CHAPTER ONE

INTRODUCTION

1.1:Intoductin:

Colorectal cancer (CRC), commonly known as colon cancer or bowel cancer, is a cancer the colon or rectum or in the appendix. Genetic analysis shows that essentially colon and rectal tumors are genetically the same cancers (Karaptis, et al. 2008).

In colorectal cancer, cells in the colon or in the rectum start to grow in an uncontrolled way, forming a lump called the primary cancer or primary tumor. Colon cancer may metastasize to the liver, lung, or other locations. Colorectal cancer affect men and women equally (Karaptis, et al. 2008).

Around 60% of cases were diagnosed in the developed world. It is estimated that worldwide, in 2008, 1.23 million new cases of colorectal cancer were clinically diagnosed, and that it killed 608,000 people (Ferlay, et al. 2010).

In the sudan according to cancer records colorectal adenocarcinoma is common causes of death in males than females in 2011 which represents163 cases and in females 112 cases, and 150 cases of males, 106 cases of females in 2012.

Colorectal cancer continues to be one of the predominant cancer in the western world and second most common cause of death in the united states (Karaptis, et al. 2008).

Risk factors for colon cancer include hereditary conditions, chronic inflammatory diseases of the colon such as Crohn's disease, a diet high in fat, smoking and alcohols, obesity and radiation therapy, it is more common in men than women (Abeloff, et al. 2013).

Diagnosis of colorectal cancer is via tumor biopsy typically done during colonoscopy, X-ray and CT scan (Computerized Tomography) and MRI (magnetic resonance imaging). Other laboratory tests including blood tests. A biopsy may be needed to confirm the diagnosis, also the use the tumor markers like AMACR (Alpha-methylacyl-CoA recemase) is essential for diagnosis and for monitoring treatment of colorectal cancer (Varmus, 2010, Pischon, et al 2006 and Marphy, et al 2010).

Many colon cancer treatment options are available for colorectal cancer, including surgery, chemotherapy, and radiation (Varmus, 2010).

AMACR is an enzyme that catalyzes the chemical reaction (Schmitz, et al.1994).

In mammalian cells, the enzyme is responsible for converting (2R)-methylacyl-CoA esters to their (2S)-methylacyl-CoA epimers and known substrates include coenzyme A esters of pristanic acid and bile acids derived from cholesterol. The enzyme is known to be localised in peroxisomes and mitochondria, both of which are known to β-oxidise 2-methylacyl-CoA esters (Schmitz, et al. 1994).

Immunohistochemical expression of AMACR marker is suitable for the localization, staging, andfollo up treatment. The levels of AMACR reflect the success of radiotherapy, surgery and chemotherapy on the patients (Schmitz, et al. 1994).

1.2:Objectives:

1.2.1:General objective:

To detect the expression of AMACR among colorectal tumors patients

1.2.2:Specific objective:

1-To correlate between AMACR expression with histopathological diagnosis and grades.

2-To correlate between age group and grades cancer

CHAPTER TWO

LITERTURE RERIEW

2.1 Anatomy and Function of colon and rectam:

2.1.1 Colon:

The colon is a 150 cm long hollow tube that is wrapped in a serosal layer. It is anatomically divided into several regions, a terminal pouch called the cecum, which is separated from the terminal ileum by the ileocecal valve, an ascending segment, a transverse segment, a descending segment, an S shaped segment called the sigmoid colon, and the rectum(Guyto and Hall, 2000).

The colon begins at the cecum, where it joins the end of the small intestine (ileum). The colon changes to rectal tissue in its last 6 inches (Guyto and Hall, 2000).

The wall of the colon consists of a mucosa, submucosa, and inner circular and outer longitudinal muscular layers. Except for the sigmoid colon and rectum, the circular muscle in the colon is covered with three bands of a longitudinal muscle that are known as taeniae coli. Because the taeniae coli do not fully surround the circular muscle, the motility of the colon is less effective and is slower than that of the small bowel. In the sigmoid colon and rectum, the longitudinal muscle completely surrounds the circular one (Guyto and Hall, 2000).

