A STUDY OF THE CLINICOPATHOLOGICAL PROFILE AND PREVALENCE OF CDX2 BIOMARKER EXPRESSION IN PRIMARY ADENOCARCINOMA OF COLON IN A TERTIARY CARE HOSPITAL AMONG INDIAN POPULATION

A DISSERTATION SUBMITTED IN PART FULFILMENT OF THE REGULATION FOR THE AWARD OF THE DEGREE OF M.D. PATHOLOGY BRANCH III.

THE TAMIL NADU DR. M.G.R. UNIVERSITY, CHENNAI, TAMIL NADU MAY-2019

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CERTIFICATE

This is to certify that this dissertation entitled "A study of the clinicopathological profile and prevalence of CDX2 biomarker expression in primary adenocarcinoma of colon in a tertiary care hospital among Indian population" is the bonafide work done by Dr. Rijo Issac N P, in part fulfillment of the rules and regulations for the M.D. Branch III (Pathology) Degree Examination of Tamil Nadu Dr. M.G.R. Medical University, to be held in May 2019.

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The candidate has independently reviewed the literature, standardized the data collection methodology and carried out the evaluation towards completion of the thesis.

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This is to certify that this dissertation entitled "A study of the clinicopathological profile and prevalence of CDX2 biomarker expression in primary adenocarcinoma of colon in a tertiary care hospital among Indian population" is a bonafide work done by me, under the guidance of Dr. Dipti Masih, in part fulfillment of the requirements for the M.D Branch III (Pathology) Degree Examination of the Tamil Nadu Dr. M.G.R Medical University, to be held in May 2019.

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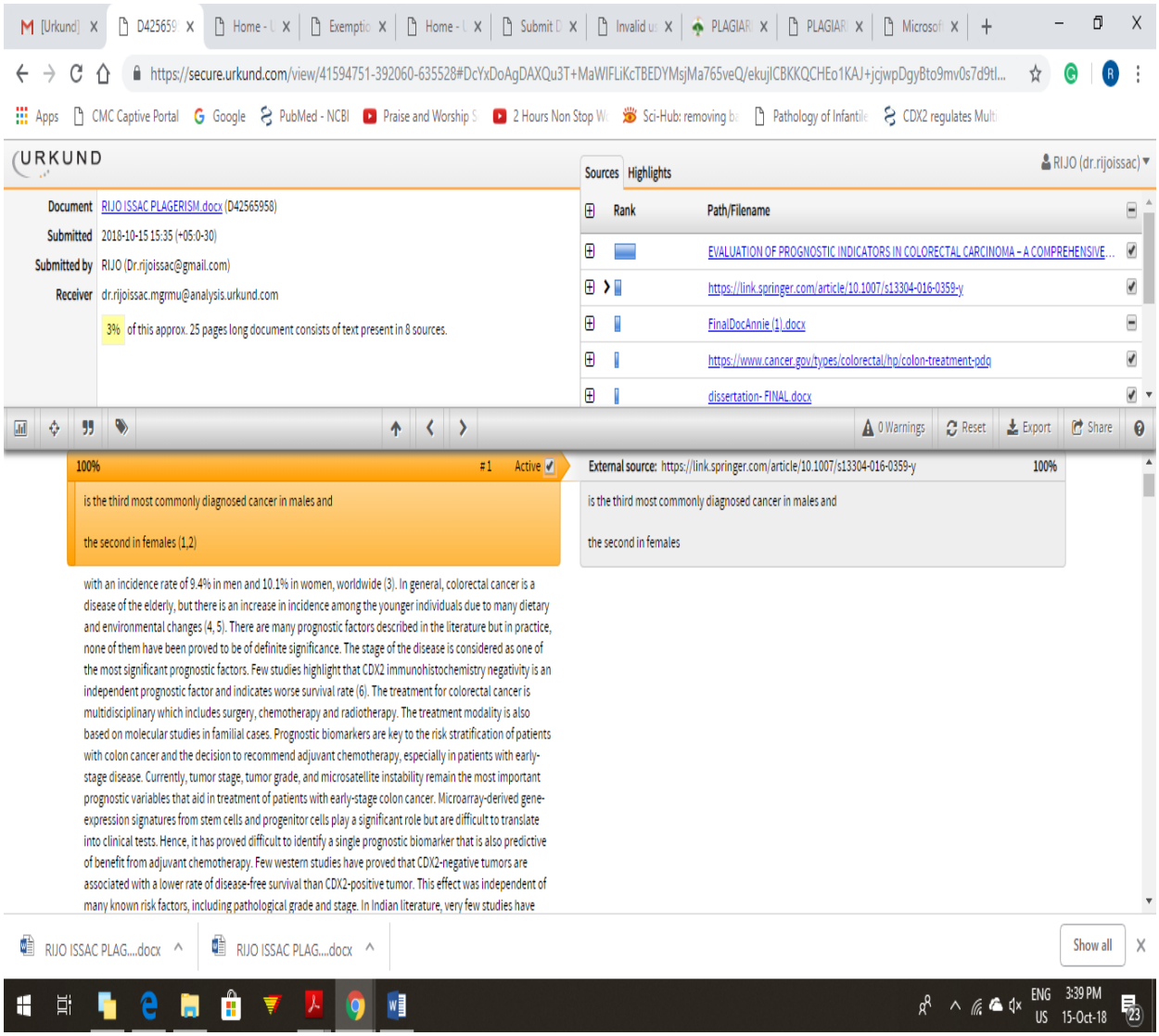
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is the third most commonly diagnosed cancer in males and
the second in females
with an incidence rate of 9.4% in men and 10.1% in women, worldwide (3). In general, colorectal cancer is a disease of the elderly, but there is an increase in incidence among the younger individuals due to many dietary and environmental changes (4, 5). There are many prognostic factors described in the literature but in practice, none of them have been proved to be of definite significance. The stage of the disease is considered as one of the most significant prognostic factors. Few studies highlight that CDX2 immunohistochemistry negativity is an independent prognostic factor and indicates worse survival rate (6). The treatment for colorectal cancer is multidisciplinary which includes surgery, chemotherapy and radiotherapy. The treatment modality is also based on molecular studies in familial cases. Prognostic biomarkers are key to the risk stratification of patients with colon cancer and the decision to recommend adjuvant chemotherapy, especially in patients with early-stage disease. Currently, tumor stage, tumor grade, and microsatellite instability remain the most important prognostic variables that aid in treatment of patients with early-stage colon cancer. Microarray-derived gene-expression signatures from stem cells and progenitor cells play a significant role but are difficult to translate into clinical tests. Hence, it has proved difficult to identify a single prognostic biomarker that is also predictive of benefit from adjuvant chemotherapy. Few western studies have proved that CDX2-negative tumors are associated with a lower rate of disease-free survival than CDX2-positive tumor. This effect was independent of many known risk factors, including pathological grade and stage. In Indian literature, very few studies have
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ACKNOWLEDGMENT

I express my deepest thanks to Dr. Dipti Masih (General Pathology) and

Dr. Anna B Pulimood (General pathology) for their guidance, support, and help for the completion of this dissertation.

I would also like extend my thanks to Dr. Mark Ranjan, Professor of General Surgery for invaluable help in the clinical aspects of my study. My sincere thanks to Mrs. Gowri Sampath, biostatistician for the statistical analysis.

I would like to thank God Almighty and my family and friends for their help in pursuing my thesis

ABBREVIATIONS

CRC – Colorectal cancer

CDX2 – Caudal -type homeobox transcription factor 2

WHO – World Health Organization

AJCC - American Joint Committee on Cancer

HNPCC - Hereditary nonpolyposis colorectal cancer

FAP - Familial adenomatous polyposis

CT - Computed tomography

PET - Positron emission tomography

MSI - Microsatellite instability

MSS – Microsatellite stable

ACF - Aberrant crypt foci

CIN - Chromosomal instability

CIMP - CpG island methylator phenotype

TGFβ - Transforming growth factor beta

MMR – Mismatch repair

VEGF - Vascular endothelial growth factor

EGFR - Epidermal growth factor receptor

PDC – Poorly differentiated clusters

CONTENTS

INTRODUCTION

Colorectal cancer (CRC) is one of the alarming and common health problem worldwide (1). It is the third most commonly diagnosed cancer in males and the second in females(1,2) with an incidence rate of 9.4% in men and 10.1% in women, worldwide (3). In general, colorectal cancer is a disease of the elderly, but there is an increase in incidence among the younger individuals due to many dietary and environmental changes (4, 5). There are many prognostic factors described in the literature but in practice, none of them have been proved to be of definite significance. The stage of the disease is considered as one of the most significant prognostic factors.

Few studies highlight that CDX2 immunohistochemistry negativity is an independent prognostic factor and indicates worse survival rate (6). The treatment for colorectal cancer is multidisciplinary which includes surgery, chemotherapy and radiotherapy. The treatment modality is also based on molecular studies in familial cases. Prognostic biomarkers are key to the risk stratification of patients with colon cancer and the decision to recommend adjuvant chemotherapy, especially in patients with early-stage disease. Currently, tumor stage, tumor grade, and microsatellite instability remain the most important prognostic variables that aid in treatment of patients with early-stage colon cancer. Microarray-derived gene-expression signatures from stem cells and progenitor cells play a significant role but are difficult to translate into clinical tests. Hence, it has proved difficult to identify a single prognostic biomarker that is also predictive of benefit from adjuvant chemotherapy. Few western studies have proved that CDX2-negative tumors are associated with a lower rate of disease-free survival than CDX2-positive tumor. This effect was independent of many known risk factors, including pathological grade and stage. In Indian literature, very few studies have been done on CDX2 expression and its correlation with clinicopathological and prognostic significance of colon cancer. This study aims to look at the prevalence of CDX2

immunohistochemistry expression in colon cancer and its correlation with clinicopathological parameters.

AIM

1) To study the prevalence of CDX2 expression by immunohistochemistry in patients diagnosed as primary colonic adenocarcinoma in our hospital from January 2015 to June 2018.

2) To correlate the expression of CDX2 immunohistochemistry in primary colonic adenocarcinoma with classification, anatomical site, differentiation, and TNM staging.

3) To study the clinicopathological features of primary colonic adenocarcinoma.

OBJECTIVE

1) To identify the total number of primary colon cancers in our institution during the period January 2015 to June 2018 from the electronic database.

2) To do a detailed clinicopathological study of all the retrieved cases.

3) To assess the expression of CDX2 in endoscopic mucosal biopsies of all cases and to correlate it with clinical and histological parameters in the corresponding resection specimens.

LITERATURE REVIEW

EPIDEMIOLOGY

Colorectal cancer (CRC) is a formidable health problem worldwide and is one of common cancer worldwide. It constitutes 9.67% of the overall 14.1 million new cancer cases globally (1). It is the third most commonly diagnosed cancer in males and the second in females(1,2). The incidence rate of CRC is 9.4% in men and 10.1% in women, worldwide(3). There is variation in incidence rates in different parts of the world and more than half of the cases of CRC and 63% of newly diagnosed CRC cases are reported from developed countries (1). CRC incidence and mortality rates vary markedly across the globe with regional differences. In the western population, colorectal cancer is one of the commonest malignancy, but in the East, cancers of the upper gastrointestinal tract (esophagus and stomach) and liver are predominant (4). There is a rapid increase in the incidence of colorectal cancer in more developed and westernized Asian countries and there is a shift in trend from older to younger age group (5). The incidence rates range from more than 40 per 100,000 people in the United States, Australia, New Zealand, and Western Europe to less than 5 per 100,000 in Africa and some parts of Asia (2).

Worldwide, annually it is about 394,000 deaths from colorectal, amounting to the fourth most common cause of death from cancer (2). But mortality rates are high in the undeveloped and developing countries because of limited resources and healthcare infrastructure. In Western countries, the mortality rates are decreasing due to early detection by screening and improved treatment of colorectal malignancy(6). The mortality rates are on the rise in Asian continent according to the WHO Mortality Database. There is the difference in incidences of colorectal cancer in many different ethnic groups in Asia (4).

Carcinoma colon is a relatively uncommon malignancy in India when compared with the western world. Indian and Malay populations have significantly lower incidences of colorectal cancer when compared with the Chinese population in Singapore and Malaysia. This difference in the incidence of colorectal cancer among races indicates genetic factors which play an important etiological role. There are conflicting results in Asian migrants to western countries. There is a significant increase in the incidence rate of colorectal cancer which is nine times higher among Asian Indian migrants to the USA. These observations suggest that genetic predisposition interacts with lifestyle modification including diet and environmental factors. In many Asian countries, there is an apparent increase in proximal colorectal cancers than distal ones. An aging population and the wider availability of colonoscopy might partly explain this increase in the incidence of proximal colorectal cancers in Asian countries. (4). In India, there is a sharp rise in incidences of all cancer due to poor to moderate living standards and inadequate medical facilities. The incidence rate of colorectal cancer is on the rise after the age of 45 years, more common in males than females and about 90% of cases are found in population over the age of 50 years. In India, the annual incidence rate for colon cancer is 4.4 per 1, 00,000 and 3.9 per 1, 00,000 in men and women respectively.

The colorectal cancer survival and prognosis is dependent mainly upon the stage of disease at the time of initial diagnosis. The survival rates range from a 90% 5-year survival rate for localized stage cancers, 70% for regional and 10% for distant metastatic cancer. In general, there is a higher survival chance for cancers diagnosed at the earlier stage. The survival rates have increased substantially since the 1960s due to progress in diagnostic and treatment services (3).

THE 2010 WHO CLASSIFICATION

According to WHO (2010), CRC is defined as a malignant tumor arising from large bowel with invasion into submucosa through muscularis mucosa. The carcinoma is subclassified as below:

1) Adenocarcinoma
    a) Cribriform-comedo type adenocarcinoma
    b) Medullary carcinoma
    c) Mucinous adenocarcinoma
    d) Serrated adenocarcinoma
    e) Signet ring adenocarcinoma
2) Adenosquamous carcinoma
3) Spindle cell carcinoma
4) Squamous cell carcinoma
5) Undifferentiated carcinoma

EMBRYOLOGY AND ANATOMY

The three germ layers in embryogenesis are ectoderm, mesoderm, and endoderm. The embryonic endoderm gives rise to intestinal epithelium during gastrulation. The endoderm undergoes extensive folding for the formation of the embryonic gut tube, following induction and molecular patterning. There are no well-known mechanisms that initiate and control epithelial reorganization and morphogenesis of villus, although crosstalk between the gut epithelium and the mesenchyme has been shown to provide both permissive and instructive cues to allow the normal development of the intestine. BMP, Hedgehog, PDGF, TGF-β, and Wnt signaling pathways are known to involve in this epithelial-mesenchymal crosstalk (7)

The large intestine is being anatomically divided into caecum with an appendix, ascending colon, transverse colon, descending colon, sigmoid colon and rectum. Except ascending and descending colon, others are considered as intraperitoneal (8).

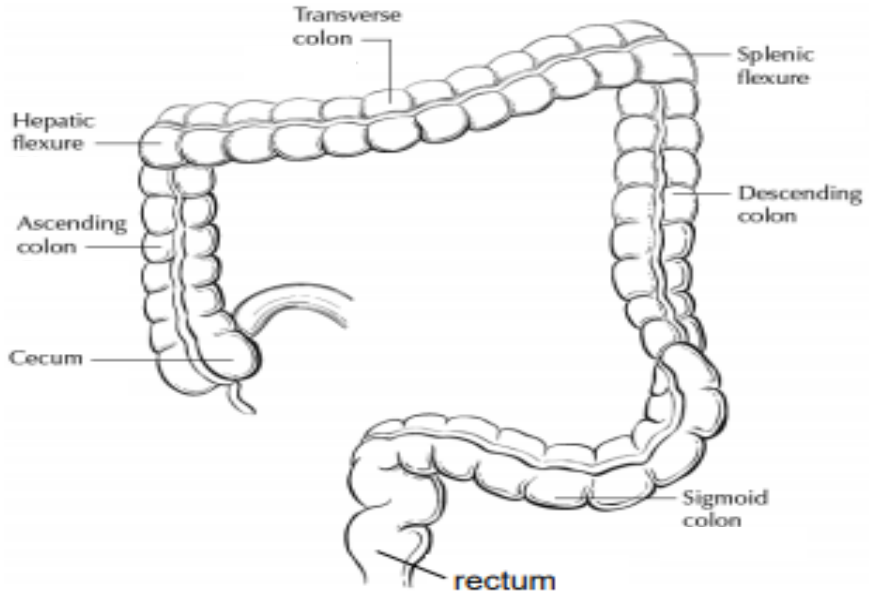


Figure 1: Anatomical subsites of colon, 8th Edition, AJCC Staging Manual

ETIOLOGY

There are several risk factors associated with the CRC incidence. Generally, risk factors are categorized into non-modifiable and modifiable or environmental factors.

NON MODIFIABLE RISK FACTORS:

1) AGE – Incidence of CRC increases with age. About 90% of colorectal cancer cases are seen in people aged 50 or older. The incidence rate in persons aged 60 to 79 years is more than 50 times higher than in those younger individuals. CRC incidence is rare in an age less than 40 years, except in those with a genetic predisposition or predisposing conditions such as chronic inflammatory bowel disease. However, colorectal cancer appears to be increasing among younger person (2,9,10).

2) ADENOMATOUS POLYPS - The precursor lesions of colorectal cancer are mainly neoplastic polyps, namely tubular, tubulovillous and villous adenomas. About 95% of sporadic colorectal cancers arise from these adenomas. There is increased risk noted for hyperplastic and adenomatous polyps which transform to carcinoma. There is a long latency period, about 5 to 10 years, is required for the development of cancer from adenomas. There is a 20% higher relative risk of developing colorectal cancer in people who have a family history of the adenomatous polyp, especially in the first-degree relatives (2,9,11)

3) INFLAMMATORY BOWEL DISEASE - Ulcerative colitis and Crohn disease are the two disease included in inflammatory bowel disease. Ulcerative colitis causes inflammation of the mucosa of the colon and rectum, whereas, Crohn disease can involve any part of the digestive tract from the mouth to the anus and causes full thickness inflammation of bowel wall. It has been estimated that the relative risk is between 4- to 20-fold in patients with inflammatory bowel disease. Schistosoma mansoni infection also aids in the development of CRC. (2, 11–13)

4) GENETIC RISK - Approximately 5 to 10% of colorectal cancers are the result of recognized hereditary conditions. Familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC), also called Lynch syndrome is the most common inherited conditions. HNPCC attribute 2 to 6% of colorectal cancers, whereas, FAP accounts for less than 1% of all colorectal cancer cases. The lifetime risk of colorectal cancer in people is approximately 70% to 80% with the known HNPCC-related mutations and the average age at colorectal cancer diagnosis is in their mid-40s but in FAP, malignant transformation of adenomas occurs as early as age 20. By age 40, almost all people with FAP will have developed cancer if the colon is not removed.

MODIFIABLE OR ENVIRONMENTAL FACTORS**:**

It broadly includes a wide range of often ill-defined factors including cultural, social and lifestyle factors. Part of evidence of environmental risk comes from studies on the migrants and their offspring. There are some other geographic factors which influencing differences in the incidence

of colorectal cancer, apart from migration, one of them is an increase in the incidence among urban residents.

1) DIETARY FACTORS - Diet has a strong and direct influence on the risk of colorectal cancer. Approximately 70% reduction is seen if there are changes in food habits. Diets especially high in fat, are considered as one of the major risk factors for colorectal cancer. The fatty diet favors the development of a bacterial flora which is capable of degrading bile salts to *N*-nitroso compounds which are potent carcinogens. High meat consumption has also been proved as one of the risk factors in the development of colorectal cancer. The meat consumption causes colon cancer more than rectal cancer. The underlying mechanisms are the production of heterocyclic amines and polycyclic aromatic hydrocarbons which have carcinogenic properties, which are formed while cooking red meat at high temperatures. Hence 'western type diet' (highly caloric food rich in animal fat) combined with a sedentary lifestyle shows an increase in CRC. In addition, a diet low in fruits, vegetables, whole grains, calcium, selenium and vitamin D have an increased risk of colorectal cancer (2,15–17).

2) PHYSICAL ACTIVITY AND OBESITY- About a fourth to a third of colorectal malignancy are related to excess body weight and physical inactivity. There are studies proving that frequency and intensity of physical activity inversely associated with risk of colorectal cancer. The risk of colorectal cancer can be reduced by a healthy diet and regular physical activity, although the evidence is stronger for colonic than for rectal disease. Due

to sustained physical activity increases oxygen uptake and raises the metabolic rate. Regular physical activity increases metabolic efficiency, reduces insulin resistance and blood pressure and also increase gut motility. The lack of physical activity leads to obesity in men and women which increases circulating estrogens and decreased insulin sensitivity, leading to increased cancer risk (2,18,19)

3) CIGARETTE SMOKING- There is proven evidence between tobacco cigarette smoking and lung cancer. But smoking also is extremely harmful to the colon and rectum. There are studies revealing that smoking contributes 12% of colorectal cancer deaths. The carcinogens found in tobacco increases the cancer growth in the colon and rectum. The formation and growth rate of adenomatous polyps, precursor lesions of colorectal cancer increases due to cigarette smoking (2,20)

4) ALCOHOL CONSUMPTION- Regular alcohol consumption is associated with an increased risk of developing colorectal cancer like cigarette smoking. One of the factors leading to the development of colorectal at a younger age is consumption of alcohol on regular basis. The reactive metabolites of alcohol like acetaldehyde can be carcinogenic. Alcohol also acts as a solvent for other carcinogenic molecules and enhancing the penetration of these molecules into mucosal cells. Also, there is the production of prostaglandins, lipid peroxidation and the generation of free radical oxygen species due to alcohol. Lastly, high alcohol consumers have diets low in essential nutrients, making tissues susceptible to carcinogenesis. (2,21)

CLINICAL FEATURES

SIGNS AND SYMPTOMS

The symptom for CRC varies according to the site, size, and extent of invasion. The common symptoms of colonic carcinoma include,

- Abdominal pain
- Bleeding per rectum
- Alteration in bowel habits
- Loss of weight and appetite
- Intestinal obstruction
- Perforation and peritonitis
- Anemia

The presenting symptoms vary according to the site whether it is a left-sided colon disease or a right-sided disease. For left side CRC, the most common symptoms include a change in bowel habit, either constipation or diarrhea. Generally, the left-sided colonic tumors proliferate in annular or constrictive fashion which leads to progressive narrowing of the bowel lumen. Moreover, the feces becomes less soft and gets impacted which leads to intestinal obstruction and perforation, especially seen in advanced cases. On the other hand, the right-sided colon cancer has a proliferative morphology which causes mild bleeding leading to iron deficiency anemia. Hence

urgent referral and evaluation are needed for iron deficiency in women who are not menstruating and men. Other non-specific symptoms include fever, malaise, weight loss and abdominal pain (22–24).

