A STUDY ON MOLECULAR EXPRESSION OF KRAS MUTATION IN COLORECTAL CARCINOMA AND ITS CORRELATION WITH CLINICOPATHOLOGICAL FINDINGS IN A TERTIARY CARE HOSPITAL"

Dissertation submitted in
partial fulfillment of the requirements for the degree of

M.D. PATHOLOGY
BRANCH- III

INSTITUTE OF PATHOLOGY
MADRAS MEDICAL COLLEGE
CHENNAI- 600003

THE TAMILNADU DR M.G.R.MEDICAL UNIVERSITY
CHENNAI

MAY 2019

CERTIFICATE

This is to certify that this Dissertation entitled **"A STUDY ON MOLECULAR EXPRESSION OF KRAS MUTATION IN COLORECTAL CARCINOMA AND ITS CORRELATION WITH CLINICOPATHOLOGICAL FINDINGS IN A TERTIARY CARE HOSPITAL"** is the bonafide original work of **DR.B.MOHANAPRIYA** requirement for M.D., (Branch-III) in Pathology examination of the Tamilnadu Dr.M.G.R Medical University to be held in May 2019.

Prof.Dr.R.Padmavathi.M.D.,

Professor of Pathology,
Institute of Pathology,
Madras Medical College,
Chennai-600003

Prof.Dr.Bharathi Vidhya Jayanthi, M.D.,
Director & Professor of Pathology,
Institute of Pathology,
Madras Medical College
Chennai-600003.

Prof.Dr.R.Jayanthi, M.D., FRCP (Glas.)
Dean,
Madras Medical College
Rajiv Gandhi Government Hospital
Chennai-600003.

DECLARATION

I, **Dr.B.MOHANAPRIYA,** solemnly declare that the dissertation entitled **"A STUDY ON MOLECULAR EXPRESSION OF KRAS MUTATION IN COLORECTAL CARCINOMA AND ITS CORRELATION WITH CLINICOPATHOLOGICAL FINDINGS IN A TERTIARY CARE HOSPITAL"** is the bonafide work done by me at the Institute Of Pathology, Madras Medical College under the expert guidance and supervision of Prof.Dr.R.Padmavathi, M.D., Professor of Pathology, Institute of Pathology, Madras Medical College. The dissertation is submitted to the Tamilnadu Dr.M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Date:
Place: Chennai **DR.B.MOHANAPRIYA**

ACKNOWLEDGMENT

I express my sincere thanks to **Prof.Dr.R.JAYANTHI, MD., FRCP(glas),** Dean, Madras Medical College and Rajiv Gandhi Government General Hospital, for permitting me to utilize the facilities of the Institution.

I take the opportunity to express my gratitude to **Prof.Dr.BHARATHI VIDHYA JAYANTHI, M.D.,** Director and Professor, Institute of Pathology, Madras Medical College, Chennai for her keen interest, constant encouragement and valuable suggestions throughout the study.

I am extremely thankful to **PROF.Dr.R.PADMAVATHI, M.D.,** Professor of Pathology,Institute of Pathology,Madras Medical College, for their valuable suggestions, constant support, advice and encouragements throughout the study.

I am truly thankful to **Prof.Dr.Sudha Venkatesh M.D., Prof.Dr.Geetha Devdas M.D.,DCP, Prof.Dr.Ramamoorthi M.D., Prof.Dr.Rama M.D., Prof.Dr.M.P.Kanchana M.D., Prof.Dr.S.Pappathi M.D., Prof.Dr.Rajavelu Indira M.D., Prof.Dr.Selvambigai M.D.,** for their valuable suggestions and encouragement throughout the study.

I express my heartfelt sincere thanks to all my Assistant Professors for their help and suggestions during the study.

I am thankful to my colleagues, friends, technicians and staff of the Institute of Pathology, Madras Medical College, Chennai for all their help and support they extended for the successful completion of this dissertation.

My sincere thanks also go to all the patients and their families who were co-operative during the course of this study.

Last but not the least, I am grateful to my family members and friends for their constant support and belief in me.

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI 600 003

EC Reg.No.ECR/270/Inst./TN/2013
Telephone No.044 25305301
Fax: 011 25363970

CERTIFICATE OF APPROVAL

To
Dr.B.Mohanapriya
Second Year Post Graduate in MD Pathology
Institute of Pathology
MMC/Chennai

Dear Dr.B.Mohanapriya,

The Institutional Ethics Committee has considered your request and approved your study titled **"A STUDY ON MOLECULAR EXPRESSION OF K – RAS MUTATION IN COLORECTAL CARCINOMA AND ITS CORRELATION WITH CLINICOPATHOLOGICAL FINDINGS IN A TERTIARY CARE HOSPITAL " - NO.17122017**

The following members of Ethics Committee were present in the meeting hold on **05.12.2017** conducted at Madras Medical College, Chennai 3

1. Prof.P.V.Jayashankar	:Chairperson
2. Prof.R.Narayana Babu,MD.,DCH., Dean,MMC,Ch-3	: Deputy Chairperson
3. Prof.Sudha Seshayyan,MD., Vice Principal,MMC,Ch-3	: Member Secretary
4. Prof.N.Gopalakrishnan,MD,Director,Inst.of Nephrology,MMC,Ch	: Member
5. Prof.S.Mayilvahanan,MD,Director,Inst. of Int.Med,MMC, Ch-3	: Member
6. Prof.Rema Chandramohan,Prof.of Paediatrics,ICH,Chennai	: Member
7. Prof. Susila, Director, Inst. of Pharmacology,MMC,Ch-3	: Member
8.Prof.K.Ramadevi,MD., Director, Inst. of Bio-Chemistry,MMC,Ch-3	: Member
9.Thiru S.Govindasamy, BA.,BL,High Court,Chennai	: Lawyer
10.Tmt.Arnold Saulina, MA.,MSW.,	:Social Scientist
11.Thiru K.Ranjith, Ch- 91	: Lay Person

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary - Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

URKUND

Urkund Analysis Result

Analysed Document:	PLAGAIRIAM MP2.docx (D42807356)
Submitted:	10/20/2018 7:03:00 AM
Submitted By:	mpriyabaskaran@gmail.com
Significance:	7 %

Sources included in the report:

final correction.docx (D31015442)
dissertation- FINAL.docx (D30995821)
Thesis.docx (D31280037)
FinalDocAnnie (1).docx (D42452481)
EVALUATION OF PROGNOSTIC INDICATORS IN COLORECTAL CARCINOMA – A COMPREHENSIVE STUD Y TO CORRELATE STAGING, GRADING AND PROLIFERATIVE INDICATORS.pdf (D42306300)
https://openarchive.ki.se/xmlui/handle/10616/40824
https://helda.helsinki.fi/handle/10138/154162
https://www.hindawi.com/journals/bmri/2010/150960/
https://link.springer.com/article/10.1186/1746-1596-5-23
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4287618/
https://bmccancer.biomedcentral.com/articles/10.1186/s12885-016-2141-4
https://academic.oup.com/ajcp/article/135/2/245/1760575

Instances where selected sources appear:

20

PLAGIARISM CERIFICATE

This is to certify that this dissertation work titled **"A STUDY ON MOLECULAR EXPRESSION OF KRAS MUTATION IN COLORECTAL CARCINOMA AND ITS CORRELATION WITH CLINICOPATHOLOGICAL FINDINGS IN A TERTIARY CARE HOSPITAL"** of the candidate **DR.B.MOHANAPRIYA** with the registration number **201613004** for the award of **M.D.PATHOLOGY** (Branch-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 and result shows **7% percentage** of plagiarism in the dissertation.

Guide & Supervisor sign with seal

ABBREVIATIONS

CRC	:	Colorectal Carcinoma
APC	:	Adenomatous Polyposis Coli
FAP	:	Familial Adenomatous Polyposis
HPE	:	Histopathological examination
KRAS	:	kirsten RAt Sarcoma virus
DNA	:	Deoxyribonucleic acid
HNPCC	:	Hereditary Non-Polyposis Colorectal Cancer
FFPE	:	Formalin Fixed Paraffin Embedded
PCR	:	Polymerase chain Reaction
MSI	:	Microsatellite Instability
MMR	:	Mismatch Repair
GTP / GDP	:	Guanosine Triphosphate/Guanosine Diphosphate
BRAF	:	Murine Sarcoma Viral Oncogene Homolog B
MAPK	:	Mitogen-Activated Protein kinase
PI3K/AKT	:	Phosphoinositidyl-3-Kinase
PTEN	:	Phosphatase and Tensin Homologue
CIMP	:	CpG Island Methylator Pathway
HRMA	:	High-resolution Melting Assay
RFLP	:	Restriction Fragment Length Polymorphism
VEGF	:	Vascular Endothelial Growth Factor

CONTENTS

Introduction

INTRODUCTION

Colorectal Carcinoma is one of the emerging cancers,which is the leading cause of cancer related deaths in developed countries. It stands as third most common malignancy in men and second most common in women worldwide.[1] The American Cancer Society estimates around 97,220 new cases of colon cancer and 43,030 new cases of rectal cancer in United states for 2018.

In India, CRC occupies the fifth most common position following breast, cervix/uteri, oralcavity and lung cancers [3].The incidence rates of colon cancer in India vary from 0.7 to 3.7 per 1Lakh population among men and 0.4 to 3/ 1 lakh among women. For rectal carcinoma it varies from 1.6 to 5.5 per 1Lakh among men and 0 to 2.8 per 1 Lakh among women.[10]

A statistical review of population based cancer registries from12 major cities of India have demonstrated that incidence of colorectal carcinoma is significantly lower in India compared to west. [4]

CRC is the carcinoma of old age occurring mostly after fifth decade of life.[2] However,its incidence is increasing in younger age especially in developing counts, mainly due to the life style and food habit changes.

Its development involves a multistep process signified by genetic alterations that have been considered to occur in a stepwise manner. Two major genetic pathways have been put forth: [5]

- Chromosomal Instability (ChieflyAPC/ß-CATENIN)
- Microsatellite Instability pathway(MSI)

Additional mutations accumulate in wide range of genes and proteins like KRAS, NRAS, BRAF,PIK3CA,MAPK,PTEN,TP53 and SMAD triggering the downstream signaling cascades in evolution of colorectal carcinogenesis. DNA mismatch repair deficiency leading to microsatellite instability and CpG island hypermethylation are other proposed pathways leading to tumorigenesis.

Among the various genetic mutations,KRAS oncogene mutation has a pivotal role associated with proliferation and decreased apoptosis.Importance of KRAS mutational status assay has been highlighted in recent years due to its theranostic significance.

Identifying KRAS mutational status in each patient is significant in order to determine the best therapy.Patients with colorectal carcinoma can fall into either of the categories:

- KRAS Wild type (WT)- which means KRAS gene in its natural,non-mutant form.They can receive monoclonal antibodies against EGFR

- KRAS Mutational type –associated with no response to targeted therapies

The development of molecular biology and genetic techniques has contributed to a better understanding of carcinogenesis predicting its evolution.Attempt of early diagnosis, selection of appropriate targeted therapies and efficient follow up can play a novel role in reducing the disease related mortalities and morbidities.

Aims and Objectives

AIMS AND OBJECTIVES

- To evaluate the expression of K-RAS mutation in colorectal carcinoma.
- To study its correlation with respect to age,histological findings and staging of carcinoma.

Review of Literature

REVIEW OF LITERATURE

HISTORICAL ASPECTS

Over 100 years ago, Dr.Alfred Warthin first suspected the hereditary colorectal disorder in the family of an affected woman who subsequently died of endometrial carcinoma.He began to research on her family ailments and in 1913, published his first report documenting a pattern of endometrial cancer and gastrointestinal cancers,particularly of gastric and colon cancers (7).In 1960's the development of colonoscope revolutionized in colorectal surgical field.In 1971,Lynch and Krush did elaborate studies on her family and showed it to be what was latter known as Lynch syndrome. Cutaneous manifestations such as sebaceous adenomas and carcinomas coming under Muir-Torre syndrome were also associated with this disorder. Knudson's two hit hypothesis suggested the basis of understanding of how tumor suppressor genes could explain the onset of familial cancers at younger age group as well as the variable penetrance. Although there is increased susceptibility,second mutations are required for producing a tumor.

Mutations in adenomatous polypsis coli(APC) gene are responsible for syndrome originally recognized in 1930's as autosomal dominant familial severe polyposis ,at present being known as Familial adenomatous polyposis(FAP) (8). Identification of hereditary and familial mutations allowed presymptomatic genetic testing in family

members leading to earlier detection of related cancers and possibly preventing them at premalignant stages increasing their survival. Technological advances now throw insight into new genetic discoveries leading to the understanding of CRC tumorigenesis.

EMBRYOLOGY OF COLON AND RECTUM

The embryonic gastrointestinal tract starts developing during fourth week of gestation.The gut is derived from endoderm and consists of Foregut,Midgut and Hindgut. Both MIDGUT and HINDGUT contributes to development of colon and rectum. Part of midgut forms a loop that is divisible into prearterial and postarterial segments.

Ascending colon develops from postarterial segment of midgut loop.As a result of rotation ,caecum(developed from caecal bud from postarterial segment of midgut loop) and ascending colon comes to lie on right side.Thus midgut forms ascending colon and proximal transverse colon,both of which receives superior mesentric artery supply.

The hindgut develops into distal transverse colon,descending colon,rectum and proximal anus.All of them receive their blood supply from the inferior mesenteric artery.

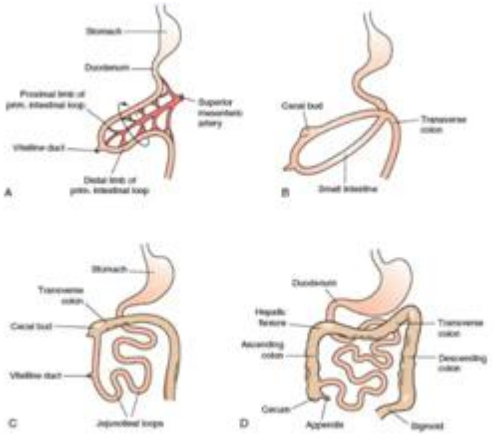


Fig-1: Rotation of Gut

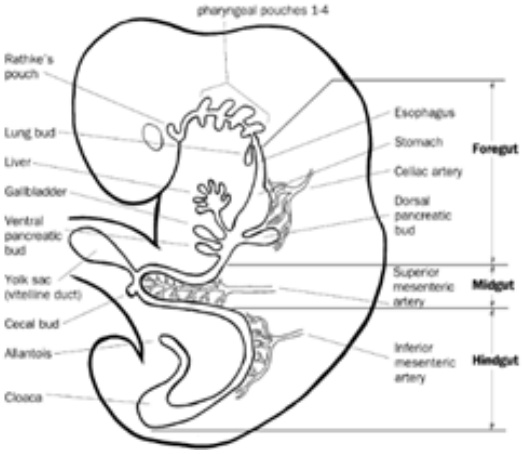


Fig-2:Embryological Development Of Gut

ANATOMY: (6)

The Large intestine is the terminal part of alimentary canal extending from distal end of ileum to anus ,with approximate distance of 1.5 meters. The general characteristics of large intestine includes - *TAENIAE COLI, APPENDICES EPIPLOICA* and *HAUSTRA OF COLON* Large intestine is subdivided into four main regions consisting of caecum, colon,rectum and anus.

***Caecum*(**Proximal right colon)-Intraperitoneal pouch which is 6x9 cm

Appendix-A vermiform diverticulum in various poitions ,usually located in lower cecum.

Ascending Colon-20 to 25cm long ,passes upwards lying retroperitoneally.When it meets right lobe of liver,it turns 90 degrees horizontally as HEPATIC FLEXURE of colon.

Transverse colon-It extends from hepatic flexure to spleen where it turns another 90 degrees inferiorly as SPLENIC FLEXURE.

Descending Colon-10 to 15 cm lying retroperitoneally.

Sigmoid colon-40cm long extending from left iliac fossa to level of S3 vertebra, surrounded by sigmoid mesocolon

Rectum-is the next 12cm structure lying retroperitoneally

BLOOD SUPPLY

The *Marginal artery of Drummond* is a clinically important collateral supply to colon.

Midgut derived structures - Middle colic,Right colic and Ileocolic arteries of superior mesenteric artery. Appendicular branch of ileocolic artery supplies appendix.

Hindgut derived structures-Left colic artery,sigmoidal ateries and superior rectal artery. Similar to arterial supply,corresponding veins drain the parts of colon and rectum.

NERVE SUPPLY

Midgut derived structures receive their sympathetic, parasympathetic and sensory supply via nerves from superior mesenteric plexus. Hindgut derived structures from inferior mesenteric plexus via splanchnic nerves.

LYMPHATICS

Lymphatics of colon drains into superior and inferior mesenteric nodes, and finally on to the cistern chyli emptying into

thoracic duct.Upper half of rectum reach pararectal nodes and lower half reach internal iliac nodes.

HISTOLOGY: [90]

There are four distinct functional layers:

1) **MUCOSA** of colon is lined by simple columnar epithelium,containing crypts of Lieberkuhn with numerous goblet cells.

2) **SUBMUCOSA** –This layer of loose collagenous connective tissue blood vessels, submucosal plexus of nerves and lymphatics.

3) **MUSCULARIS PROPRIA**- The muscle wall proper consists of smooth muscles arranged in 2 layers: *Outer longitudinal layer* and *Inner circular layer*.

4) **SEROSA**-Outer layer of loose supporting tissue lined by single layer of flattened to cuboidal mesothelial cells with fibro elastic tissue.

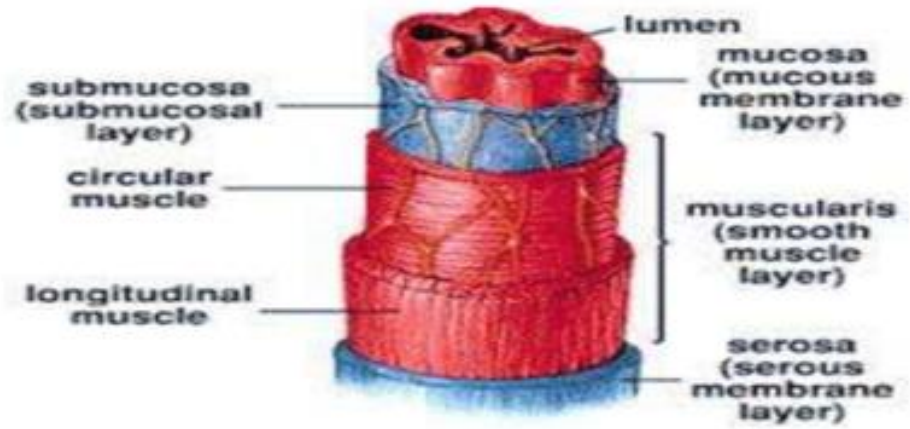


Fig-3: Layers Of Gastrointestinal Tract-Colon&Rectum

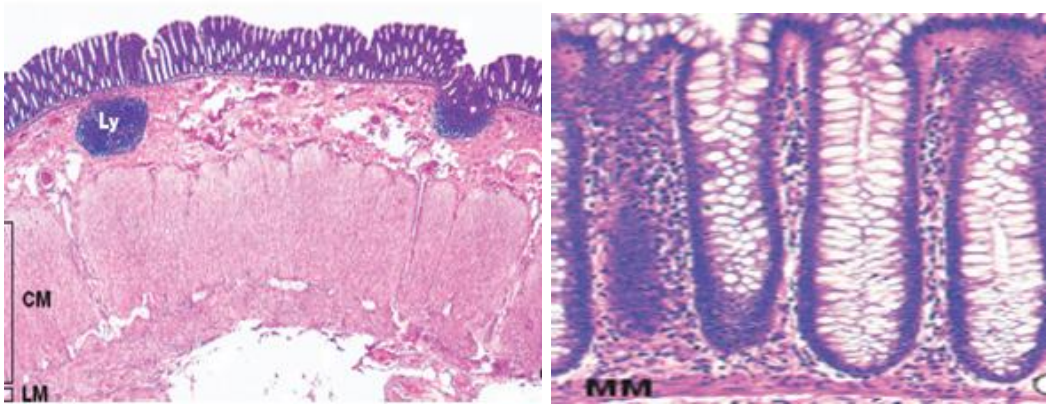


Fig-4 : Histological Layers Of Colon

EPIDEMIOLOGY:

TheAmerican cancer society has estimated number of colorectal cancer cases in United stated for 2018 .The number of new cases of colorectal cancer that can be detected are 97,220 and 43,030 new cases of rectal cancer.There is an acceleration in decline from about 2% per year prior in mid- 2000's to 3% per year from 2004-2013, which reflects detection through screening and removal of precancerous polyps. GLOBOCAN 2018 estimates over 1.8 million new colorectal cancer cases and 881,000 deaths to occur and accounting for 6.1%of carcinomas of all sites.[(9)]

This disease can be considered as a marker of socioeconomic development. In countries undergoing major development transition ,there is an increasing trend in incidence rates uniformly with increasing Human Development Index(HDI).This rise in incidence rates ,by various period-cohort study, points to the influence of dietary habits,obesity, lifestyle factors.At the same time,decline in mortality in more developed

countries are attributed to adoption of best practices in cancer treatment leading to improvements in survival.

In India,the incidence rates of colorectal carcinoma are low compared that of western world.Incidence rates of colon cancer varies from 0.7-3.7 per 1lakh among men; 0.4-3 per 1lakh among women .Rectal carcinoma incidence varies from 1.6-5.5/1lakh among men and 0-2.8/1lakh among women[(10)].

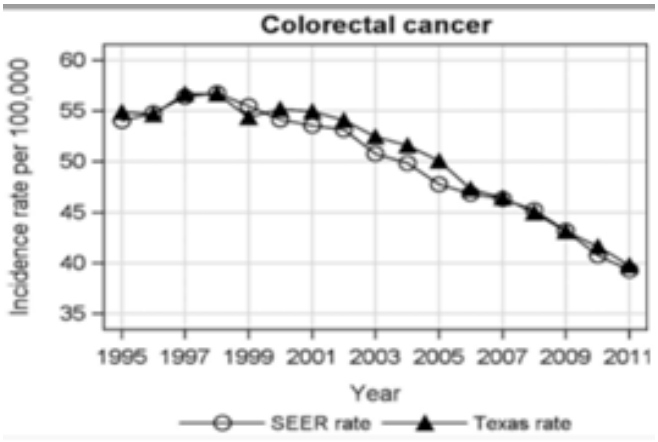


Fig-5: Seer's Trend of CRC

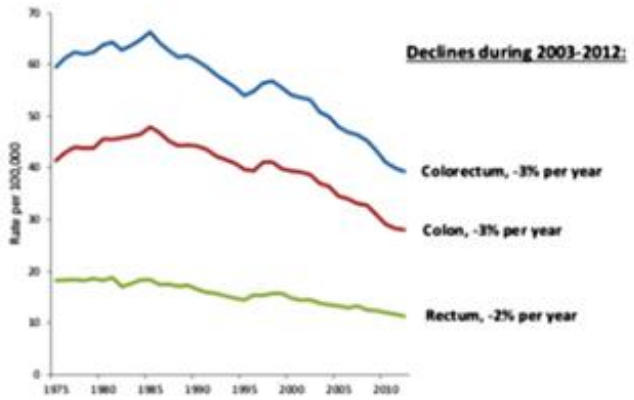


Fig 6:Seer 9 Delay-Adjusted Rates

RISK FACTORS

The etiology of colorectal carcinoma is multifactorial in nature.Notably- dietary factors, genetic factors and environmental factors .

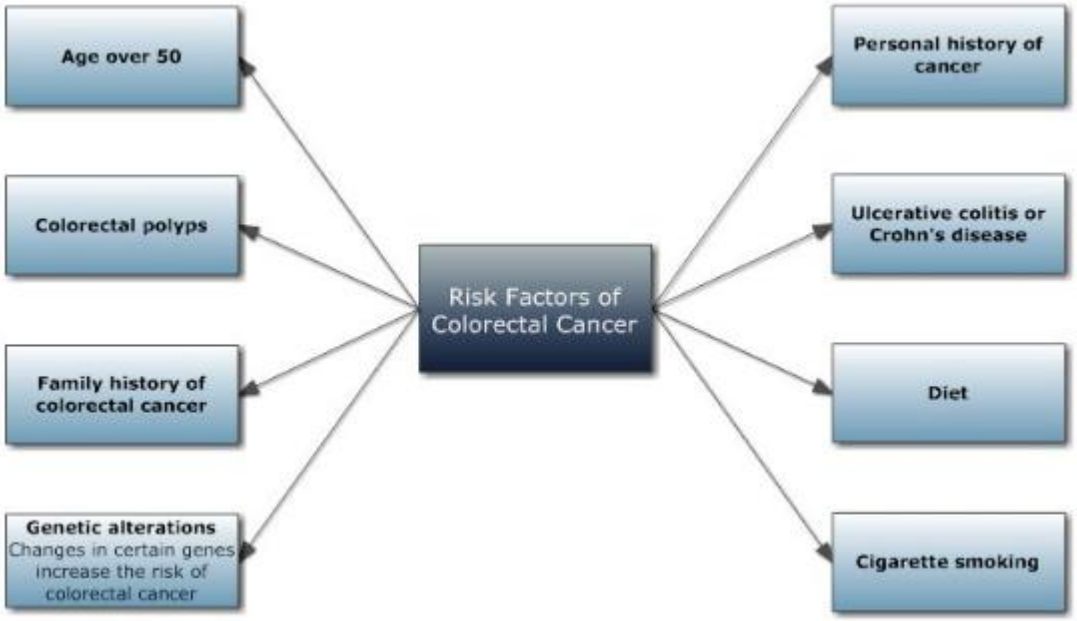


Fig-7: List Of Riskfactors For Colorectal Carcinoma

NON MODIFIABLE RISK FACTORS: [11]

1) ***Age:*** As age advances,risk of developing colorectal carcinoma also increases. SMost people are diagnosed with age more than 50 years.Patients with age older than 70 years mostly present with early-stage disease ,whereas patients of younger age diagnosed in their earlier age less than 40 years present with much aggressiveness for a given stage of presentation.[12]

2) ***Gender:*** The Role of gender in association with development of colorectal carcinoma remains unclear.Although men are more

likely to develop rectal carcinoma,there were no differences in post operative mortality between the sexes.

