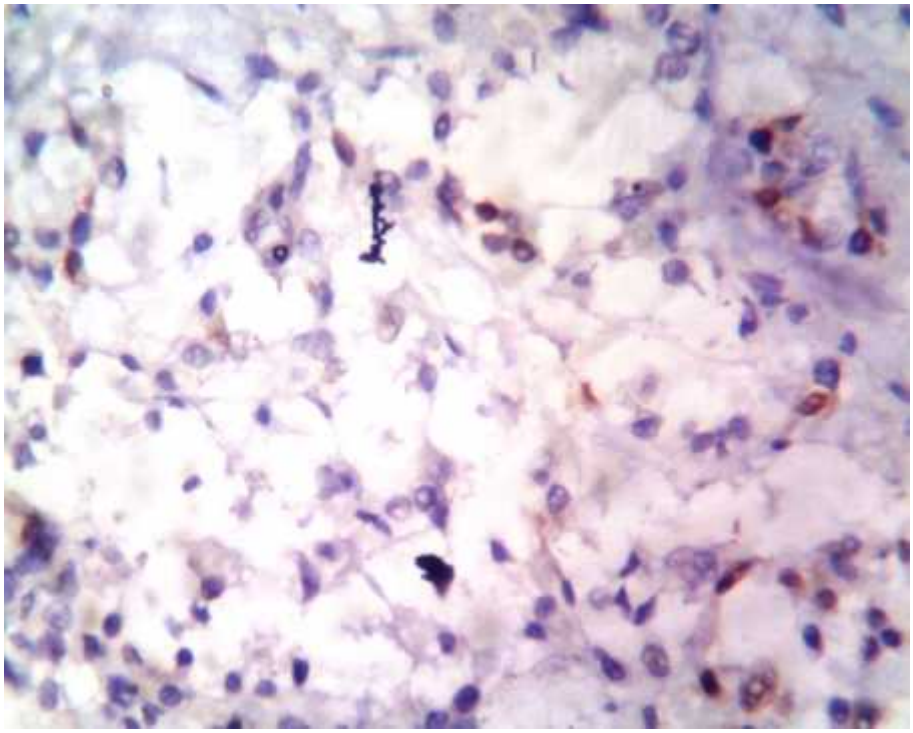

FIGURE 9: Moderately differentiated adenocarcinoma showing moderate nuclear positivity of KI-67 in the above tumor (IHC, X 400)

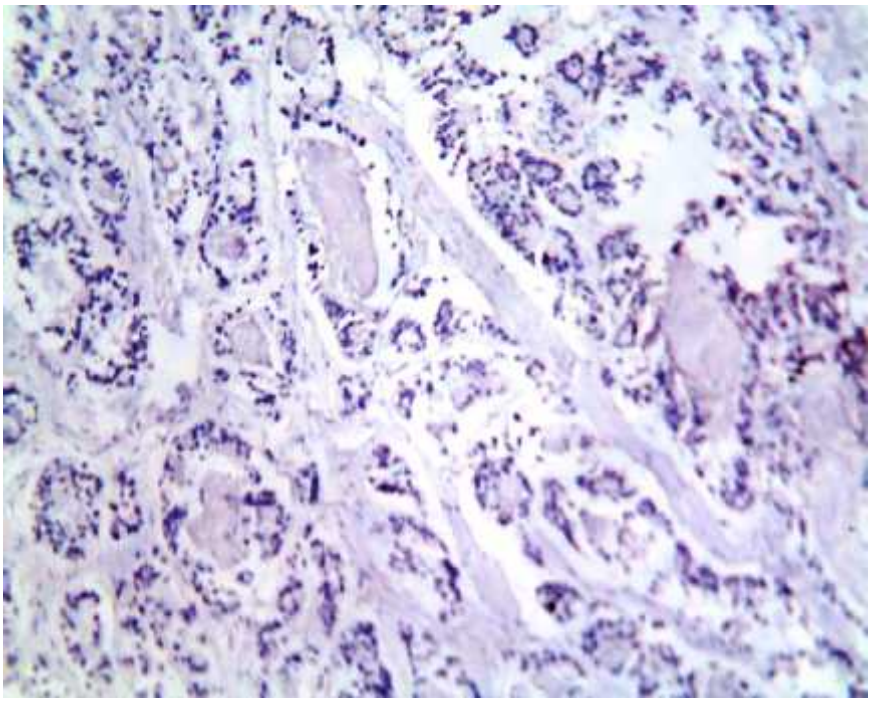

FIGURE 10: Well differentiated adenocarcinoma showing absent nuclear positivity in the above tumor (IHC, X 100)