The mucosa consists of the epithelium, the lamina propria (connective tissue), and the muscularis mucosa (smooth muscle). The epithelium consists of a single layer of epithelial cells linked together by tight junctions. The colonic mucosa differs from the small intestinal mucosa by the absence of the long villi (Gray,1984).

Consequently, the absorption surface of the colon is significantly less than that of the small intestine most of the large intestine rests inside a cavity in the abdomen called the peritoneal cavity. Parts of the colon are able to move quite freely within the peritoneal cavity as the undigested food is passing through it. As the colon heads towards the rectum, it becomes fixed to the tissues behind the peritoneal cavity, an area called the retroperitoneum (Gray, 1984).

The colon's function is threefold to absorb the remaining water and electrolytes from indigestible food matter to accept and stores food remains that were not digested in the small intestine and to eliminate solid waste (feces) from the body (Ross, et al. 1994).

2.1.2 Rectum:

The rectum is the final straight portion of the large intestine. The human rectum is about 12 centimetres long, and begins at the rectosigmoid junction (the end of the sigmoid colon), at the level of the third sacral vertebra or the sacral promontory depending upon what definition is used. The rectum presents three or more lateral curvatures, which correspond to transverse rectal folds in the interior of the gut. The rectum has neither mesentery nor haustra, and it has almost complete outer longitudinal muscular coat rather than teniae (Guyto and Hall, 2000).

The main function of the rectum is to act as a temporary storage site for fecal matter before it is eliminated from the body through the anal canal.

The rectum holds the feces until push it out of the body, through the anal canal, by having a bowel movement (Ross, et al.1994).

2.2 Colorectal diseases:

Inflammatory bowel disease (IBD) involves chronic inflammation of all or part of digestive tract.

2.2.1 Ulcerative Colitis:

In ulcerative colitis, the inner lining (mucosa) of the intestine becomes inflamed and develops ulcers. Ulcerative colitis is often the most severe in the rectal area, which can cause frequent diarrhea. (Mosley, et al. 1977).

2.2.2 Crohn's Disease:

Crohn's disease is a chronic inflammatory condition primarily involving the intestinal tract that predominantly affects young adults. The most common areas in the last part of the small intestine called the ileum and the colon (Mosley, et al. 1977).

2.2.3 Diverticular Disease:

Diverticulosis is the presence of pockets (called diverticula) in the colon wall (Mosley, et al. 1977) .

2.2.4 Irritable Bowel Syndrome (IBS):

Irritable bowel syndrome is a common intestinal muscle functioning disorder involving constipation, diarrhea, or a combination, accompanied by pain, bloating and cramps (Mosley, et al. 1977).

2.3 Colorectal cancer:

2.3.1 Definition:

Colon cancer is cancer of the large intestine (colon), the lower part of digestive system. Rectal cancer is cancer of the last several inches of the colon. Together, they're often referred to as colorectal cancers (Varmus, 2010).

All colon cancers are derived from the mucosal lining of the bowel wall. From the inside out, the bowel wall is composed of multiple layers, which include the mucosa, the submucosa, the muscularis propria (containing circular and smooth muscle layers) and the serosa. The innermost layer of the bowel wall, the mucosa, is a single layer of columnar epithelial cells,

some of which produce large amounts of mucus and are thus termed goblet cells. This is the site of the earliest genetic changes that lead to the development of cancer cells (Bresalier,1999).

A colorectal cancer forms, it begins to grow in two ways first, the cancer can grow locally and extend through the wall of the intestine and invade adjacent structures, making the mass (called the primary tumor) more of a problem and harder to remove. Second, as the cancer grows it begins the process of metastasis, shedding thousands of cells a day into the blood and lymphatic system that can cause cancers to form in distant locations. Colorectal cancers most commonly spread first to local lymph nodes before traveling to distant organs. Once local lymph nodes are involved, spread to the liver, the abdominal cavity, and the lung are the next most common destinations of metastatic spread (Varmus, 2010).