3) ***Races:*** There are racial differences in colonic cancer survival as demonstrated by several literatures.African ,American have higher incidence and lower survival rates than Caucasians and other racial groups.

4) ***Family History:*** Patients with significant family history of colonic cancer or polyps in first degree relatives are at increased risk of developing it.

5) ***Familial Adenomatous Polyposis:*** Germline mutation of APC gene referred as "gatekeeper" of colonic neoplastic progression,located on chromosome 5q21 is associated with FAP.They have relatively higher risk of developing CRC.Other syndromes with APC mutations such as Gardner's syndrome and Turcot syndrome also been associated with risk of colonic cancer. By Wilmink A BM study,it is seen that FAP accounts for 1% of CRC. **(14)**

Other inherited syndromes like Cowden's, Peutz-Jegher's and Muirr-torre syndromes also have association with colorectal carcinomas.

1) ***Hereditary Nonpolyposis Colorectal Cancer (Lynch syndrome):*** It is an inherited autosomal dominant trait caused by several DNA

mismatch (MMR)genes.Amsterdam criteria for colorectal cancer includes: [13]

a. Atleast three family members with cancers are associated with HNPCC –One must be a first degree relative.

b. Atleast two successive generations must be affected

c. Atleast one of the relatives must have been diagnosed before age of 50.

2) ***Inflammatory Bowel Disease:*** Both Ulcerative colitis and crohn's disease are prone to develop colorectal cancer.[19] There is an estimated risk between 4 to 20 folds of developing CRC.Use of NSAID'S in patients with inflammatory bowel disease was associated with protective role against colorectal cancer.

3) ***Adenomatous Polyp:*** The transformation rate of adenomatous polyps into carcinoma is around 0.25% per year.In adenomas with villous architecture (like villous and tubulovillous adenomas), high grade dysplasias ,there is a likelihood of 50% transformation to malignancy.

4) ***Endogenous Factors:*** Studies have shown that endogenous hormones, especially oestrogens may influence adenoma carcinoma sequence.Individuals with acromegaly also have increased risk.

MODIFIABLE RISK FACTORS

1) ***Dietary Factors:*** Person with high intake of fatty and cholesterol products,refined carbohydrates,red meat with little fibre intake are more likely to develop CRC..High fat intake enhances hepatic synthesis of cholesterol and bile acids which are converted to carcinogens by the intestinal bacteria. **(16)**

2) ***Obesity:*** Overweight and lack of exercise with sedentary life style are more likely related to CRC development.

3) ***Tobacco Smoking:*** It increases the risk for sessile serrated lesions and colonic carcinoma at earlier age.Even after cessation of smoking,cancer risk extends upto 20-30 years.

4) ***Alcohol:*** Consumption of alcohol is associated with higher risk of colorectal cancer development.**Tsong WH et al**. study shows the association of alcoholism with development of CRC in younger age.**(17).** Acetaldehyde and other reactive metabolites of alcohol act as carcinogenic by production of free oxygen species and it also acts as solvent for penetration of other carcinogens.

5) ***Radiation:*** Long term complication of therapautic irradiation to pelvic malignancies can cause colorectal carcinoma. **(18)**

PATHOGENESIS: [5,23]

The combination of molecular events eventually leading to colonic adenocarcinoma is heterogenous with genetic and epigenetic abnormalities.Tumorigenesis is generally considered as a multistep process where in various genetic alterations occur,eventually being reflected in abnormalities of the cellular DNA content.Based upon the genetic background ,Pathways for sporadic CRCs can be classified into:

a. Chromosomal Instability Pathway,which is activated in classic adenoma-carcinoma sequence

b. Micosatellite Instability Pathway,associated with DNA mismatch repair

c. CpG island methylator Pathway

CHROMOSOMAL INSTABILITY PATHWAY

The genes of interest involved in genetic alterations may be classified into three types:Oncogenes,tumor suppressor genes and DNA repair genes.

Proto-oncogenes act by promoting cell proliferation.Mutation of these leads to abnormal oncogenic over-expression and increased activity of protein. In normal state,tumor suppressor genes inhibit cell proliferation.Its inhibition is lost when both the alleles are inactivated

by mutations and/or epigenetic changes.DNA repair genes help in controlling rate of mutation of other genes. [20]

APC MUTATION

The adenoma-carcinoma sequence is most commonly initiated by bi-allelic APC tumour suppressor gene mutation.By studies of Otori et al [21],and Roncucci et al [22] .,APC mutations have been found in earliest lesion of pathway called microadenoma /aberrant crypt foci.APC is a key negative regulator of ß-catenin which is a component of Wnt signaling pathway. Normally,APC protein binds to ß-catenin and promotes its degradation.With loss of APC function, ß-catenin accumulates and translocates to nucleus forming a complex with DNA binding factor TCF and activates transcription of genes,including MYC and CYCLIN D1 which promotes proliferation.

KRAS MUTATION

By various literatures including **Bishehsari et al**.,Another oncogene which occurs early in adenoma-carcinoma sequence is activating mutation of KRAS. [24]

Among all the mutations in human cancers,RAS protein mutations are observed in approximately 15 to 20% of them.In some cancer types,its mutation frequency is higher.About 90% of pancreatic adenocarcinoma and cholangiocarcinoma express RAS point mutation.

50% is expressed by colonic,endometrial and thyroid carcinomas.About 30% of lung carcinomas and myeloid leukemias contain RAS mutations.

There are three RAS genes in human genome (HRAS, KRAS, NRAS). In colorectal carcinoma, mutations in KRAS are known to occur at frequency of 35-40% ,while NRAS mutations occur at frequency of 3-4% and HRAS mutations at much lower rate of <1% of patients.

MECHANISM OF ACTION OF KRAS ACTIVATION

KRAS encodes a 21-kDa protein (ras p21)which is involved in signal transduction pathways critical for normal cellular proliferation and differentiation.[20] These proteins are members of a family of membrane associated small G proteins binding to guanosine diphosphate(GDP) and guanosine triphosphate(GTP). When it is bound to GTP,ras protein becomes activated and gets inactivated when GTP is hydrolysed to GDP.They flip back and forth between these excited signal transmitting state (GTP bound) and quiescent state (GDP bound) normally.Thus,Growth factor stimulation of receptor tyrosine kinases leads to exchange of GDP for GTP and constitutive action to generate active RAS occurs by conformational changes.Subsequent stimulation of MAPK and PI3K/AKT arms of receptor tyrosine kinase downstreams the activation of cytoplasmic effectors and several other transcription factors that supports cell growth and proliferation.

RAS activation is transitory,as it has an intrinsic GTPase activity which is controlled by GTPase activated proteins(GAPs) thereby preventing uncontrolled RAS activity.Variety of distinct RAS point mutations identified markedly reduce these GAPs activity.Hence mutated RAS forms are trapped in GTP bound activated form and keeps on receiving continuous pro-growth signals.

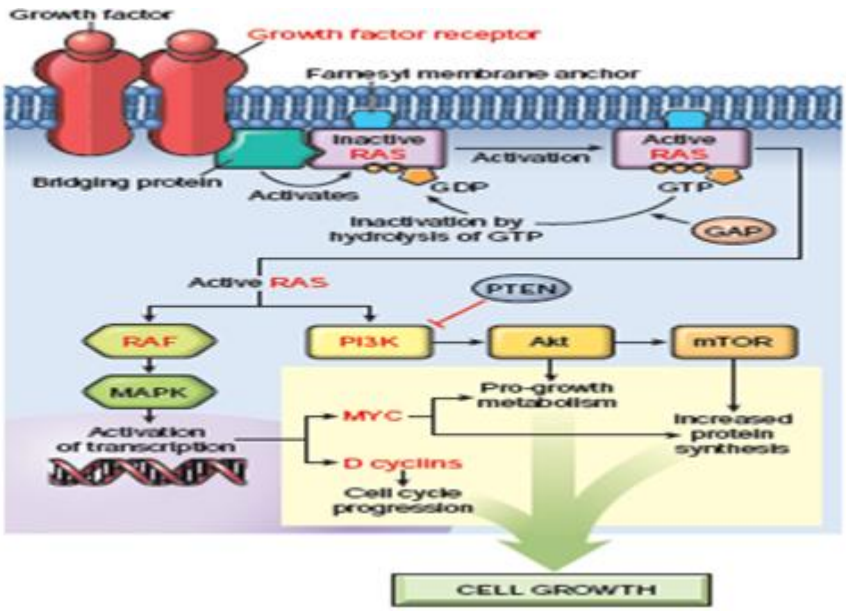


Fig-8 :Growth Factor Signaling Pathways With Ras Actvation

- According to **Vogelstein et al** [(23)].,activation of K-ras occurs in 35-42% of colorectal carcinomas.They conducted this study in 172 colorectal tumor cases representing various stages of tumor development and looked for 4 genetic alterations namely RAS gene mutations and deletions of chromosomes 5,17 and 18.RAS mutations observed in 58% of larger adenomas >1cm size. 47%in carcinomas and 9% of adenomas <1cm size.Thse 4 genetic

alterations accumulated in a fashion which paralleled clincical progression of tumor.

- By various literatures and **COSMIC resource**[25],there have been vaguely around 3000 identifiable K-ras point mutations in colorectal carcinoma.There are 61substitutions where the nucleotide change is still unclear.This COSMIC resource is the most comprehensive tool for exploring the impact of somatic mutations in human cancer.

- Study by **Brink M,et al**.[26] ,have shown that commonest mutation hot spots are CODON 12 and CODON 13 of EXON 2,with G>A transitions and G>T transversions being the predominant mutations.

- The significance of finding out K-ras mutational status lies in the fact that it is one of the most important predictors of targeted therapy using EGFR1 tyrosine kinase inhibitors.[27]

- According to **karapetis et al**.,[28] As K-ras serves as mediator between extracellular ligand binding and intracellular signal transductions from EGFR to nucleus,its mutation would defer this pathway,thereby rendering EGFR inhibitors ineffective.

TP_{53}:

$TP_{53,}$ labelled 'Guardian of genome' functions to regulate cell cycle progression,cellular senescence and DNA repair. P_{53} inhibition leads to loss of mutation involving TGF-ßreceptors which induces invasion and metastasis.

Datas of **Rodrigues et al,**[29] suggests that loss of function mutation in P_{53}, which is about 50% of colorectal carcinomas occurs as a late event in adenoma-carcinoma progression.

OTHER GENES

- Mutations in BRAF (V600E) proto-oncogene is observed in 10% of CRC cases.(30).These mutations correlate to shorter survival rates

- Identifiable tumor suppressor genes include SMAD2 and SMAD4, mutations of which allow for unrestrained cell growth.

MICROSATELLITE INSTABILITY PATHWAY: [20]

Microsatellites are types of DNA with tandem repeats usually between 1 to 5 basepairs repeated many times.In normal individuals,the length of microsatellite remains constant.These microsatellites are interspersed throughout the genome and are prone for errors during DNA duplication.The function of mismatch repair proteins(MMR) to correct these errors.In the absence of MMR function,microsatellite errors tend to accumulate.

Tumors with microsatellite instability(MSI) are further referred to as those exhibiting low levels or high levels of instability as MSI-L or MSI-H respectively.(31)Some microsatellite repeats are situated in coding or promoter regions of cell growth as like in encoding type II TGF-ß receptor and BAX pro-apoptotic protein.Mutation of TGF-ß receptor contributes to uncontrolled proliferation and loss of BAX increases survival of abnormal clones.

Notable five human MMR genes are:

- hMSH2
- hMLH1
- hPMS1
- hPMS2
- MSH6.

The majority of those associated with HNPCC have germline mutations of either hMSH2 or hMLH1.

CpG ISLAND METHYLATOR PATHWAY

Epigenetic instability in CRC is reflected as hypermethylation of loci containing CpG islands.**Toyota et al**., study was the first one to describe these class of CRC with CpG Island Methylator Phenotype(CIMP).[(32)] It has been proposed that aberrant DNA methylation arises during aging process by epigenetic drift leading to

overgrowth of tumorogenic cells.Histone modification and gene mutations involved in chromatin structure are all the postulates for this aberrant pathway[33] Further sub-classification as CIMP–H when ≥3 markers express methylation ,otherwise as CIMP-L.Study includes MINT1,MINT2,MINT3,MLH1 and $p16^{INK4a}$as markers to define the CIMP .[43]

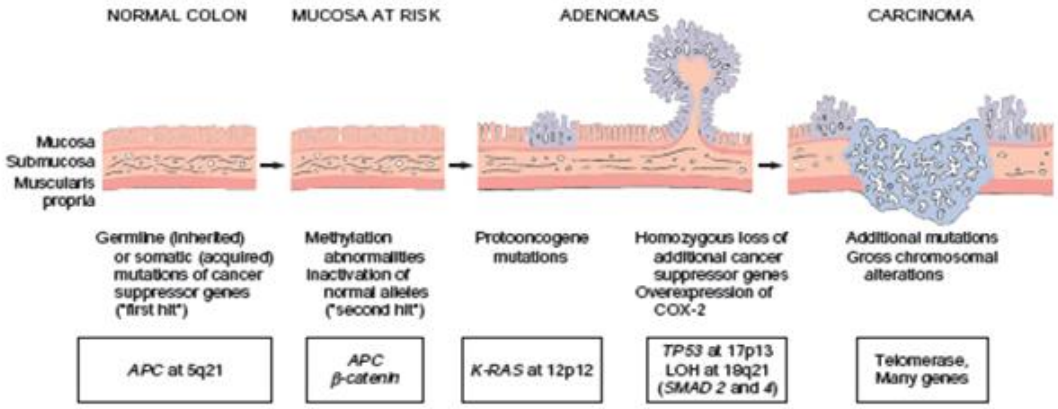


Fig-9: Molecular Pathogenesis Of Adenoma-Carcinoma Sequence

GROSS APPEARANCE OF LESIONS

Shimoda et al.[54] in his study classified lesions based on endoscopic findings:

Polypoid type	Non-polypoid type
Pedunculated Sessile	Superficial elevated Completely Flat Depressed morphology

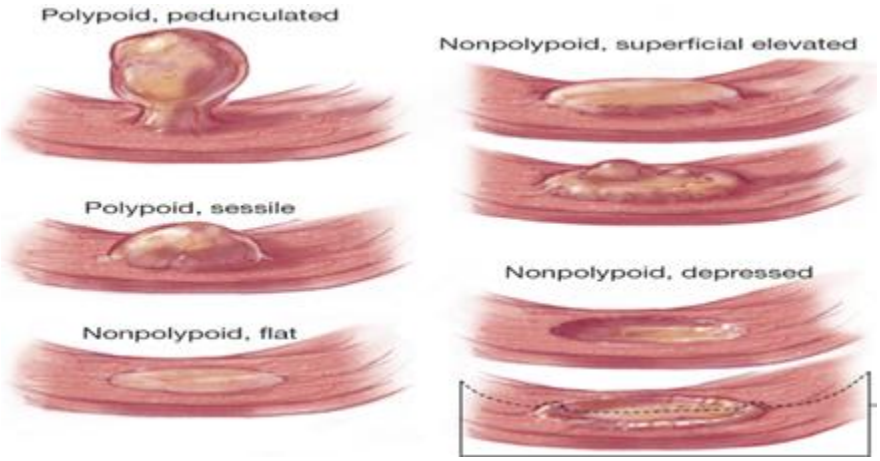


Fig 10: Macroscopic Classification Of Lesions

HISTOLOGICAL SUBTYPES OF CRC AND ITS FEATURES

ADENOCARCINOMA

Common type of large bowel tumors are well to moderately differentiated adenocarcinomas having papillary or villoglandular configuration with variable amount of mucin secretion.The tumor cells consist of columnar and goblet cells with occasional presence of neuroendocrine cells and rarely the presence of paneth cells also as mentioned in the study by **Shousa.S,**1979 **(34)**.The carcinoma elicits a desmoplastic reaction and invasion may be seen extending through all layers into pericolic fat.

The edge of tumor may show foci of residual polyp or hyperplastic change in glands,with altered mucin secretion referred to as “transitional mucosa”.The disordered glands at the advancing edge have been called as “tumor budding” which is associated with poorer prognosis within tumors of same staging .**(40)**

MUCINOUS CARCINOMA

As the name suggests, these carcinomas contain large lakes of extracellular mucin with admixture of tumor cells. According to **Connelly et al.** [35] and several other studies, mucinous foci should be atleast 50%(half) of tumor mass.These tumours occur most commonly in rectum and comprises about 15% of CRCs. They are associated with high rates of microsatellite instability.

MICROPAPILLARY PATTERN

Micropapillae are present in lacunar-like spaces and exhibit 'reverse polarity' with apical surfaces facing periphery than the center.It has a greater tendency for lymphovascular invasion and lymphnode metastasis.A minimum of 5% of tumor with micropapillary feature is required for the diagnosis of this subtype.[40] This subtype comprises about 20% of CRCs.

SIGNET RING CARCINOMA

It usually presents grossly as diffuse infiltration of wall and microscopically too tumor grows in diffuse fashion with most of the cells showing intracellular accumulation of mucin,which pushes the nucleus imparting a typical signet ring cell configuration.Pattern of spread is in the form of peritoneal dissemination and possibility of secondary deposits from primary from gastric or breast carcinoma

should always be ruled out before diagnosing it as primary signet ring cell carcinoma.[36]

SERRATED ADENOCARCINOMA

This is thought to arise from serrated adenoma neoplastic pathway,accounting for 7.5% of all colorectal carcinomas and upto 10-15% of proximal located tumors acconding to a **study** by **Makinen**.[37] It shows serrated pattern of growth with preserved polarity and no necrosis.

MEDULLARY CARCINOMA [38]

Formerly called as 'large cell carcinoma with minimal differentiation',now due to its organoid pattern, its being termed as "Medullary carcinoma".Grossly they are bulky masses with expansile growth.Microscopically they appear as sheets of malignant cells with vesicular nuclei and prominent nucleoli ,along with intraepithelial lyphocytic infiltration. Studies by **Thirunavukarusu P.et al**[39] confirms its high frequency association with MSI.

SQUAMOUS DIFFERENTIATION

In most of the cases, squamous component is associated with glandular components(Adenosquamous carcinoma),occasionally can be seen in pure form as squamous cell carcinoma.**Williams GT ,et al**[41] postulated that some of these squamous cell carcinomas may arise from areas of squamous differentiation of pre-existing polyps**.**

OTHER UNUSUAL FORMS

- ***Basaloid/ Clear cell/ Hepatoid Carcinomas:*** All these rare varieties resemble their origin of morphological counterpart.Immunohistochemical markers and biochemical markers like serum Alpha-fetoprotein will support the diagnosis.

- ***Trophoblastic differentiation-*** Immunohistochemically hCG can be demonstrated in tumor cells.

- ***Glassy cell carcinoma –*** Can be present in large bowel similar to its counterpart in uterine cervix[(42)]

- ***Rhabdoid features –***if present ,as in other sites signify aggressive behavior [(40)]

- ***Carcinosarcoma-*** This extremely rare form exhibit areas of typical adenocarcinoma merging with sarcoma.Cytokeratin positivity of sarcomatous elements is useful in establishing diagnosis.

Neuroendocrine Differentiation

It can manifest in various ways as

1) According to **Ulich et al.,** and other studies,it occurs as "scattered endocrine cells" in otherwise typical adenocarcinomas .[(44)] This kind of occurence is more common post chemotherapy or

radiotherapy suggesting that these cells may be the induced changes by these modalities.

2) In 'mixed composition' form with typical adenocarcinoma, admixed with a component of endocrine differentiation.

3) As "Neuroendocrine carcinoma" with organoid appearance composed of larger cells and small cells, being positive for Neuron specific enolase and synaptophysin.

IMMUNOHISTOLOGICAL AIDS

Though the diagnosis of CRCs are established histomorphologically, some early malignancy that have penetrated via muscularis mucosae into submucosa and presence of scattered neuroendocrine cells,paneth cells or foci of squamous differentiation which is compatible with diagnosis of adenocarcinoma can be substantiated by the use of specific immunohistochemical markers [(45)]

IHC MARKERS

CK 7 and CK 20

- Almost 80-100% adenocarcinomas typically show strong positivity for CK20.But in microsatellite unstable adenocarcinomas,there is a decreased expression of CD20.

- CK 7 is infrequently expressed in colorectal carcinomas.Although upto 13% of cases can express CK7. [(46)]

❖ Thus CK7-/CK20+ is the standard combination for diagnosing colorectal carcinoma.

Mucin

- Colorectal carcinomas can show aberrant expression of mucin ,thus making it usefulness as markers.
- Various mucins include MUC1,MUC2,MUC3,MUC4,MUC5AC and MUC6.
- MUC1-transmembrane glycoprotein overexpressed in various carcinomas like breast,colon,prostate and lung.**Al-Khayal et al**., study show that it may be used as biomarker for detection of early as well as late CRCs. **(47)**
- MUC2-Primarily expressed in colorectal goblet cells.
- MUC5AC- Predominantly expressed in mucus lining of stomach and lung.It is typically negative in CRC.If at all positive,a study by **Betge J et al** showed that Gain of aberrant MUC5AC and MUC6 expression was associated with favourable outcome of CRC. **(48)**

CDX_2:

It represents intestinal differentiation and is positive in about 75-100% of colorectal adenocarcinomas.However its not specific and

positive staining can be other list of carcinomas showing intestinal differentiation.Yet, it can distinguish colorectal carcinoma extending into bladder from primary bladder carcinoma and Metastatic CRC from mucinous bronchoalveolar carcinoma.

Villin

Intestinal brush borders are highlighted by Villin and so it appears as diffuse cytoplasmic staining with brush border accentuation in colorectal carcinomas. **(49)**

OTHER ANTIBODIES

- CARCINOEMBRYONIC ANTIGEN(CEA)
- TUMOUR-ASSOCIATED GLYCOPROTEIN-72 (TAG-72)
- FOLATE RECEPTOR –α (FR-α)

These membrane bound glycoproteins are expressed in over 80% of colorectal carcinomas with relative low expression in normal mucosa as reported by **Johnson et al(50) and Jantscheff et al (51) studies.**

- HUMAN CHORIONIC GONADOTROPIN – Increased expression is associated with aggressive behavior of tumor.
- Her2μ-EPIDERMAL GROWTH FACTOR
- CATHEPSIN B

MISMATCH REPAIR PROTEINS

For CRCs with deficiencies in mismatch repair proteins usually with characteristics of right sided location,<50 years of onset,lack of "dirty necrosis", tumor infiltrating lymphocytes,with crohns like reaction - panel of four main proteins namely MLH1,MSH2,MSH6 and PMS2 can be used .

GRADING OF ADENOCARCINOMA: [52]-- (ANNEXURE II)

STAGING SYSTEMS:

Various staging systems for colorectal carcinoma to determine the extent of spreading are as follows:-

- Duke's Classification Staging & system of Astler and Collar
- TNM staging and prognostic stage groups by AJCC (Annexure III & IV)

PROGNOSTICATION FACTORS

Factors	Good Prognosis	Poor Prognosis
Age		Very young and Very old age
Sr.CEA Levels		>5.0 ng/dl
Local extent	Focal microscopic carcinoma restricted to mucosa /submucosa	Beyond bowel wall and Metastasis
Tumor margins	Pushing margins	
Tumor budding		Isolated tumor cells or clusters >5 cells at invasive front
Vascular invasion		++
Pericolonic tumor deposits		++
Perineural invasion		++
Surgical Margins		Radial margin involvement
Tumor type	Medullary carcinoma	Mucinous/signet ring/Anaplastic
Angiogenesis		++
Expressions of:	HLA-DR/BCL2/ TGF-ßmutation MSI	KRAS mutation Fascin/ pRb & P16/ hCG Allelic loss of chr.18
Staging & grading		Increased staggings & grading

MOLECULAR STUDY OF K-RAS IN COLORECTARAL CARCINOMA

In a cohort study by **Zocche et al**(2015).,[(55)] mutational status of KRAS with 148 patients diagnosed with stage IV Colorectal carcinoma were analysed and treatment response with FOLFOX regimen were studied for a 6years period between 2008-2013.Mutational status of KRAS was determined by DNA extraction using QIAGEN kit, later on by PCR amplification and sequencing.The exclusion criteria being previous history of chemoradiotherapy and history of other malignancies within 5 years .Survival analysis was done by Kaplan-Meier analysis and comparison among groups were analysed using log-rank test.

Among total of 148 patients,48(32%)had mutated KRAS and majority (77%)were at codon12 and (23%)at codon13.The Kaplan – Meier survival analysis of individual subtypes of KRAS mutation showed that G12D subtype seemed to be associated with poor prognosis in progression free survival,while no significant results were obtained in overall survival for other subtypes in their study population.

In the present cohort study of Stage IV patients treated with FOLFOX regimen, it was observed that KRAS mutations were associated with high risk of recurrence,especially G12D mutations showed a worse clinicical outcome.Limitations of this study include inconsistent information when tracking lost patients due to transfer to

other regions and insufficient sample size.This multivariate analysis showed KRAS mutation as an independent negative prognostic factor for progression- free survival and concluded that it had an adverse impact on prognosis for metastatic CRCs.

Al-Allawi et al (2012).,[(56)] researched on KRAS gene-point mutational detection to determine its frequency in colorectal carcinoma.Study was done for two years with a total of 61 colectomy cases in Iraq population.Enrollment criteria for the study included colorectal carcinomas that are electively resected with no prior chemo and radio therapies.Sections were obtained from the tissue blocks and the one which represented the tumor best was selected for study. DNA was extracted from the Formalin Fixed Paraffin Embedded (FFPE)tissue via QIAGEN kit;KRAS point mutation detection was carried out using KRAS STRIP ASSAY kit.These readymade strips contain allele specific oligonucleotide probes as an array of parallel lines which detected 10 different KRAS mutations.

