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PROGNOSTIC SIGNIFICANCE AND CLINICOPATHOLOGICAL ASSOCIATION OF COX2 EXPRESSION IN COLORECTAL CARCINOMA OF LIBYAN PATIENTS

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIRMENT FOR MASTER DEGREE IN PATHOLOGY

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بسم الله الرحمن الرحيم

((و قل اعملوا فسيرى الله عملكم ورسوله و المؤمنون و
ستردون إلى عالم الغيب و الشهادة فينبءكم بما كنتم
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صدق الله العظيم

سورة التوبة اية 105

DEDICATED

TO

My Parents

&

Dear Family

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CONTENTS

Abbreviations

AJCC	American Joint Committee on Cancer
APC	adenomatous polyposis coli
Bcl-2	B-cell lymphoma/leukemia-2
BMI	body mass index
CD	Crohn,s disease
c-DNA	complementary deoxyribonucleic acid
CEA	carcinoembryonic antigen
CI	confidence interval
CIN	chromosomal instability
COX2	cyclooxygenase2
CRC	colorectal cancer
CT	computerized tomograghy
DCC	deleted in colorectal cancer gene
DFS	disease free survival
DNA	deoxyribonucleic acid
EGFR	epithelial growth factor receptor
EMT	epithelial –mesenchymal transion
FAP	familial adenomatous polyposis
HNPCC	hereditary non-polyposis colorectal cancer
HPF	high power field
H&E	haematoxylin and eosin
IBD	inflammatory bowel disease

IHC	immunohistochemistry
JPS	juvenile polyposis syndrome
K-ras	Kristen-rat-sarcoma
Ki- 67	proliferation factor
LOH	loss of heterozygocity
MAP	MYH- associated polyposis
MMP	matrix metalloproteinase
MMR	mismatch repair gene
m-RNA	messenger ribonucleic acid
MUC	mucin
NBI	narrow band imaging
NSAID	nonsteroidal anti-inflammatory drug
P53	(tumor suppressor gene) /protein p 53
PCR	polymerase chain reaction
PG	prostaglandin
PJP	Peutz–Jeghers polyposis
PCLE	probe-based confocal laser endomicroscopy
SEER	surveillance epidemiology and endresult program
SRCC	signet-ring cell cancer
TIMP	tissue inhibitor of metalloproteinase
TMA	tissue microarray
TNM	Tumor Node Metastasis classification
UC	ulcerative colitis

VEGFR	vascular endothelial growth factor receptor
WHO	World Health Organization

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CHAPTER I

Introduction

&

Aim of Study

1.1 ABSTRACT

Purpose: Cyclooxygenase-2 (COX-2) is generally elevated in tumors compared with normal tissue and apparently has an important role in tumor development. A number of studies have found high expression of COX-2 to be an unfavorable prognostic factor for overall survival in several cancers, in CRC is considered to play an important role in carcinogenesis and is often up-regulated in colon cancers. However, previous data on the influence of COX-2 expression on patient outcome have been conflicting. The aim of our study is to find the relation between COX-2 expression and patient outcome in Libya.

Patients and Methods: By using 83 paraffin blocks of Libyan patients with colorectal cancer on the stages (I-IV) in the period from 2007-2011, which we diagnosed and graded by Hematoxylin and Eosin staining, then immune-staining for COX-2 was preformed and evaluated, statistical analysis for the COX-2 expression and its relation to other clinico-pathological parameters was done.

Results: In our study the COX2 immunostaining of the colorectal cancer in Libyan patients showed that there is no overexpression, there was loss of the COX2 expression in relation to old patients (*P< 0.07)*, those with large tumor size (*P< 0.01)*, and with lympho/vascular invasion (*P*< 0.06), and there was no significant correlation between the COX2 expression and other patient parameters such as tumor type, grade, stage, lymph node status and metastasis. In analysis of the disease free survival of the patients in relation to COX2 expression by Kaplan-Meier, we found that patients with more COX2 expression has less DFS than those with less expression (*P< 0.11). Conclusion:* Our results implicate that COX2 overexpression related to decrease on the cancer free survival, so it has adverse effect on the outcome of colon cancer patients, which consider as an important marker in predicting outcome of patients with colorectal cancer.

Key Words: CRC, COX2, immunostaining, prognosis, disease free survival.

1.2 INTRODUCTION

Colorectal cancer (CRC) accounts for up to 9% of new malignancies worldwide, and affects more than one million people annually (Jemal et al.,2011), and represented as the fourth most common cancer in men and the second most common cancer in women worldwide (Parkin et al., 2005) and it is one of the leading causes of death from cancer worldwide, of those patients who are clinically diagnosed with colorectal cancer, 20-30% are in the advanced stage (Waisberg et al.,2009).

Previous studies have reported rapid increase in colorectal cancer incidence rates, particularly in economically transitioning countries in many parts of the world, and these increases are thought to reflect changing dietary and physical activity patterns (Cress et al., 2006).

CRC represents common tumor in developing countries, with peak incidence at 60-70 years of age. Almost all are adenocarcinoma, most frequently originating from adenomatous polyps.

Study has done in the western of Libya in 2006 in cases registered by Cancer Registry Department which set up at (African Oncology Institute (AOI) Sabratha in 2006); show cancer colon is the 4th commonest cancer in our population (10%), after breast (23%), lung(15%), prostate(17%), while cancer rectum is 6th commonest (Libya Cancer Registry, 2006).

Tumorgenesis of CRC is the result of a multistep process. In the course of this process a number of genetic alterations accumulate, which then lead to the malignant transformation of epithelial cells in the colon or rectum. There are two molecular pathways of colorectal carcinogenesis, the adenoma-carcinoma sequence and the mismatch repair MMR (or microsatellite instability) pathway. In each pathway; there is sequential accumulation of mutations in specific genes (e.g. APC and MLH1, MSH2, MSH6). The —traditional pathway, the so-called adenoma-carcinoma-sequence, described by Vogelstein and colleagues in the early 1990 (Vogelstein et al.,1990) is

characterized by an early bi-allelic inactivation of *APC* caused by alterations in the mutation cluster region (codons 1243–1567) of this gene followed by an oncogenic *K-Ras* codon 12 or 13 mutation, inactivation of the tumor suppressor gene *Tp53* at the transition from adenoma to carcinoma and chromosomal instability (Early et al.,2008).

The aetiology of CRC is multifactorial, but it appears to be influenced both by hereditary and environmental factors involving high risk and low risk genetic factors as well as environmental factors including lifestyle. The spectrum of CRC can be divided into two main groups: sporadic CRC and familial CRC. The majority of patients develop CRC on an apparently sporadic basis and are the sole family member with CRC (65-90% of all patients) (Barault et al., 2008).

People at an increased risk of colon cancer include those with either a personal or family history of colorectal cancer or polyps, individuals with a long-standing history of inflammatory bowel disease and people with familial colorectal cancer syndromes. Some of those at high risk may have a 100% chance of developing colorectal cancer.

The occurrence of CRC in some patients is related to the presence of pervious precancerous lesions, which play important role in the initiating of the tumorgenesis, Familial polyposis syndromes are characterized by the early onset of CRC development, hereditary non-polyposis (HNPCC) syndrome is characterized by multiple polyp and early onest CRC, other disease as inflammatory bowel disease (ulcerative colitis), Lynch syndrome are at increased risk of CRC.

The decrease in mortality spanning across the last decade is attributed to early detection and improved therapy. The median survival of patients with metastatic colorectal cancer (m-CRC) participating in clinical trials has improved from approximately six months to two years. This increase in survival can be attributed mainly to two treatment advances (Ferlay et al.,2007). The 5-year relative survival among CRC patients at stages II and III of all ages has been improved too (Chen et al., 2010). Early diagnosis significantly improves the chances of survival in CRC, with 5-year

survival rates for patients of all ages ranging from 95% for those diagnosed at Stage I, to 7% for those diagnosed at Stage IV (Gloeckler et al., 2003, Zhou et al.,2011).

Developed screening methods, and more people undergoing regular screening over the past few years may have contributed to a lower incidence of CRC and also an earlier diagnosis, Over the past few decades, significant progress has been made in the screening, diagnosis and treatment of CRC through advances in molecular biology, endoscopy, surgery, and chemotherapy. The recommended test for mass screening is the fecal occult blood test (FOBT) which acts as a first screen for possible malignancy, is designed to detect blood traces in the stool on a guaiac-based testing sample. Persons testing positive usually undergo colonoscopy as a more invasive but definitive examination. Newer technologies combine the guaiac-based test with tests based on molecular biology to look for cancer biomarkers in the stool (Atkin 2003, Janssens 2005). However, despite these improvements, the overall 5-year survival rate is approximately 45%. Thus, CRC remains a devastating disease and of a major global health concern (O'Connell et al.,2004).

The best form of treatment for stage I and II tumors is surgical resection which is curative in most cases, Stage III tumors receive adjuvant chemotherapy. The first treatment advance is the introduction of new cytotoxics and biological, as well as the better selection of patients. The second IS the establishment of a multidisciplinary approach treatment of metastatic colorectal cancer, improved techniques to resect metastatic disease particular in liver metastases and the development of new techniques (Jemal et al., 2008).

It is clinically relevant to identify predictive markers of cancer response to different combinations of treatments. Prior identification of patients who have a higher likelihood of responding to chemotherapy could help to select those who can benefit from the treatment. Patients with a known resistant tumors could be spared from exposure to radiation or DNA-damaging drugs that are associated with adverse side effects. Several biomarkers have been correlated with clinical staging and outcome;

and this increase the need for informative molecular markers that provide prognostic information (Huerta et al., 2006).

These biomarkers are categorized as prognostic and predictive. Predictive markers are related to the impact of the treatment on the outcome, while prognostic markers are related to the outcome independent of treatment . These considerations have prompted researchers to find biomarkers that can predict the tumor response both before and after neoadjuvant treatment (Winder et al., 2010).

As proved by many studies that the inflammatory process play important role in the carcinogenesis of many tumor, which accompanied by over or under expression of several inflammatory mediators; Cyclooxygenase-2 (COX-2; PTGS2) is considered to play an important role in colorectal carcinogenesis and is often up-regulated in colon cancers. It converts arachidonic acid to prostaglandins and related eicosanoids and promotes inflammation and cell proliferation (Buchanan et al.,2006).

Various prostaglandins are produced in a cell type-specific manner, and they elicit cellular functions via signaling through G-protein coupled membrane receptors, and in some cases, through the nuclear receptor. COX-2 utilization of arachidonic acid also perturbs the level of intracellular free arachidonic acid and subsequently affects cellular functions. In a number of cell and animal models, COX-2 is cytokine inducible. The fact that COX2 is inducible by pro-inflammatory cytokines and growth factors implies a role for COX2 in both inflammation and the control of cell growth. induction of COX-2 has been shown to promote cell growth, inhibit apoptosis and enhance cell motility and adhesion. The mechanisms behind these multiple actions of COX-2 are largely unknown (Cao et al., 2002). A large number of observations emphasize that induced prostaglandin production, particularly PGE2, is involved in cell signaling through prostanoid receptors. Suggested subtype EP2 receptor expression in colon cancer tissue to predict reduced survival (Gustafsson et al., 2007, Annika et al.,2011).

COX-2 is over expressed in the majority of human colon cancers; Supporting the importance of COX-2 in colorectal carcinogenesis (Brown et al., 2005).

Several recent epidemiologic studies suggest that the use of aspirin and other NSAIDs exerts a protective effect against colon cancer. In the Nurses 'Health Study women who used 4-6 tablets of aspirin per day for 10 years or more had a decreased incidence of colon cancer. It is suspected that this effect is via inhibition of cycloxygenase-2 (COX-2) (Liao et al., 2012).

This enzyme is over-expressed in 90% of colorectal carcinoma and 40 -90% of adenoma .How COX-2 promotes carcinogenesis is not clear. Some of its effect may be mediated by the production of prostaglandin E2(PGE2), which seems to favor epithelial cell proliferation, inhibit apoptosis, and enhance angiogenesis by enhancing the production of vascular endothelial growth factor (VEGF).

Some other previous studies are conflicting regarding prognostic significance of COX-2 in colorectal cancer with some supporting and others refuting an independent adverse effect of COX-2 over-expression. COX-2 overexpression has been positively associated with p53 alteration and inversely associated with microsatellite instability (MSI) which generally predict longer survival of colon cancer patients (Soumaoro et al., 2004).

A large prospective study of colon cancer patients suggests that COX-2 up-regulation is independently associated with a worse colon cancer-specific mortality. In addition, when compared with patients with tumors negative for both COX-2 and p53. So COX-2 over-expression is associated with poor patient outcome may have significant clinical implications considering an emerging role of COX-2 and its pathway as chemotherapeutic and chemo-preventive targets (shuji et al., 2008).

Epidemiological studies have shown that the inducible form of cyclooxygenase (COX-2) may be involved in colorectal carcinogenesis, but it is controversial whether its expression is a prognostic factor for colorectal cancer.

The aim of the study was to examine the expression of COX-2 in colorectal cancer and investigate its prognostic relevance in Libyan patients and its clinico-pathological significance in tumor outcome.

1.3 Aims of the study:

1/ Study of colorectal cancer by H&E stain to detect differentiation and grades.

2/Study the expression of immunohistochemical marker COX-2 as diagnostic and prognostic marker in colorectal cancer and find its relation to different clinicopathological parameters.

CHAPTER II

Review of Literature

2. Review of Literature

2.1 Embryology of colon

The gastrointestinal tract (GIT) extending from the bucco-pharyngeal membrane to the cloacal membrane arises initially from the endoderm of the trilaminar embryo (week 2, 3). It later has contributions from all the germ cell layers (Alan et al., 2009).

The colon develops partly from the primitive midgut (ascending colon to proximal two third of the transverse colon) and partly from the hind gut (distal one third of the transverse colon to sigmoid colon), midgut, which opens ventrally into the yolk sac. Starting at the fifth gestational week, the midgut rapidly grows and reorganizes to delineate the permanent gastrointestinal tract structures, including the colon. This progression is traditionally divided into three separate stages, in the first stage; the elongated midgut loop enters the extra-embryonic coelom into the umbilical cord, a process referred to as physiologic umbilical herniation. The superior mesenteric artery (SMA) also exits the abdominal cavity along with this bowel loop and within its corresponding mesentery, and then separates the midgut in a proximal and anterior portion, referred to pre-arterial, which carries the omphalo-mesenteric duct at its apex, and a posterior and distal portion. The herniated intestine then rotates counterclockwise by 180 degrees around the SMA axis (Yeo, 2007).

In particular, the pre-arterial segment moves posteriorly and to the left of the SMA, in the second stage at the 10^{th} week, the midgut loop returns to the peritoneal cavity from the umbilical herniation, and rotates 180^{o} counterclockwise around the pedicle formed by the mesenteric root, the cecum in the upper abdomen, descends, migrating to the right lower quadrant c/clockwise 270^{o}. In the latter weeks of the first trimaster. The process of fixation initiates with fusion of parts of the primitive mesentery, with fixation of the duodenum, and the ascending and descending parts of the colon to the posterior abdominal wall. The sympathetic innervation originates from T-8 to L-2, via splanchnic nerves and the autonomic abdomino-pelvic plexus (Bruce et al., 2007).

The arterial supply to the gut develops through consolidation and reduction of the ventral branches of the dorsal aorta that anastomose with the vessel plexuses originally supplying blood to the yolk sac. About five of these vitelline artery derivatives vascularize the thoracic foregut, and three—the celiac, superior mesenteric, and inferior mesenteric arteries—vascularize the abdominal gut (Schumpelick et al., 2000).

The intestinal endoderm layer forms the intestinal characterized in the RAD axis with the establishment of the villus-crypt axis. The pseudostratified endoderm formed of undifferentiated cells undergoes a columnar transformation accompanied with a mesodermal outgrowth. This process results in the development of structures termed villi, which form along a cranial to caudal wave. AP axis influences the RAD axis in morphologic and epithelial cellular differentiation. In late fetal life, colon epithelium shows wide and flat villi. These villi are separated by a proliferating inter-villous epithelium. As the gut develops the inter-villous epithelium is reshaped downward forming crypts. The crypt villous unit allows for a great increase in surface area for absorption. The embryonic villi will be lost in adult colonic epithelium. Human colon has a relatively flat epithelium separated regularly by crypts. The formation of these crypt-villous structures and epithelial cellular differentiation relies on reciprocal signaling between the endoderm and mesoderm (EM) (Pascal et al., 2003).

The principal function of the adult colon epithelium is to absorb water and salt. Transient formation of colonic villi is present in embryonic proximal intestine, but in human these villi are flattened by birth. The mature colon epithelium has mainly two differentiated cell types: the enterocyte and goblet cell. The colon also has endocrine cells. The goblet cells are mainly found in the midcrypt whereas the absorptive enterocytes (or colonocytes) are found at the surface (or top of the crypt); the surface between the crypts is called the “intercrypt table” and consists mainly of enterocytes. Endocrine cells are found in highest numbers at the base of the crypt (Pascal et al., 2003, Nagasaki et al., 2008).

2.2 Anatomy

The large intestine is 1.5-1.8m long, and extends from the ileum to the anus. Its size decreases gradually from the caecum, where it is approximately 7 cm in diameter, to the sigmoid, where it is approximately 2.5 cm in diameter. The large intestine lies in the infracolic part of the abdominal cavity, framing the loops of the small intestine (Anthony , 2005).

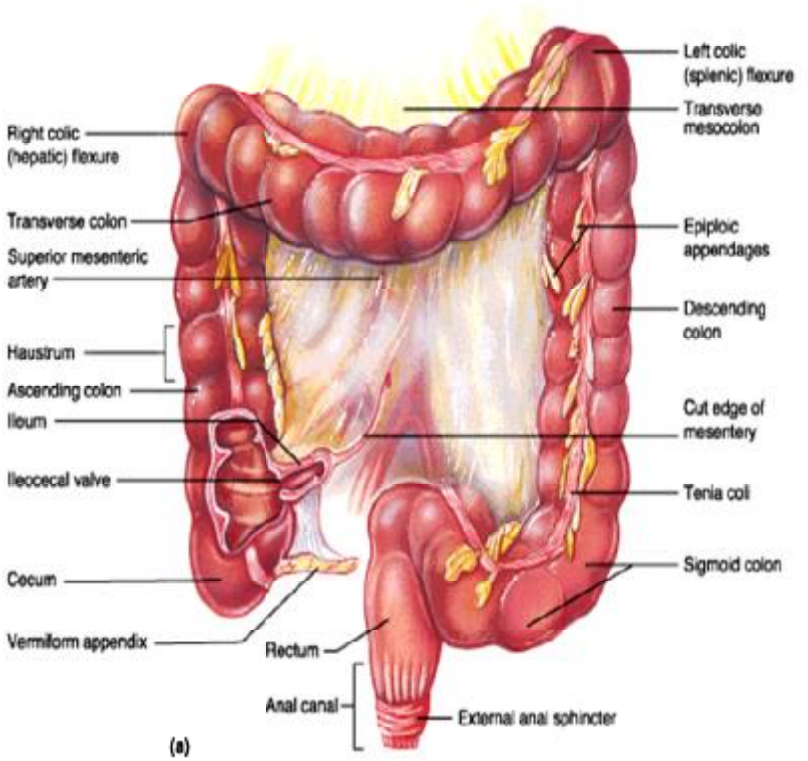


Figure. 2.1 Anatomy of the large intestine.

(Benjamin , 2001)

The colon extends superiorly from the cecum, vermiform appendix and consists of the ascending, transverse, descending and sigmoid colon. It ascending and descending

segments are retroperitoneal and its transverse and sigmoid segments are intraperitoneal.

The cecum and the colon are characterized by the teniae coli which are thickened bands of the outer longitudinal layers of muscle about 1cm each wide and referred according to their site as mesocolic tenia, omental tenia and free tenia. This anatomic point has clinical significance. Carcinomas proximal to this point are colonic; whereas distal tumors are rectal and as such may benefit from adjuvant radiation therapy.

Ascending colon

It is approximately 15 cm long and joins the caecum at the ileocaecal junction. The ascending colon is covered with peritoneum anteriorly and on both sides however, its posterior surface is devoid of peritoneum. It ascends on the right side of the abdomen to the level of the liver where it bends acutely to the left. At this point it forms the right colic or hepatic flexure and then continues as the transverse colon (Thibodeau et al., 2002).

The transverse colon

It is approximately 45 cm long that continues from the left hepatic flexure across to the left side of the abdomen to the left colic flexure. It passes in front of the stomach and duodenum and then curves beneath the lower part of the spleen on the left side as the left colic or splenic flexure and then passes acutely downward as the descending colon (Watson , 2000).

The descending colon

It is 25-30 cm along the left gutter of the peritoneal cavity with anatomical relation with kidney, ureter, iliac vessels and pelvic rim, this segment is narrower than the ascending colon.

The right colic flexure is just inferior to the right lobe of the liver, the left colic flexure occurs at the junction of the transverse and descending colon just inferior to the spleen. It higher and more posterior than the right flexure, and its attached to the diaphragm by the phrenicocolic ligament. Immediately lateral to the ascending and descending colons are the right and left paracolic gutter.

The sigmoid colon

The sigmoid colon begins above the pelvic inlet and extend to the level of vertebra SIII, where it is continuous with the rectum, its average length is 35-40 cm. It is thick and mobile and it is suspended by the sigmoid mesocolon to the lateral pelvic wall forming the inter-sigmoid recess (fossa) (William et al., 2010, Richard et al., 2012).

The rectum

Rectum is the terminal portion of the large intestine beginning at the confluence of the three tenia coli of the sigmoid colon and ending at the anal canal. Generally the rectum is 15 cm in length, is intra-peritoneal at its proximal and anterior end, and is extra-peritoneal at its distal and posterior end. The epithelial lining or mucosa of the rectum is of a simple columnar mucous secreting variety. The rectum lies in the sacro-coccygeal hollow and changes to the anal canal at the puborectal sling formed by the innermost fibers of the levator ani muscle. The rectum has a dilated middle part called the ampulla. The rectum is related anteriorly to the urinary bladder, prostate, seminal vesicles, and urethra in males and to the uterus, cervix, and vagina in females. Anterior to the rectum are the rectovesical pouch in males and the rectouterine pouch in females (Gray et al, 2000).

The colonic wall produce muscular contraction and create transverse constricting furrows which between them the colon wall bulges outward, forming sacculation known as haustraof colon with sub serosal fatty tags called omental appendices The peritoneal relation the cecum is covered completely by the peritoneum and called free colon, some part of it fixed to the posterior abdominal wall.

Vascular blood supply:

Branches from the superior mesenteric artery (SMA) supply the cecum ascending colon, and proximal two third of the transverse colon which are the ileo-colic, right colic and middle colic arteries respectively, There are several anatomic variations in the colic arteries including absent middle colic artery or absent right colic artery.

Branches from the inferior mesenteric artery supply the distal one third of the transverse, descending, and sigmoid colon which are left colic, sigmoid branches arteries. The terminal branches of these arteries entering the wall are called vasa recta.

Venous drainage of the left side of the colon through the inferior mesenteric vein to the splenic vein, and the right side of the colon through superior mesenteric vein which join the splenic vein to form the portal vein.

Lymphatic drainage of the colon starts as a network of vessels within the muscularis mucosa that drain into the extramural system through lymph channels generally follow the arterial blood vessels superior and inferior mesenteric lymph nodes. The ability of malignancies to metastasize begins once the tumor has invaded through the muscularis mucosa (Broce et al., 2008).

Nerve supply:

It is formed by important neural pathways that include parasympathetic, sympathetic, and somatic innervation to the colon, rectum, and anus. The intrinsic nervous system, also known as the enteric nervous system, is composed of the submucosal (Meissner) and myenteric (Auerbach) plexuses, which largely regulate segment-to-segment movement of the GI tract (Christensen et al.,2009).

Sympathetic stimulation that orginates from the lower six thoracic segments which give rise to fibers that join the para-vertebral ganglia and from these ganglia leave as the greater, lesser, and lumber splanchnic nerves which form the superior mesenteric

plexus, inferior mesenteric plexus, and the hypo-gastric plexus L1,L2,L3 these innervations inhibit peristalsis.

Parasympathetic innervations is from vagus nerve and sacral outflow S2-S4, the nerves emerge from the sacral outflow join the hypo-gastric plexus and innervate the colon, the fibers synapse with the ganglia of the myentric plexus of Auerbach and Meissner' plexus. Distension activates pain fibers in the splanchnic nerves (Jeffrey et al., 2008).

Histology of colon:

The colon has the same four layers that are present in most parts of gastrointestinal tracts the (a) mucosa, (b) submucosa, (c) muscularis propria, and (d) serosa.

The mucosa includes a columnar epithelium with a large number of mucus-secreting goblet cells, lamina propria, and muscularis mucosa. The submucosa contains the blood vessels and Meissner nerve plexus. The muscularis propria contains the inner circular and outer longitudinal muscles and myenteric (Auerbach) nerve plexus. Teniae coli are formed by outer longitudinal muscles. The serosa of the colon is visceral peritoneum (Gray et al., 2000).

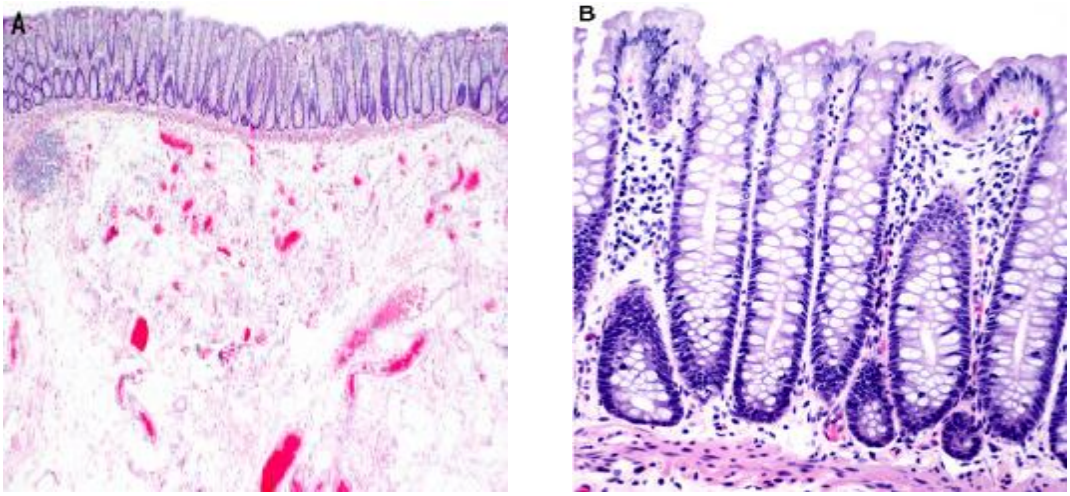


Figure.2.2 A. Normal histology of colon. B.The crypts are closely packed together and consist of columnar and many goblet cells.

(Walter et al ,2006)

Mucosa: The mucosa of colon is composed of simple columnar epithelium that contains the absorptive columnar cells and the mucus-filled goblet cells, which

increase in number towards the terminal end of the colon. The colonic glands are deep and straight and extend through the lamina propria to the muscularis mucosa. There are no villi in the colonic mucosa. The crypt of Lieberkuhn are the distinguishing histologic feature of the colonic mucosa (Victor ,2005, Fritsch et al.,2007), The luminal surface is covered by glycocalyx (glycans, enzymes, lectins, and mucin), facilitating formation of the commensal microbial ecosystem and serving as an integral barrier function. Beneath the glycocalyx is polarized columnar epithelium lining millions of regularly spaced crypts that span the depth of the lamina propria. The crypts are aligned perpendicular to and extend to the muscularis mucosa (Adegboyega et al., 2002).

It is characterized by the presence of crypts of Lieberkühn associated predominantly with goblet cells intermixed with a few absorptive and entero-endocrine cells. (glucagon-like immunoreactant) GLI /pancreatic polypeptide–like peptide (PYY) with N-terminal tyrosine amide–producing L cells predominate in the large intestine, Enterochromaffin, enterochromaffin-like, and pancreatic polypeptide– producing cells also are found. The goblet cells to the enterocytes ratio increase from cecum to rectum.

Paneth cells are scarce and normally are noted only in the proximal colon. The lamina propria of the large intestine contains solitary lymphoid follicles extending into the submucosa, these follicles are more developed in the rectum and decrease in number with age. Confluent lymphoid tissue is present in the appendix. Macrophages predominate in the subepithelial portion of the lamina propria, these cells are weakly PAS positive and are associated with stainable lipids; scattered neutrophils may be normal component but not found in the colonic surface or crypt epithelium (Piper et al., 2012).

The submucosa: Contains large blood vessels and the submucosal nerve plexus, Glands are not present, blood vessels pierce the muscularis externa and course around the circumference of the intestinal wall (Krause , 2005).

The muscularis externa: Two smooth muscle layers make up the muscularis externa which are modified, the inner circular muscle layer is continuous in the colon wall whereas the outer layer is condensed into three broad, longitudinal bands called taeniae coli, a very thin outer longitudinal muscle layer which is often discontinuous, is found between the taeniae coli. The parasympathetic ganglion cells of the myenteric nerve plexus are found between the two smooth muscle layers (Victor , 2005).

