DISSERTATION ON

EXPRESSION OF SURVIVIN IN COLORECTAL ADENOCARCINOMAS AND ITS CLINICO-PATHOLOGICAL CORRELATION

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THE TAMILNADU Dr.M.G.R. MEDICAL UNIVERSITY, CHENNAI

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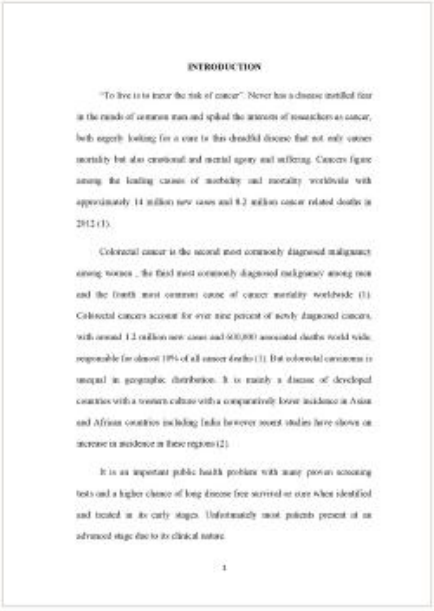

INTRODUCTION

"To live is to incur the risk of cancer". Never has a disease instilled fear in the minds of common man and spiked the interests of researchers as cancer, both eagerly looking for a cure to this dreadful disease that not only causes mortality but also emotional and mental agony and suffering. Cancers figure among the leading causes of morbidity and mortality worldwide with approximately 14 million new cases and 8.2 million cancer related deaths in 2012 (1).

Colorectal cancer is the second most commonly diagnosed malignancy among women , the third most commonly diagnosed malignancy among men and the fourth most common cause of cancer mortality worldwide (1). Colorectal cancers account for over nine percent of newly diagnosed cancers, with around 1.2 million new cases and 600,000 associated deaths world wide, responsible for almost 10% of all cancer deaths (1). But colorectal carcinoma is unequal in geographic distribution. It is mainly a disease of developed countries with a western culture with a comparatively lower incidence in Asian and African countries including India however recent studies have shown an increase in incidence in these regions (2).

It is an important public health problem with many proven screening tests and a higher chance of long disease free survival or cure when identified and treated in its early stages. Unfortunately most patients present at an advanced stage due to its clinical nature.

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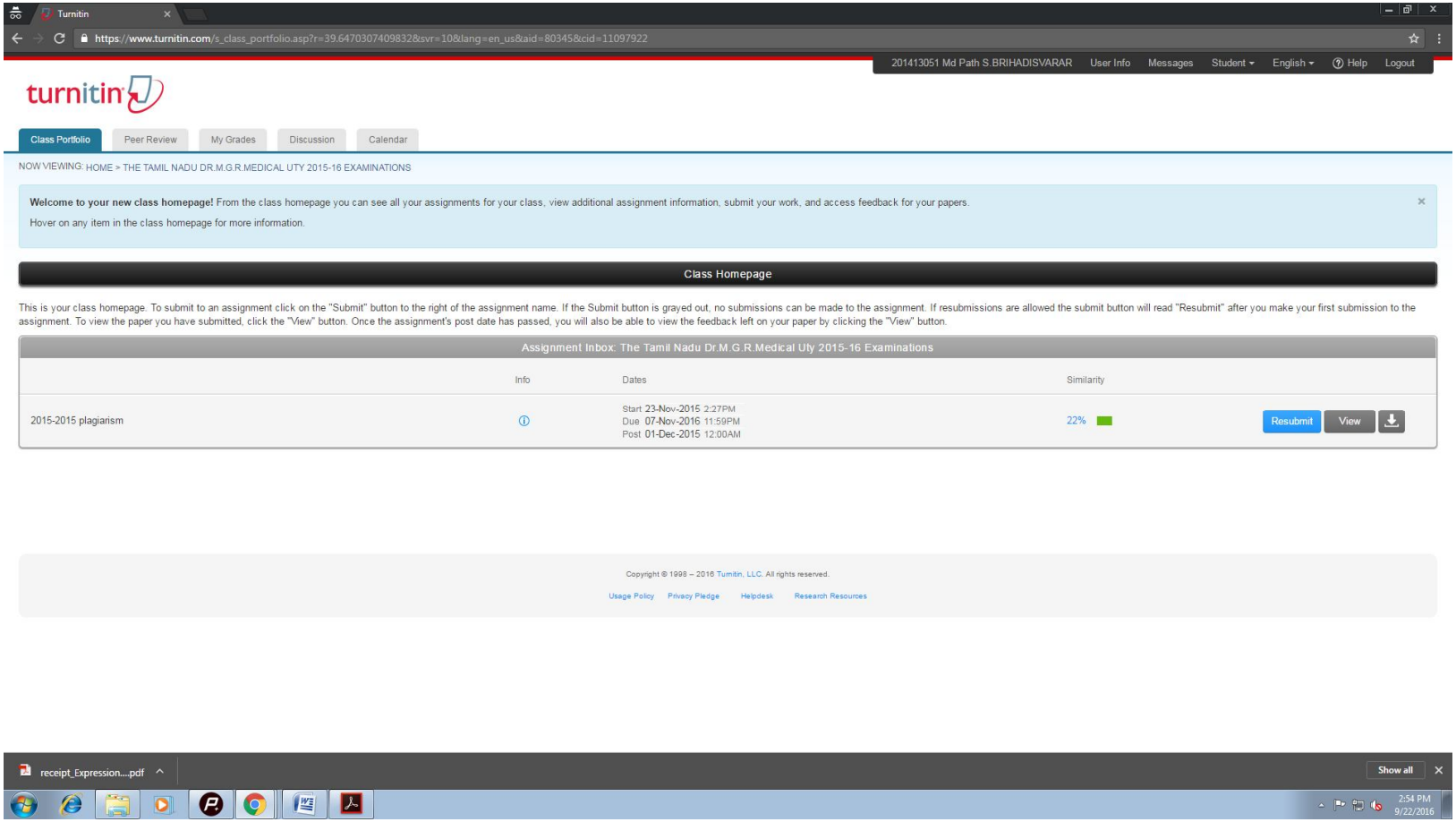
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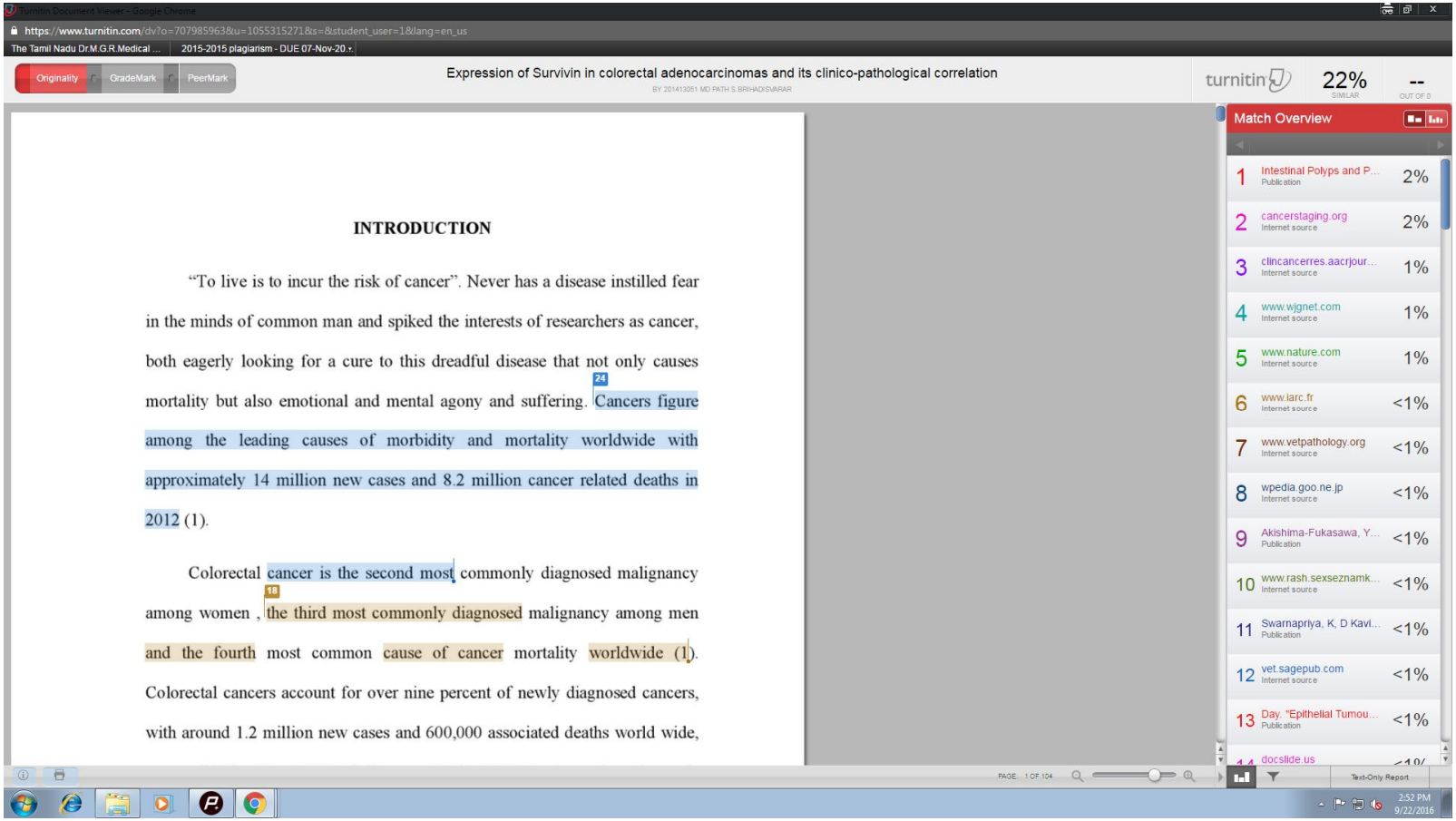
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INTRODUCTION
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Dr.S.Brihadisvarar

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LIST OF ABBREVIATIONS

AJCC	:	American Joint Committee on Cancer
ANOVA	:	Analysis of Variance
APC	:	Adenomatous Polyposis Coli
BCL2	:	B-cell Lymphoma 2
BIRC5	:	Baculoviral inhibitor of apoptosis repeat-containing 5
BRAF	:	B-Rapidly Accelerated Fibrosarcoma
CD	:	Cluster of differentiation
CDE	:	Cell cycle-dependent element
CDX2	:	Caudal type homeobox 2
CEA	:	Carcinoembryonic antigen
CHR	:	Cell cycle genes homology region
CI	:	Confidence Interval
CIMP	:	CpG island methylator phenotype
CK	:	Cytokeratin
CT	:	Computed Tomography
DAB	:	Diaminobenzidine
DCC	:	Deleted in colon cancer
DIABLO	:	Direct IAP binding protein with low pI
DNA	:	Deoxyribonucleic acid
DPC-4	:	Deleted in pancreatic cancer - 4
DPX	:	Distyrene, Plasticizer (tricresyl phosphate), xylene
EDTA	:	Ethylenediaminetetraacetic acid
EMA	:	Epithelial membrane antigen
FAP	:	Familial adenomatous polyposis
FAS	:	Apoptosis stimulating fragment
FLICE	:	FAS associated death domain like IL-1beta converting enzyme
FLIP	:	FLICE inhibitory protein
HNPCC	:	Hereditary nonpolyposis colorectal cancer
IAP	:	Inhibitor of apoptosis
IBD	:	Inflammatory bowel disease
IBM	:	International Business Machines
IHC	:	Immunohistochemistry
INCENP	:	Inner centromere protein
KRAS	:	Kirsten rat sarcoma viral oncogene homolog
MAP Kinase	:	Mitogen-activated protein kinase

MCC	:	Mutated in colorectal cancer
MIB1	:	Mindbomb E3 ubiquitin protein ligase 1
MLH1	:	mutL homolog 1
MMR	:	Mismatch repair
MRI	:	Magnetic resonance imaging
m RNA	:	Messenger RNA
MSH6	:	mutS homolog 6
MSI	:	Microsatellite instability
MUC	:	Mucin
MYH	:	mutY DNA glycosylase, earlier mutY Homolog (E. coli)
PET	:	Positron emission tomography
PI3	:	Phosphatidyl inositol 3
PMS2	:	Postmeiotic segregation increased, Saccharomyces cerevisiae, 2
PP	:	Pancreatic polypeptide
PYY	:	Peptide YY / Peptide tyrosine tyrosine
SATB2	:	Special AT-rich sequence-binding protein 2
si RNA	:	Small (or short) interfering RNA
Smac	:	Second mitochondria-derived activator of caspases
SMAD4	:	Mothers against decapentaplegic homolog 4
SPSS	:	Software Package for Social Sciences
SRCA2	:	Sarco/endoplasmic reticulum calcium ATPase 2
STAT-3	:	Signal transducer and activator of transcription 3
TGF B	:	Transforming growth factor beta
Thr	:	Threonine
TNM	:	Tumor node metastasis
TRIS	:	tris-(hydroxymethyl)aminomethane
UICC	:	Union for International Cancer Control
USFDA	:	United States food and drug administration
WHO	:	World health organization
Wnt	:	Wingless integrated

INTRODUCTION

"To live is to incur the risk of cancer". Never has a disease instilled fear in the minds of common man and spiked the interests of researchers as cancer, both eagerly looking for a cure to this dreadful disease that not only causes mortality but also emotional and mental agony and suffering. Cancers figure among the leading causes of morbidity and mortality worldwide with approximately 14 million new cases and 8.2 million cancer related deaths in 2012 [1].

Colorectal cancer is the second most commonly diagnosed malignancy among women, the third most commonly diagnosed malignancy among men and the fourth most common cause of cancer mortality worldwide[1]. Colorectal cancers account for over nine percent of newly diagnosed cancers, with around 1.2 million new cases and 600,000 associated deaths worldwide, responsible for almost 10% of all cancer deaths[1]. But colorectal carcinoma is unequal in geographic distribution. It is mainly a disease of developed countries with a western culture with a comparatively lower incidence in Asian and African countries including India however recent studies have shown an increase in incidence in these regions[2].

It is an important public health problem with many proven screening tests and a higher chance of long disease free survival or cure when identified and treated in its early stages. Unfortunately most patients present at an advanced stage due to its clinical nature.

Apoptosis is a tightly regulated pathway of cell death where the cells destined to die activate intrinsic enzymes called caspases which degrade the cells own nuclear DNA and cytoplasmic proteins[3]. Apoptosis normally takes place both during development and in adults and also helps in removal of senescent and potentially harmful cells.

Dysregulation of apoptosis by either of the two mechanisms – down regulation of proapoptotic elements and upregulation or over expression of antiapoptotic elements, confers an increased longevity upon the cell and as a consequence indirectly leads to accumulation of oncogenic mutations in a cell which should have been eliminated by apoptosis. This finally leads to the emergence of a malignant clone. Many malignancies have been shown to have dysregulated apoptosis best exemplified by over expression of bcl2, an anti-apoptotic protein in follicular lymphoma and p53 gene mutations which render the neoplastic cells resistant to intrinsic pathway of apoptosis[4,5]. So dysregulation of apoptosis plays a critical role in cancer emergence, survival and growth of the tumor.

Dysregulated apoptosis has also shown to be involved in tumor resistance to anticancer therapy. Thus targeting apoptotic pathways will induce tumor apoptosis, reduce resistance to anticancer therapies and sensitizes cancer cells to apoptosis induction by other therapies.

There are three important antiapoptotic family of proteins which include FLICE-inhibitory proteins (FLIPs), Bcl-2 family and Inhibitors of Apoptosis

Proteins (IAPs). The Inhibitors of Apoptosis (IAP) family of proteins are a group of proteins which inhibit the intrinsic pathway of apoptosis[6]. Common to all the members of this family is the presence of Baculovirus IAP repeats (BIR) in one to three copies. Survivin, the smallest member of the IAP family is a bifunctional regulator of apoptosis and cellular proliferation[7]. It is normally expressed during embryonic development, nearly undetectable in healthy adult tissue and is re expressed in most cancers including colorectal cancers[7].

AIMS AND OBJECTIVES

1. To study the expression of Survivin in colorectal neoplasia
2. To study the expression and role of Survivin in the adenoma-carcinoma sequence.
3. To study the relationship of expression of Survivin to tumor prognostic factors including-
    - Age
    - Sex
    - Histological grade
    - Ki-67 labelling index
    - Tumor Stage

REVIEW OF LITERATURE

An understanding of the normal anatomy and development is essential to know about colorectal neoplasia, their occurrence at different sites along the large intestine, various clinical presentations, factors affecting tumorigenesis, tumor survival and growth, pathways of spread including lymphatic spread and the various diagnostic and therapeutic modalities available at present.

The large intestine :

The large intestine is that part of the gastrointestinal tract which extends from the ileocaecal junction to the anus. It is about 1.5 metres long and is subdivided into the caecum with vermiform appendix located in the right iliac fossa, the ascending colon which ascends up from the caecum to the inferior surface of the liver where it makes a sharp bend called the right colic or hepatic flexure, the transverse colon which extends from the right colic flexure to the left colic flexure or the splenic flexure located inferior to the lateral end of the spleen, the descending colon which descends vertically down from the splenic flexure to the iliac crest where it curves medially towards the pelvic brim, the sigmoid colon located in the pelvis, the rectum and the anal canal which opens to the exterior.

The large intestine develops from the caudal part of the primitive gut tube which includes the post arterial segment of the mid gut, the hind gut and the proctodaeum[8].

The distal mid gut gives rise to the caecum, the appendix, the ascending colon and proximal two-thirds of the transverse colon. Caecum and appendix develop from the caecal bud which arises from the post arterial segment of the midgut loop during the 6^{th} week of gestation. The proximal portion of this bud grows rapidly to form the caecum and the distal part remains narrow and forms the appendix. The ascending colon, hepatic flexure and proximal two-thirds of transverse colon develop from the distal most part of the midgut.

There is physiological herniation of the midgut loops at 6 to 10 weeks of gestation into the extra embryonic coelom. This occurs because the body cavity at this stage of development simply cannot hold all the developing bowel loops. Hence the entire development of the midgut occurs outside the abdominal cavity where the proximal or pre arterial segment gives rise to the small intestine and the caudal post arterial segment gives rise to the right hemicolon. When present outside the abdominal cavity, the midgut undergoes a 90 degree counter clockwise rotation around the axis of the superior mesenteric artery so that the proximal limb comes to the right and the distal part of the loop comes to the left. During the 10^{th} week of gestation the intestinal loops return to the abdominal cavity. As they do so, they undergo a further 180 degree counter clockwise rotation. The proximal part of the midgut loop giving rise to the small intestine returns first passing behind the superior mesenteric artery to the left side of the abdominal cavity. The distal part of the midgut loop which at this stage is the caecal bud, reenters the abdominal cavity the last and settles more to the right side of the abdominal cavity beneath the inferior surface of

the liver. The caecum and the appendix then descend to their position in the right iliac fossa with the formation of the ascending colon and the right two thirds of the transverse colon. The mesentery of the ascending colon fuses with the parietal peritoneum and disappears, thus the ascending colon becomes retro peritoneal[8,9].