There were 50 colorectal carcinoma cases fitting into enrolled criteria of which 24(48%) had KRAS mutations. The most commonest among them were G>T transversions and G>A transitions with 41.1% each and then G>C transversions with 17.2%. Codon 12 constituted the major (89.7%) whereby the 10.3% was by Codon 13. As observed with previous literatures,this study also showed that KRAS codon 13

mutations were less frequent than Codon12.There were also double mutations and triple codon mutations observed in same patients.These multiple mutations were seen in 8% of cases.This study did not show any significant correlation of KRAS status with age,gender and site of tumor,grading,TNM staging or lymphovascular invasion. The study concluded that KRAS mutations were common among Iraqi sporadic CRC cases with comparable frequencies of western countries.

In the study of **Irani Shemirani A et al** (2011).,[57] Profile of KRAS and MSI mutations in colorectal cancer by PCR analysis was performed,whereby examining the colorectal fresh tissues and DNA extraction using Qiagen kit was done. Further amplification by PCR was carried out using specific forward and reverse primers in which 493bp region of exon 2 and 402bp in exon 3 that comprises the mutational hotspots were amplified. The amplified DNA PCR product was then subjected to direct sequencing. KRAS gene in Exon 2 and Exon 3 analysis were done in 95 cases with 48 tumor masses and 47 polyps. Patients with familial background –FAP/HNPCC were excluded.

Out of those 48 tumors,they detected 6 mutations which includes 5 mutations in codon 12 and 1 mutation in codon 13.In polyps, 2 mutations were in codon 13 and 1mutation was in codon 12.All the KRAS mutations in codon 12 were G12A. Among the tumors detected with KRAS,all except one were in colon and that one was detected in

rectum. But in contrast,among the polyps detected with KRAS mutation all yet one were in rectum and that remaining one was observed in colon.There was no significant correlation of KRAS mutation with the individual's clinical features harbouring the polyps or tumors. Furthermore,MSI status detection was done using pentaplex set of microsatellite markers.MSI was labeled when 1 or >1 marker was altered.The commonest MSI marker in the study population was NR-21.

The study gave an inference of considering Gly12Asp in exon 2 as biomarker to prediagnose susceptible individuals before the progression of tumor.

A review article by **Federico A. et al** (2009),[(58)]concluded that multiple methods are available for determining KRAS mutational status of tumor.All of these methods appear to have adequate clinical sensitivity to detect patient's unresponsiveness to EGFR inhibitors-cetuximab or panitimumab.

Various Methods used for testing KRAS mutations are summarized as tabulation in this literature:-

Methods of kras analysis	Sensitivity,% of mutant alleles	Comments
Sanger sequencing	20%	Gold standard but Time consuming
Pyrosequencing	5-10%	Ability to detect short PCR products; Detects all possible mutations and is inexpensive
PCR Clamping method	1%	Rapid, closed PCR system ;But doesn't allow to control DNA quality and efficiency of PCR amplification
Real Time PCR	1%	Detects only most common-7 mutation panels; Needs more tissue than for other methods , for analysis .
Post –PCR fluorescent melting curve analysis using specific probes	5-10%	Detects all possible mutations; rarely there is difficulty in distinguishing between mutation types

Bolton L et al (2015).,[59] study on KRAS mutation assays comparing therascreen Qiagen and Cast PCR methods to determine correlation between both was conducted testing the presence of 7 KRAS mutations in codon 12 and 13-G12A,G12D,G12R,G12C, G12V,G13D. DNA was extracted from FFPE blocks of colorectal carcinoma having >50% of neoplastic cells by using Maxwell 16 FFPE plus LEV DNA purification kit DNA extracted was quantified by Nanodrop

sphectrophotometry. Further on,the samples were subjected to ARMS-based therascreen assay and castPCR methods.

Sensitivity was calculated as proportion of KRAS mutated tumors identified by cast PCR/proportion of KRAS mutation identified by therascreen,ignoring those mutations that are detected by castPCR but not in therascreen. Specificity was determined by Wild-type KRAS tumors identified by castPCR to proportion of Wild type tumors by Therascreen. Out of 99 samples,3 of them showed discrepancies between these two mutation detected methods on initial testing.Statistical sensitivity and specificity were 95%and 98% respectively.

Sensitivity=(96/99)x100→95%; **Specificity**= (61/62)x100→98%

Inference from this study is that there was good correlation observed between these two methods. Initial discrepancies between these methods were solved by re-testing.castPCR showed comparatively lower Ct(threshold value) than therascreen,hence it can pickup even tumors with little mutant DNA present.Study showed that castPCR is a reliable assay for KRAS testing in FFPE colorectal samples.

Okayama N et al (2011).[(62)] suggested the importance of DNA amplificability and amplicon size as important for success of mutation detection tests from FFPE samples. This study evaluated the DNA amplificability levels, effects of formalin fixation, deparaffinization and

storage time in paraffin blocks for achieving success rate in KRAS mutational analysis by dideoxy sequencing .19 FFPE CRC tissue samples were obtained from a hospital in Japan during the period of 2004-2009. 10% buffered formalin was used for fixation.Sections of size 4µm and 10µm were used for H& E staining and DNA extraction respectively. Deparaffinization done with 1ml of xylene at 26^0c incubation. Then they were centrifuged to obtain the pellets,followed by DNA extraction by QIAamp kit.

Inorder to evaluate PCR amplificability of extracted DNA,12 primer sets for 9 genes having various amplicon sizes were used.PCR product analysation was done by 3% agarose gel electrophoresis and stained by ethidium bromide.PCR products were purified by ExoSAP and terminator cycle sequencing kit was used for Sequencing.

With 19 FFPE CRC samples,smaller fragments with size range of 96-278bp were amplified with PCR,whereas amplification was difficult in ≥278 bp,thereby indicating size of the amplicon and amplification property's significance to avoid DNA degradation. For PCR tests,although DNA recovery rate tended to be higher in 60 minutes xylene treatment than in 10minutes treatments,DNA extraction from 10 min.of xylene itself is sufficient.These suggest that deparaffinization time may not have any effects on DNA recovery . Also datas suggested that neither fixation nor storage time affects DNA amplification.

Tan C et al (2012).,[60] in their review study discussed on various methods of KRAS testing like Sanger sequencing with sensitivity of 20-30%,Pyrosequencing with 5% and non –sequencing methods like Real time PCR with HRMA having 5% sensitivity, RFLP with sequencing having 0.1% and COLD-PCR with sequencing having 1-2.5% sensitivities. Also concomitant analysis of other factors like BRAF,NRAS and loss of PTEN was recommended since KRAS mutations account for around 35% of nonresponsive patients that receive Anti-EGFR therapy as mentioned in **Allegra CJ et al. (2009)**.[116] In a retrospective consortium analysis among 1022 colorectal tumor DNA samples,40% harboured KRAS mutation, 4.7%-BRAF mutation, 2.64% with Nras mutation and 14.5% with PIK3CA mutation. This study provided the information that there is a lower incidence of KRAS mutational frequencies in Asian population compared to American and European nations.

This review study also mentioned about DNA fragmentation caused by improper formalin fixation,heterogenous somatic KRAS mutations, and influence of stromal cells causing False-positive KRAS mutational results.

In a study by **Aghagolzadeh P et al** (2016).,[61] in assessing molecular and cellular biomarkers of colorectal carcinoma,they have included RAS mutation studies as one of the diagnostic and predictive

markers inaddition to P_{53} ,Ki67,MSI,VEGF and DNA hypermethylation studies. Cancer related molecular and cellular markers could be classified as 4 categories:-Diagnostic markers,Prognostic markers, Predictive markers and surveillance markers. A few points stressing the importance of KRAS mutational testing in colorectal carcinoma is mentioned in this study.Different point mutations at Exon 2 commonly in Codon 12 and 13 , mutations of codon 61 in Exon 3 lead on to constitutive activation of tumorigenic pathway .Therefore, any genetic disruption of KRAS gene is one of the crucial step in development of many carcinomas including colorectal carcinoma

Andreyev HJ et al study (2001).,[63] in 'RASCAL-II' study have incorporated datas of 4268 patients at different stages of colorectal carcinoma from 42 different centres in 21 countries. Exclusion criteria for further analysis included those with missing age (n=203) and no information of Duke's staging (n=75).Perioperative deaths(n=76) are included . Also those patients providing datas only on codon 12 and not on codon 13 were excluded (n=49).This second 'RASCAL' study has been so far largest study to examine KRAS mutational status in comparison with outcome of the patient.The purpose of the study of exploring mutation at different stages of Duke's suggests that this mutation is particularly aggressive in Duke's stage C with 50% association. This study added up more details to its first study of

'RASCAL' and explored further role of KRAS mutation at different stages of colorectal carcinoma.

Study explored the effects of KRAS mutation where they detected 12 possible mutations on codon 12 & 13,with Gly→Val on codon 12 being seen in 8.6% of cases had statistically significant impact on Failure Free Survival (p-0.004) and overall survival (p-0.008)

Also,there is an suggestion from the observation that Gly→Val mutation is not only important for progression of carcinoma but also might predispose to more aggressiveness in patients with advanced CRC.

Study of **Veldore VH et al.**2014, [66] was a retrospective study in 299 unselected incidental CRC patients visiting the hospital for clinical management during the year 2009-2013.This study demonstrated the KRAS genetic abnormalities as important findings in CRC of Indian population.Seven different somatic mutations in Exon 2 region of KRAS gene was analyzed in this study. DNA extracted from FFPE blocks of tumor tissue was then screened for 7 point mutations in Codon 12 and 13 of KRAS gene using Therascreen KRAS PCR kit by Real time PCR. Statistical analysis was done to assess relationship between KRAS mutation status and variables like age,sex,tumor location and morphology. Patients were considered positive for KRAS mutation even if one out of 7 mutations was detected.

Results obtained were: Mean age of population was 55.9±12.8 years.The majority of population was male(65.2%). Neither age nor gender does seem to influence mutational subtypes of codon 12 and codon 13. There was a predominance of well-differentiated lesion (79.9%) ; Lesions primarily located in left side of colon(48.5%).KRAS mutation was obtained in 42.8% .Gly →Val followed by Gly →Cys,then Gly→Asp was the order of frequency of the mutations.Chi-Square analysis showed that there was significant correlation between KRAS mutation and well differentiated adenocarcinomas compared to other subtypes. Gly→Ala mutation was higher in rectosigmoid region. KRAS mutation is one of the recognized predictive marker in metastatic colorectal carcinoma,thereby predicting its efficacy in Anti-EGFR treatment.

Bengala et al (2010).,[(64)] demonstrated that patients with KRAS mutation had decreased rate of complete responsiveness to concomitant chemoradiation with continuous infusion of 5FU with/without oxaliplatin and capacitabine compared to wild type of KRAS (7.4% vs 19.2%). This study did a retrospective review of clinical outcome of 146 locally advanced rectal cancer (LARC) cases treated with preoperative chemoradiotherapy and their KRAS, EGFR status. Fluorescence in situ hybridization (FISH) studies were performed on selected paraffin-embedded sections having good tumor tissue for EGFR analysis.

KRAS gene analysis with 5µm thick sections obtained from FFPE blocks were transferred to an Eppendorf tube containing digestive buffer (proteinase K in 50m Tris, 1m EDTA,0.5% Tween20) with overnight incubation at 56^0 c.PCR was carried out using commercial master mix with initil denaturation at 95^0 c for 10min. was followed by 41 cycles ,annealing at 52^0 c for 1min and finally extension at 72^0 c for 2min.Exon 2 of KRAS was amplified using primers.Amplified products after quantifying with 2% gel electrophoresis was subjected to terminator cycle sequencer.This study concluded that EGFR and KRAS mutation status are neither predictive nor prognostic indicators for pathological tumor response in locally advanced cancer patients treated with prior chemoradiation.

Materials and Methods

MATERIALS AND METHODS

In this study,we performed both prospective and retrospective data analysis of patients who were diagnosed to have colorectal carcinoma over a period of 2 years from August 2016 to August 2018.The study been undertaken in Institute of Pathology,Madras Medical College and Rajiv Gandhi Government General Hospital,Chennai.

SOURCE OF DATA

During the study period,we received about 23,057 specimens for histopathological examination,of which 2603 cases belong to Gastrointestinal tract system .Out of 2603 specimens,268 specimens were lower gastrointestinal tract malignancies.In that 268 malignancy cases,250 cases were Adenocarcinoma of colorectal region which includes both colonoscopy guided small biopsy and resection specimens

INCLUSION CRITERIA

All Histopathologically proven cases of Colorectal carcinoma

EXCLUSION CRITERIA

Samples of patients with prior history of neoadjuvant chemotherapy.

METHOD OF DATA COLLECTION:

Sample size of 30 cases reported during the study period were taken for study.Detailed history regarding age,sex,type of procedure,site of tumor,imaging findings,any serological parameter if available were collected from surgical pathology records.Molecular detection of KRAS from the Formalin fixed paraffin embedded (FFPE)tissue blocks was done using Polymerase chain reaction (PCR) amplification and analysis by sanger sequencing.

DNA EXTRACTION

FFPE tissue blocks of biopsy proven colorectal carcinoma having the highest density of malignant cells are chosen by microscopic viewing of hemotoxylin and eosin stained slides.DNA extraction from theselected blocks were done using QIAamp DNA FFPE tissue kit (from Qiagen manufacturer).Steps are carried on following the manufacturer's guidelines:

- Sections from blocks are cut with 5-10µm thickness and immediately sections are placed in a 2ml microcentrifuge tube and 1ml of xylene added.Lid is closed and vortexed vigorously for 10seconds.

- Centrifuging is done at full speed for 2min. at room temperature.Later supernatant is removes by pipetting out,leaving

the pellet aside. 1ml of ethanol is added to pellet and mix by vortexing.

- Again centrifuge for 2min and then remove the supernatant,incubate the tube at 37^0/room temperature for 10minutes with opened lid.

- Now pellet is suspended with 180µl of buffer. 20µl proteinase is added and mixed by vortexing was done.Further incubations at 56^0c and 90^0c ,each for 1 hour respectively been done.

- Further more centrifugations by adding buffer to pellets are carried on and transferred to lysate column;

- Place the QIAamp MinElute column in a microcentrifuge tube,apply 20-100µl buffer ATE.Final centrifugation with buffer ATE for 5min.at room T^0 yields the DNA.

DNA QUANTIFICATION

DNA yields were quantified by using a Nanodrop 2000 spectrophotometer and concentration in ng/µl is measured.

PCR AMPLIFICATION

After standardization using best performed primer,the test was carried out with 30 samples with better DNA yield.The following 2 primers are used:

Primers	Sequence (5'→3')	Product size
FORWARD PRIMER	GGTACTGGTGGAGTATTTGATAG	247 bp
REVERSE PRIMER	GGTCCTGCACCAGTAATATGC	247 bp

Hot star Master mix was setup for the final reaction in PCR,which are tabulated later down.The reaction mixture was mixed well with 1.65µl of DNA template being added to each tube.Final volume of reaction mixture was 25µl.The mixture was well centrifuged in a microcentrifuge for 1min and tubes were placed in 96 wells of thermocycler (Applied biosystems –manufacturer).

Table Showing PCR Hot Start

Reagent	Volume
PCR buffer	2.5 µl
Forward primer	0.5 µl
Reverse primer	0.5 µl
DNTp's	2 .0 µl
Taq polymerase	0.25 µl
DNA	1.65 µl
H2O	17.6 µl
Total	25 µl

Thermal cycling conditions used for PCR

Steps	Temperature	Time	Cycles
Denaturation	95^0C	2min	1
Annealing	95^0C 59.1^0C 72^0C	30 sec 30 sec 30 sec	30
Cooling	72^0C	5 min	1

Annealing begins at 95^o C and ends at 72^oC ,lowered by 1^oC for every 4 cycles until it reaching 59.1^oC

ANALYSIS OF AMPLIFIED TARGETS BY SEQUENCING

The PCR product was checked for amplification using agarose Gel Electrophoresis. Then to 5µl of amplified PCR product,2µl of Exosap was added and subjected to sequencing reaction in ABI 3500 Genetic sequencer(Applied biosystems) and analysed using basic search tool program with available standard reference sequences (https://m.ensembl.org)

STATISTICAL ANALYSIS

The statistical evaluation was performed with IBM-SPSS statistical package for the social sciences version 20. An initial analysis of collected variables was performed. Then, Molecular expression of KRAS analyzed were correlated with clinical variables like age, gender, size and pathological variables like histological grade, stage and invasiveness of the tumor. Pearson Chi square test was used in analyzing these variables. In the present study, the P value below 0.05 is considered significant.

Observation and Results

OBSERVATION AND RESULTS

In Institute of Pathology, the total number of biopsy specimens received for histopathology from August 2016-August 2018 were 23,057 ,out of which the total number of gastrointestinal tract specimens include 2603and 268 of specimens were lower GI tract malignancy.The number of colorectal carcinomas enrolled in this study was 30 cases out of 250 cases of colorectal adenocarcinoma.

Histomorphological and molecular analysis of KRAS expression were studied and compared with literatures. For the (n=30)cases, results obtained are as follows:

AGE WISE DISTRIBUTION OF COLORECTAL CARCINOMA

Table:1

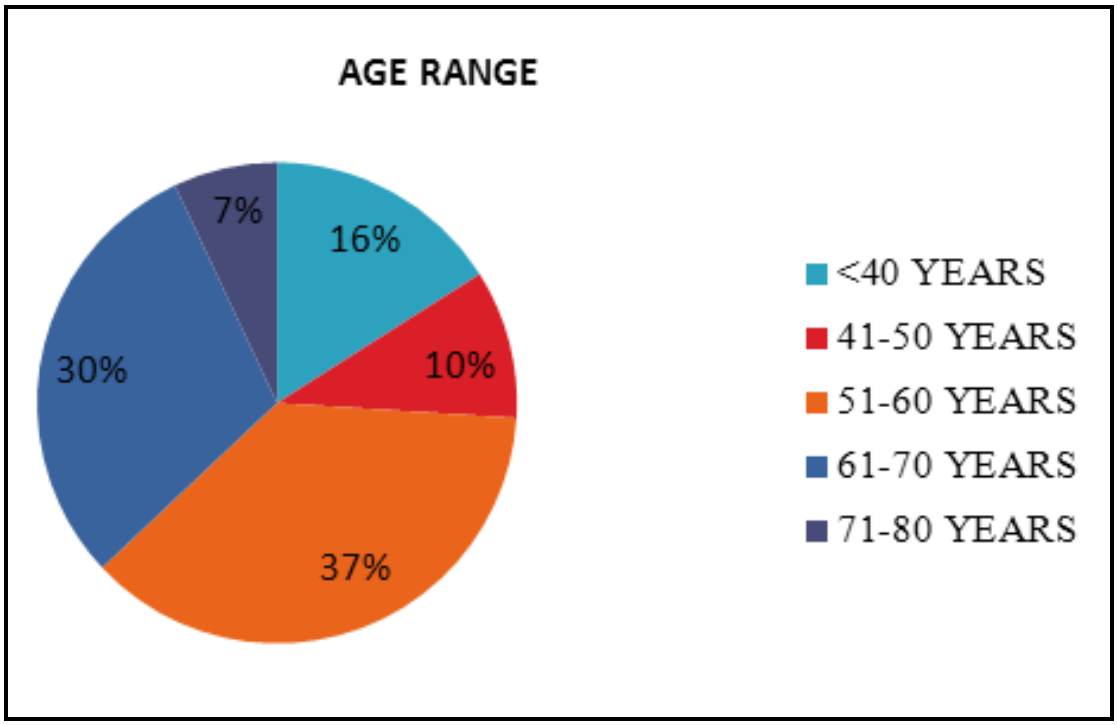
	n	Minimum	Maximum	Mean	Std. Deviation
AGE (years)	30	24.00	80.00	55.20	13.95905

Table: 2

Age range (in years)	Frequency	Percent(%)
<40	5	16.6
41-50	3	10.0
51-60	11	36.7
61-70	9	30.0
71-80	2	6.7
Total	30	100.0

The highest incidence of CRCs were in the age group of between 51-60 years(36.7%),followed by 61-70 years(30%).The mean age of presentation was 55.2 years.The youngest age of presentation in our study was 24 years and oldest age presented was 80 years.

Fig-1 : Age-wise distribution of Colorectal Carcinoma (n=30)



GENDER WISE DISTRIBUTION OF COLORECTAL CARCINOMA

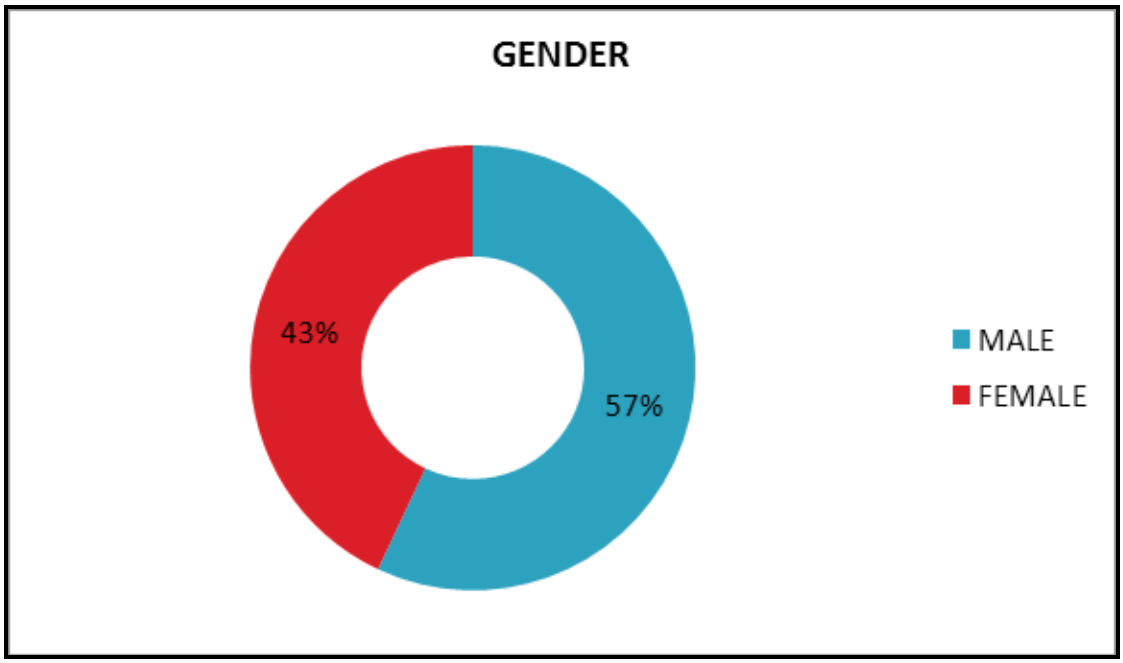
Table:3

Gender	Frequency	Percent (%)
Male	17	56.7
Female	13	43.3
Total	30	100.0

From the above table, it is observed that incidence of colorectal carcinoma in our study was comparatively more common in males (56.7%) compared to females(43.3%).

Male :Female ratio observed was 1.3:1

FIG2 : Gender wise distribution

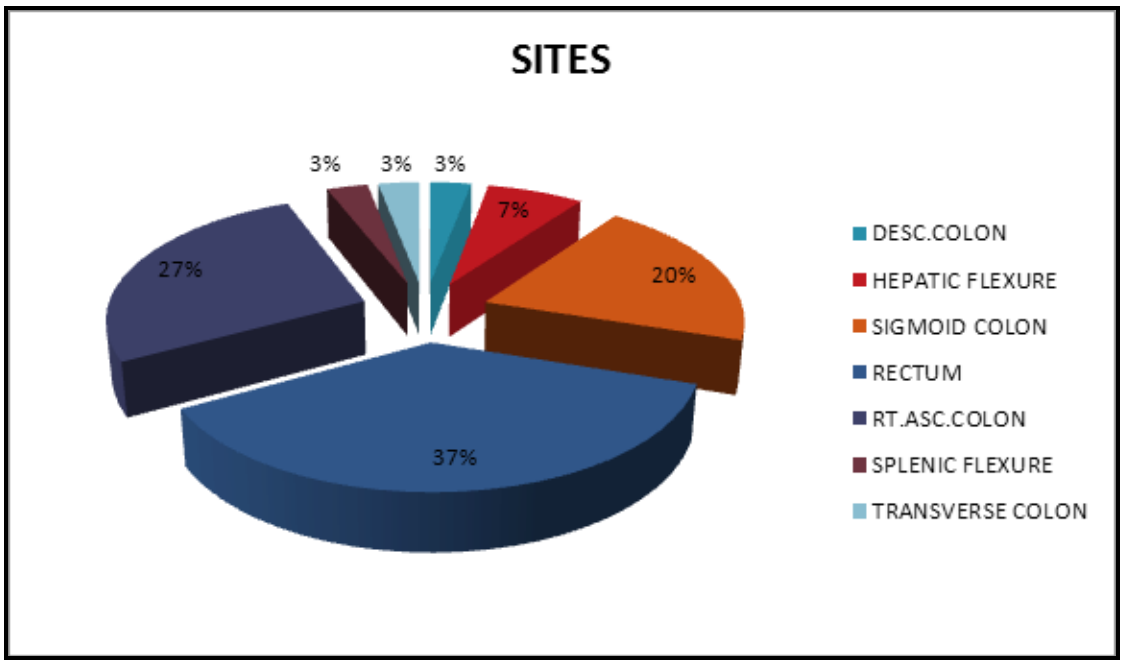


SITE WISE DISTRIBUTION OF COLORECTAL CARCINOMAS:

Table:4 –Showing distribution of tumor in various sites of colon and rectums

Site	Frequency	Percent(%)
Ascending colon(right sided)	8	26.67
Hepatic flexure	2	6.67
Transverse colon	1	3.33
Splenic flexure	1	3.33
Descending colon(Left sided)	1	3.33
Sigmoid colon	6	20.00
Rectum	11	36.67
Total	30	100.00

FIG 3: Site wise distribution of tumor



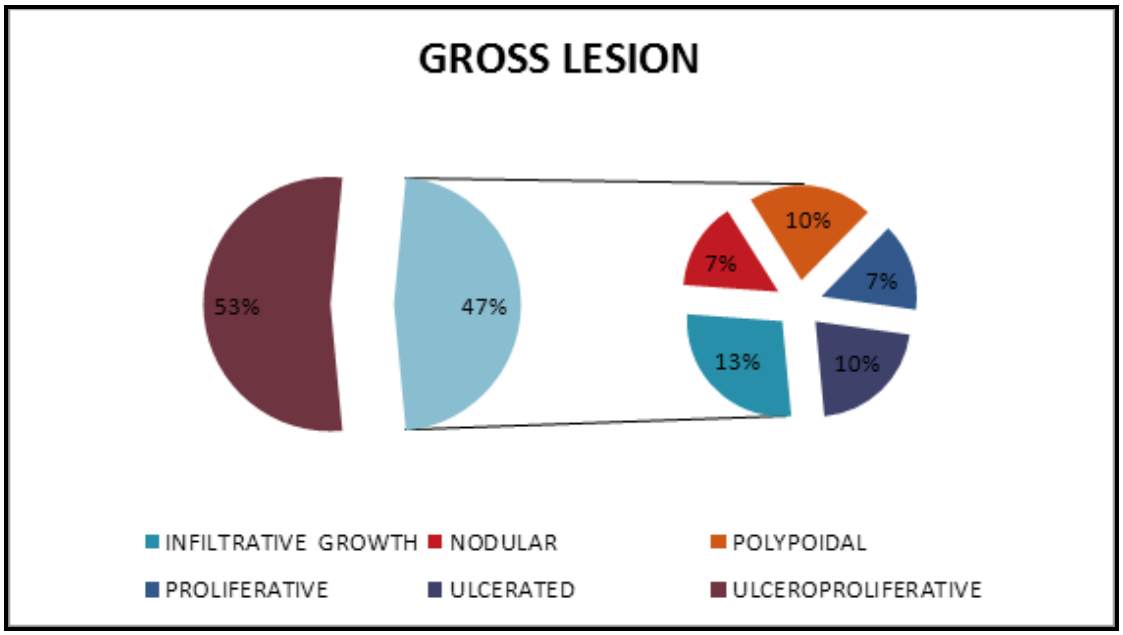
From the above pie chart ,it is inferred that majority of colorectal malignancy cases occurred in Rectum with 11 cases (37%), followed by ascending colon with 8 cases(27%).