2.3.2 Symptoms of colorectal cancer:

Symptoms of colorectal cancer are numerous and nonspecific. They include fatigue, weakness, shortness of breath, change in bowel habits, narrow stools, diarrhea or constipation, red or dark blood in stool, weight loss, abdominal pain, cramps, and bloating. other conditions such as irritable bowel syndrome (spastic colon), ulcerative colitis, Crohn's disease, diverticulosis, and peptic ulcer disease (Lynch and Chappele, 2003).

2.3.3 Risk factor of colorectal cancer:

2.3.3.1 Age:

About 90% of people diagnosed with colon cancer are older than 50. Colon cancer can occur in younger people, but it occurs much less frequently (Abeloff, et al. 2013).

2.3.3.2 A personal history of colorectal cancer or polyps:

A person who already has had colorectal cancer may develop the disease a second time, especially if the first disease was diagnosed before the age of 60 (Fung, et al. 2003).

2.3.3.3 Inflammatory intestinal conditions:

Chronic inflammatory diseases of the colon, such as ulcerative colitis and Crohn's disease can increase risk of colon cancer (Matter, et al. 2011).

2.3.4.4 Inherited syndromes:

Genetic syndromes passed through generations of family can increase risk of colon cancer. These syndromes include familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer, which is also known as Lynch syndrome (Lynch and Chappele, 2003).

2.3.3.5 Family history:

Parents, siblings, and children of a person who has had colorectal cancer are more likely to develop colorectal cancer themselves. If more than one family member has colon cancer or rectal cancer, the risk increases to about 20% (Lynch and Chappele, 2003).

2.3.3.6 Radiation therapy for cancer:

Radiation therapy directed at the abdomen to treat previous cancers may increase the risk of colon cancer (Pischon,et al. 2006).

2.3.4 Diagnosing of colorectal cancer:

2.3.4.1 Staging testes:

Identify the extent and spread of the disease essential for choosing the best treatment. staging testes, such as Computed Tomography (CT), positron emission tomography (PET) scan and x-rays help to determine deeply the cancer invaded the colon wall and whether it spread to nearly lymph nodes or organs (Varmus, 2010).

2.3.4.2 Microsatellite instability (MSI) testing:

Sometimes the tumor tissue will be tested to see if it shows changes called Microsatellite instability (MSI). This change is present in most colorectal

cancers caused by hereditary non-polyposis colon cancer (HNPCC) and can also affect some cancers in patients who do not have HNPCC (Varmus, 2010).

2.3.4.3 DNA stool tests:

Colon polyps and cancers continuously shed mutated cells that eventually make their way into stool. Analyzing these cells for genetic mutations may detect polyps and early-stage cancers (Varmus, 2010).

2.3.4.4 Surgery:

Removal of the whole colon is called a total colectomy . Removal of half of the colon is known as a hemi-colectomy. Either the left side or the right side may be removed, depending on where the cancer is. In a left hemi-colectomy the half of the transverse colon and the descending colon is removed. During a right hemi-colectomy the right half of the transverse colon and the ascending colon is removed (Varmus, 2010).

2.3.4.5 Complete blood count (CBC):

A Complete blood count (CBC) is a standard diagnostic test that determines the amount of red and white blood cells in blood. Anemia can be a sign of cancer and is often times a side effect of chemotherapy (Varmus, 2010).

2.3.5 Treatment of colorectal cancer:

Treatment depends on many things, including stage of the cancer.

Treatments may include:

2.3.5.1 Surgery:

Stage 0 colon cancer may be treated by removing the cancer cells. This is done using colonoscopy. For stages I, II, and III cancer, more extensive surgery is needed to remove the part of the colon that is cancerous. This surgery is called colon resection (Pezner, et al. 1999).

2.3.5.2 Chemotherapy:

Almost all patients with stage III colon cancer should receive chemotherapy after surgery for 6 - 8 months. This is called adjuvant chemotherapy (Pezner, et al. 1999).

2.3.5.3 Radiation:

It is usually used in combination with chemotherapy for patients with stage III rectal cancer. For patients with stage IV disease that has spread to the liver, treatments directed at the liver can be used (Pezner, et al. 1999).