IMAGING

The imaging techniques which aid in the diagnosis of CRC include computer-assisted tomography (CT), magnetic resonance imaging, transrectal ultrasonography, and positron emission tomography (PET). These imaging methods are non-invasive and aid the clinician not only in detection but also in clinical staging. Barium studies are completely replaced by CT and CT colonography. Significances of transrectal ultrasonography help to estimate the depth of invasion and the possibility of regional and distant metastasis. PET scanning and scintigraphy is used to assess the spread of the diseases (25–27)

ENDOSCOPY

Colonoscopy helps in direct visualization of the mucosal surface of the entire colon. In addition, biopsy or therapeutic removal of the identified lesion can be done by snare polypectomy, endoscopic mucosal resection for adenomas and superficial carcinoma. Use of chromoendoscopy employing dyes and confocal endoscopy aids in better visualization (26,28)

PATHOLOGY

With the rapid advancement in therapeutic intervention, the role of pathologists is inevitable in diagnoses and in the management of patients with CRC. The pathologists are responsible, not only for exact histopathological diagnosis but also in assessing pathologic staging, searching for prognostic parameters that are not included in the staging including lymphovascular and perineural invasion, analyzing surgical margins and assessing the therapeutic effect of adjuvant therapy in patients.

Histologically, the colorectal carcinomas predominantly comprise adenocarcinomas (90%) originating from epithelial cells of the intestinal mucosa. The characteristic features of adenocarcinoma are the glandular formation and are the basis for histologic tumor grading. If the tumor has >95% of glandular differentiation, then they are grouped as well differentiated adenocarcinoma. If the tumor displays 50-95% gland formation, they are grouped as moderately differentiated adenocarcinoma. If the tumor is predominantly solid in nature with <50% of gland formation, then they are grouped under poorly differentiated adenocarcinoma. The tumor grade is considered as a stage-independent prognostic variable, and poorly differentiated or high-grade histology is associated with poor patient survival (29,30).

HISTOLOGICAL VARIANTS (29)

In World Health Organization (WHO) classification, the common histologic variants of CRC are mucinous, medullary, signet ring cell, micropapillary, cribriform comedo-type, serrated, spindle cell, adenosquamous and undifferentiated. Other rare variants included are clear cell carcinoma and Paneth cell rich papillary adenocarcinoma.

Mucinous adenocarcinoma.

Adenocarcinoma with >50% of extracellular pools of mucin that contains malignant cells in acinar structure or individual cells including signet ring cells. Majority of mucinous adenocarcinomas are MSI-H and hence low-grade tumors. Mucinous adenocarcinoma with MSS and MSI-L behave as high-grade tumor(29,31)

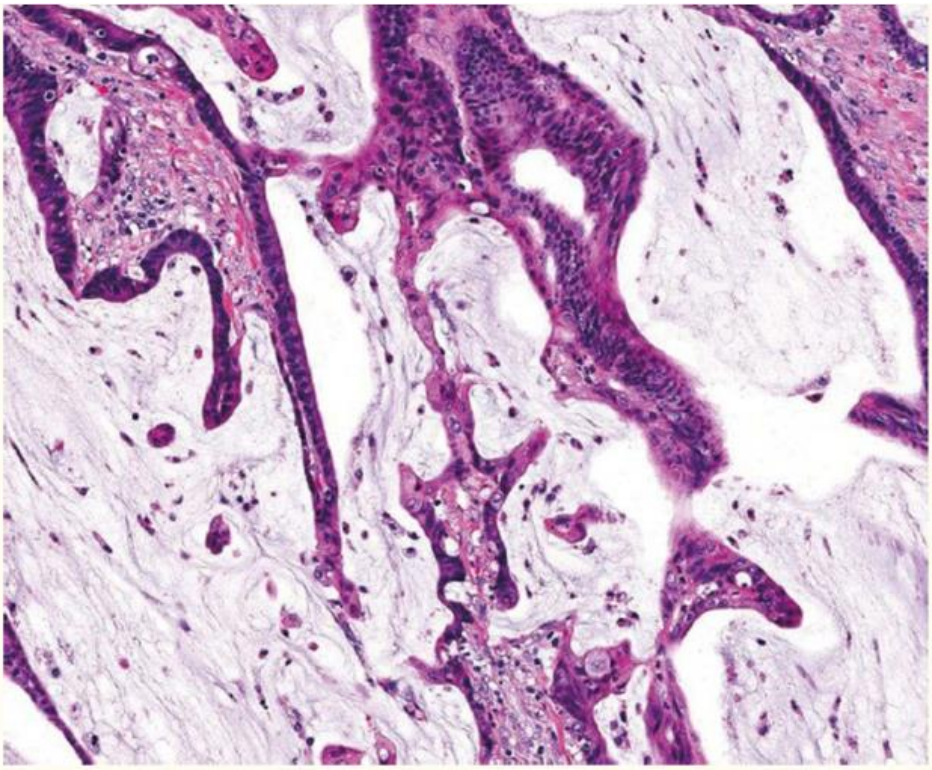


Figure 2: Mucinous adenocarcinoma (30)

Signet ring cell carcinoma

Tumors are designated as signet ring cell carcinoma by the presences of >50% of tumor cells with predominant intracytoplasmic mucin with molding and displacement of the nucleus. Large signet ring cells are known as ‘globoid cells’. This is considered to be a very aggressive variant (29,32).

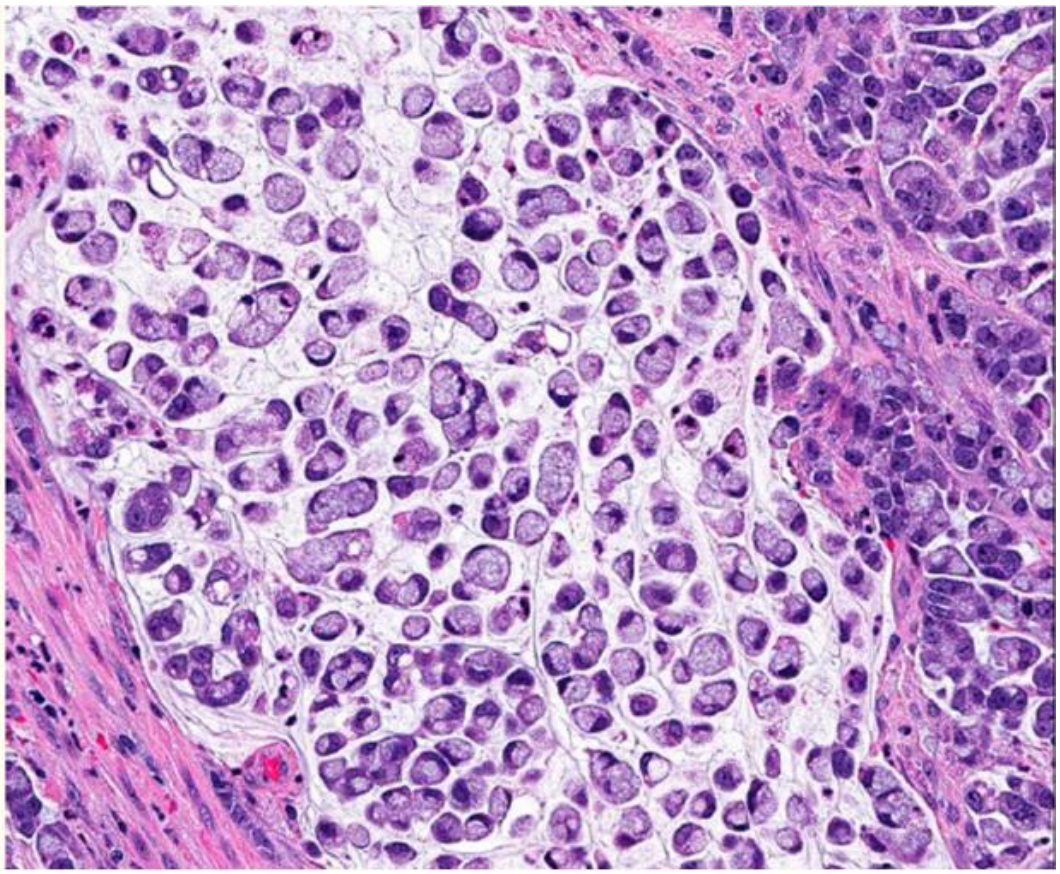


Figure 3: Signet ring cell carcinoma (30)

Medullary carcinoma

These tumors are not generally common and are characterized by sheets of malignant cells with vesicular nuclei, prominent nucleoli and abundant eosinophilic cytoplasm with prominent intraepithelial lymphocytic infiltration. Tumors are MSI-H and usually have favorable prognosis (29,33)

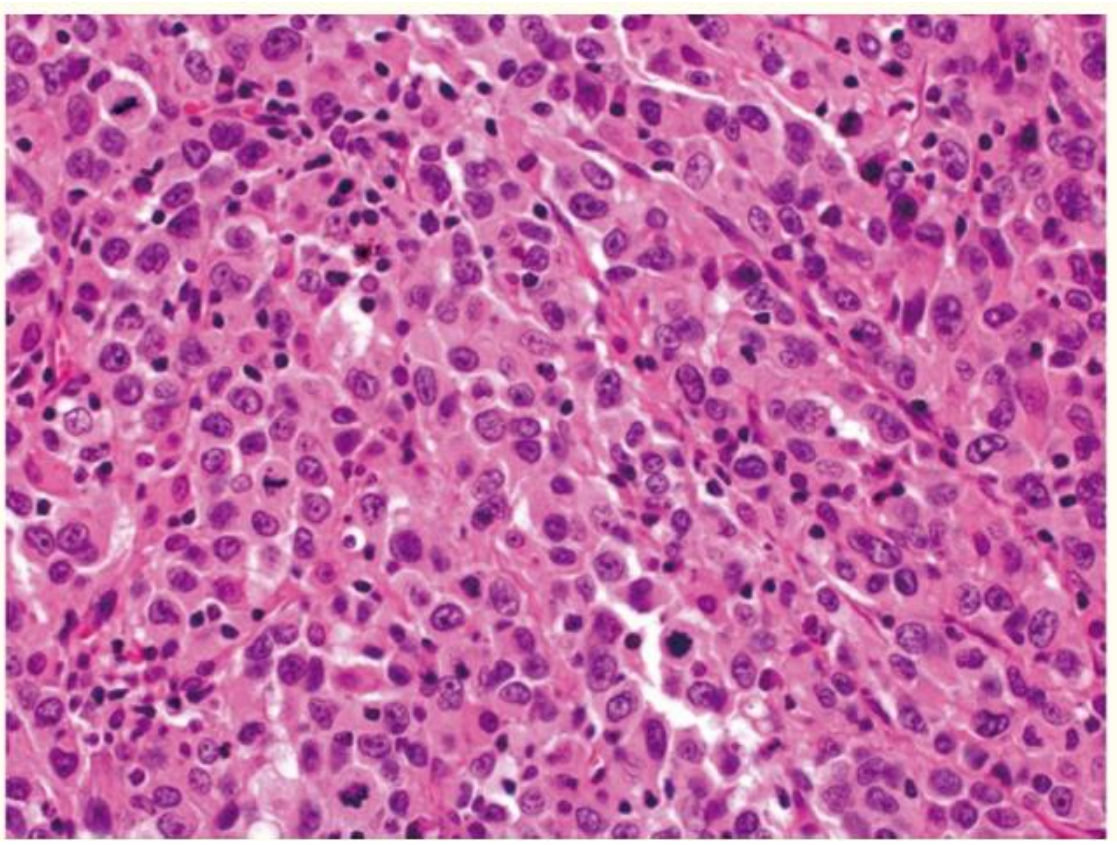


Figure 4: Medullary carcinoma (30)

Serrated adenocarcinoma

This tumor has an architectural similarity to a sessile polyp with glandular serration and low nucleus to cytoplasmic ratio. Three major patterns associated with these tumors namely serrated pattern, trabecular pattern and mucinous pattern. These tumors can be MSI-L, MSI-H, and can be associated with BRAF mutation and CpG island hypermethylation (34,35).

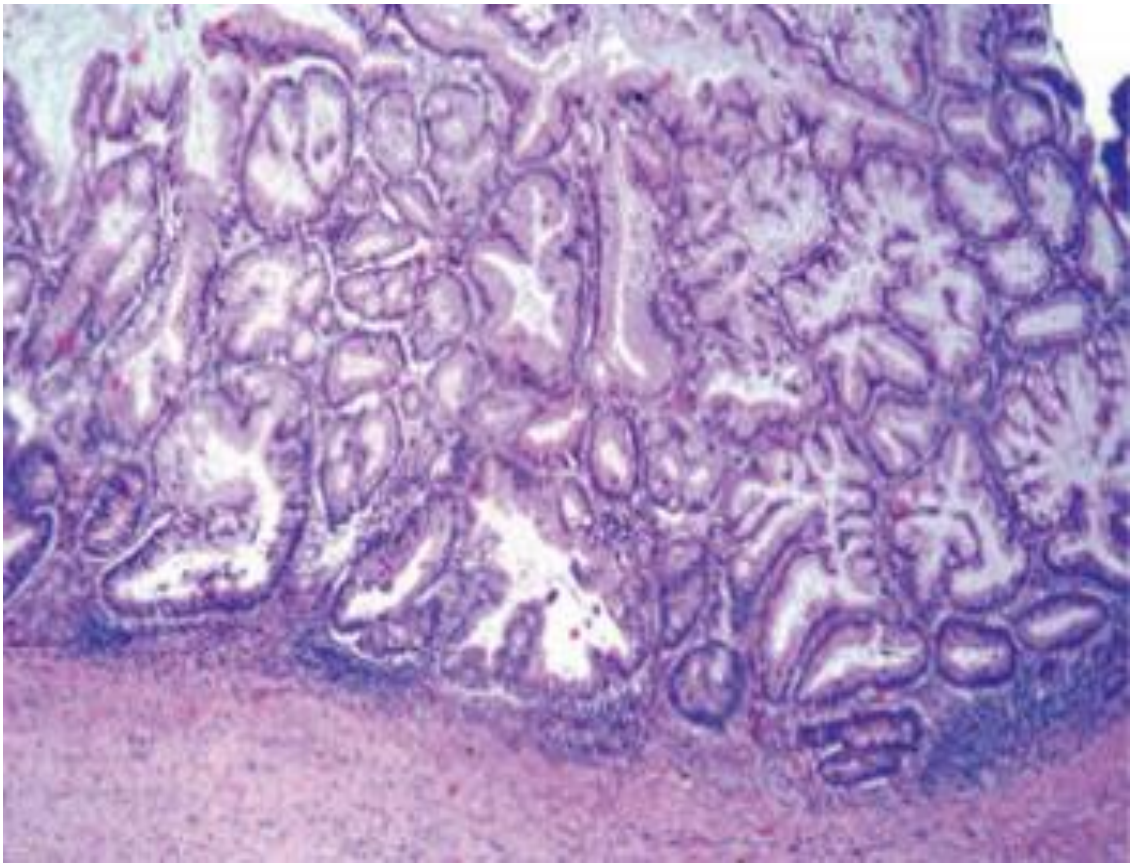


Figure 5: Serrated adenocarcinoma (30)

Cribriform comedo-type adenocarcinoma.

These rare tumors exhibit extensive large cribriform glands with central necrosis, which are usually microsatellite stable and show CpG island hypermethylation. They appear analogous to breast adenocarcinoma (36).

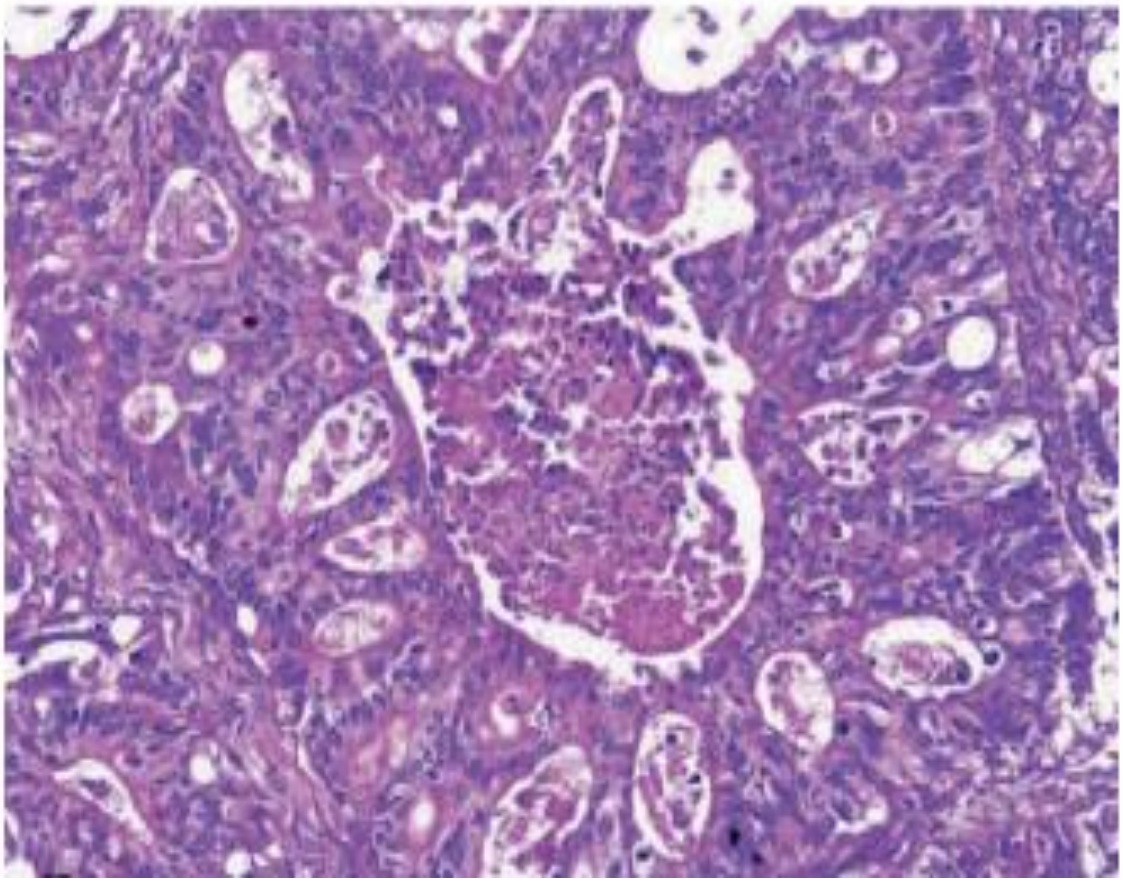


Figure 6: Cribriform comedo-type adenocarcinoma (30)

Micropapillary adenocarcinoma.

This variant appears as small clusters of tumor cells within stromal spaces mimicking vascular channels. This component is seen along with conventional adenocarcinoma. Characteristic MUC1 immunostaining is present in these tumors (37–39).

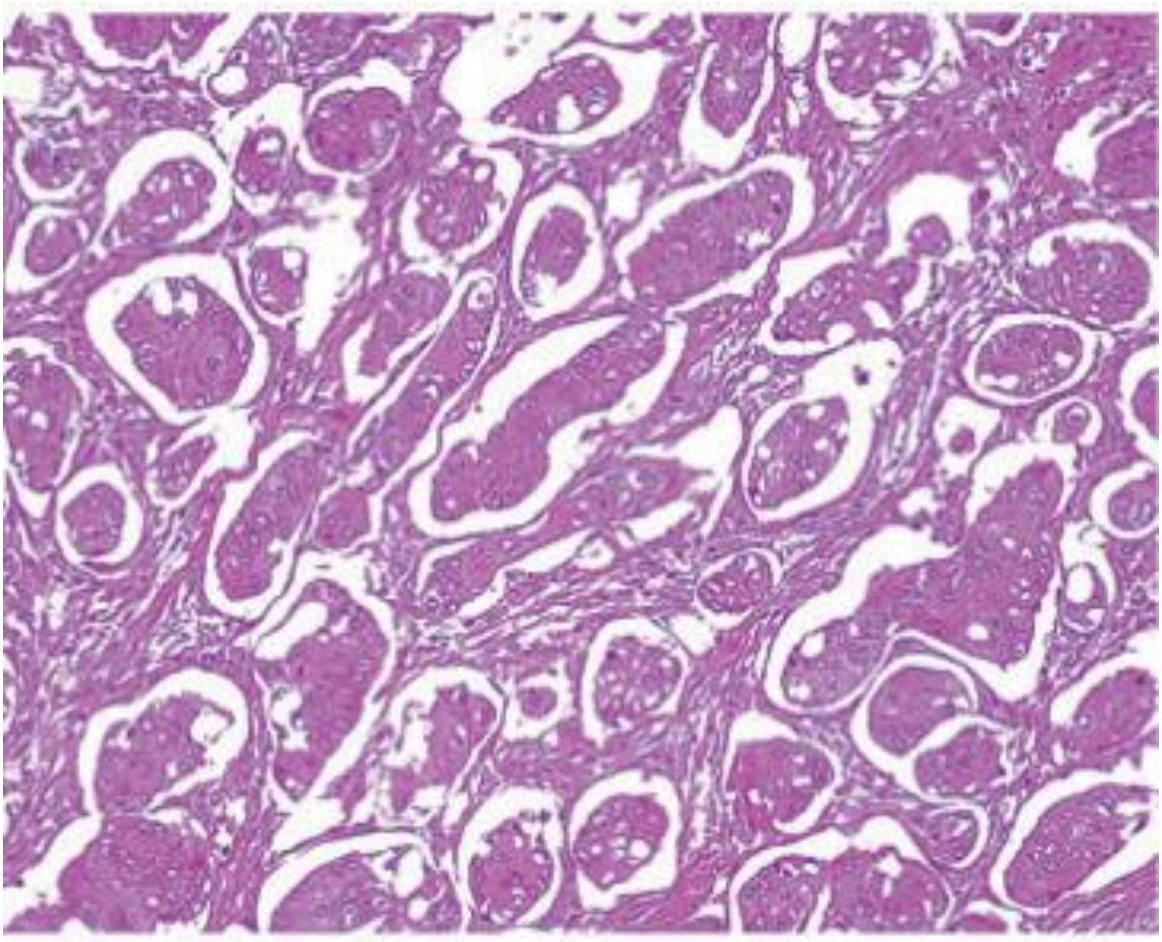


Figure 7: Micropapillary adenocarcinoma (30)

Spindle cell carcinoma

These are biphasic tumors with a spindle cell sarcomatoid component which is focally immunoreactive for keratins (40,41).