GROSS FEATURES OF COLORECTAL CARCINOMA

Table:5- depicts various types of gross lesions of colorectal tumor

Grosslesion	Frequency	Percent (%)
Ulcerated	3	10.00
Ulceroproliferative	16	53.33
Infiltrative growth	4	13.33
Proliferative	2	6.67
Nodular	2	6.67
Polypoidal	3	10.00
Total	30	100.0

Fig-4: Gross Lesions Of Colorectal Carcinoma

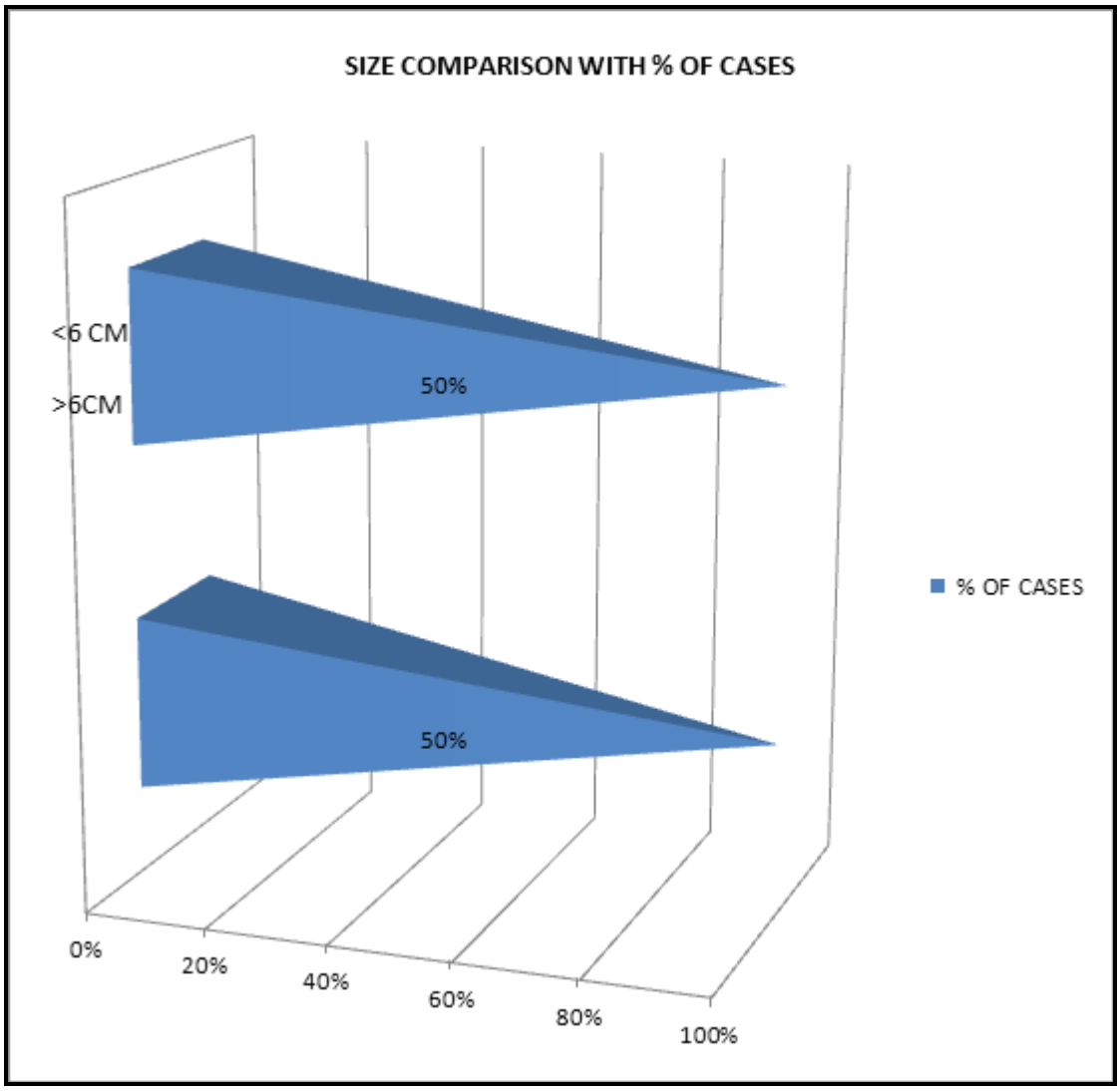


The above table and pie chart shows the gross features of colorectal adenocarcinoma.

The commonest gross feature noticed is the Ulceroproliferative type of growth (53%),followed by infiltrative growth (13%).

SIZE OF LESION

Since there is no significant cut-off size of prognostication significance, roughly % of cases of size less than and more than 6cm is taken for comparison purpose.



From the above chart,it is observed that about 50% of tumours were <6cm and another half of the percent of tumours were >6cm.

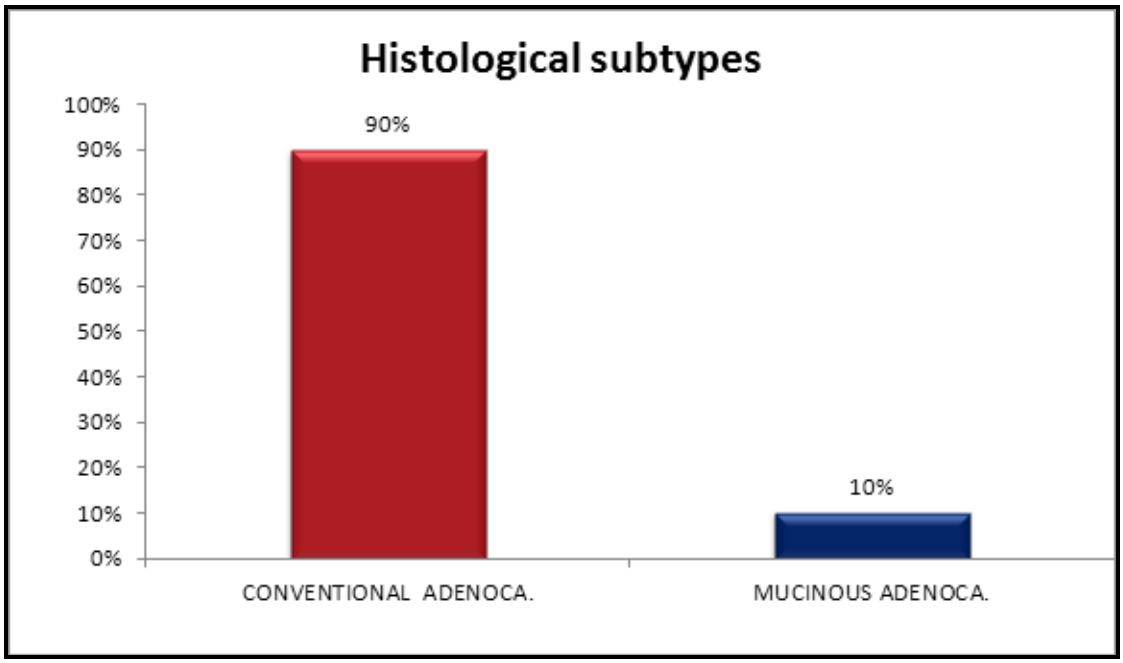
HISTOLOGICAL SUBTYPES OF COLORECTAL CARCINOMA

Table 6: Tabulation shows the histological variants of colorectal tumors of our study

Histological subtypes	Frequency	Percent
Conventional adenocarcinoma.	27	90.0%
Mucinous adenocarcinoma	3	10.0%
Total	30	100.00%

Observation made from the above table shows that 90% of cases were Conventional adenocarcinoma and 10% were mucinous type.The upcoming chart also depicts the same.

Fig 6: Histological subtypes of colorectal cancer

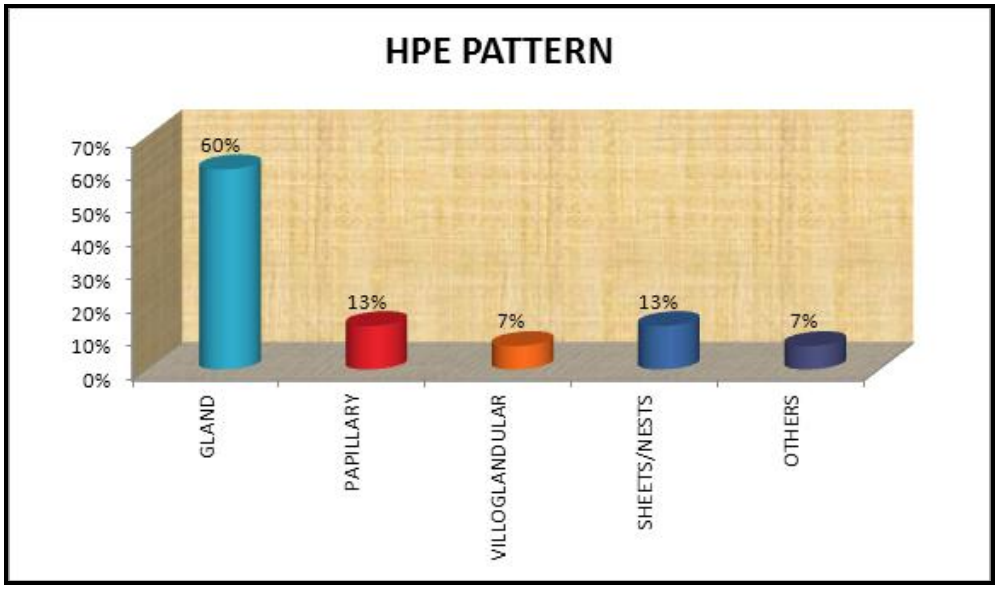


HISTOPATHOLOGICAL PATTERN

Table 7-showing Various patterns in histopathology

Pattern/configuration	Frequency	Percent (%)
Glandular	18	60.00%
Papillary	4	13.33%
Villoglandular	2	6.67%
Sheets/nests	4	13.33%
Others	2	6.67%
Total	30	100.00%

Fig:7 Chart Shows various HPE patterns in colorectal carcinoma



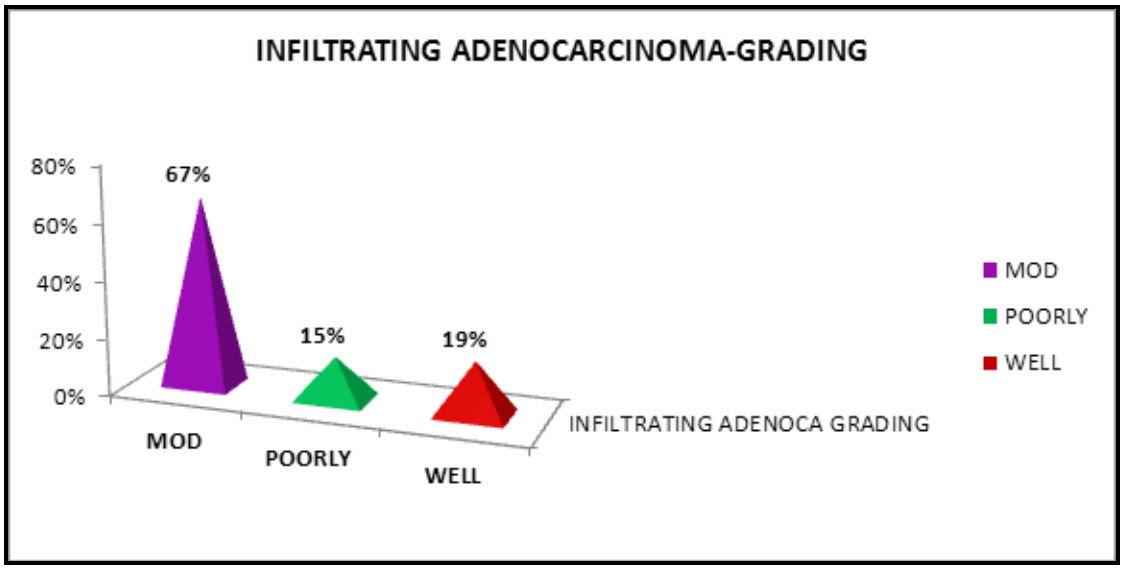
The above datas suggest that in colorectal adenocarcinoma,the major histomorphological pattern of tumor cells were Glandular configuration seen in 18 cases(60%) followed by 13% each by papillary configuration and then in sheets.Pattern to some extent can determine the grading based on differentiation of tumor, as in well differentiated adenocarcinoma mostly maintains glandular or villoglandular pattern.

GRADING OF ADENOCARCINOMA-USUAL TYPE: (N=27)

Table:8 shows % of cases in various histological gradings

Grade	Frequency	Percent(%)
Well differentiated	5	18.5%
Moderately differentiated	18	66.7%
Poorly differentiated	4	14.8%
Total	27	100.0%

Fig-8 chart depicting % of colorectal adenocarcinoma showing various degrees of grading:



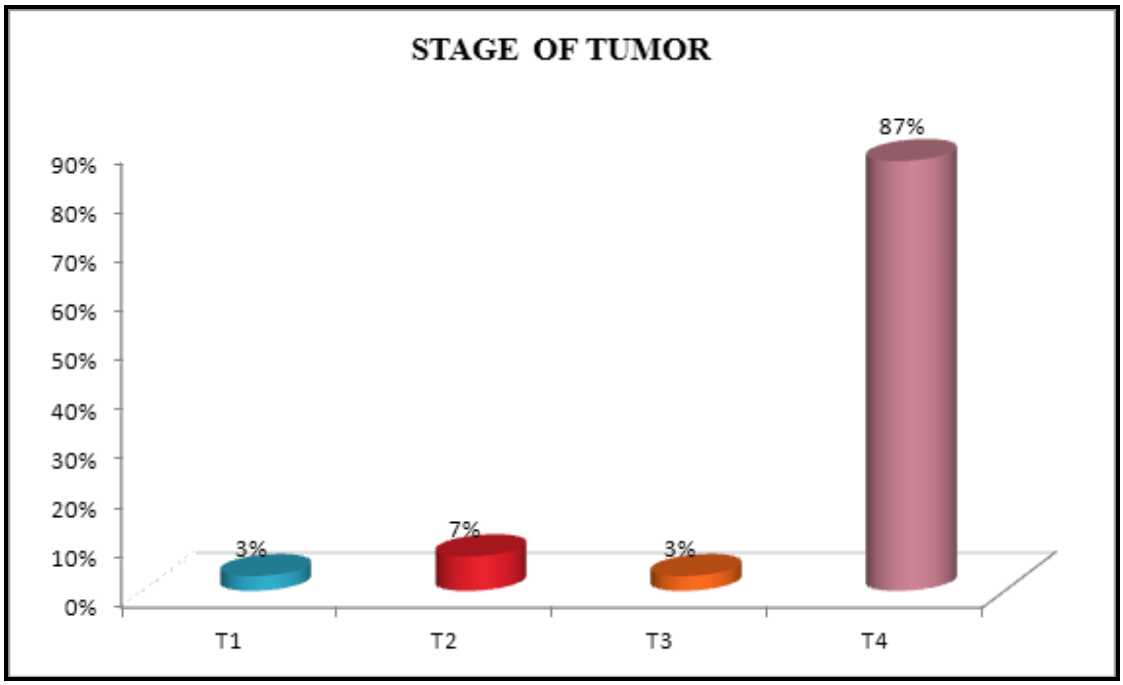
Thus it was observed that among the 27 adenocarcinoma cases, majority of them (67%) was moderately differentiated, 19% was well differentiated grade and 15% was poorly differentiated grade.

T -STAGING

Table:9 shows frequency of cases presenting with various T stages

Stage	Frequency	Percent (%)
T1	1	3.3
T2	2	6.7
T3	1	3.3
T4	26	86.7
Total	30	100.0

Fig:9 charts showing % of cases in various T stages:



From the graph above with T-stages as x-axis variables and % of cases representing in Y axis, it is seen that 87% of cases belonged to T4 stage.

LYMPHOVASCULAR & PERINEURAL INVASION

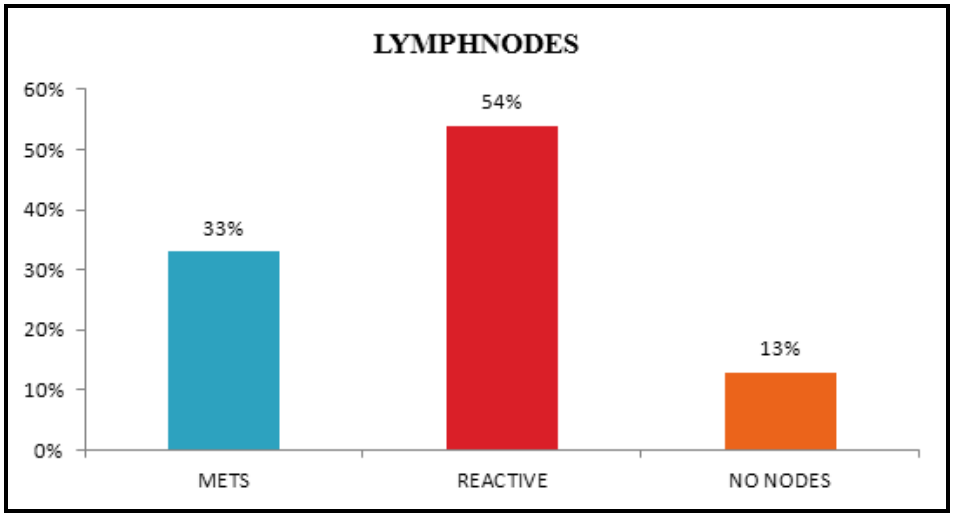
In this study,10 cases showed lymphovascular invasion and one adenocarcinoma case showed perineural invasion

LYMPHNODE STATUS

Table:10 shows the lymphnode status in colorectal observed carcinoma cases.

Lymphnodes	Frequency	Percent (%)
Metastasis	10	33.3%
Reactive	16	53.3%
No nodes	4	13.3%

Fig:10 showing % of cases with variable status of lymphnodes in colorectal carcinoma



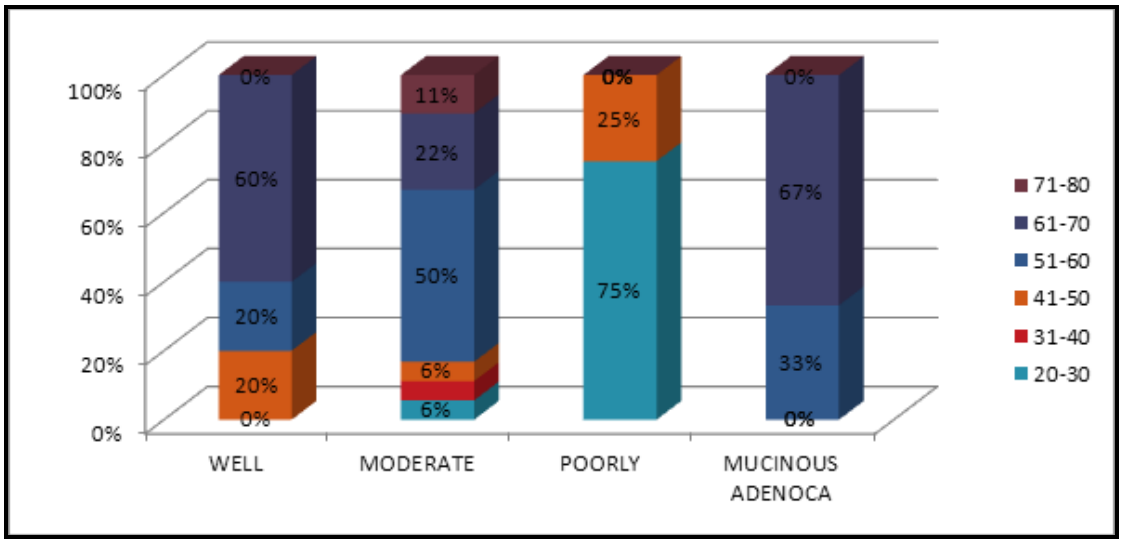
It is seen that 16 cases (54%) of lymphnodes showed reactive hyperplasia, whereas 10 cases(33%) of them showed metastatic carcinomatous deposits. In around 13% of cases nodal status cannot be assessed.

ASSOCIATION OF AGE WITH HPE DIAGNOSIS

Table:11 Crosstabulation of Age distribution with Subtypes of tumor

Age group	Infiltrating adenocarcinoma			Mucinous adenocarcinoma
	Well	Moderately	Poorly	
	N (%)	N(%)	N(%)	N(%)
20-30	0(0.0%)	1(5.6%)	3(75.0%)	0(0.0%)
31-40	0(0.0%)	1(5.6%)	0(0.0%)	0(0.0%)
41-50	1(20.0%)	1(5.6%)	1(25.0%)	0(0.0%)
51-60	1(20.0%)	9(50.0%)	0(0.0%)	1(33.3%)
61-70	3(60.0%)	4(22.2%)	0(0.0%)	2(67.7%)
71-80	0(0.0%)	2(11.1%)	0(0.0%)	0(0.0%)

Fig:11 Age wise distribution of subtypes of adenocarcinomas



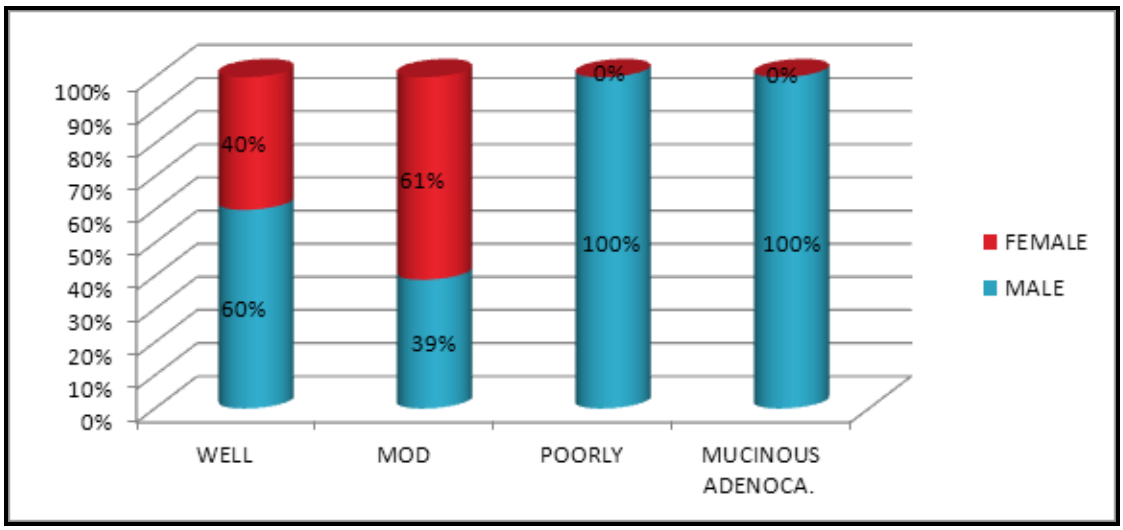
The current study shows that most of the cases fall under the age group of 51-60 years and among them majority of them are moderately differentiated grade.In older age group>60 years also moderately differentiated grade is more common than other two grades.In individuals <50 years, both poorly differentiated and moderately differentiated grades are evenly distributed,with well differentiated grade being at lowest occurance.