2.3.6 The follow-up care for colon cancer:

The cancer can come back near the original site, although this is unusual. If the cancer returns, it typically does so in a distant location such as the lymph nodes, liver, or lungs. Individuals diagnosed with colorectal cancer remain at risk of their cancer returning for up to 10 years after their original diagnosis and treatment, although the risk of recurrence is much higher in the first few years (Pezner , et al. 1999).

2.4 Tumor markers:

2.4.1 Definition:

It is substances that can be detected in higher than normal amounts in the blood, urine, or body tissues of some patients with certain types of cancer. A tumor marker may be expressedby a tumor itself, or it may be expressedby the body as a response to the tumor. Tumor marker tests are not used alone to detect and diagnose cancer because most tumor markers can be elevated in patients who don't have a tumor, because no tumor marker is entirely specific to a particular type of cancer, and because not every cancer patient has an elevated tumor marker level, especially in the early stages of cancer, when tumor marker levels are usually still normal. The marker can be a means of monitoring the success or failure of treatment. The tumor marker level may also reflect the extent (stage) of the

disease, indicate how quickly the cancer is likely to progress, and help determine the outlook (Sturgeon, et al. 2009).

2.4.2 AMACR Marker:

It is an alpha-methylacyl-CoA racemase is an enzyme that catalyzes the chemical reaction. The enzyme is responsible for converting (2R) methylacyl -CoA esters to their (2S) methylacyl -CoA epimers and known substrates include coenzyme A esters of pristanic acid and bile acids derived from cholesterol. This transformation is required in order to degrade (2R)-methylacyl-CoA esters by β-oxidation, which requires the (2S) epimer. The enzyme is known to be localised in peroxisomes and mitochondria, both of which are known to β-oxidise 2-methylacyl-CoA esters. This enzyme belongs to the family of isomerases, to be specific those racemases and epimerases acting on other compounds. The systematic name of this enzyme class is 2-methylacyl-CoA 2-epimerase (Sharma, et al. 2012).

AMACR has been to be a highly sensitive marker for colorectal cancers. Examination of colorectal cancers which is not hormone regulated, demonstrated high levels of AMACR expression in well to moderately differentiated tumors and weak expression in anaplastic colorectal cancers (Sharma, et al. 2012).

AMACR is an enzyme playing an important role in the beta-oxidation of branched-chain fatty acids and fatty acid derivatives. Altered expression levels of AMACR have been described in various cancers including colorectal cancer. AMACR immunohistochemistry was significantly associated with tumor grade, stage, non-mucinous phenotype, and left-sided tumor localization. AMACR positivity was observed in 65.8% of cancers from the right-sided colon, in 73.2% of cancers from the colon

transversum, in 81.1% of cancers from the colon descendens, and in 88.9% of the distal left-sided cancers. AMACR staining results were unrelated to clinical outcome. AMACR cannot serve as a prognostic marker in CRC (Sharma, et al. 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials:

Archive tissues blocks of colorectal tumor were used in this study.

3.2 Methods:

3.2.1 Study design:

This is a hospital based descriptive retrospective case study aimed to detect AMACR marker in colorectal tumor using immunohistochemical method.

3.2.2 Study samples:

Thirty five colorectal tissue blocks were obtained from tissues previously diagnosed with 27 colorectal cancer and 8 colorectal hyperplasia at Soba university hospital during the period from May to September2013.

Patient identification and other information were obtained from patient's file.

3.2.3 Sample processing:

One section of 5µm in thickness was obtained from each formalin fixed paraffin wax embedded tissue using rotary microtome.