The hind gut extends from the midgut to the cloacal membrane. It gives rise to the left one third of the transverse colon, the descending colon, the sigmoid or pelvic colon, the rectum and the proximal portion of the anal canal. The distal part of the rectum develops from the cloaca which opens to the exterior through the proctodaeum. The mesentery of the descending colon fuses with the peritoneum of the dorsal abdominal wall and disappears so that the descending colon becomes retroperitoneal. The mesentery of the transverse colon and that of the sigmoid colon however persist forming the transverse mesocolon and the sigmoid mesocolon respectively.

The cloaca is divided into a dorsal primitive anorectal canal and a ventral urogenital sinus by the formation of the urorectal septum. The primitive anorectal canal is in direct continuation with the hindgut tube, the rectum developing from its proximal portion. The anal canal is formed partly from the endoderm lined primitive anorectal canal proximally and caudally from the ectoderm lined proctodaeum or anal pit. The line of junction between these two is represented by the anal valves and the pectinate line. The rectum becomes retroperitoneal after the fusion of its mesentery with the posterior pelvic wall[8,9].

The blood supply of the large intestine also follows its embryological origin. The right hemicolon developing from the distal part of the midgut including the caecum, the appendix, the ascending colon and the right two thirds of transverse colon derive their blood supply from the superior mesenteric artery. Those developing from the hindgut are supplied by the inferior mesenteric artery. The rectum is supplied by the superior rectal artery a branch of the inferior mesenteric artery, the middle rectal artery a branch of the internal iliac artery and the inferior rectal artery a branch of the internal pudendal artery. The anal canal is supplied by the inferior rectal branches of the internal pudendal arteries. The venous drainage follows the arterial supply.

The lymphatic vessels follow the blood vessels in a retrograde fashion to drain into the superior mesenteric and inferior mesenteric nodes. The lymphatics from the rectum and anal canal above the pectinate line drain to the pararectal and internal iliac group of nodes, while those below the pectinate line drain into the superficial inguinal group of nodes[9].

Most of the large intestine develops from the endoderm except for the distal most portion of the anal canal inferior to the pectinate line which develops from the ectoderm.

The large intestine has, for most of its length, four layers in its wall which from lumen outward are the mucosa, the submucosa, the muscularis propria and the serosa/adventitia. The mucosa contains the lining epithelium with numerous mucus secreting goblet cells, the colonic crypts, the lamina

propria and the muscularis mucosa. The submucosa is rich in blood vessels and nerve fibres. There are also aggregates of lymphocytes forming follicles in the mucosa and submucosa. The muscularis propria has an inner circular and outer longitudinal layer of smooth muscle along with a myenteric Aurebach's plexus of parasympathetic nerves. The serosa is the outermost layer[10].

The functions of large intestine includes absorption of water and remaining absorbable nutrients, absorption of vitamins produced by the colonic bacterial flora such as vitamin K, vitamin B12, thiamine and riboflavin, compaction of faeces, lubrication of faeces by mucus secretion and storage of faeces until it can be defaecated.

COLORECTAL EPITHELIAL NEOPLASIA :

The WHO classifies colorectal epithelial tumors into the following :

1) Adenomas:
    - Tubular adenomas
    - Villous adenomas
    - Tubulo-villous adenomas
    - Serrated adenomas
2) Intraepithelial neoplasia (dysplasia) associated with chronic inflammatory diseases:
    - Low-grade glandular intraepithelial neoplasia

- High-grade glandular intraepithelial neoplasia

3) Carcinoma
    - Adenocarcinoma
    - Mucinous adenocarcinoma
    - Signet-ring cell carcinoma
    - Small cell carcinoma
    - Squamous cell carcinoma
    - Adenosquamous carcinoma
    - Medullary carcinoma
    - Undifferentiated carcinoma
4) Carcinoid (well differentiated endocrine neoplasm)
    - EC-cell, serotonin-producing neoplasm
    - L-cell, glucagon-like peptide and PP/PYY producing tumour
    - Others
5) Mixed carcinoid-adenocarcinoma
6) Others[11]

This study concentrates on the adenomas and adenocarcinomas of the colorectum.

How does a malignant tumor arise from normal epithelium? This question when put in context of colorectal adenocarcinomas has been well

explained by the multistep model of carcinogenesis known as the adenoma-carcinoma sequence. It shows the gradual and incremental acquisition of the malignant phenotype through a series of morphologically identifiable stages which includes epithelial hyperplasia followed by adenoma formation which enlarges and finally undergoes malignant transformation. These precancerous lesions gradually acquire carcinogenic mutation at each stage of their development to finally acquire all the mutations required for a cancer. This progression from adenoma to carcinomas takes 10 to 40 years depending upon multiple factors.

COLONIC NEOPLASIA :

EPIDEMIOLOGY

Colorectal carcinomas account for approximately 9% of all cancers . It is the second most common cancer among women, third most common cancer among men and is the fourth most common cause of cancer mortality in the world[1]. It shows at least a 20 fold variation in occurrence world wide, most commonly occurring in the well developed countries of North America, Europe, Australia and New Zealand, but its incidence is low in Asia and Africa[1,2]. This wide geographic differences are mostly because of varying environmental and dietary factors prevailing at these regions. Migrants from regions with a low colon cancer incidence to nations with a high risk show significant increase in their colon cancer rates indicating that environmental factors play an important

role in colon cancers[2]. The incidence rates in Asian countries including Japan, Korea and Singapore are rapidly increasing probably reflecting their acquisition of a western lifestyle . Significant differences in incidence rates also exist within continents and between different ethnic groups within a same country, indicating genetic and environmental factors at play.

In India, the annual incidence rates of colorectal carcinomas are 4.4 and 3.9 per 1,00,000 for men and women respectively, which are close to the lowest rates in the world. However the incidence of rectal cancer is higher in rural India[12]. The incidence of colorectal carcinomas increases with age. Carcinomas are rare before the age of 40 years except in individuals with a genetic predisposition or predisposing conditions .

Most colorectal adenocarcinomas develop from adenomas, their precursor lesions. Adenomas are benign glandular neoplasms originating from intestinal epithelium. Incidence rates of adenomas vary throughout the world. The reported incidence of adenomas vary from 0% to 69%[13,14]. This large variation in adenoma incidence depends on multiple factors including the geographic area, method employed for detection, age of the patient, presence or absence of hereditary colon cancer syndromes and the type of study done, whether endoscopic screening study or study of data from autopsy. Areas with a high risk of colon cancer also exhibit a higher incidence of adenomas. In western countries, the average prevalence of adenomas by endoscopic screening is about 25% , whereas autopsy studies show a mean incidence of

10 %[15] . This difference in incidence reporting by different methods could be due to various factors like geographic variations, methodological biases and other confounding factors like age of the study population (only adults or inclusion of children in the study). The incidence of adenomas shows an increase at 40 years of age and peaks at 60 to 70 years in people without colon cancer syndromes. However individuals with associated colon cancer syndromes show an increase in incidence of adenomas even before the age of 40 years. Most sporadic adenomas arise in the rectosigmoid region. As the age increases the site of occurrence of adenomas shifts from a distal to a proximal location. Thus left sided adenomas are more common in younger age groups and right sided lesions show an increased frequency in people older than 65 years . Adenomas associated with HNPCC syndrome show a right colon predominance at all age groups and those associated with FAP predominantly occur in the left colon and rectosigmoid[16].

Persons diagnosed with an adenoma have 40% to 55% chance of having additional synchronous adenomas. With increasing age, the occurrence of multiple synchronous adenomas in a person also increases.

Individuals with colorectal cancer syndromes have an increased incidence of colorectal adenomas. They are multiple, occur at a younger age than the general population and have a higher risk of malignant transformation.

ETIOPATHOGENESIS OF COLORECTAL ADENOMAS :

Colorectal adenomas are dysplastic neoplasms of intestinal epithelium with potential for transformation into invasive adenocarcinoma. They can arise sporadically or are associated with a hereditary colon cancer syndrome such as FAP, MYH polyposis, HNPCC, Muir-Torre syndrome and hereditary mixed polyposis syndromes. All adenomas share two basic features of neoplasia, dysregulated proliferation and failure to fully differentiate.

In normal colon the proliferative compartment is located at the base of the crypts. The enterocytes mature as they migrate upward in the crypt, undergo senescence which culminate in apoptosis and exfoliation near the luminal surface. This process in under the influence of the autocrine growth inhibitory, apoptosis inducing effect of TGF-β. However adenomas show a dysregulated proliferation evident by an upward shift of the proliferative compartment, demonstrable by the use of proliferation markers like Ki67 and MIB1. Mitotic figures are present throughout the entire length of the crypt. There is also an increased number of apoptotic cells and TGF-β immunoreactive cells at the luminal surface of the crypts. It indicates a reversal of epithelial cell migration and inward growth pattern directed towards the crypt base rather than toward the lumen[17]. Adenomas also show abnormal epithelial cell differentiation. The adenomatous epithelium resembles cells which are normally present at the crypt base morphologically and phenotypically. It also has incompletely differentiated goblet cells and

absorptive cells at all levels of the crypt in contrast to the pattern of progressive differentiation towards apex seen in normal crypts.

Adenomas represent clonal neoplastic proliferations of colonic epithelial cells. They begin in a single crypt and grow centrifugally by replacing the normal epithelium. As the neoplastic surface epithelium grows, new adenomatous glands are formed by infoldings of the surface epithelium. Gland proliferations predominantly occur at the surface of the crypts. Early adenomas are small growths with a benign tubular appearance. As the adenomas grow they acquire a villous histology and features of high grade dysplasia. Most adenomas progress in size very slowly. On an average, small adenomas take about 10 years to double their size[18]. Some ultimately progress to invasive cancer. However not all adenomas progress, some remain stable and some may even regress and disappear.

CLINICO-PATHOLOGIC CHARACTERISTICS OF COLORECTAL ADENOMAS :

Small adenomas measuring less than 1 cm in diameter are usually asymptomatic unless they are located in the rectosigmoid where their surface may become ulcerated due to friction from hardened stool and cause bleeding. Symptoms of larger adenomas depend upon their size and location and it includes bleeding which is seldom severe, mucous diarrhoea, constipation,

tenesmus, incontinence, prolapse and anaemia[19]. If adenomas are large enough, they may cause altered bowel habits or intussusceptions. In addition, individuals with hereditary colon cancer syndromes may show one or more extra intestinal manifestations of the disease including osteomas, epidermoid cysts, fibromas, supernumerary teeth and other dental malformations, congenital hypertrophy of the retinal pigment epithelium, desmoid tumors, thyroid cancer and a number of endocrine and other neoplasia.

Grossly, adenomas show three major patterns of growth :

1) Pedunculated polyp
2) Sessile polyp
3) Flat or depressed adenomas.

Polyp architecture depends upon the histological pattern of the adenoma – tubular, villous or tubulo-villous. Classically tubular adenomas have been described as pedunculated. However they can also be sessile. The converse holds true for villous adenomas.

Tubular adenomas tend to be spherical with a relatively smooth surface that is often divided into lobules by inter communicating clefts in larger lesions and appear redder than the surrounding mucosa.

Villous adenomas can be of three types –

1) Flat masses
2) Large lobulated sessile masses
3) Lesions with short, broad peduncles.

They tend to have a shaggy surface with obvious papillary fronds, ill defined edges and covering a wide area of mucosa.

Flat adenomas appear as plaque like lesions with vague discolouration on endoscopy, more clearly delineated after spraying the mucosa with methylene blue or indigo carmine and become highlighted and are readily identified in colectomy specimens after formalin fixation[20]. They are seldom larger than 2 cm in diameter.

Histologically, adenomas are classified into tubular, villous and tubulo-villous adenomas. This classification is based on the amount of tubular or villous component in each adenoma. The most common is the tubular adenoma[21].Tubular adenomas maintain their original crypt architecture with adenomatous epithelium replacing the normal epithelium and are composed of closely packed branching tubules that are separated by varying amounts of lamina propria. Villous adenomas show elongated finger like non-branching fronds of dysplastic epithelium extending into the colonic lumen perpendicular to the muscularis mucosae. The villi are lined by a single layer of adenomatous

epithelium with a central core of lamina propria. If an adenoma shows 75 to 80% of tubular or villous architecture, then it is classified as tubular adenoma or villous adenoma respectively, with all the lesions falling in between being classified as tubulovillous adenomas[22].

By definition all adenomas contain dysplastic epithelium. The WHO uses a two tier grading system of low grade and high grade dysplasia. The usual adenoma is considered to show low grade dysplasia.

High grade dysplasia includes both architectural and cytological changes. Architecturally the crypts show irregular branching, budding and cribriforming. Cytologically the nuclei show nucleomegaly, hyperchromasia, prominent nucleoli, stratification, loss of polarity and increased mitoses. Cytoplasmic mucin production may be reduced or absent. These dysplastic cells remain confined within the basement membrane of the crypt.

Extension of the neoplastic cells through the basement membrane into the underlying lamina propria is called an intramucosal carcinoma. Invasive carcinoma shows extension of the neoplastic cells through the muscularis mucosae into deeper layers. Differentiating between high grade dysplasia and invasive carcinoma is important as the latter has the potential to metastasize whereas the former does not.

CARCINOMATOUS TRANSFORMATION IN ADENOMAS:

Though adenomas have been shown to be precursors for most carcinomas, not all adenomas transform into carcinomas. 90 to 95 % of adenomas never turn malignant during the lifetime of an individual[23]. Factors predisposing to carcinoma development includes adenoma size, histological type, grade of dysplasia and patient age.

Most adenomas of size less than 1 cm in diameter show only low grade dysplasia and the risk of cancer developing in them is about 5% at 15 years, which becomes 27% when high grade dysplasia is present. The risk of malignant transformation in an adenoma increases with the amount of villous component present in it. Thus villous adenomas have the highest risk of malignant transformation followed by tubulovillous adenomas and tubular adenomas[24]. It is estimated that the rate of conversion of adenomas to cancer is 0.25% per year[25].

In individuals with hereditary colon cancer syndromes and adenomas, progression to cancer is inevitable without surgical intervention. FAP is the experiment of nature that provides support for the adenoma-carcinoma sequence. By the age of 30 years 75 % of FAP patients will have developed colon carcinoma unless a prophylactic colectomy is performed and this increases to 100% by 50 years of age. However patients undergoing

prophylactic colectomy are still at risk of developing malignancies at sites other than colon

THE ADENOMA-CARCINOMA SEQUENCE :

Although not all colorectal carcinomas arise from adenomas and not all adenomas turn malignant, the precancerous nature of adenomas is widely agreed upon and adenomas constitute the obligate precursor lesion for most colorectal carcinomas. The adenoma-carcinoma sequence is a multi step model of colorectal carcinogenesis which explains the gradual and incremental acquisition of the malignant phenotype through a series of morphologically identifiable stages which includes epithelial hyperplasia followed by adenoma formation which enlarges and finally undergoes malignant transformation. Molecular analyses of each of these stages have shown that the precancerous lesions have fewer mutations than adenocarcinomas and a tendency to acquire incremental mutations at various stages of carcinogenesis.

Various facts and studies show the precancerous nature of adenomas. They include:

1. Prevalence rates of adenomas and carcinomas in countries with varying amounts of colon cancer risk show a correlation between the two.
2. Increased incidence of carcinomas in individuals with adenomas.

3. Similar distribution of adenomas and carcinomas in various geographic regions.
4. Similar anatomic distribution of adenomas and carcinomas.
5. Coexistence of adenoma and carcinoma in the same lesion.
6. Residual adenomas found in patients with cancer.
7. Production of both adenomas and carcinomas in laboratory animals.
8. All patients with FAP eventually develop carcinoma if adenoma bearing colon is not removed.
9. Absence of carcinoma in situ outside the area of adenoma.
10. Denovo carcinoma is extremely rare.
11. Endoscopic removal of adenomas reduces the expected incidence of cancer by 85%.
12. Areas of direct transition from adenoma to carcinoma are present.
13. In vitro growth of adenomatous cells results in cell populations that acquire features of carcinomas.
14. Similar chromosomal constitution of adenomatous and carcinomatous tissues.
15. Antigenic relatedness between adenomas and carcinomas.
16. Similar enzyme patterns in adenomas and carcinomas which are different from normal mucosa and hyperplastic polyps.
17. DNA content of benign adenomas is intermediate between normal colon and cancer.
18. Similar oncogenes are found in some adenomas and carcinomas.

COLORECTAL ADENOCARCINOMAS –

ETIOLOGY AND RISK FACTORS :

Multiple factors are involved in the etiology of colorectal adenocarcinomas. Both environmental and constitutional factors are implicated and the precise composition of various factors varies from one individual to another.

A) DIETARY FACTORS :

a) Fat and animal proteins :

Multiple studies have shown that a high fat diet and animal protein intake particularly red meat favors the development of colon cancer [26]. A strong correlation exists between national per capita fat consumption and colon cancer. The mechanisms by which high fat diet is associated with increased risk of colon cancer are as follows:

- Fat as a source of calories, results in long term weight gain, an identified risk factor for colon cancer .
- As a surrogate marker for red meat intake which is a strong and independent risk factor for colon cancer[26].
- Diets high in fat and meat associate with formation of hydroxyl radicals which lead to oxidative DNA damage of colonocytes and their subsequent malignant transformation .

b) Fibers, fruits and vegetables :

An inverse relationship exists between colon cancer and intake of fruits, vegetables and dietary fibers. The mechanisms by which fiber may protect against colon cancers include :

- Increases fecal mass.
- Decreases mucosal contact time with potential carcinogens.
- Binds various reactive compounds.
- Dilutes intestinal contents.
- Blocks free radical formation.
- Substitutes for dietary fat.
- Reduces time for bacterial conversion of bile acids.
- Has direct anti toxic effect against carcinogens.
- Increases production of hydrogen, methane and short chain fatty acids.
- Adsorbs organic and inorganic substances including bile salts.
- Decreases hydroxylation of bile acids.

c) Micronutrients :

In addition to fibers, green leafy vegetables and fruits are a rich source of antioxidants, folate, micronutrients like carotenoids and ascorbate and non nutrients such as flavonoids, isothiocyanates and indoles that possess potent anti carcinogenic properties[27]. Consumption of vitamins A,C,D and E and the

micronutrients calcium, selenium also reduces colon cancer risk. Garlic is the vegetable with the strongest inverse association with colon cancer risk[28].

d) Relationship with energy balance :

Reduced physical activity, obesity and positive energy balance have a positive correlation with incidence of colon cancers. In addition insulin resistance also appears to play a role. Patients having type 2 diabetes have a three fold increase in colon cancer risk.

B) ALCOHOL CONSUMPTION :

Alcohol significantly increases the risk of rectal cancer but has only a weak association with colon cancer . Also alcohol consumption increases the risk of adenomas and cancers due to abnormal DNA methylation[29].

C) SMOKING :

The incidences of both colorectal adenomas and carcinomas are significantly increased with smoking and tobacco use. Cancer risk increases significantly with pack years and earlier age of first use. Smoking may act as a tumor initiator and is also linked with microsatellite instability, CpG Island Methylator Phenotype (CIMP) and BRAF mutations in colon cancers[30,31].