The serosa is incomplete in the colon, as the ascending and descending portions of the colon is attached to adjacent structures by an adventitia and contain large pendulous lobules of fat called appendices epiploicae (Krause , 2005).

2.3 Epidemiology:

2.3.1 The Incidence and mortality:

Colorectal cancer is the third most common cancer in the world. An estimated 1.24 million people worldwide were diagnosed with colorectal cancer in 2008 (Ferlay et al., 2009).

Colorectal cancer incidence worldwide is noticeably higher in men than in women (1.4: 1.0). In both sexes there are ten-fold differences in incidence between the different regions of the world.

Colorectal cancer ranks among the three most common cancers in terms of both cancer incidence and cancer-related deaths in most western industrialized countries. Thus, every year nearly one million people worldwide develop colorectal cancer. Lifetime risk of colorectal cancer may reach 6% of the population in the Western industrialized countries (Jemal et al., 2006).

The highest colorectal cancer incidence rates in 1998-2002 were observed in registries from North America, Oceania, and Europe, including Eastern European countries. These high rates are most likely the result of increases in risk factors associated with "Westernization" such as obesity and physical inactivity. In contrast, the lowest colorectal cancer incidence rates were observed from registries in Asia, Africa, and South America. Colorectal cancer mortality rates have declined in many longstanding as well as newly economically developed countries; however, they continue to increase in some low-resource countries of South America and Eastern Europe (Center et al, 2009).

From 2005-2009, the median age at diagnosis for cancer of the colon and rectum was 69 years of age. Approximately 0.1% was diagnosed under age 20; 1.1% between 20

and 34; 4.0% between 35 and 44; 13.4% between 45 and 54; 20.4% between 55 and 64; 24.0% between 65 and 74; 25.0% between 75 and 84; and 12.0% 85+ years of age.

The age-adjusted incidence rate was 46.3 per 100,000 men and women per year. These rates are based on cases diagnosed in 2005-2009 from 18 SEER geographic areas (Howlader et al., 2011).

Colorectal cancer remains the third leading cause of cancer deaths in the United States. The incidence, mortality, and screening vary by race/ethnicity, with African Americans/Blacks and Hispanics being disproportionately represented. Early detection through screening prolongs survival and decreases mortality (William , 2012).

Without preventive actions, about 6% of Americans will develop colorectal cancer sometime in their lives. Recent research, however, has contributed to a growing consensus that early detection methods can prevent a substantial proportion of the suffering and mortality from colorectal cancer (TIM et al., 1997).

CRC is the third most common cancer in the UK (2009), accounting for 13% of all new cases. It is the third most common cancer among men , accounting for 14% of all new cases of cancer in males and it is the second most common cancer in women in the UK (2009), accounting for 12% of all new cases. In 2009, there were 41,142 new cases of bowel cancer in the UK giving a male: female ratio of 12:10. Almost two-thirds (64% in 2009) of all bowel cancers are cancers of the colon and over one-third (36%) are cancers of the rectum (including the anus). Most rectal cancer cases occur in men (60%), while colon cancer cases are approximately evenly divided between men and women (53% male) (UK National statistics, 2011).

Cancer registration in Northern Africa is still limited. In Libya, colon cancer prevalence accounting for about 10% of the total cancer cases and was ranked the 2nd after lung cancer (19%) in male, and also the 2nd after breast cancer (26%) in female. In a study on the incidence of cancer in eastern part of Libya conducted in a total of 997 cases diagnosed in 2003 registered in the oncology and pathology department/ Benghazi

Universith, it is relatively frequent in Benghazi. This is in contrast with the Globocan 2002 estimates. Moreover, the incidence of colorectal cancer in Benghazi is closer to that reported in other North African cancer registries. The higher incidence rates are probably due to dietary factors, variations in economic status and the diffusion of endoscopic procedures, especially in urban areas of eastern Libya. Colorectal cancer was most frequent between 60-75 yrs in male and 45-60 yrs in female, and 20% of patients had less than 45 yrs (Mufid et al., 2007).

A study has done in the western of Libya in 2006 on cases registered by Cancer Registry Department which set up at the African Oncology Institute (AOI) in Sabratha in 2006; show cancer colon is the 4th commonest cancer in our population (10%), after breast (23%) ,lung(15%), prostate(17%), while cancer rectum is 6^{th} commonest. CRC are seen more frequently in younger population with peak incidence for cancer colon in forties and cancer rectum in fifties. This age distribution in younger age may be an environmental, dietary, or genetic effect. This however may have an effect in survival data as young age cancers might be more aggressive.

The age-adjusted incidence rate for colorectal cancers in western Libya is closer to global incidence with 20.1 per 100,000 for males and 14.6 per 100,000 for females which is higher than reported from other countries of southern Mediterranean region (Libya Cancer Registry, 2006).

International incidence variation:

Colorectal cancer (CRC) is common in the Western world and usually ranks high in incidence and mortality among malignancies in those countries. Two observations have led researchers to look for diet and lifestyle as explanatory factors of risk for CRC. First ecological studies comparing large populations have shown that rates of CRC differ dramatically among countries, varying by as much as 10-fold, from low-incidence areas in Asia and Africa, to much higher rates in northern Europe and the United States. Second studies have shown that migrants from low-risk areas to high-risk Western countries experience rapid increases in CRC risk within the same generation (Monroe et al., 2003).

This great variation which takes place in the frequency of this disease over geographic areas of all sizes. Colorectal cancer is common in most countries of North America and Europe, is rare in Asia and is particularly uncommon in Africa. Internationally, the variation in colon cancer is 60-fold, and within Europe there is a 4-fold difference in the incidence of colon cancer between areas with the highest and lowest rates. For cancer of the rectum, variation internationally is 18-fold and within Europe it is 3-fold. Within the United Kingdom, colon cancer is uniformly higher in the Scottish Cancer Registry Regions than in their counterparts in England and Wales, with the North and South clearly demarcated by a striking difference in colon cancer incidence in both sexes, examination of international mortality rates for colorectal cancer demonstrates remarkable differences in trends over time between countries. In countries where colorectal cancer mortality rates were initially low, rates have increased substantially. In many countries where rates circa 1950 were moderately high, they have increased slightly or become stabilized. However, in countries such as Scotland, Canada, England and Wales and the United States, where rates were initially high, there have been gradual falls in mortality over time (Boyle et al., 2006).

Globally, the age-standardized incidence rate (ASR) of CRC is 20.1 per 100,000 males and 14.6 per 100,000 females. As mentioned earlier, there are notable differences between CRC incidence rates in more developed versus less developed countries. In the developed parts of the world, the ASR is 40.0 in males and 26.6 in females in less developed areas, the rates are 10.2 and 7.7, respectively.

The highest ASRs in males are observed in Australia/New Zealand (48.2) followed by North America (44.4) and Western Europe.

On the other end of the scale, the rates in South-Central Asia (42.9) and Central Africa (2.3) are lowest .Incidence-to-mortality ratios also differ substantially between developed and less developed countries.

The rate ratio varies from in North America (indicating 2.9 incident cases for every death 2.9 from CRC) to 1.0 in Central and North Africa (indicating that for every new case of CRC, there is a death from this cancer) (Autier et al.,2003).

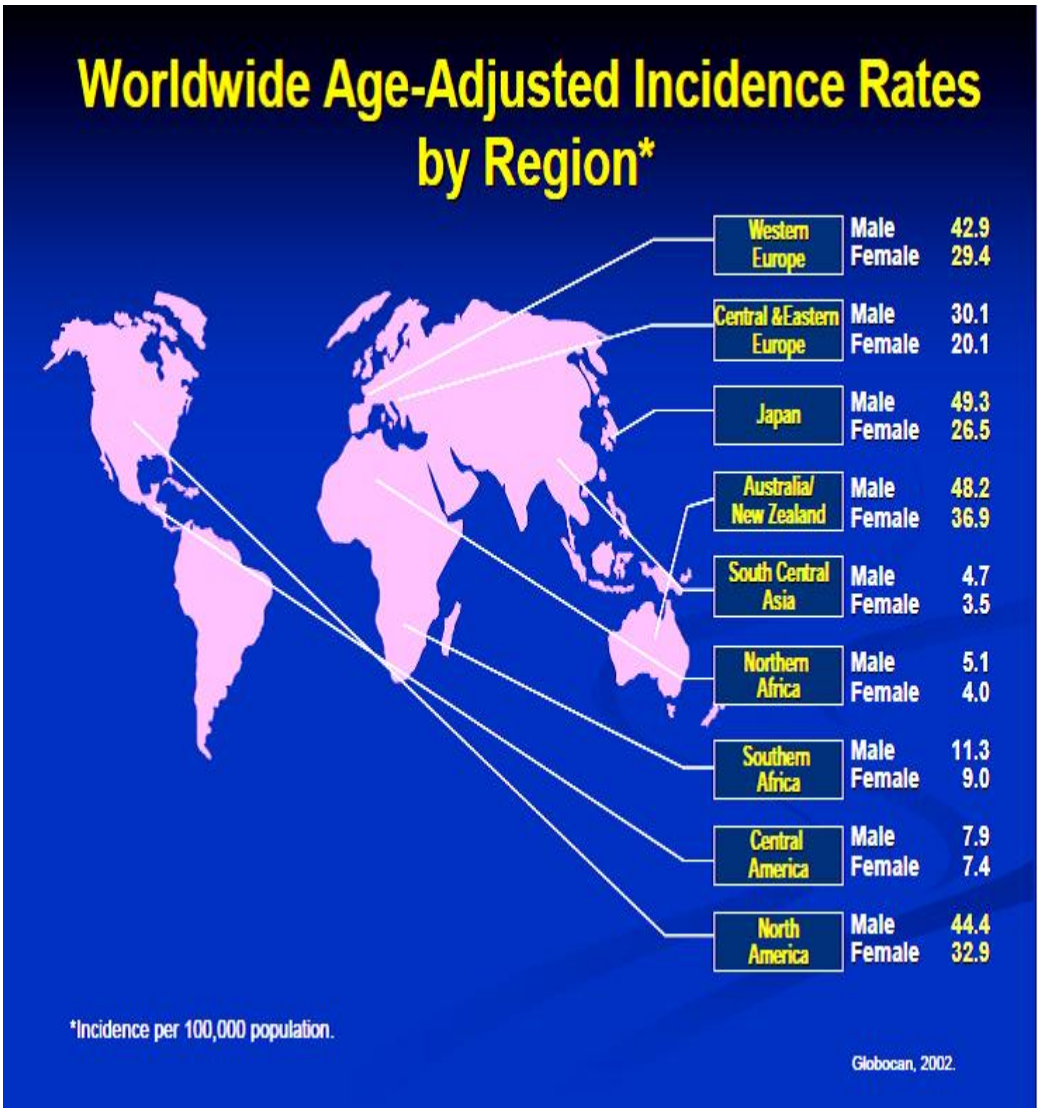


Figure 2.3. International incidence variation of colorectal cancer(Ferly et al,2009).

MORTILITY:

Bowel cancer is the fourth most common cause of cancer death worldwide, estimated to be responsible for almost 610,000 deaths in 2008 (around 8% of the total). Last estimated new cases and deaths from CRC in 2012 were as, new cases: 143,460, deaths: 51,690 (ACS, 2012).

Bowel cancer mortality rates are lowest in Middle Africa and South-Central Asia and highest in Central and Eastern Europe, with a six-fold variation in male mortality rates between the regions of the world, and a five-fold variation in female rates (Ferlay et al .,2010).

In the United States for example, the five-year survival rate for tumors in the ascending colon is about 63%. In the transverse colon, the survival rate is about 59%, and in the descending colon it's about 66% (Donna, 2008).

Colon cancer survival rates also vary by country. While the overall five-year survival for colon cancer in America is 62% it is 43% in Europe. Quality of care may be one reason, but another could be colon cancer screening programs. In general, the earlier colon cancer is detected, the easier it is to treat.

2.3.2 Risk factors

Environmental risk factors

1. Diet. The etiology of colorectal cancer (CRC) involves the interaction of cell molecular changes and environmental factors, with a great emphasis on diet components. Several risk factors are commonly found in western diets, such as high concentrations of fat and animal protein, as well as low amounts of fiber, fruits and vegetable. Many studies found a counteractive effect of fibers on neoplasia induction, especially in relation to fermentable fibers (wheat bran and cellulose), high consumption of fruits was associated with a 32% reduction in the risk of CRC, while high intake of cereal fiber did not lower risk of CRC (Milly , 2007).

Red meat, processed meats, and perhaps refined carbohydrates are also implicated in CRC risk. Current recommendations for decreasing the risk of CRC include dietary measures such as increased plant food intake. The consumption of whole grains, vegetables and fruits and reduced red meat intake (Campos et al, 2005).

Asian populations have changed from traditional to westernized diets, with increased red meat intake. They are suggested to be particularly susceptible for the adverse effects of red meat on the development of colorectal cancers. Red meat intake may modestly increase the risk of colon cancer in middle-aged Japanese, although the highest quantity of red meat consumption could be considered moderate by western standards (Takachi et al, 2011).

Immigrants from low- to high-incidence areas provided important evidence that lifestyle factors and diet changes may influence the development of this malignancy (Martinez , 2005).

McKeown- Eyssen and Giovannucci noted the similarity of the risk factors for colorectal cancer and those for insulin resistance and suggested that insulin resistance leads to colorectal cancer through the growth-promoting effect of elevated levels of

insulin, glucose, or triglycerides which lead to increased growth of colon cancer precursor lesions and the development of colorectal cancer (Bruce et al., 2000).

Meta-analysis studies have suggested that magnesium intake may be associated with a decreased risk of colorectal cancer (Che et al., 2012). Every 100-mg/d increase in magnesium intake was associated with 13% lower risk of colorectal adenomas and 12% lower risk of colorectal cancer (Wark et al., 2012).

Recent epidemiological and experimental studies support the association of vitamin D deficiency with the high risk of colorectal cancer. In which the calcium normally affect on induced differentiation, controls the detoxification metabolism and cell phenotype, sensitizes cells to apoptosis and inhibits the proliferation of cultured human colon carcinoma cells (Pereira et al., 2012).

2.Lifestyle. The risks for colon cancer are far higher in industrialized nations than less developed countries. A western lifestyle, being sedentary, smoking, and having excess weight have all been associated with increased risk for CRC cancer. (However, about 75% of cases occur without a known predisposing factor).

3.Alcohol consumption. Alcohol intake is associated with a significantly increased risk of colorectal cancer but the risk seems to be reduced when wine is included in the alcohol intake. A study has been done in the association between total alcohol intake and colorectal cancer during a mean follow up of 14.7 years; show Drinkers of more than 14 drinks of beer and spirits a week, but not wine, had a risk of 3.5 (1.8-6.9) of rectal cancer compared with non-drinkers, while those who drank the same amount of alcohol but including more than 30% of wine had a risk of 1.8 (1.0-3.2) of rectal cancer (Pedersen et al., 2003), other study in Japan prove the same result (Mizoue et al ,2006).

4.Cigarette smoking. Considerable evidence suggests that cigarette smoking is associated with a higher risk of CRC cancer. A study proved exposure to tobacco

products early in life is associated with a higher risk of developing colorectal neoplasia (Martinez, 2005).

Meta-analysis research of 36 studies to find the association between smoking and colon and rectal cancer in terms of incidence and mortality and include daily cigarette consumption, duration, pack-years and age of initiation. Relative to nonsmokers, current and former smokers had a significantly increased risk of CRC incidence and mortality (Liang et al., 2009).

CRC risk remained increased for about 25 years after quitting smoking, and the pattern of decline in risk varied by cancer subsite (Gong et al., 2012).

5.Medical condition: Adenomatous Polyps. People who have had adenomatous polyps (adenomas) have an increased risk of developing colorectal cancer. When these polyps are detected during colorectal screening, as colonoscopy, they can be removed before they turn cancerous.

Inflammatory Bowel Disease. Inflammatory bowel diseases include Crohn's disease and ulcerative colitis,.The long-term inflammation caused by these chronic disorders can increase the risk for CRC; particularly with all ulcerative life as Inflammatory bowel disease (IBD) is not the same as irritable bowel syndrome (IBS) that does not increase CRC risk.

Diabetes; Many studies have identified an association between type 2 diabetes mellitus and colon cancer. Both diseases share common risk factors of obesity and physical inactivity, but diabetes itself is a risk factor for CRC, DM patient or a family history of DM are associated with increased risk of CRC (Limburg et al., 2006, Takahari et al., 2009).

Previous research found that stages II and III CRC patients with DM had poorer (Disease free survival) DFR than those without. The resultant hyper-insulinemia up regulates insulin-like growth factor-1 (IGF-1) binding to its receptor to suppress

apoptosis, promote cell proliferation, and may induce expression of VEGF (Kaulfuss et al., 2009). This may also promote metastasis and contribute to poor prognosis.

Homocystinuria; A study was done to assess the relation of occurrence of colon adenoma and adenocarcinoma in women proved that according to subgroup analysis by gender, plasma homocysteine concentration was not associated with adenoma in males; however, a high plasma homocysteine concentration has significantly increased the risk of adenoma as well as advanced adenoma in females. Hyperhomocysteinemia is a risk factor for colorectal adenoma in women (Lim et al., 2012).

Cholecystectomy. Abnormal bile acid metabolism may predispose both to CRC and cholelithiasis. After cholecystectomy, increased quantities of secondary bile acids have been detected in the feces and may have a role in colonic carcinogenesis (Davi et al., 2011).

Genetic risk factors:

1. Hereditary. The two most common inherited colorectal cancer syndromes are hereditary nonpolyposis colorectal cancer (HNPCC) and familial adenomatous polyposis (FAP). They can affect either sex, and the children of people who carry these genes have a 50% chance of inheriting the disease-causing gene.

Hereditary non-polyposis colorectal cancer (HNPCC), an autosomal-dominant syndrome, accounts for 2-5% of all colorectal carcinomas, and it is the most common form of inherited colon cancer (Ladabaum et al., 2011).

Colorectal cancer in patients with hereditary nonpolyposis colorectal cancer (HNPCC) presents at an earlier age than in the general population, it is affected at least two generation in the same family, and is characterized by an increased risk of other cancers, such as endometrial cancer and, to a lesser extent, cancers of the ovary,

stomach, small intestine, hepatobiliary tract, pancreas, upper urinary tract, prostrate, brain, and skin (Zhang , 2008).

Two other, milder hereditary colorectal syndromes, known as attenuated familial adenomatous polyposis (AFAP) and MYH-associated polyposis, less is known about these two recently discovered syndromes.

2.Race. Black-Americans have the highest risk of being diagnosed with and dying from colorectal cancer. Among Caucasians, Jews of Eastern European (Ashkenazi) descent have a higher rate of colorectal cancer. Asian Americans/Pacific Islanders, Hispanics/Latinos, and American Indians/Alaska Natives have a lower risk than Caucasians.

2.Age. The risk of developing colorectal cancer increases as one ages, the disease is more common in people over the age of 50, and the chance of developing colorectal cancer increases with each decade. However, CRC has also been known to develop in younger people as well (Patel, 2009, Gairdiello, 2008). Affected individuals develop carcinomas mostly at relatively advanced age (mean age of 70 years). Approximately 10-35% of all cases show familiar clustering of CRC, and only a proportion can be explained by known highly penetrate syndromes such as Lynch syndrome, familial adenomatous polyposis (FAP), Peutz-Jeghers syndrome (PJS), Juvenile polyposis syndrome (JPS), Cowden disease (CD) and MUTYH-associated polyposis (MAP). The majority of these syndromes are caused by autosomal dominant genetically inherited risk factors. Thus far, only one syndrome (MAP) shows an autosomal recessive mode of inheritance (Early et al., 2008).

3.Genetic mutation. Genetic mutations have been identified as the cause of inherited cancer risk in some colon cancer–prone families; these mutations are estimated to account for only 5% to 6% of all CRC cases overall. It is likely that other undiscovered genes and background genetic factors contribute to the development of familial CRC in conjunction with non-genetic risk factors. All gene mutations known to cause a predisposition to CRC are inherited in an autosomal dominant fashion. At

least one example of autosomal recessive inheritance, *MYH*-associated polyposis (MAP), has been identified (Burt et al., 1996).

The diagnosis of hereditary non-polyposis colorectal cancer (HNPCC) at the molecular level relies on the presence of a deleterious germline mutation in one of the mismatch repair (MMR) genes (Hampel et al., 2005).

Hereditary CRC has two well-described forms: FAP (including an attenuated form of polyposis (AFAP)), due to germline mutations in the *APC* gene, and Lynch syndrome (LS) (also called hereditary nonpolyposis colorectal cancer (HNPCC)), which is caused by germline mutations in DNA MMR genes. Many other families exhibit aggregation of CRC and/or adenomas, but with no apparent association with an identifiable hereditary syndrome, and are known collectively as familial CRC (Glanz et al., 1999).

4.Family history. About 20 - 25% of CRC occur among people with a family history of the disease. 75% of cases are due to other causes People who have more than one first-degree relative (sibling or parent) with the disease are especially at high risk. The risk is even higher if the relative was diagnosed with colorectal cancer before the age of 60. About 5 - 10% of patients with colorectal cancer have an inherited genetic abnormality that causes the disease. Syndromes associated with genetic mutations include familial adenomatous polyposis and hereditary non-polyposis colorectal cancer.

5.Gender. Sex significantly influences the clinical and pathological characteristics of CRC. These include differences in incidence and mortality rates, clinical presentations including age, emergency surgery for complications from CRC, screening participation rates, site, stage and treatment utilization, histopathology and survival (Jenn et al., 2010).

Research has shown that in general, men are more likely to have colon polyps and colon tumors than women. The older we get, the bigger the gender gap gets. A study published in the American Journal of Gastroenterology found that men 69 and older

were much more likely to have colorectal polyps and tumors than women in the same age group. Gender is more susceptible to colorectal cancer also depends on the location of the tumor. It was found that men tend to get rectal cancer and left-sided colon cancers more often than women and women tend to get right-sided colon cancer more often than men (DeCosse et al., 2006).

Large-scale population-based studies such as the Women's Health Initiative have shown a significant reduction in both the risk and rate of developing CRC in post-menopausal women treated with combined hormone replacement therapy (HRT), and both pregnancy and the oral contraceptive pill are associated with a reduced CRC risk. Taken together, these data suggest that estrogens and/or progestins have a protective effect against colorectal carcinogenesis, although the molecular mechanisms behind these observations are not yet fully understood. The effects of estrogens are mediated by estrogen receptors (ERs), of which two (ERα and ERβ) exist, with ERβ being the predominant ER expressed in CRC (Campbell-Thompson et al., 2001, La Vecchia et al., 2009).

A study analysis of age- and sex-specific incidence and mortality of CRC in the US and 10 other large countries from different parts of the world indicate that the lower incidence and mortality among women quite consistently translates to an age difference of approximately 4–8 years at which comparable levels of risk are reached (Brenner et al., 2007).

The proportion of cancer in the distal colon and rectum is considerably lower among women than among men; therefore, the sex difference in distal CRC occurrence is even larger than the sex difference in overall CRC occurrence (McCashland et al., 2001).

2.4 Molecular Basis of colorectal carcinogenesis

Accumulated genetic and epigenetic changes underlie the development of neoplasia of the colon. This multistep process leads to the transformation of normal colonic epithelium to colon adenocarcinoma. During this process, somatic mutations accumulate and determine the final phenotypic characteristics of the colorectal tumor (Diep et al., 2006). ,

Colorectal cancer (CRC) progression can occur through one of the chromosomal instability (CIN), microsatellite instability (MSI) pathway, or CpG island methylator phenotype (CIMP). Early adenomatous changes are secondary to loss of APC, KRAS loss initiates the formation of larger adenomas in the CIN pathway followed by 18qLOH, mutations in TP53 are a late change. Sporadic MSI tumors are commonly part of the serrated neoplasia pathway and BRAF mutations are more common finding.

Table. 2.1 The Molecular Classification of Colorectal cancer

	CIN	MSI	CIMP
Feature	Aneusomy +LOH	Replication error	Gene silencin
Genes	BUB1, AURKA,APC,p53	MSH2,MLH1,MSH6	BRAF,p53,p21
Status	Present /absent	L/H	0/L/H
Pathology	_____	HNPCC,mucinous,signet-ring cell,crohn,TILs necrosis, high tumor grade	Serrated histological features

(Diep et al., 2006)

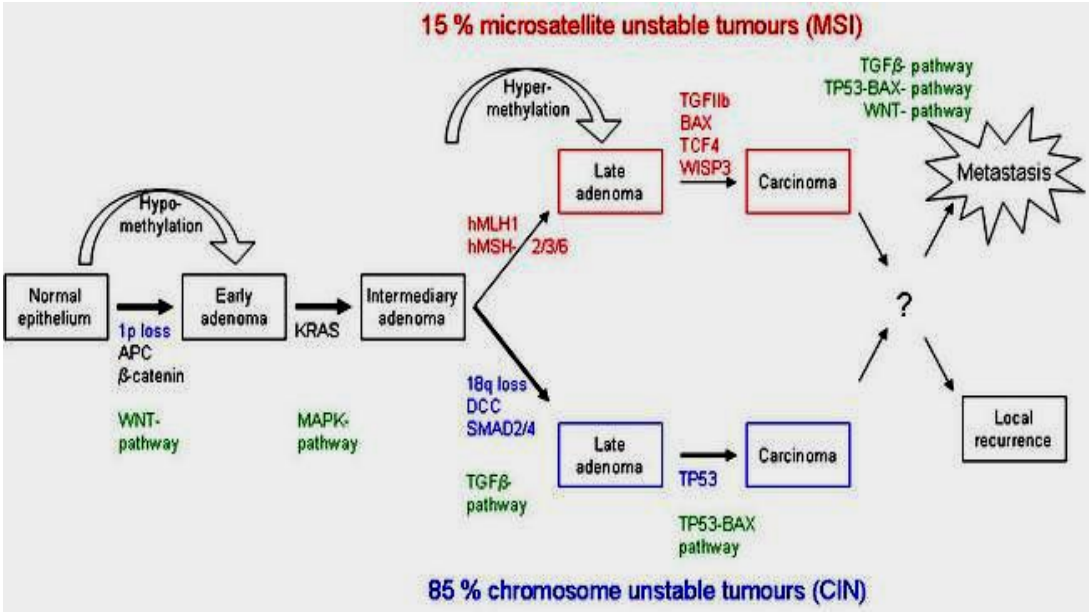


Figure. 2.4. Molecular carcinogenesis of CRC. (Myutan, et al, 2011)

Chromosomal Instability

Chromosomal instability (CIN) is a predominant pathway characterized by chromosomal copy number variation including chromosomal gains, physical losses, and copy neutral loss of heterozygosity (cnLOH). These tumors show aneuploidy, which is the equivalent of a gross amount of CIN. In general, carcinomas with CIN present with losses of chromosomes 17p and 18q, and gains at 8q, 13q, and 20 that occur at early stages during the transition from adenoma to carcinoma (Diep et al., 2006), CIN tumors are characterized by aneuploidy, multiple chromosomal rearrangements and an accumulation of somatic mutations. CIN tumors have a poor prognosis compared to MSI tumors (Myutan et al., 2011).

Epigenetic alterations are thought to be precursor events in tumor progression through the serrated, alternate Vogelstein model. It is believed that CRC may arise from at least three interlinked mechanisms. CIN is the most commonly found in CRC accounting for up to 80% of cases (Nakao et al., 2004).

The classic Vogelstein report, which describes the step by step mutational process starting from a small adenoma to invasive cancer, is the theoretical basis for our understanding of the CIN pathway, CRC progresses through activating mutations in oncogenes or deactivation of tumor suppressor genes. This leads to a selection of clonal tumor cells which continue to divide through a growth advantage inactivating mutations in APC and activating mutations in KRAS are thought to be early changes in the Vogelstein sequence. Mutations in p53 and TGF-β have been described as late changes in tumorgenesis (Kinzler et al., 1998).

Microsatellite Instability

The second pathway is MIN or MSI, which is characterized by tumor cells with small deletions and insertions in coding and non-coding stretches of short repetitive DNA sequences distributed throughout the genome. Accumulation of these mutations leads to frameshifts within coding sequences and the subsequent inactivation of genes, thereby contributing to tumor development and progression. These tumors are diploid or near-diploid (Marjo, 2008).

MSI has been found in 15% of CRC and is characterized by the inactivation of the Mismatch Repair Genes (MMR); which leads to a change in length of DNA microsatellites due to the insertion or deletion of repeating units (Grady, 2004).

This phenomenon is caused by defects in MMR genes such as MLH1, MSH2, or MSH6, or methylation of the MLH1 promoter MSI are the cause of hereditary CRC but are also found in sporadic cancers. In sporadic cases of MSI the MMR gene activity is silenced by promoter methylation of the hMLH1 gene. Several genes affected by MSI have been identified including TGF-β, those encoding regulation of cell proliferation, cell cycle or apoptosis and DNA repair. MSI represents a unique pathway for tumor development that does not involve loss of heterozygosity (Walther et al., 2008).

There is strong evidence that sporadic colorectal carcinomas with MSI are frequently poorly differentiated, right-sided, and associated with a prominent inflammatory infiltrate (Chao et al.,2000) , and more common in female patients (Samowitz et al., 2001).