3.2.3.1 Immunohistochemical tissue processing:

Monoclonal antibodies by modified new indirect Dako technique as follow:

Section required for immunohistchemistry was retrieved by water bath retrieval technique 95c^{o} for 40 minutes, then immunostained using monoclonal antibodies by indirect decxtraine polymer technique as follows:

Section was dewaxed in hot plate oven and cleared in two changes of xylene for two minutes, then hydrated through descending concentrations of ethanol (100%,90%,70%50%) and water two minutes, then retrieved by water bath retrieval technique (citrate buffer) for fourty minutes, then treated with endogenous hydrogen peroxide blocker solution for ten minutes, then washed in phosphate buffer saline (PH7.4) for five minutes, then treated with primary antibody AMACR (Dako code IS060) for thirty minutes, then rinsed in phosphate buffer saline (PH7.4), then teated with

secondary antibody for thirty minutes, then rinsed in phosphate buffer saline(PH7.4), then treated with DAB for five minutes, then washed in phosphate buffer saline(PH7.4) for five minutes, then counterstained with Mayer's haematoxylin for one minutes, then washed and blued in running tap water for ten minutes, then dehydrated through ascending concentrations of ethanol (50%,70%,90%,100%), then cleared in xylene and mounted in DPX mountant (Bancroft and Marily, 2002).

3.2.4 Result interpretation:

All quality control positive and negative control measures were adopted during samples processing for the assessment of immunohistochemical results.

3.2.5 Statistical analysis:

The data were analyzed using version 15.0 SPSS computer program frequencies, means and chi_ square test values were calculated.

3.2.6 Ethical consideration:

Hospital administration agreements were taken ethically for archive samples.

CHAPTER FOUR

RESULTS

A total of 35 samples of patients, with colorectal tumor were investigated by immunhistochemistry method, 27of them were malignant colorectal tumors representing (77.1%), and the remaining 8 (22.9%), were benign as indicated in table (4.1).

The age of the study population ranged 19 to 78 years old with mean age of 52 years. Most patients were older than 40 years representing 29 (82.9%) and the remaining 6 (17.1%) were younger than 40 years as indicated in Table(4.2).

The description of sex as showed in Table (4.3), most patients were male representing 19 (54.3%) and the remaining 16 (45.7%) were female.

The description of tumor grade revealed that well differentiated tumor in 4 (14.8%) patients, moderately differentiated tumor in 21 (77.8%) patients and poor differentiation were observed in 2 (7.4%) patients as indicated in Table (4.4).

As mentioned in table (4.5), malignant colorectal tumors revealed positive expression of AMACR in 14 (40.0%) patients and negative expansion of AMACR in 13 (37.1%) patients, while all benign colorectal tumor showed negative expansion of AMACR in 8 (22.9%) patients, this result show significant statistical association (P value 0.009), as indicated in Table (4.5).

Correlation between AMACR expression and tumor grade revealed that well differentiated tumor showed positive expression was 1 (3.7%) patient and negative expression of AMACR were 3 (11.1%) patients, moderately differentiated tumor showed positive expression of AMACR were 13 (48.1%) patients and negative expression of AMACR were 8 (29.6%) patients and all poor differentiated tumor showed negative expression of AMACR were 2 (7.4%) patients, this result showed insignificant statistical association (P value 0.125), as indicated in Table (4.6).

Table (4.1) Distribution of sample among the study population

Sample	**Frequency**	**Percent**

Malignant	27	77.1
Benign	8	22.9
Total	35	100%

Table (4.2) Distribution of age among the study population

Age group (year)	**Frequency**	**Percent**

Less than 40 year	6	17.1
40_60 year	19	54.3
61_80 year	10	28.6
Total	35	100%

Table (4.3) Distribution of sex among the study population

Sex	**Frequency**	**Percent**
Male	19	54.3

Female	16	45.7
Total	35	100%

Table (4.4) Distribution of cancer grade among malignant colorectal tumors

Tumor grade	Frequency	Percent
Well differentiated tumor	4	14.8
Moderate differentiated tumor	21	77.8
Poor differentiated tumor	2	7.4
Total	27	100%