Adenosquamous carcinoma

These tumors are rare and show features of adenocarcinoma and squamous cell carcinoma, either mixed or separate areas in the same tumor (30).

Undifferentiated carcinoma.

These are rare tumors which lack morphological, immunohistochemical and molecular biological evidence of differentiation beyond that of epithelial tumors. Some of these tumors are MSI-H (30).

GRADING

Generally, colorectal carcinoma is graded as well-, moderately, poorly and undifferentiated adenocarcinoma on the basis of percentage of the glandular formation. Undifferentiated carcinoma is designated for malignant tumors with no glandular formation, mucin production, neuroendocrine, squamous or sarcomatoid differentiation. Clinically, the tumors are classified as high grade and low grade. Other morphological variants are not graded because of their own prognostic significance. If the tumors are heterogeneous, grading depends upon the least differentiated component (29,34,42,43).

Grade 1 – well differentiated (>95% gland formation)

Grade 2 – moderately differentiated (50-95% gland formation)

Grade 3 – Poorly differentiated (<50% gland formation)

Grade 4 – Undifferentiated (no gland formation or mucin, no squamous or Neuroendocrine differentiation)

PATHOLOGY STAGE (AJCC Staging Manual, 8th Edition)

Primary Tumor (pT)

___ pTX: Primary tumor cannot be assessed
___ pT0: No evidence of primary tumor
___ pTis: Carcinoma in situ, intramucosal carcinoma (involvement of lamina propria with no extension through muscularis mucosae)
___ pT1: Tumor invades the submucosa (through the muscularis mucosa but not into the muscularis propria)
___ pT2: Tumor invades the muscularis propria
___ pT3: Tumor invades through the muscularis propria into pericolorectal tissues
___ pT4: Tumor invades the visceral peritoneum or invades or adheres to adjacent organ or structure
___ pT4a: Tumor invades through the visceral peritoneum (including gross perforation of the bowel through tumor and continuous invasion of tumor through areas of inflammation to the surface of the visceral peritoneum)
___ pT4b: Tumor directly invades or adheres to adjacent organs or structures

Regional Lymph Nodes (pN)

___ pNX: Regional lymph nodes cannot be assessed
___ pN0: No regional lymph node metastasis
___ pN1: One to three regional lymph nodes are positive (tumor in lymph nodes measuring ≥0.2 mm), or any number of tumor deposits are present and all identifiable lymph nodes are negative
___ pN1a: One regional lymph node is positive
___ pN1b: Two or three regional lymph nodes are positive
___ pN1c: No regional lymph nodes are positive, but there are tumor deposits in the subserosa, mesentery, or nonperitonealized pericolic, or perirectal/mesorectal tissues.
___ pN2: Four or more regional lymph nodes are positive
___ pN2a: Four to six regional lymph nodes are positive
___ pN2b: Seven or more regional lymph nodes are positive

Distant Metastasis (pM) (required only if confirmed pathologically in this case)

___ pM1: Metastasis to one or more distant sites or organs or peritoneal metastasis is identified
___ pM1a: Metastasis to one site or organ is identified without peritoneal metastasis
___ pM1b: Metastasis to two or more sites or organs is identified without peritoneal metastasis
___ pM1c: Metastasis to the peritoneal surface is identified alone or with other site or organ metastases

AJCC Stage Groupings

Stage 0	Tis	N0	M0[#]
Stage I	T1	N0	M0
	T2	N0	M0
Stage IIA	T3	N0	M0
Stage IIB	T4a	N0	M0
Stage IIC	T4b	N0	M0
Stage IIIA	T1-T2	N1/N1c	M0
	T1	N2a	M0
Stage IIIB	T3-T4a	N1/N1c	M0
	T2-T3	N2a	M0
	T1-T2	N2b	M0
Stage IIIC	T4a	N2a	M0
	T3-T4a	N2b	M0
	T4b	N1-N2	M0
Stage IVA	Any T	Any N	M1a
Stage IVB	Any T	Any N	M1b
Stage IVC	Any T	Any N	M1c

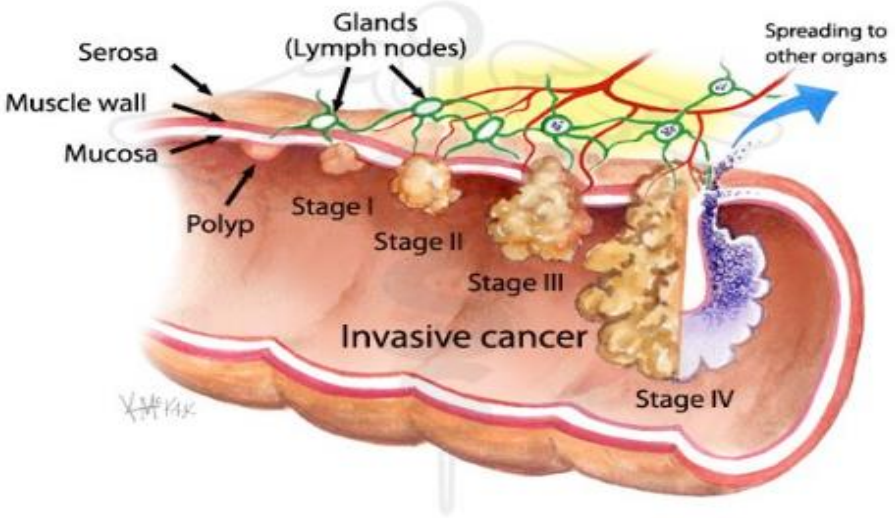


Figure 8: Stages of colon cancer

PRECURSOR LESIONS

Aberrant crypt foci (ACF)

ACF are abnormal crypt clusters seen on staining colorectal mucosa with methylene blue or chromoendoscopy. There are two main types: those which resembles hyperplastic polyps (common) and others with dysplasia (rare). Increased numbers of ACF has a close association with neoplasia.

Adenomas

According to WHO (2010) adenomas are defined by the presence of dysplastic epithelium. Enlarged, hyperchromatic nuclei, varying degree of nuclear spindling and stratification and loss of polarity are considered as features of dysplasia. Depending upon architectural complexity, the extent of nuclear stratification and pleomorphism, dysplasia can be grouped into the low and high grade. Grossly these adenomas are polypoidal, either sessile or pedunculated. A few ones have flat or depressed surface. There are mainly three architectural patterns, tubular, villous and tubulovillous types. Clinical significance of adenomas is their association with occurrence of synchronous and metachronous carcinoma. Adenomas of size larger than 1cm, extensive villous architecture, and high-grade dysplasia will be mostly associated with an invasive component or can transform into malignancy (44–46).

Serrated lesions

A heterogenous group of lesions morphologically characterized by serrated architecture in the epithelial component. They include hyperplastic polyp, sessile serrated adenoma/ polyp and traditional serrated adenomas (30)

Juvenile polyp.

Usually, juvenile polyps are seen in children. These lesions are characterized by abundant stroma composed of edematous, granulation tissue that surrounds cystically dilated glands containing mucin. Dysplasia is very uncommon in sporadic cases. In juvenile polyposis syndrome often have frond-like growth pattern with less stroma and many proliferated small glands, microtubular pattern and has increased the risk for dysplasia and malignant transformation (47,48).

Puetz- Jeghers polyp

These are hamartomatous polyps arising in the gastrointestinal tract, mainly in the small intestine, along with mucocutaneous melanin pigmentation. These polyps are a component of Puetz-Jeghers syndrome (49).

GENETIC SUSCEPTIBILITY

CRC cases with familial clustering are approximately 10-35% in which only <6% have high-risk germline mutation which predisposes to CRC associated syndromes. Familial adenomatous polyposis and Lynch syndrome (non-polyposis) are considered as high-risk genetic diseases.

Majority of these syndromes are autosomal dominant except MUTYH- associated polyposis which is autosomal recessive (50,51).

Tumours should undergo further molecular testing in the following situations:
Colorectal cancer diagnosed in a patient who is less than 50 years of age.
Presence of synchronous, metachronous colorectal, or other HNPCC-associated tumours,* regardless of age.
Colorectal cancer with the MSI-H** histology*** diagnosed in a patient who is less than 60 years of age.
Colorectal cancer diagnosed in one or more first-degree relatives with an HNPCC-related tumour, with one of the cancers being diagnosed under age 50 years.
Colorectal cancer diagnosed in two or more first- or second-degree relatives with HNPCC-related tumours, regardless of age.

*HNPCC-related tumours include colorectal, endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, and brain tumours, sebaceous gland adenomas and keratoacanthomas in Muir–Torre syndrome and carcinoma of the small bowel.
**MSI-H refers to changes in two or more of the five National Cancer Institute-recommended microsatellite markers.
***Presence of tumour infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern.

MOLECULAR PATHOLOGY

There are distinct pathological features, molecular signatures and natural histories for colorectal cancer and its precursors. There are mainly three pathway discovered which includes chromosomal instability (CIN), microsatellite instability (MIS) and CpG island methylator phenotype (CIMP) pathway. The predominant pathway (~ 85%) is contributed by the CIN pathway. Due to the understanding of the molecular pathology underlying colorectal carcinogenesis, a multi-tier approach is implemented for reducing the burden of CRC through earlier diagnosis of cancer and the detection and removal of benign polyp precursors. Patients with a past history of colorectal neoplasia or symptomatic patient are subjected to colonoscopy for diagnosis and surveillance.

Patients with familial colorectal syndromes are identified and managed by combined endoscopic and molecular test. Genomic instability is critical in carcinogenesis which enhances the neoplastic evolutionary process. The development of new mutations would be too slow for a carcinogenesis without genomic instability.

THE CHROMOSOMAL INSTABILITY (CIN) PATHWAY

About 70%-85% of colorectal cancers develop through the CIN pathway. In the CIN pathway, significant molecular aberrations occur through the accumulation of structural or numerical chromosomal abnormalities. Dysplastic aberrant crypt focus is the earliest identifiable lesion in this pathway, which is a microscopic mucosal lesion that precedes the development of polyp. The CIN pathway and its accompanying adenoma-carcinoma sequence have laid a foundation for the molecular classification of colorectal carcinogenesis but it is now proven that colorectal cancer can also develop by other pathways.

The mutation associated with the CIN pathway include *APC* gene, *KRAS* oncogene, loss of chromosome 18q and chromosome 17p deletion, which contains the tumor suppressor gene *TP53* (53–55)

APC, important tumor suppressor gene, plays a vital role in the CIN pathway. Mutation in APC gene truncate APC protein and prevents binding of APC to β-catenin which releases the suppression Wnt-signaling pathway. The purpose of *Wnt signaling* is to regulate growth, apoptosis, and differentiation. Loss of functional APC might also Influence the regulation of mitosis

contributing to CIN. APC mutation is seen in approximately 80% in early adenoma, 60% of colonic and 82% of rectal cancers.

KRAS (12p12) is one of the other important gene involved in the CIN pathway. *KRAS gene* encodes a GTP-binding protein which, when mutated, can cause inherent GTPase activity loss and thus constitutive signaling through the RAS-RAF-MEK-ERK pathway. *KRAS* is not particular to the CIN pathway, but *KRAS* has a significant role in the CIMP pathway as well. About 35-42% of colorectal cancer and in a similar number of advanced adenomas reveals *KRAS* mutations.

SMAD2 and *SMAD4*, located at18q21.1, are involved in the TGF-β signaling pathway, which regulates growth as well as apoptosis. About 60% of colorectal cancer is associated with allelic loss of these genes. Generalized juvenile polyposis syndrome due to germline mutation of *SMAD4* can cause colorectal cancer.

Lastly, impairment of *TP53* (17p13) by the allelic loss of 17p is usually a late event in the traditional pathway, from adenoma to adenocarcinoma. Either mutation or loss of heterozygosity causes *TP53* abnormalities which leads to the advancing histological stage of the lesion. About 50% of adenomas with invasive foci and 50-75% of colorectal cancer have impaired function of *TP53*. The p53, guardian of the cell cycle, increase the expression of cell-cycle genes and slow down the cell cycle, providing sufficient time for DNA repair. The p53 protein induces pro-apoptotic genes if the genetic damage is too high (52,53,56,57)

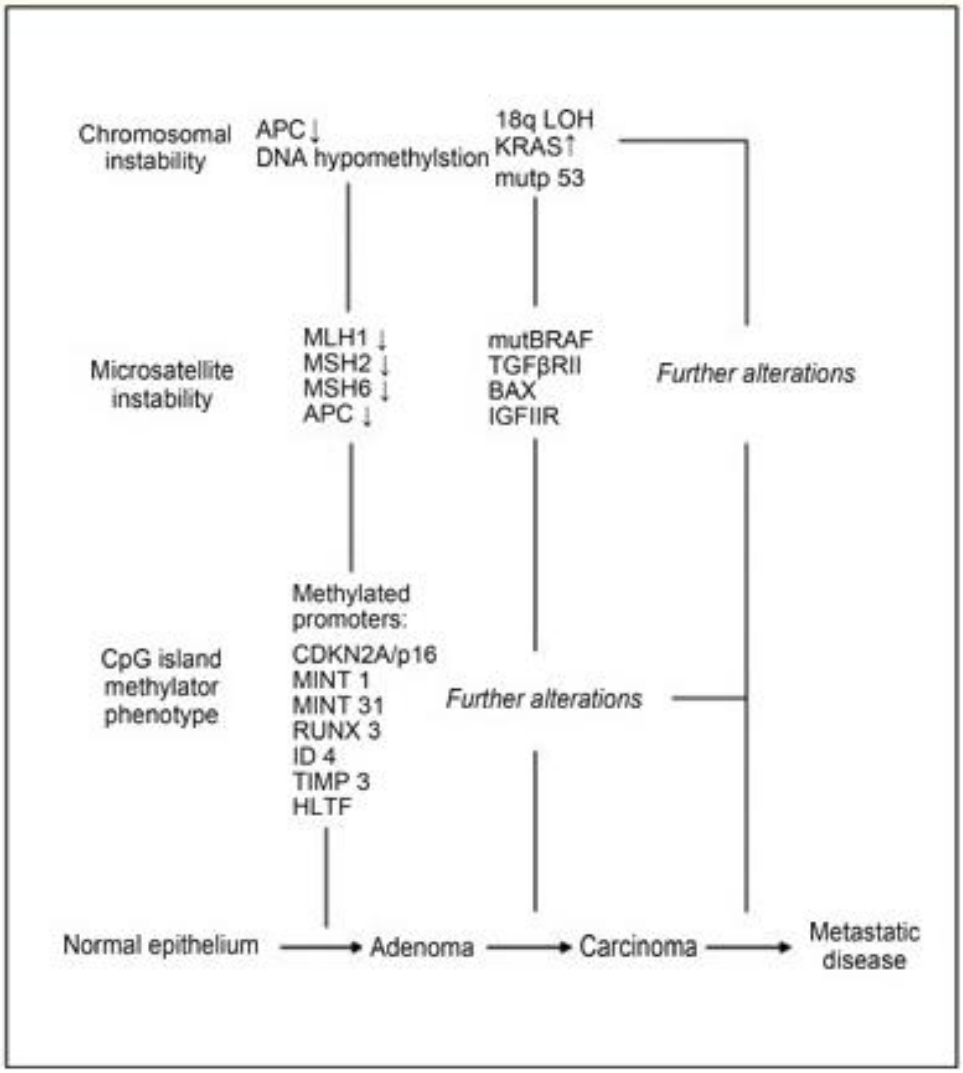
MICROSATELLITE INSTABILITY (MSI) PATHWAY

Microsatellites are certain nucleotide repeat sequences which are seen scattered throughout the genome. The discrepancy in the nucleotide repeat numbers found within these regions in tumor leading to microsatellite instability. Mismatch repair (MMR) dysfunction is caused when copying these repeat sequences by DNA polymerase enzyme, resulting in MSI. MMR system is composed of seven proteins, at least, including, MSH2, MSH3, MLH1, MLH3, MSH6, PMS1, and PMS2. MSH2 and MLH1 are main in the mismatch repair machinery and these form five functional heterodimeric proteins (MSH2-MSH6, MSH2-MSH3, MLH1-PMS1; MLH1-MLH3, MLH1-PMS2). In HNPCC, mutations in *MLH1, MSH2*, *MSH6,* and *PMS2*, have been seen. MSI-high (MSI-H) or considerable MSI is when MSI ≥2 (40%) of the five specified sites, MSI low (MSI-l), when MSI at one site, whereas microsatellite stable (MSS) when no instability is seen at these sites (52,57–59).

CpG ISLAND METHYLATOR PHENOTYPE PATHWAY (CIMP)

The second most common pathway to sporadic CRCs (15%). CIMP pathway leads to epigenetic instability by methylating the promoter regions of tumor suppressor genes such as *MLH1* and thus epigenetically inactivate expression of these genes. Currently, colorectal cancers with CIMP-positivity are defined by a panel of CpG island methylation marker that are classified on the basis

of stipulated thresholds to tumors having DNA methylation or not. There is no Gold-standard technique for methylation categorization for diagnosing CIMP. The recommended panel is of five markers including CACNA1G, IGF2, NEUROG1, RUNX3, and SOCS1. CIMP-positive tumors show predominantly cancers with a percent of methylated reference (PMR) of ≥10 at three or more gene promoter sites are categorized as CIMP positive. These tumors show association with BRAF and KRAS mutation. Few authors classify CIMP-positive group into CIMP low (or CIMP2) and CIMP-high (or CIMP1) group. CIMP2 colorectal tumors show a closer association with KRAS rather than BRAF mutation (52,57)



Studies on molecular pathology including CIN, MSI, and CIMP which often overlap in molecular tumor subtypes, has significant effects on prognosis. Initially, the tumors were categorized as MSI or MSS tumors. The MSS subgroup was further classified as CIN-only, CIMP-only, CIN+CIMP, and triple negatives ones. The MSI tumors show the lowest frequency for *KRAS* and *APC* mutations, the second lowest for *p53* mutations, and the highest for *BRAF V600E* mutations. The CIN-only tumors exhibited the lowest frequency for *BRAF V600E* mutations and the highest for *p53* mutations. These molecular subtypes based on MSI, CIMP, *BRAF*-mutation, and *KRAS*-

mutation status has a close association with patient survival outcome. The highest five-year disease specific survival (89.5%) is for the MSI-H tumors, followed by the MSI-L/MSS tumors (82.5%) without CIMP or *KRAS* and *BRAF* mutations, and the tumors with *KRAS* mutations (72.4%) only. The worst survival (49.2%) is for the tumors with CIMP and *BRAF* mutations. CIMP associated tumors have poor disease-free survival and overall survival rate irrespective of the MSI status.

The clinical significance of classifying tumors according to mutations are essential in determining the treatment regimen. The main line for treatment is the combination of 5-fluorouracil (5-FU) or capecitabine along with oxaliplatin or irinotecan. Targeted therapies for epidermal growth factor (EGFR) and vascular endothelial growth factor (VEGF) inhibitors have been found effective as both first and second line for CRC treatment. Studies suggest that anti-EGFR treatment is ineffective in tumors with codon 12 and 13 mutations in *KRAS*. Henceforth, anti-EGFR inhibitors are considered as contraindicated in those patients with mutant *KRAS. The BRAF*V600E mutation has poor disease-specific survival, which has resistance to anti-EGFR therapies even in the wild-type *KRAS* tumors (34,52,53,57,58,60)

PROGNOSTIC FACTORS

There are many prognostic factors which indicate a high risk for progression, recurrence, and resistance to therapy. Some of the significant prognostic markers are described below.

TUMOR MORPHOLOGY.

Tumor size- The tumor size has a direct association with the prognosis of CRC. Some studies indicate that if the primary tumor size is equal or more than 6.5 cm (≥ 6.5cm) is considered as bad

prognostic factor. Tumor size is considered a strong and independent risk factor in metachronous tumors. Large colonic tumors are generally associated with microsatellite instability (MSI). But tumor size has less significance when compared to the tumor stage (61)

Tumor budding- Studies have shown that tumor budding is considered as one of the strong adverse prognostic factors in colorectal cancer. Generally, tumor budding is a morphologic phenomenon seen at the invasive tumor front. It is characterized by isolated or small clusters of tumor cells (<5 cells) which separated from the main tumor and migrate into desmoplastic stroma for a short distance. It represents localized tumor dedifferentiation which is recognized as a significant component in the metastatic process.