D) OCCUPATIONAL FACTORS :

Rectal and sigmoid cancers are associated with occupations in which dusts or fumes are inhaled for a long period of time. An increased cancer risk is

found in workers exposed to wood, metal dusts, plastics, fumes, organic solvents, cement, fibreglass and asbestos. Also individuals with sedentary jobs are more likely to develop colorectal cancer than those with active jobs.

E) ASSOCIATION WITH DIVERTICULOSIS :

There is an increased incidence of left sided colon cancer in patients with diverticular disease. This is especially true in western population but is less common among Asian population[32]. It has been shown that adenomas and diverticulae both increase in migrant population, possibly due to dietary influences. However it is unlikely that the diverticulae themselves predispose to neoplastic transformation.

F) SOCIOECONOMIC FACTORS AND URBANISATION :

Colorectal cancer incidence is higher among urban population than in the rural population, presumably due to dietary and lifestyle differences[33].

G) INFLAMMATORY BOWEL DISEASE :

Individuals with IBD, both ulcerative colitis and Crohn's disease have an increased risk of developing colorectal adenocarcinomas. The incidence of colon cancer is 4 to 20 times higher than the general population. Colon cancer affects 11.2% of patients with ulcerative colitis and 4.8 % of patients with Crohn's disease. The cancer risk positively correlates with disease duration and anatomical extent of the disease[34,35]. The average age at the time of diagnosis of colorectal cancer is 10 to 20 years earlier than those arising in patients

without IBD. The mean duration of disease to diagnosis of cancer is 15 years in Crohn's disease and 18 years in ulcerative colitis.

H) RADIATION :

A minority of colon cancers have radiation as their etiological factor. Women undergoing radiotherapy for gynaecological cancers have a relative risk of 2 to 3.6 for developing colorectal cancers. Rectal tumors arise more commonly in patients treated with radiation for prostatic, cervical and uterine cancers.

I) SCHISTOSOMIASIS :

Patients with Schistosoma japonicum infection have an increased incidence of colorectal neoplasia compared to the normal public. The colorectal neoplasia develop in a background of schistosomal colitis and are often preceded by dysplasia. The parasitic ova can be found admixed with the tumor. The carcinomas are often multicentric and arise at an earlier age[36].

J) URETEROSIGMOIDOSTOMY AND OTHER SURGICAL PROCEDURES :

There is a 500 time increase in risk of colon cancer in patients who have undergone ureterosigmoidostomy. Activation of fecal carcinogens by the diverted urine causes chronic inflammation and increase cell proliferation, predisposing to adenoma and carcinoma formation. Adenomas develop 10 to 20 years following ureterosigmoidostomy and carcinomas can arise as late as

53 years later[37]. Other procedures with an increased risk of colorectal carcinomas include ileal conduits, ileostomy, colostomy and peptic ulcer surgeries.

K) GENETIC FACTORS :

Familial forms of colorectal cancers can be classified into three categories :

a. Polyposis syndromes.
b. Hereditary colon cancer syndromes.
c. Carcinoma in individuals appearing to be sporadic but with other family members having colon cancer.

Hereditary polyposis account for about 1% of all colorectal carcinomas, HNPCC accounts for upto 5% and perhaps 30% or more of sporadic carcinomas may be inherited[38].

a) HEREDITARY POLYPOSIS SYNDROMES:

It includes Familial adenomatous polyposis (FAP) syndrome and its variants, attenuated FAP and MYH polyposis syndrome.

Familial adenomatous polyposis (FAP) syndrome :

It is an autosomal dominant disorder caused by mutation of the APC gene located on chromosome 5q21 which is an important negative regulator of

the Wnt signalling pathway[39]. Every cell in an FAP patient has an inactive APC allele. An alteration in the other allele gives rise to intestinal and extra intestinal manifestations of the disease.

Various combinations of the extra intestinal manifestations have been given different names. Gardner syndrome has colonic polyps, epidermoid cysts, osteomas and desmoids whereas Turcot syndrome was diagnosed if the patient had colonic polyps with brain tumors. But FAP, Gardner syndrome and Turcot syndrome all represent variations of the same disease rather than distinct entities resulting from the same underlying genetic abnormality with variable expressivity.

Atleast 100 polyps are necessary for the diagnosis of classical FAP but much more can be present. In addition to polyps which can be pedunculated or sessile, flat adenomas are also seen in FAP. The earliest lesion in FAP is the microscopic adenoma in a grossly normal appearing mucosa.

10 to 30% of FAP patients have no family history and represent new germline mutations of the APC gene. Adenomas are not present at birth in FAP patients. They start developing at the time of puberty and progressively increase with time . The adenomas almost always involve the rectosigmoid. By the time a patient comes for colectomy he or she may have hundreds to tens of thousands of polyps.

Progression to cancer is inevitable. By age 30, around 75% of FAP patients will develop colon cancer and 100% by the age of 50 years unless a

prophylactic colectomy is performed. Colorectal carcinomas arising in the setting of FAP may be multifocal and more frequently develops on the left side of the colon.

Attenuated adenomatous polyposis is a less severe form of FAP with a lesser number of adenomas, usually less than 100 and an increased frequency of flat adenomas. However these patients sustain a high risk of colorectal cancer. The cancers usually develop 15 years later than in patients with classic FAP and approximately 10 years earlier than individuals with sporadic colorectal cancer.

MYH polyposis syndrome :

It is an autosomal recessive syndrome caused by mutation of the human base excision repair gene MYH located on chromosome 1. The precise colon cancer risk associated with MYH is yet to be determined, however it is probably similar to FAP. The mean age of adenoma and carcinoma diagnosis is 46 and 49.7 years respectively[40].

b) HEREDITARY COLON CANCER SYNDROMES :

These include Hereditary Non Polyposis Colorectal Cancer syndrome (HNPCC) and Muir –Torre syndrome.

HNPCC or Lynch syndrome is due to germline mutations in the DNA mismatch repair genes, most commonly MLH1, MSH2, MSH6 AND PMS2 resulting in a high level of microsatellite instability. HNPCC is five times more common than FAP and represents the most common form of inherited

colorectal cancer[41]. The following are the features of Hereditary Non Polyposis Colorectal Cancers:

- Autosomal dominant inheritance
- Cancer development at younger age (45 years)
- Tumors tend to originate proximal to the splenic flexure
- Tendency to develop multiple tumors
- Abundant mucin secretion by the tumor
- Tumor infiltrating lymphocytes
- Poor differentiation
- Lack of dirty necrosis
- Presence of a Crohn's disease like inflammatory reaction
- Diploid nature of the tumors
- Association with tumors of other sites especially endometrium, urothelium and stomach

MUIR-TORRE SYNDROME : It is an autosomal dominant hereditary disorder with a variant phenotype that consists of skin tumors of sebaceous differentiation and visceral malignancies. 50% of patients have colorectal cancers. The median age of onset of colorectal cancer is 50 and they are more often located in the proximal colon[42].

L) ASPIRIN AND OTHER NONSTEROIDAL ANTI INFLAMMATORY DRUGS:

Several studies have shown that aspirin and other nonsteroidal anti inflammatory drugs have a protective effect against colorectal adenomas and carcinomas. They prevent the development, progression and have also been demonstrated to cause regression of colorectal adenomas and carcinomas[43]. This is believed to be due to inhibition of cyclooxygenase-2(COX-2), which is highly expressed on 90% of colorectal carcinomas and 40 to 90% of adenomas.

M) OTHER FACTORS :

Other factors which may play a role in colorectal cancers include pernicious anemia, diabetes mellitus, celiac disease, skin tags and AIDS, all of which show an increased colorectal cancer risk.

CLINICAL FEATURES :

Colorectal carcinomas develop over a long period of time hence the symptoms may be absent or are slowly progressive that the patient fails to notice them, or may present with minimal symptoms. 5% to 12.5% of patients may remain asymptomatic. The symptoms and signs may be related to the gastrointestinal tract or they may be constitutional in nature. Weight loss, malaise and symptoms of anaemia commonly occur but are often disregarded by the patient due to their non specificity.

The clinical presentation depends upon whether the tumor is located in the right or left side of the colon and whether they are early or advanced. Cancers of the caecum and ascending colon are often polypoidal or flat in nature and the stool is soft in this location. Hence right sided lesion tend to remain clinically silent as they seldom cause obstruction or melena.

Changes in bowel habits affects 22% to 58% of the patients and are commonly associated with neoplasms of the left colon. These changes are often minimal but progressive. They include diarrhoea, a sense of incomplete rectal emptying and incontinence. As the tumor grows and encircles the bowel wall, constipation, obstipation and other signs of bowel obstruction appear progressively[44].

50% of patients present with rectal bleeding which varies in severity from a very subtle degree to frank hematochaezia. Most cases of noticeable hematochaezia occurs with left sided colorectal cancers. Another 50% of patients present with abdominal pain which is more likely to occur in tumors involving colon rather than those involving the rectum. Pain often occurs in advanced cancers when they invade the serosa or adjacent tissues. It can also be due to varying degrees of bowel obstruction proximal to a constricting carcinoma. Occasionally sigmoid carcinomas present as acute abdomen due to bowel obstruction and perforation causing acute peritonitis. Peritonitis can also result due to perforation of bowel by a deeply invasive tumor. Small bowel

obstruction is encountered when the small bowel loops become adherent to or are invaded by the tumor[44,45].

Locally advanced carcinomas invade through the bowel wall and a colo-colic fistula may develop. Uncommonly a clinically silent but advanced carcinomas, usually of the caecum may present as an abdominal mass or hepatomegaly due to metastases[45]. Unusual presentations of colorectal cancers include the development of gluteal abscesses or colocutaneous fistulae, pyrexia of unknown origin or pyogenic arthritis. In addition to these, colorectal carcinomas arising in specific settings of polyposis syndromes, hereditary colon cancer syndromes and inflammatory bowel disease have additional intestinal and extra intestinal manifestations specific to the disease process.

PATHOLOGICAL FEATURES :

GROSS FEATURES –

The gross appearance of colorectal carcinomas can be :

- Polypoidal
- Fungating or exophytic
- Ulcerating
- Stenosing
- Diffusely infiltrative
- Flat superficial

The polypoid type of carcinoma forms exophytic intraluminal masses with little surface ulceration. It is generally nodular, lobular or papillary and often has residual adenomas.

One third of colorectal adenocarcinomas are fungating. Large fungating lesions often occur in the caecum and ascending colon. Fungating lesions are basically papillary lesions with extensive ulceration which destroys the underlying papillary architecture leaving behind the residual exophytic component. Although these may occupy a large part of the colonic lumen, they rarely cause obstruction. The intraluminal tumor volume often exceeds the intramural tumor volume.

About two thirds of all colorectal carcinomas are ulcerating. These invade deep into the wall of the colon. Carcinomas of descending and transverse colons are usually ulcerative and infiltrative, progressively involving the entire circumference of the colon eventually forming annular constricting tumors. These tumors show the characteristic “apple core” or “napkin ring” appearance on barium contrast radiographs. Their surface appearance resembles infiltrative carcinomas. These cause bowel wall thickening due to tumor infiltrating the muscularis propria and are firm due to the desmoplastic response that they elicit. With continued growth the tumor invades through the colonic wall and involves contiguous structures. Necrosis and ulceration in a tumor may cause perforation and peritonitis.

Infrequently encountered are the diffusely infiltrating type of carcinomas resembling linitis plastica of the stomach. The involved colonic wall is thickened and rigid. Few adenocarcinomas show a flat or superficial pattern of growth. These arise from flat adenomas and grossly appear as flat plaques with extensive intramural invasion. About 10% of colorectal cancers have a mucoid appearance on cut surface.

MICROSCOPIC FEATURES :

90% to 95% of all colorectal malignancies are adenocarcinomas[46]. The defining feature of adenocarcinomas is their invasion through the muscularis mucosae into the submucosa and deeper. They are usually easily identifiable gland forming well to moderately differentiated adenocarcinomas. 25% are well differentiated, 60% are moderately differentiated and 15 % are poorly differentiated[46]. Tall malignant columnar cells with a high mitotic rate line irregular glands of varying sizes. The malignant cells show cytological anaplasia however some tumors may be so well differentiated that the only evidence of malignancy may be the presence of glands infiltrating the bowel wall. Many adenocarcinomas show a strong desmoplastic response.

In many tumors the superficial tumor and the deeper infiltrative part are histologically similar. In others the deeper tumor may differ histologically from the surface. Approximately 10% of colorectal adenocarcinomas produce mucin. The designation mucinous adenocarcinoma is used when more than 50% of the tumor is composed of mucin where malignant cells can be seen floating in

pools of mucin. Exophytic carcinomas may exhibit a papillary architecture composed of malignant cells, however the invasive component is more commonly glandular. Very rare tumors show a prominent papillary pattern even in the invasive component. Occasional colorectal adenocarcinomas may mimic urinary bladder villous adenomas[47].

The adenocarcinomas invade through the wall in an expanding or an infiltrative pattern. The expanding part consists of nodular aggregates of malignant glands. The infiltrative part is composed of small glands and individual malignant cells infiltrating through the bowel wall.

The edge of the tumors may show residual adenomatous mucosa especially in smaller tumors and they tend to be well differentiated.

TUMOR STROMA :

The stroma in colorectal adenocarcinomas vary from little or no stroma to obviously scirrhous tumour. The tumor can elicit variable desmoplastic response in the stroma. Prominent stromal elastosis occurs in some tumors. Peritumoral lymphocytic infiltrates have been associated with prognosis and they signify an ongoing immune response potentially directed against the tumor cells . Cytotoxic T lymphocytes constitute the majority of the infiltrating cells. Studies have shown that patients having peritumoral lymphocytic infiltrates have a favourable prognosis[48].

SPECIAL HISTOLOGICAL TYPES:

- Cribriform comedo-type adenocarcinoma
- Medullary carcinoma
- Micropapillary carcinoma
- Mucinous adenocarcinoma
- Serrated adenocarcinoma
- Signet ring cell carcinoma[11]

HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL FEATURES :

Histochemically, most colorectal adenocarcinomas are positive for mucin stains. Immunohistochemically MUC1 and MUC3 are expressed in conventional adenocarcinomas, also MUC13 is expressed in poorly differentiated tumors[49].

Colorectal adenocarcinomas are positive for cytokeratin (CK). They are commonly positive for CK20 and negative for CK7, a feature that is greatly helpful in differentiating adenocarcinomas of colon from that of other sites. However aberrant expression of CK7 is possible in poorly differentiated carcinomas[50].

Colorectal adenocarcinomas express CEA, and are usually strongly positive for the transcription factors CDX-2 and SATB2[50,51]. Strong immunoreactivity for p53 is observed in most cases, as is the nuclear staining for beta catenin.

Other markers consistently expressed by colorectal carcinomas include villin, cathepsin B, neuropilin-1, SRCA2 and cadherin-17.

MOLECULAR GENETIC FEATURES – THE GENETIC MODEL OF COLORECTAL CANCER:

Based upon the genetic background, colorectal carcinomas can be classified into three types.

1) Chromosomal instability pathway :

This explains the classical adenoma-carcinoma sequence. Carcinomas arise from the accumulation of activation of oncogenes and the inactivation of tumor-suppressor genes that initially cause adenomatous polyps, some of which then acquire additional mutations and become malignant. The molecular pathway may vary in different colorectal carcinomas.

The earliest event involves inactivation of the APC gene leading to the formation of dysplastic crypts and polyps. KRAS mutations occur in larger polyps transforming them into adenomatous lesions. Further 18q loss and mutations of DCC (Deleted in Colon Cancer) and DPC-4 (Deleted in Pancreatic Cancer-4) occur late in the sequence. Finally inactivation of p53 by mutation or 17p loss heralds development of high grade dysplasia and invasive carcinoma[52,53,54].

2) Microsatellite instability pathway :

Microsatellite instability (MSI-H) has been found to be an important alternate pathway in 15% to 20% of colorectal carcinomas[54,55]. The microsatellite instability is caused by DNA mismatch repair (MMR) deficiency. The DNA mismatch repair genes can be inactivated by germline and somatic mutations.

Mutational inactivation of the MMR genes usually occurs as a second hit in patients already carrying germline mutations of MMR genes and is associated with HNPCC syndromes.

Tumors associated with microsatellite instability frequently tend to be mucinous or poorly differentiated type, with a prominent host response, a circumscribed growth pattern and a more right sided location.

3) CpG Island Methylator Phenotype (CIMP) pathway :

It is associated with MLH1 hypermethylation resulting from epigenetic inactivation of MMR genes. The tumors associated with this pathway often initially present as serrated adenomas, show hypermethylation of numerous genes (CIMP) and also show BRAF mutations[52,53,54].

Oncogenes that are commonly mutated in colorectal adenocarcinomas are KRAS, BRAF, PIK3 and CTNNB1 (beta catenin gene).

Tumor suppressor genes that are commonly involved in colorectal adenocarcinomas are TP53, APC, DPC4/SMAD4, DCC and MCC.

PROGNOSTIC FACTORS :

The prognosis of colorectal adenocarcinomas is related to a number of clinical and pathological parameters. The most important among them is the tumor stage. Particularly the presence of visceral peritoneal invasion and lymph node metastasis are critical.

Predictors of poor outcome include extramural venous invasion, lymphovascular invasion, an infiltrative (as against expansile or circumscribed) tumor border, tumor budding at the invasive front, perineural invasion, mucinous carcinoma, signet ring carcinoma, CEA levels greater than 5 ng/mL, tumor multiplicity, perforation and aberrant expression of pRB and P16[56].

Features associated with a favourable prognosis include tumor infiltrating lymphocytes, a Crohn-like reaction, peritumoral lymphoid response and BCL-2 protein expression[56].

HISTOLOGICAL GRADING OF COLORECTAL ADENOCARCINOMAS:

Adenocarcinomas are graded on the basis of the extent of glandular differentiation. The percentage of tumor showing well formed glands is used to define the grade. Well differentiated or grade 1 tumors exhibit glandular structures in more than 95% of the tumor, moderately differentiated or grade 2 adenocarcinoma has 50% to 95% glands, poorly differentiated or grade 3 adenocarcinoma has 5% to 50% glands and undifferentiated grade 4 carcinoma

has less than 5% glands. Mucinous adenocarcinoma and signet ring carcinoma are considered poorly differentiated (grade 3)[57].

STAGING OF COLORECTAL CANCERS :

Pathologists have been using Dukes classification[58], devised in 1932 and Astler-Coller classification[59], devised in 1954 for many years. However in order to have a universal standardized form of reporting, these two classifications are now being rapidly replaced by the TNM system, as suggested by the AJCC and UICC. It is based on the depth of tumor invasion, regional lymph node status and the presence or absence of metastasis.

The following is the AJCC (7^{th} edition) TNM classification of colorectal carcinomas[60] :

TUMOR (PRIMARY TUMOR)

TX – Primary tumor cannot be assessed.

T0 – No evidence of primary tumor.

Tis – Carcinoma in situ : intraepithelial or invasion of lamina propria.

T1 – Tumor invades submucosa.

T2 – Tumor invades muscularis propria.

T3 – Tumor invades through the muscularis propria into pericolorectal tissues.

T4a – Tumor penetrates to the surface of visceral peritoneum.

T4b – Tumor directly invades or is adherent to other organs or structures.

REGIONAL LYMPH NODE :

NX – Regional lymph nodes cannot be assessed.

N0 – No regional lymph node metastasis.