A minority of colorectal carcinoma harbor DNA mismatch repair defects and manifest a phenotype in which there is a high frequency of instability at microsatellite sequence tracts. MSI is observed in essentially all colorectal cancers arising in patients with hereditary nonpolyposis CRC and in ~10 to 15% of apparently sporadic colorectal cancers. Defects in mismatch repair function are thought to increase the rate at which cells acquire the mutations critical in malignant transformation (Kinzler et al., 1996).

In one study, MSI was correlated with carcinomas of the medullary subtype (Ruschoff et al., 1997); MSI is in form part of the presentation of Lynch syndrome, or hereditary nonpolyposis CRC (Hendriks et al., 2006).

A study in stage II and III CRC showed that patients with high microsatellite instability (MSI-H) had improved survival, and that patients with MSI were more likely to exhibit better recurrence-free survival than those with microsatellite stable (MSS) phenotypes. Additional studies have analyzed the relationship between MSI and CRC prognosis and concluded that CRC patients exhibiting MSI had a significantly better prognosis compared to those with intact MMR but did not benefit from the administration of 5-flurouracil (5-fu) therapy in the adjuvant setting (Lim et al., 2004, Popat et al., 2005).

MSI can be detected in tumors by a number of complementary approaches. Using the polymerase chain reaction (PCR) to amplify specific microsatellite repeats, the presence of instability can be monitored through a comparison of the length of repeats obtained from normal DNA (typically extracted from adjacent normal mucosa cells) with those from the DNA extracted from the tumor cells. A reference panel of 5–10 microsatellite loci is used to diagnose MSI cases (Umar et al., 2004).

CpG island methylator phenotype (CIMP)

DNA methylation is recognized as one of the most common gene alterations in human tumors including CRC (Laird, 2005). A subset of CRC exhibit promoter methylation at multiple sites and are referred to as the CpG island methylator phenotype (CIMP). The CIMP is observed in 30% of CRC, this has been hypothesis as an early contributor to CRC progression (Yifan et al., 2012).

Both hyper and hypo-methylation of DNA play a role in CRC tumorogenesis (Matsuzaki et al., 2005). Before the entity of CIMP was identified, CRC was classified into either MSI or CIN in origin. It now apparent that some tumors are neither MSI nor CIN and that hypermethylation of DNA is a common finding on them. Sporadic MSI tumors are secondary to CIMP related silencing of the MMR gene MLH1 the difficulty producing a standardized marker and the unclear distinction between the CIMP tumors and sporadic MSI tumors has meant that the clinical importance of CIMP tumors is difficult to quantify. CIMP can be divided into CIMP-High (CIMP-H) and CIMP-Low (CIMP-L) groups. The CIMP-H tumors are associated with the BRAF mutation whereas CIMP-L are associated with KRAS mutations (Barault et al., 2008), Activation of oncogenes including KRAS, BRAF and PIK3CA affects intracellular signalling pathways and has been associated with CIMP and MSI (Shuji et al., 2011).

Several genetic alterations that contribute to initiation and progression of colorectal tumors have been identified, and they include mutation of specific oncogenes such as K-ras, and tumor suppressor genes, such as p53 and APC (Marra, 1995, Fearon , 2001).

Alterations commonly seen in typical colorectal carcinomas, including increased p53 and β-catenin immunoreactivity, K-ras gene mutations, microsatellite instability, and loss of heterozygosity of markers on chromosomes 5q, 17p, and 18q.

The loss of heterozygosity on the long arm of chromosome 5q, 17p, and/or 18 (18qLOH) is the most common genetic alteration in CRC. SMAD4 and deleted in Colorectal Cancer (DCC) are two important tumor suppressor genes found on the long

are of chromosome 18 (Fearon, 2001), and this deletion results in tumorgensis via the TGFβ pathway (Alberts et al., 2008). Studies have shown that 18qLOH is an indicator of poor prognosis in early stage CRC, which has not been proven by multi-variate studies against other biomarkers, making 18qLOH an unlikely independent prognostic marker. Furthermore, 18qLOH has associations with CIN (Alhopuro et al., 2005, Rowan et al.,2005). The retention of the SMAD4 diploidy results in a three-fold higher benefit from 5-Fl (5-FU) chemotherapy (Boulay et al., 2002).

Mutations in the tumor suppressor gene TP53 are found in almost half of CRC (Lacopetta, 2003). Mutations in different domains of the gene lead to a variable prognosis. TP53 mutations are found more commonly in distal CRC (Russo et al, 2002).

Proximal tumors found to have mutations in TP53 were more likely to exhibit lymphatic invasion and be more responsive to 5-FU therapy. Mutation in exon 5 of the TP53 gene is associated with a poorer outcome (Russo et al., 2005) .Individuals with wild type TP53 have a superior survival rate with 5-FU therapy in rectal cancer (Lacopetta , 2003), at present there is no strong data to support the role of TP53 as a prognostic or predictive marker in CRC.

CRC presentation can be one of three types which are sporadic, inherited, and familial (Fig. 2.5).

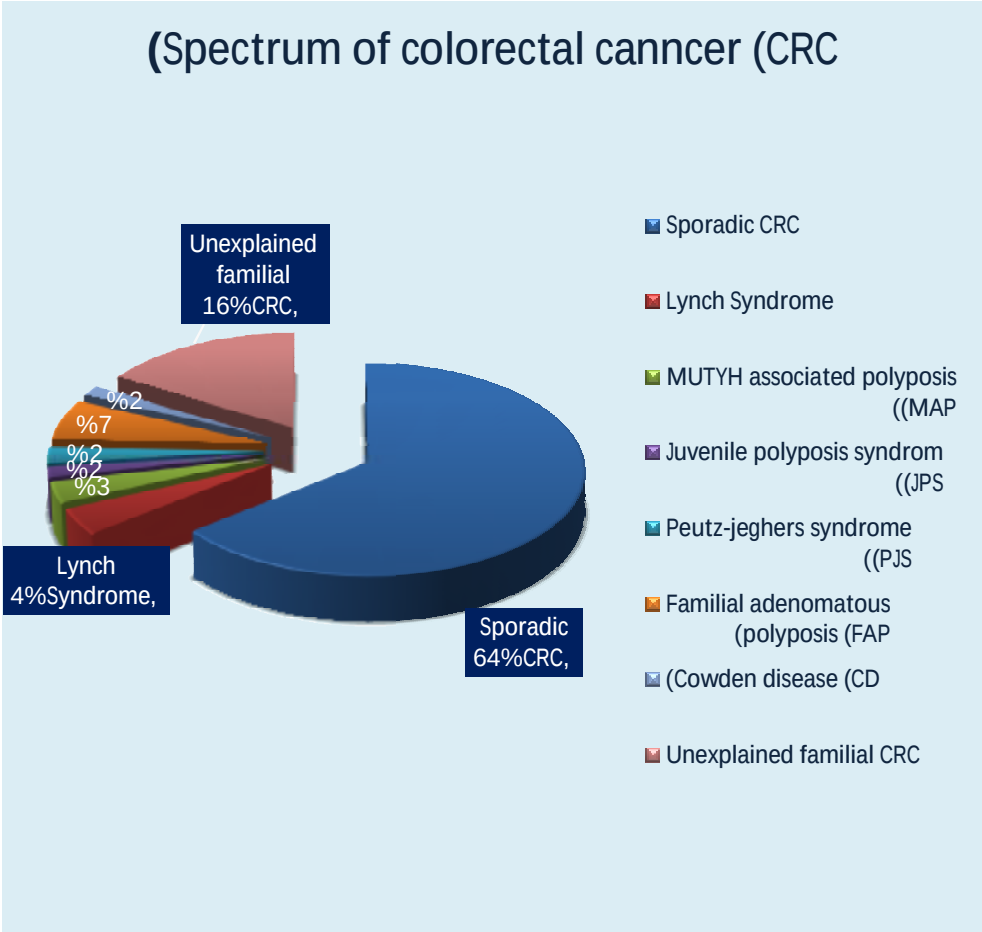


Figure 2.5. Spectrum of colorectal cancer (CRC)
Colorectal cancer can be divided into two main groups: sporadic CRC (65-90% of all patients) and familial CRC (10-35% of all patients). Up to 5% of CRC can be explained by these hereditary syndromes (Marjo, 2008).

Sporadic disease, in which there is no family history, accounts for approximately 65 percent of all CRCs. It is most common over the age of 50, and dietary and environmental factors have been etiologically implicated, Fewer than 10 % of patients have a true inherited predisposition to CRC, and these cases are subdivided according to whether or not colonic polyps are a major disease manifestation. The diseases with

polyposis include familial adenomatous polyposis (FAP), MUTYH associated polyposis (MAP), and the hamartomatous polyposis syndromes (eg, Peutz-Jeghers, juvenile polyposis), while those without polyposis are referred to as hereditary nonpolyposis CRC (HNPCC, Lynch syndrome, familial colorectal cancer type X (Wirtzfeld et al., 2001, Lindor 2009).

So the benefit from studying the different molecular carcinogesis of tumors and mutated genes can play a central role in early detection of predisposing patient to the cancer. Molecular tumor testing can be applied to direct germline gene testing as a cost effective approach in index patients of these families. Subsequently, these patients will be screened for the presence of a germline defect in the known high risk genes (MLH1, PMS2, MSH2, MSH6, or MUTYH), after identification of the underlying gene defects causing a high risk of CRC, pre-symptomatic testing can be offered to these families and screening options can be discussed in mutation carriers and individuals at risk who choose not to be tested. CRC families without identified mutations are due to either an undetected defect in known genes or the single high risk gene not yet having been identified as a target for mutations. Alternatively, the high risk for CRC could be the result of a combination of gene variations, with each contributing a low level of risk.

By using the Mutation Analysis of APC, K-Ras, B-Raf and CTNNB1 gene marker panel it could be shown that 65% of the serrated lesions and 61% of the adenomas carried at least one of the four genes in a mutated form. Based on its excellent performance in detecting mutations in sporadic pre-neoplastic and neoplastic lesion of the human colon and rectum.

Various reports have confirmed this initial finding and have associated hypermethylation of tumor suppressor genes and hypomethylation of oncogenes to tumorigenic processes (Esteller 2008, Gargiulo and Minucci, 2009). Hypomethylation phenomena may convey diverse effects upon living (epithelial) cells, including an increase in genome instability, over-expression of a variety of genes and loss of imprinting of particular genes such as IGF2, the latter of which has indeed been implicated in the pathogenesis of colorectal cancer (Cui et al., 2003). Next to global hypomethylation discrete hypermethylation targeting promoter regions of specific genes, has also frequently been observed in various cancer types, including colorectal cancer (Esteller, 2008). Many of the genes affected by hypermethylation are involved in cell cycle regulation, DNA repair, apoptosis, angiogenesis, invasion and adhesion. Promoter hypermethylation of the MLH1, APC, RB1, VHL, MGMT, GSTP1 and BRCA1 genes, represent paradigmatic cancer-related epigenetic silencing events (Esteller 2000, Feinberg and Tycko, 2004).

Interestingly, it was found that sporadic and inherited cancers may exhibit similar DNA methylation patterns (Esteller et al., 2001) and many genes that are mutated in familial cancers have also been found to be hypermethylated, mutated or deleted in sporadic cancers. The DNA mismatch repair gene MLH1, for example, can be inactivated by hypermethylation or mutation in both iinherited and non-inherited colorectal cancers (Esteller et al., 2001).

2.5 Clinico –pathological classification of colorectal tumor

2.5.1 Tumor types.

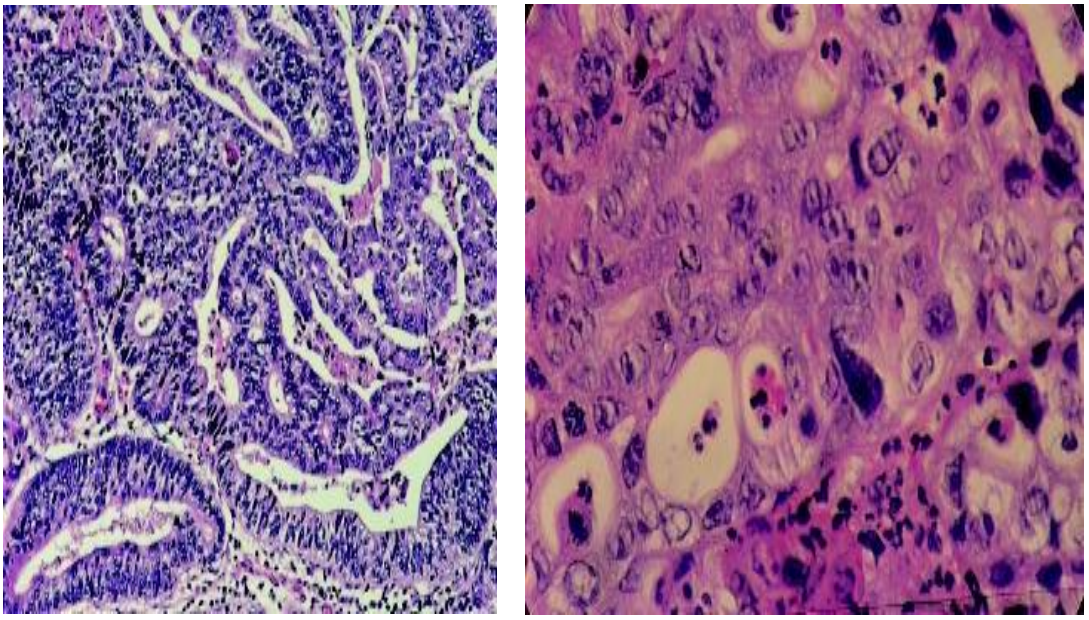
The World Health Organization (WHO) has identified histological typing of tumors of the colon and rectum as following: (Kang et al., 2007).

Table 2.2 The histological types of CRC.

Epithelial origin tumors	Non –epithelial orgin tuomrs
1.Adenoma: Tubular /tubule-villous/ Serrated.	Lipoma Leiomyoma. Leiomyosarcoma.
2.Intra-epithelial dysplasia: Low grade glandular IEN. High grade glandular IEN.	Gastro-intestinal stromal tumor. Angiosarcoma. Kaposi sarcoma. Melanoma.
3.Carcinoma: Adenocarcinoma. Mucinous adenocarcinoma. Signet-ring cell adenocarcinoma. Small cell adenocarcinoma. Squamous cell carcinoma. Adenosequamous. Medullary carcinoma. Carcinoid tumor. Enterochromaffin EC producing tumor. Mixed carcinoma. Undifferentiated tumor.	Malignant Lymphoma: - Marginal zone cell lymphoma. Mantle cell lymphoma. - DLCL. - Burkitt lymphoma. Others

Adenocarcinoma

The majority of malignant tumors in the colon and rectum are gland- forming adenocarcinoma (95%). Conventional adenocarcinoma is characterized by glandular formation, which is the basis for histological tumor grading. In well differentiated adenocarcinoma >95% of the tumor is gland forming. Moderately differentiated adenocarcinoma shows 50-95% gland formation. Poorly differentiated adenocarcinoma is mostly solid with <50% gland formation. In practice ,most colorectal adenocarcinomas (~70%) are diagnosed as moderately differentiated. Well and poorly differentiated carcinomas account for 10% and 20% respectively.



A B

Figure 2.6. A.Colonic adenocarcinoma, well differentiated and forming glandular structures, without intervening stroma (H&E, × 10; courtesy of Dr. Arnold . Szporn). B.poorly differentiated, with pleomorphic nuclei (H&E, × 40; courtesy of Dr. Arnold (Szporn)

Mucinous adenocarcinoma

Mucinous adenocarcinoma (MA) is diagnosed when more than 50% of the tumor comprises a mucinous pattern upon histological examination (Chew et al., 2010). MA makes up 6 to 20% of all colorectal cancers (Xie et al., 2009), and differs from non-mucinous adenocarcinoma (NMA) with regard to its clinicopathological characteristics, distinct genetic profiles, and pathogenic pathways. It carries a worse prognosis than the usual type of adenocarcinoma.

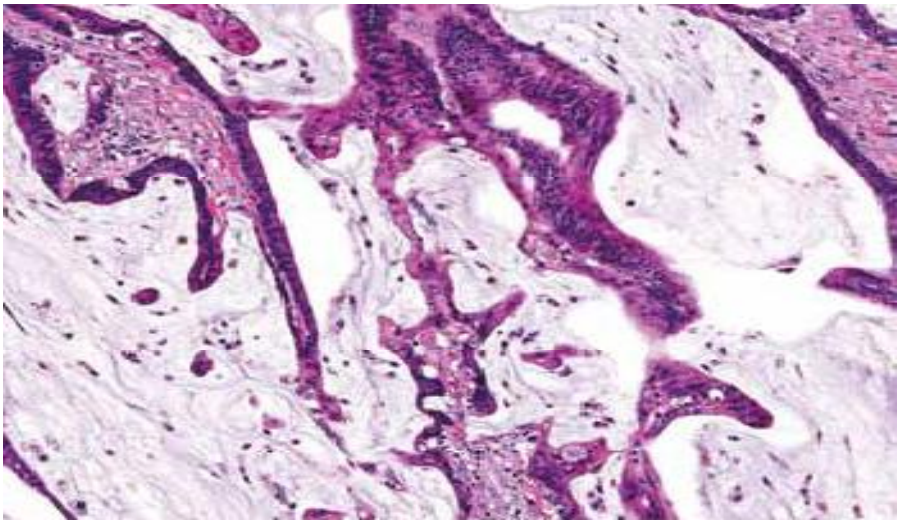


Figure .2.7. Mucinous adenocarcinoma showing abundant Extracellular mucin (H&E ×20),(Metthew, 2012).

It has been suggested that the presence of excessive mucin may facilitate tumor growth by dissecting tissue planes or prevent immunological recognition of tumor cells by interfering with inflammatory response. MAC is common with patients of young – age sporadic colorectal cancer and hereditary nonpolyposis CRC (Chiang et al., 2010). It has been reported that both MUC1 and MUC2 are commonly investigated in MAC, which their up regulation has been correlated with a higher incidence of LN, liver metastasis, worse prognosis (Lugli et al ., 2007) .

Signet –Ring cell adenocarcinoma

Signet-ring cell carcinoma (SRCC) is a rare type of adenocarcinoma characterized by mucin-secreting cancer cells that contain intracytoplasmic mucin, which pushes its nucleus to the peripheral side, showing its characteristic morphological appearance (Vinod et al., 2011), SRCC comprises approximately 0.1-2.6% of colorectal cancer, mostly observed in younger age groups (< 40 yrs) and is more common in female. The tumor is most common in the proximal colon and often presented in advanced stage (Fuki et al., 2006).

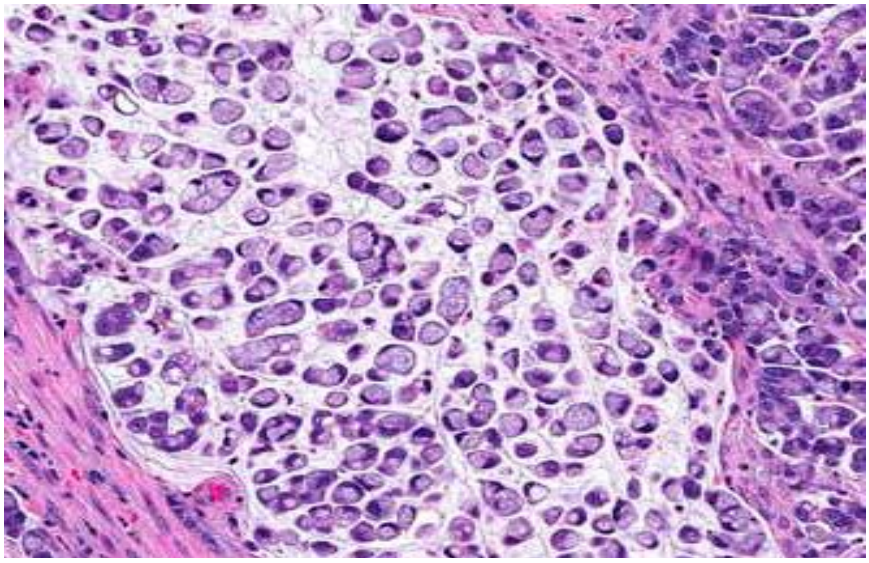


Figure .2.8. Signet ring cell carcinoma (H&E, ×40)(Metthew ,2012).

High-level microsatellite instability, loss of heterozygosity (LOH) at four loci, CpG island methylation phenotype based on seven loci, *BRAF*V600E mutation and *KRAS* mutation in signet ring cell carcinoma were compared with mucinous and conventional adenocarcinomas (Sanjay et al., 2012).

Other types of colon tumor are uncommon and have the same features of their histological types elsewhere in the body.

2.5.2 Staging of colorectal carcinoma:

In 1932 the British pathologist Cuthbert Dukes (1890-1977) devised a famous classification system for colorectal cancer and several different forms of the Dukes classification were developed.

Dukes' A: Invasion into but not through the bowel wall (90% 5-y survival).

Dukes' B: Invasion through the bowel wall but not involving lymph nodes (70% 5-y survival).

Dukes' C: Involvement of lymph nodes (30% 5-y survival).

Dukes' D: Widespread metastases.

(Kang et al., 2007).

Definitive staging can only be done after surgery has been performed and pathology reports reviewed. An exception to this principle would be after a colonoscopic polypectomy of a malignant pedunculated polyp with minimal invasion. Preoperative staging of rectal cancers may be done with endoscopic ultrasound. Adjunct staging of metastasis include Abdominal Ultrasound, MRI, CT, PET scanning, and other imaging studies.

The tumor, node, metastasis (TNM) staging system of the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC) is now the standard for colorectal cancer staging recommended by the College of American Pathologists, the Royal College of Pathologists, the Commission on Cancer of the American College of Surgeons, and the National Cancer Institute (Common Data Elements) (Greene et al.,2002, Sobin et al.,2009).

The TNM staging system is also widely used by national, regional, and local tumor registries in the United States and internationally In the TNM system (Compton ,2000), the TNM system incorporates both clinical and pathologic of staging approaches and

can encompass the newest and most technically advanced methodologies in either realm. Because the TNM system can be applied to the preoperative evaluation of patients, this system, more so than the pathologically based Dukes classification or its variations, is more meaningful and helpful to clinicians, especially in the setting of preoperative patient management to provide prognostic information useful for deciding the best treatment options for the patients (Gramont, 2005).

T – Primary Tumor

Tx Primary tumor cannot be assessed.

T0 No evidence of primary tumor.

Tis In situ: intraepithelial dysplasia.

T1 Tumor invades submucosa.

T2 Tumor invades muscularis propria.

T3 Tumor invades subserosa.

T4 Tumor invades other organs.

N – Lymph Nodes

Nx lymph nodes cannot be identified .

N0 No lymph nodes metastasis.

N1 Metastasis to 1-3 local lymph nodes

N2 Metastasis to more than 4 lymph nodes.

M – Distant Metastasis

M0 No distant metastasis.

M1 Distant metastasis

Table. 2.3 AJCC/UINCC stage grouping

TNM				Modified Astler-coller	Dukes
Stage 0	Tis	N0	M0	N/A	N/A
Stage I	aqT1	N0	M0	Stage A	A
	T2	M0	M0	Stage B1	A
Stage IIA	T3	N0	M0	Stage B2	B
Stage IIB	T4	N0	M0	Stage B3	B
StageIIIA	T1,T2	N1	M0	Stage C1	C
StageIIIB	T3,T4	N1,N2	M0	Stage C2,C3	C
StageIIIC	Any T	N2	M0	Stage C1,C2,C3	C
Stage IV	Any T	Any N	M1	Stage D	N/A

- 5-year survival rates

T1=97%
T2=90%
T3=78%
T4=63%
Any T; N1; M0=66%
Any T; N2; M0=37%
Any T; N3 M0=12%
Any M1 =4%
(Yasuda et al., 2001, Wittekind et al., 2003).

Accuracy of staging is directly proportional to the aggressiveness of surgical resection and nodal identification in this group of patients without nodal metastases also show a survival advantage when a greater number of nodes are identified, indicating the positive effect of the greater magnitude of mesenteric resection (Goldstein 2002; Le voyer et al., 2003).

Other studies have suggested additional benchmarks for nodal excision. Although some have supported the concept of "upstaging" patients with Stage I or II colorectal cancer using immunohistochemical identification of nodal involvement with or without sentinel node assessment (Yasuda et al., 2001).

Pathologic classification is based on gross and microscopic examination of the resection specimen of a previously untreated primary tumor. Clinical classification (c-TNM) is based on evidence acquired through a variety of techniques that include but are not limited to physical examination, radiologic imaging, endoscopy, biopsy, and surgical exploration, and both of them complete the other. TNM parameters representing a combination of clinically and pathologically derived data (eg , pT1, pN0, cM0) are used when only partial pathologic data are available. This is often the case because distant metastatic status is commonly unconfirmed pathologically (p-MX).

Table.2.4. Additional staging can include venous and lymphatic involvement

Venous invasion	Lymphatic invasion
V0 No venous invasion	L0 No lymphatic vessel invasion
V1 Microscopic venous invasion	L1 Lymphatic venous invasion
V2 Macroscopic venous invasion	

2.5.3. Grading system

The grading of colonic adenocarcinoma is based on the proportion of tumor composed of well-defined glands relative to that of displaying solid sheets of tumor cells poorly formed glands, or infiltrating individual tumor cells. The grading of colorectal carcinoma pertains only to adenocarcinoma of the usual type. Signet-ring cell carcinoma and small cell carcinoma are always classified as poorly differentiated. Some authors consider mucinous adenocarcinoma (where mucin is equal or greater than 50% of the total surface area in the slides) as poorly differentiated.

The most commonly used system of grading is as per the guidelines of the American Joint Commission on Cancer. As per their standards, the following are the grading categories :

GX Grade cannot be assessed.

G1 Well differentiated (Low grade) in which more than 50% of the neoplastic glands resemble the normal colonic gland, preserving their lumen with somewhat basal nuclei.

G2 Moderately differentiated (Intermediate grade) the neoplastic glandular tissues are less regular with obliteration of the lumen.

G3 Poorly differentiated (High grade) the tumor is highly different from the normal colonic glandular tissue, with formation of solid or sheet of the malignant cells.

G4 Undifferentiated (High grade).

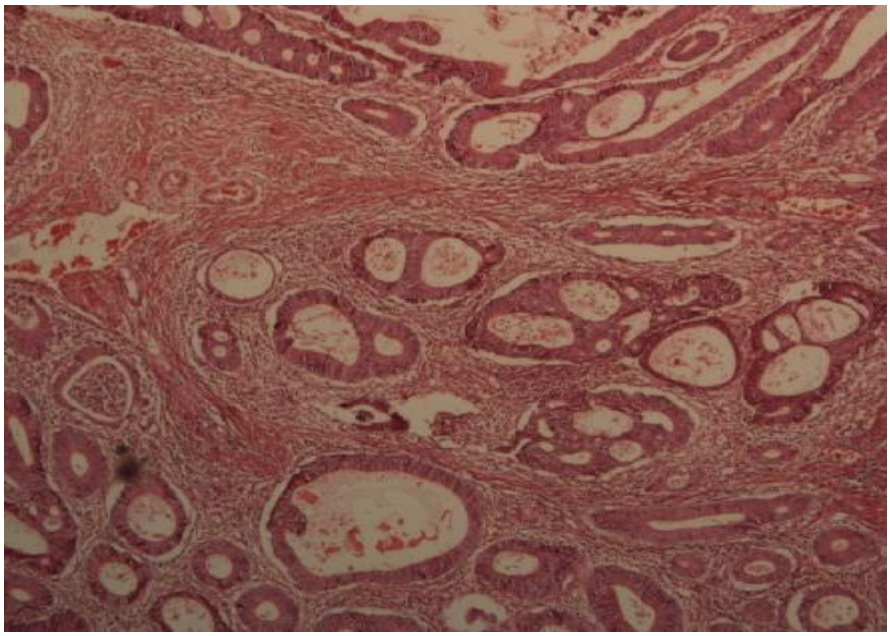


Figure 2.9. Well differentiated colon cancer (G1, H&E, 20X);(Pathology Department/Benghazi/University).