Tumor grade – Poorly differentiated and undifferentiated (grade 4) tumors are considered as important prognostic factors. Usually, these tumors have an aggressive behavior and have an increased chance for recurrence and metastasis. Poorly differentiated clusters (PDC) are defined as clusters containing more than or equal to five cancer cells (≥5 cells) present at tumor invasive front without any glandular differentiation. PDC is considered an evolution of tumor bud. They are strongly associated with lymphovascular invasion and lymph node metastasis. Histological variants like signet ring cell carcinoma, mucinous adenocarcinoma (in absence of MSI-H), small cell carcinoma and adenosquamous carcinoma have a poorer prognosis. Medullary-type associated with diploid status, MSI-H has reduced nodal involvement and has improved survival (55,62,63).

Tumor stage – AJCC tumor node metastasis (TNM) stage remains the gold standard of prognostic factors in colorectal cancer. Initially, the TNM staging system was implemented to predict the prognosis of the disease, but now, they aid in planning treatment protocols also. For stage III

disease adjuvant therapy is accepted but for stage II tumors adjuvant therapy is not yet recommended as a treatment protocol.(23, 34, 52, 55)

Lymphovascular and perineural invasion – These parameters are significant prognostic factors. Extramural venous and lymphatic invasion leads to the formation of micro-metastases and causes metastatic tumor deposits. They also indicate a higher risk of regional recurrence after surgical resection and nodal involvement. They are also associated with decreased overall survival in metastatic disease (63,65)

Lymph node involvement – This indicates tumor metastasis, upgrades stage of the tumor and renders a bad prognosis. They also play an important role in deciding treatment protocol, particularly adjuvant chemotherapy. Patients with node-negative disease have a better 5-year survival rate of 70%-80%. Hence obtaining adequate numbers of lymph nodes is considered a crucial role for accurate staging(63,66).

Tumor-infiltrating lymphocytes – There is an improvement of overall and disease-specific survival rate if the tumor and peritumoral stroma are infiltrated by many CD3 and CD8 T cells (63).

Other potential predictors are extend of resection, margin involvement, bowel obstruction or perforation, age older than 70 years, preoperative and postoperative serum carcinoembryonic antigen (CEA) levels, preoperative carbohydrate antigen 19-9 (CA19-9) levels, tumor location and infiltrative growth pattern (64,67,68).

PROGNOSTIC AND PREDICTIVE GENES

Many studies suggest molecular or immunohistochemical prognostic markers for CRC but none of them are adopted into routine practice. The lack of marker utility is due to the complexity of CRC with molecular subgroups which have different biological behavior but often shares markers. At present, mutations involving BRAF, PIK3CA, allelic imbalance at 18q and overexpression of osteopontin, CXCL12 and CD133 has a poorer prognosis (69–71).

Predictive gene and biomarkers indicate the likelihood of tumor resistance or response to therapies. Hence specific targeted chemotherapeutic agents can be used for the therapy. Most of CRC express EGFR on the cell surface, hence use of anti-EGFR (cetuximab & panitumumab) are effective for a treatment regimen. However, EGFR signals work through RAS/RAF pathway. Hence, if there is a mutation in the downstream KRAS, the tumors will be unresponsive for the anti-EGFR therapy. Therefore, testing for KRAS mutation (codon 12/13, 61 & 146) is essential for CRC therapy. Mutation of PIK3CA and loss of PTEN also are adverse predictive markers. Expression of epi- and amphiregulin ligands and EGFR amplification are favorable predictive markers. MSI-H has a poor response to 5-fluorouracil and oxaliplatin. In the absence of mismatch repair mechanism, administration of these agents do not cause apoptosis and causes resistance. Administration of irinotecan has a favorable outcome in MSI-H tumors. Hence detection of these predictive genes is now becoming an inevitable part of the treatment of CRC (56,58,60,72).

CAUDAL-TYPE HOMEOBOX TRANSCRIPTION FACTOR 2 (CDX2).

The human *Cdx1* and *Cdx2* genes are mammalian homologs of *Drosophila* homeobox-containing gene *Caudal* (73) located in chromosomes 13q12-13 (74). These genes encode transcription factors which are expressed largely restricted to the epithelium of the gut mainly throughout the small and large intestine. Studies also reported that *Cdx*1 gene expression exhibit a gradient, the highest level caudally, whereas *Cdx2* expression is maximum at proximal colon and reduces caudally. *Cdx* genes play an important role in epithelial positional along the rostral-caudal axis of the gastrointestinal tract. Many in vitro studies have shown that CDX2 overexpression in gut epithelial cells results in growth arrest along with upregulation of several genes associated with differentiation of intestinal epithelium (73–75). Intestinal differentiation by CDX2 is by activating transcription of intestine-specific proteins, like MUC2, isomaltase, carbonic anhydrase I and sucrose (74,75). The current paradigm is that *Cdx2* Is considered as a master "control gene" regarding intestinal epithelial differentiation (75). In rat and mice, disease models exhibit decrease In *Cdx2* expression as the tumor upgrades in humans, that is when the tumor grade increases expression of *Cdx2* decreases (73,74). Hence, there is an inverse correlation between tumor grade to CDX2 staining (74). High-grade adenomas display reduced CDX2 expression when compared with low-grade adenomas or even normal tissue. This reduction is not completely attributed to mutation of the *Cdx* alleles but small deletions within both *Cdx2* alleles is noted in replication error human colorectal cancer (73). Hamartomatous polyps arise when there is one copy of the *Cdx2* gene is eliminated in mice (75), hence proving tumor suppressor quality of this gene (76). Poorly differentiated colon cancer has a higher proportion of CDX2-negative cases than differentiated

counterparts. The CDX2 expression is graded according to the percentage of positive cells as 0(negative), 1+ (<25%), 2+ (25-75%) and 3+ (>75%) (76).

There is an association with lymph node metastasis. In invasive front of carcinoma, if CDX2 is negative leads to transient tumor cell differentiation defect that triggers dissemination of tumor cells through the blood and lymphatic vessels. Therefore, CDX2 plays a pivotal role in adenoma growth and malignant transformation and also the progression of colorectal cancer (74). High levels of CDX2 is also seen in neuroendocrine tumors derived from intestinal epithelium, hence these also play an important role in normal neuroendocrine cell differentiation. Other than intestinal adenocarcinomas, CDX2 nuclear staining is also seen in gastroesophageal adenocarcinomas (20%), ovarian adenocarcinomas and uterine endometriosis (20% & 4.3%), mucinous adenocarcinomas (20%) and prostatic adenocarcinoma (1%). Other significant uses of CDX2 staining are in the workup of metastatic tumors at other sites and aids in well-differentiated neuroendocrine tumor differential diagnosis (75).

Regarding the prognosis of CRC, CDX2 negative tumors are associated with bad prognosis with regards to five-year disease-free survival period. For CDX2 negative tumors have a lower rate of survival regardless of low or high pathological grade. Some studies have shown that stage II CDX2 negative tumors have a lower rate of five-year disease-free survival than stage II CDX2-positive tumors with respect to overall survival. CDX2 status not only aids in assessing the prognosis but also helps in planning treatment regimens. Few studies reveal a higher rate of disease-free survival for CDX2 negative tumors with adjuvant chemotherapy in stage 2 CRC (77). Prognostic biomarkers are those parameters which aid in risk stratification and in decision to recommend

adjuvant chemotherapy. At present, tumor grade, tumor stage, lymphovascular and perineural invasion, lymph node metastasis and microsatellite instability are significant prognostic variables in CRC for development of treatment algorithms. Previous studies had shown that CDX2 negative tumors are associated with several adverse prognostic variables like an advanced stage, poor differentiation, vascular invasion, BRAF mutation, and CIMP-positive status. Hence CDX2 is considered as an independent prognostic factor in CRC (77).

TREATMENT

The medical treatment of colorectal cancer was not well defined with little or no progress until the early 1990s. The establishment of effective adjuvant chemotherapy and the treatment of advanced cancer has improved gradually since then. Over the past few years, colorectal cancer has had many new developments in adjuvant chemotherapy. Most of these new treatments are still undergoing phase III trials and have not yet been included in the standard protocol for treatment. However, if these therapies are proved effective, they will open a new door for cancer treatment in the future (78).

Currently, the treatment protocol is dependent on the stage of the disease and the main goal is to remove the tumor with adequate clearance. Surgery is the primary treatment modality by which this is achieved. The usual surgical approaches for colon carcinoma include total, partial, or segmental colonic resection. Generally speaking, Stage 0 & I tumors require only resection and no further adjuvant therapy.

Stage 0:

In stage 0 CRC, the tumor is limited to the colon wall and the main aim is to remove the tumor with adequate margin. If the tumor is polypoid and small in size, the mode of surgery implemented is polypectomy, usually done endoscopically. If the tumor is larger, removing a segment of the colon (fractional colectomy) is required. There is no increased benefit to the administration of chemotherapeutic agents. On the other hand, these agents may cause other adverse effects to the patients.

Stage I:

Stage I colon disease refers to those tumors that have infiltrated into the colonic wall, up to the submucosa and have not spread outside the colon. The standard treatment is surgery by removing the affected segment of colon and neighboring lymph nodes. Adjuvant therapy is not recommended.

Stage II:

Stage II colon tumors may be of a larger size, but are still limited to the colonic serosa and have not yet spread to the regional lymph nodes. The mainstay of treatment is surgery, either segmental colectomy along with regional lymph node dissection (incomplete colectomy) or total colectomy. This decision is based on the size and location of the disease. Lymph node dissection is considered adequate when a minimum of twelve lymph nodes is found.

Adjuvant chemotherapy is needed if the tumor has a higher risk for local recurrence or metastasis. The increased risk is seen in tumors with a large size, lymphovascular invasion, perineural

invasion and those involving the serosa. A few studies have shown that CDX2 immunohistochemistry negativity is an indication for adjuvant therapy.

Stage III:

Stage III colon tumors are those that have spread into the regional lymph nodes but had not yet metastasized. Surgery followed by adjuvant chemotherapy is the standard treatment for tumors of this stage. For adjuvant chemotherapy, either the 5-Fluorouracil, oxaliplatin or capecitabine regimens are utilized frequently. However, in recent days, chemotherapeutic agents are more often decided based on mutational studies. Radiation treatment and/or chemotherapy might be used for individuals who are not amenable to surgery.

Stage IV:

Stage IV colon tumors are those that have metastasized to various local or distant organs and tissues. The liver is one of the most common sites for metastasis in colon cancer. Other sites of metastatic disease include lungs, peritoneum, and distant lymph nodes. The majority of advanced colonic tumors are not amenable to surgery. In a few cases, however, surgery is still performed in order to prevent or manage complications. These surgeries may be colectomies, redirecting colostomies, or de-bulking procedures. In addition, radiotherapy and palliative chemotherapy may be implemented for stage IV disease if the need arises. Metastasectomy may be done for solitary, resectable liver or lung metastasis (80–84).

Fluorouracil has been the cornerstone of colorectal medical treatment for nearly 40 years. Following this, an increased disease-free and overall survival in stage III colon cancer were seen when a combination of fluorouracil and levamisole was administered. Subsequently, many studies have shown that the combination of fluorouracil with folinic acid has a similar benefit to fluorouracil with levamisole, with less toxicity. Further fluorouracil and folinic acid are administered for a period of six months, as opposed to fluorouracil and levamisole combination which is given for a year. The fluorouracil and folinic acid has been shown to increase the five-year survival rate by an average of 5-10%, which represents a 25-35% reduction in mortality, and has now become a part of the standard treatment protocol.

For stage II disease, the role of adjuvant chemotherapy has been inconsistent and chemotherapy is administered depending on other parameters like tumor size, depth of tumor, tumor differentiation and lymphovascular invasion. (78)

Irinotecan and oxaliplatin are two newer drugs which are in phase III trials and have been to report to double the chemotherapy response rate and increase the progression-free survival. Oxaliplatin has also proved to downsize liver metastases, hence enabling surgeons to perform a curative resection in patients whose tumor was previously considered inoperable. Bevacizumab, an anti-VEGF (vascular endothelial growth factor) monoclonal antibody has recently been licensed for the first-line treatment of metastatic colon cancer by the US Food and Drug Administration. Cetuximab, a new antibody to the epidermal growth factor receptor (EGFR) is an upcoming chemotherapeutic agent for colorectal cancer. (78).

The monoclonal antibodies have an immunomodulatory activity which acts through the host's immune system to fight against the tumor cells. When immunotherapy is administered along with

conventional chemotherapy, there is a synergistic effect on cancer cells, inducing tumor cell death, eliminating regulatory T cells, and enhancing tumor cell sensitivity to lysis by cytotoxic T lymphocytes. These therapies are still under phase III trials(78,79).

JUSTIFICATION FOR THIS STUDY

Colorectal cancer is one of the leading cause for mortality and morbidity worldwide and also in the Indian subcontinent and the rate of incidence is on the higher side, especially in young adults. Early detection and treatment have a major role in overall survival and disease-free survival rates. There are many prognostic factors and genetic biomarkers in order to assess the course of the disease. Recent studies show that CDX2 biomarker negative tumors had a bad histological prognostic factor and also a worse survival outcome. There are a few studies on CDX2 biomarker expression in association with a prognosis of colorectal cancer and yet another study has shown that stage2 CDX2 negative tumors had benefitted from adjuvant therapy. Hence, this study aims to assess clinical and histopathological features of colonic malignancy and to analyze the immunohistochemical expression of CDX2 and correlate the latter with histological factors of prognostic importance like the stage, grade of the tumor, lymphovascular invasion and lymph node status in the resection specimens of colonic adenocarcinoma in a tertiary care referral hospital.

MATERIALS AND METHODS

STUDY SETTING

This study was conducted in the department of General pathology on 148 consecutive mucosal biopsies with sufficient tumor and corresponding resection specimens of colonic adenocarcinoma diagnosed from January 1, 2015, to June 30, 2018. The clinicopathological details were retrieved from the electronic database and were reviewed systematically. The hematoxylin and eosin stained slides were reviewed for classification, grading, and staging. Immunostaining for CDX 2 was performed on freshly cut sections. The positive staining was graded for intensity and percentage of positive tumor cells. CDX 2 expression of the tumor cells were correlated with age, gender, clinical features, anatomical site, classification, grading and staging of the tumor including metastasis.

RESEARCH BUDGET PLAN

Institutional review board (IRB) Minutes number: **10624** approved our study. Interdepartmental collaboration between General Pathology and General Surgery significantly improved the quality of our research. The institutional Fluid Research grant account number (**22 Z 294**) was used to cover the costs of immunohistochemical staining.

SAMPLE SIZE

The aim of the study is to know the prevalence of CDX 2 expression in colonic adenocarcinoma by immunohistochemistry and to correlate the expression CDX 2 with the clinicopathological parameters including stage and grade of colon cancer. Patients who had consecutive mucosal biopsies with sufficient tumor and corresponding resected specimens of colonic adenocarcinoma diagnosed in our hospital from January 1, 2015, to June 30, 2018, were recruited into our study. Preliminary analysis showed that the sample size required was 144 cases to meet the objectives of the study.

INCLUSION CRITERIA

All patients diagnosed as primary adenocarcinoma of the colon from January 1, 2015, to June 30, 2018, on endoscopic mucosal biopsies and had resection following that performed in our institution.

EXCLUSION CRITERIA

1) Patients who had undergone biopsy for recurrent tumors.

2) Patients with secondary metastatic disease to the colon.

3) Primary colon malignancy diagnosed on endoscopic biopsy and did not have a resection following a diagnostic biopsy.

4) Inadequate tissue for immunohistochemistry.

DATA SOURCES/MEASUREMENT

The clinical details of the patients were obtained from the charts retrieved from the Medical Records Department and from the electronic database.

QUANTITATIVE VARIABLES

The variables analyzed in this study are listed in the proforma (see Annexure 1).

Immunohistochemical marker were graded according to the score provided in the literature review.

STATISTICAL ANALYSIS

All study data was analyzed at first for descriptive statistics relating to demography and prevalence. Pearson's Chi-square test was used to assess associations, using the SPSS software, where a P value of <0.05 was considered statistically significant

METHODOLOGY

This study utilized 148 matched mucosal biopsies and the corresponding surgical resections of the patients who were diagnosed with colonic adenocarcinoma in the Department of Pathology from January 1, 2015, to June 30, 2018. The slides and blocks of these cases were retrieved from the Archives. CDX2 immunohistochemistry (clone DAK-CDX2) using Detection Kits, in combination with a VENTANA automated slide stainer Benchmark XT, reduced the possibility of human error and inherent variability resulting from manual pipetting and manual reagent application. The technical procedure as described in Appendix-1.

RESULT

A total of 214 cases were included which were mucosal biopsy proven resection specimen reported as adenocarcinoma colon from January 2015 to June 2018. The archived slides and blocks were retrieved from the pathology records, Department of General Pathology. Of these cases, 148 cases were included in the study according to the inclusion and exclusion criteria. Thirty-three cases were eliminated as the mucosal biopsies were slides and blocks from elsewhere, seven cases had slides for mucosal biopsy but did not have a block and two cases had very scanty tumor, difficult to evaluate in mucosal biopsy while taking further sections for immunohistochemistry. One of the cases of adenocarcinoma colon was eliminated according to exclusion criteria since it was metastatic disease. Twenty-three cases were not included because of lack of block for further immunohistochemical examination. The clinicomorphological and immunohistochemical features were analyzed for all 148 cases.

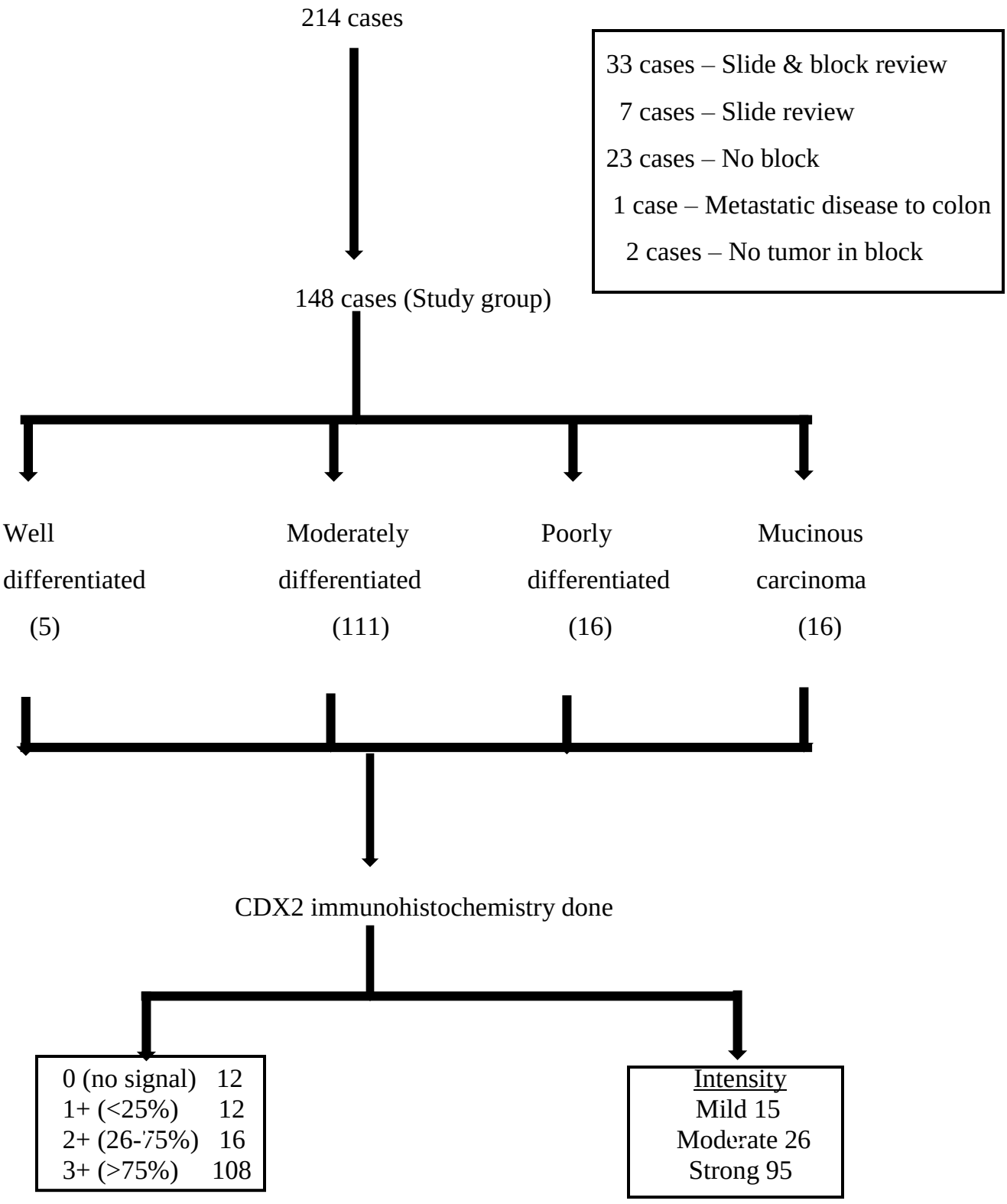


Fig 35: Selection of cases included under study

AGE

The median age at diagnosis for all cases of colonic adenocarcinoma was 53 years (26-88 years) with a standard deviation of 12.33. The youngest patient was 26 years and the oldest patient was 88 years old (Fig 9).