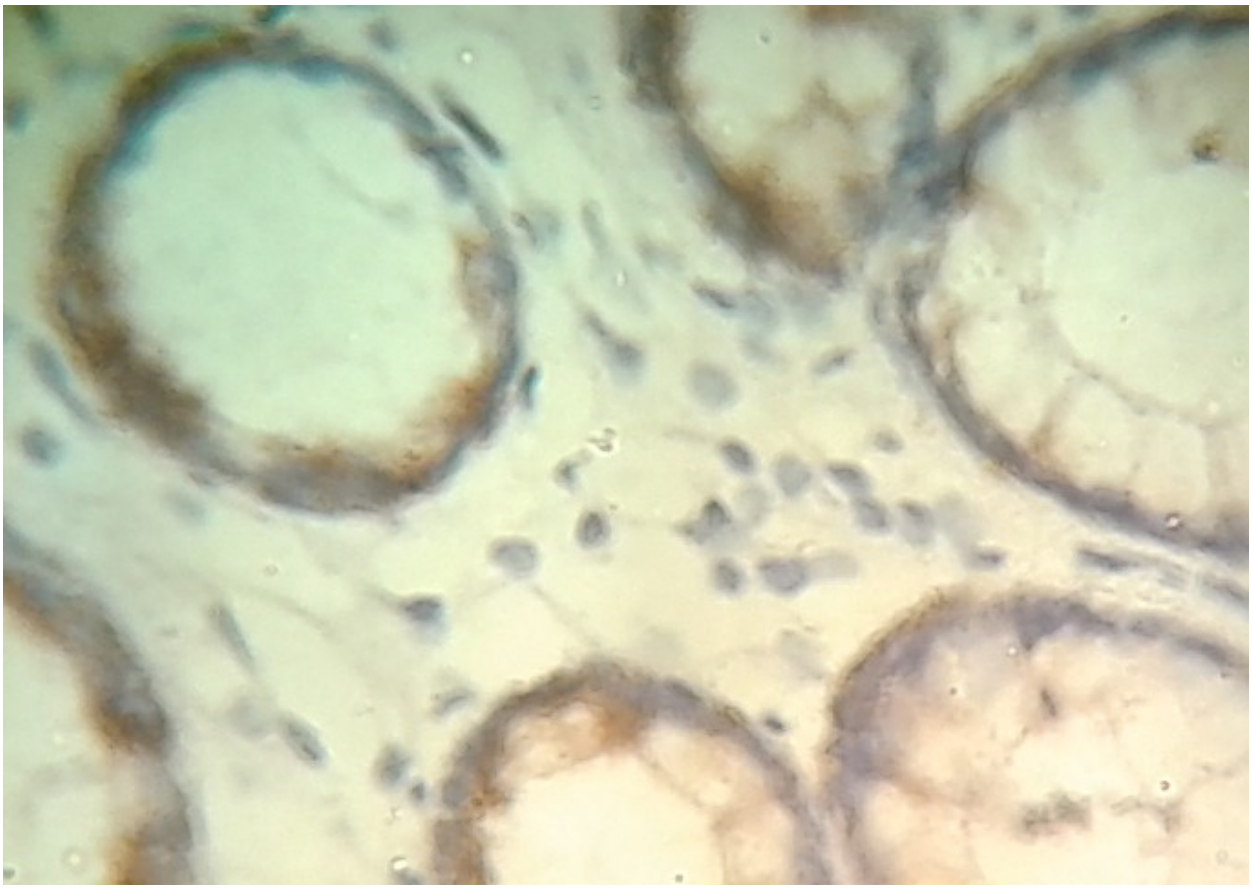
Table (4.5) Immunohistochemical expression of AMACR among the study samples

Sample	**AMACR**				**Total**		**P Value**
	Positive		Negative				
	N	%	N	%	N	%	
Malignant	14	40.0%	13	37.1%	27	77.1%	0.009
Benign	0	0.0%	8	22.9%	8	22.9%	
Total	14	40.0%	21	60.0%	35	100%	