ANNEXURE - I

WHO CLASSIFICATION OF HISTOPATHOLOGIC TYPES OF COLORECTAL CARCINOMA

Adenocarcinoma, not otherwise specified

Mucinous adenocarcinoma

Signet ring carcinoma

Small cell carcinoma

Micropapillary adenocarcinoma

Serrated adenocarcinoma

Cribriform comedo-type adenocarcinoma

Adenosquamous carcinoma

Squamous cell carcinoma

Medullary carcinoma

Undifferentiated carcinoma

ANNEXURE - II

WHO CLASSIFICATION OF MALIGNANT LYMPHOMAS OF THE COLON AND RECTUM

1. Marginal zone lymphoma of MALT type
2. Mantle cell lymphoma
3. Diffuse large B-cell lymphoma
4. Burkitt lymphoma
5. B –cell lymphoma unclassifiable

ANNEXURE - III

PROCEDURE FOR IMMUNOHISTOCHEMISTRY AS GIVEN BY MANUFACTURER (PATH N SITU)

1. Cut 3mm sections on charged slides and incubate at 60-70 ^{0}C for 1 hour.
2. Deparafinize by 2 changes of xylene 15 minutes each.
3. Hydrate through descending grades of alcohol as follows:
    - Absolute alcohol – two changes ,5 minutes each
    - 90% alcohol – 5 minutes
    - 70% alcohol – 5 minutes
    - Wash in distilled water , two changes , 2 minutes each
4. Antigen retrieval for 15 -20 minutes in MERS. pH of retrieval buffer may be either 6,8 or 9.5 according to the marker.
5. Wash in distilled water , two changes, 2 minutes each
6. Wash in PBS /TBS for 2 minutes
7. Do endogenous peroxidise blocking by adding H2O2 on the section, keep for 5 minutes. Wash in the wash buffer for 2 minutes , twice
8. Add primary antibody and keep for 30 minutes in a moist chamber. Then wash in wash buffer 2 times , 2 minutes each
9. Add Polyexcel Target binder reagent and keep for 12 minutes. Wash in two changes of buffer, 2 minutes each.

10. Add Polyexcel HRP and incubate for 12 minutes. Wash with buffer, 2 minutes two changes.
11. Add working DAB chromogen (1mlDAB Buffer + 1 drop DAB Chromogen, mix well) and keep for 2-5 minutes, then wash in distilled water.
12. Counterstain with Hematoxylin for 30 seconds, wash with water
13. Dehydrate (70%, 90%, and absolute), clear (xylene) and mount as usual.

S. NO	AGE	SEX	IP NO	TUMOUR LOCATION	TUMOUR SIZE	DIAGNOSIS	MARGINS	NODAL STATUS	HER 2	KI-67
1	45	FEMALE	66208	SIGMOID COLON	3X3X2	MODERATELY DIFFERENTIATED ADENOCARCINOMA	FREE	Could'nt be assessed	NEGATIVE	HIGH
2	40	MALE	45137	SIGMOID COLON	10X3.5X1	MUCINOUS ADENOCARCINOMA WITH TRANSMURAL INFILTRATION	FREE	2 OUT OF 2 - REACTIVE CHANGES	NEGATIVE	LOW
3	40	FEMALE	56246	RECTUM	2X1X1	MODERATELY DIFFERENTIATED ADENOCARCINOMA	FREE	1 OUT OF 2- METASTATIC	NEGATIVE	LOW
4	50	FEMALE	56255	ASCENDING COLON	8x5x3	WELL DIFFERENTIATED ADENOCARCINOMA INFILTRATION	FREE	2 OUT OF 9 - METASTATIC	NEGATIVE	LOW
5	52	FEMALE	8816	SIGMOID COLON	7X4X2.5	INVASIVE SQUAMOUS CELL CARCINOMA	CIRCUM- INV	1 NODE - REACTIVE CHANGES	NEGATIVE	LOW
6	62	MALE	13195	RECTUM	2.5X2.4X(	MODERATELY DIFFERENTIATED ADENOCARCINOMA	CIRCUM & RM - FREE	Could'nt be assessed	NEGATIVE	LOW
7	40	FEMALE	12611	SIGMOID COLON	3x1.5x1	WELL DIFFERENTIATED ADENOCARCINOMA	1 MARGIN-INVOLVED	2 OUT 2- REACTIVE CHANGES	3+	LOW
8	53	MALE	13477	SIGMOID COLON	2.5X2X1	MODERATELY DIFFERENTIATED ADENOCARCINOMA	FREE	Could'nt be assessed	3+	HIGH
9	28	MALE	16992	RECTO-SIGMOID	3X1X1	MUCINOUS ADENOCARCINOMA	FREE	1 NODE- REACTIVE CHANGES	NEGATIVE	LOW
10	45	MALE	23510	RECTUM	4.5x3x1	MODERATELY DIFFERENTIATED ADENOCARCINOMA	FREE	1 OUT OF 7- METASTATIC	NEGATIVE	LOW
11	52	MALE	34384	RECTUM	4X3X1	WELL DIFFERENTIATED ADENOCARCINOMA	FREE	3 OUT OF 3 - REACTIVE CHANGES	NEGATIVE	LOW
12	51	FEMALE	36715	RECTUM	10X6X4	WELL DIFFERENTIATED ADENOCARCINOMA	ANAL VERGE & DISTAL RM-	2 OUT OF 6 - METASTATIC	NEGATIVE	LOW