Table 1: Age distribution in colon cancer

AGE (IN YEARS)	NUMBER OF CASES (n=148)
<20	0
21-30	1
31-40	17
41-50	48
51-60	34
61-70	37
71-80	7
>80	4

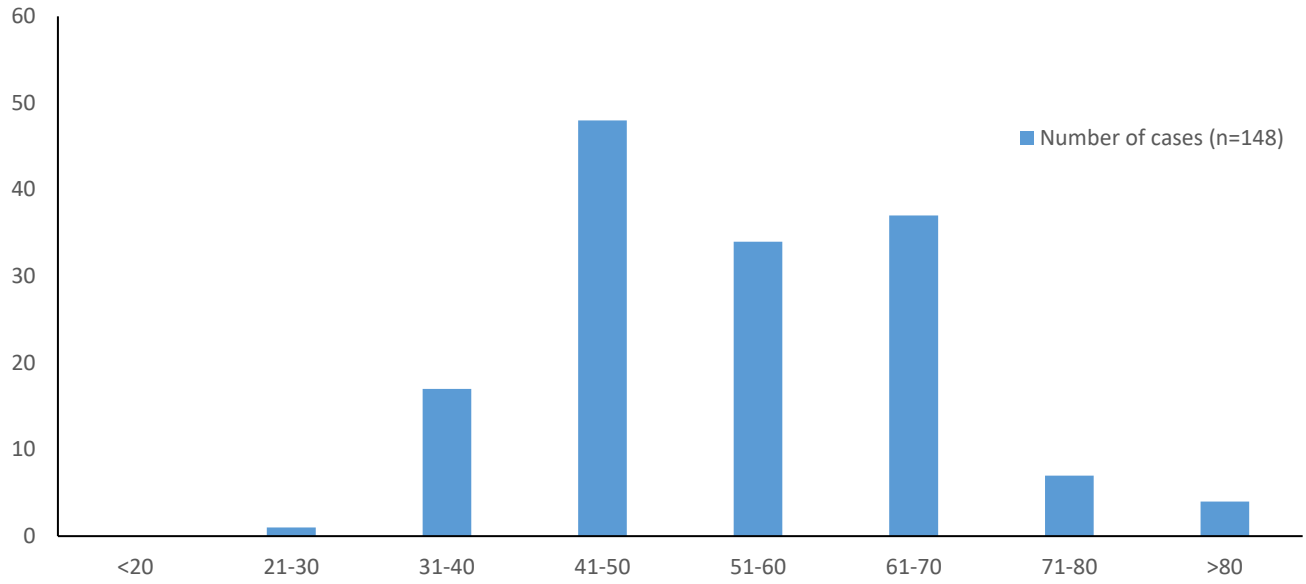


Figure 9: Graphical representation of age distribution in colon cancer

GENDER

Of the 148 cases, 106cases were males, 42 were females. The distribution of cases showed a male preponderance. (Fig.10)

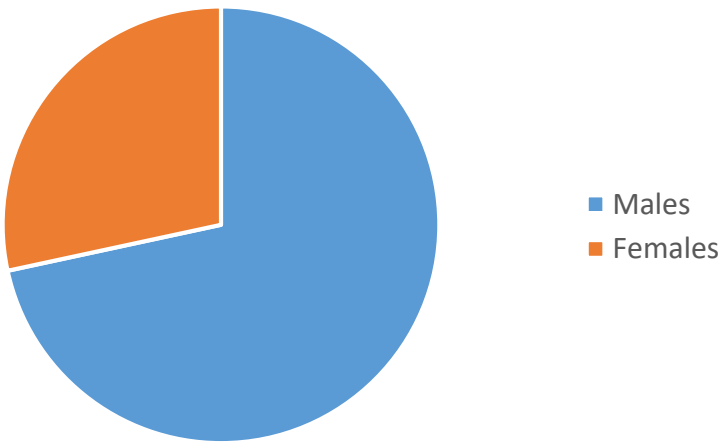


Figure 10: Gender distribution in colon cancer

CLINICAL FEATURES

Most of the patients presented with abdominal pain as a major symptom (27%). The rest of the patients presented with constitutional symptoms like loss of appetite and weight, altered bowel habits, bleeding per rectum and anemia. A minority of patient presented with perforation peritonitis and obstruction. (Table 2)

Table 2: Clinical presentations of Colon cancer

CLINICAL PRESENTATION	NUMBER OF PATIENTS (n=148)
Abdominal pain	40
Loss of appetite & weight	28
Altered bowel habit	27
Bleeding per rectum	26
Anemia	15
Tenesmus	7
Abdominal mass	3
Perforation peritonitis	1
Intestinal obstruction	1

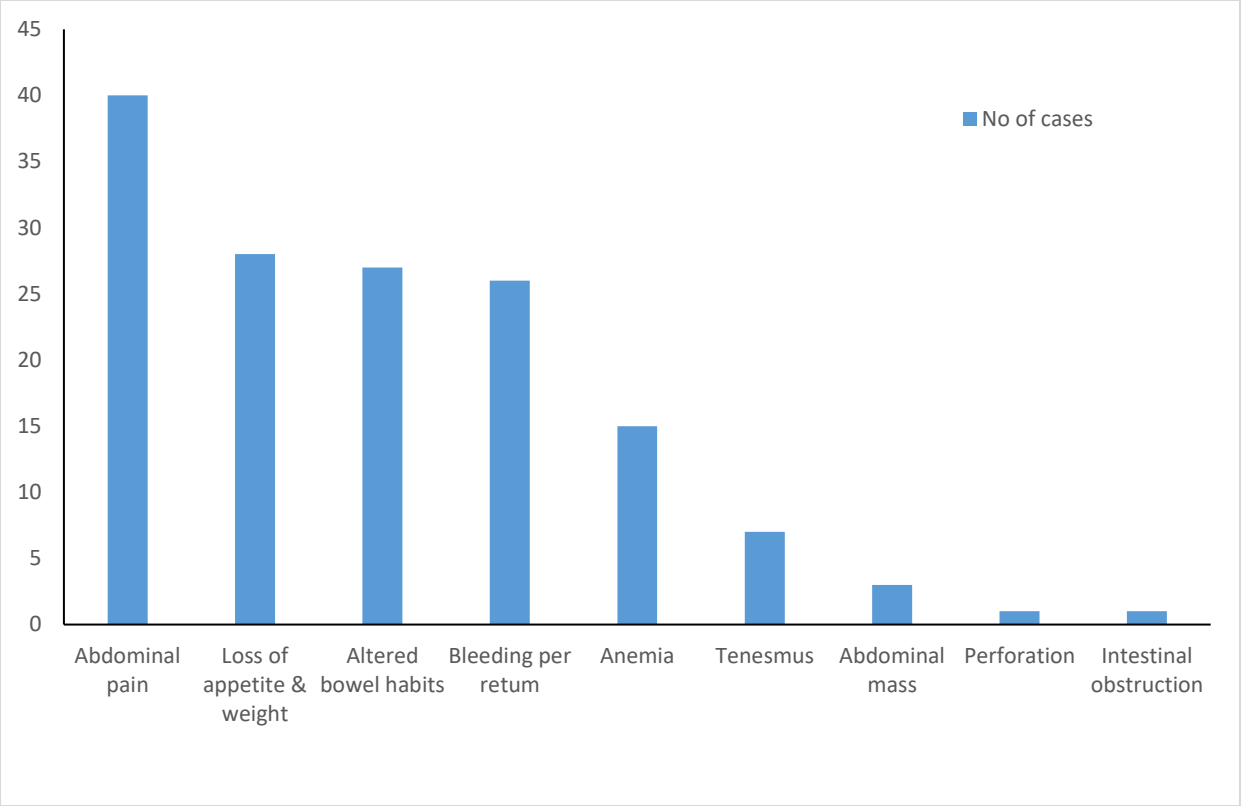


Figure 11: Graphical representation of the clinical presentation of Colon cancer

SITE

The most common site involved was the ascending colon (48), followed by sigmoid colon (43). The least involved site was descending colon. Hence, in this study the right sided colon cancer was found to be more when compared to left side colon cancers.

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Table 3: Anatomical sites of colon cancer

SITE	NUMBER OF PATIENTS (n=148)
CAECUM	33
ASCENDING COLON	48
TRANSVERSE COLON	17
DESCENDING COLON	7
SIGMOID COLON	43

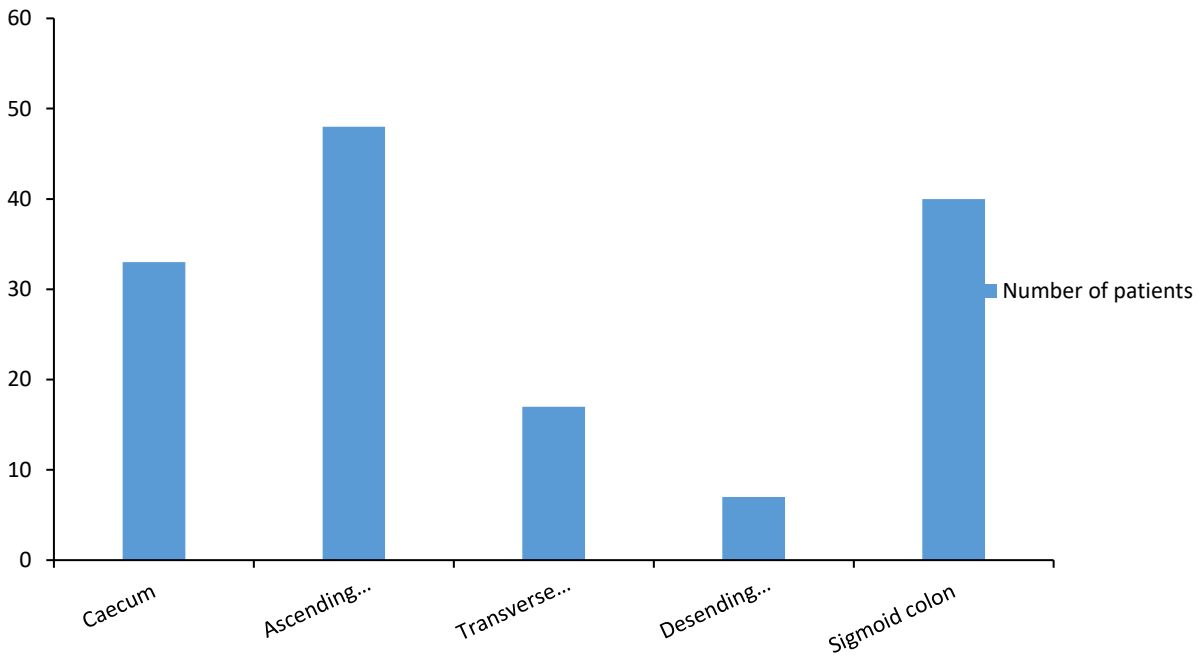

Figure 12: Graphical representation of anatomical sites of colon cancer

TUMOR SIZE

The tumor size ranges from 1cm to 13cm in maximum dimension with a median of 5.5cm and standard deviation of 2.25.

TUMOR GROSS MORPHOLOGY.

Ulceroproliferative tumors (104) are the most common gross morphology out of the other five types encountered in this study. The least one is annular, constrictive type (5).

Table 4: Gross morphology of colon cancer

GROSS MORPHOLOGY	NUMBER OF PATIENTS (n=148)
Ulceroproliferative	104
Polypoid	26
Ulcerative	13
Annular constriction	5

TUMOR HISTOLOGY

The 148 cases were classified into one of the following histological types according to the WHO - Well differentiated, moderately differentiated, poorly differentiated and mucinous carcinoma. Majority of the tumor were classified as moderately differentiated adenocarcinoma (111 in number). The poorly differentiated and mucinous carcinoma had 16 cases each.

Table 5: Histological subtypes of colon cancer

HISTOLOGY	NUMBER OF PATIENTS (n=148)
Well-differentiated adenocarcinoma	5
Moderately differentiated adenocarcinoma	111
Poorly differentiated adenocarcinoma	16
Mucinous carcinoma	16

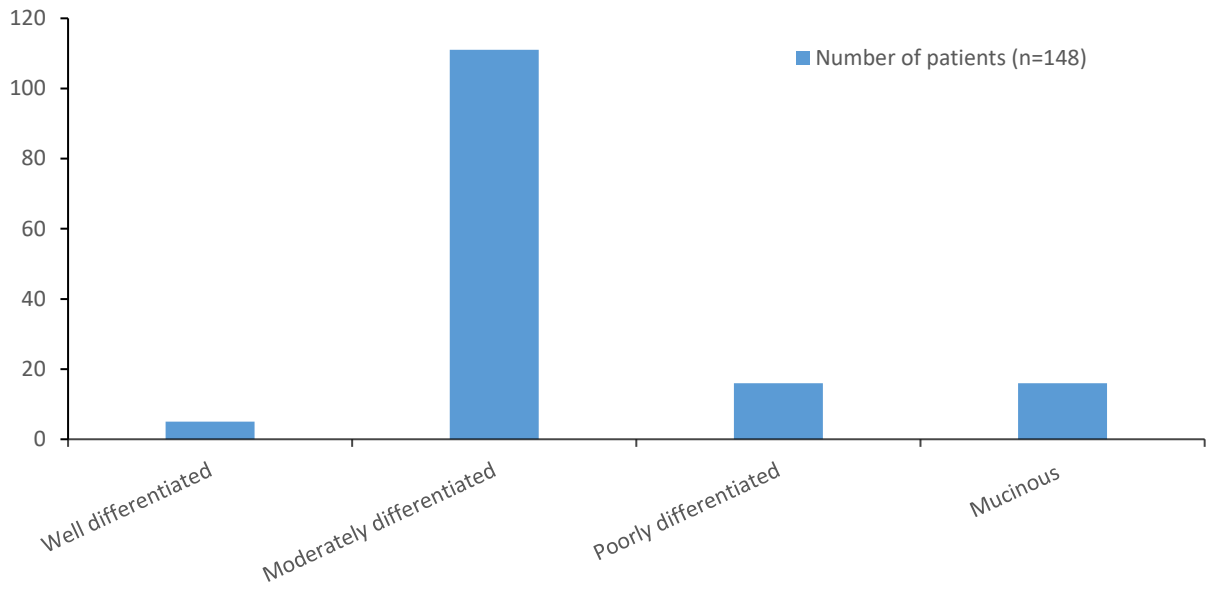

Figure 13: Graphical representation of the histological subtypes of colon cancer.

PATHOLOGICAL STAGING

PRIMARY TUMOR

Primary tumor invasion depends on the depth of tumor infiltration. pT3 constituted about 59% of total cases, followed by T4a (22%) and pT1 (1%) had the least cases.

Table 6: Primary tumor invasion

PRIMARY TUMOUR INVASION	PERCENTAGE OF CASES
pT1	1
pT2	11
pT3	59
pT4a	22
pT4b	7

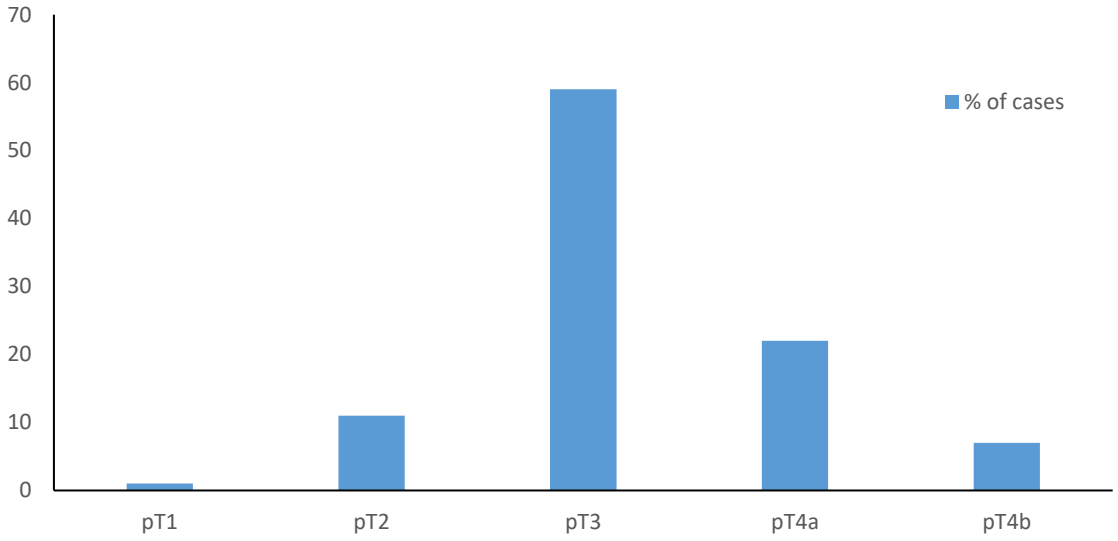

Figure 14: Graphical representation of primary tumor invasion.

REGIONAL LYMPH NODES

Most of the tumor did not have regional lymph node metastasis (52%). Among the remaining lymph node metastasis cases, pN1b (2-3 regional lymph node metastasis) comprises 18% of cases.

Table 7: Regional lymph node metastasis.

REGIONAL LYMPH NODES METASTASIS	PERCENTAGE OF CASES
pN0	52
pN1a	14
pN1b	18
pN1c	8
pN2a	12
pN2b	5

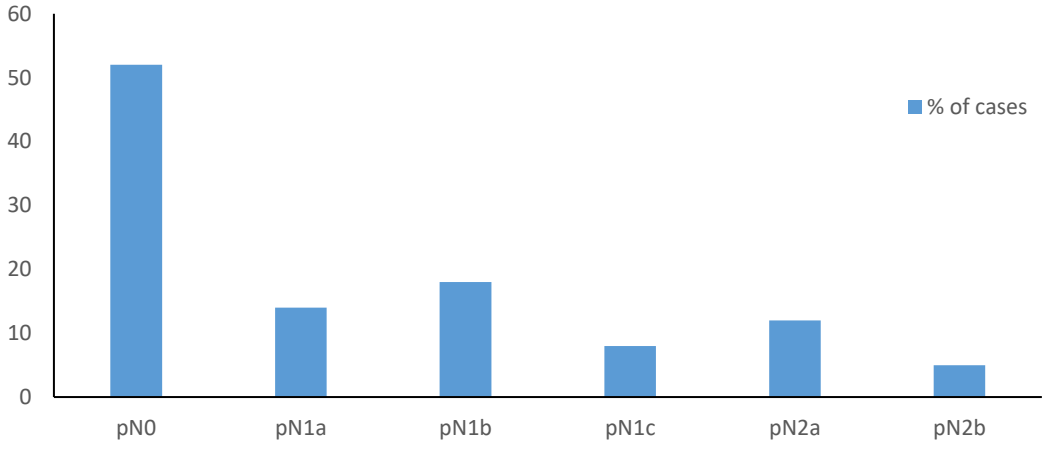

Figure 15: Graphical representation of regional lymph node metastasis

METASTASIS

Out of 148 cases, 12 cases had distant organ metastasis. Nine out of twelve cases have liver metastasis (75%) and remaining three cases have lung metastasis (25%).

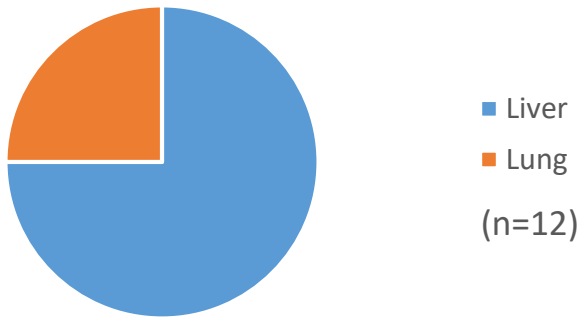

Figure 16: Metastatic colon cancer site distribution

STAGE

The majority of cases with colon cancer were in Stage II and in Stage III status. Only 12 cases were found to have Stage IV disease in which metastasis was to liver and lung.

Table 8: Stage of colon cancer

STAGE	NUMBER OF CASES (n=148)
I	14
II	62
III	60
IV	12

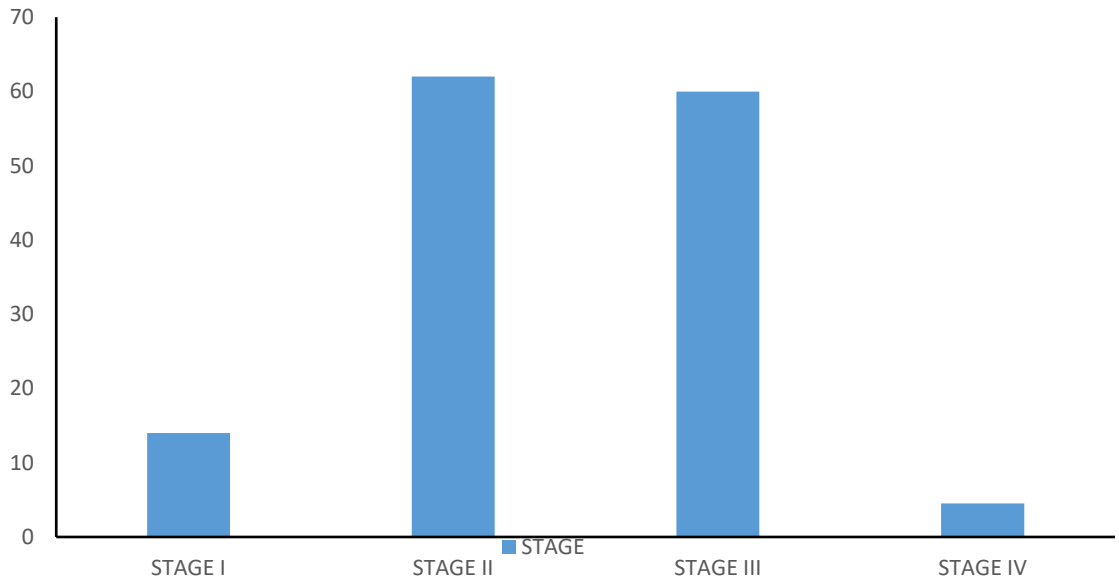

Figure 17: Graphical representation of the stage of colon cancer

LYMPHOVASCULAR AND PERINEURAL INVASION

On histological examination, only fifty percentages of cases had a lymphovascular invasion. But the major proportion of cases do not have perineural invasion (83%). Rest 17% of the cases show perineural invasion.