ASSOCIATION OF GENDER WITH HPE DIAGNOSIS

Table 12: Comparison of Gender with subtypes of tumor

Sex	Well.diff	Moder.	Poorly	Mucinous adenocarcinoma
	N(%)	N(%)	N(%)	N(%)
MALE	3(60.0%)	7(38.9%)	4(100%)	3(100.0%)
FEMALE	2(40.0%)	11(61.1%)	0(0%)	0(0%)

Fig-12: Cylindrical chart depicting correlation of gender with various types and grades of colorectal carcinoma.



From above observation,it is seen that among Men,Adenocarcinomas occurred with majority being moderately differentiated grade,followed by poorly differentiated and well differentiated grades.There were also 3 cases of mucinous carcinoma among male gender category.In females also, moderately differentiated grade comprised highest category than others.

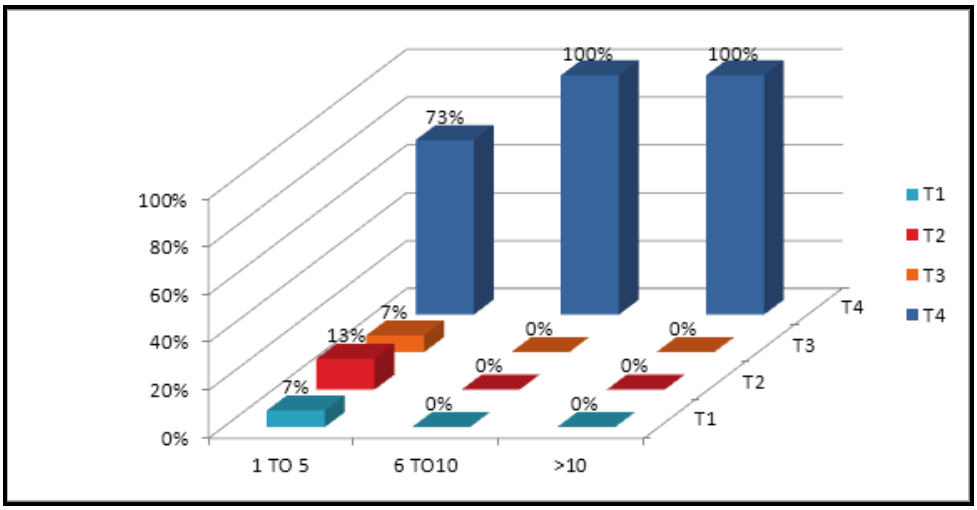
CORRELATION OF TUMOR SIZE WITH STAGE OF TUMOR

Table 13:Size and T stage Crosstabulation

Size(in cm)	T stage				Total
	T1	T2	T3	T4	
1-5	1	2	1	11	15
	(6.7%)	(13.3%)	(6.7%)	(73.3%)	(100.0%)
6-10	0	0	0	14	14
	(0%)	(0%)	(0%)	(100%)	(100%)
>10	0	0	0	1	1
	(0%)	(0%)	(0%)	(100%)	(100%)

Pearson Chi-Square= 4.615,p=0.594

Fig 13: Bar diagram demonstrating T stage comparison with size of tumor.



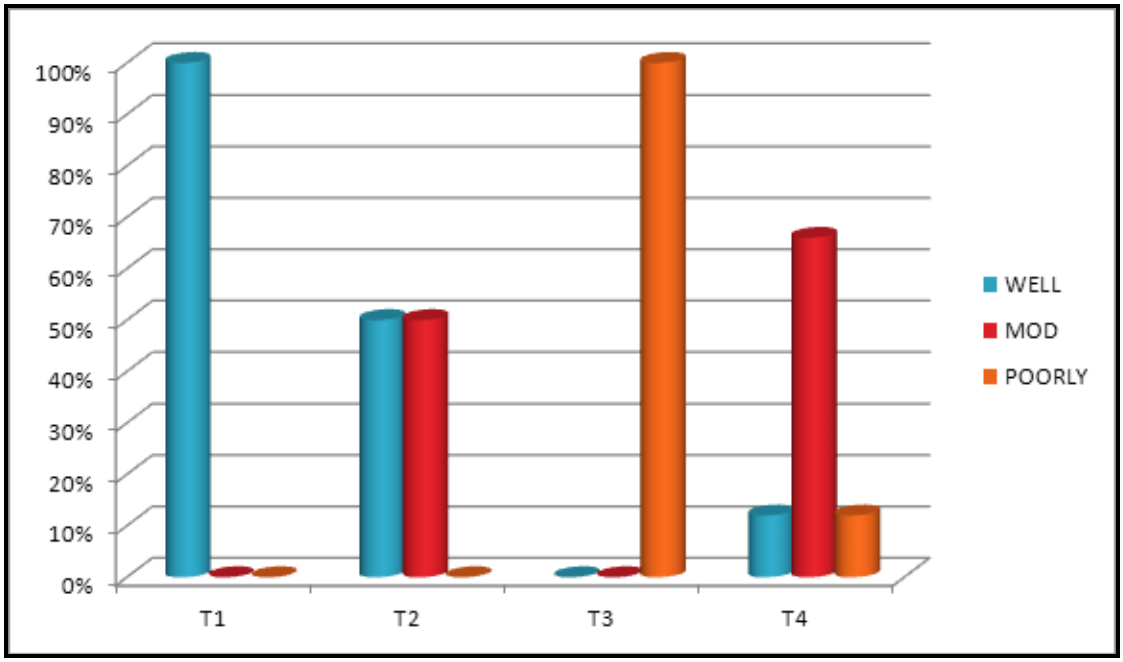
Most of the tumors ,about 26 cases fall under T4 staging with tumor invasion into serosa and periserosal pad of fat.Of which 14 cases were having the size in the range of 6-10cm,11 cases fall in 1-5cm size and 1 cases with >10 cm .And highest number of cases of all stages were in the size range of 1-5cm.There was no significant correlation between size and stage of tumor (p=0.594).

CORRELATION OF TUMOR GRADE WITH STAGING

In our study ,it was inferred that majority of T4 cases (17 cases) falls in moderately differentiated grade; 1 case of T3 stage tumor was a poorly differentiated ; All T1 cases were well differentiated and in T2 tumors 50% were well-differentiated.

Thus earlier stage cancers were mostly well differentiated in our study.There is no correlation between grade and stage of tumor (chi square = 13.994 p=0.123).

Fig 14:Chart shows the correlation between T staging and histological grading.



EXPRESSION OF KRAS MUTATION BY PCR & SEQUENCING

Molecular expression of KRAS by PCR amplification and sanger sequencing method is detected for the Codons 12 & 13,in Exon 2.

The following mutations are screened in this assay:

GLY12ALA(G12A),GLY12ASP(G12D),GLY12ARG(G12R),

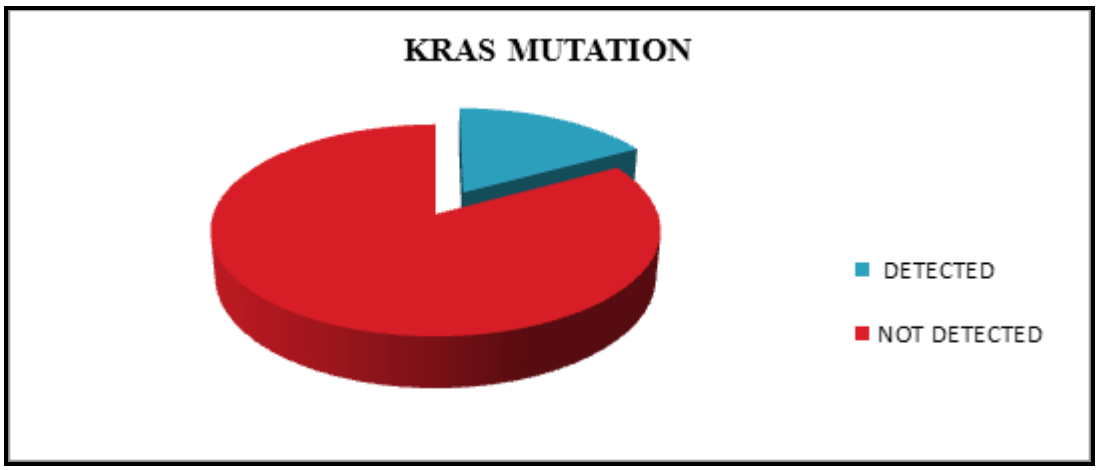
GLY12CYS(G12C),GLY12SER(G12S),GLY12VAL(G12V),

GLY13ASP(G13D)

Table 14: showing KRAS expressional status

KRAS MUTATION	Frequency	Percent (%)
Detected	5	16.7%
Not Detected	25	83.3%
Total	30	100.0%

Fig 15: Pie chart showing kras mutated and wild types



In the study,it was observed that 5 cases (16.7%) showed KRAS mutation and remaining 25 cases(83.3%) were KRAS Wild type.

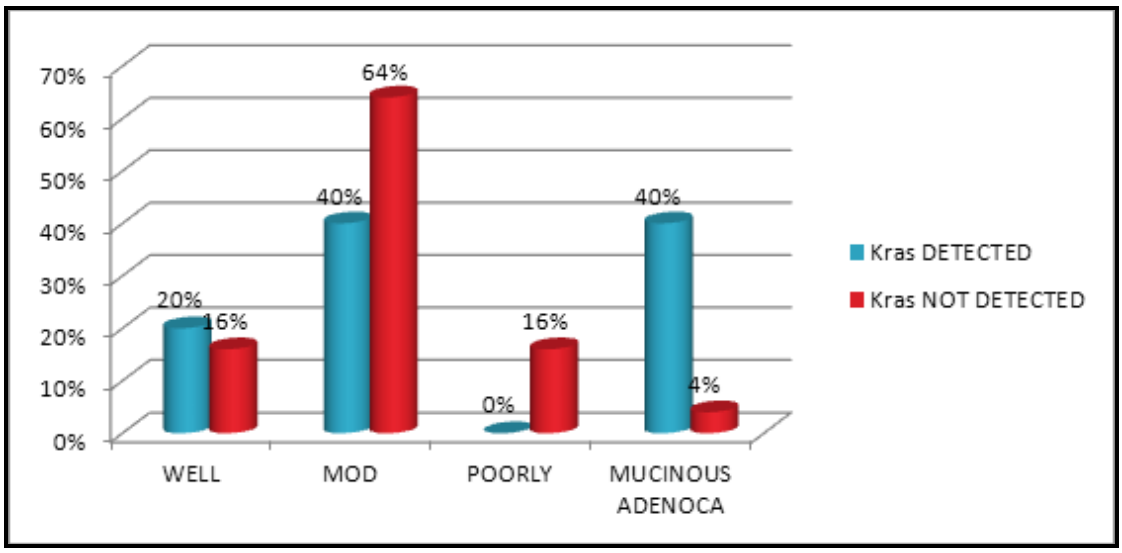
CORRELATION OF HPE DIAGNOSIS WITH KRAS EXPRESSION

Tab:15 HPE Diagnosis & KRAS Mutation Correlation

Grade	KRAS mutation		Total
	Detected	Not detected	
Mucinous Adenoca	2(40.0%)	1(4.0%)	3(10.0%)
Well Differentiated	1(20.0%)	4(16.0%)	5(16.7%)
Moderately Differentiated	2(40.0%)	16(64.0%)	18(60.0%)
Poorly Differentiated	0 (0%)	4(16.0%)	4 (33.3%)
Total	5(100.0%)	25(100.0%)	30(100.0%)

Pearson Chi-Square=6.640; P=0.084

Fig 16 :Molecular KRAS Expression status with HPE diagnosis of tumor.



As seen from the above clustered cylinder chart, 40% (2 cases) of mucinous adenocarcinoma showed KRAS mutation; 20%(1 case) of well differentiated adenocarcinoma showed KRAS positivity, 40%(2 cases) of moderately differentiated adenocarcinoma showed KRAS positivity.There was no significant statistical correlation between grading of adenocarcinoma and KRAS expression as p value >0.05 (p=0.084).

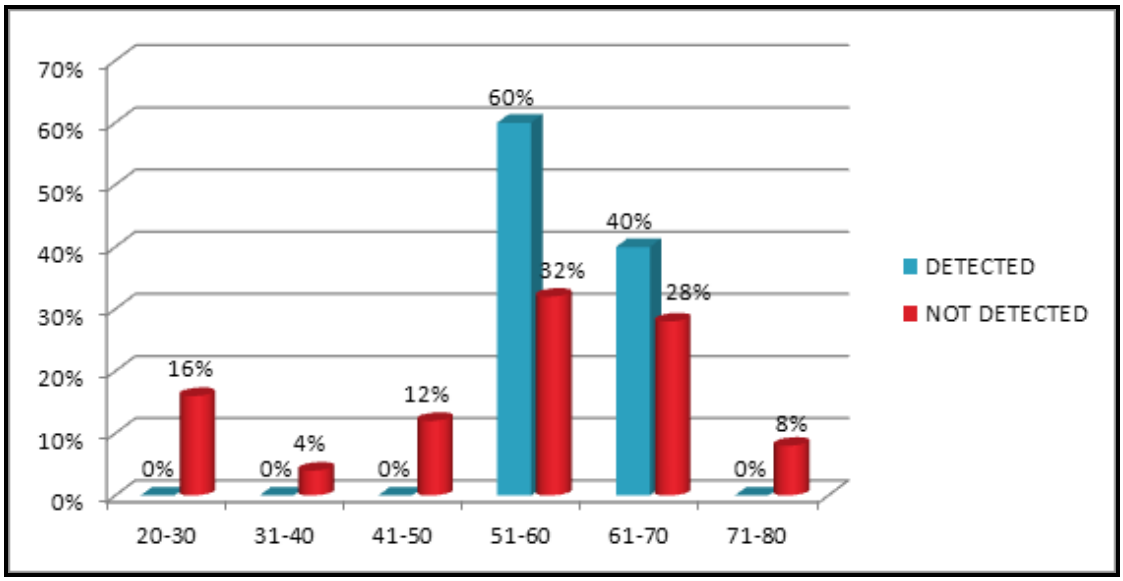
CORRELATION OF AGE WISE DISTRIBUTION OF KRAS EXPRESSION

Table 16 :shows KRAS expression status with that of age distribution

Age Group	KRAS Mutation				Total No.of cases(%)
	Detected		Not Detected		
	No.of cases	Percent%	No.of cases	Percent%	
20-30 Years	0	0%	4	16%	4(13.3%)
30-40 Years	0	0%	1	4%	1(3.3%)
41-50 Years	0	0%	3	12%	3(10%)
51-60 Years	3	60%	8	32%	11(36.7%)
61-70 Years	2	40%	7	28%	9(30%)
Above 70 Years	0	0%	2	8%	2(6.7%)
Total	5	100.00%	25	100.00%	30(100%)

Pearson Chi-Square=3.091 p= 0.686

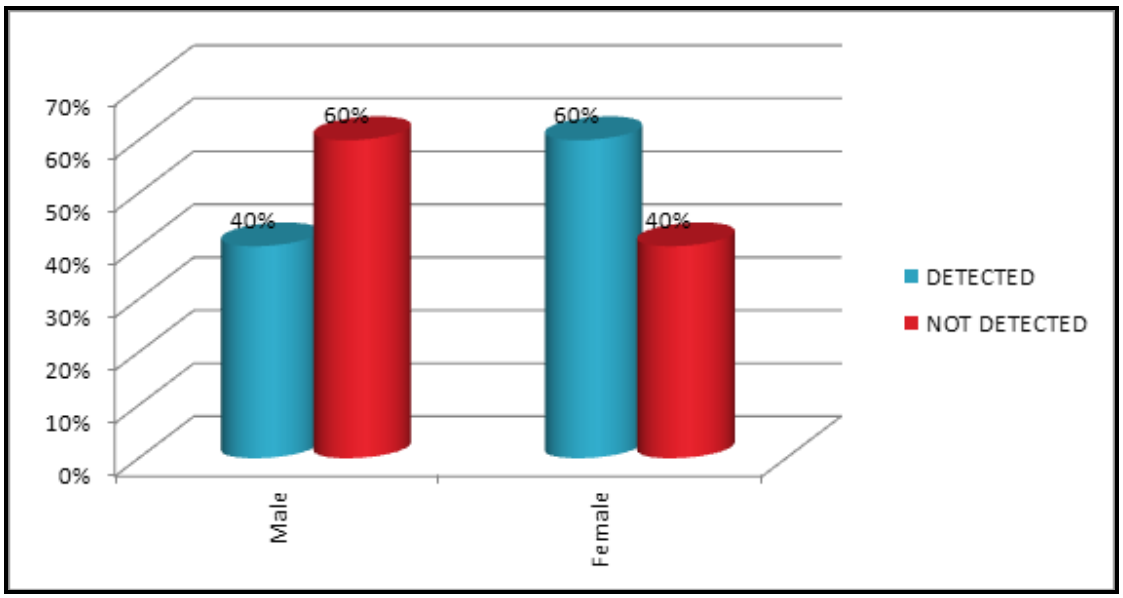
Fig 17: chart depicting correlation of kras mutational status with age



Among the 5 KRAS mutation positive cases, 3cases belonged to age group of 51-60 years and 2 cases fall in 61-70 years age group.There was no statistical correlation seen between KRAS expression and age of the patient (p=0.686).

CORRELATION OF KRAS EXPRESSION WITH GENDER

Fig 18: Chart showing the correlation between sex amd KRAS expression



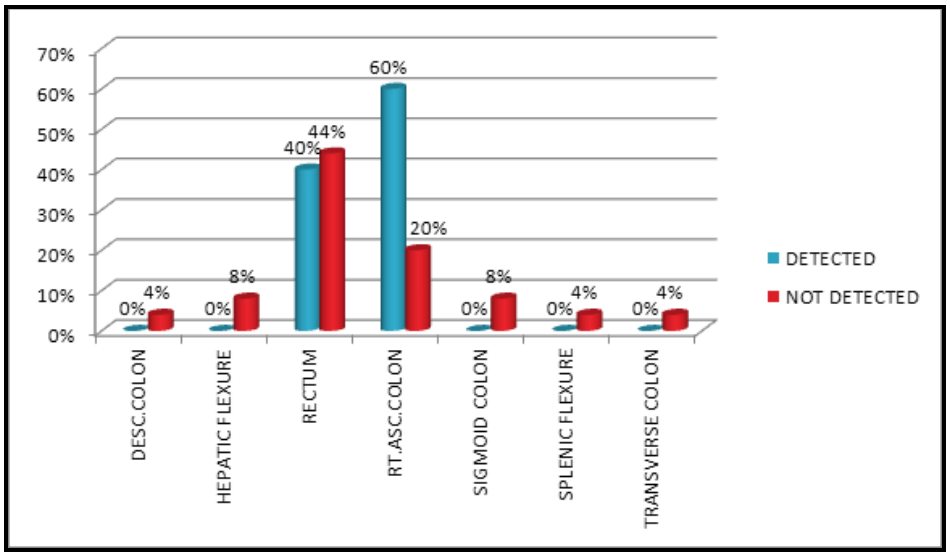
From the above chart, it was observed that among the KRAS mutated types, 60% were females and 40% were males.No statistical correlation was obtained between KRAS expression and gender.(Pearson Chi-Square=0.679 ; p= 0.410).

CORRELATION BETWEEN KRAS EXPRESSION AND TUMOR LOCATION

Table 17: Upcoming table conveys the association between KRAS expression status with the various locations of colorectal carcinoma

SITE	KRAS Mutation				Total No.of cases(%)
	Detected		Not Detected		
	No.of cases	Percent%	No.of cases	Percent%	
Asc.colon	3	60%	5	20%	8(26.7%)
Hepatic flexure	0	0%	2	8%	2(6.7%)
Transverse colon	0	0%	1	4%	1(3.3%)
Splenic flexure	0	0%	1	4%	1(3.3%)
Desc.colon	0	0%	1	4%	1(3.3%)
Sigmoid colon	0	0%	4	8%	4(6.7%)
Rectum	2	40%	11	44%	13(30%)
Total	5	100%	25	100%	30(100%)

Fig 19: chart showing expression of KRAS at various tumor locations.



In this study,it was observed that 60% of KRAS mutation detected cases were ascending colon growth(right sided colon) and 40% of them were rectal growth. No statistical correlation obtained between these two variables (Pearson Chi-Square=0.429 p= 0.807).

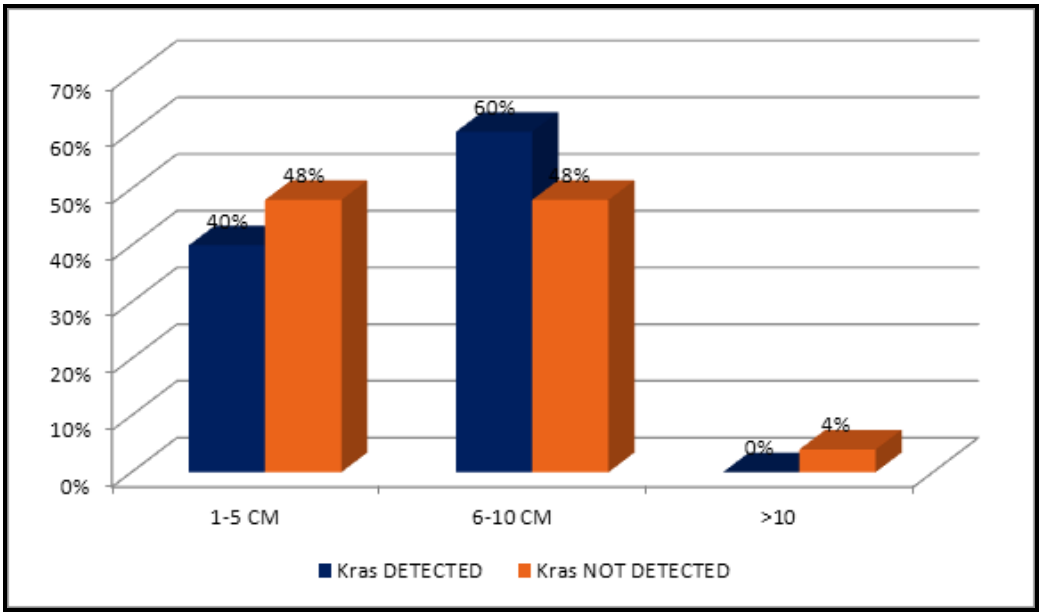
ASSOCIATION OF SIZE DISTRIBUTION WITH KRAS EXPRESSION

Table 18: Showing the distribution of size with KRAS expression

SIZE	KRAS detected		KRAS not detected	
	N	%	N	%
1-5 cm	2	40.0%	12	48.0%
6-10 cm	3	60.0%	12	48.0%
>10	0	0.0%	1	4.0%
Total	5	100.0%	25	100.0%

Chi square 0.377 ; p= 0.828

Fig 20: Chart displaying the KRAS expression in varying sizes of tumor (in Cm)



The above bar diagram represents that most of the cases fall under age group of 6-10 years,with 3 cases showing KRAS mutation and 12 cases are of KRAS wild type. Remaining 2 KRAS mutated cases come under age category of 1-5 years. Correlation between Tumor sizewith KRAS expression was found to be of no statistical significance (p=0.828).

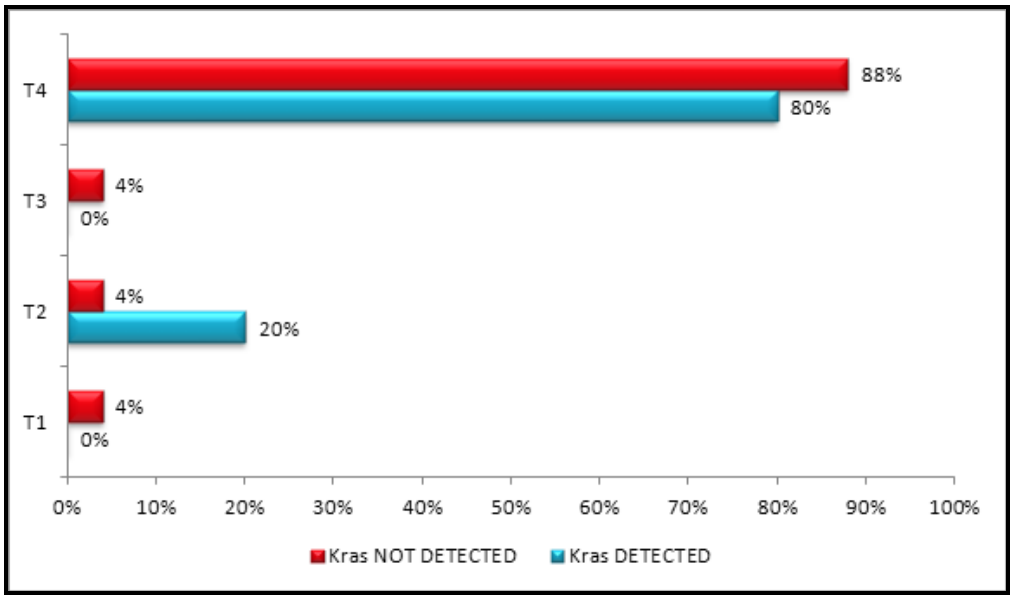
CORRELATION OF "T" STAGING WITH KRAS EXPRESSION

Table19: shows the association between KRAS expression with the depth of invasion of tumor ('T' staging)

T stage	KRAS Detected		KRAS not detected	
	N	%	N	%
T1	0	0.0%	1	4.0%
T2	1	20.0%	1	4.0%
T3	0	0.0%	1	4.0%
T4	4	80.0%	22	88.0%
Total	5	100.0%	25	100.0%

Chi square = 2.031; p= 0.730

Fig 21 :Showing Depth of invasion ('T' Staging) with KRAS expression status



Most of KRAS mutated types (80%) were T4 stage -ie.,shows invasion into serosa and periserosal fat. Other 20% of KRAS mutated type falls under T2 stage invading the muscularis propria.