N1 – Metastasis in 1- 3 regional lymph node(s).

N1a – Metastasis in 1 regional lymph node.

N1b – Metastasis in 2 - 3 regional lymph nodes.

N1c – Tumor deposit(s) in the subserosa, mesentery, or nonperitonealized pericolic or perirectal tissues without regional nodal metastasis.

N2 – Metastasis in 4 or more regional lymph nodes.

N2a – Metastasis in 4 – 6 regional lymph nodes.

N2b – Metastasis in 7 or more regional lymph nodes.

METASTASIS :

M0 – No distant metastasis.

M1 – Distant metastasis.

M1a – Metastasis confined to one organ or site (e.g., liver, lung, ovary, nonregional node).

M1b – Metastasis in more than one organ/site or the peritoneum.

STAGE GROUPING :

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

Table 1 : Stage grouping of colorectal adenocarcinomas

(MAC – Modified Astler-Coller staging)

INVESTIGATIONS FOR SCREENING, DIAGNOSIS AND STAGING :

1) SCREENING AND EARLY DETECTION OF COLORECTAL CANCER :

This is a type of secondary prevention which involves detecting the disease process before it becomes symptomatic. Cancers detected during screening have a more favourable stage. There is a reduction in cancer mortality due to –

a) Early identification of curable cancers

b) Identification and removal of premalignant lesion including adenomatous polyps.

c) Subsequent surveillance to detect recurrence early.

Screening can be applied to -

a) General population having a higher baseline incidence of colorectal carcinomas, especially in individuals above 50 years of age.

b) High risk individuals – patients having polyposis or hereditary colon cancer syndromes, inflammatory bowel disease and individuals having relatives with colon cancer.

Methods employed for screening include fecal occult blood testing, double contrast barium enema, sigmoidoscopy, colonoscopy and a combination of these.

2) COLONOSCOPY:

Colonoscopy is the preferred and most accurate modality for diagnosis of colorectal carcinomas. It has the advantage of accurate localisation of the lesion, identification of synchronous lesions, facility to biopsy suspicious lesions and removal of polyps[61]. The disadvantage is that it is an invasive procedure and needs bowel preparation. For lesions identifiable endoscopically, tissue sampling methods include biopsy, brushings, polypectomy, mucosal resection and sub mucosal dissection.

Incomplete colonoscopy : Approximately 11 to 12% of individuals undergoing colonoscopy end up having an incomplete colonoscopy due to the inability of the colonoscope to visualise the tumor or the mucosa proximal to the tumor. The reasons for an incomplete colonoscopy includes poor preparation, tortuous colon, partial or complete obstruction by the tumor and patient intolerance.

3) FLEXIBLE SIGMOIDOSCOPY:

It is not considered an adequate or complete investigation for colorectal carcinoma diagnosis unless there is a palpable mass involving the rectum.

4) CT COLONOGRAPHY :

It is also known as virtual colonoscopy. It uses an imaging modality which could be conventional spiral CT, helical CT or magnetic resonance to capture images of the colon. A sophisticated computer software then processes

these images and finally provides a computer simulated image of the colon from an endoscopic view point. This investigation needs prior bowel preparation as fecal material may mimic polyps. CT colonography is useful in cases of incomplete colonoscopy[62]. By virtue of being a non invasive technique, it is now being evaluated as the initial diagnostic test in suspected colorectal cancer.

5) PILLCAM :

This consists of a capsule with inbuilt video recording apparatus which is swallowed by the patient. It has been approved by the EMA in Europe and the USFDA for colon cancer screening and in persons who have had an incomplete colonoscopy without obstruction.

6) BARIUM ENEMA :

It can be used to investigate patients with symptoms and signs of colorectal carcinomas. Double contrast barium enemas can be employed and is widely available compared to other diagnostic modalities. However the diagnostic yield is low. If a lesion is detected by barium enema, a colonoscopy must be done to establish the histological diagnosis and to search for synchronous lesions [63].

7) TUMOR MARKERS :

Numerous serum markers are associated with colorectal carcinomas. Most notable among these is Carcino Embryonic Antigen (CEA). However

none of the tumor markers are specific for colorectal cancer. There are numerous conditions which are associated with elevation of these tumor markers including cigarette smoking, liver disease, peptic ulcer disease, diverticulitis, chronic obstructive pulmonary disease, diabetes and other acute or chronic inflammatory states. Further these tumor markers have a low sensitivity for early stage disease and are not of value in screening and diagnosing colorectal cancers.

However CEA levels can be used for follow up of patients diagnosed with colorectal cancers and hence, measuring pretreatment CEA levels is now recommended. Elevated CEA levels which do not fall following surgery indicates residual disease. Rising CEA levels during followup after surgery indicates recurrence or metastasis and requires further investigation. Also serum CEA levels have a prognostic utility in newly diagnosed patients and those with CEA levels of more than 5ng/mL have a poorer prognosis[64].

8) BLOOD BASED INVESTIGATIONS for early detection of colorectal cancer or their recurrence following treatment are being developed at present. Sept9 and the Gemini test to name a few of these tests are under active development.

9) CT, MRI AND PET SCANS :

Preoperative CT or MRI scans of abdomen and pelvis demonstrate the tumor, its extension, regional lymphnodes, metastatic deposits, and tumor related complications like obstruction, perforation and fistula formation. This is

necessary for pretreatment staging of the disease. Occasionally colorectal cancer may be incidentally detected by these investigations done for an unrelated cause.

Positron emission tomography (PET) scans are useful for –

- Localising the tumor in patients with post treatment elevation of CEA levels
- Localising occult disease in individuals where colorectal carcinoma is strongly suspected clinically.
- Evaluation of patients for resection of isolated liver metastasis.

10) OTHER INVESTIGATIONS FOR LOCOREGIONAL STAGING :

Digital rectal examination and endoscopic ultrasound assist in locoregional staging of the disease and this determines further treatment course - local excision, radical resection or preoperative therapy.

SURVIVIN :

Survivin is a member of the Inhibitor of Apoptosis (IAP) family of proteins. The IAPs are a family of highly conserved cell death inhibitors with homologues found in both invertebrates and vertebrates. These were first discovered in Baculoviruses where they were found to inhibit the host cell apoptosis in response to the viral infection. IAP has nine family members

which are X-linked IAP, cIAP1, cIAP2, neuronal apoptosis inhibitor protein, melanoma IAP, IAP-like protein 2, Livin, Apollon, and Survivin. Common to all IAPs is the presence of Baculovirus IAP Repeat (BIR), a 70 amino acid motif, in one to three copies which is essential for their function. Survivin was discovered due to its structural homology to other IAP family of proteins in human B-cell lymphoma.

MOLECULAR ORGANISATION AND STRUCTURE :

Survivin is the smallest member of the IAP family of proteins. It is encoded by the BIRC5 gene spanning 14.7 kb on telomeric position of human chromosome 17q25 [65]. It is a 142 aminoacid containing 16.5 kDa protein containing only a single BIR domain characterized by a conserved zinc-coordinating Cys/His motif at the N-terminal half of the protein. It also contains an elongated C-terminal alpha helix comprising 42 amino acids [66].

X-ray crystallography studies showed that Survivin molecules are identified as homodimers in solution. Two molecules of human Survivin coming together to form a bowtie-shaped dimer through a hydrophobic interface, with the –C terminus alpha helices protruding from the core dimer [67].

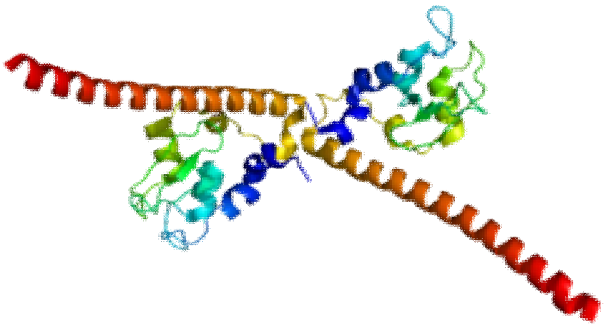

Figure 1 : Structure of Survivin

SURVIVIN EXPRESSION AND REGULATION:

Survivin expression in normal adult cells is found to be dominant only in the G2/M phase of mitosis. The expression of Survivin is controlled at multiple levels including gene transcription, post transcriptional differential splicing, post translational modification, sequestration and degradation [68].

Regulation at the level of gene transcription involves the canonical CDE/CHR boxes in the Survivin promoter, which act as potential G1 repressor elements. Survivin transcription increases during G1 phase and peaks during G2-M phase of the cell cycle. Other upregulators of Survivin transcription include Nuclear Factor kappa B, activated indirectly by growth factors via the phosphatidylinositol 3-kinase/Akt pathway, insulin like growth factor I/mTOR signalling which up-regulates Survivin by rapid increase in mRNA translation, members of the Ras oncogene family, signal transducer and activator of transcription -3(STAT-3), and the antiapoptotic factor Wnt-2. The Survivin gene is repressed at the transcriptional level by wild type p53 and p75 [68,69].

Four different isoforms are formed by the alternative splicing of Survivin mRNA . These isoforms include full length Survivin, Survivin-2B, Survivin-Delta-Ex-3 and Survivin-3B. Each of these isoforms have different patterns of expression, sub cellular localisation and anti apoptotic efficacies resulting in an additional level of complexity in the levels of Survivin expression, functionality and regulation.

Survivin has a short half life of 30 minutes. Post translational modifications which increase Survivin stability and half life, increases the levels of Survivin at specific phases of the cell cycle for example, the mitotic phosphorylation of Survivin on Thr34 by p34cdc2-Cyclin B1 increases the stability of Survivin during metaphase.

Degradation of Survivin by the ubiquitin – proteasome pathway contributes to the cell cycle periodicity by keeping the levels of Survivin low during interphase. Binding to Heat Shock Protein 90 increases the stability of Survivin and reduces its degradation thereby increasing cellular levels of Survivin.

FUNCTIONS :

The two main functions of Survivin are :

1) Regulation of cell division.
2) Inhibition of apoptosis.

Role of Survivin in cell division:

Survivin is essential for mitosis and cytokinesis. It has a transcriptionally controlled expression at the G2/M phase and functions during a narrow window of time.

With its expression during mitosis, Survivin localises to various components of the mitotic apparatus including centrosomes, microtubules of metaphase, anaphase spindle and remnants of the mitotic apparatus suggesting that Survivin has an important role in microtubule dynamics and maintenance of normal bipolar mitotic apparatus[70].

Survivin has also been shown to form complexes with molecules involved in regulation of cytokinesis including Aurora B kinase, Inner centromere protein (INCENP) and Borealin/Dasra which indicates that survivin also functions as a subunit of the chromosomal passenger complex which is essential for proper chromosome seggregation and cytokinesis [71].

The discrepancy regarding Survivin's cytoplasmic versus nucleoplasmic location and its function as a regulator of microtubule dynamics and also as a chromosomal passenger protein have been explained by three possibilities :

1) Existence of two pools of Survivin, one localised to centrosome and microtubules involved in maintaining a normal bipolar mitotic apparatus and the other localised to kinetochores involved in cytokinesis.
2) The differentially spliced isoforms could mediate different functions during cell division.
3) Various Survivin pools provide a continuum in the dynamic regulation of the mitotic spindle checkpoint.

Role of Survivin in apoptosis :

That Survivin is involved in inhibition of apoptosis like other members of the IAP family of proteins has been demonstrated by three types of experimental evidence :

1) Over expression of Survivin is associated with inhibition of cell death via the intrinsic or extrinsic apoptotic pathways.
2) Transgenic expression of Survivin resulted in apoptosis inhibition in vivo.
3) Molecular antagonists of Survivin including antisense, ribozymes, siRNA sequences or dominant negative mutants resulted in enhanced caspase dependent cell death and anticancer activity in vivo.

The mechanisms by which survivin inhibits apoptosis are yet to be completely understood. It was initially postulated that survivin like other IAPs selectively bind to and promote the degradation of active caspase-3, caspase-7 and

caspase-9. However it was later established that Survivin lacked the structural motifs necessary for binding to caspases that was present in other IAPs [72]. Other studies have shown that Survivin inhibits active caspase 9 but requires a cofactor, the Hepatitis B X-interacting protein. Survivin also associates with X-linked IAP, increasing its stability, resulting in the synergistic inhibition of caspase-9. Survivin binds to and inhibits Smac/DIABLO. This Smac/DIABLO is a mitochondrial released proapoptotic protein which binds other IAPs preventing them from inhibiting caspases. Thus unlike other IAPs which directly target and inhibit initiator and executioner caspases, Survivin has a more sophisticated mechanism of action and targets the multimolecular processes involved in caspase-9 activation [72,73]. This involves cooperation with other molecules like Hepatitis B X-interacting protein , X-linked IAP and also interaction of Survivin with Smac/DIABLO.

Role of Survivin in cancer :

What makes Survivin clinically intriguing is its differential distribution in cancers compared to its limited expression in normal, terminally differentiated tissues. Survivin is normally expressed in embryonic and fetal tissues but is undetectable in terminally differentiated normal adult tissues with the exception of thymus and CD34+ bone marrow stem cells[65]. In contrast most human cancers have been shown to overexpress Survivin. Genome-wide searches have confirmed the differential expression of survivin in tumors compared to normal tissues. The overexpression of Survivin is consistently

associated with more aggressive tumors, increased rates of recurrence, resistance to therapy and poorer prognosis than tumors that are negative for Survivin.

The mechanisms by which Survivin expression is deregulated in cancers include :

- Amplification of Survivin locus on chromosome 17q25
- Demethylation of Survivin exons
- Increased promoter activity
- Increased upstream signalling in the PI3-kinase or MAP kinase pathways [69,74,75].

In addition, upregulation of Survivin expression in cancers is cell cycle independent, unlike normal cells.

The role of Survivin in cancer is much more than simple inhibition of apoptosis. Survivin has the important function of regulating the mitotic spindle check point. Its dysregulation and overexpression allows cells with spindle defects and misaligned kinetochores to proceed through mitosis. In addition to its direct role in tumorigenesis, Survivin is also implicated in tumor angiogenesis. This is supported by the fact that Survivin is highly expressed in endothelial cells during remodelling and proliferative phases of angiogenesis. Survivin has also been shown to inhibit cell death induced by several anti-cancer agents thereby contributing to therapy resistance. Studies have also

shown that inhibition of Survivin reduces tumor growth potential and sensitizes them to chemo, radio and immunotherapeutic agents.

Exploitation of Survivin for cancer therapy:

The differential over expression of Survivin in cancer and its near absence in normal adult tissue makes Survivin a promising and novel target for cancer therapy. Several experiments targeting Survivin expression are currently under investigation. Three strategies have shown promising results:

1) Generation of antigen specific immune response against Survivin bearing cancer cells for potential cancer vaccination strategies.
2) Use of molecular antagonists of Survivin including antisense, ribozyme and siRNAs.
3) Pharmacological inhibition of mitotic phosphorylation of Survivin on Thr34 to mimic the dominant negative phenotype.

These studies have demonstrated that interference with Survivin expression and function resulted in tumor growth suppression and increased tumor cell apoptosis, alone or in combination with other anti cancer treatments by reducing tumor cell resistance to these therapeutic agents. In addition there is also the potential role of inhibition and regression of tumor-associated angiogenesis[76,77].

Ki-67 :

Ki-67 is a non histone nuclear protein encoded by the MKI67 gene located on human chromosome 10[78]. The Ki-67 protein was originally identified by Gerdes et al in the early 1980s using the prototype monoclonal antibody Ki-67. It was produced by immunizing mice with nuclei of Hodgkin lymphoma cell line L428. It was named after the researcher's location, Ki for Kiel University, Germany and the 67 label referring to the clone number on the 96-well plate.

Expression and localisation :

Ki-67 expression varies during different phases of the cell cycle. It is expressed during all the active phases of the cell cycle including G1, S, G2 and Mitotic phases but not during the resting G0 phase. Thus Ki-67 is strictly associated with cellular proliferation and is used as a marker for the same[79].

Ki-67 levels are low during the G1 and S phases, rises to its peak levels during mitosis and during the later half of mitosis which includes anaphase and telophase, a rapid decrease in Ki-67 levels occurs. During interphase, Ki-67 antigen is exclusively detected in the cortex and dense fibrillar components of the nucleolus whereas during mitosis, it relocates to the surface of the condensed chromosomes.

Functions: Ki-67 is associated with and is necessary for cellular proliferation. Recent studies have shown that Ki-67 functions as a structural/scaffolding protein that is necessary for the assembly of the perichromosomal compartment on condensed chromosomes during mitosis. It also determines the nature of behaviour of many important nucleolar components during mitosis[79]. Ki-67 in addition is associated with the transcription of ribosomal RNA. Inactivation of Ki-67 protein leads to inhibition of ribosomal RNA synthesis[80].

Applications and uses :

Ki-67 by virtue of its expression in only the proliferating cells and its absence in the resting cells makes it an excellent marker for cellular proliferation. It is indeed used to measure the growth fraction of a given cell population. This is done by immunostaining the tissue with monoclonal Ki-67 antibody and measuring the percentage of cells which are positive for Ki-67, also known as the Ki-67 labelling index. The fraction of Ki-67 positive cells in a tumor is often correlated with the clinical course of the disease with tumors having a higher Ki-67 index having been shown to have an aggressive course and a poorer prognosis. The best studied examples in this context include carcinomas of the brain, breast, prostate, nephroblastoma and neuroendocrine tumors.

Hiroshi Kawasaki et al studied Survivin and Ki-67 expression in human colorectal tumors including 43 hyperplastic polyps, 171 adenomas with low grade dysplasia, 42 adenomas with high grade dysplasia and 60 colorectal

adenocarcinoma cases using immunohistochemical staining. It was concluded that the immunoreactivity of Survivin significantly increased in the transition from adenomas with low grade dysplasia to adenomas with high grade dysplasia and carcinoma showing that Survivin plays an important role in colorectal tumorigenesis. It was also noted that transition from normal epithelium to adenoma and carcinoma was associated with increased Ki-67 expression and there was a significant correlation between Survivin and Ki-67 expression [81].

Lian-Jie Lin et al studied 188 samples of colorectal tumors including 30 normal epithelia, 41 adenomas with low grade dysplasia, 30 adenomas with high grade dysplasia and 87 colorectal carcinomas of which 33 were well differentiated, 28 were moderately differentiated and 26 were poorly differentiated adenocarcinomas respectively. The positive rate of Survivin expression increased significantly from normal epithelium to adenoma with low grade dysplasia, to adenoma with high grade dysplasia and carcinoma. No correlation was observed between carcinoma differentiation or grade and Survivin expression. The study concluded that Survivin expression plays an important role in colorectal carcinogenesis and has a role in the early stage of the transition sequence [82].

Ulrike Gerlach et al investigated 100 cases which was composed of 41 normal mucosa samples, 18 dysplastic epithelial samples and 41 invasive carcinomas. It was concluded that Survivin expression increased from normal

to dysplasia to invasive carcinoma. Survivin expression also correlated with tumor differentiation, with Survivin expression increasing as the grade increased from well differentiated to moderately differentiated to poorly differentiated carcinomas [83].