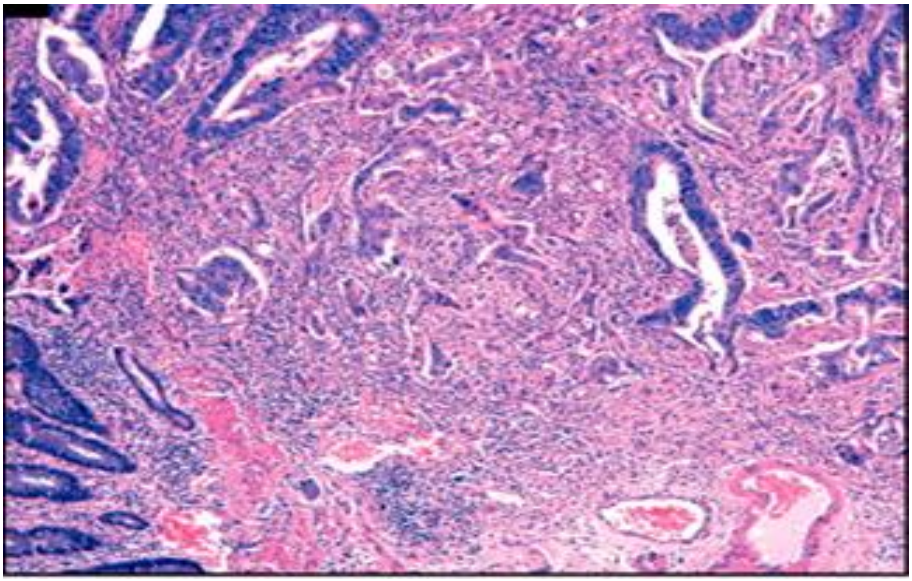


Figure 2.10. Moderately differentiated colon cancer (G2, H&E, 20X);(Hideki et al , 2010)

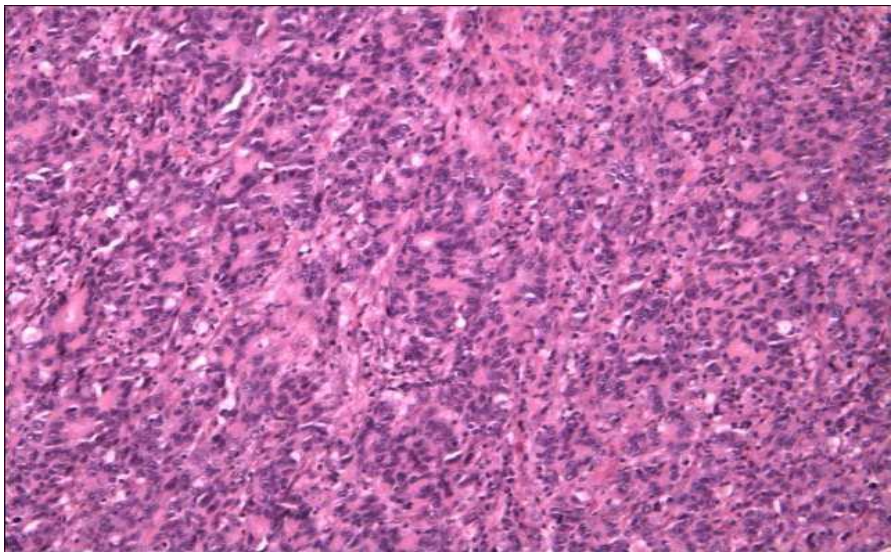


Figure 2.11. Poorly differentiated colorectal cancer (G3, H&E, ×20);(Hideki et al, 2010).

2.6. SCREENING

The identification of a well-defined premalignant lesion, the adenomatous polyp together with the good survival associated with early disease, make CRC an ideal target for screening. Evidence indicates that significant reduction in CRC mortality can be achieved by screening (Munteanu et al., 2008).

CRCs are among the very few cancer sites where screening and early detection are both feasible and proven to reduce mortality. The recommended test for mass screening is the fecal occult blood test FOBT which acts as a first screen for possible malignancy is designed to detect blood traces in the stool on a guaiac-based testing sample. Persons testing positive usually undergo colonoscopy as a more invasive but definitive examination. Newer technologies combine the guaiac-based test with tests based on molecular biology to look for cancer biomarkers in the stool. More direct methods for detecting colonic premalignant and malignant tumors include the use of colonoscopy or flexible sigmoidoscopy. An exciting new CRC screening option is virtual colonoscopy (VC), which, by screening out persons without neoplasia, allows colonoscopy to be reserved for those requiring therapeutic intervention (Atkin 2003; Janssens ,2005).

Common screening methods are digital rectal examination (DRE), sigmoidoscopy (usually flexible sigmoidoscopy, using a flexible endoscope, but more rarely the older rigid sigmoidoscopy, using a rigid endoscope), lower gastrointestinal series (barium enema), colonoscopy, and virtual colonoscopy.

Standard colonoscopy has been used for identification of colonic lesions. However, standard endoscopic inspection by itself cannot reliably distinguish between neoplastic and non-neoplastic lesions (Sikka et al., 2008, Rastogi et al., 2009).

Narrow Band Imaging (NBI) is a novel diagnostic approach highlighting blood vessel structures on polyps which are an indicator for future cancer risk especially in small polyps (less than 10 mm). Thus, all visualized lesions need to be removed during colonoscopy to be evaluated by histopathology, this approach remains the gold standard for final diagnosis (Winawer et al., 2006).

With almost half of all polyps being hyperplastic , the standard approach results in a large proportion of unnecessary polypectomies, which increases time, risk, and cost of colonoscopy with unnecessary follow-up, so many new techniques has been developed as Probe-based confocal laser endomicroscopy (pCLE) allows in *vivo* imaging of tissue at micron resolution, a probe-based confocal laser endomicroscopy system is a new tool that allows cellular and subcellular micron-level imaging of colonic mucosa during endoscopy without requirement of the use of a designated endoscope which show higher sensitivity in comparison with other method, may replace the need for ex vivo histological confirmation of small polyps (ANNA et al.,2010).

2.7 Precancerous lesion of the colon

A polyp is a mass that protrudes into the lumen of the gut. Traction on such a mass may create a stalked, or pedunculated, polyp. Alternatively, the polyp may be sessile, without a definable stalk. Polyps may be formed as the result of abnormal mucosal maturation, inflammation, or architecture. These polyps are non-neoplastic and do not have malignant potential. Those polyps that arise as the result of epithelial proliferation and dysplasia are termed adenomatous polyps or adenomas. They are true neoplastic lesions and are precursors of carcinoma. Hyperplastic polyps are the most common polyps of the colon and rectum. When single, they do not have malignant potential. However, a lesion known as sessile serrated adenoma, which has some similarities with hyperplastic polyps, may have malignant potential (Kumar, 2007).

Epithelial polyps of the colon and rectum have been classified on the basis of their malignant potential as non-neoplastic lesions; hyperplastic or metaplastic polyps, and neoplastic adenomas. And on the basis of histological type into tubular, tubulovillous, villous. The distinctive feature was the presence of cytologic dysplasia or intraepithelial neoplasia (IEN).

Some high risk factors for cancer were identified from adenomas, such as the size of the polyp, histological type and the presence of high grade dysplasia (Saini et al., 2009), even analysis of some biomarker and receptors can play role in prediction of the polyp behavior, as on a pervious study investigate the expression and clinical relevance of TLR in colorectal polyps. TLR7 expression was lower in both hyperplastic and tubulovillous adenoma polyps from patients who developed CC. TLR9 expression was decreased in hyperplastic and villous polyps from patients who developed CC (Noemi et al., 2012).

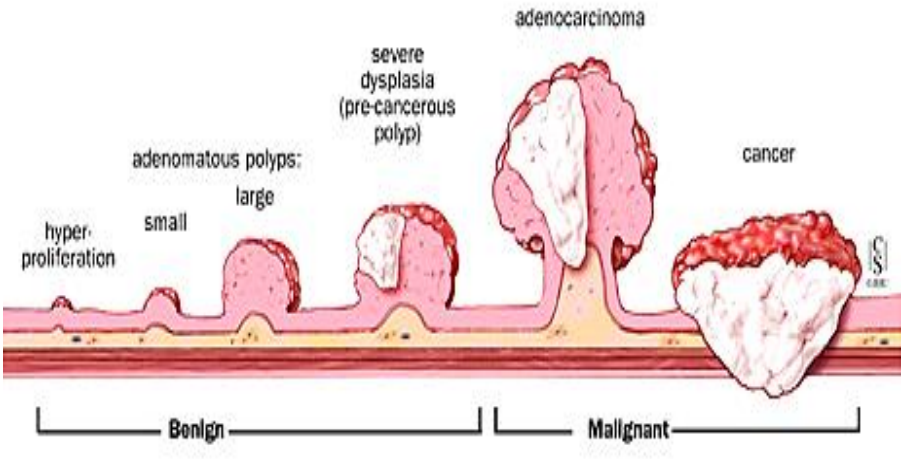


Figure 2.12. An illustration showing progression from polyp to cancer.

Colon polyps are not commonly associated with symptoms. Occasionally rectal bleeding, and on rare occasions pain, diarrhea or constipation may occur because of colon polyps. Results from previous studies have shown that colonic polyps are more common in men than in women and increase in frequency with increasing age (Saini et al., 2009).

Colon polyps are a concern because of the potential for colon cancer being present microscopically and the risk of benign colon polyps transforming over time into malignant ones. Since most polyps are asymptomatic, they are usually discovered at the time of colon cancer screening. The polyps are routinely removed at the time of colonoscopy either with a polypectomy snare or with biopsy forceps. If an adenomatous polyp is found with sigmoidoscopy or if a polyp is found with any other diagnostic modality, the patient must undergo colonoscopy for removal of the polyps. Even though colon cancer is usually not found in polyps smaller than 2.5 cm in diameter, all polyps found are removed since the removal of polyps reduces the future likelihood of developing colon cancer. When adenomatous polyps are removed, a repeat colonoscopy is usually performed in three to five years. Complications of

colonic polyps include bleeding, obstruction, diarrhea, and development of cancer (Munteanu et al., 2008).

Recent updates of the National Polyp Study and the U.S. Multi-Society Task Force recommend that patients be identified as low risk (one or two tubular adenomas smaller than 1.0 cm long or low grade dysplasia) or high risk (three or more adenomas, one of them being larger than 1.0 cm long, villous or tubulovillous histology or high grade dysplasia). Low risk patients should undergo another colonoscopy in five years or more, while high risk patients should be submitted to a new colonoscopy in three years, as long as all polyps are properly removed. According to guidelines of the American Gastroenterology Association and the American College of Gastroenterology, low risk patients should be re-evaluated in five years (Saini et al., 2009).

Colon polyps are divided into three histological subtypes based on their glandular architecture: tubular, tubulovillous, and villous. The risk of malignancy increases with both the size of the polyp and the degree of villous component (Emeester et al., 2001). Adenomas was based on structural and cytology modifications. They were classified according to the presence of 0 to 25% of villous tissue for tubular adenoma; 25 to 75% of villous lesions, as tubulovillous, and above 75%, as villous (Kudo et al., 2008).

Tubular adenoma contain a complex branching architecture of the glandular tissue and are the most common type (85%) and are typically smaller measuring < 10mm (Judy 2008).

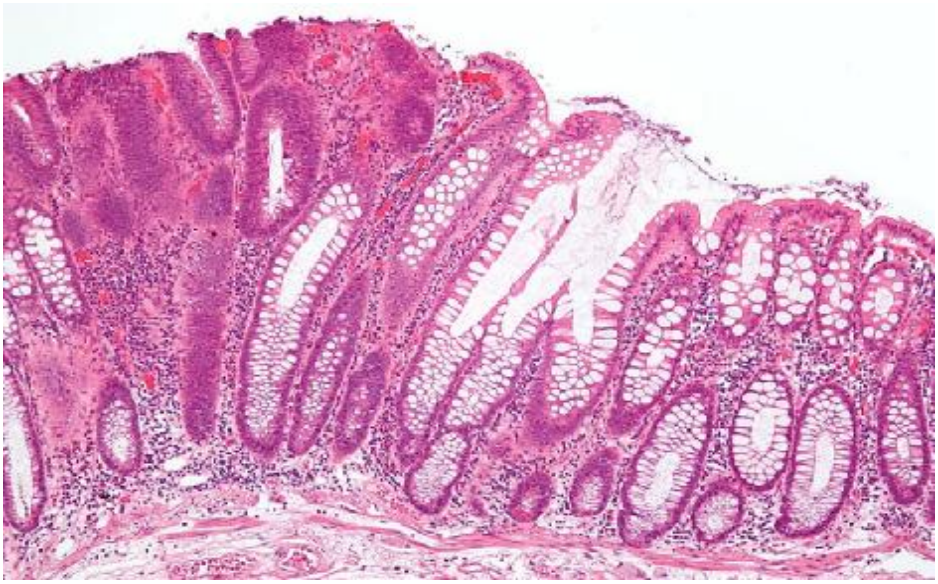


Figure .2.13. Histological appearance of tubular adenoma. (Judy 2008).

Tubulovillous adenomas contain both tubular and villous glandular architecture and clinically behave according to the majority component, having a higher risk of malignant degeneration when more villous are present. It account approximately for 10%, and often larger than tubular adenoma > 10mm (Yee, 2009).

Villous adenoma contain short, straight glands extending to the muscularis mucosa and are associated with a 10-times greater incidence of malignancy than other types, approximately 40% of villous adenoma will harbor infiltrating carcinoma, usually at the base of the lesion. It is uncommon and constitutes 5% of all adenoma. They occur with increasing frequency in older individuals and are often larger in size, with approximately 75% measuring > 2cm long, common location are the rectum and cecum. The concept of advanced adenoma includes adenomatous polyps that are ≥ 10mm long, contains any villous component, high or invasive carcinoma (Judy, 2008, Yee, 2009).

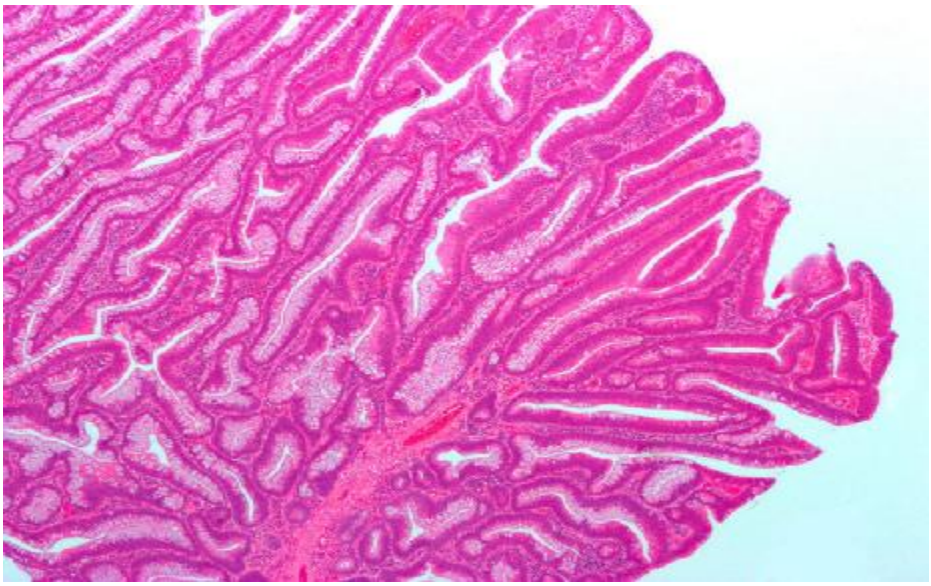


Figure 2.14. Histological features of villous adenoma.(Judy 2008).

The association between COX2 and adenoma has been studied in previous research and showed no significant correlations were found between the expression of COX2 and histology (tubular vs tubulovillous), localization (proximal vs distal) and morphology (sessile Vs pedunculated) of the adenomas. Both stromal and epithelial COX-2 expressions were higher in larger (≥4mm long) compared with smaller (≤4 mm long) adenomas (Carmela et al., 2005).

Polyps are traditionally divided by their behavior as benign or malignant as follows: hyperplastic polyps, adenomas, and polyposis syndromes.

According to the latest WHO classification of serrated polyps, polyps are classified according to the morphology as hyperplastic polyps (HPs), sessile serrated adenomas/polyps (SSA/Ps), and traditional serrated adenomas (TSAs) (Snover et al., 2010, Arzu et al., 2012) and classified according to the malignant potential as the following table (Helmut , 2006).

Table 2.4 Types of colonic polyps

Neoplastic polyp	Non-Neoplastic polyp
Adenoma	Peutz-Jeghers polyp
Carcinoid tumor	Juvenile polyp
Non-epithelial tumor (lipoma, leiomyoma, hemangioma, lymphangioma)	Hyperplastic polyp
	Benign lymphoid polyp
	Inflammatory polyp

Serrated colorectal polyps are a heterogeneous group of mucosal gastrointestinal lesions. Although they are highly prevalent in western populations, their prevalence is not clear in the Arab region. Except from a study in Saudi Arabi showed similar prevalence to that seen in the western world (Rana, 2009).

Specific lifestyle and dietary factors appear to be associated with increased prevalence of hyperplastic polyps, such as cigarette smoking, alcohol consumption, obesity and low folate intake. Other factors such as NSAIDs intake, high calcium intake and hormone replacement therapy were found to reduce the risk of their occurrence.

The meaning of serration should be explained. All serrated polyps should show some degree of infolding of the crypt epithelium, which leads to the characteristic saw-

toothed appearance in longitudinal sections and the satellites appearance on cross-sections of the crypts.

Hyperplastic polyp (HP):

HPs are by far the most common serrated polyps (80–90%) They occur most often in the distal part of the colon and rectum. Grossly, these are slightly elevated lesions with a diameter of usually less than 5 mm, microscopically; hyperplastic polyps are characterized by elongated crypts with serrated architecture in the upper half of the crypts. There is no cytological atypia or intraepithelial neoplasia (Torlakovic et al., 2003).

The risk of malignant progression for most of the small distally located HPs in the colon and the rectum is very low. In contrast, an HP with a diameter of more than 10 mm and a localization in the proximal colon should be completely removed because some case studies and studies with small cohorts suggest that at least some HPs have malignant potential (Azimuddin et al., 2000). The occurrence of multiple hyperplastic polyps within the hyperplastic polyposis syndrome is associated with colorectal carcinomas (Jeevaratnam et al., 1996).

Traditional serrated adenoma

The traditional serrated adenoma (TSA) is the rarest variant of serrated lesions (1–6%). It has been known since 1990 by the term “serrated adenoma” as a rare variant of adenomas (1%) (ACS, 2012). Grossly, TSAs are pedunculated or villous polyps, which are more common in the left side than in the right side of the colon in mostly elderly patients (60%). By definition, the TSA microscopically shows IEN (90% LG-IEN and 10% HG-IEN).

It has been demonstrated that hyperplastic polyps/serrated lesions without the presence of IEN are, in fact, clonal epithelial proliferations with underlying genetic alterations mainly in KRAS (Ajioka et al., 1998) and BRAF (Preto et al., 2008), and the

normal shedding of the epithelium in the polyp is inhibited by activated/mutated RAS or RAF (Kambara et al., 2004).

Sessile serrated adenoma (SSA)

The term SSA/P with dysplasia has replaced the category of mixed hyperplastic /adenomatous polyps (MPs). With a frequency of 15–20%, the SSA is the second most common form of serrated polyps. Grossly, SSAs are flat or slightly elevated lesions typically >5 mm in diameter and localized in the right part of the colon. The microscopic characteristic of the SSA is hyper-serration and dilatation of the crypts (with reduced stroma and back-to-back positioning of the dilated crypts) with T- and L-shaped branching at the crypt base (Jass 2007, Kudo et al., 2008).

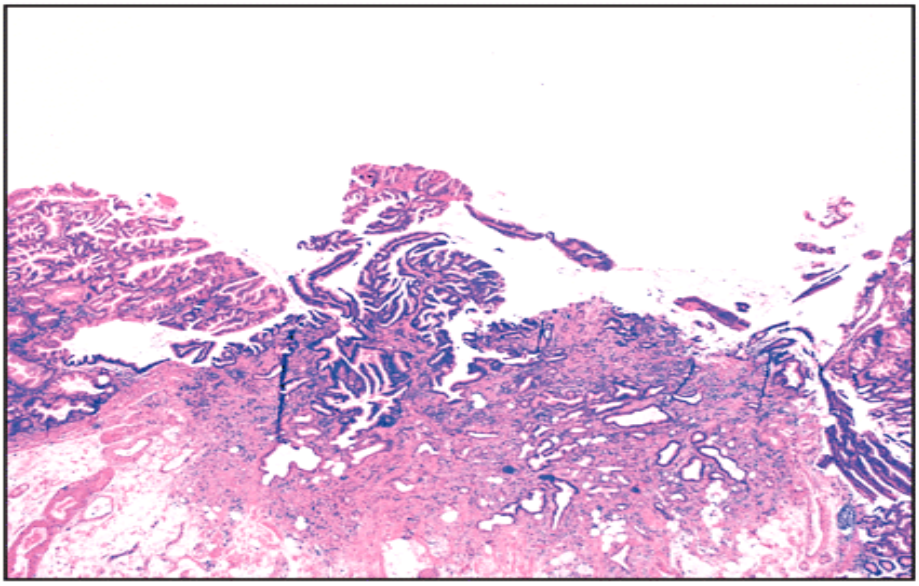


Figure 2.15. Sessile serrated adenoma (Kudo et al.,2008)

Sessile serrated adenoma with invasive adenocarcinoma. Low-power magnification of a sessile serrated polyp/adenoma with an area of invasive carcinoma arising in the center of the polyp (H&E, x20).

Administration of selective photosensitizer precursor hexaminolevulinate (HAL) H enema induces selective lesion fluorescence and increases the lesion detection rate in patients with colorectal adenoma and early carcinoma (Mayinger et al., 2008).

As early as 1999, Iino and Jass found hyperplastic/serrated polyps preceding microsatellite instability MSI colorectal carcinomas indicating that serrated polyps are involved in the carcinogenesis of a subgroup of colorectal carcinomas (Lino et al., 1999). On the other hand, Jass et al. demonstrated that "classical" adenomas are most likely not the precursor lesions of sporadic colorectal carcinomas with high (type 1, according to Jass et al.,2007) and low (type 2 according to Jass et al.,2007) microsatellite instability (MSI-H and MSI-L) since BRAF-mutations and CpG-island methylation which are frequently detected in these carcinomas were only very infrequently observed in adenomas.

Lymphoid polyp

Benign lymphoid hyperplasia in the colon is a condition that is not uncommonly biopsied in the course of screening colonoscopy. In the rectum these lesions have been termed 'rectal tonsils reactive lymphoid nodules may accompany a variety of conditions, including viral infections (Jason, 2007), histologically, well-formed germinal centers may be seen. When a lymphoid population is identified on colon biopsy, lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) must be excluded and immunohistochemical stains are helpful in this regard (Kojima et al., 2005).

Inflammatory polyp

Crohn's disease and ulcerative colitis are idiopathic inflammatory bowel diseases believed to result from abnormal local immune responses against unknown microbes and/or self antigens in the intestine.

Crohn's disease:

It is associated with HLA-DR7 and -DQ4 alleles, and with mutations in the NOD2 gene, which encodes an intracellular sensor of microbes results from a chronic T cell-

mediated inflammatory reaction involving IFN-γ-producing TH1 cells and, perhaps IL-17-producing TH17 cells manifested by chronic inflammation with granulomas, ulcers, and strictures caused by fibrosis, involving the terminal ileum and colon consequences include fistula formation, abdominal abscesses, intestinal obstruction, and increased risk of carcinoma.

Ulcerative colitis:

It is associated with HLA-DRB1 manifested by superficial ulcers in the colon without granulomas or extensive fibrosis, The nature of the pathologic immune response is unknown; the most serious complication is the increased risk of carcinoma.

Familial adenomatous polyposis (FAP)

Familial polyposis syndromes are uncommon autosomal dominant disorders. Their importance lies in the propensity for malignant transformation and in the insights that such transformation has provided in unraveling the molecular basis of CRC. Individuals with familial adenomatous polyposis (FAP) typically develop 500 to 2500 colonic adenomas that carpet the mucosal surface, a minimum number of 100 is required for the diagnosis. Multiple adenomas may also be present elsewhere in the alimentary tract, including almost a 100% lifetime incidence of duodenal adenomas. Most polyps are tubular adenomas. Occasional polyps have villous features. Polyps usually become evident in adolescence or early adulthood. The risk of colonic cancer is virtually 100% by midlife, unless a prophylactic colectomy is performed. The genetic defect underlying FAP has been localized to the APC gene on chromosome 5q21; Gardner syndrome and the much rarer Turcot syndrome seem to share the same genetic defect as FAP. These syndromes differ from FAP with respect to the occurrence of extra-intestinal tumors in the latter two, osteomas, gliomas, and soft tissue tumors, to name a few.

Peutz–Jeghers polyp, also known as hereditary intestinal polyposis syndrome, is an autosomal dominant genetic disease characterized by the development of benign

hamartomatous polyps in the GIT and hyperpigmented macules on the lips and oral mucosa (James et al., 2005) .Peutz –Jeghers syndrome has an incidence of approximately 1 in 25,000 to 300,000 births (Jerry et al., 2008).

Most patients will develop flat, brownish spots (melanotic macules) on the skin, especially on the lips and oral mucosa, during the first year of life, and a patient's first bowel obstruction due to intussusception usually occurs between the ages of six and 18 years. The cumulative lifetime cancer risk begins to rise in middle age. Cumulative risks by age 70 for all cancers, gastrointestinal (GI) cancers, and pancreatic cancer are 85%, 57%, and 11%, respectively. In 1998, a gene was found to be associated with the mutation on chromosome 19, the gene known as STK11 (LKB1) is a possible tumor suppressor gene (Boardman et al., 1998). It is inherited in an autosomal-dominant pattern which means that anyone who has PJS has a 50% chance of passing it onto their children, assuming that their spouse does not have the disease.

Mismatch repair cancer syndrome (MMRCS) is a condition associated with biallelic DNA mismatch repair mutations. It is also known as Turcot syndrome after Jacques Turcot who described the condition in 1959, under the name "constitutional mismatch repair-deficiency" (CMMR-D), it has been mapped to MLH1, MSH2, MSH6 or PMS2 (Kratz et al., 2009). Although these are the same genes mutated in the condition known as Lynch syndrome or hereditary nonpolyposis colorectal cancer, the mutations are biallelic in CMMR-D (Wimmer et al, 2008). The term "childhood cancer syndrome" has also been proposed Café-au-lait macules have been observed (Kruger et al., 2008).

Juvenile polyposis syndrome is a syndrome characterized by the appearance of multiple polyps in the gastrointestinal tract, usually in a child, adolescent or young adult. The majority of the polyps found in Juvenile Polyposis Syndrome are non-neoplastic, hamartomatous, self-limiting and benign, there is an increased risk of adenocarcinoma.

Juvenile Polyposis Syndrome can occur sporadically in families or be inherited in an autosomal dominant manner.

Two genes associated with Juvenile Polyposis Syndrome are BMPR1A and SMAD4 (Howe et al., 1998). Gene testing may be useful when trying to ascertain which non-symptomatic family members may be at risk of developing polyps, however having a known familial mutation would be unlikely to change the course of treatment. A known mutation may also be of use for affected individuals when they decide to start a family as it allows them reproductive choices.

While mutations in the gene PTEN were also thought to have caused Juvenile polyposis syndrome, it is now thought that mutations in this gene cause a similar clinical picture to Juvenile polyposis syndrome but are actually affected with Cowden syndrome or other phenotypes of the PTEN hamartoma tumor syndrome (Stoler et al., 2009).

Lynch syndrome (HNPCC or hereditary nonpolyposis colorectal cancer) is an autosomal dominant genetic condition which has a high risk of colon cancer have about an 80% lifetime risk for colon cancer (Kastrinos et al., 2009) , as well as other cancers at an early age including endometrium, ovary, stomach, small intestine, hepatobiliary tract, upper urinary tract, brain, and skin. The increased risk for these cancers is due to inherited mutations that impair DNA mismatch repair, HNPCC can be divided into Lynch syndrome I (familial colon cancer) and Lynch syndrome II (HNPCC associated with other cancers of the GIT or reproductive system, most of the genetic defects identified being attributable to mutations in two genes, MSH2 and MLH1 (Lagerstedt et al., 2007).

A small proportion of cases are caused by germ-line mutations in two other MMR genes, MSH6 and PMS2, the complete loss of MMR function in tumors leads to increased mutations at microsatellite sequences resulting in the microsatellite instability–high (MSI-H) phenotype, although numerous studies report MSI-H Lynch syndrome cases that lack mutations in known MMR genes (Van der Klift et al., 2005, James et al., 2009).

HNPCC is responsible for approximately 2% to 7% of all diagnosed cases of colorectal cancer. The average age of diagnosis of cancer in patients with this syndrome is 44 years old, as compared to 64 years old in people without the syndrome (Lindor, 2009).

The utility of immunohistochemical detection of DNA mismatch repair proteins in screening colorectal cancer for hereditary nonpolyposis CRC (HNPCC) is being widely investigated. Currently, in both research and clinical settings, a 4-antibody panel that includes the 4 most commonly affected proteins (MLH1, MSH2, MSH6, and PMS2) is being used generally (Shia et al., 2009).

Research criteria for defining Lynch syndrome (LS) families were established by the International Collaborative Group (ICG) meeting in Amsterdam in 1990, and are known as the Amsterdam criteria.

These criteria provide a general approach to identifying LS families, but they are not considered comprehensive.

Table 2.5. Showing Amestrdam criteria for Lynch syndrome.

Amsterdam criteria I (Vaasen et al., 1991)	Amsterdam criteria II (Vasen et al., 1999)	Revised Bethesda criteria (Umar et al., 2004)
1.One member diagnosed with CRC before age 50 years.	1.There should be at least three relatives with a LS-associated cancer (CRC or cancer of the endometrium, small bowel, ureter, or renal pelvis.	1.CRC diagnosed in an individual younger than 50 years.
2.Two affected generations.	2.One should be a first-degree relative of the other two.	2. Presence of synchronous, metachronous colorectal, or other LS-associated tumors
3.Three affected relatives, one of them a first-degree relative of the other two.	3.At least two successive generations should be affected.	3. CRC with MSI-high (MSI-H) pathologic associated features diagnosed in an individual younger than 60 years. [Note: Presence of tumor-infiltrating lymphocytes, Crohn-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern.
4.FAP should be excluded.	4. At least one should be diagnosed before age 50 years	4. CRC or LS-associated tumor* diagnosed in at least one first-degree relative younger than 50 years
5.Tumors should be verified by pathological	5.FAP should be excluded in the CRC cases	5.CRC or LS-associated tumor diagnosed at any age in two first-degree or second-degree relatives.