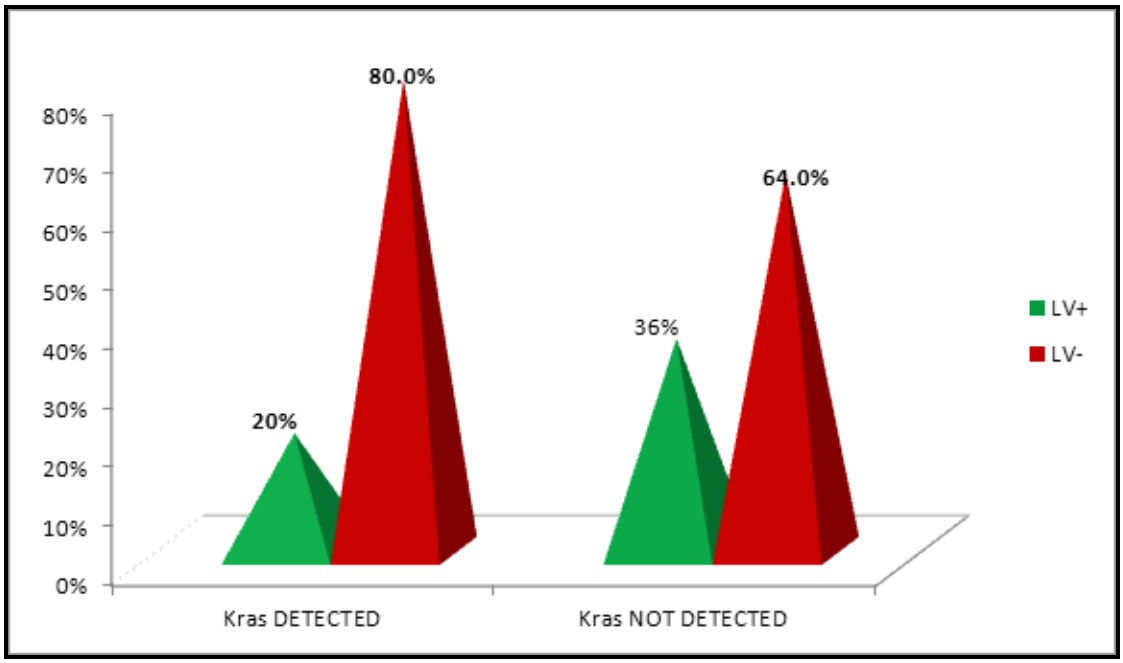
CORRELATION OF ANGIOLYMPHATIC INAVASION WITH KRAS EXPRESSION

Table 20:gives the datas about correlation between angiolymphatic invasion of tumour with KRAS molecular expression:

LV Invasion	Detected		Not detected	
	N	%	N	%
LV+	1	20.0%	9	36.0%
LV -	4	80.0%	16	64.0%
Total	5	100.0%	25	100.0%

Pearson chi square =0.480 ;p= 0.787.

Fig 22: Depiction of KRAS mutational status with lymphovascular invasion



The above pyramidal chart gives us the inference that in our study,Angiolymphatic invasion is seen in 20% of KRAS mutation detected cases and 36% of KRAS wild type showed Lymphovascular invasion.There was no significant correlation between angiolymphatic invasion of tumor with KRAS expression.(p=0.787).

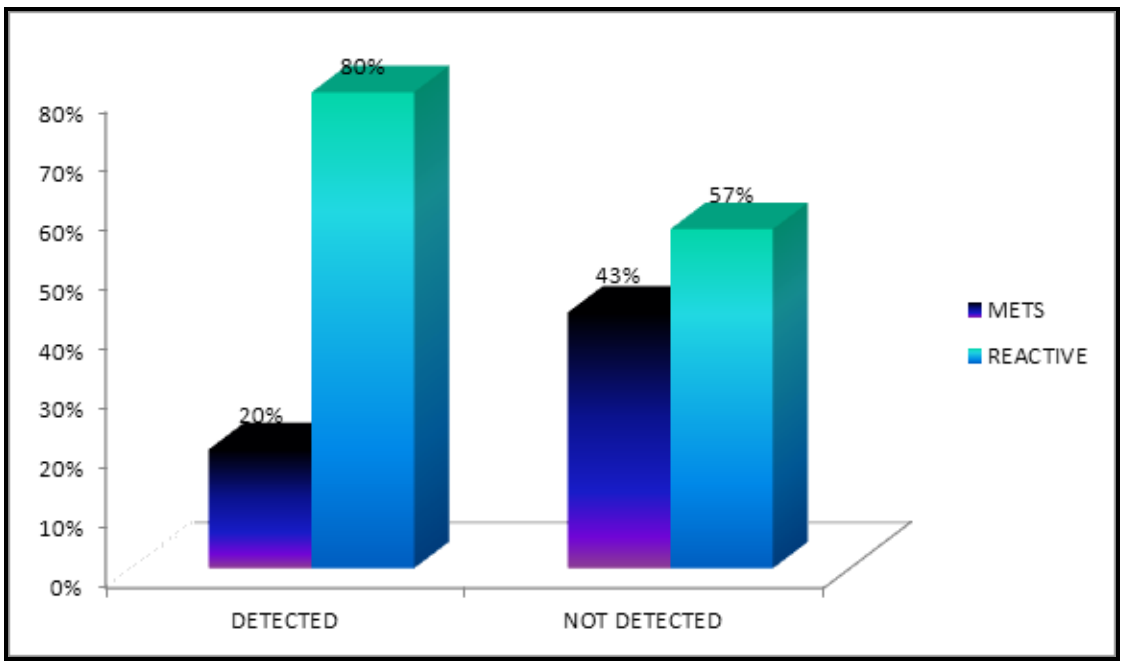
LYMPHNODE STATUS ASSOCIATION WITH KRAS EXPRESSION

Table 21 : →Conveys the lymphnodal status of tumor with KRAS expression

Lymphnode status	KRAS detected	KRAS wild type	Total
Metastasis	1 (20%)	9 (42.9%)	10 (38.5%)
Reactive	4 (80%)	12 (57.1%)	16 (61.5%)
Total	5 (100%)s	21 (100%)	26 (100%)

Pearson chi square = 0.891 P= 0.640

Fig 23 : Lymphnodal status with KRAS expression in tumor tissue



In our study, among the KRAS Mutated cases only one case showed lymphnode metastasis and other 4 showed features of reactive hyperplasia .Majority of cases (21 cases) fall under KRAS wild type. No significant correlation was seen between these two variables,as p=0.640.

Colour Plates

GROSS IMAGES

Fig 3: Mucinous carcinoma of

HISTOPATHOLOGY IMAGES

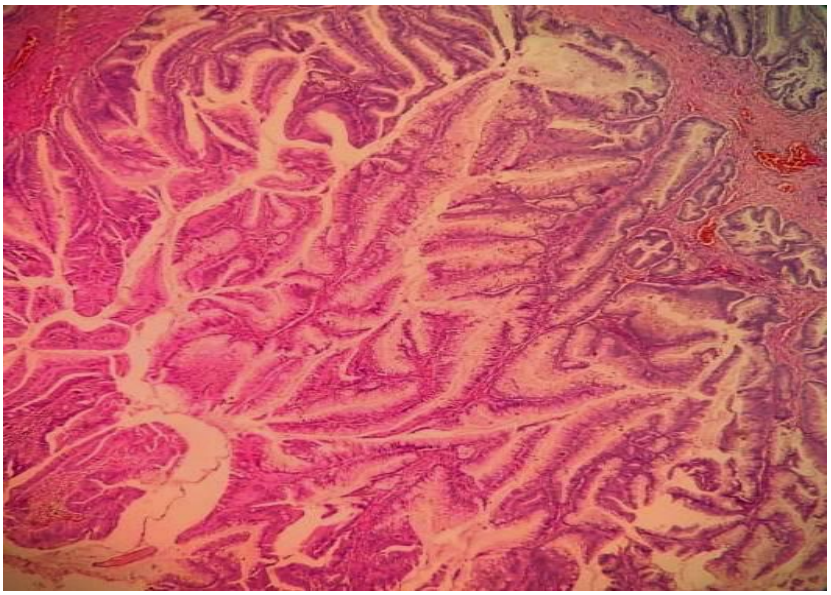


Fig 4: Infiltrating adenocarcinoma-well Differentiated type(100x)

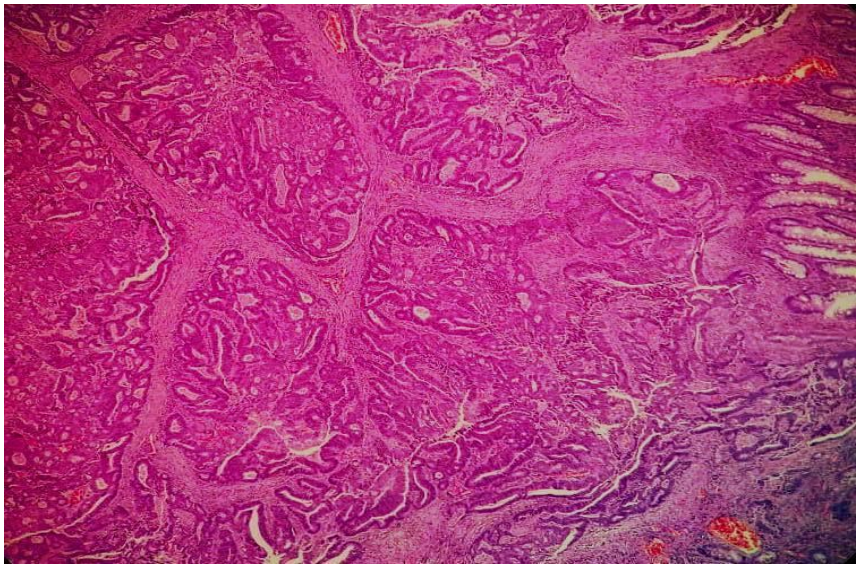


Fig 5: Infiltrating adenocarcinoma-Moderately differentiated type(100x)

Fig 8: Muscle invasion by tumor cells

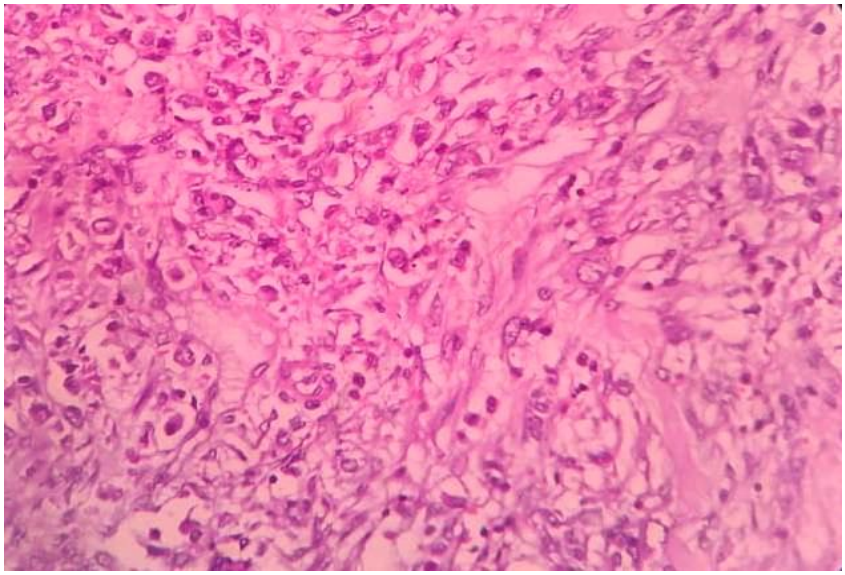


Fig 6:Poorly differentiated Adenocarcinoma(400x)

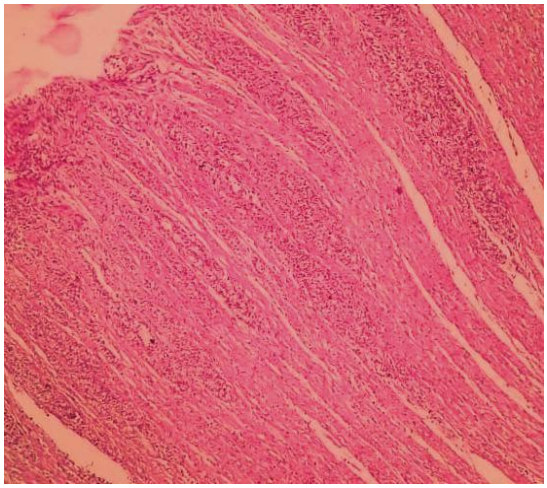


Fig 7: Adenocarcinoma-showin
muscularis propria invasion (10(

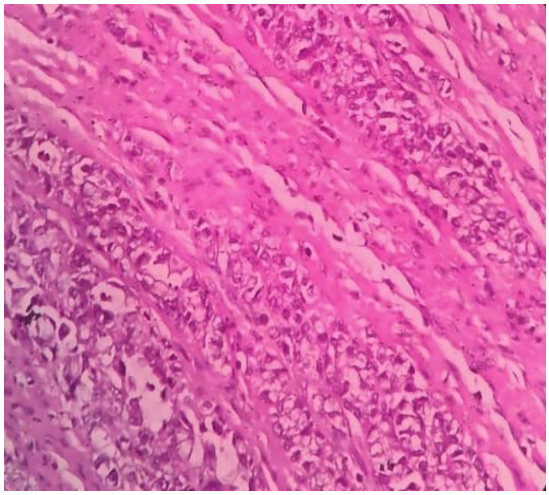


Fig 8: Muscle invasion by
tumor cells (400x)

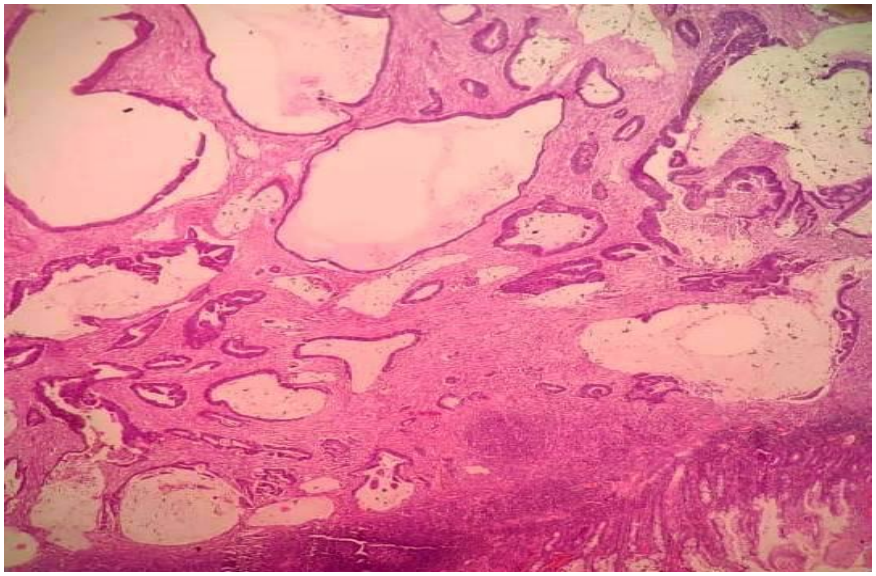


Fig 9: Mucinous adenocarcinoma of colon

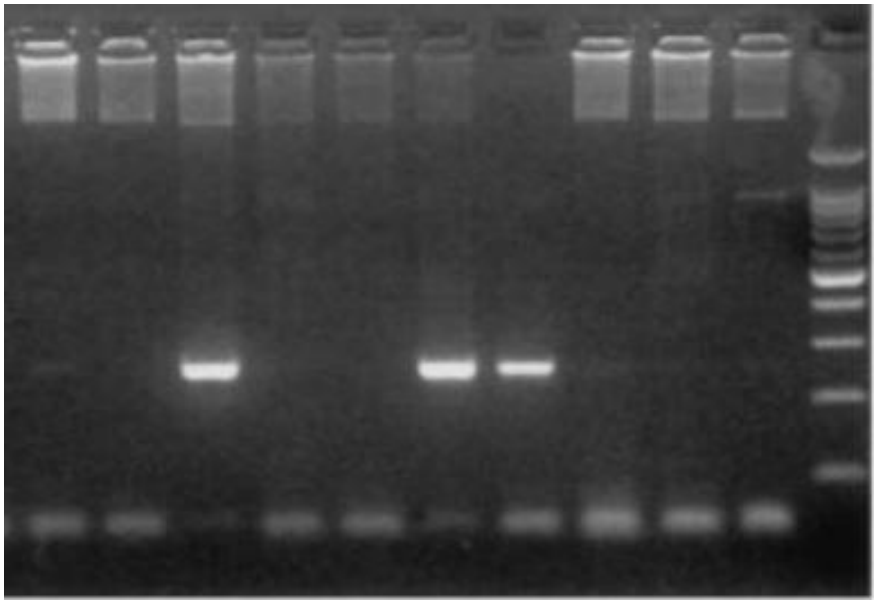


Fig 10: Gel electrophoresis showing amplified DNA –PCR product

- LANE 3,6,7 –AMPLICON OF PCR PRODUCT at 247bp
- LANR 11--100bp LADDER

Positive (B_X.No: 7174)-KRAS MUTATED:

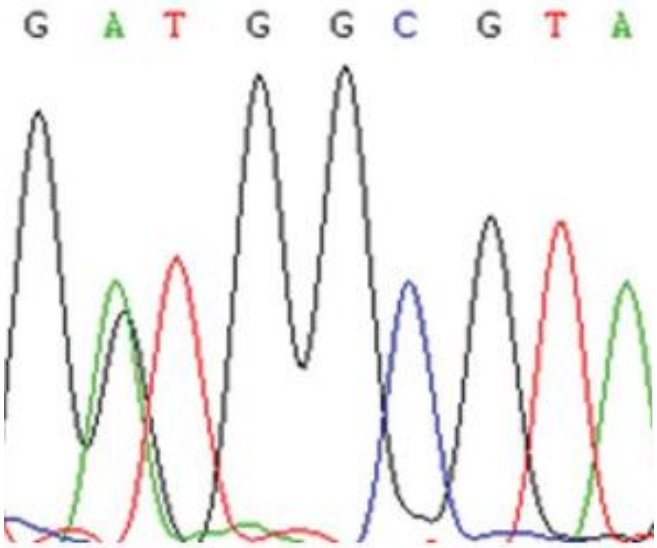


Fig 11: Positive KRAS Mutation in

Negative-KRAS WILD TYPE:

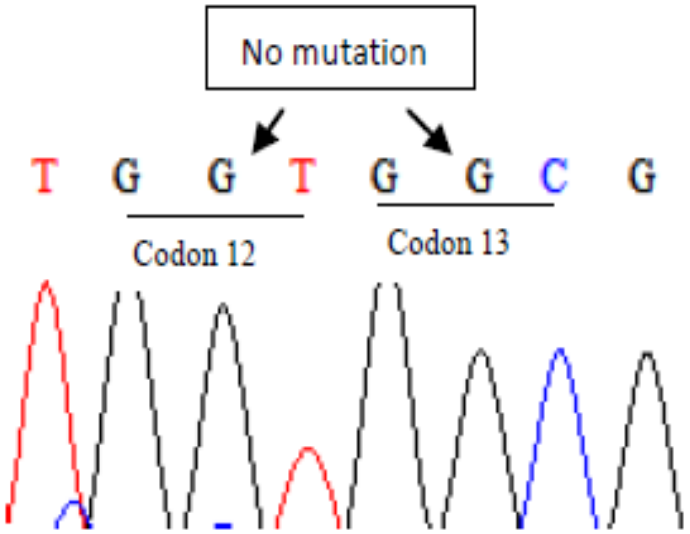


Fig 12: Negative-KRAS wild type

Discussion

DISCUSSION

Colorectal carcinoma has now become one of the commonest cancer worldwide.As by various literatures,like **Guraya et al(2006).**,[65] there is an evidence of a paradigm shift in its incidence among younger people < 50 years,as compared to old age due to various riskfactors ,to ascertain ,dietary factors with high animal protein and non modifiable familial risk factors owing to genetic alterations.Hence there is necessity for early diagnosis with the aid of evolving technologies and molecular assays .

As Rajiv Gandhi Government General hospital is a tertiary referral centre,we receive a wide range of lower gastrointestinal malignancies which was around 268 cases during our study period of 2 years,of which 250 were proven to be colorectal adenocarcinomas.

In view of due significance of molecular assays in both prognostication and theranostication ,present study is done to analyse the KRAS expression in colorectal carcinomas and 30 FFPE blocks were studied for KRAS expression which was correlated with its clinicopathological variables.

AGE WISE INCIDENCE OF COLORECTAL CARCINOMAS

The mean age of presentation in this study was 55.2 years ±13.95. This is in concordance with various studies of **Veldore VH et al**,**2014**[66] , **Nekalson et al,2008** [67] and **Devadass clement et al,2016** [68]

Table 1: Comparison of Mean age with various studies

Studies	Mean age of presentation
Veldore VH et al,2014	55.9 ± 12.8
Nekalson et al,2008	58.2 ± 12.5
Devadass clement et al,2016	52.3 ± 13.2
Current Study	**55.2 ± 13.9**

In most of the age groups,moderately differentiated carcinoma(Grade 2) was the commonest Grade.A study by **T.Patra et al.2018** [69] also showed similar finding.

GENDER WISE COMPARISON

The incidence of colorectal carcinoma in our study was more in males (56.7%) compared to females(43.3%) and male:female ratio was 1.3:1 .This was in concordance with other studies by **Quddus et al. 2012** [70] ; **peedikayil et al .2009** [87]

Table-2: Comparison showing Male: Female ratio

Studies	Male:Female ratio
Quddus et al,2012	1.5:1
Peedikayil et al,2009	2.1:1
Current Study	**1.3:1**

These studies shows that females were affected at lower rates and 5 year survival rate is also better than males, possibly attributed to the oestrogen hormones.The risk of post-menopausal women becomes the same as that of men at that age group.

HISTOLOGICAL SUBTYPES OF CRC

In the present study,adenocarcinoma-usual type comprised 90% and 10% by mucinous type. This is in par with a study by **Fatemeh Hajmanoochehri et al**.2014 [(71)] and **Manmeet kaur Gill et al**.2011[(73)] where majority of cases included conventional type of adenocarcinoma.

Table:3 showing frequencies of adenocarcinomas in various studies

Studies	Conventional adenocarcinoma %
Fatemeh Hajmanoochehri et al	87.5%
Manmeet Kaur Gill et al	77.5%
Current Study	**90%**

LOCATION OF CRC

According to many literatures,its seen that part of colon distal to Splenic flexure were considered to be left sided colon and those proximal to splenic flexure were taken as right sided colon. Rectum was the highest site of occurance of carcinoma with11 cases(37%) in present study; This in in concurrence with other studies by **T.patra et al**.**2018** [(69)] , where 46.2% of carcinomas occurred in rectum and **Fatemeh Hajmanoochehri et al.2014** [(71)] **,** where 55% were rectal carcinoma.

Table 4- shows the commonest tumor location incomparison with other studies

Studies	Jung MK et al. [72]	T.Patra et al.[69]	Current Study
Commonest site(%)	Left colon (66.2%)	Left colon (63.1%)	Left colon(60%)

The right and left sided colon carcinomas differ in presentation of symptoms and aggressiveness .Right sided colonic carcinomas present with bleeding,anemia and is more aggressive than left sided colonic cancer.Left side colonic cancer presents usually as palable obstructive mass.

COMPARISON OF HISTOLOGICAL GRADES OF CRC

In current study,most of the cases were of to moderately differentiated grade (66.7%), and this is in concordance with a study by **Uzma nabi et al**.2010 [74] , however were discordant as compared with studies of **Manmeet Kaur Gill etal**.2011[73] and Veldore et al. [66]

Table -5, shows % of various histological grades in different studies

Grades	Well diff (Gr-I)	Mod.diff (Gr-II)	Poorly diff (Gr-III)
Uzma nabi et al	15%	62%	23%
Manmeet Kaur Gill et al	51.6%	41.94%	6.4%
Veldore et al	80%	13%	17%
Current Study	**18.5%**	**66.7%**	**14.8%**

Grossly Ulceroproliferative lesions were commonest (53.3%) in the present study and Histologically, glandular pattern were predominant in tumors with 60% .Angiolymphatic invasion was observed in 10cases and 1 case showed perineural invasion

T staging observation in comparison with other studies -Table 6 depicts it.

Stage T	Carvalho et al.2017[75]	Balta AZ et al.2014 [76]	**Current Study**
T1	2%	3%	**3.3%**
T2	10%	16.6%	**6.7%**
T3	66%	69.2%	**3.3%**
T4	22%	10.5%	**86.7%**

In the current study, majority of cases (86.7%) fall in T4 stage,with invasion into serosa and periserosal fat. This was in discordance with other studies by Carvalho et al. and Balta AZ et al., whereby T3 stage was present in majority of cases ,as observed from the table above.

It is also observed from the present study that larger the size of the tumor ,there is a higher tendency of tumors to fall in Stage T4 as compared to lesser size. In Size distribution of 1-5 cm,out of 15 cases → 11 cases(73.3%) were T4 stage ,Under 6-10 cm range → all 14 cases(100%) were T4 stage, in >10cm size,only one case presented which also falls in T4 stage.

From the study proposed by Balta AZ et al. [76], it was found that progression of tumor stage was accompanied by the increase in tumor size.

MOLECULAR EXPRESSION OF KRAS BY PCR AND SANGER SEQUENCING METHOD

Initially there were some difficulties in DNA extraction and isolation from the FFPE blocks selected for PCR assay .Around 5 blocks from which DNA extraction done proved to be in vain owing to poor yield of DNA quantity and hence these samples were rejected.After optimization of procedures, 30 other FFPE blocks were subjected to DNA isolation and PCR processing .Most likely attribute to poor DNA yield and failure in seqsequent steps could be the DNA fragmentation. [77,78]

It is postulated in the study by **Domagala P et al.2012** [79] that DNA fragmentation is caused by formation of DNA –protein cross links and deactivation of nucleases over time in formalin solution.

In Present study, 5 out of 30 cases (16.7%) showed KRAS mutation and other 25 cases (83.3%) were of KRAS wild type. Among the 5 mutated KRAS cases all of them showed mutational changes in Codon 12 .