Hai-Yan Tan et al conducted a study involving 48 cases of colorectal carcinomas. It was found that Survivin expression was very weak in normal epithelial cells, partially expressed in precancerous tissues. But significant positive expression of Survivin was present in cancerous tissues showing that Survivin expression could be an early event in colorectal carcinogenesis. Further Survivin correlated with the pathological grade, lymph node status and Dukes stage of colorectal carcinomas. No significant correlation was found between Survivin expression and age, gender and tumor diameter [84].

Alfred King-Yin Lam and colleagues conducted a study in which 51 cases of colorectal carcinomas were studied for Survivin expression and found that colorectal carcinomas had much higher Survivin levels than non-tumor tissue. Survivin levels correlated with the size of the tumor. No relationship was found between Survivin expression and differentiation, tumor site, age and gender. It was concluded that Survivin is important in the pathogenesis of colorectal adenocarcinomas [85].

Yin-Yu Lee and colleagues performed a study of 119 colorectal samples which consisted of 24 colorectal adenomas, 10 normal epithelia and 18 well differentiated, 50 moderately differentiated and 27 poorly differentiated

carcinomas respectively. Survivin expression was studied using immunohistochemistry and was found that the percentage of cells immunostained for Survivin were higher in adenomas and carcinomas compared to normal colonic epithelium which was statistically significant. It was concluded that Survivin scores correlated with AJCC staging of colorectal adenocarcinomas and also with shorter survival [86].

Woong Na et al did immunohistochemical analysis of survivin in 622 colorectal tissue samples including 529 colorectal adenocarcinomas, 40 normal mucosa, 34 adenomatous polyps and 59 metastatic lymph nodes. In colorectal adenocarcinomas, it was established that Survivin expression correlated with tumor location (higher in rectum vs colon), AJCC stage and lymph node metastasis. Stage wise Survivin expression significantly correlated with overall and disease free survival with increased Survivin expression having lower survival rates. No correlation was found between age, gender, tumor size and histological grade [87].

Rei Chong Xi and colleagues studied the expression of Survivin in 61 primary colorectal carcinomas along with 32 adenomas and 17 normal colorectal mucosa samples using immunohistochemical and molecular biological techniques. It was concluded that Survivin expression in colorectal carcinomas correlated with lymphatic invasion, metastasis, recurrence and poor prognosis. However no relation was found between Survivin expression and age, gender, histological differentiation and TNM stage of the tumors [88].

A.I.Sarela et al studied the expression of Survivin in 144 colorectal carcinomas and 86 adjoining histologically normal mucosa samples. The study concluded that expression of Survivin in the primary tumor was associated with a significantly higher risk of death due to recurrent cancer and the 5 year survival rate of Survivin positive patients was significantly lower than that of Survivin negative patients [89].

Saleh Hussain and colleagues studied the expression of Ki-67 in 52 colorectal carcinomas and 56 adenomas and concluded that higher Ki-67 values are associated with histopathological parameters of tumor grade and stage and an inverse relationship exists between Ki-67 expression and colonic neoplasia[90].

Miao-Xia Lin et al studied Ki-67 expression in 60 colorectal cancers, 30 adenomas and 20 normal mucosal tissues and found that Ki-67 expression was significantly higher in colorectal carcinomas than in adenomas and normal tissue and Ki-67 overexpression correlated with tumorigenesis, stage and metastasis [91].

Yan-Lei Ma et al showed in their study that Ki-67 scoring showed significant positive correlation with tumor stage and differentiation [92].

Anway Sen et al concluded in their 2015 study that Ki-67 labelling index showed a strong correlation with histopathological grade of colorectal carcinomas. However no correlation was found between Ki-67 expression and tumor stage [93].

Xia-Bin Li et al studied 275 cases of colorectal adenocarcinomas for Survivin polymorphisms and the expression of survivin and Ki-67 using immunohistochemistry and concluded that there was a significant positive correlation between the expression of Survivin and Ki-67 [94].

MATERIAL AND METHODS

The study was conducted during the period from January 2015 to December 2015. It was carried out on specimens obtained from patients with confirmed histopathological diagnosis of colorectal adenomas and adenocarcinomas. The study was approved by the Ethical committee of Government Stanley Medical College and Hospital.

STUDY DESIGN : Retrospective and comparative study.

STUDY POPULATION :

CASES : The study sample comprised of 90 cases, 30 each of normal colonic mucosa, colorectal adenoma and colorectal adenocarcinoma. Cases were chosen from the Department of Surgical Gastoenterology, Government Stanley Medical College and Hospital. Age, sex, tumor site, histological grade and tumor stage were obtained for all cases. All the 90 cases were screened for Ki-67 and Survivin expression through immunohistochemical assay.

INCLUSION CRITERIA –

Patients with colorectal adenomas and adenocarcinomas.

EXCLUSION CRITERIA :

Chemotherapy and/or radiotherapy prior to sampling.

Recurrent and metastatic adenocarcinomas.

Cancer types other than adenocarcinomas.

METHOD OF DATA COLLECTION :

A total of 90 cases, 30 each of normal colonic mucosa, colorectal adenoma and colorectal adenocarcinoma were studied at random and selected, applying the inclusion and exclusion criteria. The tissues so obtained were processed and sections were cut at 5 micron thickness. Hematoxylin and eosin staining of the sections were done and analysed. The clinico-pathological characteristics including age, sex, tumor site, histological diagnosis (normal/adenoma/adenocarcinoma), histological grade (adenomas- low grade/high grade dysplasia ; adenocarcinoma – well/moderately/poorly differentiated) and adenocarcinoma stage were obtained for all the cases.

METHOD OF TISSUE PREPARATION:

10% neutral buffered formalin was used for fixing the specimens. The tissues were processed in various grades of alcohol and xylol using automated histokinette. Paraffin blocks were prepared and section of 5 micron thickness were cut in semiautomatic microtome using disposable blades and stained with hematoxylin and eosin. Suitable blocks were chosen for immunohistochemistry.

IMMUNOHISTOCHEMICAL STAINING FOR SURVIVIN AND Ki-67 :

IHC was performed on the selected blocks for Survivin and Ki-67. Primary rabbit monoclonal anti-Survivin antibody (clone – EP119, Isotpe : IgG, PathnSitu) and primary mouse monoclonal anti-Ki-67 antibody (clone –

GM001, Isotype : IgG1, PathnSitu), with reactive lymph node as positive control were used. Sections for immunohistochemistry were also cut in semiautomatic microtome using disposable blades. Slides coated with chrome alum were used. Sections were subjected to antigen retrieval using pressure cooker technique using TRIS EDTA (pH 9.2) buffer solution and then treated by HRP (horse radish peroxidase) polymer technique.

HRP POLYMER TECHNIQUE

The coated slides were taken through the following stages -

1. Overnight incubation (first at 70 degree Celsius for one hour, then at 40-45 degree Celsius for overnight).
2. Xylene- 2 changes, 15 minutes each.
3. Graded alcohol- first with absolute alcohol- 2 changes, 5 minutes each. Then for 3 minutes with 90% alcohol and finally for 3 minutes with 70% alcohol.
4. Distilled water rinse for 2-5 minutes.
5. Antigen retrieval with pressure cooker.
6. Wash with tap water gradually.
7. TRIS buffer wash for 5 minutes, 2 changes.
8. Treatment with peroxidase block –for inhibiting endogenous peroxidases in the tissue for 10 minutes.

9. TRIS buffer wash for 5 minutes, 2 changes .
10. Application of primary antibody for 45 minutes.
11. TRIS buffer wash for 5 minutes, 2 changes.
12. Application of superenhancer for 15 minutes which enhances the final reaction product by increasing the sensitivity of antigen antibody reaction.
13. TRIS buffer wash for 5 minutes, 2 changes.
14. Application of SS label –secondary antibody from the goat with the tagged horse radish peroxidase enzyme for 15 minutes.
15. TRIS buffer wash for 5 minutes, 2 changes.
16. Application of DAB (Diaminobenzidine) chromogen for 5 minutes –this is cleaved by the enzyme to give the colored product at the antigen sites.
17. Wash in distilled water for 5 minutes.
18. The slides were counterstained with hematoxylin.
19. Air dried, dehydrated, cleared and mounted with DPX (Distyrene dibutyl pthalide in Xylol).

SCORING CRITERIA FOR SURVIVIN AND Ki-67 :

The mean percentage of cells positive for the expression of Survivin and Ki-67 was determined in at least 5 areas at 400-fold magnification. This scoring was performed in a blinded fashion.

OBSERVATION AND RESULTS:

Category of the participants (Normal vs adenoma vs adenocarcinoma) was the primary outcome variable. The survivin and Ki67 expression values were considered as the primary outcome variables. The clinico-pathological factors like age, gender, malignancy related factors like grade of carcinoma, etc. were considered as other explanatory variables. Initially descriptive analysis of explanatory and outcome variables was done using mean and standard deviation for quantitative variables, frequency and proportion for categorical variables. The association between the explanatory and outcome variables was done by comparing the mean Survivin and Ki-67 values across the groups. The mean differences and their 95% CI were presented. ANOVA was used to assess the statistical significance of the association. IBM SPSS version 21 was used for statistical analysis. IHC for Survivin and Ki-67 showed nuclear positivity.

Results:

A total of 90 participants were included in the analysis. The proportion of participants below 40 years were 10.0% and the proportion of participants above 40 years were 90.0%.The proportion of males constituted 60% of the study subjects. The remaining 40% of the participants were females respectively. (Table 2)

Table 2: Descriptive analysis of age groups in study group (N=90)

Age groups	Frequency	Percentage
<40 years	9	10.0
>40 years	81	90.0
II.Gender		
Male	54	60
Female	36	40

Chart 1 : Pie chart for age groups in study group (N=90)

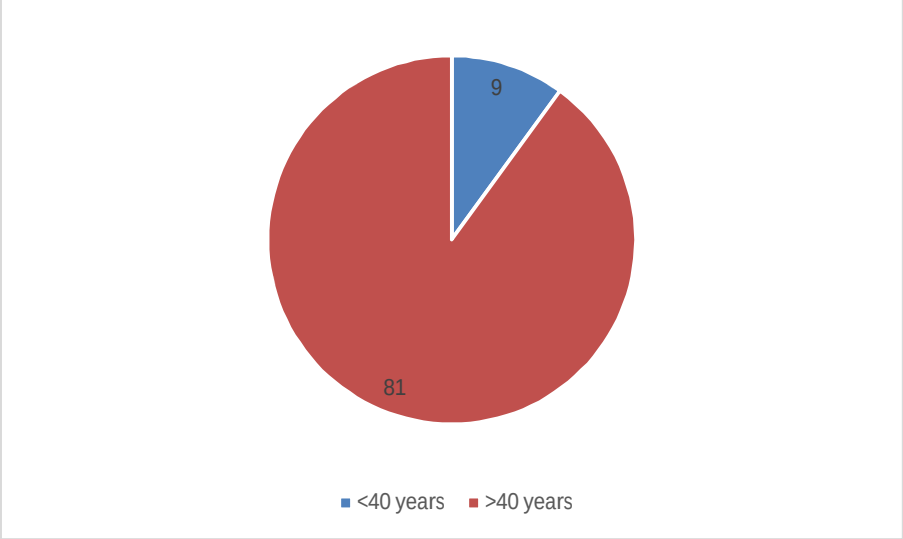

Chart 2:Bar chart for gender in study group(N=90)

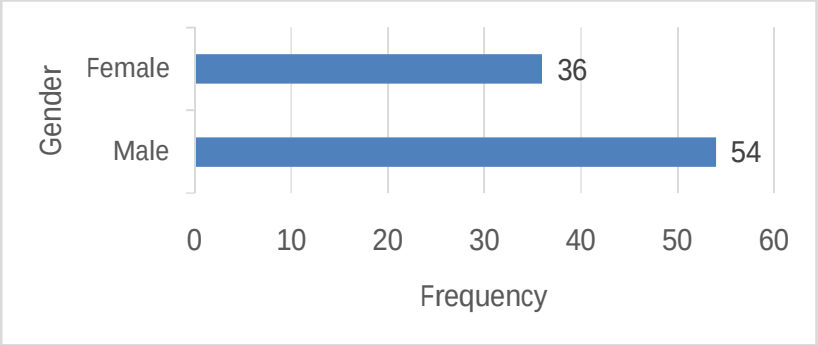

Table 3: Descriptive analysis of final diagnosis in study group (N=90)

Final diagnosis	Frequency	Percentage
Normal	30	33.3
Adenoma	30	33.3
Adenocarcinoma	30	33.3

The number of participants who were normal was 30(33.3%) and 30(33.3%) had adenoma in study population. The proportion of subjects with adenocarcinoma was 30(33.3%) in the study population. (Table 3)

Chart 3: Bar chart of final diagnosis distribution in study group (N=90)

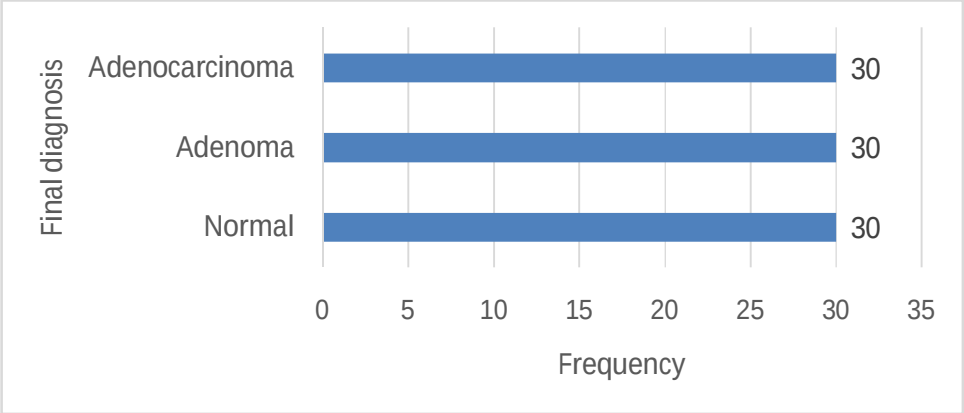

Table 4 :Descriptive analysis of tumor site in study group (N=60)

Tumor site	Frequency	Percentage
Rectum	23	38.3
Right hemicolon	15	25.0
Left hemicolon	10	16.6
Sigmoid colon	12	20.0

The number of participants who had tumor in the rectum was 23 (38.3%). Tumor was present in right hemicolon in 15 (25.0%) people. The proportion of subject with tumor location in left hemicolon and sigmoid colon were 16.6% and 20.0% respectively in study population. (Table 4)

Chart 4: Bar chart for tumor site in study group (N=60)

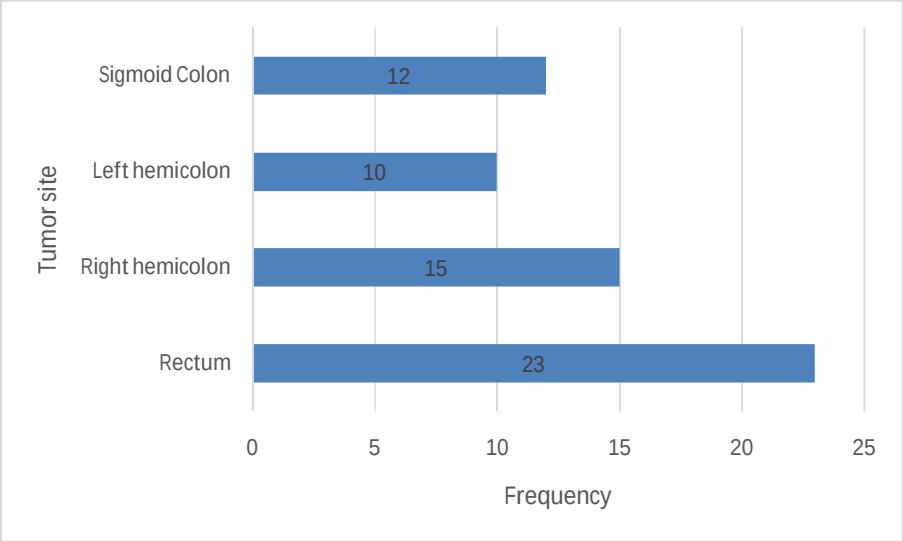

Table 5: Descriptive analysis of carcinoma grade, stage and tumor stage in study group (N=30)

Parameter	Frequency	Percentage
Carcinoma Grade		
1	10	33.3
2	12	40.0
3	8	26.6
II.Stage		
I	13	43.3
II A	4	13.3

II B	2	6.66
II C	2	6.66
III A	5	16.6
III B	3	10.0
III C	1	3.33
III.Tumor stage		
T1	1	3.33
T2	17	56.6
T3	7	23.3
T4a	3	9.99
T4b	2	6.66

Out of 30 subjects with adenocarcinoma, the grade of carcinoma was 1, 2 and 3 in 33.3%, 40% and 26.6% of the participants respectively. Tumor stage was stage 1 in 43.3% of participants, 8 participants had stage II malignancy and the remaining 9 participants had stage III malignancy. Majority of the participants belonged to T2 and T3 stage. (Table 5)

Chart 5: Pie chart of carcinoma grade distribution in study group (N=30)

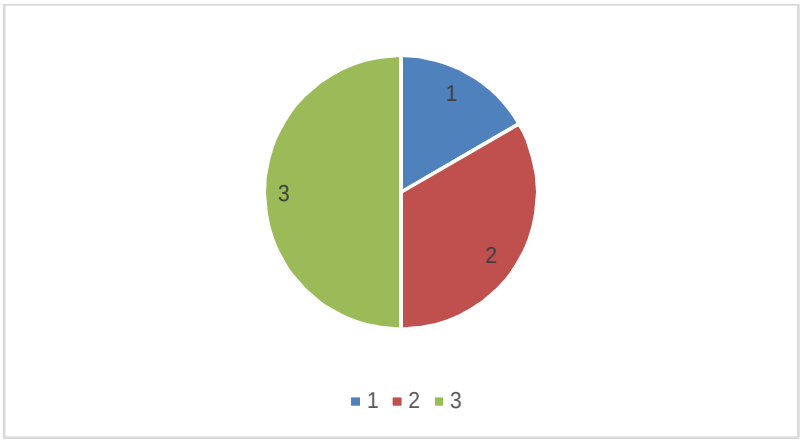

Chart 6: Bar chart of stage distribution in study group (N=30)

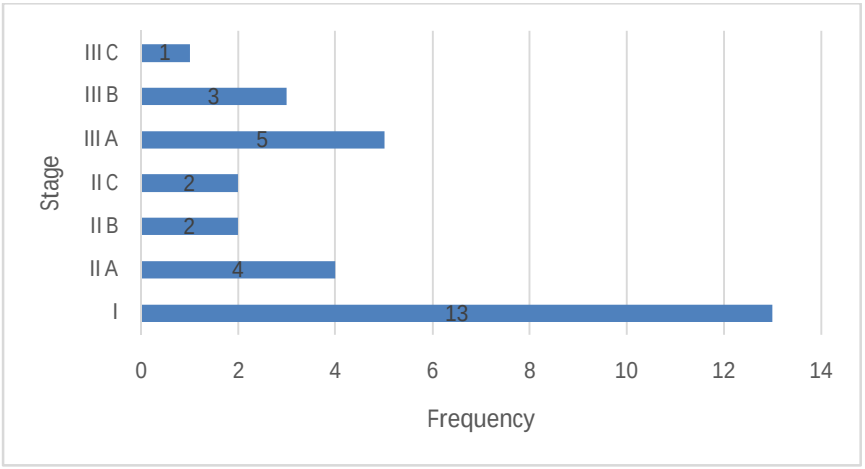

Chart 7: Bar chart of tumor stage distribution in study group (N=30)

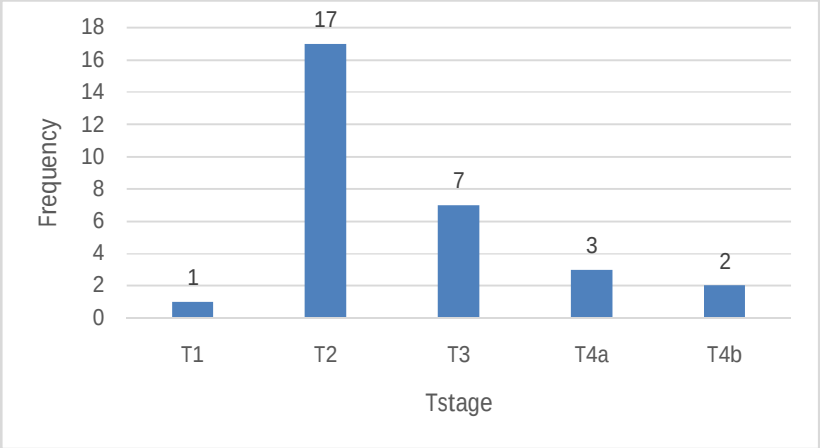

Table 6: Descriptive analysis of parameters in study group (N=90)

Parameter	Mean±STD	Median	Max	Min	95% C.I.for EXP(B)	
					Lower	Upper
Ki67	50.19 ± 18.28	47.40	84.20	25.80	43.37	57.03
Survivin	40.41 ± 12.72	39.15	77.10	21.80	35.66	45.17

The mean Ki-67 was 50.19±18.28 and the mean Survivin expression was 40.41±12.72 in study population (Table 6).