2.8. Diagnosis

The symptoms and signs of colorectal cancer depend on the location of tumor in the bowel, and whether it has metastasized. The classic warning signs include, worsening constipation, blood in the stool, weight loss, fever, loss of appetite, and nausea or vomiting in someone over 50 years old. While rectal bleeding or anemia are high-risk features in those over the age of 50, other commonly described symptoms including weight loss and change in bowel habit are typically only concerning if associated with bleeding. A change in bowel habits, such as diarrhea, constipation, or narrowing of the stool, that lasts for more than a few days (Adelstein et al., 2011).

Patients who have distal rectal carcinomas may present with spotting of blood in their stool, particularly during the initial phases of polyp or malignant tumor development. Even when digital examination is carefully performed, the physician often may miss a frond-like, premalignant villous tumor, such lesions can be totally resected before the epithelial transformation progresses to malignancy and it often can be removed with sphincter preservation, but, some investigational methods aside, only if the diagnosis is made before the tumor invades the rectal wall (Rustum et al., 1997).

Imaging and blood tests

Imaging include CT scan, CT with portography, CT-guided needle biopsy, abdominal, endorectal, intraoperative USS, MRI, chest x-ray, positron emission tomography PET scan, angiography.

Complete blood count (CBC), Some people with colorectal cancer become anemic because of prolonged bleeding from the tumor.

Liver enzymes : Checking liver function test with other techniques is important to role out liver metastasis.

Tumor markers, CRC cells sometimes make substances, like carcinoembryonic antigen (CEA) and CA 19-9, that are released into the bloodstream, blood tests for these tumor markers are used most often along with other tests to monitor patients who already

have been diagnosed with or treated for colorectal cancer, they may help show how well treatment is working or provide an early warning of a cancer that has returned.

These tumor markers are not used to screen for or diagnose CRC because the tests can't tell for sure whether or not someone has cancer. Tumor marker levels can sometimes be normal in a person who has cancer and can be abnormal for reasons other than cancer. For example, higher levels may be found in the blood of some people with ulcerative colitis, non-cancerous tumors of the intestines, or some types of liver disease or chronic lung disease. Smoking can also raise CEA levels.

2.9. Right- and left-sided colonic cancer - different tumor entities

There has been conflicting information regarding the relationship between cancer location and mortality. In (Meguid et al.,2008) reported a 4% increase in mortality for right-sided compared with left-sided colon cancers by using the Surveillance Epidemiology and End Results Program (SEER) database. More recently, (Benedix et al., 2010) queried the database created by the German multicentered observational study, Colon/Rectal Carcinoma (Primary Tumor), and found an even larger increase (12%) in mortality for right-sided compared with left-sided colon cancers (Jennifer et al., 2011).

Differences have been noted in the following characteristics: right-sided colon cancers are more likely to be exophytic, to be diploid, and to have mucinous histology, high microsatellite instability, CpG island methylation, can spread more readily to LNs or peritoneal carcinomatosis. Whereas left-sided colon cancers are often infiltrating lesions, present with obstructive symptoms, have chromosomal instability, and are more often aneuploid .Analysis of tumor specimens also has shown a difference in gene expressions between tumors in the right and left colon. However, it is unclear whether these biologic differences translate into meaningful differences in mortality (Birkenkamp et al.,2005).

Right-side tumors at a high risk for relapse exhibit elevated expression of cell cycle control genes and elevated Wnt signaling. On the other hand, relapse-prone left-side

tumors show elevated expression of genes that promote stromal expansion and reduced expression of tumor suppressor genes that initiate Wnt signaling. Single gene prognostic biomarkers are found separately for right-side and left-side disease (Kerry et al., 2012).

Treatment

Surgery is the mainstay in the management of patients with early stage of colon cancer, the primary goal is a wide resection of the primary tumor with all loco-regional lymph nodes. Optimal surgery by experienced colorectal surgeons should be performed, an adequate number of lymph nodes should be recovered (at least 12) and resection margins have to be free Laparoscopic resection gives similar oncologic outcome compared to laparotomy and has less postoperative morbidity in experienced surgical hands (Cutsem et al., 2009).

A significant proportion of patients presenting with stage I, II, or III disease on TNM classification (75% of patients) can be cured by surgical intervention, with U.S. 5-year survival rate figures of 93.2%, 82.5%, and 59.5%, respectively, compared with only 8.1% for stage IV disease (Gill et al., 2004). Following resection, there is a considerable risk for tumor recurrence in patients with stage III and high-risk stage II disease, which can be significantly reduced by treating with 5-fluorouracil (5-FU)-based adjuvant chemotherapy (Andre et al., 2007).

Current standard treatment for m-CRC includes a fluoropyrimidine backbone either intravenously or orally with oxaliplatin and/or irinotecan with the incorporation of the biological targeted therapies bevacizumab, cetuximab, or panitumumab. The choice of a treatment regimen is highly directed by the planned strategy: resectable or potentially resectable if tumor shrinkage/control exists versus non-resectable metastases and by the need for an aggressive treatment or not (Jemal et al., 2008).

5-FU remain the mainstay of therapeutic options in the treatment of advanced CRC, with response rates of 20% to 25%. The introduction of newer agents such as irinotecan, leucovorin and oxaliplatin (FOLFOX), a regimen that is associated with a higher 5-year disease-free and overall survival compared with 5-FU alone in stage III CRC patients. In addition, FOLFOX has been shown to significantly reduce recurrence rates and increase overall survival in high-risk stage II CRC patients (Andre et al., 2009).

In combination with 5-FU has increased response rates to 40% to 50% in advanced disease and improved overall survival (Douillard et al., 2000).

In patients with stage IV or metastatic CRC (mCRC), treatment goals are mainly palliative and the 5-year survival rate is less than 10%. With 5-FU adjuvant treatment, overall survival has been shown to be around 12 months.

The development of monoclonal antibodies targeting the epidermal growth factor receptor or VEGF has demonstrated additional clinical benefit for patients with metastatic disease (Cunningham et al., 2004).

However, many patients succumb to their disease, and a significant proportion will experience severe chemotherapy-associated toxicities while deriving little or no benefit. To improve the treatment of CRC, efforts must be directed toward the identification of patients who are likely to respond to a specific therapy, those who will experience severe toxicities, and those who will benefit from chemotherapy in the adjuvant setting (Peter et al., 2007).

In the absence of adjuvant therapy, approximately 50% of colon cancer patients with resectable disease are cured by surgery alone, whereas 50% relapse. Using adjuvant chemotherapy following surgery rescues approximately 15% of patients from the relapsing group (Bosman et al., 2009), in current practice, the majority of colon cancer patients receive treatment unnecessarily, either because they were cured or because they will relapse despite treatment (Sabine et al., 2010).

Celecoxib, a selective COX-2 inhibitor, has been reported to exert chemopreventive and antitumor effects on colon cancer, celecoxib may be able to affect epithelial-mesenchymal transition (EMT), a critical process involved in cancer cell invasiveness and metastasis and then proposed to be relevant for cancer progression.

Celecoxib inhibits basal and EGF-stimulated proliferation, hypoxia-related HIF-1α recruitment/stabilization as well as hypoxia- and EGF-dependent activation of ERK and PI3K. Interestingly, celecoxib prevented EMT-related changes, as shown by

modifications of β-catenin intracellular localization or vimentin and E-cadherin levels, as well as HT-29 invasiveness induced by hypoxia, EGF, or hypoxia plus EGF. Finally, experiments performed on SW-480 colon cancer cells (i.e., cells lacking COX-2) exposed to hypoxia, used here as a stimulus able to induce EMT and invasiveness, revealed that in these cells celecoxib was ineffective. Celecoxib has the potential to negatively affect induction of EMT and increased invasiveness of colon cancer cells as elicited by different signals originating from tumor microenvironment, hypoxia and EGF. Moreover, these effects are likely be related to the pharmacological inhibitory effect exerted on COX-2 activity (Bocca et al., 2012).

Some studies have proved that revealed higher COX-2 expression in chemoresistant CRC cells and tumor xenografts. In vitro, the combination of either aspirin or celecoxib with 5-fluorouracil (5-FU) was capable of improving chemosensitivity in chemorefractory CRC cells (Mahbuba et al., 2012).

Radiation, while a combination of radiation and chemotherapy may be useful for rectal cancer, its use in the treatment of colon cancer is not routine due to the sensitivity of the bowels to radiation.

Follow-up

Despite optimal primary treatment, with adequate surgery with or without adjuvant chemotherapy, 30%–50% of patients with colon cancer will relapse and die of their disease. Detecting relapse in advance is the main goal of surveillance after primary treatment, but this is clinically meaningful only if it improves survival, following treatment of colon cancer, periodic evaluations may lead to the earlier identification and management of recurrent disease. The impact of such monitoring on overall mortality of patients with recurrent colon cancer is limited by the relatively small proportion of patients in who localized, potentially curable metastases are found. To date, no large-scale randomized trials have documented the efficacy of a standard, postoperative monitoring program (Labianca et al., 2010).

CEA is a serum glycoprotein frequently used in the management and follow up of patients with colon cancer. A review of the use of this tumor marker suggests the following:

1. A CEA level is not a valuable screening test for colorectal cancer because of the large numbers of false-positive and false-negative reports.

2. Postoperative CEA testing should be restricted to patients who would be candidates for resection of liver or lung metastases.

3. Routine use of CEA levels alone for monitoring response to treatment should not be recommended.

4. History and physical examination and CEA determination are advised every 3–6 months for 3 years and every 6–12 months at years 4 and 5 after surgery [II, B].

5. Colonoscopy must be performed at year 1 postoperatively and thereafter every 3–5 years looking for metachronous adenomas and cancers [III, B].

6. CT scan of chest and abdomen every 6–12 months for the first 3 years can be considered in patients who are at higher risk for recurrence [II, B.]

7. CEUS (contrast enhancement ultrasound scan) could substitute for abdominal CT scan [III, C]. (Gan et al., 2007 ,Van et al., 2009)

2.10. Prognosis

Prognostication of newly diagnosed CRC predominantly relies on stage as defined by the UICC-TNM and American Joint Committee on Cancer classifications. Tumor extent, lymph node status, tumor grade and the assessment of lymphatic and venous invasion are still the most important morphological prognostic factors, evidence suggests those tumor budding and tumor border configurations are important.

Survival is directly related to detection and the type of cancer involved, but overall is poor for symptomatic cancers, as they are typically quite advanced. A survival rate for early stage detection is about 5 times than that of late stage cancers, patients with a tumor staged (TNM stage Tis, N0, M0) have an average 5-year survival of 100%, while those with an invasive cancer T1 or T2 cancer have an average 5-year survival of approximately 90%. Those with a more invasive tumor yet without node involvement (T3-4, N0, M0) have an average 5-year survival of approximately 70%, Patients with (any T, N1-3, M0) have an average 5-year survival of approximately 40%, while those with distant metastases (any T, any N, M1) have an average 5-year survival of approximately 5% (Elizabeth et al., 2008).

According to the American Cancer Society statistics in 2006, over 20% of patients present with metastatic (stage IV) colorectal cancer at the time of diagnosis, and up to 25% of this group will have isolated liver metastasis that is potentially resectable. Lesions which undergo curative resection have demonstrated 5-year survival outcomes now exceeding 50% (Simmonds et al., 2006).

Although CRC must be seen as a tumorous biologic entity, the prognosis for colon cancer and rectal cancer individually differs considerably. The most important reason is certainly the great difference in locoregional tumor failure, which is significantly higher for rectal cancer, whereas wide resection margins are always possible for colonic tumors, lateral margins of clearance for rectal carcinomas are often limited because of

the anatomy of the small pelvis. In addition, adjuvant therapy regimens for colon cancer and rectal cancer differ substantially (Michael et al., 2000).

Based on the International Union against Cancer (UICC)/American-Joint Committee on Cancer (AJCC) tumor stage, complete tumor removal (R0 resection) is essential for local tumor control and long-term survival (Fleming et al., 1997).

Many prognostic factors have large effect on local recurrence and long-term survival, a multidisciplinary group of clinical (including the disciplines of medical oncology, surgical oncology, and radiation oncology), pathologic, and statistical experts in CRC reviewed all relevant medical literature and stratified the reported prognostic factors into categories that reflected the strength of the published evidence demonstrating their prognostic value , prediction of the prognostic factor for the patient determines treatment and plays a key role in selecting patients for clinical trials (Myutan et al., 2011).

There is some difference in the prognostic factors in relation to the patient age; More than 70% of deaths from CRC in the Western world occur in patients >65 years old. The fact that the outcome in this group of patients is often worse may be attributed to more advanced stage at diagnosis and often, less aggressive treatment and decreased use of second- and third-line therapies Elderly patients fit enough to be eligible for clinical trials appear to derive a similar benefit from the treatment (Pallis et al., 2010).

In general, fit elderly patients tolerate the chemotherapy quite well, but some studies reported that elderly patients may have more specific toxicity from cytotoxics: e.g. increased neutropenia in patients >75 years when treated with irinotecan and an increase in bevacizumab-related arterial thrombosis in patients >70 year. Co-morbidity and socio-economic status further impact treatment availability and prognosis (Folprecht, et al, 2008).

The prognostic factors for young patients with colon cancer are age, surgical procedure, radical resection, blood transfusion, pathological type, diameter of tumor,

depth of tumor invasion, lymph node metastasis and distant metastasis; the independent prognostic factors are only blood transfusion and lymph node metastasis (Han et al., 2006).

As a consistent fraction of patients with locally advanced colonic carcinoma experience a relapse, one of the reasons accounting for this failure may be the difficulty in correctly stratifying patient groups with locally advanced disease in different risk categories. Missing additional features may account for remarkable prognostic differences between patients in the same risk category, This differentiation can be detected by survey a number of prognostic markers can help to obtain the best line of therapy among different patients in the same stage (Puppa et al., 2007).

Predictive Marker

General clinical and biochemical factors. Patient and tumor variables are strong prognostic factors and as such often predict efficacy of the chemotherapeutic treatment of mCRC, the development of genetic biomarkers can be used in combination with clinicopathological staging plays a key role in selecting patients for clinical trials.

Hemoglobin and WBC levels, LDH levels, time since diagnosis, low number of organs involved, performance status, and age are predictive of response and survival (Saltz et al.,2001). Extensive and symptomatic peritoneal carcinomatosis is often related to a lower chance of response to an antitumor therapy, on top of its prognostic significanse, skin rash: One of the more consistent clinical parameters is the association found between the development of skin toxicity mainly rash, and response on EGFR-targeting agents (Freyer et al., 2000).

Many molecular predictive marker play important role in detect the best regime of treatment, some study has even been reported that a KRAS mutation is associated with a deleterious effect when patients are treated with an oxaliplatin based backbone in combination with an anti-EGFR antibody. The knowledge that patients with mutated

KRAS do not respond to monoclonal antibody treatment in the metastatic setting has been an important step forward in attempting to tailor medication to the individual. This has led to careful genotyping of patients and identifying those with wild type-KRAS (WT-KRAS) for cetuximab and panitumumab therapy, leading to a reduction in chemotherapy toxicity and cost-effectiveness.

MSI is a potential predictor of treatment response to 5-FU and prognosis of disease when used in conjunction with TNM staging (George et al., 2007).

In colon cancer studies in the adjuvant setting, genomic profiling identified a 23-gene signature reported to predict recurrence in colon cancer patients with Dukes' B disease, yielding 78% prognosis prediction accuracy (Wang et al., 2004). This was validated in an independent study that yielded a 67.7% mean prognosis profile (Chao et al., 2000) and identified a 30-gene expression profile that produced highly variable prediction accuracy across training and validation sets, microarray expression profiling is able to predict, to some extent, prognosis in stage B colon cancer patients and that re-sampling techniques should be used to objectively assess the performance of microarray-based prognosis predictors (Barrier et al., 2006).

2.10.1. Traditional Prognostic Factors:

Category I

Tumor site

The local primary site and extent of tumor assessed pathologically, a colonic primary tumor is a positive prognostic marker compared to a rectal tumor (Kohne et al., 2002).

Lymph nodes involvement

Metastasis to regional lymph nodes as determined by pathologic assessment is among the factors that most strongly predict outcome following surgical resection and has been shown to be the most important independent prognostic factor for the outcome of patients (Shepherd et al., 1997).

Methods of lymph node examination for micrometastatic disease and the biologic significance of metastasis identified by these methods currently lack validation, It has been shown that 12 to 15 negative lymph nodes predict for regional node negativity (Fleming et al., 1997,Ratto et al., 1999).

Distant Metastasis

Metachronous metastases have a better outcome compared to synchronous metastases. Metastatic spread confined to the liver shows improved overall survival rates as opposed to spread to multiple organ sites. In contrast, patients with liver metastases show a shorter survival compared to lung metastases, peritoneal metastases are related to a worse outcome. The higher the number of metastatic sites; the worst the prognosis (Tournigand et al., 2004).

Vascular Invasion

Blood or lymphatic vessel invasion has an independent adverse impact on patient outcome. This association has also been shown in many studies (Sternberg et al.,

2006). Lymphatic invasion is a weaker prognostic factor compared to venous invasion. It is associated with higher rates of recurrence and decreased survival (Ishida et al, 2004).

Surgical margin

Residual tumor following surgery with curative intent especially as it relates to positive surgical margins carry poor prognosis.

Preoperative CEA

Preoperative CEA has been advocated as prognostic for disease free survival as well as persistent disease after surgery (Hampel et al., 2005). Baseline levels of CEA prior to treatment in the metastatic setting also seem to have some prognostic relevance, preoperative elevation of CEA elevation (more than 5 ng /mL), signifies increased risk of neoplastic recurrence and reducing survival expectancy (Strambu et al.,2011).

Biochemical Markers

Other biochemical prognostic markers should optimally be combined together, pretreatment blood count values with low hemoglobin levels and high white blood cell (WBC) counts depict poor prognosis. Increased alkaline phosphatase baseline levels are considered one of the strongest poor prognostic factors for survival in m-CRC (Graf et al., 1991, Mitry et al., 2004).

Lactate dehydrogenase (LDH) participates in anaerobic glycolytic metabolism in tumor cells and is postulated as a biomarker for high angiogenic tumors. High base line LDH measurements predict poor prognosis. High levels of serum bilirubin and low levels of serum albumin also represent poor prognosis (Koukourakis et al., 2005).

Category IIA

Tumor grade

Colorectal adenocarcinoma graded into three histological grades, well, moderately and poorly differentiated, or undifferentiated (anaplastic tumor). Accurate grading plays an important in prediction patient survival.

Category IIB

Histological type. The internationally accepted histological classification proposed by the WHO is recommended by the college of American Pathologist (Compton, 2000). The histological type is one of the prognostic factors for patients with colorectal cancer. Patients with different papillary adenocarcinoma have the best outcome. Patients with moderately-differentiated and mucinous adenocarcinoma have a moderate outcome, patients with signet-ring cell poorly-differentiated adenocarcinoma have a poor prognosis (O'Connell et al., 2005), and medullary carcinoma is prognostically favorable , as is mucinous carcinoma when associated with microsatellite instability (Compton, 2003).

Host lymphoid response to tumor ; Lymphatic infiltration of tumor or peritumoral tissue is indicative of an immunologic response to the invasive malignancy and has been shown to be a favorable prognostic factor (Takemoto et al ., 2004) and lack of an inflammatory reaction at the tumor edge is associated with unfavorable prognosis (Losi et al ., 2006). A study concern in the prognostic significance of immune criteria was compared with that of the tumor extension criteria , Growth of the primary tumor and metastatic spread were associated with decreased intratumoral immune T-cell densities. 60% of patients with high densities of CD8+ cytotoxic T-lymphocyte infiltrate presented with stage Tis/T1 tumor, whereas no patients with low densities presented with such early-stage tumor so univariate analysis showed that the immune score was significantly associated with differences in disease-free, disease-specific, and overall survival (Bernhard et al., 2011).

Category III

Tumor size and gross tumor configuration (infiltrating or bushing) and tumor budding, The classification of patients into prognostic subgroups is improved with the addition of tumor border configuration to TNM stage, an irregular infiltrating pattern of growth, is a poor prognostic factor and may predict liver metastasis (Losi et al., 2006) . In particular, patients with stage II disease characterized by an infiltrating tumor border have poor clinical outcome and represent a subset of lymph node-negative patients who could be considered for adjuvant therapy (Zlobec et al.,2009). Tumor budding is a specific feature at the tumor border and it is defined as the presence of isolated cancer cells or clusters scattered in the stroma at the invasive margin of the tumor (Ueno et al., 2002) .

2.10.2. Biological prognostic marker

1.The development of CRC through microsatellite instability (MSI) is attributed to the hereditary HNPCC syndrome patients and to about 15% of sporadic CRC patients (Vilar et al., 2010). MSI-H patients have a phenotype characterized by right-sided location and a relatively early stage at diagnosis. In a study in 607 patients at ages ≤ 50 diagnosed with CRC, 17% were found to have high MSI that was associated with a significant survival advantage regardless of standard prognostic factors, including stage (Gryfe et al., 2000).

2.K-ras/BRAF mutation status is an important factor to influence the clinical outcome of CRC, Although K-ras and BRAF belong to the same growth signaling pathway, the clinical outcome of CRC with K-ras mutation is different from that of CRC with BRAF mutation. The genetic profiles of MSI and K-ras, BRAF mutation status could be prognostic biomarkers for CRC and could be important to realize personalized medicine for patients with CRC. MSI cancer would rarely reveal metastatic potential in primary CRC, and non-MSI CRLM with K-ras or BRAF mutations would lead to a poor prognosis after curative surgery (Nagasaka et al., 2008).

K-ras, a proto-oncogene, encodes a GTP-ase that is involved in facilitating cellular response to extracellular stimuli. Point mutations within the K-ras gene have been found in about 40% of CRC, resulting in constitutive activation of downstream signaling pathways and resistance to inhibition of cell surface receptor tyrosine kinases, most notably EGFR (Roth et al., 2010) , The prognostic significance of K-ras mutations on recent large studies showed no prognostic role of K-ras mutations in stage III and II/III colon cancer (Artale et al.,2008, Ogino et al., 2009, Lievre et al., 2010).

As a predictive marker in the adjuvant setting, most studies report no association between K-ras mutations and response to standard chemotherapy in all stages of CRC. However, K-ras mutation status has emerged as a predictive marker to identify patients with m-CRC that may benefit from EGFR inhibitors, the introduction of the K-

ras mutation status for the prediction of resistance to anti-epidermal growth factor receptor (EGFR) antibodies (Chun, et al, 2009).

BRAF encodes a serine-threonine protein kinase that acts as downstream effectors of the K-ras signaling pathway. Various studies have revealed that an activating mutation of BRAF (BRAF V600E) occurs in about 34% to 70% of sporadic MSI-H CRCs and about 10% of unselected CRCs (Samowitz, et al, 2005).

In the adjuvant setting, BRAF mutation status appears to be a valid prognostic marker. Several studies, including two large retrospective series, have shown that BRAF mutations were associated with poor clinical outcome, especially in patients with MSS/MSI-L colon cancer, and a significantly high cancer-specific mortality (Ogino, et al, 2009, Roth, et al, 2010).

3.Loss of heterozygosity (LOH) has been implicated as an important mechanism of tumor suppressor gene inactivation. Chromosome 18q allelic loss is one of the well-studied molecular prognostic biomarkers, occurring in up to 70% of CRC, many retrospective studies have demonstrated that LOH 18q is associated with poor survival in advanced stage II and III CRC patients (Popat et al., 2005); there are limited studies in the literature on the predictive nature of LOH 18q with CRC treatment.

4.P53, a tumor suppressor gene, P53 protein binds to the regulatory sequences of a number of target genes to initiate a program of cell cycle arrest, DNA repair, apoptosis, and angiogenesis (Westra et al., 2005).

It is mutated in about 40% to 60% of CRC, TP53 in CRC often associated with the CIN phenotype and inversely correlated with the MSI tumor phenotype (Munro et al., 2005). TP53 mutation was associated with lower overall survival. TP53 protein expression and gene mutation have been associated with poor prognosis in colon cancer patients, although other studies report no prognostic value (Russo et al., 2005).

5.Thymidylate synthase (TS) plays an essential role in DNA synthesis by catalyzing the reductive methylation of deoxyuridylate (dUMP) to thymidylate (d-TMP). Inhibition of

TS by 5-FU (pyrimidine analog) blocks d-TMP production, and therefore rapidly shuts off DNA synthesis and repair, triggering apoptosis. Several studies, including a meta-analysis, have shown that high TS expression is associated with poorer overall and disease-free survival in CRC patients (Allegra et al., 2003). Many studies have shown that high TS expression is associated with longer survival in CRC patients receiving 5-FU-based adjuvant therapy. TS expression is currently not recommended for routine use as a prognostic or predictive marker in CRC (Edler et al., 2002).

6. VEGF. Patients with over-expression of VEGF-mRNA demonstrated poorer survival. Thus, VEGF is associated with the progression, invasion and metastasis of CRC. VEGF has become a promising target for therapeutic intervention (Ishigami et al., 1998, EL-Khoueiry et al., 2009).

7. Endothelial Growth Factor Receptor (EGFR). Oncogenic activation of intracellular signalling pathways downstream of EGFR has a major role in colorectal carcinogenesis but has also been reported to be an important mechanism of resistance to anti-EGFR antibodies. Among the activating mutations found in CRC, tumor with K-ras mutations, which are found in approximately 40% of the cases, have been widely demonstrated as a major predictive marker of resistance to cetuximab or panitumumab, therefore, opening the way to individualized treatment for patients with mCRC. Other oncogenic mutations, such as BRAF or PIK3CA mutations or loss of PTEN expression, may also be additional interesting predictive markers of response to anti-EGFR monoclonal antibodies but required further evaluation before being incorporated in clinical practice (Lievre et al., 2010).

BRAF mutation status – for anti-EGFR treatment: BRAF mutations have a strong prognostic significanse in chemo-refractory m-CRC, the evidence is accumulating for a predictive role of BRAF mutations as a marker for resistance and testing can be considered before an anti-EGFR antibody is considered. However, in earlier lines of treatment routine testing of BRAF mutations cannot currently be recommended (Saltz, et al, 2001).

8.Cytokeratin (CK). The relationship between primary colon cancer and occult nodal metastases (OMs) detected by cytokeratin immunohistochemistry (CK-IHC) is unknown. A study sought to investigate the correlation of clinicopathologicl features of colon cancer with OMs and to identify predictors of OM, Adverse primary pathologic colon cancer characteristics correlate with OMs. A study show at that patients with negative nodes on H&E and stage T3/T4 colon cancer, lymphovascular invasion, or high tumor grade, consideration should be given to performing cytokeratin (CK-IHC). The detection of OMs in this subset may influence decisions regarding adjuvant chemotherapy and risk stratification (Nabil et al., 2010).

The prognostic heterogeneity of advanced stages as stage III disease was addressed in the last TNM edition by stratifying patients into three sub-stages (IIIA–C), adopting criteria based on the depth of the intestinal wall involvement and the number of metastatic lymph nodes (Greena et al.,2008).

Table 2.6. Summary of prognostic biomarkers for colorectal cancer

Biomarker	Alteration	General comments
Microsatellite instability	MSI-H	MSI-H or dMMR tumors are associated with longer DFS and OS. Evidence favors MSI-H as a strong prognostic biomarker. NCCN and ASCO guidelines recommend testing for MSI status on stage II CRC.
K-ras	Mutation	Prospective studies regarding the prognostic value of *K-ras* mutation are inconsistent.
BRAF	Mutation	Several studies demonstrate that *BRAF* mutation status is associated with shorter OS in MSS/MSI-L and *K-ras* WT tumors; however, only the NCCN recommends *BRAF* mutation testing in *KRAS* WT mCRC.
Loss of heterozygosity 18q	LOH 18q	Prospective studies regarding the prognostic value of LOH 18q are inconsistent. The ongoing E5202 will help provide additional data.
TP53	Mutation	Prospective studies regarding the prognostic value of *TP53* mutation are inconsistent. EGTM and ASCO recommend against *TP53* mutation analysis for prognosis.
Thymidylate synthase	Overexpression	Prospective studies regarding the prognostic value of thymidylate synthase overexpression are inconsistent.
VEGF	Overexpression	Data on VEGF expression status are limited; therefore, more studies are needed to determine VEGF expression status as a prognostic biomarker.

ASCO American Society of Clinical Oncology; CRC colorectal cancer; DFS disease-free survival; dMMR defective mismatch repair; EGTM European Group of Tumor Markers; LOH loss of heterozygosity; mCRC metastatic colorectal cancer; MSI microsatellite instability; MSI-H microsatellite instability-high; MSI-L microsatellite instability-low; MSS microsatellite instability-stable; NCCN National Comprehensive Cancer Network; OS overall survival; WT wild typ.