S. NO	AGE	SEX	IP NO	TUMOUR LOCATION	TUMOUR SIZE	DIAGNOSIS	MARGINS	NODAL STATUS	HER 2	KI-67
13	75	FEMALE	45129	ASCENDING COLON	5X4X3	MUCINOUS ADENOCARCINOMA INVOLVING UPTO SEROSA	FREE	6 OUT OF 6- METASTATIC	NEGATIVE	LOW
14	51	FEMALE	4389	ASCENDING COLON	5x2x1.5	MUCINOUS ADENOCARCINOMA WITH TRANSMURAL	FREE	3 OUT OF 3 - REACTIVE CHANGES	NEGATIVE	LOW
15	61	MALE	52852	ASCENDING COLON	3X3X3	MUCINOUS ADENOCARCINOMA INVOLVING UPTO SEROSA	FREE	7 OUT OF 7- METASTATIC	NEGATIVE	LOW
16	45	MALE	75657	CAECUM	8X6X3	MODERATELY DIFFERENTIATED ADENOCARCINOMA	FREE	3 OUT OF 8 - METASTATIC	NEGATIVE	INTER MEDIATE
17	53	MALE	32555	RECTUM	6x4x1	MODERATELY DIFFERENTIATED ADENOCARCINOMA	FREE	10 OUT OF 10 - REACTIVE	NEGATIVE	LOW
18	70	MALE	18725	SIGMOID COLON	4x2x1	MODERATELY DIFFERENTIATED ADENOCARCINOMA	FREE	4 OUT OF 4- REACTIVE	NEGATIVE	LOW
19	53	FEMALE	17287	RECTUM	3X3X1.5	MODERATELY DIFFERENTIATED ADENOCARCINOMA	FREE	4 OUT OF 4- REACTIVE	NEGATIVE	LOW
20	40	MALE	75200	RECTUM	4X3X3	WELL DIFFERENTIATED ADENOCARCINOMA	RADIAL - INVOLVED	1 OUT OF 6 - METASTATIC	NEGATIVE	LOW
21	60	FEMALE	73233	ASCENDING COLON	7.5x6.5x3	POORLY DIFFERENTIATED ADENOCARCINOMA	RADIAL - INVOLVED	15 OUT OF 15 - METASTATIC	NEGATIVE	HIGH
22	70	FEMALE	84924	SIGMOID COLON	6.5X3X1	WELL DIFFERENTIATED ADENOCARCINOMA	FREE	Could'nt be assessed	NEGATIVE	HIGH
23	40	FEMALE	52276	RECTUM	5X2.5X2	WELL DIFFERENTIATED ADENOCARCINOMA	FREE	Could'nt be assessed	NEGATIVE	HIGH
24	68	FEMALE	14821	DESCENDING COLON	7X4X2	WELL DIFFERENTIATED ADENOCARCINOMA	RADIAL - INVOLVED	3 OUT OF 4 - METASTATIC	NEGATIVE	LOW
25	55	FEMALE	15269	RECTUM	4X4X1	MODERATELY DIFFERENTIATED ADENOCARCINOMA RECTUM	RADIAL - INVOLVED	2 OUT OF 4 - METASTATIC	NEGATIVE	LOW
26	50	MALE	20193	RECTOSIGMOID	8X6X3	MODERATELY DIFFERENTIATED ADENOCARCINOMA	FREE	1 OUT OF 9 - METASTATIC	NEGATIVE	LOW