Table (4.6) Correlation between AMACR expression with cancer grade

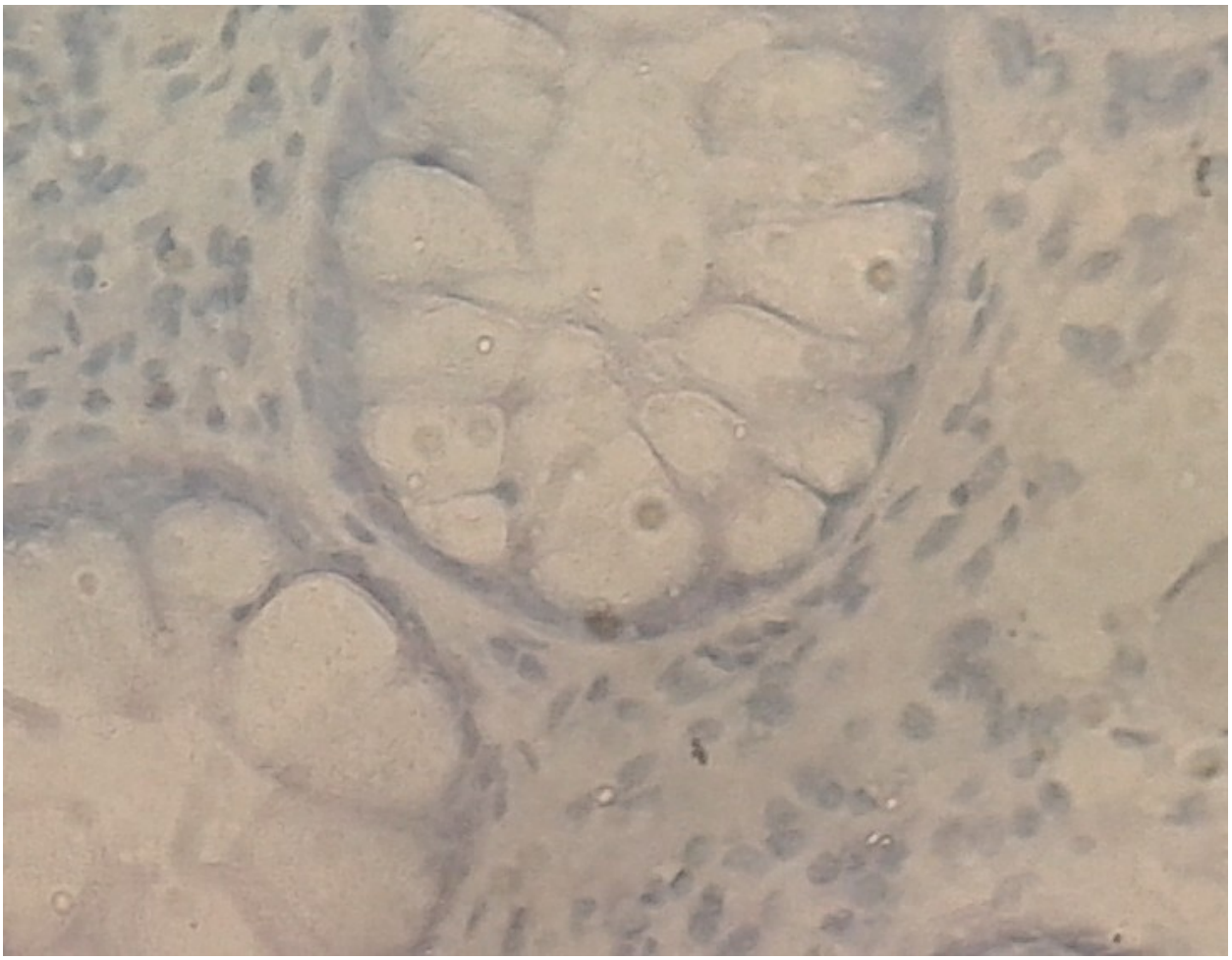
Grade	AMACR	Total	P

	Positive		Negative				Value
	N	%	N	%	N	%	
Well differentiated tumor	1	3.7%	3	11.1%	4	14.8%	0.125
Moderate differentiated tumor	13	48.1%	8	29.6%	21	77.8%	
Poor differentiated tumor	0	0.0%	2	7.4%	2	7.4%	
Total	14	51.9%	13	48.1%	27	100%	



Microphotography (4.1):

Colon adenocarcinoma moderately differentiated showed cytoplasmic positive expression of AMACR (40X).