9.CDX2 loss in CRC is independently associated with female gender, CIMP-high, high-level LINE-1 methylation, high tumor grade, and advanced stage. CDX2 loss may be

associated with poor prognosis among patients with a family history of colorectal cancer (Yoshifumi et al., 2009).

10.Ki67 antigen expression is one of the most widely used markers to evaluate the proliferation of tumor cells had significant prognostic value for colon cancer but could not be used alone to clearly discern among groups of patients with different prognosis, a multivariate analysis that over expression of Ki-67 carry poor prognosis (Ishida et al., 2004, Yifan et al., 2012).

11.SMAD4 loss is a poor prognostic indicator. The retention of the SMAD4 diploidy results in a three-fold higher benefit from 5-Fluorouracil therapy (5-FU) chemotherapy (Boulay et al., 2002).

12.P21-activated kinase 1 (PAK1) is a cyclin-dependent kinase upon which activation results in cell cycle arrest at the G1- to S-phase transition in mammals (Yifan et al., 2012). Its expression is predominantly induced by p53, and it is considered a mediator of the tumor-suppressor activity of p53. Tumors with positive pre-therapeutic p21 expression showed a better local tumor response, P21 is associated with colon cancer progression and metastasis, downregulation of PAK1 in colon cancer cells reduces total β-catenin level, as well as cell proliferation (Zhu et al., 2012).

14.E-cadherin; Loss of E-cadherin–mediated adhesion allows detachment from the primary site, impairment of cell adhesion, invasion of adjacent normal tissue, and distant dissemination. In CRC, aberrant E-cadherin expression has been correlated with tumor size, histopathologicl characteristics and differentiation. Furthermore, deregulation of E-cadherin is an early event in colorectal carcinogenesis (Markowitz et al., 2009).

15**β**-catenin ; Beta-catenin, a central molecule of the Wnt-signaling pathway. The Wnt/β-catenin signaling pathway is known to be activated in many malignancies, including colorectal cancer (Ishimoto et al., 2010). Epithelial cancers including the CRC are diseases driven by a small set of self renewing cells, termed cancer stem cells (CSC)

or cancer-initiating cells, which are distinct from the bulk of the cells in the tumor. Initially identified in hematopoietic tumors. CSCs share all the fundamental traits of stem cells-self renewal by asymmetric division, reduced proliferation and differentiation and resistance to apoptosis (Dick , 2008); Recent studies have reported the pivotal role of Wnt/β-catenin signaling pathway in the regulation of epithelial stem cell self renewal (Reya et al., 2001, Korkaya et al., 2009).

Expression of beta-catenin in CRC showed an association with better differentiation and earlier staging. Dys-regulation of Wnt/β-catenin signaling has been implicated in colon carcinogenesis. This signaling pathway also plays a critical role in regulating the proliferation of CSCs suggesting that Wnt/β-catenin signaling is intricately involved in the growth and maintenance of colonospheres of CSC (Shailender et al., 2010).

Epithelial-mesenchymal Transition (EMT), MET is believed to participate in the establishment and stabilization of distant metastases by allowing cancerous cells to regain epithelial properties and integrate into distant organs (Yang et al., 2008). Cancer cells employ developmental processes to gain migratory and invasive properties, the most known EMT markers include (E-cadherin/N-adherin/vimentin/fibronectin/actin/MMPs) and CD44.

16.CD44 is the transmembrane adhesion receptor for hyaluronan (HA) and plays a central role in the remodeling and degradation of HA that leads to cell migration, as well as to cancer invasion and metastasis. CD44 is highly expressed in primary and metastatic colon cancer but lowly expressed in normal tissues. CD44 is downregulated E-cadherin expression, upregulated N-cadherin, α-actin, vimentin, fibronectin and MT1-MMP, and inhibited the formation of the membrane-associated E-cadherin-β-catenin complex, which resulted in cell invasion and migration (Cho et al., 2012), The downregulation of the standard CD44 isoform (CD44s) in colon cancer is postulated to result in increased tumorigenicity (Suniti et al.,2011).

17.HuR is an mRNA stability factor that binds to the AU-rich element-containing 3' untranslated region of the transcript. HuR over-expression is associated with increased

tumor growth, increased cytoplasmic HuR expression occurs in colorectal cancer where it may contribute to the increased COX-2 expression observed during tumorigenesis. Some studies show that positive cytoplasmic HuR immunostaining is correlated with high COX-2 immunoreactivity in colon mucosa of FAP patients and in sporadic colorectal carcinomas (Lodewijk et al., 2008, Song et al., 2009).

18.MMP protein family is overexpressed by some tumor cells and is thought to enhance the tumor metastatic potential. Extracellular matrix (ECM) degradation is required for invasion and metastasis formation in colorectal carcinoma.

The probable role of MMPs in vivo is to degrade most components of the extracellular matrix (ECM). MMP-2, in particular, has a high activity against insoluble elastin which is a highly cross-linked ECM component of elastic connective tissues such as blood vessels. The ability of MMP-2 to degrade vascular basement membranes indicates a potential to facilitate hematogenous metastases. The levels of MMP-2 mRNA and protein are significantly increased in human CRC liver metastases compared with normal liver tissues. Pervious data showed that MMP-2 positivity in CRC tumors was associated with early recurrence and metastases (Oshima et al., 2008).

A study examined MMP-9 expression in tumors from CRC 360 patients who underwent bowel resection for stage II, III, IV tumor. Negative MMP-9 expression levels correlated with longer survival time as evaluated by disease-free survival and disease-specific survival. The detection of MMP-9 expression may be valuable in finding patients who are at high risk of developing disease recurrence (Riyad et al., 2010).

19.Vimentin, a major constituent of the intermediate filament family of proteins, is ubiquitously expressed in normal mesenchymal cells and is known to maintain cellular integrity and provide resistance against stress. It is recognized as a marker for epithelial–mesenchymal transition (EMT). Vimentin is overexpressed in various epithelial cancers; including colon cancer. Vimentin's overexpression in cancer

correlates well with accelerated tumor growth, invasion, and poor prognosis (Shirahata et al., 2009, Arun et al., 2011).

COX2

Cyclooxygenase-2 (COX-2, PTGS2) is the inducible isoform of cyclooxygenase, the enzyme that catalyzes the rate-limiting step in prostaglandin synthesis from arachidonic acid which converts arachidonic acid to prostaglandins and related eicosanoids, and promotes inflammation and cell proliferation (Brown et al., 2005, Buchanan et al., 2006) .

Various prostaglandins are produced in a cell type-specific manner. and they elicit cellular functions via signaling through G-protein coupled membrane receptors, and in some cases, through the nuclear receptor. COX-2 utilization of arachidonic acid also perturbs the level of intracellular free arachidonic acid and subsequently affects cellular functions. In a number of cell and animal models, induction of COX-2 has been shown to promote cell growth, inhibit apoptosis and enhance cell motility and adhesion. The mechanisms behind these multiple actions of COX-2 are largely unknown (Cao et al., 2002). A large number of observations emphasize that induced prostaglandin production, particularly PGE2, is involved in cell signaling through prostanoid receptors, suggested subtype EP2 receptor expression in colon cancer tissue to predict reduced survival (Gustafsson et al., 2007, Annika et al., 2011).

Structurally, the two cyclooxygenase enzymes, COX-1 and COX-2, are homodimers of two 70-kDa subunits related by a C2-axis of symmetry. Each monomer consists of a globular catalytic domain, a membrane-binding domain, and an epidermal growth factor (EGF) type domain. The catalytic domain of each COX monomer has two distinct active sites, a cyclooxygenase site and a heme-containing peroxidase site. The membrane-binding domain consists of a series of amphipathic α helices with several hydrophobic amino acids exposed to a membrane monolayer (Dong et al., 2011).

COX-1 and COX-2 are bifunctional enzymes that carry out two consecutive chemical reactions in spatially distinct but mechanistically coupled active sites. Both the cyclooxygenase and the peroxidase active sites are located in the catalytic domain, which accounts for approximately 80% of the protein. The catalytic domain is

homologous to mammalian peroxidases such as myeloperoxidase. The COX site converts arachidonic acid (5,8,11,14-eicosatetraenoic acid or AA) to the hydroperoxy endoperoxide product prostaglandin G2 (PGG2) via two highly regiospecific and stereoselective oxygen additions and two cyclization reactions . The hydroperoxide intermediate PGG2 then migrates to the peroxidase site, where it is reduced to the final product PGH2. The membrane-binding domain in each monomer consists of four amphipathic helices (helices A-D) arranged in a box-like configuration that anchors the protein to one leaflet of the lipid bilayer. The relatively large space enclosed by these four helices has been referred to as the “lobby” region . This membrane-associated domain provides a logical pathway for fatty acid substrates to travel from the membrane into the cyclooxygenase active site through a “gate” constriction, consisting of arginine, tyrosine and glutamate residues (Arg-120, Tyr-355 and Glu-524 using the ovine COX-1 numbering). This gate region engages the carboxylate of fatty acid substrates in a charge-reinforced hydrogen bond network that anchors the substrate in the active site (Kristina et al., 2006).

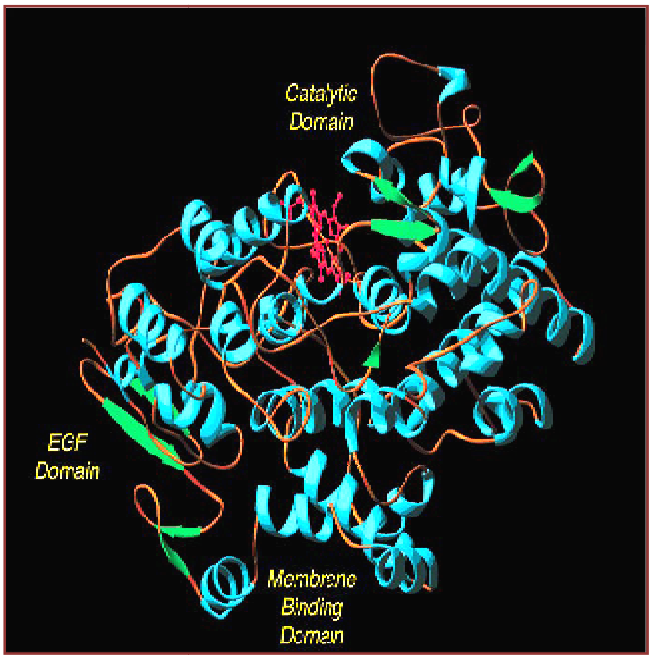


Figure 2. 16. Domain structure of COX enzymes(Dong et al.,2011).

COX-2 is overexpressed in the majority of human colon cancers supporting the importance of COX-2 in colorectal carcinogenesis (Zhang et al., 2002, Soumaoro et al., 2004).

Compelling evidence from genetic and clinical studies indicates that COX-2 upregulation is a key step in carcinogenesis. Overexpression of COX-2 has been shown to occur at multiple stages of colon carcinogenesis allowing for elevated prostaglandin synthesis to occur in the tumor microenvironment (Oshima et al.,2008), which is sufficient to cause tumorigenesis in animal models and inhibition of the COX-2 pathway results in reduction in tumor incidence and progression (Cao et al., 2002).

Toll-like receptor 4 (TLR4) and its role in CAC. TLR4 normally is expressed at low levels in the intestinal mucosa, whereas it is up-regulated in patients with IBD as in acute colitis. TLR4 is a potent inducer of Cox-2 expression. TLR4 also may be important for evasion of tumor surveillance altogether. These data raise the intriguing possibility that TLR4 promotes colon cancer in the setting of chronic inflammation and COX2 overexpression.

TLR4-dependent tumorigenesis also was associated with activation of EGFR signaling. These findings are significant because both Cox-2 and EGFR have been linked to the development of colon tumors (Masayuki et al., 2007).

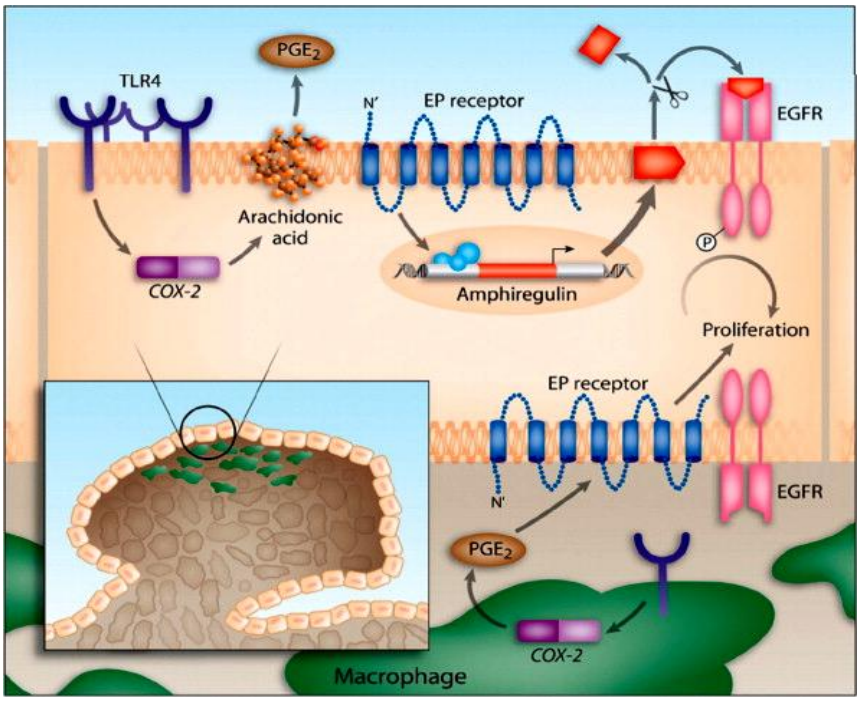


Figure.2.17. Model of TLR4-mediated colon carcinogenesis. TLR4 expression is increased in chronic intestinal inflammation. TLR4 signaling in response to LPS induces Cox-2 expression and PGE2 production. PGE2 through its receptors (ep) can act in a paracrine or autocrine fashion on colonocytes to stimulate the expression and release of amphiregulin, an EGFR ligand. EGFR signaling is associated with increased proliferation of colonocytes. Likewise, TLR4 expression in tumor-associated macrophages also may respond to LPS by inducing Cox-2 and PGE2, which then may act on the epithelium to stimulate proliferation of colonocytes (Masayuki et al., 2007).

A large body of evidence from population based studies, case control studies, and clinical randomized trials have demonstrated that aspirin and COX-2 selective inhibitors reduce risk of recurrent adenoma among high-risk patients (Arber et al., 2006, Flossmann et al., 2007). The regular use of NSAIDs over a 10–15 year period reduces the relative risk of developing CRC by 40–50%, Potential for application of non-steroidal anti-inflammatory drugs as well as the recently developed COX-2 specific inhibitors in cancer clinical practice has drawn tremendous attention in the past few

years. Inhibition of COX-2 promises to be an effective approach in the prevention and treatment of cancer, especially colorectal cancer. Both primary and secondary prevention with cyclooxygenase (COX) inhibitors demonstrate and confirm decreased incidence of colorectal carcinoma in both retrospective and randomized patient cohorts (Cahlin et al., 2005).

Furthermore, use of NSAIDs leads to regression of pre-existing adenomas in patients with familial adenomatous polyposis (Wolfe et al., 1999).

As many other human cancers are reported to have elevated levels of COX-2 and overproduce PGs, there is great interest in evaluating the role of NSAIDs for prevention and treatment strategies for other cancers such as breast, stomach, pancreas, urinary tract, lung, and prostate. However, the prolonged use of NSAIDs is associated with side effects such as nausea, dyspepsia, gastritis, abdominal pain, peptic ulcer, GI bleeding, and/or perforation of gastroduodenal ulcers (Vane et al., 1998). It was hypothesised that NSAIDs exert their anti-inflammatory and antitumour effects through inhibition of the inducible COX-2 (Grover et al., 2003), while unwanted side effects of these drugs such as damage to the gastric mucosa and GI bleeding are thought to arise from the inhibition of the constitutive COX-1.

Several factors of immune response as serpin peptidase inhibitor and inducible metric oxide synthase 2 with antitumoral activities were either up- or down-regulated in normal colon tissue from patients with tumors of high COX-2 express.

Despite the well-accepted role of COX-2 in tumor development, studies are conflicting regarding prognostic significance of COX-2 in colorectal cancer with some supporting (Gustafsson et al., 2007) and others (Fux et al., 2005, Yamac et al.,2005, Lim et al., 2008) refuting.

COX-2 overexpression has been positively associated with p53 alteration (Ogino et al., 2006), and inversely associated with microsatellite instability (MSI), (CIMP) and BRAF

mutation, which generally predicts longer survival of colon cancer patients (Popat et al., 2005).

Moreover, COX-2 and p53 appear to regulate each other in a complex manner (Benoit et al., 2006). Thus, effect of COX-2 on patient survival can possibly be confounded by p53 alteration, MSI and other related molecular events.

Investigation into the value of COX-2 as a predictive marker is especially important given the ready availability of drugs with COX-2 inhibitory activity such as celecoxib and aspirin. Recently, it was shown in a large prospective study of 1279 patients with stage I to III CRC that regular aspirin use after diagnosis was associated with a lower risk of CRC-specific mortality, especially among patients who had primary tumors that overexpressed COX-2 (Chan et al., 2009).

Large difference in gene expression was observed when comparing tumor tissue with high COX-2 expression to normal colon mucosa tissue from the same patients. A large number of genes with altered expression appeared also in colon cancer tissue of tumors with high COX-2 expression when compared to tumors with low COX-2 expression. Expression of COX-2 in normal colon mucosa tissue was significantly increased in patients with tumors of high COX-2 expression compared with mucosa from patients with low COX-2 expression in tumors. Highly expressed genes in tumor tissue with high COX-2 expression were associated to cell motility, cell structure, muscle proteins, and energy homeostasis while down-regulated genes in such tumors seemed to be related to tumor antigens. However, COX-2 expression predicted survival at borderline significance in a larger patient material (Greenhough et al., 2001).

CHAPTER III

Result

&

Discussion

3.1 Patients and Methods

The records of all diagnosed colorectal cancer cases between January 2007 to December 2011 based on availability of representative paraffin blocks were retrieved in the files of Histopathology Department, Benghazi University, 83 Libyan patients (42 male, 41 female) were diagnosed with CRC, for each patient, we collect the following data: age, gender, date of diagnosis, histological grade, stage and type of the tumor, tumor size, metastasis, vascular invasion, and also we reviewed the patients files on the Oncology Department, Benghazi Medical Center, for gathering further information as the surgical intervention, initial presentation and the follow up (the months of oncology clinic visit and the last state of the patient dead or alive).

Paraffin blocks and the H&E stained slides of these patients have were collected. Diagnosis and the grading was confirmed by an expert pathologist to detect the histological type of the tumor (adenocarcinoma, mucinous or signet ring) and grade (well, moderately, poorly differentiated tumor) and according to the TNM system the tumor stage obtained from the oncologist description.

The following table summarizes the clinicopathological parameters of the studied patients.

Table 3.2.1. Clinicopathological parameters of the studied patients

Data	No. of the patients	(%)
Age (yrs)		
< 55 years	40	(48.1%)
≥ 55 years	43	(51.8%)
Gender		
Male	42	(50.6%)
Female	41	(49.3%)
Localization		
Ill-defined	3	(3.6%)
Rt colon	17	(20.4%)
LT colon	40	(48.1%)
Rectum	23	(27.7%)
Histopathological type		
Adenocarcinoma	74	(89.1%)
Mucinous adenocarcinoma	7	(8.4%)
Signet ring carcinoma	2	(2.4%)
Lymph node involovment		
Nx	16	(19.2%)
N0	34	(40.9%)
N+	33	(39.7%)
Metastasis		
Mx	4	(4.8%)
M0	58	(69.8%)
M+	21	(25.3%)
Histological grade		
Grade I	25	(30.1%)
Grade II	50	(60.2%)
Grade III	8	(9.6%)
Lympho/vascular invasion		
Yes	77	(92.7%)
No	6	(7.2%)
Primary tumor status		
T1	1	(1.2%)
T2	17	(20.4%)
T3	48	(57.8%)
T4	14	(16.8%)
Tx	3	(3.6%)
Survival months		
< 35 months	40	(48.1%)
≥ 35 months	43	(51.8%)
Recurrence during follow up		
Yes	7	(8.4%)
No	76	(91.5%)
Unkown	0	(%0)

COX2 Immunostaining

Formalin-fixed, paraffin-embedded primary colorectal tumor tissue was obtained from 83 patients. Sections were cut serially at 5 mm for routine HE staining and for immune-histochemical (IHC) analysis. IHC analysis was done using an automatic system (Bench-Mark XT; Ventana Medical Systems, Inc. Tucson, AZ, USA). This fully automated processing of bar code-labeled slides included baking of the slides, solvent-free de-paraffinization, antigen retrieval in a cell conditioning buffer CC1 (Mild: 36 min conditioning and standard: 60 min conditioning), incubation with (the monoclonal anti-COX2 antibody (clone ECH-6; Ventana Medical Systems), for 32 min, at 37 _C. Application of ultra-ViewTM Universal DAB (a biotin-free, Multimer-based detection system for the specific and sensitive detection of rabbit IgG, mouse IgM, and rabbit IgG primary antibodies). UltraView DAB includes: ultraView Universal HRP, ultraView Universal DAB Inhibitor, ultraView Universal DAB Chromogen, ultraView Universal DAB H_2O_2, and ultraView Universal DAB Copper. Counterstaining with hematoxylin (2021) took 4 min, and post-counterstaining with bluing reagent (2037) took four mintes as well. After staining, the sections were dehydrated in ethanol, cleared in xylene, and covered with Mountex and cover slips.

Cytoplasmic COX-2 expressed in the tumor as absent, weak, moderate, or strong staining compared with adjacent normal colonic epithelium. Inflammatory cells served as internal built-in positive controls, Cells were considered positive for COX-2 when distinct cytoplasmic yellow to brown staining was identified. When immunostaining intensity was moderate or strong, tumors were classified as cancers with COX-2 overexpression. When immunostaining intensity was weak or absent, tumors were classified as cancers with negative COX-2 expression. The extent and intensity of the staining were recorded on a scale from 0 to +++; +++ implied strong staining that was maximally intense throughout the specimen, and 0 implied negative staining. When dichotomized for statistical risk assessment [odds ratio (OR) calculation], negative (-)

and weak (+) staining were defined as low expression, whereas moderate (++) and intense (+++) staining were included in the high expression category.

Evaluation of COX2 staining:

Evaluation of the stained slides was performed with a light microscope at the magnification of 4x, 10x, 40x, blinded by the information on tumor grade, stage or clinical outcome, as the COX2 show cytoplasmic staining so, cytoplasmic staining was also graded into four categories: (0) Negative, no detectable staining(no brown), (1) Weak, but still detectable staining (light brown), (2) Moderate, clearly positive but still weak (dark brown) (3) Heavy staining (intense brown) ,(Elzagheid et al, 2006).

The cytoplasmic index was calculated with both the intensity of the staining and the fraction of positively- stained cells taken into account using the following formula:

$I = 0 * f0 + 1 * f1 + 2 * f2 + 3 * f3$

Where the staining index and f0-f3 are the fractions of the cells showing a defined level of staining intensity (from 0 to 3). Theoretically, index scores could vary between 0 and 3.

Statistical analysis:

Statistical analyses were performed using the SPSS® (SPSS, Inc., Chicago, USA) and STATA (Stata Corp., TX, USA) software packages (SPSS for Windows, version 12.0.1 and STATA/SE 9.1). Frequency tables were analyzed using the Chi-square test, with the likelihood ratio (LR) or Fisher's exact test being used to assess the significance of the correlation between the categorical variables. Differences in the means of continuous variables were analyzed using non-parametric tests (Mann-Whitney) or Kruskall-Wallis for 2- and K-independent samples respectively.

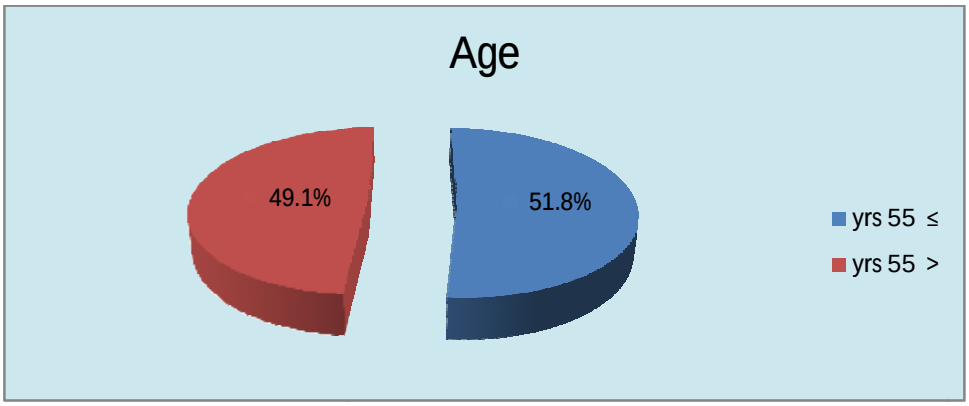
Analysis of variance was only used to derive the mean values (and 95% CI) of each individual stratum. Univariate survival analysis for the outcome measure [disease-specific survival (DSS) and disease-free survival (DFS)] was based on the Kaplan-Meier method, with log-rank (Mantel-Cox) comparison test. To assess the value of COX-2 as an independent predictor, multivariate survival analysis was performed, using the COX proportional hazards regression model, controlling for the confounding by: age, sex, tumor localization, tumor stage, grade (for DFS), and recurrence as additional variable for DSS. In all tests, $p < 0.05$ was regarded as statistically significant.

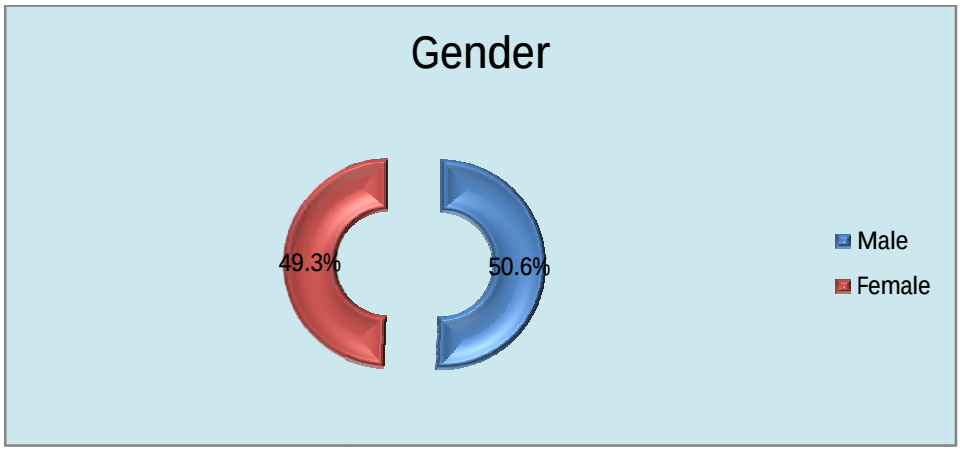
The log-rank test was used to examine the significance of the differences between the curves. Univariate and multivariate analyses were carried out on all patients and on subgroups classified by patient age (below and above 55 years) and by lymph node status. The results were analyzed with the SAS system for Windows release 6.12 (SAS Institute, Inc., Cary, NC, USA).

3.2 Results

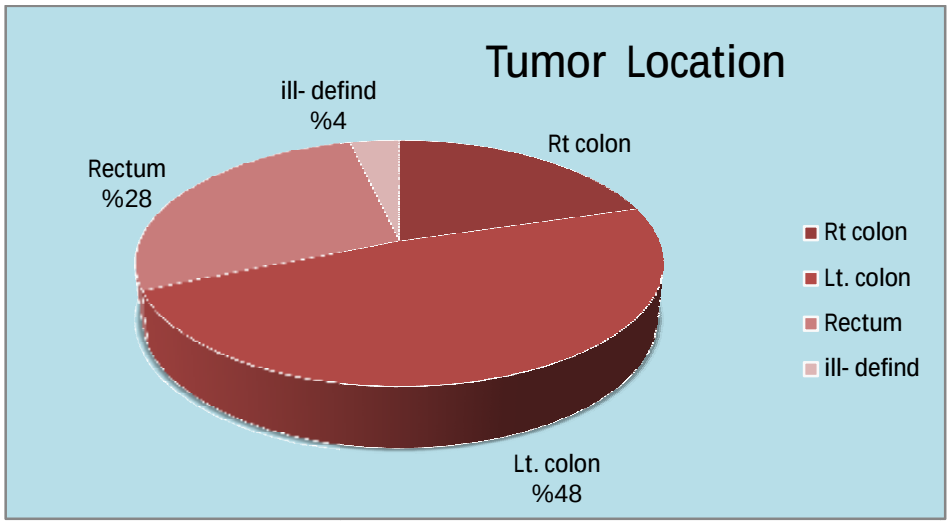
3.2.1 Clinicopathological results

A total of 102 patients' collected specimen. Only 83 were included in the study as the availability of the processed immunostained slide. Among these 83 patients 40 of them (48.1%) were 55 years while 43 patients (51.8%) were 55 years or older, the mean age was 55 years, The number of the male patients 42 (50.6%) are slightly more than female patients 41 (49.3%).