Present study revealed no significant correlation between KRAS mutations and clinicopathological variables like age ,gender,site and size of tumor.Also T staging and grading of tumor,angiolymphatic

invasion ,lymphnodal status exhibited no signification correlation with KRAS mutation ($p>0.05$).

COMPARISON OF KRAS MUTATED FREQUENCIES

In a study of KRAS mutation in colorectal cancer by **Niraj Kumari et al**.**2013**[80] made on Indian population, 18.5% were KRAS mutated types and it also showed that Codon 12 had higher frequencies of mutations(64.7%) than codon 13(35.3%). It was in concordance with the present study frequencies too.

From **Patil H et al .2013** [81] study in Indian cohort's KRAS mutation analysis ,it was observed that KRAS mutations was found in 20.5% .There was significant association ($p<0.05$) between KRAS mutations ,age and tumor differentiation; Whereas no significant association was observed between KRAS mutations and gender ($p>0.05\%$)

Sameer et al. [82] study was done to identify KRAS gene mutations in colorectal cancer patients among kashmiri population.Tissue samples were collected from series of 53 patients undergoing respective surgery for CRC.Results showed that 22.64% of population presented with KRAS mutations with 61.5% occurring in codon 12 and 38.5% in codon 13. KRAS mutations in both codon 12 and 13 were common in mucinous type of carcinoma (38.1%) compared to non mucinous type (15.2%). This was not in concordance with current

study where KRAS mutation in mucinous carcinoma type was 40% and remaining 60% by non mucinous adenocarcinomas.

AGE DISTRIBUTIONAL CORRELATION WITH KRAS MUTATIONAL STATUS

In the current study,among 5 KRAS mutation positive cases,3 cases fall in 51-60 years and 2 cases under 61-70 years, with all cases > 50 years of age.

Thus, it was observed that older the age, higher is the frequency of KRAS mutation compared to younger age group from our study; Though there was no significant correlation found between these two variables.

As per **Liu et al**. [84] study,Mean age of presentation of mutated KRAS was 60.5± 11.7 years and there was no significant correlation between age and KRAS mutational status in their study.

CORRELATING THE KRAS MUTATIONAL STATUS WITH GENDER: (AS SHOWN IN TABLE-7)

Studies	Male (KRAS mutated%)	Female (KRAS mutated %)
Phipps AI et al [83]	45%	55%
Zocche et al [55]	44.7%	55.3%
Niraj Kumari et al [80]	70.6%	29.4%
Current Study	**40%**	**60%**

Thus in observation with other studies by Phipps AI et al,Zocche et al .,our study showing higher incidence of KRAS mutation among female gender appeared to in concordance;Yet few studies like niraj kumari et al showed male predominance (70.6%) of KRAS mutation.

CORRELATION OF KRAS MUTATION EXPRESSION WITH LOCATION OF TUMOR

The current study revealed that 60% of KRAS mutation detected were ascending colonic growth and 40% of them were rectal growth.There was no statistical correlation between site of tumor and KRAS mutation (p=0.807) . As there were thoughts from few literatures that right sided colonic growth are aggressive than left sided colorectal growth, Our study with being KRAS mutation higher on right side prompts us further insights of correlation between tumor aggressiveness with KRAS mutational status.

Also a study by **Loree JM et al**.[(85)]with 1,876 patients of colorectal cancer compared mutation analysis according to location.They stressed the significance of precise tumor location rather than classifying as right and left sided colonic carcinomas.Mutations prevalence differed by sides and locations for KRAS,TP_{53}, BRAF,PTEN ,PIK4CA,SMAD4 within the right and left sided tumors.

In a study by**Liu et al.2011** [(84)] with 217 cases of CRC with KRAS mutation testing, the distribution of KRAS mutated cases were

observed as follows: 37% were right sided colon , 33% were left sided colon, 13% by rectal tumors and other sites of colon (like hepatic/ splenic flexures) included 25%. There was no statistical significance between tumor site and KRAS mutation in this study which was alike our study.

Table-8 -shows KRAS exprseeion in association with histological tumor gradings

KRAS Mutated in	Li J et al [86]	Al-Allawi et al [56]	Current Study
Well diff (Gr-I)	22.54%	25%	**33.33%**
Moderately Diff (Gr-II)	52.62%	62.5%	**66.67%**
Poorly diff (Gr-III)	24.84%	12.5%	**0%**

CORRELATING THE EXPRESSION KRAS MUTATION WITH HISTOLOGICAL GRADING

Current study showed that most of KRAS mutated cases were of moderately differentiated grade(66.67%) followed by well differentiated type (33.33%).This is in par with studies by Li J et al. 2014[84] and Al-allawi et al .[56] However there is no significant correlation seen between histological grade of tumor and KRAS mutational status.

CORRELATING THE EXPRESSION OF KRAS WITH 'T' STAGING

In current study KRAS mutation were seen in 80% of T4 cases, whereby depth of invasion of tumor is into serosa and periserosal

fat.Remaining 20% of T2 tumors were mutated. KRAS mutation shows predilection for tumors of T4 stage as per our study.

Kim HS et al.2016 [87] study picturised that among the KRAS mutant types,most of them were of T3 staging as against our study results.However,There is no statistical significance between 'T' staging and KRAS mutational status.

In **Liu et al** [84] study ,T3 staged tumors were most commonly associated with KRAS mutated cases .Both T3 and T4 stage tumors(T3 + T4=60%) frequently show KRAS mutation as compared to T1(20%) and T2(0%) tumors.P value was 0.495 ,hence no statistical significance was obtained between these two.

CORRELATING EXPRESSION OF KRAS WITH ANGIOLYMPHATIC INVASION AND LYMPH NODAL STATUS

It was observed that among the tumors which showed lymphovascular invasion,most of them were KRAS Wild type (90%) and p value was not statistically significant(p=0.787). Also most of cases with Nodal metastasis (9 out of 10 cases) were KRAS wild type .

Table-9: showing correlationof KRAS expression with angiolymphatic invasion

Studies	LV Invasion +		Nodal Metastasis +	
	KRAS +	**KRAS (wild)**	**KRAS +**	**KRAS (wild)**
Al-Allawi et al [56]	54.2%	38.5%	50%	50%
Niraj kumari et al [80]	11.8%	18.6%	35.3%	52%
Liu et al [84]	27%	73%	32%	68%
Current Study	**10%**	**90%**	**20%**	**80%**

From above observations from Al-Allawi et al, Niraj kumara et al and Liu et al,it was inferred that both angiolymphatic invasion and nodal metastasis were less in mutated KRAS in comparison to wild-type KRAS .These were in concordance with our study results. This is of theranostically significant as most of nodal metastatic patients fall into KRAS wild type,hence they can be Sufficed with anti-EGFR therapy if needed at later stages.

Summary

SUMMARY

This two years study on “Molecular expression of KRAS mutation in colorectal carcinoma and its correlation with clinicopathological findings” was done in Madras medical college and Rajiv Gandhi Government General hospital ,with sample size of 30 Formalin Fixed Paraffin Embedded (FFPE) blocks of biopsy proven colorectal carcinoma cases.

- Out of these 30 cases,27 were of Conventional adenocarcinoma and 3 were mucinous carcinoma.
- The mean age of presentation was 55.2 years and youngest age of presentation was 24 years.
- Males constituted 56.7% and females accounted for 43.3% of cases ,with
- Male : Female ratio of 1.3:1 .
- The Commonest site of tumour occurence was rectum followed by ascending (Right sided) colon.
- Majority of tumours showed ulceroprolifrative growth grossly and histologically glandular pattern was the commonest.
- Half of the tumors were < 6cm and another 50% of tumors were >6cm

- Histologically most of the tumours (66.7%) were of moderately differentiated grade (Grade-II)

- Individuals >50 years show predominantly moderately differentiated tumours and in <50 years both moderately and poorly differentiated grades seen.

- Most of the cases (86.7%) belonged to “T4”stage with invasion to serosa and periserosal pad of fat.

- KRAS mutation detection by PCR and sanger sequencing yielded 5 KRAS mutation positive cases(16.7%) and remaining 25 were KRAS Wild type.

- There were also difficulties in standardization of DNA isolation and PCR cycling procedures as 5 of the samples initially showed poor DNA yield probably due to DNA fragmentation attributed to formalin over-soakage.

- Among the 5 KRAS mutated cases, 2 of them were mucinous carcinoma, another 2 were moderately differentiated adenocarcinomas and one case was well differentiated type.

- There was no significant correlation between expression of KRAS and histological grading of tumours.

- All 5 KRAS mutated cases were of age group >50 years. There is no statistical significance between KRAS expression and age of patient.

- Majority of KRAS mutated cases resided in right sided(ascending colon) ,followed by rectum and no correlation was found between location of tumor and KRAS expression

- Most KRAS mutated types were 'T4 stage' . No significant correlation seen between T stage of tumor and KRAS expression

- Tumours showing angiolymphatic invasion and nodal metastasis were less common in KRAS mutated type compared to KRAS wild type.

- Thus the expression of KRAS gene showed no significant correlation with any of the clinicopathological parameters .

- The absence of significant correlation of KRAS status with different variables are shared by most other literatures.

However,though there is no signification correlation of KRAS expression as such, some of our results were in concordance with other studies .Variables like older age of presentation, histologically tumours of moderately differentiated grade being the commonest , KRAS wild type cases exhibiting angiolymphatic and nodal metastasis higher than mutated types were all in par with most of the literatures.

Limitations of the Study

LIMITATIONS OF THIS STUDY

- Present study has relatively small sample size (n=30 cases). Hence, Larger clinical trials are required to confirm these findings.

- Also the present study included only Codon 12 and Codon 13 of Exon 2 ,which is the commonest of KRAS mutations in colorectal carcinoma. However,analysis of these mutations alone are not sufficient to decide on Anti-EGFR therapy ,with several other mutational spots being present.

Conclusion

CONCLUSION

Colorectal carcinoma ,though at one pole poses an impending threat of increased estimated rates of incidences in future, boon of advanced molecular technologies and regular screening methodologies at other pole prevents the human era falling into the trap of demise. This study evaluated the expression of KRAS gene in a hospital based setup, hence may not reflect its exact incidence in the community level. KRAS ,one of the significant genes involved in the multistep tumorigenesis of colorectal carcinoma ,plays an inevitable role in imparting aggressiveness to tumor flourishment and reduced apoptosis, thereby associated with poor survival .Mutations can occur in plenty number of exons and codons within it, hence many hidden novel mutations could throw a light to various targeted therapies,although codon 12 and 13 of exon 2 are the commonest.

The utmost implication of knowing KRAS mutational status in colorectal carcinoma patients has been recently augmented by the fact that it is one of the important predictors of resistance to targeted therapy by Epidermal Growth Factor Receptor tyrosine kinase inhibitors like cetuximab and penitusumab (Anti-EGFR therapy).

Thus these mutational studies aids the oncologists to decide on mode of treatment and assess the prognosis.Current study attempted to study the KRAS mutational status and to favour us, many of the patients were of KRAS wild type , paving a way for targeted therapy in case if at all needed.

Bibliography

BIBLIOGRAPHY

1) Fleming M, avula S,Tatishchev SF,Wang HL.Colorectal carcinoma: Pathological aspects.J Gastrointest Oncol 2012;3(3):153-173.

2) Sharma D,Singh G.Clinico-pathological profile of colorectal cancer in first two decades of life:A retrospective analysis from tertiary health center.Indian J Cancer.2017 Apr-Jun;54(2):397-400.

3) Ferlay J,Seorjomataram I,Dikshit R,Eser S,Mathers C,Rebelo M et al.Cancer incidence and mortality worldwide:sources,methods and major patterns in GLOBOCAN 2012.Int J Cancer.2015 Mar 1;136(5):E359-86

4) Meyer B,Are C.Current status and Future Directions in Colorectal Cancer.Indian J Surg Oncol.2017 Dec;8(4):455-456.

5) Kumar V,Abbas AK,Aster JC.Robbins and Cotran Pathologic Basis of Disease,9e.South asia edition.Vol II.Chapter 17: 811-814

6) Drake,Richard L.Vogl,Wyne.Mitchell,Adam W.M. Gray's anatomy for students,2nd edition.Chapter 4:307-313

7) Schlussel AT,Gagliano RA,Setto-Donlon S,et al.The evolution of colorectal cancer genetics-Part1;from discovery to practice.Journal of Gastrointestinal Oncology.2014;5(5);326-335.

8) Dukes C.The hereditary factor in polyposis ontestine ,or multiple adenomata.Cancer review.1930;5:241-255

9) Bray F,Ferlay J,Soerjomataram I,SiegelRL,Torre LA,Jemal A.Global cancer statistics 2018:GLOBOCON estimates of incidence and mortality worldwide for 36 cancers in 185 countries.CA Cancer J Clin.2018 sep12.

10) Mohandas KM,Desai DC.Epidemiology of digestive tract cancers in India.V.large and small bowel.Indian J gastroenterol.1999;Jul-sep 18(3):118-21.

11) Amersi F,Agustin M,Ko CY.Colorectal Cancer:Epidemiology ,Risk Factors and Health Services.*Clinics in Colon and Rectal Surgery*.2015;18(3):133-140

12) Paraf,F.&Jothy,S.Colorectal cancer before the age of 40.Dis Colon Rectum (2000)43:1222

13) Silva Roberta Vasconcelos e et al.Hereditary nonpolyposis colorectal cancer identification and surveillance of high-risk families.clinics.2005.60(3),251-256.

14) Wilmink ABM.Overview of the epidemiology of colorectal cancer.Dis Colon Rectum.1997;40(4):483-493

15) Bethesda, MD . National Institutes of Health What you Need to know about Cancer of the Colon and Rectum. U.S.Department of Health and Human services and National Institutes of Health; 2006.

16) Hill MJ.Bacteria and etiology of colonic cancer.Cancer :1974 sep;34(53):suppl: 815-18

17) Tsong WH,Koh WP,Yuan JM,Wang R et al.Cigarattes and alcohol in relation tocolorectal cancer:the Singapore Chinese health study.British J .Cancer.2007;96(5): 821-827

18) Sasaki H,Ishihara S,Hata K,et al.Radiation –asociated colon cancer:A case report.Molecular and clinical Oncology.2017;6(6):817-820.

19) Dyson JK,Rutter MD.Colorectal cancer in inflammatory bowel disease:What is the real magnitude of the risk?World Journal of Gastroenterology:WJG.2012;18(29): 3839-3848.

20) Leslie A,Carey FA,Pratt NR and Steele RJC.The colorectal adenoma-carcinoma sequence;British Journal of Surgery.2002;89:845-860

21) Otori K,Oda Y,sugiyama K et al.High frequency of K-RAS mutations in human olorectal hyperplastic polyps. GUT .1997;40:660-663

22) Ronucci L,Pedroni M,Vaccina F et al.Aberrant crypt foci in colorectal carcinogenesis.cell and Crypt dynamics,Cell proliferation .Vol 33;issue 1

23) Vogelstein B,Fearon ER,Hamilton SR,et al.Genetic alterations during colorectal tumor development .N Engl J Med.1988; 319:525-32.

24) Bishehsari F,Maahdavinia M,Malekzadeh R, Verginelli F,Catalano T,Sotoadeh M et al. K-ras mutation in colorectal carcinomas from Iran and Italy:influence of microsatellite status and country of origin.Ann Oncol.2006;17:vii91-vii96

25) Catalogue of somatic mutations in cancers.Available from : www.Sanger.ac.uk/genetics/CGP/cosmic

26) Brink M,de Goeij AF,Weijenberg MP,Roemen GM,Lenties MH,Pachen MM et al. K-ras oncogene mutations in sporadic colorectal cancer in the Netherlands cohort study.carcinogenesis.2003 Apr;24(4):703-10

27) Jancik S,Drabek J,Radzioch D and Hajduch M .Clinical relevance of KAS in Human Cancers.Journal of Biomedicine and Biotechnology,Vol.2010:ID 150960

28) Karapetis CS,Khambata Ford S,Jonker DJ,O'Calighan CJ,Tu D,Tebbutt NC et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer.N Engl J.Med.2008;359:1757-65

29) Rodrigues NR,Rowan A,Sumith ME,Kerr IB,Bodmer WF et al.P_{53} mutations in colorectal cancer.Proc Natl Acad.Sci USA.1990 Oct;87(19):7555-9.

30) Barras.BRAF mutation in colorectal cancer:An update. Biomarkers in Cancer.2015:7(51)9-12

31) Dietmaier W,wallinger S,Bocker T,Kullmann T,Fishel R,Ruschoff J.Diagnostic microsatellite instability:definition and correlation with mismatch repair protein expression.Cancer Res 1997;57:4749-56

32) Toyota M,Ahuja N,Ohe-Toyota M,Herman JG Baylin SB,Issa JP.CpG island methylator phenotype in colorectal cancer.Proc Natl Acad.Sci USA.1999;96;8681-6

33) Grady WM,Markowitz SD.The Molecular pathogenesis of colorectal cancer and its potential application to colorectal cancer screening;Dig Dis Sci.2015 March;60(3); 762-772.

34) Shousha S.Paneth cell –rich papillary adenocarcinoma and a mucoid adenocarcinoma occurring synchronously in colon.A light and electron microscopic study.histopathology 1979,3:489-501

35) Connelly JH,Robey-cafferty SS, Cleary KR.Mucinous carcinoma of the colon and rectum.An analysis of 62 stage B and C lesion.Arch Pathol Lab Med.1991,115: 1022-1025

36) Balthazar EJ,Rosenberg HD,Davidian MM.Primary and metastatic scirrhous carcinoma of the rectum.Am J Roentgenol 1979,132:711-715

37) M akinen MJ.Colorectal serrated adenocarcinoma.histopathology 2007,50:131-150

38) Thirunavukarasu p.et al .Medullary carcinoma of the large intestine:a population based Analysis.Int J Oncol.2010 oct;37(4):901-7.

39) Lanza G,Gafa R,Matteuzzi M,Santini A.Medullary type poorly differentiated adenocarcinoma of the large bowel :a distinct clinicopathologic entity characterized by microsatellite instability and improved survival.J clin Oncol 1999,17:2429-2438

40) Sternberg's Diagnostic surgical pathology ,5th edition,Lippincott Williams &wilkins,2010.Chapter 34:1400

41) Williams GT ,Blackshaw AJ,Morson BC.Squamous carcinoma of the colorectum and its genesis.J Pathol.1979,129:139-147.

42) Aru A,Rasmussen LA,Federspiel B,Horn T.Glassy cell carcinoma of colon with human chorionic gonadotropin production:A case report with immunohistochemical and ultrastructural analysis.Am J surg Pathol 1996,20:187-192.

43) Goel A,Nagasaka T,Arnold CN,et al.CpG island methylated phenotype and chromosome instability are inversely correlated in sporadic colorectal cancer.Gastroenterology 2007;132:127-128.

44) Ulich TR,Cheng L,Glover H,Yan K,Lewin KJ.A Colonic adenocarcinoma with argentaffin cells .An immunoperoxidase study demonstrating the presence of numerous neuroendocrine products.cancer 1983;51:1483-1489.

45) Vrabie CD,Ceausu M,Petrescu A et al;The usefulness of immunohistochemistry in sporadic colorectal cancer;Romanian journal of morphology and embryology 2008,49(4):525-535

46) Lee MJ,Lee HS,Kim EH et al.Expression of mucins and cytokeratins in primary carcinomas of the digestive system.Modern Pathology.2003;16:403-410.

47) Al-khayal K,Abdulla M,Al –obaid O.,Zubaidi A et al.Differential expression of mucins in middle eastern patients with Colorectal carcinoma.Oncology letters.2016: 12.1: 393-400

48) Betge J,Schneider NI,Harbaum et al.MUC1, MUC2, MUC5A1, MUC6 in colorectal cancer:Expression profiles and clinical significance .Virchows Archiv.2016;469(3): 255-265

49) Dabb's Diagnostic immunohistochemistry,third edition.Chapter 14:514-517

50) Johnson VG,Schlom J,Paterson AJ,Bennett J,Magnani JL,Colcher D.Analysis of a human tumor associated glycoprotein(tag-72)identified by monoclonal antibody b72.3. Cancer Res.1986;46(2:850-857)

51) Jantscheff P,Terracciano L,Lowy A,Glatz-Krieger K,Grunert F,Micheal B ,et al. Expression of $CEACAM_6$ in resectable colorectal cancer:a factor of independent prognostic significance.J Clin Oncol.2003;21(19:3638-3646)

52) AJCC Cancer staging manual ,8^{th} edition.ISBN 978-3-319-40618-3: 268-269

53) Rosai and Ackerman's Surgical pathology,10^{th} edition,Chapter 11:770-771

54) Shimoda T,Ikegami M,Fujisaki J,Matsui T,Aizawa S. Early colorectal carcinoma with special reference to its development de novo.Cancer,1989;64:1138-46

55) Zocche DM ,Ramirez C,Fantao FM et al.Global impact of KRAS mutation patterns in FOLFOX treated metastatic colorectal cancer.Frontier in Genetics.2015;6:116

56) Al-Allawi NA,Ismaeel AT,Ahmed NY,Merza NS.The frequency and spectrum of K-ras mutations among Iraqi patients with sporadic colorectal carcinoma.Indian Journal of Cancer.2012; 49(1):163-168

57) Shemirani AI,Haghighi MM,Milanizadeh S,Taleghani MY,Fatemi SR,Damavand B, et al. The role of Kras mutations and MSI status in diagnosis of colorectal cancer. Gastroenterology and Hepatology From Bed to Bench.2011;4(2):70-75

58) Federico A.Monzon,ShujiOgino M,Elizabeth H,Hammond,Kevin C.Halling, et al. The role of KRAS mutation testing in the management of patients with metastatic colorectal cancer.Archives of pathology&Laboratory Medicine.Oct 2009;133(10):1600-1606

59) Bolton L,Reiman A,Lucas K,Timms J,Cree IA. KRAS mutation analysis by PCR:A comparison of two methods.PLoS ONE.2015; 10(1):e0115672.s

60) Tan C,Du X. KRAS mutation testing in metastatic colorectal cancer.*World J Gastroenterol* 2012;18(37);5171-5180.

61) Aghagolzadeh P,Radpour R. New trends in molecular and cellular biomarker discovery for colorectal cancer.World J Gastroenterol 2016 July 7;22(25):5678-5693

62) Okayama N,Nishioka M,Hazama S,Sakai K,Suehiro Y.et al. The importance of evaluation of DNA amplificability in KRAS mutation testing with dideoxy sequencing using formalin –fixed and paraffin –embedded colorectal cancer tissues.Jpn J Clin Oncol 2011;41(2)165-171

63) Andreyev HJ,Norman AR,Cunningham D,et al. Kirsten ras mutation in patients with colorectal cancer.British Journal of Cancer.2001;85(5):692-6

64) Bengala C,Bettelli S,Bertolini F,sartori G,Fontana A,Malavasi N et al. Prognostic role of EGFR gene copy number and KRAS mutation in patients with locally advanced rectal cancer treated with preoperative radiotherapy.Bristish Journal of Cancer.2010;103: 1019-1024

65) Guruya SY,Eltinay OE. Higher prevalence in younger population and rightward shift of Colorectal carcinoma. Saudi Med J.2006, Sep ;27(9):1391-3

66) Veldore VH,Rao MR,Prabhudesai SA. Prevalance of KRAS mutation in metastatic colorectal cancer:A retrospective observational study from India. Indian Journal of Cancer.2014;5(4):531-537.

67) Neklason DW,Kerber R A. et al., Common familial colorectal cancer linked to chromosome 7q31:a genome wide analysis; cancer Res.2008 nov 1;68(21):8993-8997

68) Devadass Clement W, Usha M,Patil Shilpa T,Vijaya V;Clinicopathological evaluation of colorectal carcinoma: unicenter study. Indian Journal of Pathology,research &practice.2016;5(3):265-270

69) Patra T,Mandal S,Alam N,Murmu N.Cliniopathological trends of colorectal carcinoma patients in a tertiary cancer centre in eastern India. Clinical epidemiology and global health .2018;6:39-43

70) Quddus MA,Alimunzzaman M,Rahman MZ,Alam H. Clinicopathological study of 50 cases colorectal carcinoma at tertiary care hospital. J Shaheed Suhrawady medical College.2012;4(2):53-56

71) Fatemah Hajmanoochehri, Asefzadeh S,Kazemifar AM . Clinicopathological features of colon adenocarcinoma in Qazvin,Iran: A 16 year study.Asian Pacific Journal of cancer prevention.2014;15(2):951-955

72) Jung MK,Shin US,Sung SJ et al.Is the location of tumor another prognostication factor for patients with colon cancer .Annals of Coloproctology.2017 Dec;33(6):210-218

73) Gill M K,Kalpana Jain,Manjeri M,Tanveer kaur. Expression of Her2/Neu in colon carcinoma and its correlationwiththe histological grades wuth lymphnodal status. Journal of clinical & diagnostic research.2011 Dec;5(8):1564-1568.

74) Uzma Nabi, Nagi A H,Riaz S, sami W.Morphological evaluation of colorectal carcinoma with grading,staging and histological types.Journal of Pakistan medical association.2010;60(12).

75) Carvalho,This Inacio de,Paulo cezar N, at al.Analysis of gene expression FGFR and KRAS ,microRNA 21 and micro RNA -203 in patients with colon and rectal cancer and correlation with clinal outcome and prognostication factor. Acta Cir.Bras .2017;32(3)

76) Balta A Z, Ozdemir Y, Sucullu et al. Can horizontal diameter of colorectal tumor predict prognosis?. Turkish Journal of surgery.2014;30(3);115-119

77) sundstrom et al.KRAS analysis in colorectal carcinoma:Analytical aspects of pyrosequencing and allele-specific PCR in clinical practice. Bio med Center2010;10:660

78) Mittempergher L,de Ronde JJ, Nieuwland et al .Gene expression Profiles from formaline fixed paraffin embedden breast cancer tissue are largely comparable to fresh frozed matched tissue. PloS one.2011;6:e17163

79) Domagala P ,Hybiak J,Sulzyc-Bielicka V et al.KRAS mutation testing in colorectalcancer as an example of the pathologist's role in personalized targeted therapy:A practical approach;POL J Pathol 2013;3:145-164

80) Niraj Kumari ,Narendra Krishnani,Balraj Mittal .KRAS mutations in colorectal cancer and its histological correlation in Indian population.American Journal of clinical pathology.2013; 140(suppl1):A190

81) Patil H ,Korde R,Kapat A. KRAS gene mutation in correlation with clinicopathological features of colorectal carcinomain Indian patient cohort,Med Oncol.2013;30(3):617

82) Sameer et al. mutation pattern of K-ras gene in colorectal cancer patients of Kashmir: A report.Indian Journal of cancer. July 2009;46(3)

83) Phipps I,Buchanan DD, Makar KW., et al. KRAS mutation status in relation to colorectal cancer survival:the Joint impact of correlated tumor markers;British Journal of Cancer.2013; 108:1757-1764.