MARGINS

Proximal and distal margins were not involved in 148 cases.

IMMUNOHISTOCHEMISTRY

CDX2 immunohistochemistry was negative in 12 cases out of 148 tumor cases (8%). Remaining 136 cases (92%) showed positivity for CDX2 immunohistochemistry but varied in cell proportion and intensity. CDX2 biomarker positive cases were subcategorized into 1+ (<25%), 2+ (26-75%) & 3+ (>76%) according to percentage of positive cells. The intensity of CDX2 positive also varied from mild, moderate and strong. Majority of tumor cases were 3+ (73%) and strong intensity (70%).

Table 9: Distribution of colon cancer by the percentage of positive cells.

GRADING ON % CDX2 POSITIVE CELLS	PERCENTAGE OF CASES N=148)
0	8%
1+ (<25%)	8%
2+ (26-75%)	11%
3+ (>75%)	73%

Table 10: Distribution of colon cancer by the intensity of positive cells.

INTENSITY OF CDX2 POSITIVITY	PERCENTAGE OF CASES (N=136)
MILD	11%
MODERATE	19%
STRONG	70%

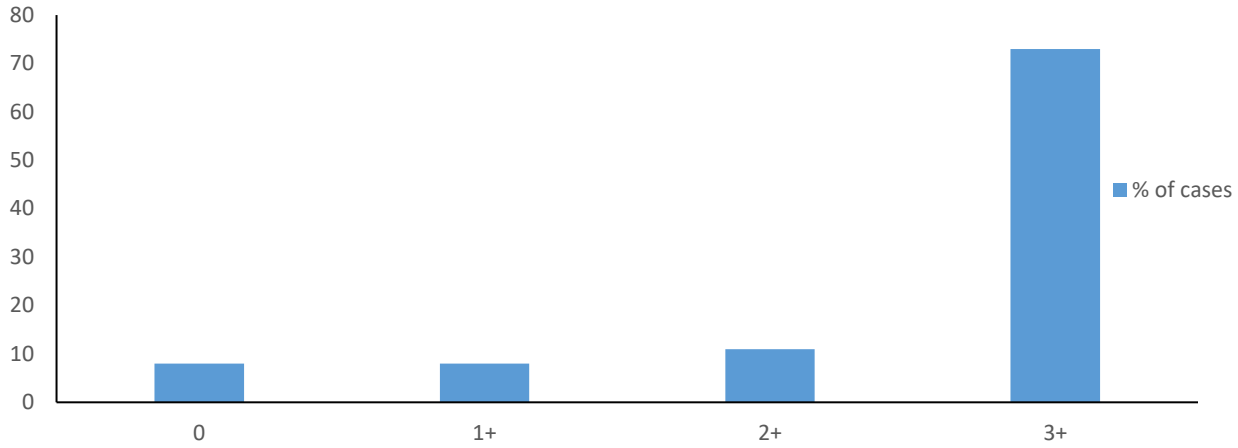

Figure 18: Graphical representation of colon cancer by the percentage of positive cells.

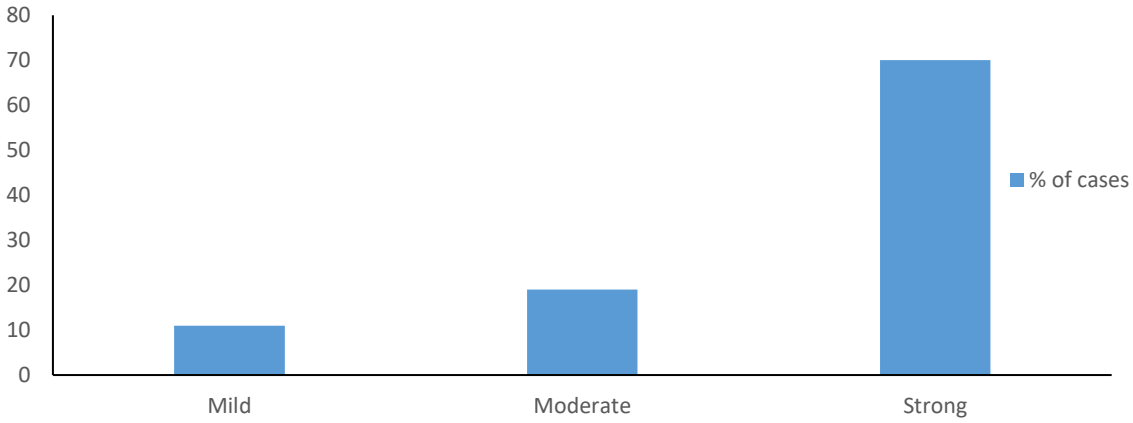

Figure 19: Graphical representation of colon cancer by the intensity of positive cells.

CDX 2 IMMUNOHISTOCHEMISTRY STUDY

CDX2 AND DEMOGRAPHY

On immunohistochemical analysis, CDX2 was positive in 136 cases and negative for 12 cases. In our study, CDX2 immunohistochemistry negativity was compared with demographic and histopathological features. The CDX2 expression was found to be negative more often in men than in women, however, the number of men with colon cancer was proportionately higher than in women in our study. There were no statistical significances found between CDX2 expression with patient's age, gender and clinical features in our study.

CDX2 AND TUMOUR MORPHOLOGY

Tumor location, size, and gross appearances were also compared with CDX2 immunohistochemistry expression. Most of the CDX2 negative tumors were located in the sigmoid colon (41.67%) and were of the ulceroproliferative morphology (91.67%) grossly. All these parameters were found to be statistically insignificant. CDX2 negativity was observed mostly in poorly differentiated adenocarcinoma (12.5%) and mucinous carcinoma (12.5%) when compared with moderately differentiated adenocarcinoma (7.2%). When the depth of tumor invasion was assessed, the majority of the cases of CDX2 immunohistochemistry were found to be negative in pT3 (41.67%), and pT4b (25%) stages. Among the cases with regional lymph node metastasis, CDX2 immunohistochemistry negativity was noted to be slightly higher in pN2a and pN2b (25% each) cases when compared to pN0 (8.33%) though it was not statistically significant.

CDX2 AND TUMOUR METASTASIS

Though eight out of twelve cases with lymphovascular invasion (66.67%) were negative for CDX2 immunohistochemistry, there was no statistical significance noted. Similarly, ten out of twelve cases with perineural invasion (83.33%) were negative for CDX2 immunohistochemistry which was not statistically significant. Among tumors with liver metastasis, 28.6% of cases were CDX2 negative and among tumors with lung metastasis, 66.7% of cases were CDX2 negative but had no statistical significance.

Table 11: Association between CDX2 status and clinical parameters

	Total cases n=148(100%)	CDX2 positive 136 (92%)	CDX2 negative 12(8%)	P value (Significant<0.05)
Age				0.532
<40	18(12.16)	17(12.5)	1(8.34)	
>40	130(87.83)	119(87.5)	11(91.66)	
Gender				0.348
Male	106(71.62)	96(70.59)	10(83.33)	
Female	42(28.38)	40(29.41)	2(16.67)	
Clinical features				
Abdominal pain	91(61.49)	85(62.5)	6(50)	0.394
LOW & LOA	66(44.59)	59(43.38)	7(58.33)	0.318
Bleeding PR	64(43.24)	59(43.38)	5(41.67)	0.908
Altered bowel habit	62(41.89)	58(42.65)	4(33.33)	0.531
Anemia	35(23.65)	31(22.79)	4(33.33)	0.410
Tenesmus	12(8.11)	10(7.35)	2(16.67)	0.257
Abdominal mass	8(5.41)	7(5.15)	1(8.33)	0.640
Perforation	2(1.35)	2(16.67)	0	
Intestinal obstruction	1(0.68)	1(0.74)	0	

LOW loss of weight, LOA loss of appetite, PR per rectum

Table 12: Association between CDX2 status with tumor

	Number of cases n=148(100%)	CDX2 positive 136 (92%)	CDX2 negative 12(8%)	P value (Significant<0.05)
Tumor size				0.324
<6.5cm	103(69.59)	93(68.38)	10(83.33)	
>6.5cm	45(30.41)	43(31.62)	2(16.67)	
Tumor location				
Caecum	33(22.3)	31(22.79)	2(16.67)	0.625
Ascending colon	48(32.43)	45(33.09)	3(25)	0.566
Transverse colon	17(11.49)	16(11.77)	1(8.33)	0.434
Descending colon	7(4.73)	6(4.41)	1(8.33)	0.540
Sigmoid colon	43(29.05)	38(27.94)	5(41.67)	0.315
Tumor pattern				NA
Polypoid	26(17.57)	26(19.12)	0	
Ulcerative	13(8.78)	13(9.56)	0	
Ulceroproliferative	104(70.27)	93(68.38)	11(91.67)	
Annular	5(3.38)	4(2.94)	1(8.33)	
Tumor histology				NA
Well differentiated	5(3.38)	5(3.68)	0	
Moderately differentiated	111(75)	103(75.74)	8(66.67)	
Poorly differentiated	16(10.81)	14(10.29)	2(16.67)	
Mucinous carcinoma	16(10.81)	14(10.29)	2(16.67)	
Tumor invasion				NA
pT1	2(1.35)	2(1.47)	0	
pT2	17(11.49)	15(11.03)	2(16.67)	
pT3	87(58.78)	82(60.29)	5(41.67)	
pT4a	32(21.62)	30(22.06)	2(16.67)	
pT4b	10(6.76)	7(5.15)	3(25)	
Lymph node metastasis				NA
pN0	77(52.03)	76(55.88)	1(8.33)	
pN1a	20(13.51)	18(13.24)	2(16.67)	
pN1b	26(17.57)	25(18.38)	1(8.33)	
pN1c	8(5.41)	6(4.41)	2(16.67)	
pN2a	12(8.11)	9(6.62)	3(25)	
pN2b	5(3.38)	2(1.47)	3(25)	

NA – Not applicable

Table 13: Association between CDX2 status with tumor stage

	Number of cases n=148(100%)	CDX2 positive 136 (92%)	CDX2 negative 12(8%)	P value (Significant<0.05)
				0.425
STAGE I	14(9.46)	12(8.82)	2 (16.67)	
STAGE II	62(41.89)	59(43.38)	3(25)	
STAGE III	60(40.54)	55(40.44)	5 (41.67)	
STAGE IV	12(.8.11)	10(7.35)	2(16.67)	

Table 14: Association between CDX2 status with tumor invasion

	Number of cases n=148(100%)	CDX2 positive 136 (92%)	CDX2 negative 12(8%)	P value (Significant<0.05)
Lymphovascular invasion	74(50)	66(48.53)	8(66.67)	0.228
Perineural invasion	25(16.89)	15(11.02)	10(83.33)	0.409

Table 15: Association between CDX2 status with tumor metastasis

	Number of cases n=12(100%)	CDX2 positive 8(83%)	CDX2 negative 4(17%)	P value (Significant<0.05)
Metastasis				0.371
Liver	9(75)	7(87.5)	2(50)	
Lung	3(25)	1(12.5)	2(50)	

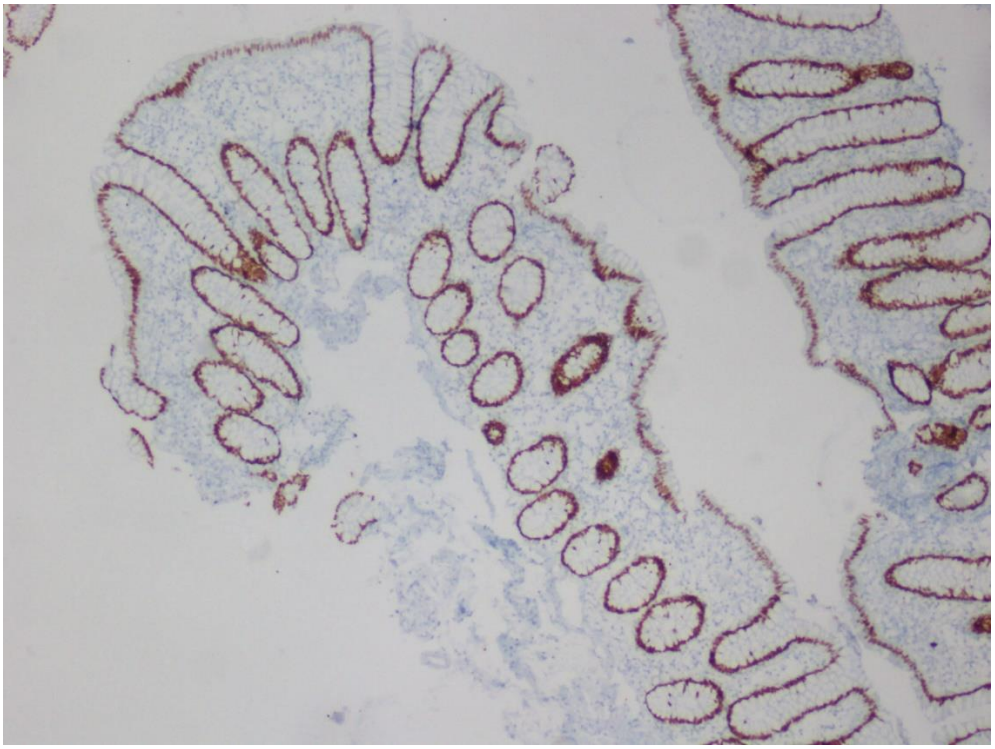

Figure 20: CDX2 immunohistochemistry positive control (10X magnification)

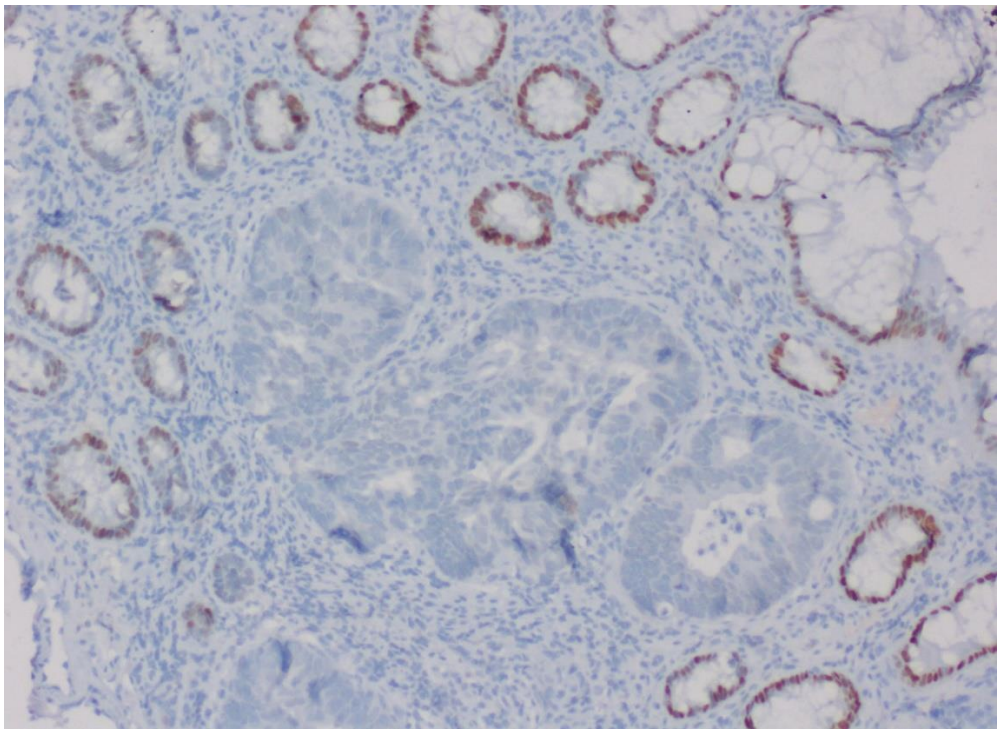

Figure 21: CDX2 immunohistochemistry negative tumor

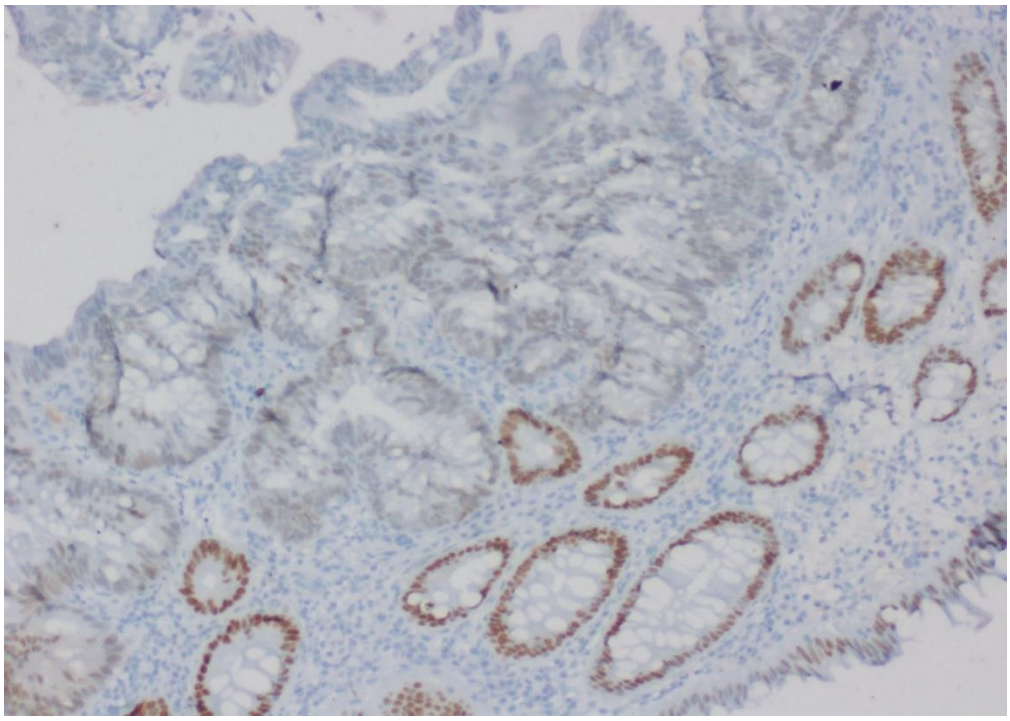

Figure 22: CDX2 immunohistochemistry positive tumor with mild intensity

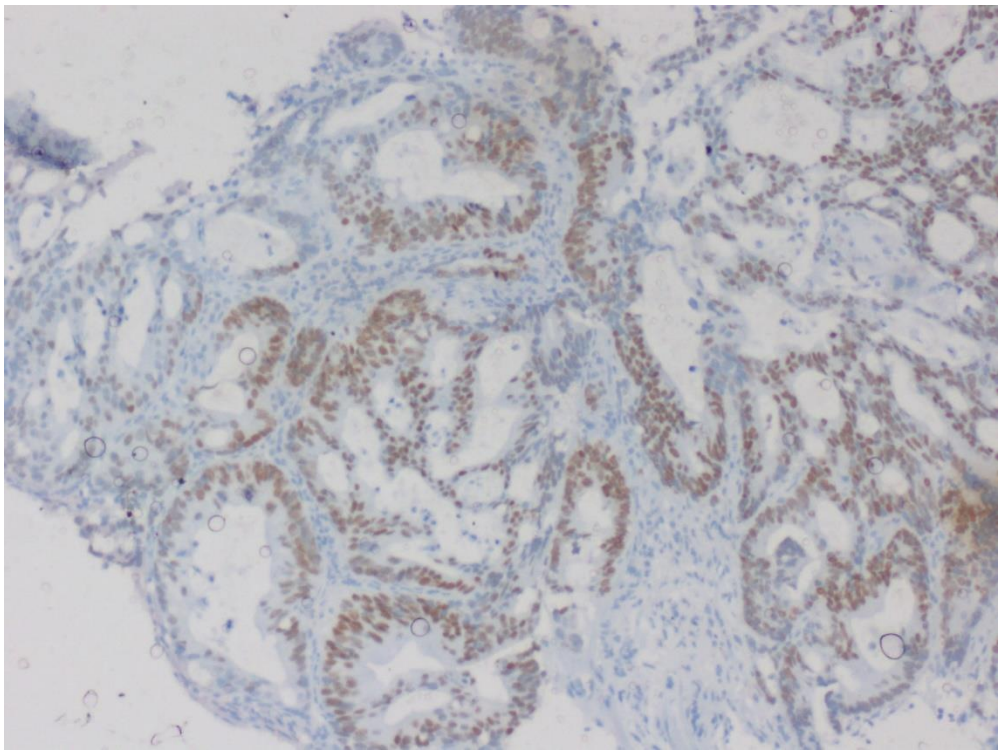

Figure 23: CDX2 immunohistochemistry positive tumor with moderate intensity

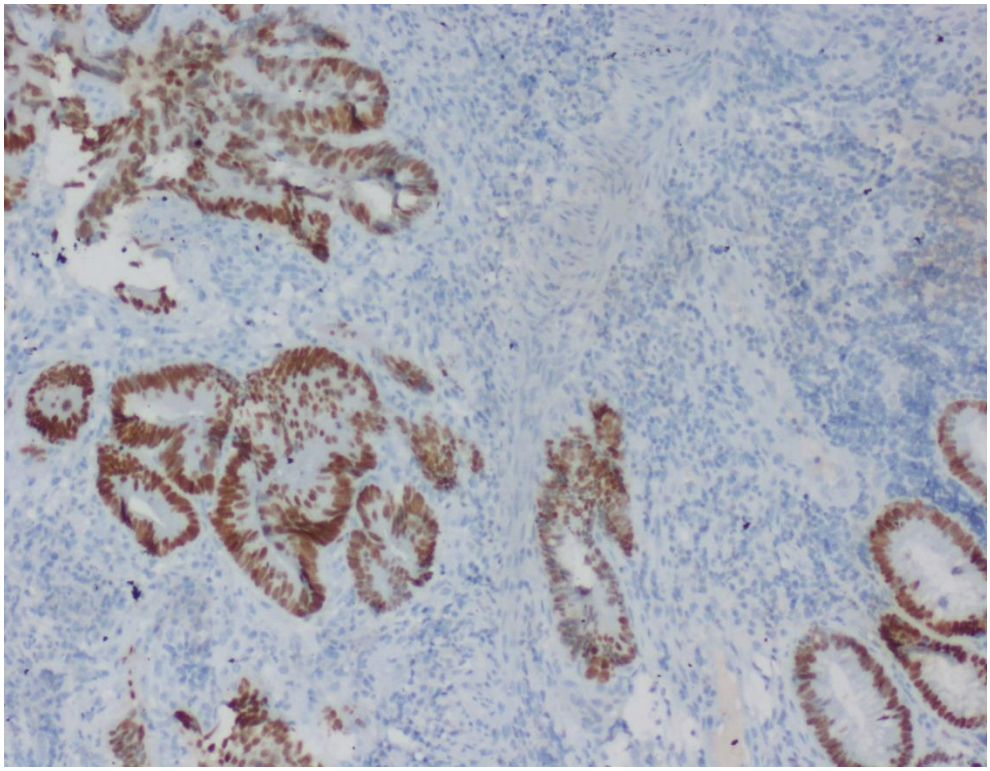

Figure 24: CDX2 immunohistochemistry positive tumor with strong intensity

DISCUSSION

In our study, CDX 2 immunohistochemistry was correlated with clinicopathological features, including grading and staging of colonic adenocarcinoma. 148 cases of colonic adenocarcinoma were included from January 2015 till the end of June 2018.