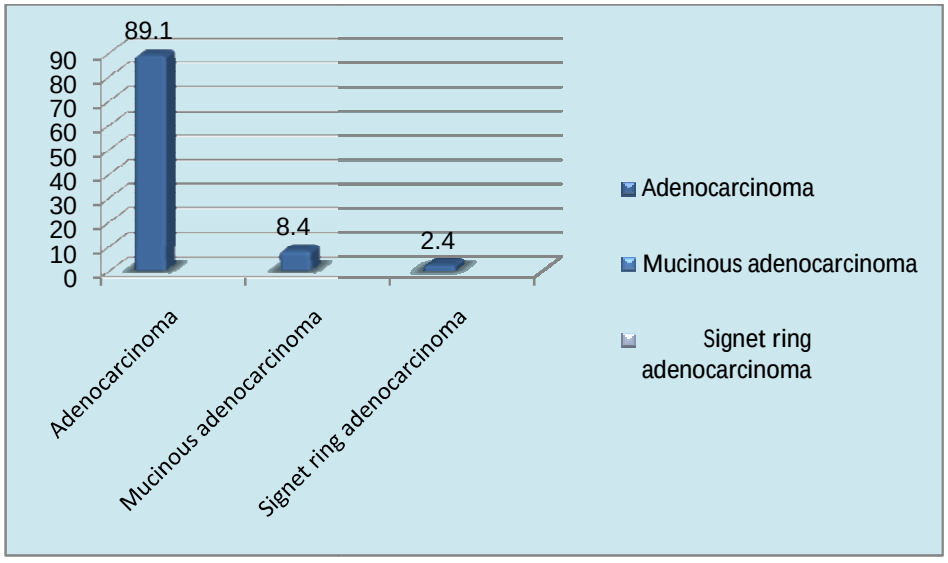




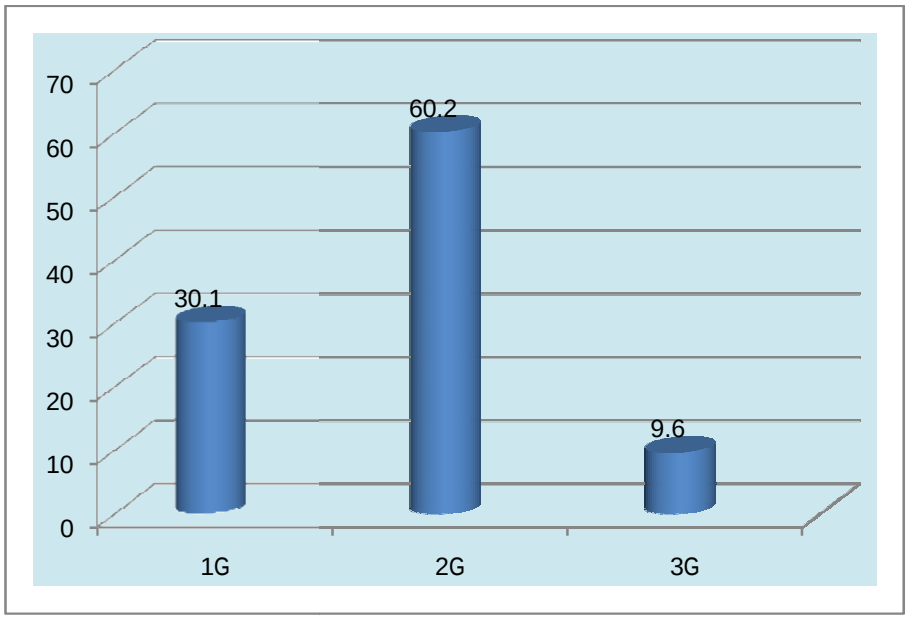
Most of the patients were having tumor on the left Colon 40 (48.1%), 23 on the rectum (27.7%) and 17 of the cases on the Rt. colon (20.4%), the rest 3 patients; their tumor location was not included in their data.



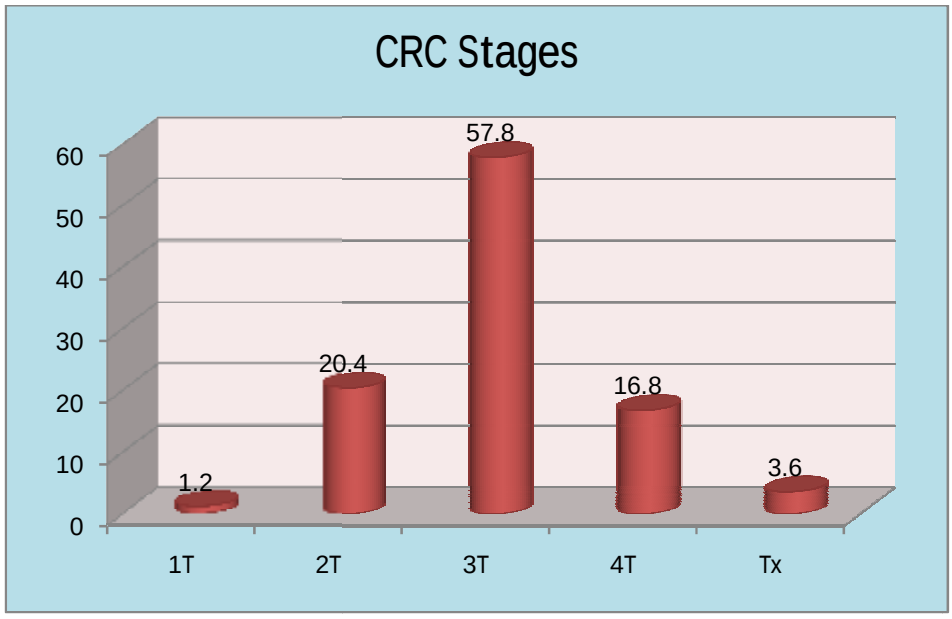
The most diagnosed histological type is adenocarcinoma with about 74 (89.1%), then mucinous adenocarcinoma seven patients (8.4%), and only two cases with signet ring cell adenocarcinoma (2.4 %).



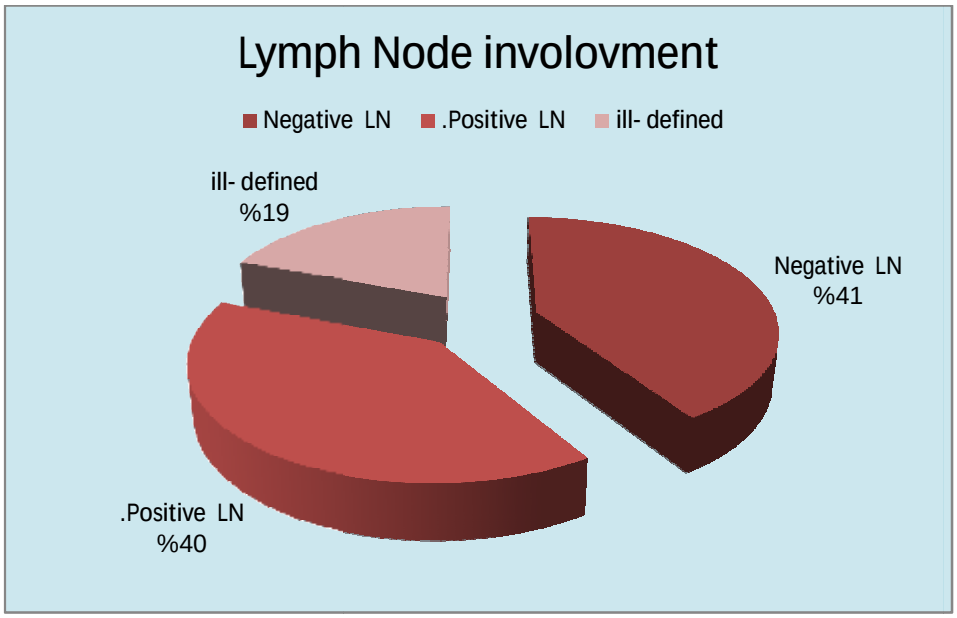
The histological grade of the patients are mainly moderate grade G2 50 (60.2%), well-differentiated G1 25 (30.1%), and poor differentiated G3 8 (9.6%) .



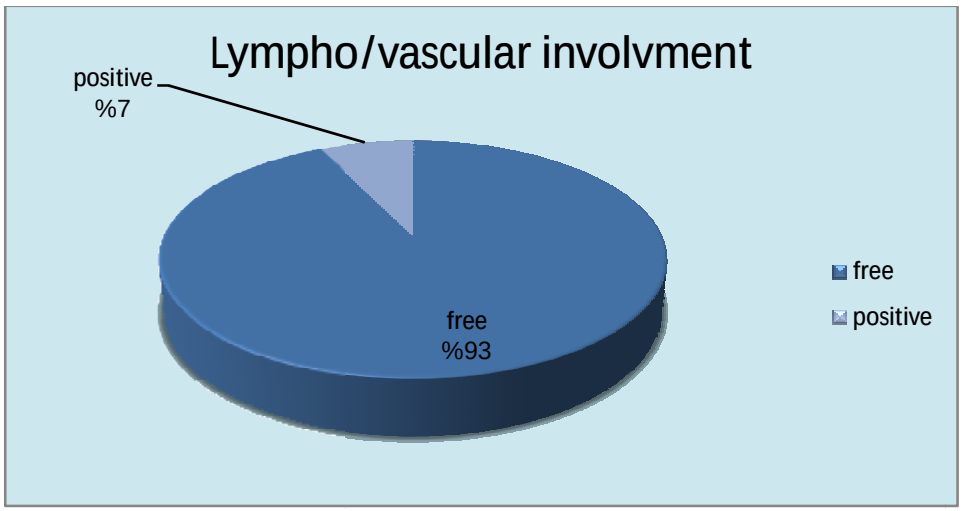
Among the 83 patients, 48 of them (57.8%) were of T3 stage by the (TNM classification), 17 (20.4%) of T2, 14 (16.8%) of T4, and only one patient of stage T1 (1.2%), the rest 3 patients their stage were not identified (3.6%).



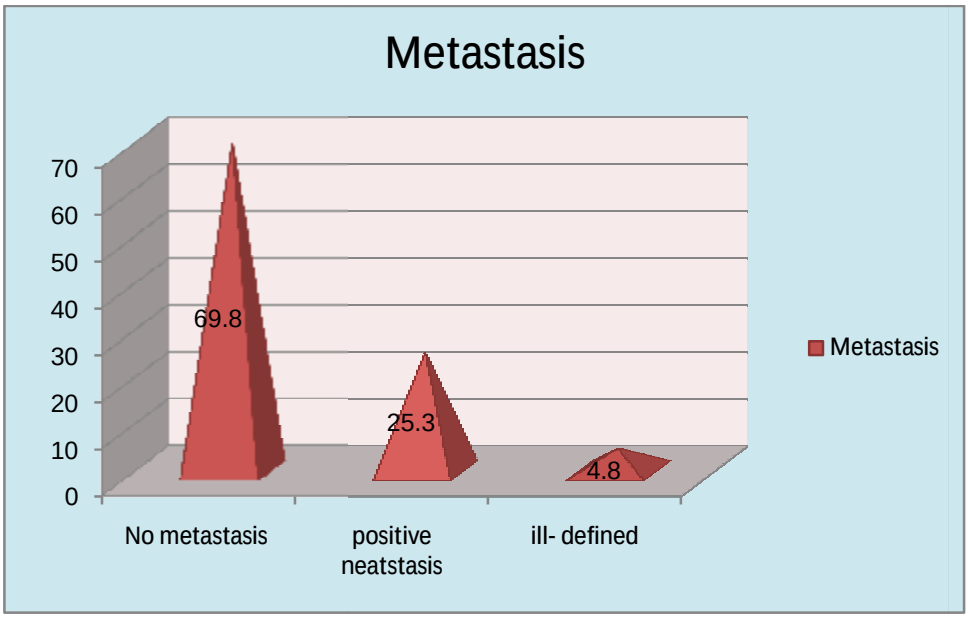
The number of the patients having positive lymph node metastasis at the time of the diagnosis were 33 (39.7%), and about 34 (40.9%) of them without lymphatic spread, 16 patients (19.2%) their lymphatic status were not detected.

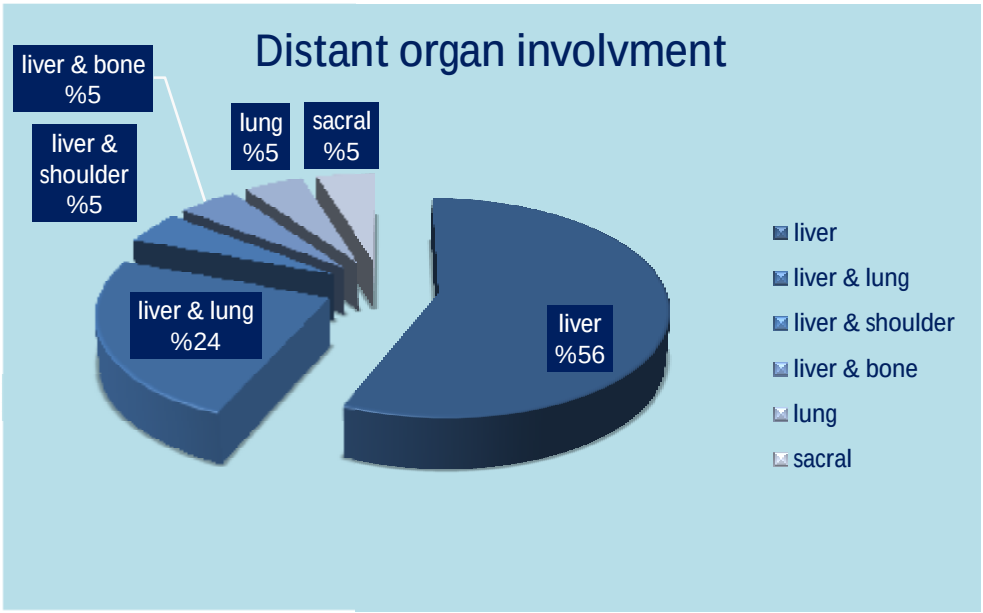


Lympho/vascular invasion was present on six patient s only (7.2%) and free on the rest 77 (92.7%).



Most of the patients presented without distant metastasis 58 (69.8 %), four patients (4.8%) were unknown wheather they have metastasis, 21 (25.3%) patients had distant organ involvement mainly liver alone 12 (14% of the total), both liver and lung 5 (6%), both liver and bone one patient (1.2%) . The same for lung alone, sacral involvement, both liver and shoulder.





The overall duration of the follow up was four years . At the end of the follow up only one patient died (1.2%), and the rest 82 were still alive (98.7%), 76 patients (91.5%) have no recurrence and only 7 (8.4%) relapse.

3.2.2 Statistical results

COX-2 expression profiles

A total of 83 patients with CRC were included in the study, but nine patients of them were excluded from the immunostaining result because of technical defect in tissue processing.

Normal colorectal mucosa showed no or weak COX-2 expression, but weak cytoplasmic expression was detected in a few inflammatory mononuclear cells. In cancer cells, COX-2 expression appeared as yellow-brown staining and was observed mainly in the cytoplasm and occasionally in the nuclear envelope. Expression was high in 51 (68.9 %) cases and low or absent in 23 cases (31%).

The following table 3.2.2. show the number of the cases which included in the immunostaining study:

Table 3.2.2 key features of CRC patients

Features	Number of cases (%)
Age	
< 55 ys	37 (50.0%)
≥55 ys	37 (50.0%)
Gender	
Male	36 (48.6%)
Female	38 (51.4%)
LN status	
+ve	30 (50.8%)
-ve	29 (49.2%)
Lympho/vascular invasion	6 (8.1%)
+ve	68 (91.9%)
-ve	
Stage	
T1	1 (1.4 %)
T2	17 (23.9%)
T3	42 (59.2%)
T4	11 (15.5%)
Grade	
Well	22 (29.7%)
Moderate	46 (62.2%)
poor	6 (8.1%)
Location	
Rt. colon	13 (18.1%)
Lt. colon	38 (52.8%)
Rectum	21 (29.2%)
Recurrence	
Yes	6 (8.1%)
No	68 (91.9%)
Diagnosis	
Adenocarcinoma	66 (89.2%)
Mucinous ad.	7 (9.5%)
Signet-ring cell ad.	1 (1.4%)
Tumor size	
< 5cm	51 (68.9%)
≥ 5 cm	23 (31.1%)

Correlation of COX-2 expression with the clinicopathological features

The associations between COX-2 expression and clinicopathological features are presented in the Table 3.2.3.

Table3.2.3. Expression of COX2 in Libyan CRC patients as related to clinico-pathological data and disease outcome. The relationship was preformed on different cut-off points, Figures presented are p- value of relation between clinico-pathological features and COX2 at different cut-off points.

Feature	Mean (1.24)	Median (1.40)	0,1 vs 2,3	0 vs 1,2,3
Age	0.234	0.351	0.079	0.645
Gender	0.315	0.236	0.550	0.387
Tumor size	0.010	0.025	0.037	0.221
Tumor location	0.248	0.373	0.215	0.396
Grade	0.832	0.979	0.986	0.336
Stage (T)	0.579	0.495	0.886	0.978
Diagnosis	0.441	0.546	0.324	0.900
Lymphovascular invasion	0.150	0.055	0.090	0.064
Lymph node (N)	0.673	0.514	0.861	0.520
Status at end point	0.419	0.353	0.499	0.726
Recurrence	0.759	0.518	0.426	0.347

Significant and border- line results are shown in BOLD.

Gender, grade, tumor location, recurrence and status at end point had no significant relationship with the expression of COX2. However, the present study showed that, the age of the patient, the tumor size and the vascular invasion were significantly associated with COX2 expression, as we found less expression being more common in large tumor size (> 5 cm) (9/23) which expressed less COX2 than smaller size tumor (<5cm) (36/51) (*P< 0.01),* and with the presence of lympho/vascular invasion there was less expression (*P*< 0.055) in that (1/6) of tumor with the invasion tested positive for COX2 expression.

There were borderline association between age of the patient and expression of COX2 (*P< 0.07);* 43% of older age group ≥ 55yrs tested positive for COX2, whereas 65% of the cases with no COX2 expression.

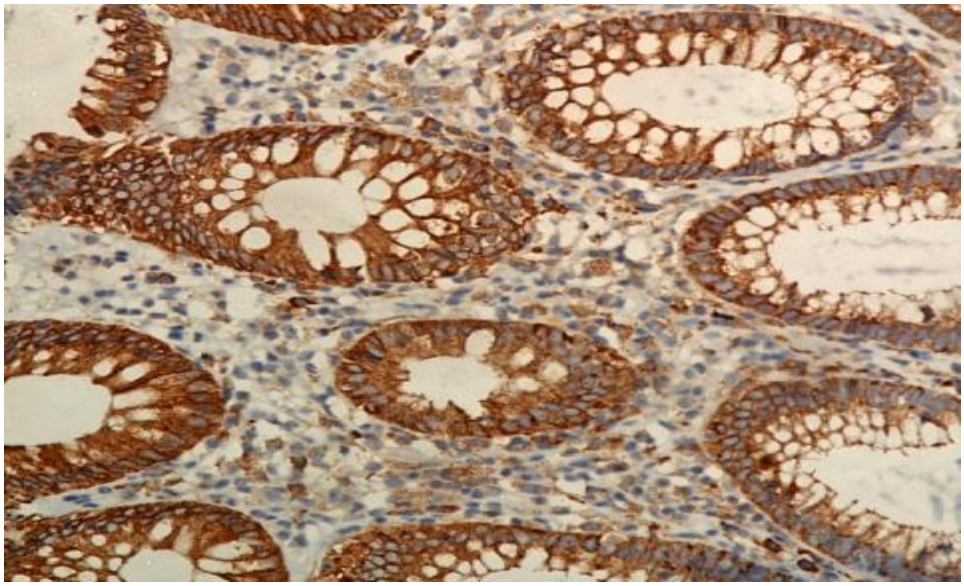


Figure. 3.1. COX2 cytoplasmic expression in normal colonic epithelium (20X HPF, obtained from our result).

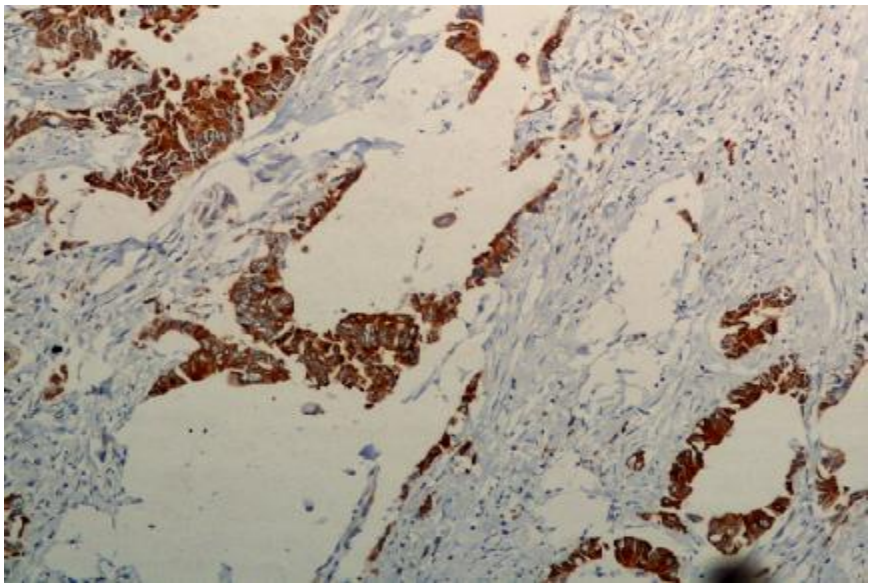


Figure 3.2. Strong cytoplasmic COX2 expression in colorectal cancer (20X HPF , obtained from our result)

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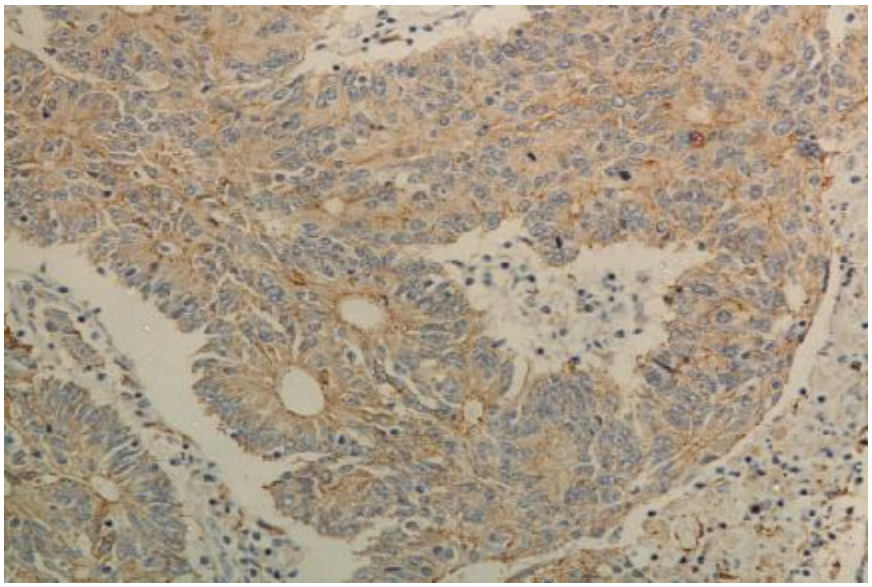

Figure 3.3. Moderate cytoplasmic COX2 expression in colorectal cancer (20X HPF, obtained from our result).

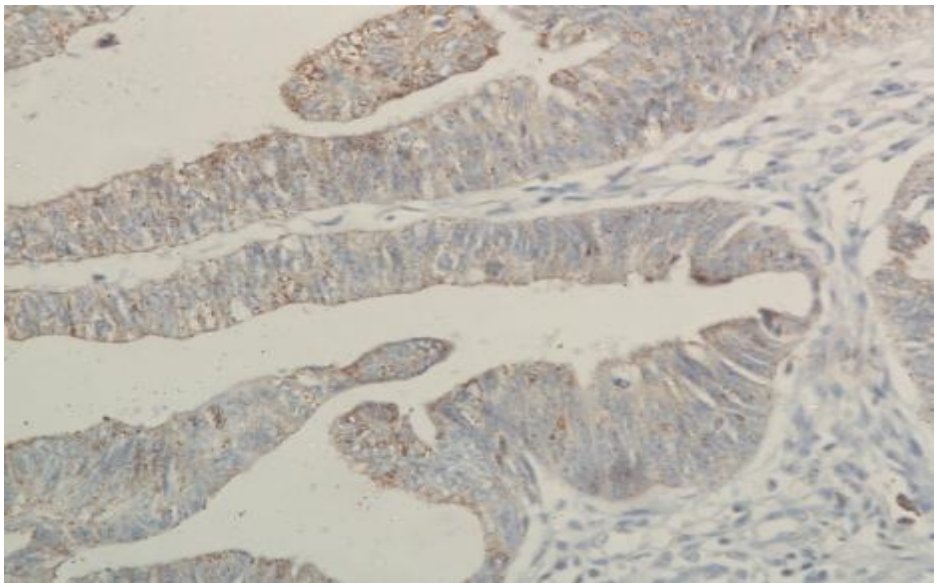

Figure 3.4. Weak COX2 cytoplasmic expression in colorectal cancer (20X HPF, obtained from our result).

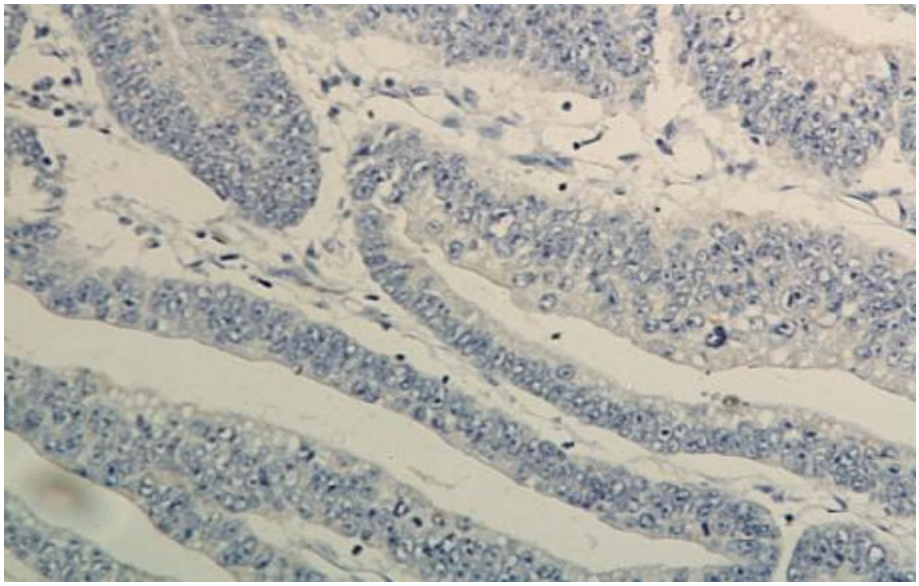

Figure 3.5. Negative COX2 expression in colorectal cancer (20X HPF, obtained from our result

Survival analysis

In Kaplan- Meier survival analysis, there was no clear significant difference in DFS between patients with COX2 positive tumor and those with COX2 negative tumor (*P= 0.11) (at 0,1 vs 2,3 as cut-off point)*, 85% of patients with negative COX2 express tumor at 3 years follow up showed longer disease free survival in comparison with 65% of patients express positive COX2 tumor.