Table 7: Comparison of mean age with Survivin across study groups (N=90)

Age	Mean	Mean difference	P value	95% CI	
				Lower	Upper
<40 years	14.17±10.96	6.62	0.319	-6.50	19.74
>40 years	20.79±19.40				

The mean Survivin expression was 14.17 in < 40years age group and 20.79 in > 40 years, with a mean difference of 6.62 which was statistically not significant. (P value 0.319)

Table 8: Comparison of gender with mean Survivin across study groups (N=90)

Gender	Mean	Mean difference	P value	95% CI	
				Lower	Upper
Male	19.92	0.52	0.898	-8.60	7.55
Female	20.45				

The mean Survivin expression was 19.92 in male and 20.45 in female, with a mean difference of 0.52 which was statistically not significant. (P value 0.898) (Table 8)

Table 9: Comparison of mean Survivin with final diagnosis across study groups (N=30)

Final diagnosis	Mean	Mean difference	P value	95% CI	
				Lower	Upper
Normal	0.010±0.03				
Adenoma	19.98±8.80	-19.97	<0.001	-25.60	-14.34
Adenocarcinoma	40.41±12.72	-40.40	<0.001	-46.03	-34.77

The mean Survivin expression was 0.01±0.03 in normal people, 19.98±8.80 with adenoma and 0.41±12.72 with adenocarcinoma, which was statistically significant. (P value <0.001) (Table 9).

Table 10: Comparison of mean Ki-67 with final diagnosis across study groups (N=30)

Final diagnosis	Mean	Mean difference	P value	95% CI	
				Lower	Upper
Normal	7.41±1.60				
Adenoma	17.89±4.08	-10.48	<0.001	-17.32	-3.64
Adenocarcinoma	50.29±28.38	-42.78	<0.001	-49.63	-35.94

The mean Ki-67 was 7.41±1.60 with normal, 17.89±4.80 with adenoma and 50.29±28.38 with adenocarcinoma, which was statistically significant. (P value <0.001) (Table 10)

Table 11: Comparison of mean Survivin with carcinoma grade

Carcinoma	Mean	Mean difference	P value	95% CI	
				Lower	Upper
Grade 1	27.59±4.43				
Grade 2	40.59±5.23	-13.00	<0.001	-6.15	-19.85
Grade 3	56.18±9.12	-28.59	<0.001	-21.00	-36.18

The mean Survivin expression was 27.59±4.43 in people with carcinoma grade 1, 40.59±5.23 in people with grade 2 and 56.18±9.12 with carcinoma grade 3 which was statistically significant. (P value <0.001) (Table 11)

Table 12: Comparison of mean Ki-67 with carcinoma grade

Carcinoma	Mean	Mean difference	P value	95% CI	
				Lower	Upper
Grade 1	29.79±1.26				
Grade2	51.48±2.02	21.66	<0.001	-14.84	-28.49
Grade3	73.81±2.57	44.02	<0.001	-36.35	-51.58

The mean Ki-67 was 29.79±1.26 in people with carcinoma grade 1, 51.48±2.02 in people with grade 2 and 73.81±2.57 with carcinoma grade 3, which was statistically significant. (P value <0.001) (Table 12)

Table 13: Comparison of mean Survivin across different stages (N=30)

Survivin	Mean Survivin	Mean difference	P value	95% CI	
				Lower	Upper
Stage 1& 2	37.66±10.38	9.18	0.069	-0.77	19.13
Stage 3	46.84±15.84				

The mean Survivin expression was 37.66±10.38 in people at stage 1& 2 and 46.84±15.84 in people at stage 3 with a mean difference of -9.18±15.84 (95% CI 0.77 to -19.13) which was statistically significant. (P value 0.06). (Table 13)

13. Curve Estimation: For Ki-67 and Survivin

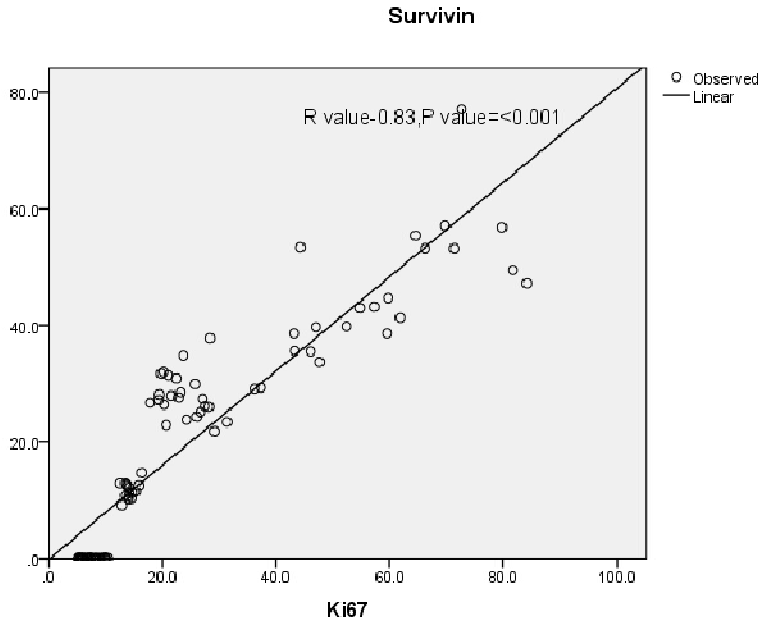

Figure 2 : Curve estimation for Survivin and Ki-67

The curve estimation for correlation between Survivin and Ki-67 had an R value of 0.83 which shows a strong correlation between Survivin and Ki-67 expression which was statistically significant.

COLOUR PLATES

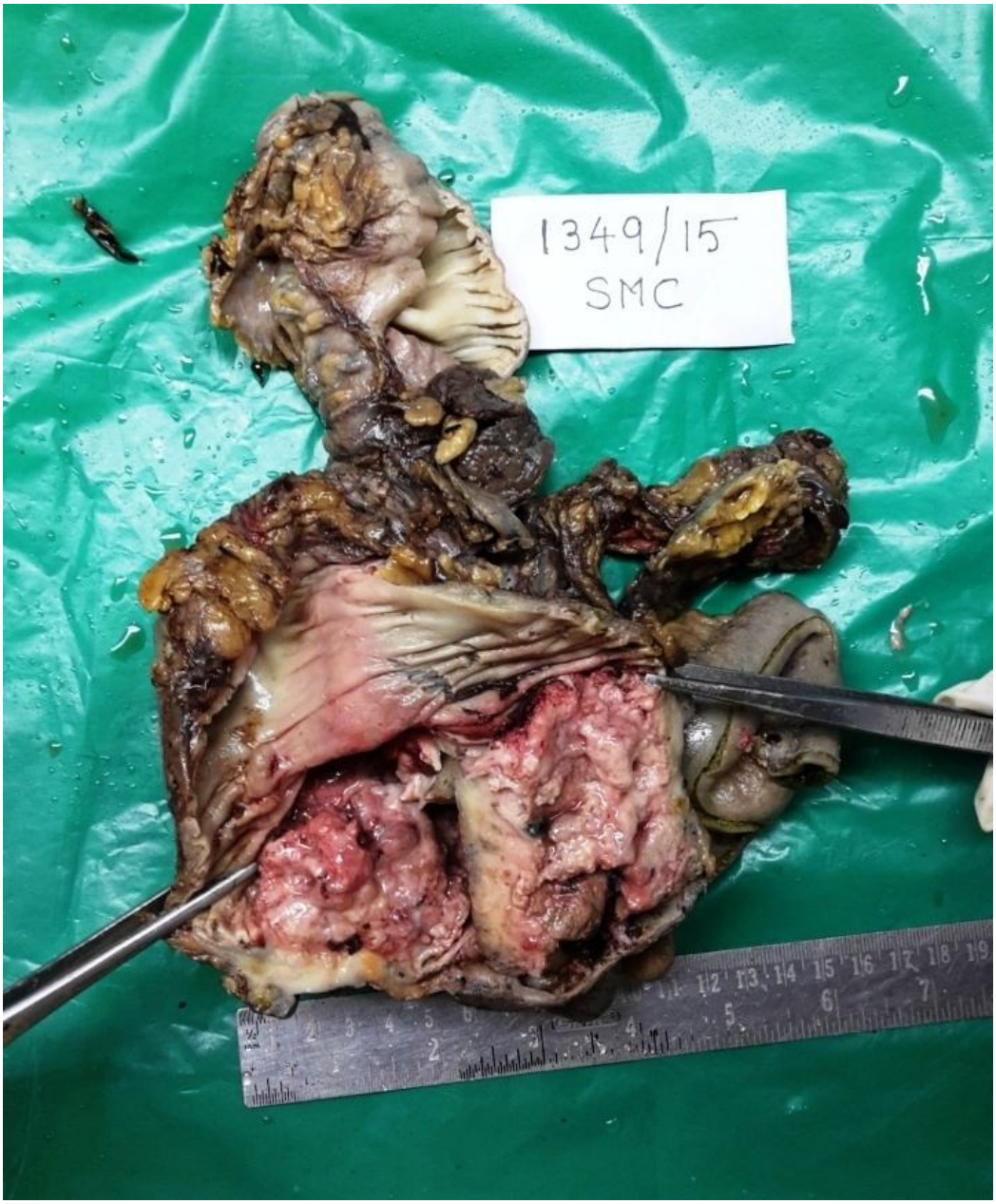

Figure 3 : Gross appearance of an ulcero-proliferative growth involving the caecum.

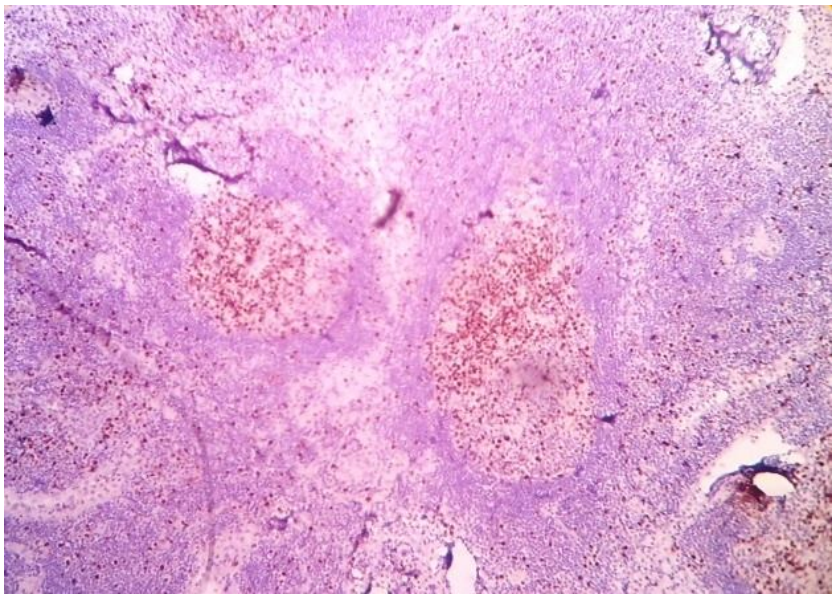

Figure 4 : Survivin control (Reactive lymph node).

Nuclear positivity in germinal centre cells (100 X).

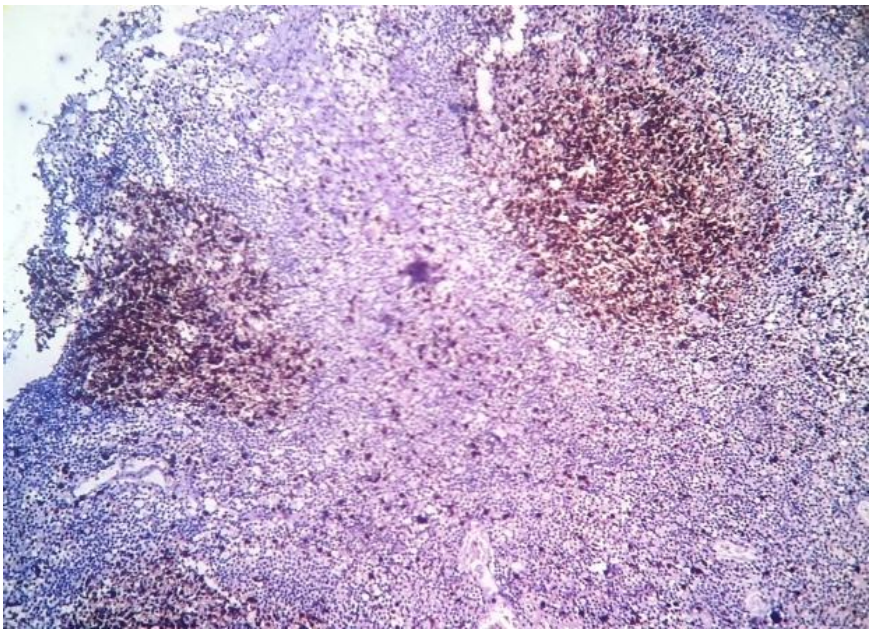

Figure 5 : Ki-67 control (Reactive lymph node).

Nuclear positivity in germinal centre cells (100 X).

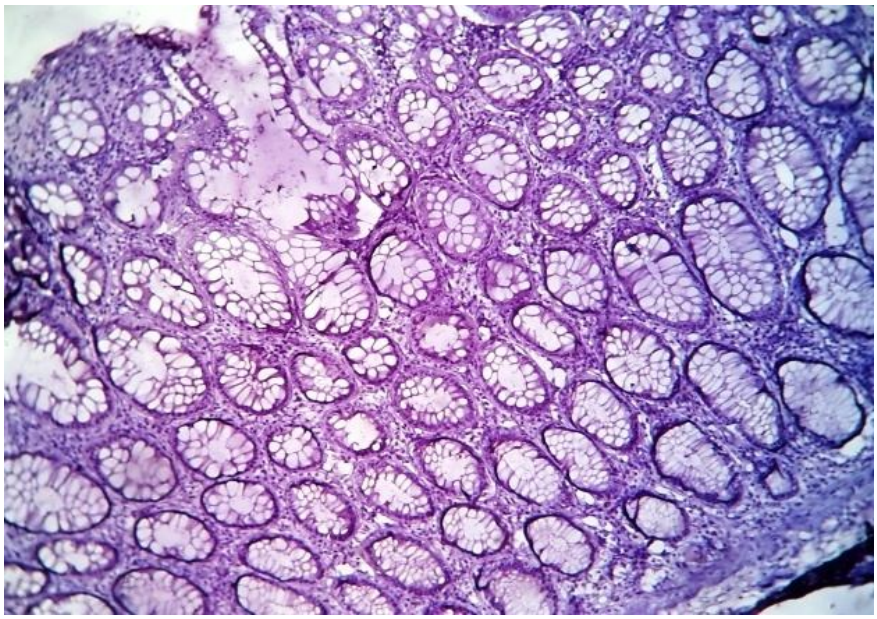

Figure 6 : Survivin expression in normal colonic mucosa (Nil) (100X)

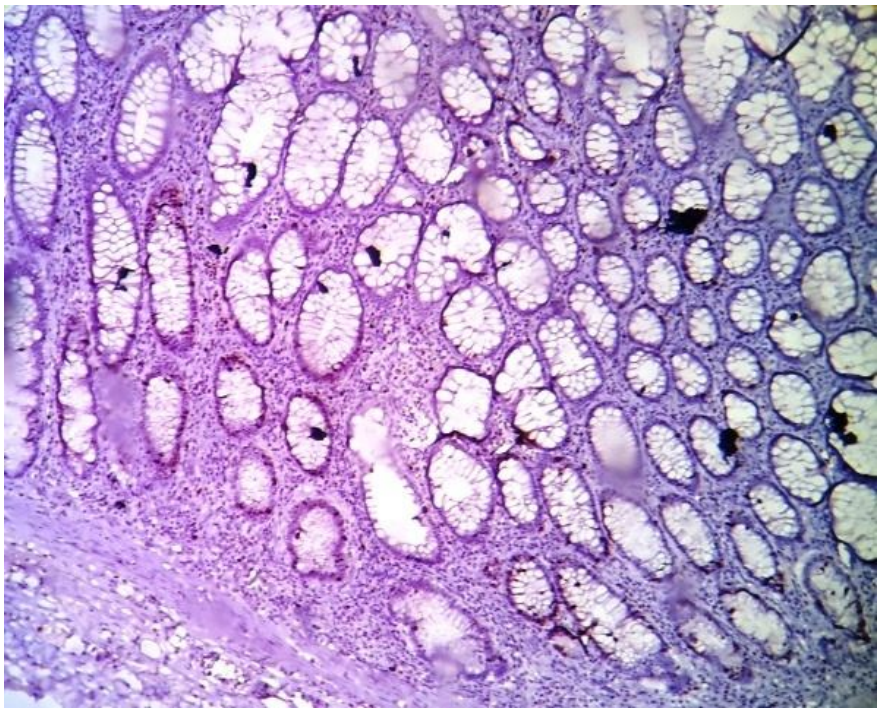

Figure 7 : Ki-67 expression in normal colonic mucosa, at the base of crypts. Nuclear positivity in 5.2% of mucosal epithelial cells (100X).