AGE

Globally, the incidence of CRC is higher in the population ≥65 years of age (1). In our study, the median age of diagnosis was 53 years with the youngest patient of 26 years. This was in keeping with the Indian studies published earlier, for example, a study by Patil PS et al, in which the mean age at diagnosis was 47 years (2). This was in contrast with many of the published western studies (1, 3-5). The incidence of colon cancer among the young in India may be due to a large proportion of the young population with a broad-based population pyramid (2). Change in lifestyle and environmental factors may also play an important role in this shift of trend. There is no significant association eluted when age was compared with CDX2 immunohistochemistry expression in this study.

GENDER

In our study, there was a male preponderance (70.59%) which was in concordance with many western and Indian studies (1-5). However, there was no statistical correlation between CDX2 immunohistochemistry expression and gender found in this study.

CLINICAL FEATURES

The most common symptom was abdominal pain (61.49%) in our study which was not in line with other Indian and western studies which project bleeding per rectum as the commonest symptoms(2). The logical explanation for this disparity could be due to a higher number of right-sided colon cancer found in our study than the left-sided ones which usually present as bleeding per rectum. The CDX2 immunohistochemistry expression was not found to be statistically significant with any of the symptoms of colon cancer.

TUMOR PATHOLOGY

In our observation, right-sided colon cancer, predominantly involving ascending colon cancer, was found to be significantly more prevalent compared to the left-sided colon cancers as published in the western literature (83, 84). This is in contrast to the Indian studies in which left-sided colon cancers were found to be more common. This could be partly explained by the fact that screening sigmoidoscopies would have mainly targeted precursor lesions in the left colon which are easily accessible. Poor preparations and incomplete evaluations during colonoscopy would have had a bigger impact on right-sided tumors. The third reason could be due to the inclusion of rectal cancer along with colonic cancers in the Indian studies. Conventional moderately differentiated adenocarcinoma formed the major proportion of tumors in our study because of increased incidence of colonic adenocarcinoma among the older age group, unlike the studies by Patil PS et al (2) and Patra et al (87) in which signet ring cell carcinomas and mucinous adenocarcinoma formed significantly greater proportion respectively in the younger population. Most of the CDX2

immunohistochemistry negative tumors were poorly differentiated adenocarcinoma and mucinous carcinoma, pT3 (41.67%) and pT4 (41.67%). Fifty percentage of CDX2 negative tumors were of the pN2 stage (88–91). Hence, our study revealed a direct correlation between CDX2 immunohistochemistry negativity and depth of tumor invasion, lymph node metastasis, and tumor grading, although it was not statistically significant.

TUMOR METASTASIS

In our study, the percentage of cases which had metastasis was approximately 8.1% which may be due to prompt screening and early diagnosis of cancer. Among metastasis, the commonest site was found to be liver (75%) which was congruent with previous studies (2, 88). CDX2 negative status was common in cancers with liver and lung metastasis but had no statistical significance with CDX2 immunohistochemistry negativity.

CONCLUSION

- Colon cancer cases had a median age of 53 years with a male preponderance.
- The most common site of occurrence of colon cancer was right-sided colon (ascending colon).
- The most common clinical presentation was abdominal pain followed by loss of appetite, altered bowel habit and bleeding per rectum.
- The prevalence of CDX2 expression in colon cancer in our population was found to be 8%.
- CDX2 immunohistochemistry negativity was found to be increased in patients above 40 years of age, in males, in left-sided colon cancers and in poorly differentiated adenocarcinoma and mucinous carcinoma, though these associations were not statistically significant.
- CDX2 negative status was more common among tumors with higher pathological TNM stage and those with liver and lung metastasis.
- The liver was found to be the commonest site for metastatic colon cancer though it had no significant statistical correlation with CDX2 immunohistochemical expression.

LIMITATION

Since the incidence of poorly differentiated adenocarcinoma, mucinous carcinoma, and signet ring carcinoma was low compared to the moderately differentiated adenocarcinoma in our study, it was difficult to correlate CDX2 immunohistochemical negativity with tumor grade and to prove the aggressive behavior of CDX2 negative tumors.

The CDX2 expression could be affected due to intratumoral heterogeneity, according to an Indian study, which reported a lower expression in the invasive front of the tumor compared to the tumor center. Hence an accurate evaluation of the CDX2 expression may not have been possible in small endoscopic mucosal biopsies. Multiple site mucosal biopsies would have been ideal for proper assessment of CDX 2 expression.

The prognosis of colon cancer was not compared with CDX2 immunohistochemistry expression in our study since CDX2 has been proven to be an independent prognostic factor, irrespective of tumor grade and stage in many of the studies. Therefore, the relevance of CDX2 immunohistochemistry negativity would be clearer when compared with survival and mortality.

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APPENDIX

APPENDIX 1 - Protocol for automated immunostaining:

1. Paraffin-embedded tissue sections were cut at the 4µ thickness and floated in poly L lysine coated slides incubated overnight at 37degree Celsius.

2. These slides were then treated with 4% milk solution for 10 minutes to eliminate the hydrophobic effect and give a positive charge to the slides.

3. Then the slide labels were barcoded and the labeled slides were loaded in Ventana Benchmark XT autostainer (a fully automated autostainer).

4. Individual protocols have been devised in the software attached to the machine for each marker. Specific protocols were selected according to the marker.

5. A standard protocol was used for most of the markers with minimal variation for few individual markers. The steps included in the protocol were as follows.

a) Deparaffinisation

b) Liquid coverslip application

c) Heat-induced antigen retrieval by treating with a standard CC1 solution (pH patent for the company) for one hour at 90 degree Celsius.

6. Then the primary antibody was added and incubated for 40 min at 37 degree Celsius.

7. Then the secondary antibody was added and incubated for 8 minutes.

8. Finally, the slides were counterstained with hematoxylin and incubated for 8 min, followed by incubation with the bluing agent for 4 min.

9. From antigen retrieval till counterstaining, in between every step, the slides were washed with reaction buffer. The whole processing is automated. Then the slides were brought to 80% alcohol (2 changes) to remove the liquid coverslip and then dried and mounted in DPX.

ANTIBODY	CLONE	DILUTION	SOURCE
MONOCLONAL MOUSE ANTI-HUMAN CDX 2	DAK-CDX2 Std 40	1:75	DAKO

APPENDIX 2 - Proforma

A study of the clinicopathological profile and prevalence of CDX2 biomarker expression in primary adenocarcinoma of colon in a tertiary care hospital among Indian population

1. ID NO- 2. AGE 3. SEX 4. MUCOSAL BIOPSY NO: 5.RESECTION BIOPSY NO:

5. CLINICAL FEATURES:

BLEEDING PER RECTUM	Y/N	LOSS OF WEIGHT & APPETITE	Y/N
ABDOMINAL PAIN	Y/N	TENESMUS	Y/N
ANAEMIA	Y/N	ALTERED BOWEL HABITS	Y/N
ABDOMINAL MASS	Y/N	INTESTINAL OBSTRUCTION	Y/N
PERFORATION	Y/N		

6. PAST HISTORY

a) ANY OTHER MALIGNANCIES:

b) ANY MALIGNANCIES IN FAMILY:

7. MACROSCOPIC FINDINGS

7.1 ANATOMICAL SITE

a) CAECUM	Y/N	e) SPLENIC FLEXURE	Y/N
b) ASCENDING COLON	Y/N	f) DESCENDING COLON	Y/N
c) HEPATIC FLEXURE	Y/N	g) SIGMOID COLON	Y/N
d) TRANSVERSE COLON	Y/N		

7.2 GREATEST TUMOUR DIMENSION: cm

7.3 PATTERN OF GROWTH: a) POLYPOIDAL b) ULCERATIVE c) ULCEROPROLIFERATIVE
f) ANNULAR/ CONSTRICTION e) DIFFUSE

8. MICROSCOPIC FINDINGS

8.1 HISTOLOGICAL TUMOUR DIFFERENTIATION

a) GRADE1 WELL DIFFERENTIATED (>95% OF TUMOUR COMPOSED OF GLANDS) ☐
b) GRADE 2 MODERATELY DIFFERENTIATED (50-95% OF TUMOUR COMPOSED OF GLANDS) ☐
c) GRADE3 POORLY DIFFERENTIATED (<50% OF TUMOUR COMPOSED OF GLANDS) ☐
d) SIGNET RING CELL CARCINOMA ☐
e) MUCINOUS CARCINOMA ☐
f) OTHERS:

8.2 TUMOR INVASION

a) pT0: No evidence of primary tumor ☐
b) pTis: Carcinoma in situ, intraepithelial (no invasion of lamina propria) ☐
c) pTis: Carcinoma in situ, invasion of lamina propria/muscularis mucosae ☐
d) pT1: Tumor invades submucosa ☐
e) pT2: Tumor invades muscularis propria ☐
f) pT3: Tumor invades through the muscularis propria into pericolorectal tissues ☐
g) pT4a: Tumor penetrates the visceral peritoneum ☐
h) pT4b: Tumor directly invades or is adherent to other organs or structures ☐

8.3 REGIONAL LYMPH NODES

a) pN0: No regional lymph node metastasis ☐
b) pN1a: Metastasis in 1 regional lymph node ☐
c) pN1b: Metastasis in 2 to 3 regional lymph nodes ☐
d) pN1c: Tumor deposit(s) in the subserosa, or pericolic or perirectal tissues without regional lymph node metastasis ☐
e) pN2a: Metastasis in 4 to 6 regional lymph nodes ☐
f) pN2b: Metastasis in 7 or more regional lymph nodes ☐

8.4 LYMPHOVASCULAR INVASION Y/N

8.5 PERINEURAL INVASION Y/N

8.6 MARGINS INVOLVED - a. PROXIMAL Y/N b. DISTAL Y/N

8.7 DISTANT METASTASIS Y/N IF YES, SITE- …….

9 CDX2 IMMUNOHISTOCHEMISTRY

9.1 PERCENTAGE OF POSITIVE CELLS:

a) 0 (NO SIGNAL) ☐ c) 2+ (26-75%) ☐
b) 1+ (<25%) ☐ d) 3+ (>75%) ☐

9.2 INTENSITY OF POSITIVE CELLS

a) MILD ☐
b) MODERATE ☐
c) STRONG ☐

APPENDIX 3 - Institutional Review Board Approval

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

Dr. B.J. Prashantham, M.A., M.A., Dr.Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Anna Benjamin Pulimood, M.B.B.S., MD., Ph.D.,
Chairperson, Research Committee & Principal

Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

June 24, 2017

Dr. Rijo Issac N P,
PG Registrar,
Department of Pathology,
Christian Medical College,
Vellore – 632 002.

Sub: **Fluid Research Grant NEW PROPOSAL:**
A study of the clinic pathological profile and CDX2 biomarker expression of primary adenocarcinoma of colon in a tertiary care hospital among Indian population.
Dr. Rijo Issac N P, Employment Number: 21288, Post Graduate Registrar Department of Pathology. Dr. Dipti Masih Employment Number: 32530, Dr. Anna Pulimood, Professor, Department of General pathology, Dr Mark Ranjan Jesudason, Professor and Head, Department of General surgery unit II.Mrs. Mahasampath Gowri.S, Employment No: 33418, lecturer, Department of Biostatistics.

Ref: IRB Min. No. 10624 [OBSERVE] dated 03.04.2017

Dear Dr. Rijo Issac N P,

I enclose the following documents:-

1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Biju George, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board

Cc: Dr. Dipti Masih, Dept. of Pathology, CMC, Vellore

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
Tel: 0416 – 2284294, 2284202 Fax: 0416 – 2262788, 2284481 E-mail: research@cmcvellore.ac.in

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

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Ref: IRB Min. No. 10624 [OBSERVE] dated 03.04.2017

Dear Dr. Rijo Issac N P,

The Institutional Review Board (**Blue**, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "A study of the clinic pathological profile and CDX2 biomarker expression of primary adenocarcinoma of colon in a tertiary care hospital among Indian population" on April 03rd 2017.

The Committee reviewed the following documents:

1. IRB Application format
2. Waiver of Consent.
3. Proforma.
4. Cvs of Drs. Anna Pulimood, Dipti, Mark Ranjan and Rijo Issaac.
5. No. of documents 1 – 3.

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on April 03rd 2017 in the CK Job Hall, Christian Medical College, Bagayam, Vellore 632002.

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
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Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Name	Qualification	Designation	Affiliation
Dr. Biju George	MBBS, MD, DM	Professor, Haematology, Research), Additional Vice Principal , Deputy Chairperson (Research Committee), Member Secretary (Ethics Committee), IRB, CMC, Vellore	Internal, Clinician
Dr. B. J. Prashantham	MA(Counseling Psychology), MA (Theology), Dr. Min (Clinical Counselling)	Chairperson, Ethics Committee, IRB, Director, Christian Counseling Centre, Vellore	External, Social Scientist
Dr. Ratna Prabha	MBBS, MD (Pharma)	Associate Professor, Clinical Pharmacology, CMC, Vellore	Internal, Pharmacologist
Dr. Rekha Pai	BSc, MSc, PhD	Associate Professor, Pathology, CMC, Vellore	Internal, Basic Medical Scientist
Dr. Jayaprakash Muliyil	BSC, MBBS, MD, MPH, Dr PH (Epid), DMHC	Retired Professor, CMC, Vellore	External, Scientist & Epidemiologist
Mr. C. Sampath	BSc, BL	Advocate, Vellore	External, Legal Expert
Ms. Grace Rebekha	M.Sc., (Biostatistics)	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
Dr. Sowmya Sathyendra	MBBS, MD (Gen. Medicine)	Professor, Medicine III, CMC, Vellore	Internal, Clinician
Dr. Santhanam Sridhar	MBBS, DCH, DNB	Professor, Neonatology, CMC, Vellore	Internal, Clinician
Dr. Thomas V Paul	MBBS, MD, DNB, PhD	Professor,Endocrinology, CMC, Vellore	Internal, Clinician
Dr Sneha Varkki	MBBS, DCH, DNB	Professor, Paediatrics, CMC, Vellore	Internal, Clinician
Dr. Sathish Kumar	MBBS, MD, DCH	Professor, Child Health, CMC, Vellore	Internal, Clinician

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Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Dr. Anuradha Rose	MBBS, MD, MHSC (Bioethics)	Associate Professor, Community Health, CMC, Vellore	Internal, Clinician
Dr. Ajith Sivadasan	MD, DM	Professor, Neurological Sciences, CMC, Vellore	Internal, Clinician
Dr. Visalakshi, J	MPH, PhD	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
Mrs. Pattabiraman	BSc, DSSA	Social Worker, Vellore	External, Lay Person
Dr. Mathew Joseph	MBBS, MCH	Professor, Neurosurgery, CMC, Vellore	Internal, Clinician
Dr. Shyam Kumar NK	MBBS, DMRD, DNB, FRCR, FRANZCR	Professor, Radiology, CMC, Vellore	Internal, Clinician

We approve the project to be conducted as presented.

Kindly provide the total number of patients enrolled in your study and the total number of withdrawals for the study entitled: "A study of the clinic pathological profile and CDX2 biomarker expression of primary adenocarcinoma of colon in a tertiary care hospital among Indian population" on a monthly basis. Please send copies of this to the Research Office (research@cmcvellore.ac.in).

Fluid Grant Allocation:

A sum of 1,00,000/- INR (Rupees One Lakh Only) will be granted for 2 years. 50,000/- INR (Rupees Fifty Thousand only) will be granted for 12 months as an 1st Installment. The rest of the 50,000/- INR (Rupees Fifty thousand only) each will be released at the end of the first year as 2 nd Installment

Yours sincerely,

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board

Dr. BIJU GEORGE
MBBS., MD., DM.
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

IRB Min. No. 10624 [OBSERVE] dated 03.04.2017

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
Tel: 0416 – 2284294, 2284202 Fax: 0416 – 2262788, 2284481 E-mail: research@cmcvellore.ac.in