Microphotograghy (4.2):

Rectal adenocarcinoma moderately differentiated showed cytoplasmic negative expression of AMACR (40X).

CHAPTER FIVE

DISCUSSION

Colorectal cancer, commonly known as colon cancer or bowel cancer is the third most commonly diagnosed cancer starts in a small area but can spread to other parts of the body to form metastatic tumors (karaptis, et al. 2008).

In this study out of thirty-five samples of patients with colorectal tumor were investigated by immunhistochemical method, 27 of them were malignant colorectal tumors representing (77.1%), and the remaining 8 (22.9%), were benign. The age of the study population ranged between 19 to 78 years old with mean age of 52 years. Most patients were older than 40 years representing 29 (82.9%) and the remaining 6 (17.1%) were younger than 40 years. This means older than 40 years are more susceptible for colorectal tumor. This study compatible with Abeloff, et al (2013), who reported that the condition is rare in people under 40 years and the majority of cases are diagnosed in age over 55 years old. Also compatible with Marphy, et al (2010), who reported that the colorectal cancer appear mainly after the age of 50 years.

Regarding sex, that males are more affected by colorectal cancer than females representing (54.3%) and (45.7%) respectively. This is attributed to increase smoking and consumption of alcohol in males than females. This result supported by Pischon, et al (2006) and Marphy, et al (2010), they reported that the incidence of colorectal cancer in appear in males higher than females.

The description of tumor grade revealed that well differentiated tumor in 4 (14.8%) patients, moderately differentiated tumor in 21 (77.8%) patients and poor differentiated tumor is observed in 2 (7.4%) patients, this study compatible with Compton, et al (2000), who reported that the most colorectal adenocarcinomas

(70%) are diagnosed as moderately differentiated tumor. Well and poorly differentiated carcinomas account for 10% and 20%, respectively.

Malignant colorectal tumors revealed positive expression of AMACR in 14 (40.0%) patients and negative expansion of AMACR in 13 (37.1%) patients, while all benign colorectal tumor showed negative expression of AMACR 8 (22.9%) patients, this result show significant statistical association (P value 0.009). This result supported by Virchows (2008), who reported that altered expression levels of AMACR have been described in various cancers including colorectal cancer.

Based on this study the statistical association between AMACR expression and tumor grade showed insignificant association (P value 0.125). It revealed that well differentiated tumor showed positive expression in (3.7%) patient and negative expression of AMACR in 3 (11.1%) patients, moderately differentiated tumor showed positive expression of AMACR in 13 (48.1%) patients and negative expression of AMACR in 8 (29.6%) patients and all poor differentiated tumor showed negative expression of AMACR 2 (7.4%) patients, this study incompatible with Kuefer, et al (2002), who reported that high levels of AMACR expression in well to moderately differentiated tumor and weak expression in anaplastic colorectal cancer.

Conclusion and Recommendations

Conclusion:

On the basis of this study we concluded that:

AMACR expression association with malignant tumor with not affected by histological grade of tumors.

Most cases of colorectal carcinoma in this study appear above 40 years old.

Most cases of colorectal carcinoma in this study the male were affected more than female.

Recommendations:

On the basis of this study we recommended that:

Carry out another similar study with large sample size and additional parameters.

Also carry out another similar study with types, locations and scoring stages of colorectal adenocarcinoma.

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APPENDIX

Appendix

Materials and instruments used for processing and staining of the specimens

Include:

Disposable gloves.

Rotary microtome.

Microtome knives.

Coated slides.

Cover glasses.

Dry oven.

Water bath.

Coplin jars.

Humidity chamber.

Ethanol (100%, 90%, 70%, 50%).

Xylene.

Eosin.

Mayer’s haematoxylin.

Citrate buffer (PH 6.8).

Phosphate buffer (PH 7.4).

Primary antibody (AMACR).

Secondary antibody.

Substrate-Chromogen.