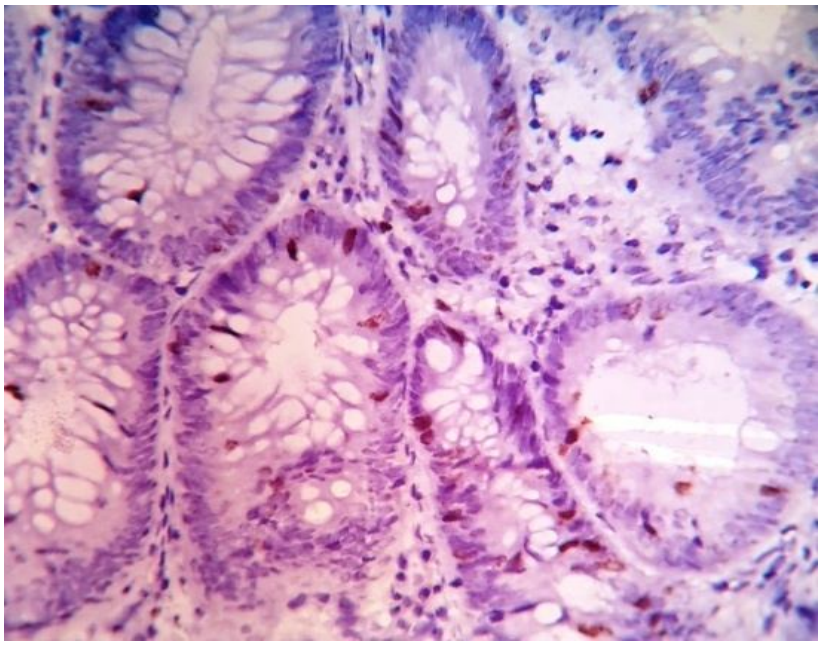

Figure 8 : Survivin expression in adenoma with low grade dysplasia. Nuclear positivity in 11.2% of cells (400X).

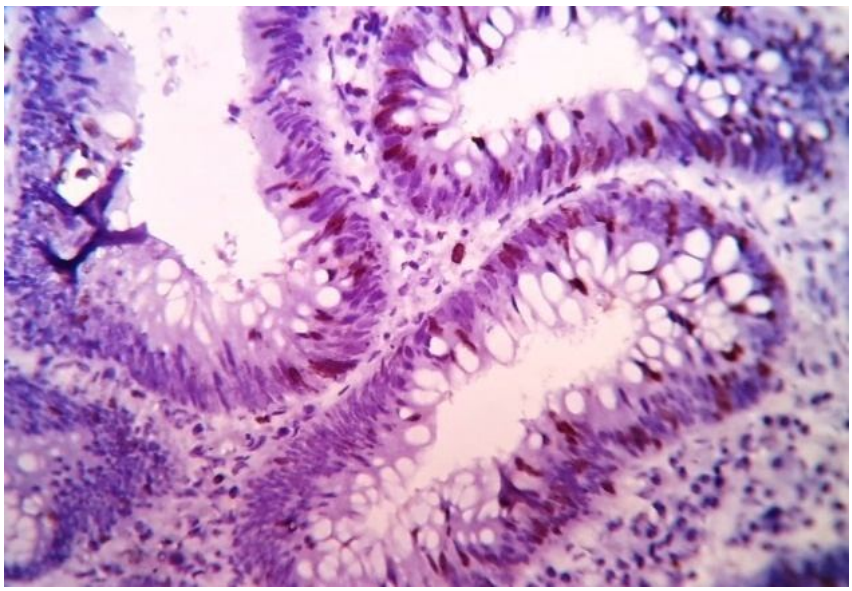

Figure 9 : Ki-67 expression in adenoma with low grade dysplasia. Nuclear positivity in 14.2% of cells (400X).

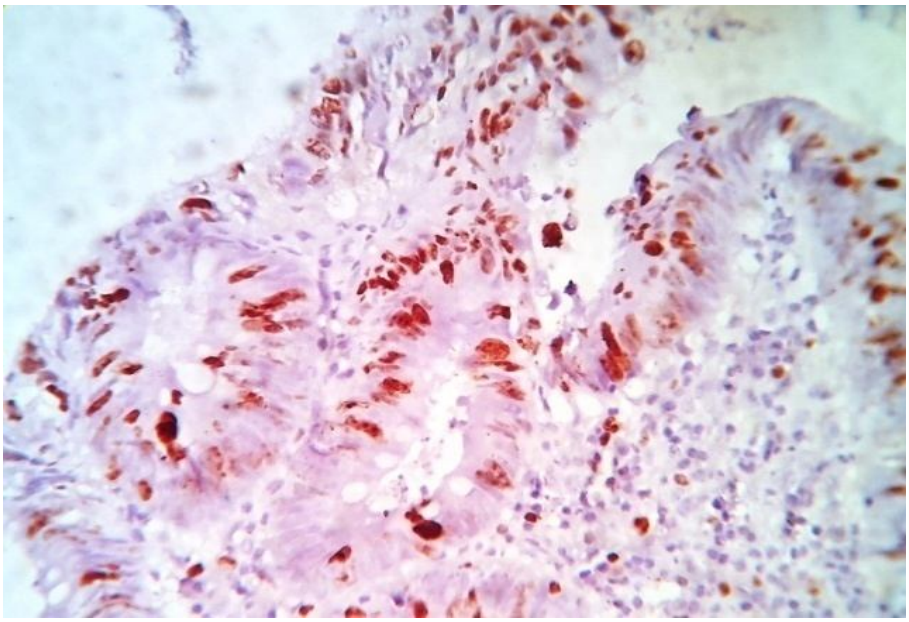

Figure 10 : Survivin expression in adenoma with high grade dysplasia.
Nuclear positivity in 23.8% of cells (400X).

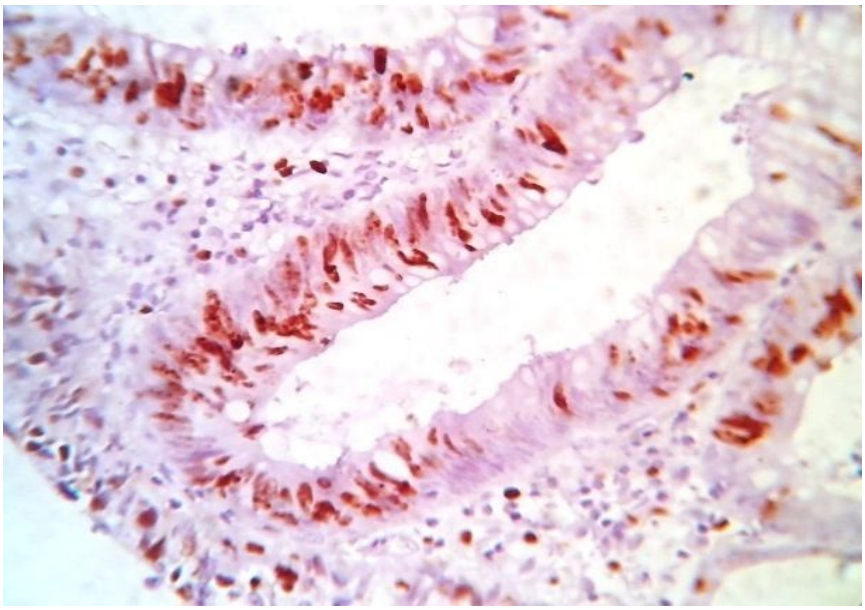

Figure 11 : Ki-67 expression in adenoma with high grade dysplasia.
Nuclear positivity in 24.3% of cells (400X).

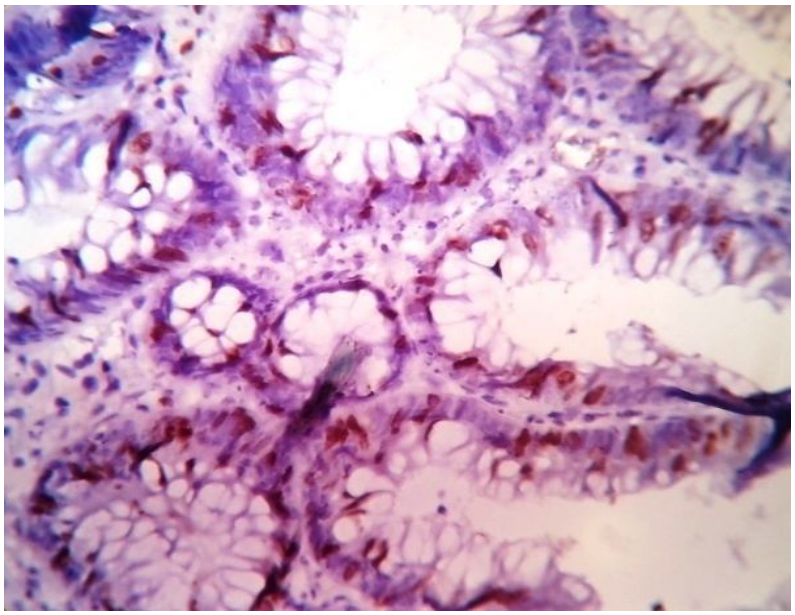

Figure 12 : Survivin expression in well differentiated adenocarcinoma. Nuclear positivity in 29.3% of neoplastic cells (400X).

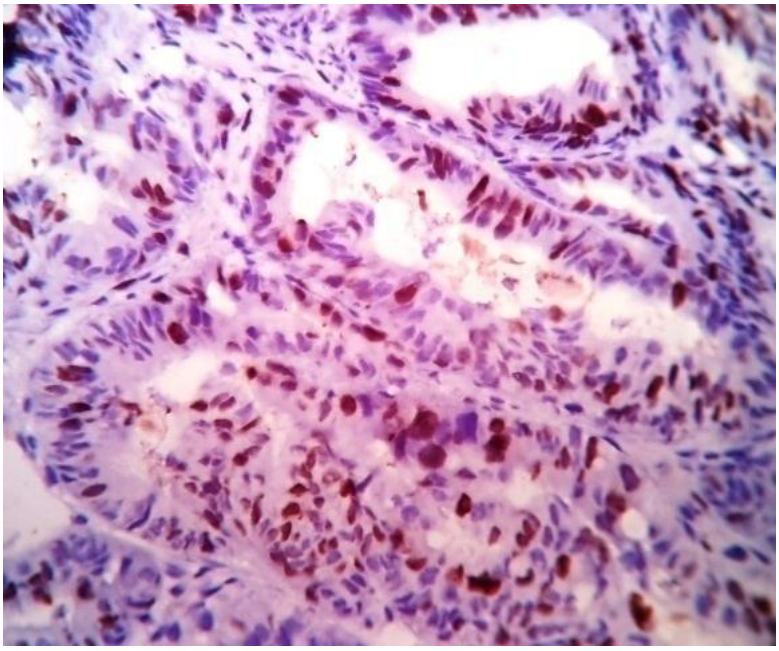

Figure 13 : Ki-67 expression in well differentiated adenocarcinoma. Nuclear positivity in 37.4% of neoplastic cells (400X).

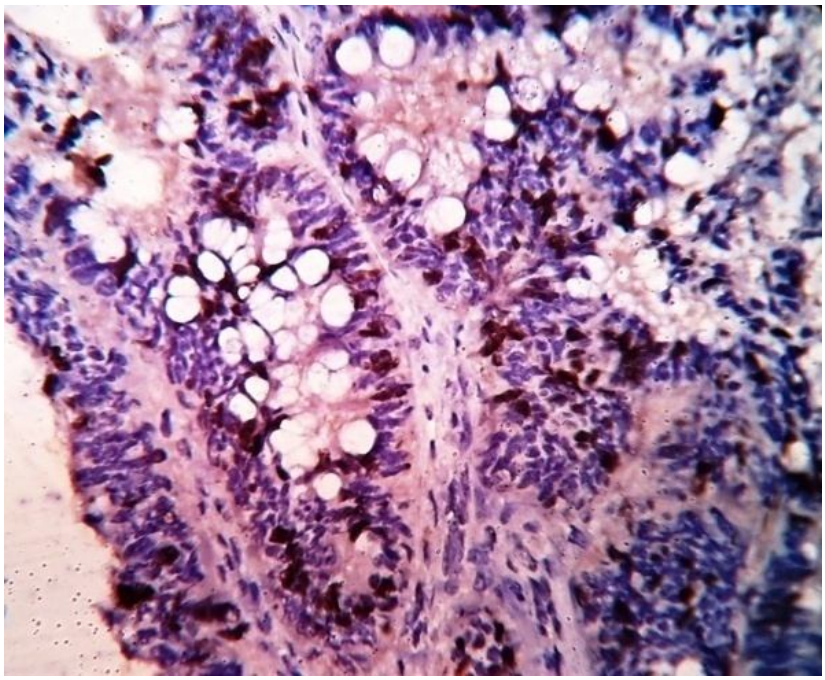

Figure 14 : Survivin expression in moderately differentiated adenocarcinoma. Nuclear positivity in 43.2% of tumor cells (400X).

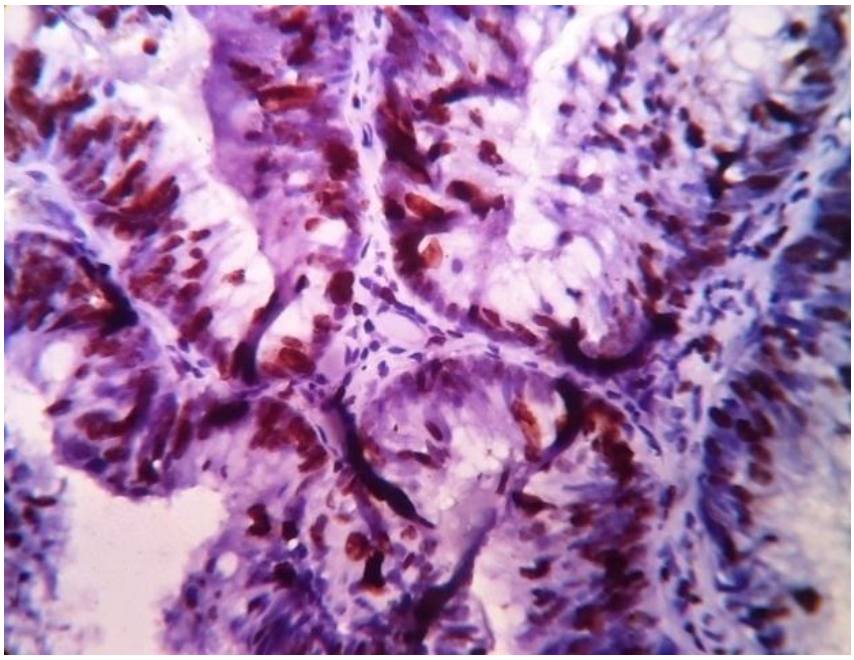

Figure 15 : Ki-67 expression in moderately differentiated adenocarcinoma. Nuclear positivity in 53.4% of tumor cells (400X).

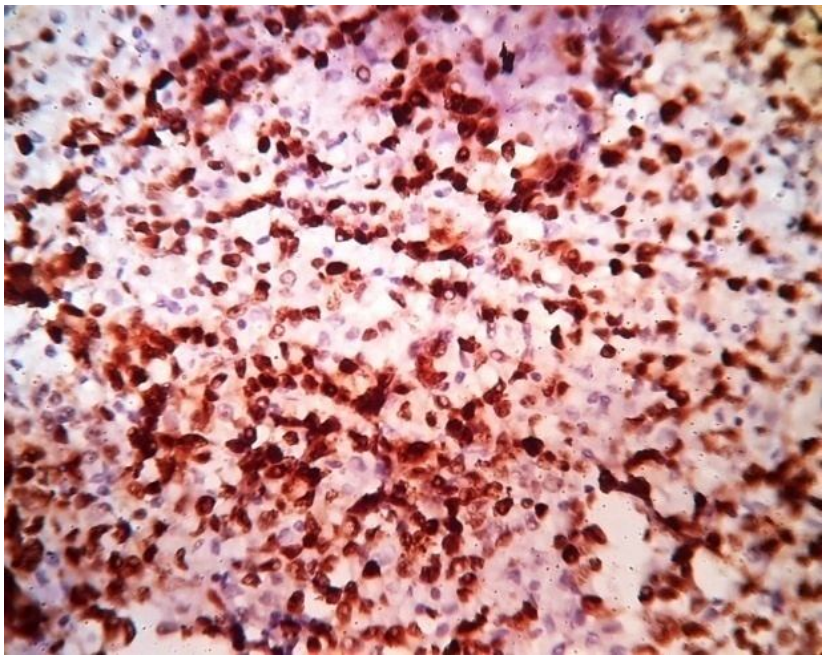

Figure 16 : Survivin expression in poorly differentiated adenocarcinoma. Nuclear positivity in 72.7% of tumor cells (400X).

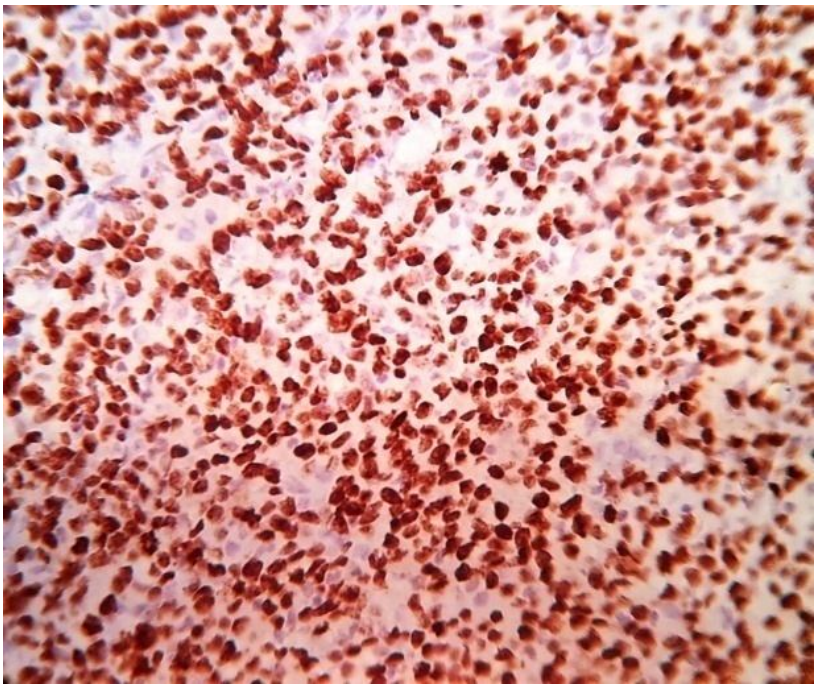

Figure 17 : Ki-67 expression in poorly differentiated adenocarcinoma. Nuclear positivity in 84.2% of tumor cells (400X).

DISCUSSION

1) Age, gender and site distribution :

The present study includes 90 cases which is comprised of 30 samples of normal colorectal epithelium, 30 cases of colorectal adenomas and 30 cases of colorectal adenocarcinomas.

The age group of patients included in the study varied from less than 20 years to more than 70 years with most of the patients being above 40 years of age. 60 percentage of the patients were males and 40 % were females. In most of the patients, the tumor was located in the rectum (38.3%), followed by right hemicolon (25%), left hemicolon (16.6%) and sigmoid colon (20%).