APPENDIX 4 - Thesis Data

	age	sex	mucbiop	resbiops	bleed	abdp	anae	abdm	perf	wtlos	tene	bow	intest	maligfa	cae	ascco	hepa	tran	splen	descol	sigm	greattu	grow	histtun	tumor	lymph	lymphov	perineu	distant	distar	immuno	intesit	stage
2	75	2	15203/16	16611/16	1	1	2	2	2	1	2	1	2		2	2	2	2	2	2	1	7	3	5	6	3	2	1	2		1	1	6
3	62	2	16793/16	20041/16	2	2	1	2	2	1	2	2	2		1	2	2	2	2	2	2	4.4	3	2	6	1	2	2	2		3	2	2
4	48	1	16912/16	18099/16	1	1	2	2	2	1	2	2	2	SISTER -	2	2	2	2	2	2	1	7	3	2	5	1	2	2	2		0		1
5	67	2	17269/18	19498/16	1	2	2	2	2	1	1	1	2		1	1	2	2	2	2	2	8	3	2	6	3	2	2	2		3	2	6
6	35	1	17455/16	19623/16	2	1	1	2	2	1	2	2	2		2	2	1	2	2	2	2	7	3	2	6	3	2	2	2		2	2	6
7	44	1	20005/16	21175/16	1	1	1	2	2	2	2	1	2		2	1	1	2	2	2	2	7	1	2	6	1	2	2	2		3	2	2
8	58	2	18713/16	20883/16	1	1	2	2	2	2	2	1	2		2	1	2	2	2	2	2	7.8	3	2	6	1	2	2	2		3	3	2
9	43	1	20920/16	22597/16	2	1	2	1	2	2	2	2	2		2	2	1	2	2	2	2	8	3	2	7	3	2	2	2		3	3	6
10	65	1	20931/16	22031/16	1	1	2	2	2	1	2	1	2		2	1	2	2	2	2	2	6	3	2	7	3	2	2	1	LUNG	3	3	8
11	58	2	25285/16	29392/16	2	2	1	2	2	1	2	2	2		2	2	1	2	2	2	2	4.2	3	2	6	2	2	2	2		3	3	6
12	49	2	27984/16	31503/16	2	1	2	2	2	1	2	1	2		2	2	2	2	1	2	2	5.5	1	2	7	3	2	2	2		2	2	6
13	79	1	49384/15	5735/16	2	2	1	2	2	1	2	2	2		1	2	2	2	2	2	2	6	1	2	6	1	2	2	2		3	3	2
14	69	1	219/16	2522/16	2	2	2	2	2	1	2	2	2	BROTHI	2	1	2	2	2	2	2	5	3	5	6	3	2	2	2		2	2	6
15	45	1	49622/15	829/16	2	1	1	2	2	1	2	2	2		2	2	1	2	2	2	2	8.8	3	2	6	1	2	1	2		3	3	2
16	59	1	49631/15	1747/16	2	1	1	2	2	2	2	1	2		1	1	2	2	2	2	2	8	3	2	6	1	2	2	2		3	3	2
17	67	2	3500/16	6271/16	1	2	2	2	2	2	2	2	2		2	2	2	2	2	2	1	6	3	5	6	1	2	2	2		3	2	2
18	49	2	4418/16	5268/16	1	2	2	2	2	1	1	1	2		2	2	2	2	2	2	1	3	1	2	6	2	2	2	2		3	3	6
19	59	2	2083/16	12397/16	1	2	2	2	2	1	2	2	2		2	2	2	2	2	2	1	2.7	4	2	5	2	2	2	2		3	3	5
20	46	2	23195/16	25831/16	2	1	2	2	2	1	2	1	2		2	2	2	2	2	2	1	2.5	1	2	7	1	2	2	2		3	2	3
21																																	
22	56	2	27044/16	33619/16	2	2	2	2	2	1	2	2	2		1	2	2	2	2	2	2	5	3	2	7	3	1	1	2		3	2	6
23	33	1	32874/16	35141/16	2	1	2	2	2	2	2	1	2		2	2	1	2	2	2	2	5.2	3	2	6	1	2	2	2		2	3	2
24	38	1	34048/16	36528/16	1	2	2	2	2	1	2	1	2		2	1	2	2	2	2	2	3.5	1	1	7	1	2	2	2		3	3	3
25	32	1	34329/16	35738/16	2	1	2	1	2	2	2	2	2		1	1	2	2	2	2	2	12	3	5	7	1	2	2	2		3	3	3
26	33	2	3015/16	5399/16	1	2	2	2	2	2	2	1	2		2	1	2	2	2	2	2	4.5	3	3	6	3	1	1	2		2	1	6
27	68	2	43314/16	44752/16	2	2	1	1	2	1	2	2	2		2	2	2	1	2	2	2	12	3	2	6	1	2	2	2		0		2
28	37	1	44793/16	46180/16	1	1	2	2	2	1	1	1	2		2	2	2	2	2	2	1	5	3	2	8	1	1	2	1	LIVER	0		8
29	63	1	5327/16	7024/16	1	2	2	2	2	2	2	1	2		2	2	2	2	2	2	1	6.3	3	2	6	3	1	2	2		3	3	6
30	40	1	44973/16	46410/16	2	2	2	2	2	1	2	1	2	FATHER	2	1	2	2	2	2	2	5	3	2	6	1	2	2	2		3	2	2
31	52	2	41987/16	44018/16	1	1	2	2	2	2	2	1	2		2	2	2	2	2	2	1	4	2	2	8	5	1	1	2		1	1	7
32	61	1	40679/16	44022/16	2	2	2	2	2	1	2	1	2	MOTHE	2	2	2	2	2	2	1	6.3	3	2	7	1	2	2	2		3	2	3
33	40	1	38804/16	44458/16	2	1	1	2	2	2	2	2	2		2	2	2	1	2	2	2	5.5	3	2	7	2	1	1	1	LIVER	3	3	8
34	61	2	36962/16	46916/16	2	2	1	2	2	1	2	2	2		2	1	2	2	2	2	2	3.2	3	2	5	1	2	2	2		3	2	1
35	67	1	31245/16	32179/16	2	1	2	2	2	1	2	1	2		2	2	2	2	2	2	1	4	3	2	7	4	1	2	2		3	3	6
36	44	1	21301/16	24009/16	2	1	2	2	2	2	2	2	2		2	2	1	2	2	2	2	5	3	2	5	1	2	2	2		2	1	1
37	41	1	12424/16	14748/16	1	2	2	2	2	1	2	1	2		2	2	2	2	2	2	1	3	3	2	6	3	1	2	1	LIVER	2	2	8
38	69	2	11981/16	14539/16	2	1	2	2	2	2	2	2	2	SIBLING	2	1	2	2	2	2	2	8	1	5	6	1	2	2	2		3	3	2
39	51	1	9001/16	11946/16	2	1	2	2	2	1	2	1	2		2	1	2	2	2	2	2	7.5	1	2	6	1	2	2	2		3	2	2
40	47	1	8846/16	11081/16	1	1	1	2	2	1	2	1	2		2	2	1	1	2	2	2	2	1	2	6	1	2	2	2		1	1	2
41	48	1	8847/16	15280/16	2	1	2	1	2	1	2	1	2		2	2	2	1	2	2	2	6	3	3	7	1	2	2	2		3	3	3
42	51	1	7250/16	11505/16	2	2	2	2	1	2	2	2	2		1	1	2	2	2	2	2	7	3	5	8	5	1	2	2		0		7
43	33	1	6305/16	9274/16	2	1	2	2	2	2	2	2	2		2	1	1	2	2	2	2	3.2	3	2	6	5	1	1	2		3	2	7
44	63	1	5327/16	7024/16	1	2	2	2	2	2	2	1	2		2	2	2	2	2	2	1	6.3	3	2	6	3	1	2	2		3	3	6
45	54	1	395/16	1904/16	2	2	2	2	2	1	2	1	2		2	2	1	1	2	2	2	5	1	2	6	1	2	2	2		3	3	2
46	53	1	4952/16	8370/16	1	1	2	2	2	2	2	1	2		2	2	2	1	2	2	2	7	3	2	6	2	1	2	2		3	3	6
47	39	2	31099/17	31870/17	2	1	2	2	2	2	2	2	2		1	2	2	2	2	2	2	3.6	3	2	7	6	1	1	2		2	2	7
48	45	1	29124/17	31250/17	1	2	2	2	2	1	2	1	2		2	2	2	2	2	1	2	8	3	5	6	3	1	1	2		3	3	6
49	45	1	33037/17	34100/17	2	1	1	2	2	2	2	2	2		2	1	2	2	2	2	2	5.5	3	2	6	1	2	2	2		3	3	2
50	57	1	48249/16	558/17	2	1	2	2	2	1	1	2	2		2	2	2	2	2	2	1	1	2	2	6	4	1	1	2		3	3	6
51	62	1	35450/17	36901/17	2	1	1	2	2	2	2	1	2		2	2	2	1	2	2	2	6	3	2	6	1	2	2	2		3	3	2
52	49	2	29455/17	30737/17	2	1	2	2	2	1	2	2	2		2	2	1	2	2	2	2	5.5	3	2	7	1	2	2	2		3	3	3
53	69	2	21264/17	29716/17	1	2	1	2	2	1	2	1	2		2	2	2	2	2	2	1	5	3	1	6	1	1	1	2		3	3	2
54	47	1	26715/17	30876/17	1	1	2	2	2	1	2	2	2		2	1	1	2	2	2	2	7	3	2	6	1	1	2	2		3	3	2
55	47	1	27451/17	29266/17	2	1	2	2	2	2	2	2	2		1	2	2	2	2	2	2	8.8	3	2	7	1	1	2	2		2	1	3
56	60	2	24180/17	28711/17	2	1	2	2	2	1	2	1	2		1	1	2	2	2	2	2	5	3	2	6	3	1	1	2		3	3	6

57	45	1	14612/17	24472/17	1	2	2	2	2	1	2	1	2		2	2	2	2	2	2	1	6	3	2	6	2	1	2	1	LIVER	3	3	8
58	57	2	20152/17	24867/17	2	1	2	2	2	2	2	2	2		2	2	1	2	2	2	2	6.2	3	2	5	2	1	2	1	LIVER	2	3	8
59	68	2	12441/17	14769/17	1	2	1	2	2	2	2	2	2		2	1	2	2	2	2	2	5.8	1	2	6	1	1	2	2		3	3	2
60	45	2	11807/17	15058/17	2	1	2	2	2	2	2	2	2		2	2	2	1	2	2	2	10.6	3	2	6	1	2	2	2		3	2	2
61	50	1	12636/17	15315/17	2	1	2	2	2	2	2	2	2		2	2	1	2	2	2	2	7	4	2	6	5	1	1	2		0		7
62	69	2	11643/17	14574/17	2	1	2	1	2	2	2	2	2		2	2	2	2	2	2	1	6	3	2	6	5	1	2	2		3	3	6
63	81	1	6316/17	12546/17	1	2	2	2	2	2	1	2	2		2	2	2	2	2	2	1	3.2	3	2	6	1	1	2	2		3	2	2
64	47	2	14259/17	15845/17	2	1	2	2	2	2	2	2	2		1	1	2	2	2	2	2	4	3	2	6	1	2	2	2		3	2	2
65	66	2	9426/17	16259/17	1	1	1	2	2	2	2	1	2		2	2	2	2	1	2	2	6.5	1	2	6	6	1	1	2		1	3	7
66	48	1	19014/17	21838/17	2	1	2	2	2	1	2	2	2		1	1	2	2	2	2	2	8	3	3	8	2	1	2	2		0		7
67	53	1	8794/17	10234/17	1	1	2	2	1	1	2	1	2		2	2	2	2	2	2	1	9	3	3	7	5	1	2	1	LIVER	0		8
68	41	1	18587/17	22366/17	2	1	2	2	2	2	2	2	2		2	2	2	1	2	2	2	8.5	3	2	6	1	2	2	2		3	2	2
69	52	1	19987/17	22515/17	1	1	2	2	2	1	2	1	2		1	1	2	2	2	2	2	9.5	3	3	8	4	1	2	2		3	3	7
70	40	1	21458/16	22962/17	1	2	2	2	2	1	2	2	2		2	1	1	2	2	2	2	6	3	2	6	1	1	2	2		3	3	2
71	66	1	11442/16	12406/17	2	1	1	2	2	2	2	2	2		2	1	2	2	2	2	2	4.5	3	2	5	1	2	2	2		3	3	1
72	62	1	3374/17	4730/17	1	1	2	2	2	2	1	1	2	DAUGH	2	2	2	2	2	2	1	7	3	2	6	2	1	2	2		1	1	6
73	50	2	6740/17	8089/17	1	2	2	2	2	2	1	2	2		2	2	2	2	2	2	1	3.4	2	2	7	1	2	2	2		3	3	3
74	53	1	7072/17	8548/17	2	2	2	2	2	1	1	1	2		2	2	2	2	2	2	1	3.4	3	1	6	1	2	2	2		3	3	2
75	62	1	2431/17	9516/17	2	1	1	2	2	2	2	2	2		2	2	1	2	2	2	2	4	3	2	7	5	1	2	2		3	3	7
76	34	1	14985/17	19613/17	1	1	2	2	2	2	2	1	2		2	1	2	2	2	2	2	8	3	5	7	3	1	2	2		3	3	6
77	52	1	13291/17	14887/17	1	1	1	2	2	2	2	2	2		1	1	2	2	2	2	2	8.5	3	3	6	1	2	2	2		3	3	3
78	52	2	11195/17	14091/17	1	2	2	2	2	2	2	2	2		2	2	2	2	2	2	1	4	3	2	5	1	2	2	2		3	3	1
79	83	1	6365/17	6534/17	1	1	2	2	2	2	2	2	2		2	2	2	2	2	2	1	3.5	1	2	5	1	2	2	2		3	3	1
80	56	1	3977/17	6544/17	1	1	2	2	2	2	2	2	2		2	1	1	2	2	2	2	2.8	2	2	5	2	1	2	2		3	3	5
81	65	1	613/17	5585/17	1	1	2	2	2	2	2	2	2		2	2	2	2	2	2	1	4.5	3	1	5	1	2	2	2		3	3	1
82	70	2	15822/17	17093/17	2	1	2	2	2	1	2	2	2		2	2	2	1	2	2	2	3.5	3	2	6	3	1	2	1	LIVER	3	3	8
83	58	2	43437/17	47754/17	2	2	1	2	2	2	2	2	2		1	2	2	2	2	2	2	6.5	3	2	6	2	1	2	2		3	3	6
84	47	2	42262/17	44613/17	2	1	2	2	2	2	2	1	2		2	1	2	2	2	2	2	5	3	2	6	2	1	2	2		3	3	6
85	67	1	47395/17	48476/17	1	1	1	2	2	2	2	2	2		2	2	1	2	2	2	2	5	3	2	7	1	1	2	2		3	3	3
86	79	1	49384/15	5735/16	2	2	1	2	2	1	2	2	2		1	2	2	2	2	2	2	6	1	2	6	1	2	2	2		3	3	2
87	76	1	19070/18	20977/18	2	1	2	2	2	2	2	2	2		1	2	2	2	2	2	2	5	3	2	7	3	1	1	2		3	3	6
88	47	1	45801/17	540/18	2	1	1	2	2	2	2	1	2		2	2	2	2	2	1	2	2	3	2	6	4	1	1	2		3	3	6
89	74	1	47695/17	2615/18	2	1	1	2	2	2	2	2	2		2	2	2	2	2	2	1	2.5	3	2	6	4	1	2	2		3	3	6
90	66	2	13521/16	17024/16	2	2	1	2	2	1	2	2	2		2	2	2	2	2	2	1	6.8	3	2	6	3	1	2	2		0		6
91	55	1	14178/18	16128/18	1	1	2	2	2	2	2	1	2		2	1	2	2	2	2	2	2.5	3	1	4	1	2	2	2		3	3	1
92	76	1	46566/14	14722/16	2	1	2	2	2	2	2	1	2		2	2	2	2	2	2	1	6	3	2	7	2	1	1	2		3	3	6
93	34	2	2580/16	8232/16	1	2	2	2	2	1	1	1	2		2	2	2	2	2	2	1	2	3	5	5	1	2	2	2		3	3	1
94	54	1	13367/16	15620/16	1	1	2	2	2	1	2	2	2		2	2	2	2	1	2	2	4.2	3	2	6	1	2	1	2		3	2	2
95	88	1	35461/15	38355/15	2	2	1	2	2	1	2	2	2	UNCLE-	1	2	2	2	2	2	2	7	1	5	8	3	1	2	2		3	3	7
96	50	1	35828/15	37548/15	2	1	2	2	2	2	2	1	2	MOTHE	1	1	2	2	2	2	2	4	3	2	6	3	1	2	1	LIVER	3	3	8
97	54	1	41383/15	42584/15	1	1	1	2	2	2	2	2	2		2	1	2	2	2	2	2	7.5	4	2	6	4	1	2	2		2	1	6
98	50	1	36006/15	37389/15	1	2	2	2	2	2	2	2	2		2	2	2	2	2	2	1	2.2	2	2	6	2	1	2	2		3	2	6
99	55	2	39099/15	39885/15	2	1	2	2	2	1	2	2	2		2	1	2	2	2	2	2	5	1	2	6	2	1	2	2		3	3	6
100	64	1	3499/15	7775/15	2	1	2	2	2	2	2	2	2		2	2	1	2	2	2	2	8.5	3	3	6	1	2	2	2		3	3	2
101	50	1	6269/15	10180/15	2	1	1	2	2	1	2	2	2		1	1	2	2	2	2	2	4	3	3	7	3	1	1	2		3	3	6
102	46	1	6620/15	13800/15	2	1	2	2	2	2	2	1	2		2	1	2	2	2	2	2	7.5	3	2	7	1	2	2	2		3	3	3
103	43	1	8713/15	11280/15	2	2	1	2	2	2	2	2	2		2	1	2	2	2	2	2	7.8	1	2	6	1	2	2	2		3	3	2
104	54	2	11279/15	15658/15	1	1	2	2	2	2	2	2	2		2	2	2	2	2	2	1	5	2	2	6	2	1	2	2		3	3	6
105	50	1	1512/15	4712/15	1	1	2	2	2	2	1	1	2		2	2	2	2	2	2	1	4.5	3	2	6	3	1	2	2		1	1	6
106	66	1	2261/15	6163/15	2	1	1	2	2	2	2	2	2		2	1	2	2	2	2	2	5	3	2	6	1	2	2	2		0		2
107	35	2	34836/15	36779/15	2	1	2	2	2	1	2	2	2		2	1	2	2	2	2	2	3.2	2	2	7	5	1	1	2		3	3	7
108	48	1	35014/15	38044/15	2	1	2	2	2	2	2	2	2		1	1	2	2	2	2	2	5	1	2	6	2	1	2	2		3	2	6
109	64	1	34059/15	36374/15	2	2	1	2	2	1	2	2	2		2	2	2	1	2	2	2	5	3	2	6	1	2	2	2		0		2
110	47	1	26727/15	28412/15	1	1	2	2	2	2	2	1	2		2	2	2	2	2	1	2	5	1	5	6	6	1	2	2		3	3	7
111	60	1	26728/15	27928/15	1	2	2	2	2	2	1	1	2		2	2	2	2	2	2	1	6	3	2	7	2	1	2	2		0		6
112	33	1	33705/15	35584/15	2	1	1	1	2	1	2	2	2	MOTHE	1	2	2	2	2	2	2	5.9	3	5	6	3	2	2	2		2	3	6

114	45	1	12991/15	16315/15	2	1	2	2	2	2	2	2	2		1	2	2	2	2	2	2	4	1	2	6	1	2	2	2		3	3	2
115	47	1	15735/15	17162/15	1	1	2	2	2	1	2	2	2		1	1	2	2	2	2	2	4	4	2	6	1	2	2	2		1	3	2
116	47	1	19245/15	22208/15	2	2	1	2	2	2	2	2	2		2	2	2	1	2	2	2	5.5	3	2	6	1	2	2	2		3	3	2
117	52	1	26725/15	34438/15	1	2	2	2	2	2	2	2	2		2	2	2	2	2	2	1	4	3	2	6	1	2	2	2		2	1	2
118	48	1	22774/15	24204/15	2	1	2	2	2	1	2	1	2		1	2	2	2	2	2	2	5	1	3	6	3	1	2	2		1	1	6
119	46	1	23382/15	26302/15	1	2	2	2	2	2	2	1	2		2	2	2	2	2	1	2	7.5	3	5	5	1	1	2	2		0		1
120	59	2	21444/15	22203/15	2	1	2	1	2	1	2	2	2		2	2	2	1	2	2	2	8.5	2	3	7	6	1	2	2		3	3	7
121	26	1	19778/15	20130/15	2	1	2	2	2	2	2	2	1		2	2	2	2	2	2	1	6	1	2	7	1	2	2	2		2	1	3
122	50	2	49759/14	3792/15	2	1	2	2	2	1	2	2	2		1	1	2	2	2	2	2	6	3	2	8	5	1	2	2		1	2	7
123	46	1	49275/14	12294/15	2	1	2	2	2	1	2	2	2		2	1	2	2	2	2	2	1	3	3	5	1	2	2	2		2	1	1
124	67	1	45561/14	2785/15	1	2	2	2	2	1	2	2	2		2	2	2	2	2	2	1	2.7	1	2	6	1	2	2	2		1	1	2
125	46	2	40672/17	41447/17	1	2	2	2	2	2	2	2	2		2	2	1	2	2	2	2	4.5	2	5	5	4	2	2	2		3	3	5
126	56	1	35639/17	43015/17	2	1	2	2	2	2	2	1	2		2	2	1	2	2	2	2	9	3	5	6	1	2	2	2		3	3	2
127	61	1	45699/17	79/18	1	2	2	2	2	2	2	1	2		2	2	2	2	2	2	1	4.4	3	2	5	2	1	2	2		1	3	5
128	61	1	48536/17	367/18	2	2	1	2	2	2	2	1	2	FATHER	1	1	2	2	2	2	2	4.6	3	2	7	3	1	2	2		2	2	6
129	69	1	48361/17	11409/18	2	2	2	2	2	2	2	1	2		2	1	2	2	2	2	2	2.5	1	2	4	1	2	2	2		3	3	1
130	69	1	15924/18	20582/18	2	1	2	2	2	2	2	1	2		2	2	2	2	1	1	2	5	3	2	6	1	2	2	2		3	3	2
131	44	1	11775/18	12234/18	1	2	2	2	2	2	2	1	2		2	2	2	2	2	2	1	4	3	2	6	1	2	2	2		3	3	2
132	66	2	11383/18	15135/18	1	2	2	2	2	2	2	1	2		2	2	2	2	2	2	1	2.5	2	3	7	6	1	1	2		1	1	7
133	58	2	8097/18	10278/18	2	1	2	2	2	1	2	2	2		2	2	2	1	2	2	2	10	3	3	7	1	1	2	2		3	3	3
134	49	1	4182/18	7508/18	2	1	2	2	2	1	2	2	2	MOTHE	2	2	1	2	2	2	2	5	4	2	6	1	2	2	2		3	3	2
135	41	1	22069/18	23355/18	1	1	2	2	2	2	2	1	2		2	2	2	2	2	1	2	5.5	3	2	6	1	2	2	2		3	3	2
136	50	1	18837/18	22689/18	1	2	2	2	2	1	2	1	2		2	2	2	2	2	2	1	12.5	3	3	7	1	1	2	2		3	3	4
137																																			
138	51	1	18470/18	21826/18	1	1	2	2	2	2	2	1	2		2	1	2	2	2	2	2	13	3	2	6	1	2	2	2		3	3	2
139	55	1	16741/18	20994/18	1	1	2	1	2	2	2	2	2		2	2	2	1	2	2	2	7	2	2	6	5	1	2	1	LUNG	3	3	8
140	33	1	17187/18	19883/18	2	2	2	2	2	1	2	2	2		2	2	2	2	1	2	2	6.7	3	2	8	1	1	2	2		3	3	4
141	42	1	10301/18	11785/18	2	1	2	2	2	1	2	1	2		1	2	2	2	2	2	2	9	1	3	5	1	1	2	2		3	3	1

141	42	1	10301/18	11785/18	2	1	2	2	2	1	2	1	2		1	2	2	2	2	2	2	9	1	3	5	1	1	2	2		3	3	1
142	69	1	47393/18	4093/18	2	2	2	2	2	1	1	1	2		2	2	2	2	2	1	2	6.2	3	2	6	1	2	2	2		3	3	2
143	65	1	256/18	7082/18	1	2	2	2	2	2	2	2	2		1	1	2	2	2	2	2	5.5	3	2	6	1	2	2	2		3	2	2
144	35	1	2218/18	4940/18	2	1	2	2	2	2	2	2	2		1	1	2	2	2	2	2	5.5	3	3	7	2	1	1	1	LIVER	3	3	8
145	58	1	3993/18	16274/18	2	1	2	2	2	2	2	2	2		2	1	2	2	2	2	2	5	3	2	8	4	1	1	2		3	3	7
146	64	2	5433/18	9420/18	2	2	2	2	2	1	2	2	2		1	2	2	2	2	2	2	5	3	2	6	3	1	2	2		3	3	6
147	47	1	8904/18	11655/18	1	1	2	2	2	1	2	2	2		2	2	1	2	2	2	2	10	3	2	8	1	1	1	2		3	3	4
148	83	1	41131/17	1338/18	1	2	2	2	2	1	2	1	2		2	2	2	2	2	2	1	4	3	2	6	1	2	2	2		3	3	2
149	66	1	45984/17	2561/18	1	1	2	2	2	1	2	2	2	BROTH	1	1	2	2	2	2	2	7.5	2	2	6	5	1	1	1	LUNG	3	3	8
150	60	1	4598/18	15284/18	1	2	2	2	2	2	2	1	2		2	2	2	2	2	2	1	3.5	3	2	6	1	1	2	2		3	3	2
151	76	1	11495/18	13294/18	2	2	1	2	2	2	2	2	2		1	2	2	2	2	2	2	3.5	1	2	5	1	2	2	2		3	3	1