S. NO	AGE	SEX	IP NO	TUMOUR LOCATION	TUMOUR SIZE	DIAGNOSIS	MARGINS	NODAL STATUS	HER 2	KI-67
27	76	MALE	36512	HEPATIC FLEXURE	3.5X3X1	WELL DIFFERENTIATED ADENOCARCINOMA	FREE	10 OUT OF 10 - REACTIVE	NEGATIVE	HIGH
28	65	FEMALE	59474	RECTUM	0.8X0.5X(	WELL DIFFERENTIATED ADENOCARCINOMA		Could'nt be assessed	NEGATIVE	HIGH
29	55	FEMALE	68591	RECTOSIGMOID	5X2X1	MODERATELY DIFFERENTIATED ADENOCARCINOMA	RADIAL - INVOLVED	9 OUT OF 9 - REACTIVE	NEGATIVE	HIGH
30	60	FEMALE	23510	RECTUM	3CC	WELL DIFFERENTIATED ADENOCARCINOMA		Could'nt be assessed	NEGATIVE	HIGH
31	38	FEMALE	11624	RECTUM	1X1X0.5	MODERATELY DIFFERENTIATED ADENOCARCINOMA		Could'nt be assessed	NEGATIVE	HIGH
32	74	FEMALE	20472	RECTUM	4x3.5x1	MODERATELY DIFFERENTIATED ADENOCARCINOMA	FREE	1 OUT OF 1 - REACTIVE	1+	LOW
33	17	FEMALE	39794	DESCENDING COLON	3X3X1	SIGNET RING CELL ADENOCARCINOMA	FREE	4 OUT OF 4 - METASTATIC	NEGATIVE	LOW
34	61	MALE	48532	CAECUM	9X9X5	MODERATELY DIFFERENTIATED ADENOCARCINOMA	FREE	3 OUT OF 4 - METASTATIC	NEGATIVE	LOW
35	30	MALE	76008	RECTUM	7X5X1	MODERATELY DIFFERENTIATED ADENOCARCINOMA	RADIAL - INVOLVED	2 OUT OF 3 - METASTATIC	NEGATIVE	LOW
36	45	FEMALE	72894	ASCENDING COLON	7X3.5X2.5	MODERATELY DIFFERENTIATED ADENOCARCINOMA	FREE	Could'nt be assessed	NEGATIVE	LOW
37	60	MALE	31655	SIGMOID COLON	2X1.5X1	MODERATELY DIFFERENTIATED ADENOCARCINOMA	INVOLVED	Could'nt be assessed	NEGATIVE	LOW
38	65	MALE	68973	ASCENDING COLON	7X5.5X2	MODERATELY DIFFERENTIATED ADENOCARCINOMA	FREE	1 OUT OF 1 - REACTIVE CHANGES	3+	HIGH
39	60	FEMALE	31548	RECTUM	4.5X3.5X3	MODERATELY DIFFERENTIATED ADENOCARCINOMA	DISTAL-INVOLVED	Could'nt be assessed	3+	HIGH
40	65	MALE	70893	RECTUM	3.3X3X1	MODERATELY DIFFERENTIATED ADENOCARCINOMA	RADIAL - INVOLVED	Could'nt be assessed	NEGATIVE	HIGH

S. NO	AGE	SEX	IP NO	TUMOUR LOCATION	TUMOUR SIZE	DIAGNOSIS	MARGINS	NODAL STATUS	HER 2	KI-67
41	55	MALE	46264	ASCENDING COLON	12X7.5X6	WELL DIFFERENTIATED ADENOCARCINOMA	ONE-INVOLVED	2 OUT OF 2-REACTIVE CHANGES	NEGATIVE	LOW
42	52	FEMALE	57470	TRANSVERSE COLON	7.5X6X5.5	WELL DIFFERENTIATED ADENOCARCINOMA	PROXIMAL AND DISTAL - FREE	1 OUT OF 11-METASTATIC	3+	HIGH
43	51	FEMALE	57426	DESCENDING COLON	8X6.5X4	WELL DIFFERENTIATED ADENOCARCINOMA	PROXIMAL AND DISTAL-FREE	1 OUT OF 1 - REACTIVE CHANGES	NEGATIVE	LOW