2) Carcinoma grade and stage distribution :

Out of the 30 adenocarcinomas that were studied, 10 were well differentiated, 12 were moderately differentiated and 8 were poorly differentiated. AJCC staging was used. 13 were stage I, 8 were stage II and 9 belonged to stage 3. Of the 8 stage II tumors, 4 belonged to stage IIA, 2 belonged to stage IIB and 2 belonged to stage IIC. 5 of the stage III tumors were of stage IIIA, 3 were of stage IIIB and 1 was of stage IIIC.

3) Comparison of Survivin expression with age :

In the present study 9 out of 90 patients (10%) were below 40 years of age and 81 out of 90 (90%) were above 40 years of age. The mean differences of survivin expression between those below 40 years and those above 40 years was -6.62, which was statistically not significant.

Hai-Yan Tan et al in 2005 observed in his study that comparison of survivin expression with age of the patient was statistically not significant [84].

Alfred King-Yin et al and Woong Na et al found that no correlation existed between survivin expression and patient's age [85].

The study conducted by Ren Chong Xi and colleagues in 2011 also showed no significant correlation between survivin expression and age [88].

4) Comparison of Survivin expression with gender :

In the present study, 54 patients (60%) were male and 36 (40%) were female. Statistical analysis showed that the difference between mean expression between males and females was statistically not significant.

Hai-Yan Tan and colleagues in their study conducted during 2005 found no significant correlation between survivin expression by the tumor and gender[84].

In 2009 a study was conducted by Woong Na et al which showed that survivin expression by colorectal neoplasia did not correlate with the gender [87].

Similarly Ren Chong Xi and colleagues in their 2011 study concluded that no correlation existed between gender and survivin expression by colorectal adenomas and carcinomas [88].

5) Survivin expression in normal epithelium vs adenomas vs adenocarcinomas (The adenoma-carcinoma sequence):

In the present study, Survivin expression was minimal to absent in normal colonic mucosa, gradually increased in adenomas from low grade dysplasia to high grade dysplasia and was maximally expressed in adenocarcinomas. The differences in mean survivin expression between normal mucosa and adenomas and between adenomas and adenocarcinomas was statistically significant (P value less than 0.001).

This observation of minimal to absent survivin expression in normal colonic epithelium and its significantly higher expression in adenomas and adenocarcinomas makes survivin a potentially exploitable target of anti-cancer

therapy with maximal targeting of the tumor and minimal damage to the normal epithelium. Also the significant increase observed in survivin expression from normal mucosa to adenoma to adenocarcinoma suggests that survivin has an important role in colorectal tumorigenesis and malignant transformation of adenomas (the adenoma-carcinoma sequence).

This result of the present study correlates with that of Hiroshi Kawasaki et al who in their 2001 study of colorectal neoplasia which included 43 hyperplastic polyps, 171 adenomas with low grade dysplasia, 42 adenomas with high grade dysplasia and 60 carcinomas concluded that the immunoreactivity of Survivin significantly increased from hyperplastic polyps to adenomas with low grade dysplasia and adenomas with high grade dysplasia and carcinomas which showed that survivin played an important role in the malignant transformation of adenomas [81].

In their 2003 study, Lian-Jie Lin and colleagues inferred that the positive rate of survivin increased in transition from normal epithelium to adenoma with low grade dysplasia to adenoma with high grade dysplasia and carcinoma concluding that survivin expression is related with the early stage of colorectal carcinogenesis and plays an important role in the adenoma-carcinoma sequence[82].

6) Comparison of Ki-67 expression between normal epithelium, adenomas and adenocarcinomas :

The Ki-67 expression was predominantly present at the base of the colonic crypts in normal epithelium as against the expression at the surface of the adenomas. In the present study, the percentage of cells expressing Ki-67 increased from normal epithelium to adenoma to adenocarcinoma. The differences in mean Ki-67 expression between normal epithelium, adenomas and adenocarcinomas were statistically significant.

The results of the present study correlates with that of Yan-Lei Ma et al who in their 2010 study on colorectal carcinomas concluded that Ki-67 was overexpressed in colorectal carcinoma relative to normal colorectal tissues [92].

Miao-Xia Lin et al studied the Ki-67 expression in 60 colorectal adenomas and 20 normal mucosal tissues and observed that Ki-67 expression was higher in colorectal carcinomas than in adenomas and normal epithelium which was statistically significant (P value less than 0.05), concluding that Ki-67 over expression correlated with colorectal tumorigenesis. Similar observations were made in the present study [91].

The inference of the present study is similar to that of Saleh Husain A et al, conducted in the year 2000 which concluded that an inverse relation exists

between Ki-67 expression and colonic neoplasia (including adenomas and adenocarcinomas) [90].

7) Comparison between Survivin expression and the differentiation of colorectal adenocarcinomas:

The present study includes 30 cases of colorectal adenocarcinomas of which , 10 were well differentiated (grade1), 12 were moderately differentiated (grade2)and 8 were poorly differentiated (grade3)adenocarcinomas. The difference of mean survivin expression between grade 1 and grade 2 adenocarcinomas was -13 and that between grade 2 and grade 3 was -28.59, both of which were statistically significant (P value less than 0.001). A significant correlation is observed between survivin expression and grade of the adenocarcinoma.

Ulrike Gerlach and colleagues reached similar conclusions in their 2006 study on colorectal carcinomas which showed that survivin expression correlated significantly with tumor differentiation [83] .

The results of the present study are in agreement with, Hai-Yan Tan et al who in their study conducted in 2005 comprising 48 cases of colorectal adenocarcinomas concluded that Survivin expression correlated with the pathological grade of the tumor [84].

In contrast to the observations made in the present study, Ren Chong Xi et al in 2011 concluded that there was no correlation between survivin expression and histological differentiation of the tumor [88].

Likewise Woong Na et al who studied survivin expression in 529 cases of colorectal adenocarcinomas concluded that there was no significant correlation between carcinoma grade and survivin expression[87].

Lian-Jie Lin and colleagues concluded that no correlation was observed between histological grade of the adenocarcinoma and expression of survivin [82].

8) Comparison between Ki-67 expression and differentiation of colorectal adenocarcinomas :

In the present study, there was a significant correlation between Ki-67 expression and grade of the adenocarcinomas (P value less than 0.001). Similar results were obtained by Saleh Hussain A et al in 2000 who concluded that Ki-67 values are associated with carcinoma grade [90].

Similarly Anway sen et al in 2015 also concluded that the Ki-67 labelling index showed strong correlation with the histopathological grade of the carcinoma [93].

9) Comparison between Survivin and Ki-67 expression in colorectal neoplasia :

Upon assessing the correlation between Survivin expression and Ki-67 expression in colorectal neoplasia including adenomas and adenocarcinomas using curve estimation, the resulting R value turned out to be 0.83 which signifies a strong and significant correlation between the two (P value less than 0.001).

Hiroshi Kawasaki and colleagues had similar conclusions in their 2001 study of colorectal neoplasia. The study showed that the expression of Survivin positively correlated with Ki-67 labelling index and the correlation was statistically significant [81].

Xia-Bin Li et al studied 275 cases of colorectal carcinomas during 2012 for survivin polymorphisms and had findings similar to the present study. They concluded that there was a significantly positive correlation found between the expression of Survivin and Ki-67 in colorectal carcinomas [94]. Similarly Wei-Chang Chen and colleagues in their study during 2004 concluded that survivin expression correlated with Ki-67 proliferation index [95].

10) Comparison between survivin expression and stage of colorectal adenocarcinomas :

On comparing the survivin expression between stage I and II adenocarcinomas and stage III adenocarcinomas it was found that the difference in mean survivin expression between the two groups was -9.84 which was statistically significant showing that survivin expression correlates with the stage of colorectal adenocarcinomas. This result of the present study is similar to that of Ying-Yu Lee et al who studied 95 cases of colorectal adenocarcinomas and 24 adenoms for survivin expression finding that survivin expression correlated with the AJCC stage of the tumor [86]. Similarly Hai-Yan Tan and colleagues demonstrated a significant correlation between survivin expression and Dukes stage of colorectal carcinomas [84].

In 2009, Woong Na and colleagues also concluded in their study involving 529 colorectal adenocarcinomas that there was a significant correlation between AJCC stage of the tumor and the expression of survivin [87].

On the contrary one study conducted by Wei-Chang Chen et al in 2004 and another conducted by Ren Chong Xi et al in 2011 concluded that there was no significant correlation between the stage of colorectal adenocarcinomas and their survivin expression[88,95].

CONCLUSION

The aim of the present study was to examine the expression of Survivin, a novel member of the Inhibitor of apoptosis family of proteins, in colorectal neoplasia, its role in the transition sequence from normal to adenocarcinoma and its association with various clinico-pathological characters of adenocarcinomas. The following are the conclusions of the present study :

- The expression of survivin is negligible to absent in normal colonic epithelium.
- Survivin expression showed a significant increase from normal mucosa to adenoma to adenocarcinoma. This signifies that survivin plays an important role in all stages of the adenoma-carcinoma sequence, which includes the early event of adenoma formation from normal epithelium and its malignant transformation.
- The expression of survivin showed significant correlation with the differentiation of adenocarcinoma.
- Expression of survivin had a strong correlation with Ki-67 expression by the tumors.
- Survivin expression in colorectal adenocarcinomas also showed a significant positive correlation with the AJCC stage of the tumors, being highly expressed in stage III tumors as compared to stage I and stage II tumors.

- These results highlight the association of Survivin expression with malignant behaviour of colorectal adenocarcinomas and Survivin could prove to be a new biomarker for aggressiveness and prognostic information in these tumors.
- There was however no correlation between survivin expression and the age and gender of the patient.
- The finding of absent to minimal expression in normal colonic epithelium and significantly higher expression in adenocarcinomas makes survivin an attractive and potential therapeutic target, which when implemented will result in maximal targeting of cancerous and also pre cancerous tissues with minimal damage to the surrounding normal mucosa.

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MASTER CHART

S.No.	Diagnosis	Age	Sex	Tumor Site	Tumor Size	Carcinoma Grade	Stage	Tumor stage	Ki67 %	Survivin %
1	Normal	78	M	_	_	_	_	_	6.2	0
2	Adenocarcinoma, grade 2	78	M	Right hemicolon	6x5x3	2	III B	T3	47.7	33.7
3	Adenoma with high grade dysplasia	68	M	Rectum	3x3x1	_	_	_	22.9	27.6
4	Normal	53	F	_	_	_	_	_	6.1	0
5	Adenocarcinoma, Grade 3	53	F	Right hemicolon	6x5x4	3	III B	T3	79.8	56.8
6	Normal	45	M	_	_	_	_	_	6.8	0
7	Adenocarcinoma, grade 2	45	M	Right hemicolon	5x5x4	2	II B	T4a	47.1	39.7
8	Normal	58	F	_	_	_	_	_	5.6	0
9	Adenocarcinoma, grade 2	58	F	Rectum	4x3x2.5	2	I	T2	43.3	35.6
10	Adenoma with high grade dysplasia	50	M	Rectum	0.4x0.2x0.2	_	_	_	23.7	34.8
11	Adenoma with low grade dysplasia	66	M	Left hemicolon	2x1x1	_	_	_	14.1	10.2
12	Normal	62	M	_	_	_	_	_	8.1	0
13	Adenocarcinoma, grade 2	62	M	Left hemicolon	11x9x4	2	III A	T2	61.9	41.3
14	Adenoma with low grade dysplasia	28	F	Rectum	1x1x0.5	_	_	_	12.6	12.9
15	Adenocarcinoma, Grade 3	66	M	Rectum	4.5x4.5x3.5	3	II A	T3	64.6	55.4
16	Normal	66	M	_	_	_	_	_	8.6	0
17	Normal	75	F	_	_	_	_	_	9.8	0
18	Adenocarcinoma, grade 2	75	F	Right hemicolon	6x5x2.5	2	III A	T2	46.1	35.5
19	Normal	66	M	_	_	_	_	_	7.4	0
20	Adenocarcinoma, grade 1	66	M	Right hemicolon	10x4.5x4.5	1	I	T2	25.8	29.9
21	Adenoma with high grade dysplasia	25	M	Rectum	3x2x1	_	_	_	19.8	31.7
22	Adenoma with low grade dysplasia	62	M	Sigmoid Colon	1x0.5x0.5	_	_	_	14	10.6
23	Normal	59	M	_	_	_	_	_	8.3	0
24	Adenocarcinoma, Grade 3	59	M	Rectum	6x4.5x4.5	3	III C	T4a	72.7	77.1
25	Adenoma with low grade dysplasia	58	M	Left hemicolon	1x1x1	_	_	_	15.3	11.5
26	Normal	58	F	_	_	_	_	_	6.1	0
27	Adenocarcinoma, grade 1	58	F	Sigmoid Colon	6x5x5	1	I	T2	28.4	37.8

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28	Adenoma with high grade dysplasia	45	F	Sigmoid Colon	0.8x0.5x0.5	_	_	_	17.9	26.7
29	Normal	72	M	_	_	_	_	_	7.3	0.1
30	Adenocarcinoma, grade 2	72	M	Sigmoid Colon	6x5x2.5	2	II C	T4b	59.8	44.7
31	Adenoma with low grade dysplasia	60	F	Rectum	0.5x0.5x0.5	_	_	_	14.2	11.2
32	Adenoma with low grade dysplasia	49	M	Sigmoid Colon	1x0.5x0.5	_	_	_	16.4	14.7
33	Adenoma with high grade dysplasia	59	M	Rectum	1x0.5x0.5	_	_	_	24.3	23.8
34	Adenoma with high grade dysplasia	53	M	Rectum	0.3x0.3x0.3	_	_	_	21.1	31.4
35	Normal	64	F	_	_	_	_	_	7.9	0
36	Adenocarcinoma, grade 1	64	F	Right hemicolon	7x5.5x2	1	I	T2	28.2	26
37	Adenoma with low grade dysplasia	27	F	Right hemicolon	1.5x1x0.5	_	_	_	13.5	10.8
38	Normal	72	F	_	_	_	_	_	5.9	0
39	Adenocarcinoma, grade 2	72	F	Sigmoid Colon	6x4x4	2	II C	T4b	57.3	43.2
40	Adenoma with high grade dysplasia	74	F	Sigmoid Colon	0.8x0.5x0.3	_	_	_	20.7	22.9
41	Adenoma with high grade dysplasia	45	F	Rectum	1x1x0.5	_	_	_	20.2	32
42	Adenoma with high grade dysplasia	40	M	Rectum	0.5x0.5x0.5	_	_	_	22.5	30.9
43	Normal	65	M	_	_	_	_	_	6.2	0
44	Adenocarcinoma, grade 2	65	M	Rectum	7.5x5x3	2	II B	T4a	43.2	38.6
45	Adenoma with low grade dysplasia	24	M	Rectum	1x0.5x0.5	_	_	_	15.9	12.5
46	Normal	26	F	_	_	_	_	_	6.9	0
47	Adenocarcinoma, grade 1	26	F	Rectum	3x3x2	1	I	T2	26.7	25.1
48	Adenoma with high grade dysplasia	60	M	Right hemicolon	0.4x0.4x0.4	_	_	_	19.4	27.2
49	Adenoma with high grade dysplasia	42	F	Left hemicolon	0.2x0.2x0.2	_	_	_	23.2	28.5
50	Adenoma with high grade dysplasia	10	M	Left hemicolon	1x0.5x0.5	_	_	_	26.1	24.3
51	Adenocarcinoma, grade 1	53	F	Right hemicolon	2x2x1	1	I	T2	29.2	21.8
52	Normal	49	F	_	_	_	_	_	5.9	0
53	Adenocarcinoma, Grade 3	49	F	Left hemicolon	5.8x5x4.5	3	III A	T2	69.8	57.1
54	Adenocarcinoma, Grade 3	60	F	Rectum	4x3.5x3	3	II A	T3	71.4	53.2
55	Adenocarcinoma, grade 1	74	F	Rectum	4.5x2.5x2	1	I	T2	37.4	29.3
56	Adenocarcinoma, grade 1	46	M	Sigmoid Colon	5x3x2	1	III A	T2	31.4	23.4

57	Normal	64	M	_	_	_	_	_	9.9	0.1
58	Normal	56	M	_	_	_	_	_	5.2	0
59	Adenoma with high grade dysplasia	62	F	Rectum	1x1x1	_	_	_	20.3	26.4
60	Adenoma with low grade dysplasia	59	F	Sigmoid Colon	0.7x0.5x0.3	_	_	_	13.7	12.7
61	Normal	60	M	_	_	_	_	_	6.6	0
62	Adenocarcinoma, grade 1	60	M	Rectum	2.5x2.5x1	1	I	T2	36.2	29.1
63	Normal	65	F	_	_	_	_	_	5.7	0
64	Adenoma with low grade dysplasia	64	M	Sigmoid Colon	0.5x0.3x0.2	_	_	_	14.8	11.3
65	Adenoma with high grade dysplasia	48	F	Rectum	2.5x2x1.5	_	_	_	21.6	27.9
66	Adenoma with low grade dysplasia	55	M	Left hemicolon	2x1x1	_	_	_	13.5	12.9
67	Normal	62	M	_	_	_	_	_	9.5	0
68	Adenoma with low grade dysplasia	38	M	Right hemicolon	1.5x1x0.5	_	_	_	14.7	10.3
69	Normal	15	M	_	_	_	_	_	6.8	0
70	Adenoma with high grade dysplasia	55	M	Right hemicolon	2x2x1	_	_	_	19.5	28.1
71	Normal	56	M	_	_	_	_	_	9.1	0
72	Adenocarcinoma, grade 1	56	M	Left hemicolon	5x3x2	1	I	T2	27.1	27.4
73	Normal	64	M	_	_	_	_	_	10.5	0
74	Adenocarcinoma, grade 2	64	M	Rectum	4.5x3x2	2	II A	T3	44.3	53.4
75	Adenoma with low grade dysplasia	45	M	Left hemicolon	0.5x0.5x0.5	_	_	_	12.9	9.1
76	Normal	60	F	_	_	_	_	_	7.5	0
77	Adenocarcinoma, Grade 3	60	F	Right hemicolon	7x5x5.4	3	I	T2	66.3	53.2
78	Adenoma with low grade dysplasia	44	M	Right hemicolon	0.3x0.3x0.3	_	_	_	13.8	12.4
79	Normal	65	F	_	_	_	_	_	5.3	0.1
80	Normal	52	M	_	_	_	_	_	8.5	0
81	Adenocarcinoma, grade 2	52	M	Right hemicolon	6x3x1.5	2	II A	T3	54.8	43
82	Adenoma with low grade dysplasia	60	M	Left hemicolon	2x2x1	_	_	_	14.2	12.2
83	Adenocarcinoma, Grade 3	61	M	Sigmoid Colon	6.5x6x5	3	III B	T3	84.2	47.2
84	Normal	60	M	_	_	_	_	_	9	0
85	Adenocarcinoma, grade 2	60	M	Sigmoid Colon	4x4x1.5	2	I	T2	52.4	39.8

86	Normal	60	F	_	_	_	_	_	10.1	0
87	Adenocarcinoma, Grade 3	60	F	Right hemicolon	9x5x4	3	III A	T2	81.7	49.5
88	Adenocarcinoma, grade 1	62	M	Rectum	2.5x2x0.8	1	I	T1	27.5	26.1
89	Normal	48	F	_	_	_	_	_	5.5	0
90	Adenocarcinoma, grade 2	63	M	Rectum	2.5x2.3x1	2	I	T2	59.6	38.6

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