84) Liu X,Jakubowski M,Hunt JL. KRAS gene mutation in colorectal cancer is correlated with increased proliferation and spontaneous apoptosis. Am J Clin Pathol.2011Feb; 135 (2):245-52.

85) Loree JM,Pereira AL,Lam M. et al. Classifying colorectal cancer by tumor location rather than sidedness highlights a acontinuum in mutation profiles and consensus molecular subtypes;Clin Cancer Res.2017;24(5):1062-72

86) Li J,Guo BC,Sun LR. Et al. TNM Staging of colorectal cancer should be reconsidered by T stage weighting.world j Gastroenterol .2014 May 7;20917):5104-12

87) Kim HS,Heo JS,Lee J,Lee JY et al.The impact of KRAS mutations on prognosis in surgically resected colorectal cancer patients with liver and lung metastasis;A retrospective analysis.BMC Cancer 2016;16:120

88) Peedikayil MC,Prem Nair,Seena S.M ,Lakshmi radhakrishnan .et al; Colrectal cancer distribution in 220 indian patients indergoing

colonoscopy;Indian journal of Gastroenterology 2009(Nov-Dec);28(6):212-215

89) Lievre A, Bachet JB, Le Corre D, Boige V, Landi B et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res.2006 Apr 15;66(8):3992-5

90) Young,O'Dowd and Woodford. Wheater's Functional Histology:A Text and Colour Atlas ,6e.2013;Chap 14:270-275

91) Saraga E,Bautista D,Dorta G,Chaubert P,Martin P,Sordat B,et al. Genetic heterogeneity in sporadic colorectal adenomas. J Pathol.1997 Mar;18(3):281-6

92) Gonzalez R S,Washington K,Chanjuan Shi. Current applications of molecular pathology in colorectal carcinomas.Applied Cancer Research. 2017; 37:13

93) Velho S,Moutinho C,Cirnes L,Albuquerque C,Hamelin R,Schmitt F,et al.BRAF,KRAS and PIK3CA mutations in colorectal serrated polyps and cancer:primary or secondary genetic events in colorectal carcinogenesis? .BMC Cancer.2008 Sep 9;8:255

94) Kesari MV,Gaopande VL,Joshi AR, et al.Immunohistochemical study of MUC1,MUC2 and MUC5AC in colorectal carcinoma and review of literature. Indian J Gastroenterol.2015 Jan;34(1):63-7

95) Valtorta E,Misale S,Sartore-Bianchi A,Nagtegaal ID,Paraf F,Lauricella C, et al. KRAS gene amplification in colorectal cancer and impact on response to EGFR-targeted therapy. Int J Cancer.2013 sep 1;133(5):1259-65.

96) Monzon FA,Ogino S,Hammond ME,Hailing KC,Bloom KJ,Nikiforova MN. The role of KRAS mutation testing in the management of patients with metastatic colorectal cancer.Arch Pathol lab Med.2009 Oct;133(10):1600-6

97) Raponi M,Winkler m,Dracopoli NC. KRAS mutation predict respoSnse to EGFR inhibitors.Curr opin Pharmacol 2008 Aug;8(4):413-418

98) Ghazi S,Berg E,Lindblom A,Lindfross U. Clinicopathological analysis of colorectal cancer:a comparison between emergency and elective surgical cases. World J Surg Oncol.2013 Jun 11;11:133.

99) Sagap I, Elnaim ALK,Hamid I, Rose IM. Surgeons' Evaluation of colorectal cancer resections against standard HPE protocol-auditing the surgeons.The Indian Journal of Surgery.2011; 73(3):194-198.

100) Ansari R,Mahdavinia M, Sadjadi A,Nouraie M,kamangar F, et al.Incidence and age distribution of Colorectal cancer in

Iran:results of a population-based cancer registry. Cancer Lett.2006 Aug 18;240(1):143-7

101) Armaghany T,Jon D,Wilson,Ouyen Chu and Glenn Mills.Genetic alterations in colorectal cancer.Gastrointestinal Cancer Res.2012 Jan-Feb;5(1):19-27

102) Zhang J,Zheng J,Yang Y,Lu J,Gao J,et al.Molecular spectrum of KRAS,NRAS,BRAF and PIK3CA mutations in Chinese colorectal cancer patients:analysis of 1,110 cases. Scientific reports.5:18678

103) Wilson,P.M., Labonte,M.J. &Lenz,H.J.Molecular markers in the treatment of metastatic colorectal cancer. Cancer J ;16:262-272.

104) De Roock ,W. et al. KRAS wild –type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab.Ann Oncol.2008;19:508-515

105) Neumann J, Zeindl-Eberhart E, Kirchner T and Jung A.Frequency and type of KRAS mutations in routine diagnostic analysis of metastatic colorectal cancer.Pathol Res Pract. 2009; 205,858-862.

106) Span M,Moerkerk PT,De Goeij AF,Arends JW.A detailed analysis of KRAS point mutations in relation of tumor progression and survival in colorectal cancer patients.Int J Cancer.1996;69:241-5

107) Estrada P,Rojas-Atencio A,Zabala W,Borjas L,Soca L,Urdaneta K,et al. Clinicopathological associations of KRAS mutations in Venezuelan patients with colo-rectal cancer.Invest Clin 2009;50:55-63

108) Capella G,Cronauer-Mitra S,Peinado MA,Perucho M.Frequency and spectrum at codon 12 and 13 of the KRAS gene in human tumors.Environ Health Perspect.1991;93:125-131.

109) Bisht S,Ahmed F,Sawaimoon S,Bhatia S,Das BR.Molecular spectrum of KRAS,BRAF and PIK3CA gene mutation:Determination of frequency distribution pattern in Indian colorectal carcinoma.Med Oncol.2014;31:124

110) Phang T,Shi C,Wee A,Ngoi S ,Li B,Lee H,et al. KRAS mutation in colorectal carcinomas from Singapore .Int J Oncol.1995;6:191-5.

111) Sirisena ND,Deen K,Mandawala DEN,Herath P,Dissanayke VHW.The pattern of KRAS mutations in metastatic colorectal cancer:a retrospective audit from srilanka. BMC Research Notes.2017;10:392

112) Dinu D,Dobre M,Panaitescu E,et al.Prognostic significance of KRAS gene mutations in colorectal cancer-preliminary study.Journal of Medicine and Life.2014;7(4):581-587.

113) van Krieken JH, Jung A,Kirchner T,et al.KRAS mutation testing for predicting response to anti-EGFR therapy for colorectal carcinoma:proposal for an European quality assurance program.Virchows Arch 2008;453:417-431

114) Baas JM,Krens LL,Guchelaar HJ,Morreau H, et al.Concordance of predictive markers for EGFR inhibitors in primary tumors and metastases in colorectal cancer: a review.Oncologist 2011;16:1239-1249.

115) Whitehall V,Tran K,Umapathy A,Grieu F,Hewitt C,Evans TJ,et al.A multicenter blinded study to evaluate KRAS mutation testing methodologies in the clinical setting. J Mol Diagn 2009;11:543-52

116) Allegra CJ,Jessup JM,Somerfield MR,Hamilton SR,Hammond EH,Hayes DF ,et al.American Society of clinical Oncology provisional clinical opinion:testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. J Clin Oncol.2009 Apr 20;27(12):2091-6

117) Sung JJ,Lau JY,Goh KL,Leung WK,Asia pacific working group on colorectal cancer.Increasing incidence of colorectal cancer in Asia:Implications for screening. Lancet Oncol 2005;6:871-6

118) Fearon ER,Vogelstein B. A genetic model for colorectal tumorigenesis.Cell 1990;61:759-67.

119) Jass J R.Molecular heterogeneity of colorectal cancer:Implications for cancer control.SurgOncol.2007;16:S7-S9.

120) Sharma S,Kelly T K,Jones P A. Epigenetics in cancer. Carcinogenesis 2010;31:27-36

Annexures

ANNEXURE-I

WHO CLASSIFICATION OF TUMORS OF COLON AND RECTUM

EPITHELIAL TUMORS

Premalignant Lesions

- **Adenoma**
  - -Tubular
  - -Villous
  - -Tubulovillous
- **Serrated lesions**
  - -Hyperplastic polyp
  - -Traditional serrated adenoma
  - -Sessile serrated adenoma/polyp
- **Hamartomas**
  - -Juvenile polyp
  - -Peutz-Jeghers polyp
  - -Cowden associated polyp

Carcinomas

- **Adenocarcinoma**
  - -Mucinous carcinoma
  - -Medullary carcinoma
  - -Micropapillary carcinoma
  - -Cribriform comedo type
  - -Serrated adenocarcinoma
  - -Signet ring cell carcinoma
  - -Squamous cell carcinoma
  - -Spindle cell carcinoma

-Basaloid (cloacogenic)carcinoma

-Clear cell carcinoma

-Hepatoid adenocarcinoma

-Undifferentiated carcinoma

- **Neuroendocrine neoplasms**

-Neuroendocrine tumor(NET) -- NET G1 Carcinoid /NET G2

-Neuroendocrine carcinoma(NEC) –Large cell NEC/Small cell NEC

- Mixed adeno neuroendocrine carcinoma
- EC cell,Serotonin producing NET
- L cell,glucagon like peptide producing,and PP/PPY producing NETs

Mesenchymal Tumors

- Lipoma
- Leiomyoma
- Hemangiomas
- Angiosarcoma
- Schwannoma
- Gastrointestinal stromal tumor
- Kaposi sarcoma
- Leiomyosarcoma

Lymphomas

- Marginal zone B-cell Lymphoma (MALT)Type
- Mantle cell lymphoma
- Burkitt lymphoma
- Diffuse large B-cell lymphoma

Metastatic Tumors

ANNEXURE-II

HISTOLOGICAL GRADING OF ADENOCARCINOMA

Grading	Differentiation	Features
Grade X	Grade cannot be assessed	
Grade-I	Well differentiated tumor	Simple tubules,nuclear polarity easily discerned, Uniform sized nuclei
Grade-II	Moderately differentiated tumor	Simple/complex/slightly-irregular tubules ,nuclear polarity lost
Grade-III	Poorly differentiated tumor	Absence of glands,with Solid like pattern
Grade-IV	Undifferentiated tumor	

ANNEXURE-III

AJCC STAGING:[52]

Primary Tumour(T)

- T_{X--} Primary tumour cannot be assessed
- T_0-- No evidence of primary tumour
- T_{is}- Carcinoma in situ: intraepithelial or invasion of lamina propria
- T_{1--} Tumour invades submucosa
- T_{2--} Tumour invades muscularis propria
- T_{3--} Tumour invades through muscularis propria into pericolorectal tissues
- T_{4a}- Tumour penetrates to the surface of visceral peritoneum
- T_{4b}-Tumour directly invades or is adherent to other organs or structures.

Regional Lymph Nodes(N)

- N_x- Regional lymph nodes cannot be assessed
- N_0- No regional lymph node metastasis
- N_1- Metastasis in 1 to 3 regional lymph nodes
- N_2- Metastasis in 4 or more regional lymph nodes

Distant Metastasis(M)

- M_0-No distant metastasis
- M_1-Distant metastasis
- M_{1a}-Metastasis confined to one organ or site
- M_{1b}-Metastasis in $\geq$1 organ/site

ANNEXURE -IV

AJCC PATHOLOGIC STAGE GROUPS

STAGE 0	Tis N_0M_0
STAGE I	$T_1N_0M_0$ $T_2N_0M_0$
STAGE II	$T_3N_0M_0$ $T_{4a/4b}$ N_0 M_0
STAGE III	Any T N_1M_0 Any T N_2M_0
STAGE IV	Any T Any N M_1

Master chart

S.No	Bx.NO	AGE	SEX	AGE RANGE	SITE	GROSS LESION	SIZE	HPE-PATTERN	INFILTRATION	TYPE	GRADE	INVASION	LYMPHNODES	STAGE	KRAS MUTATION
1	7170/18	60	F	51-60	RT.ASC.COLON	ULCERO PROLIFERATIVE	4.5X3.5X1CM	SHEETS/NESTS/GLANDS	SEROSA	INFILTRATING ADENOCA.	MOD	LV -/PN-	7NODES-REACTIVE	pT4aN0Mx	DETECTED
2	7174/18	65	F	61-70	RECTUM	PEDUNCULATED POLYP	4X3.5X4CM	VILLOGLANDULAR	MUSCULARIS PROPRIA	INFILTRATING ADENOCA.	WELL	LV-/PN-	4NODES-REACTIVE	T2N0MX	DETECTED
3	4019/18	60	F	51-60	SIGMOID COLON	ULCEROPROLIFERATIVE	8X4X2CM	GLAND/PAPILLAE	SEROSA	INFILTRATING ADENOCA.	MOD	LV+/ PERINEURAL -	1/3-METS	T4aN1MX	NOT DETECTED
4	4287/18	80	F	71-80	RECTOSIGMOID	ULCEROPROLIFERATIVE	6X3X1CM	PAPILLAE/GLANDS	SEROSA	INFILTRATING ADENOCA.	MOD	LV+/PERINEURAL -	2/8-METS	T4aN1bMX	NOT DETECTED
5	4475/18	55	F	51-60	RT.ASC.COLON	ULCEROPROLIFERATIVE WITH POLYP	6X5X3CM	GLAND/PAPILLAE	SEROSA	INFILTRATING ADENOCA.	MOD	LV-/PN-	10 NODES-REACTIVE	T4aNXMX	DETECTED
6	4667/18	55	M	51-60	RT.ASC.COLON	ULCERATED	7X3X3CM	GLANDS	SEROSA	INFILTRATING ADENOCA.	MOD	LV-/PN-	4NODES-REACTIVE	T4aNXMX	NOT DETECTED
7	4703/18	57	M	51-60	RT.ASC.COLON	ULCEROPROLIFERATIVE	7X5X4CM	PAPILLAE/GLANDS	SEROSA	INFILTRATING ADENOCA.	MOD	LV-/PN-	11NODES-REACTIVE	T4aN0MX	NOT DETECTED
8	5182/18	65	M	61-70	RT.ASC.COLON	PROLIFERATIVE	8X4X6CM	GLANDULAR/PAPILLAE	SEROSA	INFILTRATING MUCINOUS ADENOCA.		LV-/PN-	2NODES-REACTIVE	T4aNXMX	NOT DETECTED
9	7442/18	45	F	41-50	RECTUM	INFILTRATING	4X3X3CM	GLANDULAR	SEROSA	INFILTRATING ADENOCA.	MOD	LV-/PN-	NO NODES	T4aNxMX	NOT DETECTED
10	7476/18	50	M	41-50	RECTUM	POLYPOIDAL	2X2X1.5CM	POLYPOIDAL	SUBMUCOSA	INFILTRATING ADENOCA.	WELL	LV-/PN-	12NODES-REACTIVE	T1NXMX	NOT DETECTED
11	7497/18	30	F	21-30	SIGMOID COLON	INFILTRATING	3.5X2X0.5CM	GLAND	SEROSA	INFILTRATING ADENOCA.	MOD	LV-/PN-	12NODES-REACTIVE	T4aN0MX	NOT DETECTED
12	7657/18	60	F	51-60	RT.ASC.COLON	ULCEROPROLIFERATIVE	6X4.5X3.5CM	VILLOGLANDULAR	SEROSA	INFILTRATING ADENOCA.	WELL	LV+/PERINEURAL -	3/23-METS	T4aN1bMX	NOT DETECTED
13	7706/18	56	M	51-60	RT.ASC.COLON	ULCERPROLIFERATIVE	10X6X6CM	GLANDS	PERISEROSAL PAD OF FAT	MUCINOUS ADENOCA.		LV+/PERINEURAL -	1/14-METS	T4aN1aMX	DETECTED
14	7776/18	47	M	41-50	DESC.COLON	ULCERPROLIFERATIVE	10X6.5X4CM	SHEETS/NESTS	SEROSA	INFILTRATING ADENOCA.	POORLY	LV-/PN-	NO NODES	T4aNXMX	NOT DETECTED

15	7856/18	67	M	61-70	RECTUM	ULCEROPROLIFERATIVE	3X1.5CM	CRIBRIFORM	SEROSA	INFILTRATING ADENOCA.	MOD	LV-/PN-	2NODES-REACTIVE	T4aNXMX	NOT DETECTED
16	1306/18	26	M	21-30	RECTUM	ULCERATED	4X4X2.5CM	GLANDS	SEROSA	INFILTRATING ADENOCA.	POORLY	LV-/PN-	NO NODES	T4aNXMX	NOT DETECTED
17	1317/18	72	F	71-80	RECTUM	ULCERATED	2.5X1.5CM	GLANDS	SEROSA	INFILTRATING ADENOCA.	MOD	LV-/PN-	NO NODES	T4aNXMX	NOT DETECTED
18	1318/18	60	F	51-60	RT.ASC.COLON	PROLIFERATIVE	3X2X2CM	GLAND	SEROSA	INFILTRATING ADENOCA.	MOD	LV-/PN-	4NODES-REACTIVE	T4aNxMX	NOT DETECTED
19	1453/18	56	F	51-60	RECTOSIGMOID	NODULAR	3X2X2CM	GLANDS	SEROSA	INFILTRATING ADENOCA.	MOD	LV-/PN-	4NODES-REACTIVE	T4aNXMX	NOT DETECTED
20	1573/18	62	M	61-70	HEPATIC FLEXURE	CIRCUMFERENTIAL INFILTRATIVE GROWTH	6X3X2CM	GLAND,PAPILLARY	SEROSA	INFILTRATING ADENOCA.	MOD	LV+/PERINEURAL -	1/12-METS	T4aN1aMX	NOT DETECTED
21	2240/18	53	M	51-60	RECTUM	ULCEROPROLIFERATIVE	4X2.5X5CM	PAPILLAE/GLANDS	MUSCULARIS PROPRIA	INFILTRATING ADENOCA.	MOD	LV+/PERINEURAL -	3/10 METS	T2N1bMX	NOT DETECTED
22	1075/18	38	M	31-40	SIGMOID COLON+BLADDER INFILTRATION	ULCEROPROLI/ NODULARITY	8X8X6CM	GLANDS	SEROSA	INFILTRATING ADENOCA.	MOD	LV+/PERINEURAL -	5NODES-REACTIVE	T4bNXMX	NOT DETECTED
23	1161/18	65	M	61-70	HEPATIC FLEXURE	ULCEROPROLIFERATIVE	2.5X1CM	GLANDS	SEROSA	INFILTRATING ADENOCA.	MOD	LV-/PN-	5NODES-REACTIVE	T4aNXMX	NOT DETECTED
24	6964/18	65	F	61-70	RECTUM	ULCEROPROLIFERATIVE	6X4X2CM	IRREGULAR GLANDS	SEROSA	INFILTRATING ADENOCA.	MOD	LV+/PERINEURAL -	1/2-METS	T4aNXMX	NOT DETECTED
25	6774/18	24	M	21-30	SPLENIC FLEXURE	ULCEROPROLIFERATIVE	16.5X7.5X2.5CM	SOLID SHEETS,SIGNETRING	SEROSA	INFILTRATING ADENOCA.	POORLY	LV+/PERINEURAL -	1/12-METS	T4aN1MX	NOT DETECTED
26	8877/17	60	F	51-60	RECTUM WITH LIVER METS	ULCEROPROLIFERATIVE	4X4X3CM	GLANDULAR/PAPILLARY	SEROSA+	INFILTRATING ADENOCA.	MOD	LV-/PN-	6NODES-REACTIVE	T4aNXM1	NOT DETECTED
27	3159/17	66	M	61-70	SIGMOID COLON WITH ILEAL INFILTRATION	ULCEROPROLIFERATIVE	6.5X6CM	GLANDS	PERISEROSAL PAD OF FAT	INFILTRATING ADENOCA.	WELL	LV-/PN-	4NODES-REACTIVE	T4bNXMX	NOT DETECTED
28	6695/17	65	M	61-70	TRANSVERSE COLON	ULCEROPROLIFERATIVE	3X3X1CM	GLANDULAR	PERISEROSAL PAD OF FAT	INFILTRATING ADENOCA.	WELL	LV+/PERINEURAL -	1/6-METS	T4bNXMX	NOT DETECTED

29	6589/17	65	M	61-70	RECTUM+POSTERIOR BLADDER INFILTRATION	INFILTRATING	10X7X3CM	PAPILLAE/GLANDS,SIGNETRING	SEROSA	INFILTRATING MUCINOUS ADENOCA.		LV-/PN-	6NODES-REACTIVE	T4bNXMX	DETECTED
30	8275/17	27	M	21-30	RECTUM	ULCEROPROLIFERATIVE	3X2X2CM	SHEETS/NESTS,SIGNET RING	MUSCULARIS PROPRIA	INFILTRATING ADENOCA.	POORLY	LV+/PN +	6/10-METS	T3N2aMx	NOT DETECTED

INFORMED CONSENT FORM

Title of the study : **"A study on molecular expression of K-RAS mutation in Colorectal carcinoma and its correlation with clinicopathological findings in a tertiary care hospital"**

Name of the Participant :

Name of the Principal(Co-Investigator) :

Name of the Institution : Madras Medical College / Rajiv Gandhi Govt. General Hospital, Chennai-03.

Name and address of the sponsor / agency (ies) (if any) :

Documentation of the informed consent

I _____ have read the information in this form (or it has been read tome). Iwas free to ask any questions and they have been answered. I am over 18 years of age and,exercisingmyfree power of choice, hereby give my consent to be included as a participant in **"A study on molecular expression of K-RAS mutation in Colorectal carcinoma and its correlation with clinicopathological findings in a tertiary care hospital"**

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study in which the resected colorectal tumors will be subjected to Molecular analysis and immunohistochemistry examination.
4. I have been explained about my rights and responsibilities by the investigator. I have the right to withdraw from the study at any time.
5. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
6. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.
7. I have understood that my identity will be kept confidential if my data are publicly presented
8. I have had my questions answered to my satisfaction.
9. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For adult participants:

Name and signature / thumb impression of the participant (or legal representative if participant incompetent)

Name _____ Signature_____ Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____ Signature_____ Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____ Date_____

ஆராய்ச்சி தகவல் தாள்

ஆராய்ச்சி தலைப்பு : பெருங்குடலில் ஏற்படும் புற்றுநோயில் கே-ராஸ் (K-RAS) என்னும் புற்றணு விகாரத்தின் மூலக்கூறு வெளிப்பாடு மற்றும் அவற்றின் நோயியல் தன்மைகளோடு ஒப்பிடும் ஓர் பகுப்பாய்வு.

ஆய்வாளர் : மரு.B. மோகனப்பிரியா,
இரண்டாம் ஆண்டு,
நோய்குறியியல் துறை,
சென்னை மருத்துவக் கல்லூரி,
சென்னை - 600003.

தங்களது பெருங்குடலில் உள்ள புற்றுநோய் கட்டி (அறுவை சிகிச்சை செய்யப்பட்ட கட்டி.) இங்கு பெற்றுக்கொள்ளப்பட்டது.

இராஜீவ் காந்தி அரசு பொது மருத்துவமனைக்கு வரும் நோயாளிகளிடம் இருக்கும் பெருங்குடலில் ஏற்படும் புற்றுநோய் பற்றி ஒரு ஆராய்ச்சி இங்கு நடைபெற்று வருகின்றது.

பெருங்குடலில் ஏற்படும் புற்றுநோயை மூலக்கூறு நோயறிதல் நுட்பங்கள் கொண்டு கண்டறிவதே இந்த ஆய்வின் நோக்கமாகும்.

நீங்களும் இந்த ஆராய்ச்சியில் பங்கேற்க நாங்கள் விரும்புகிறோம். இந்த ஆராய்ச்சியில் உங்களுடைய திசுக்கள் மற்றும் இரத்தம் எடுத்து சில சிறப்புப் பரிசோதனைக்கு உட்படுத்தி அதன் தகவல்களை ஆராய்வோம். அதனால் தங்களது நோயின் ஆய்வறிக்கையோ அல்லது சிகிச்சையோ பாதிப்புக்குள்ளாகாது என்பதையும் தெரிவித்துக் கொள்கிறோம்.

முடிவுகளை அல்லது கருத்துகளை வெளியிடும் போதோ அல்லது ஆராய்ச்சியின் போதோ தங்களது பெயரையோ அல்லது அடையாளங்களையோ வெளியிட மாட்டோம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களுடைய விருப்பத்தின் பேரில் தான் இருக்கிறது. மேலும் நீங்கள் எந்நேரமும் இந்த ஆராய்ச்சியில் இருந்து பின்வாங்கலாம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த சிறப்புப் பரிசோதனைகளின் முடிவுகளை ஆராய்ச்சியின் போது அல்லது ஆராய்ச்சியின் முடிவின் போது தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த ஆய்வை பற்றிய சந்தேகங்களுக்கு தொடர்பு கொள்ள வேண்டியவர் : மரு.B. மோகனப்பிரியா, செல் : 9677253606

பங்கேற்பாளர் கையொப்பம்..... இடம் :..... தேதி :.....

பங்கேற்பாளர் பெயர் மற்றும் விலாசம் .....

ஆராய்ச்சியாளர் கையொப்பம்..... இடம் :..... தேதி :.....