Crosstab
Count

		HER2			
		negative	1+	3+	P value
AGE_GRP	1	3	0	0	
	2	11	0	1	
	3	23	1	4	0.857

Crosstab
Count

		HER2			
		negative	1+	3+	P value
GENDER	1	17	0	2	
	2	20	1	3	0.646

Crosstab
Count

		HER2			
		negative	1+	3+	P value
TUM_SIZ	1	22	1	3	
	2	15	0	2	0.715

Crosstab
Count

		HER2			
		negative	1+	3+	P value
TUM_LOC	1	10	0	2	
	2	9	0	2	
	3	15	1	1	
	4	3	0	0	0.791

Crosstab
Count

		HER2			
		negative	1+	3+	P value
HIS_TYP	1	12	0	2	
	2	17	1	3	

3	1	0	0	
4	5	0	0	
5	1	0	0	
6	1	0	0	0.992

Crosstab
Count

		HER2			
		negative	1+	3+	P value
HIS_GRD	1	12	0	2	
	2	17	1	3	
	3	8	0	0	0.656

Crosstab
Count

		KI67			
		low	ntermediat	high	P value
AGE_GRP	1	3	0	0	
	2	8	1	3	
	3	17	0	11	0.308

Crosstab
Count

		KI67			
		low	ntermediat	high	P value
GENDER	1	14	1	4	
	2	14	0	10	0.22

Crosstab
Count

		KI67			
		low	ntermediat	high	P value
TUM_SIZ	1	16	0	10	
	2	12	1	4	0.307

Crosstab
Count

		KI67			
		low	ntermediat	high	P value
TUM_LOC	1	7	1	4	
	2	8	0	3	
	3	11	0	6	
	4	2	0	1	0.821

Crosstab
Count

		KI67			
		low	ntermediat	high	P value
HIS_TYP	1	8	0	6	
	2	13	1	7	
	3	0	0	1	
	4	5	0	0	
	5	1	0	0	
	6	1	0	0	0.693

Crosstab
Count

		KI67			
		low	ntermediat	high	P value
HIS_GRD	1	8		6	
	2	13	1	7	
	3	7		1	0.512

Crosstab
Count

		HER2			
		negative	1+	3+	P value
LYMP	1	15		1	
	2	12	1	2	
	3	10		2	0.601

Crosstab

Count

		KI67			
		low	ntermediat	high	P value
LYMP	1	13	1	2	
	2	12		3	
	3	3		9	0.004

AGE_GRP

		Frequency	Percent
Valid	1	3	2.44
	2	12	9.76
	3	28	22.76
	Total	43	34.96

GENDER

		Frequency	Percent
Valid	1	19	15.45
	2	24	19.51
	Total	43	34.96

TUM_LOC

		Frequency	Percent
Valid	1	12	9.76
	2	11	8.94
	3	17	13.82
	4	3	2.44
	Total	43	34.96

TUM_SIZ

	Frequency	Percent

Valid		1	26	21.14
		2	17	13.82
	Total		43	34.96

NOD_STAT

		Frequency	Percent
Valid	1	15	12.20
	2	16	13.01
	Total	31	25.20

HER2

		Frequency	Percent
Valid	negative	37	30.08
	1+	1	0.81
	3+	5	4.07
	Total	43	34.96

KI67

		Frequency	Percent
Valid	low	28	22.76
	intermedia	1	0.81
	high	14	11.38
	Total	43	34.96

HIS_TYP

		Frequency	Percent
Valid	1	14	11.38
	2	21	17.07
	3	1	0.81
	4	5	4.07
	5	1	0.81

		6	1	0.81
	Total		43	34.96

HIS_GRD

		Frequency	Percent
Valid	1	14	11.38
	2	21	17.07
	3	8	6.50
	Total	43	34.96

LYMP

		Frequency	Percent
Valid	1	16	13.01
	2	15	12.20
	3	12	9.76
	Total	43	34.96