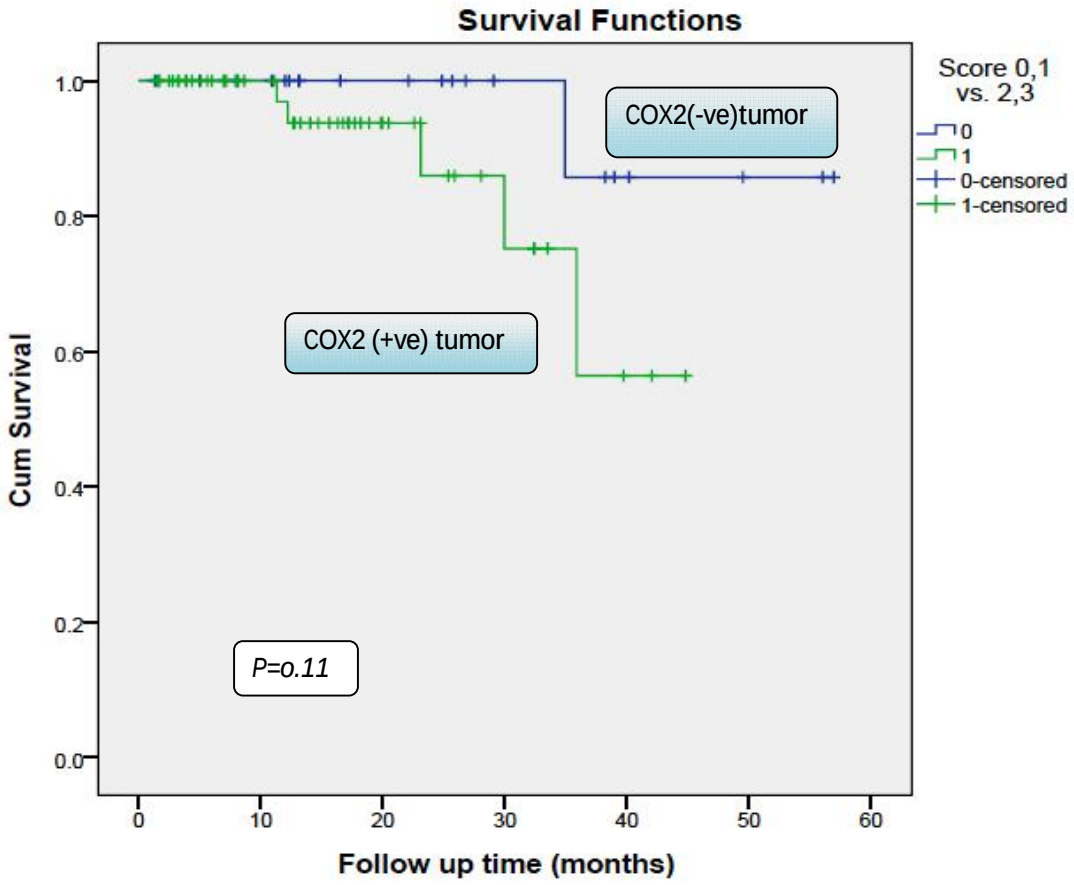

Figure 3.6 Disease- free survival (DFS) related to COX2 expression below and above tire (0,1 Vs 2,3 as cut-off point).

At another cut-off point (0 vs 1,2,3), we find that tumor with more COX2 expression has less DFR than other with tumor negative for the COX2 expression; although these finding there was no statistical significant (*P= 0.211)*, in which approximately 99% of the negative COX2 tumor has more DFR in compare to 63% of the positive COX2 tumor at four years of follow up.

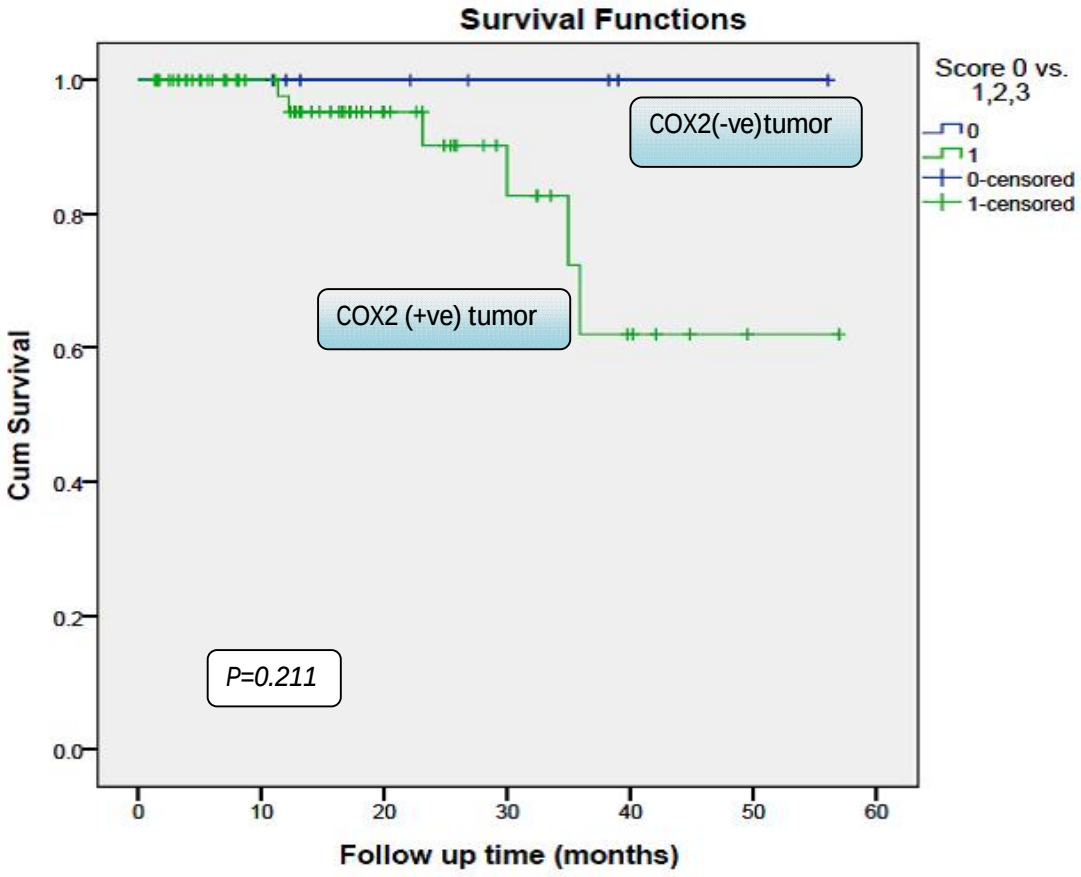

Figure 3.7. COX2 expression as determinant of disease free survival (DFS) in Kaplan-Meier analysis of CRC patients.

To assess the value of COX-2 as an prognostic factor, a multivariate survival analysis was done, using the COX proportional hazards regression model controlling for confounding by age, sex, tumor localization, stage, grade, (for DFS), and recurrence as additional variable for DSS. In the final multivariate model, patient gender with (*P =0.002)* means that female patients have more DFR than male patients.

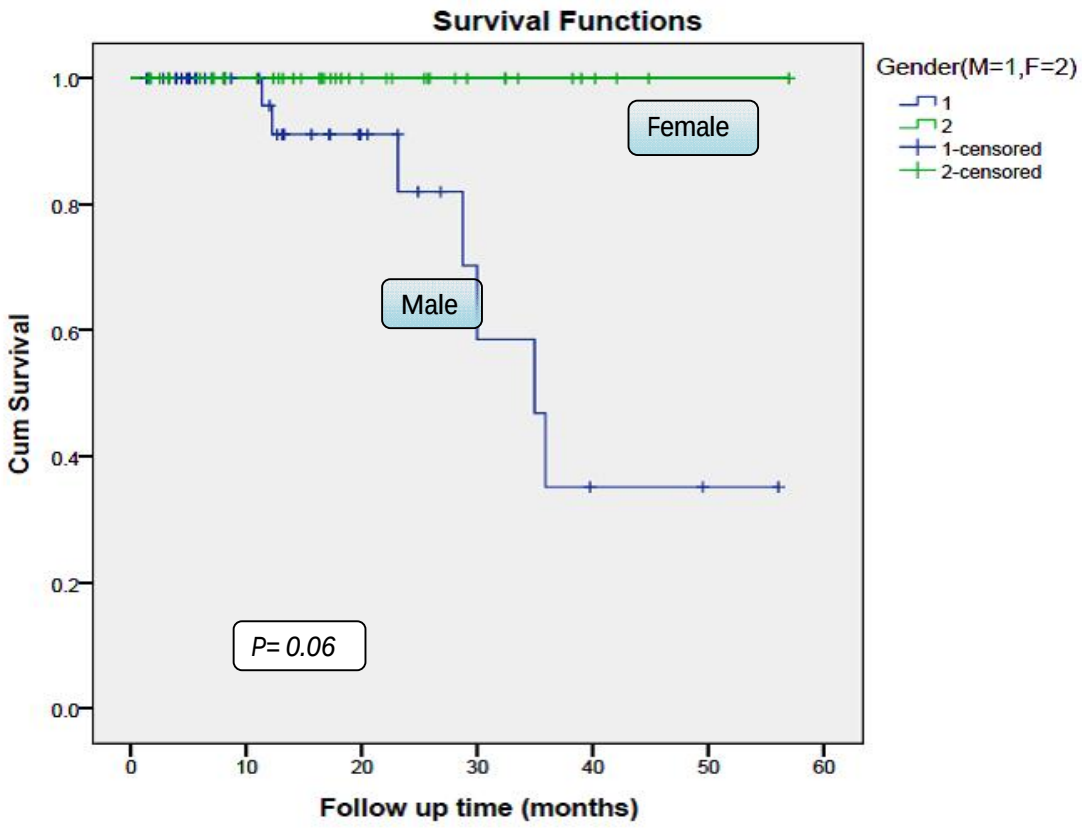

Figure 3.8. Disease free survival predicted by gender of patient.

The survival analysis in relation to the tumor location by the Kaplan-Meier analysis showed that a difference (*P< 0.066)* in DFR between different tumor location, left colon show more DFR than right colon and rectum.

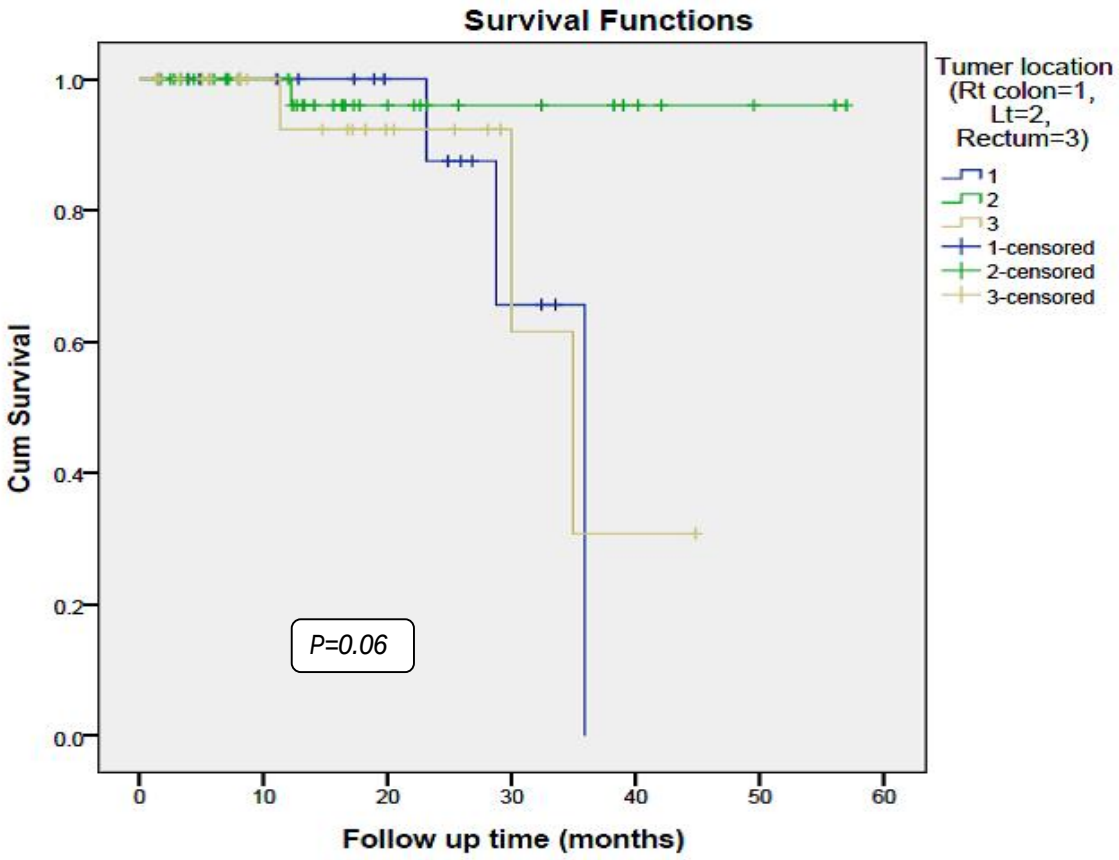

Figure.3.9. Tumor location as variant of DFR in Kaplan-Meier analysis of CRC patients.

DISSCUSION

Every year in the world, there is more than one million cases of CRC are diagnosed and 600,000 patients die of the disease, making it the second leading cause of death from cancer in adults. The disease progress from benign adenomatous polyp, which develops into advanced adenoma with high grade dysplasia and then progress to an invasive cancer and this occur on the basis of molecular changes and genetic alteration that affect on the normal cell proliferation and differentiation. Much attention has been focused on the involvement of cyclooxygenase COX in tumor development and progression (Soumaoro et al., 2004).

The clinical behavior of a CRC results from interaction at many levels. The challenge nowadays are to understand the molecular basis of individual susceptibility to CRC and to determine factors that initiate the development of the tumor, its progression, determine its responsiveness to different therapeutic agents; and all of these require further specification of the tumor behavior by study its expression to different prognostic and predictive biomarkers (Sanford et al., 2009).

COX-2 pathway is important in cancer development because it is involved in the regulation of various critical cellular processes such as tumor progression, metastases, angiogenesis, and chemotherapy resistance (Peter et al., 2009). Elevated COX-2 expression has been associated with poor prognoses in CRC and other cancers, such as breast, head and neck, lung, and cervix carcinomas (Konno et al., 2002).

The aim of the current study were to concern on the issues related to prognosis of colorectal cancer in Libyan patients and assess the value of COX-2 expression as prognostic marker and find its relation to the traditional prognostic clinicopathological parameters of the patients (Shuji et al., 2008), we found that COX2 overexpression related to decrease on the cancer free survival, so it has adverse effect on the outcome of colon cancer patients and as the best of my knowledge this is the first study for the COX2 expression of Libyan patients.

COX2 has cytoplasmic expression on the malignant cell, although many of the studied specimens showed also cytoplasmic expression on the normal colonic tissue and some inflammatory cells (Dominique et al., 2003, Sung et al., 2009).

In our study, 68.9% of the primary CRCs have high to moderate COX-2 expression, whereas previous studies have shown that COX-2 was overexpressed in 56 -80 % of CRCs (Lim et al., 2010 ,Jaudah et al., 2012).

COX-2 has been examined as a prognostic biomarker in cancer, previous studies are conflicting regarding prognostic significance of COX-2 in colorectal cancer with some (Soumaoro et al., 2004, Gustafsson et al., 2007) supporting and others (Zhang et al., 2002, Wu et al., 2003, Lim et al., 2008) refuting independent adverse effect of COX-2, some other suggest that the expression of COX-2 protein has no significant impact on the outcome of patients with colorectal cancer (Zafirellis et al., 2008). These discrepant results are likely due to differences in patient cohorts, COX-2 detection methods, criteria of evaluation for COX-2 overexpression, and multivariate survival analysis models. Our current study has comprehensively examined the effect of COX-2 on patient survival on dependent of clinical characteristics.

The prognostic relation of COX2 has been studied previously ,and many of them showed that positive expression of COX-2 was more common among advanced stage tumors than in early stage tumors (Lim et al., 2010) who showed that COX-2 expression was correlated with the depth of invasion and advanced tumor stage, and this somewhat correlate with results of the current study ($P < 0.45$), in which 59.2% were presented on stage III (T3), 24% on T2 and 15.5% on T4, suggesting that COX2 participating in tumor progression.

Many studies confirm independent prognostic features of COX2. It may be possible that COX-2 and p53 regulate each other to form a feedback loop. Thus, it may not be surprising to find a significant interactive effect of COX-2 and p53 alterations on patient survival (Ogino et al., 2006, Shuji et al., 2008).

One of the most important observations of the present study is that linking COX-2 expression with disease outcome, i.e. disease recurrence and length of DFS, we found that patients with negative COX2 expression has more survival rate 85% at 4- years interval, in which those with overexpression has 55% DFR as shown in figure 3.7. This is in agreement with the study by (Wan et al., 2009, Jaudah et al.,2012), DFS time being significantly shorter for patients with high expression of COX-2 compared with low expression. These important results suggest that selective COX-2 inhibitors might be useful chemopreventive agents, not only in growth of the primary tumor but also for prevention of hematogenous metastasis of CRC. When adjusted for other potential predictors in multivariate Cox regression model, COX-2 expression lost its value as a significant independent predictor of DFS.

The age of the patients at presentation play an important role in the long term outcome, Colorectal cancer predominantly occurs in the elderly, some study showed that less than 3% of the patients are younger than 40 years (Charles et al., 2011), in study done in Egypt is also found that 38% of the cases are less than 40 years (Ahmed et al., 2002), in Saudi Arabia same conclusion has been obtained (Ibrahim et al, 2002) and 20% of patients had less than 45 yrs (Mufid et al.,2007), in our study 40% were younger than 55 year which is represent the mean age of our patients, which indicate an early onset of the tumor in our population than other developed countries .

Previous studies showed that young patients develope more aggressive behavior of the tumor, Young patients had less stage I or II disease, more stage III or IV disease, and worse-grade (poorly differentiated or anaplastic) tumors, found that young colon cancer patients tend to have later-stage and higher-grade tumors (O'Connell et al., 2004), young age is modestly associated with poorer progression free survival, but not overall survival or response rate in treated patients with a CRC, and young patients have more nausea but less diarrhea and neutropenia with chemotherapy in general. Young versus older patients derive the same benefits from combination

chemotherapy. Absent results of a clinical trial, standard combination chemotherapy approaches are appropriate for young patients with a CRC (Charles, 2011).

Several studies have also shown that old age is an independent prognostic factor associated with poor prognosis in CRC patients (Butfalari et al., 2006). population-based study shows that young rectal cancer patients seem to have equivalent overall and stage-specific survival as older patients (O'Connell et al.,2006), Our data on the current study showed that loss of COX2 expression more frequently detected on older age group (22/37), in which (29 /37) on younger group and that is not matched with many of pervious study, some other study show no correlation between COX2 expression and the age of the patient (Xiong et al.,2004, Lim et al., 2008, Jaudah et al.,2012).

The higher postoperative morbidity rate in the older age patient group is because of the significant enhancement in common postoperative complications. (Schiffmann et al.,2006) also revealed that the worse prognosis was in older CRC patients, and with the significantly higher American Society of Anesthesiologists (ASA) classifications. Hence, older age is associated with poor overall survival, but not cancer-specific survival, in CRC patients. Actually, patient age has a decisive impact on the short-term postoperative (LIE Chun et al., 2009).

CRC is the third most common cancer in men and the second in women. Worldwide, as has been concluded by many previous studies. The CRC is more common in males than females (Melissa et al., 2009). In our current study result the male patients are more predisposed to cancer than females. This can correlate with difference in lifestyle or hormonal effect (Campbell-Thompson et al., 2001, La Vecchia et al., 2009).

Many studies proved strong relation between COX2 overexpression and tumor metastasis as they found that the expression of COX-2 was upregulated from normal cells (17%) to primary tumors (72%) and to metastases (100%) (Hong et al., 2002, Yao et al,.2005). The expression of COX-2 is strongly correlated with recurrence of CRC, especially with blood-borne metastasis, but in our data analysis there is no evidence of

such strong relation (P < 0.514), even though the lymphovascular correlation with the enzyme expression has a little significant result (P< 0.055). Previous studies have shown that overexpression of COX-2 induced angiogenesis and invasion, possibly due to enhanced expression of metalloproteinases.

Sheehan et al.,1999, Fujita et al.,1998) indicated that greater COX-2 expression was correlated with more advanced Dukes' stage and larger tumor size, other study on breast cancer prove strong relation with large tumor size and COX2 overexpression (Nassar et al., 2007), in our study we found less COX2 expression with increased tumor size with significance (P < 0.010) which is controversial with other studies, and this can explained by the facts that proved by some studies as the COX-2 expression is more in early stages than late stages other show no association was found between COX-2 expression and tumor size (Sevin et al.,2010, Jing et al., 2012).

(Dimberg et al.,1999) analyzed 39 colorectal cancers using Western blot and indicated that the overexpression of COX-2 protein was higher in tumors located in the rectum than in those located in the colon, but (Yao et al.,2005) show no relation between COX2 expression and tumor location.

CHAPTER IV

Conclusions

&

Recommendation

4.1 Summary and Conclusions

Tumors with negative COX2 expression have more survival than positive tumors.

We found that COX2 overexpression related to decrease on the cancer free survival, so it has adverse effect on the outcome of colon cancer patients and as the best of my knowledge this is the first study for the COX2 expression of Libyan patients.

Colorectal cancer has high incidence in our population as the second tumor in both male and female, with more incidences in male patient.

Early onset of the tumor as several Arab countries, in comparison to data from other developed countries.

Most of Libyan patients presented on stage III –IV, which indicate inadequate screening program.

COX2 expression in our study show no significant correlation with patient sex, tumor grade, location, stage, metastasis or recurrence.

COX2 expression was less in large tumor size.

Results suggest that COX-2 expression play a significant role in the prognosis of CRC in Libyan patient as used in combination with other prognostic markers.

4.2 Recommendation

General

*Establishment of electronic archives to facilitate collection of data for further studies in the future.

*Provision of the National cancer registry of Libya, to give more precise information.

*Provision of the National Guidline in histopathology reporting.

*The members of Oncology, Pathology, Surgon and Radiology must work as one team for more productive result.

Specific

*Studies must do with large number of cases and longer follow up period.

*Obtaining more accrute clinicopathological data regarding tumor margin, lymph nodes status, lymphovascular invasion and follow up.

*Accrute staging of the tumor based on the TNM system.

*Further studies to prove the significant effect of cyclooxgenase-2 inhibitors as antitumor agent in colorectal cancer among Libyan patients.

*Study the dependent relation between COX2 and p53 in promoting CRC carcinogenesis in Libyan patients.

Chapter V

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REFERANCES

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الملخص العربي

القولون هو الجزء السفلي من الجهاز الهضمي، ويسمى أيضاً الأمعاء الغليظة أو المصران الغليظ. وهو عبارة عن آخر خمسة إلى ستة أقدام من الأمعاء في حين أن آخر 20-25 سم من القولون هي المستقيم.

سرطان القولون والمستقيم:
يعتبر سرطان القولون والمستقيم ثاني أسباب الوفيات بالسرطان بعد سرطان الرئة في المجتمعات الغربية. أما في المجتمع العربي فهو أقل انتشاراً ولكن ما يزال من العشرة الأكثر حدوثاً حسب الإحصائيات المسجلة في بلادنا. ويحدث سرطان القولون والمستقيم غالباً في المسنين وهو نادر الحدوث قبل سن الأربعين مع أنهم ليسوا بمأمن منه، وهناك العديد من الحالات المسجلة في مجتمعنا في أعمار صغيرة ما بين العشرين والثلاثين. وبشكل عام، فإن تواتر الحدوث متساو بين الذكور والإناث مع أن ثمة بعض الاختلافات في نسبة توزيع هذا السرطان بين كلا الجنسين، فسرطان المستقيم أكثر حدوثاً عند الذكور بينما يعتبر سرطان القولون أكثر شيوعاً عند الإناث. وينشأ أكثر من 70% من أورام الأمعاء الغليظة في المستقيم والقولون السيني ويحدث 10% منها في الأعور ويتوزع الباقي في ما تبقى من الأجزاء الأمعاء الغليظ.

مرض سرطان القولون يبدأ على شكل زوائد لحمية حميدة، غير سرطانية , هذه الزوائد تبقى حميدة لمدة تقدر بحوالي 10 سنوات وفي هذه الأثناء لا يشكو المريض من أي شكوى في بطنه (بدون أعراض). بعد انقضاء هذه المدة تتحول بعض هذه الزوائد (5-10% من الزوائد) إلى خلايا سرطانية والتي تبدأ بالنمو التدريجي حتى تصبح سرطانا خبيثا ومع مرور الوقت يمكن أن يبدأ الورم الخبيث بالانتشار خارج جدار القولون إلى العقد الليمفاوية أو إلى أجزاء بعيدة في الجسم مثل الكبد وغيره. المشكلة أن أعراض هذا المرض تظهر متأخرة جداً بعد تحوله إلى سرطان مما يقلل من فرصة الشفاء التام منه؛ ولذلك ينبغي أن لا ينتظر المريض ظهور أعراض مثل وجود دم في البراز أو آلام في البطن أو تغيّر في نمط التبرز حتى يراجع طبيبه . وإنما عليه أن يقوم بالكشف المبكر للبحث عن زوائد اللحمية الحميدة واستئصالها أولا بأول حتى لا تتحول في المستقبل إلى أورام سرطانية خبيثة، وبهذه الطريقة يمكن تفادي مرض سرطان القولون والشفاء التام منه في أكثر من90% من الحالات

قد تظهر بعض الأعراض لدى تأثر القولون أو المستقيم بداء ما. وفيما يلي قائمة بعلامات تنذر باحتمال وجود مشكلة بالقولون أو المستقيم:

- **إسهال أو إمساك .**
- **دم في البراز أو عليه (سواء كان لون الدم فاتحاً أو شديد القتامة)**
- **البراز أرفع بكثير عن المعتاد .**
- **انزعاج عام بالبطن (انتفاخ، امتلاء، مغص)**
- **آلام غازية متكررة .**
- **الشعور بأن الأمعاء غير فارغة تماما, فقدان الورن والشعور بالتعب العام**

بإمكان إجراء "تنظيراً" يعاين من خلاله المستقيم ونهاية أسفل القولون، بعض هذه المناظير صلبة، والبعض الآخر مرنة تتيح للطبيب معاينة أعلى القولون. ويتم اكتشاف 50% من حالات سرطان القولون والمستقيم بواسطة الفحص بالمنظار .
عقب هذه الخطوات الأولية من الفحص، قد يطلب الطبيب بعض الفحوص المخبرية وفحوصاً أخرى. فقد يطلب

الطبيب من المريض عينة براز لمعرفة ما إذا كان هناك دم في البراز، ثم يتم إرسال العينة إلى المختبر لفحصها. وفي بعض الأحيان قدر يرغب الطبيب في معاينة طول القولون بأكمله، حيث يستخدم منظاراً للقولون عبارة عن أنبوب مرن ورفيع ذي إضاءة في آخره. وفي حال تم العثور على ورم سيقوم الطبيب بأخذ عينة صغيرة لفحصها في المختبر. ويدعى هذا الإجراء بالخزعة حيث أنه الطريقة الوحيدة الأكيدة لمعرفة ما إذا كان الورم سرطاناً.
وفي حالات عديدة يستطيع الطبيب استخدام منظار القولون لإزالة الورم برمته. كما قد يطلب الطبيب إجراء التصوير الإشعاعي بحقنة الباريوم. وهو عبارة عن تصوير القولون بالأشعة السينية عقب إعطاء حقنة شرجية سائل الباريوم الكثيف إلى الأمعاء. يظهر الباريوم شكل القولون في التصوير السيني مما يساعد الطبيب على معاينة الأورام، أو أي مناطق يشتبه إصابتها، ولم تظهر في الفحوص الأخرى.
إذا كان الورم حميداً تتم إزالته على الأرجح دون حدوث مزيد من المشاكل.

مراحل سرطان القولون: Ducke's classification

"مرحلة 0: مبكراً جداً سرطان على الطبقةِ الأعمق للأمعاء
"المرحله الأولى: السرطان في الطبقاتِ الداخليةِ للقولون
"المرحلة الثّانية: إنتشارَ السرطانُ عبر حائطُ عضلة القولون
"المرحلة الثّالثة: انتَشارَ السرطانُ إلى العُقد اللمفاويةِ
"المرحلة الرّابعة: السرطان الذي ا نتشرَ إلى الأعضاء الأخر

ملخص البحث:

دراسة العوامل السريرة و المرضية المختلفة التي تتعلق بالإصابة بسرطان القولون و مدى أهميتها كعوامل تنبؤية وتأثيرها على علاج المرضى ومدى امتثالهم للشفاء منه.

هذه الدراسة تعتمد على دراسة مدى قدرة الانسجة الورمية على أخذ الصبغة المناعية بواسطة انزيم السيكلوكسجينيز و الذي لديه قدرة كبيرة على تحفيز نمو الخلايا السرطانية , و علاقة هذا الأنزيم بالعوامل التنبؤية التقليدية .

المرضى و طرق الدراسة:

تعتمد هذه الدراسة على عينات شمعية من سرطان القولون مأخوذة مسبقا من 83 مريض ليبي بالمنطقة الشرقية و موجودة بقسم علم الامراض بجامعة بنغازي و التي تم تشخيصها في الفترة من سنة 2007 وحتى 2011 ,و متابعة ملفاتهم الطبية بقسم الأورام بمركز بنغازي الطبي . جهزت هذه العينات على شرائح خاصة وتم صبغها (بصبغة مناعية خاصة بإنزيم السيكلوكسيجينز) , ومن ثم تقييم مدى استجابة النسيج للصبغة واختبار النتائج احصائيا.

نتائج البحث:

بدراسة النتائج الإحصائية تبين أن أنزيم الدراسة كلما كانت نسبة وجوده أكثر بالنسيج الورمي , كلما قلت فرصة المريض في الشفاء التام من المرض , مع وجوده بنسبة أقل كلما زاد حجم الورم , عمر المريض , او انتشار الورم الوعائي.

وعليه فإن إنزيم السيكلوكسيجينزا يمكن اعتباره عامل مهم ومساعد للتنبؤ في مجال سرطان القولون.

جامعة بنغازي

كلية الطب

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دراسة للحصول على درجة الماجستير في علم الأمراض

للطبيبة : رانيا عوض الشريف

بكالوريوس طب و جراحة

تحت لإشراف

الدكتور : عبد الباسط بو حميدة

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الدكتور : آدم إبراهيم الزغيد

قسم علم الأمراض

جامعة بنغازي- كلية الطب

بنغازي – ليبيا

2013