Sudan University of Science and Technology

College of Graduate Studies

Immunohistochemical Detection of Human Epidermal Growth Factor Receptor 2 Among Colorectal Tumor Patients

الكشف النسيجي الكيميائي المناعي عن الواسمة الورمية HER2 لدى مرضى أورام القولون و المستقيم

A Dissertation Submitted for Partial Fulfillment for Requirements of M.Sc Degree in Histopathology and Cytology

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2015

الآية

بسم الله الرحمن الرحيم

قال تعالى:

(فَقُلْتُ اسْتَغْفِرُوا رَبَّكُمْ إِنَّهُ كَانَ غَفَّارًا (١٠) يُرْسِلِ السَّمَاءَ عَلَيْكُمْ مِدْرَارًا (١١) وَيُمْدِدْكُمْ بِأَمْوَالٍ وَبَنِينَ وَيَجْعَلْ لَكُمْ جَنَّاتٍ وَيَجْعَلْ لَكُمْ أَنْهَارًا (١٢))

صدق الله العظيم

سورة نوح الآية (١٠-١٢)

DEDICATION

To my father,

To my mother,

To my Husband,

To my Daughter,

To my brothers, sisters, colleagues and friends

I dedicate this study.

Acknowledgement

I would like to thank Allah for giving me the knowledge and support me to complete this research.

Special thanks to my supervisor Dr. Abu Elgasim Abass Awad Alkareem who has provided me with all program needed for this study as well as sufficient aid support in finishing this project.

Thanks to all my teachers in histopathology and cytology department at Sudan University of science and Technology.

My precious, friends and every bodies whom I love.

ABSTRACT

This is a hospital based descriptive retrospective study conducted at Wad madani hospital (Jazeera State) and Sudan University of Sciences and Technology, College of Medical Laboratory Sciences during the period from January to May 2015, aimed to detect Human Epidermal Growth Factor Receptor 2 (HER2) in colorectal tumors patients using immunohistochemistry.

Thirty paraffin block samples were collected from patients samples previously diagnosed as colorectal tumor (20 of them were malignant colorectal samples and 10 of them were benign colorectal samples) using simple random collection method. The paraffin blocks were cut by rotary microtome, then stained by immunohistochemical method using modified new indirect Dako technique for detection of HER2. The data obtained was analyzed using SPSS program version 15.0.

The age of the patients ranged between 17 to 80 years old with mean age of 49 year. The study revealed that most patients were older than 40 years representing of 22 (73.3%) and the remaining 8 (26.7%) were younger than 40 years.

Out of thirty patients, the majority of patients were males representing 17 (56.7%) and the remaining 13 (43.3%) were females.

HER2 expression showed positive result among malignant colorectal tumors in 9 (30.0%) patients and negative expression in 11 (36.7%) patients, while all benign colorectal tumors gave negative expression, this result showed significant statistical association (P value 0.009).

HER2 expression showed positive expression well differentiated tumor in 2 (10.0%) patient and negative expression in 5 (25.0%) patients, moderately differentiated

tumor positive expression in 7 (35.0%) patients and negative expression in 6 (30.0%) patients.

The study concluded that the HER2 expression is associated with malignant colorectal tumors, with no association with the grade of cancer, this result showed insignificant association (P value 0.125).

المستخلص

أجريت هذه الدراسة الوصفية التراجعية في مستشفى ود مدني (ولاية الجزيرة) وجامعة السودان للعلوم والتكنلوجيا, كلية علوم المختبرات الطبية في الفترة من يناير إلى مايو٢٠١٥م للكشف عن واسمة الأورام HER2 في مرضى أورام القولون والمستقيم باستخدام كيمياء الأنسجة المناعية.

تم جمع العينات بواسطة الطريقة العشوائية وتقطيع ثلاثون نسيج مثبت بالفورملين مغمور بشمع البرافين من عينات مرضى تم تشخيصهم مسبقا على أنهم مصابون بأورام القولون والمستقيم (٢٠ منهم أورام القولون والمستقيم الخبيثة و١٠ منهم أورام القولون والمستقيم الحميدة) بواسطة طريقة كيمياء الأنسجة المناعية باستخدام طريقة داكو المعدلة غير المباشرة. واستخدم برنامج الحزمة الإحصائية للعلوم الاجتماعية النسخة ١٥,٠ لتحليل البيانات.

كانت أعمار المجموعة تحت الدراسة تتراوح بين ١٧-٨٠ عام بمتوسط عمر ٤٩ سنة. أظهرت الدراسة أن معظم المصابين كانت أعمارهم أكثر من ٤٠ سنة وكان عددهم ٢٢ مريضا بنسبة (٧٣,٣%) و٨ مصابا بنسبة (٢٣,٧%) كانت أعمارهم أقل من ٤٠ سنة.

كان معدل الإصابة عند الذكور أعلى من الإناث ممثلا ١٧ (٥٦,٧%) مريضا و١٣ (٤٣,٣%) مريضا من الإناث.

أظهرت الدراسة أن إفراز HER2 موجب الظهور في أورام القولون والمستقيم الخبيثة ٩ (٣٠%) وسالب الظهور في ١١ (٣٦,٧%) بينما كل أورام القولون والمستقيم الحميدة كانت سالبة الظهور، كانت هنالك بين HER2 وتشخيص الانسجة (القيمة الاحتمالية ٠,٠٠٩).

أظهرت الدراسة أن إفرازHER2 موجب الظهور في سرطان جيدة التمايز في ٢ (١٠%) وسالب في ٥ (٢٥%) وموجب الظهور في سرطان متوسطة التمايز ٧ (٣٥%) وسالب الظهور في ٦ (٣٠%) وفي حين أن جميع عينات السرطان ضعيف التمايز كان التعبير سلبيا، لم تكن هنالك علاقة بين HER2 والمرحلة التي تم فيها تشخيص المرض.

خلصت الدراسة إلى أن إفراز HER2 له علاقة مع أورام القولون والمستقيم الخبيثة، مع عدم وجود علاقة بينه وبين درجة تمايز الورم (القيمة الاحتمالية ٠,١٢٥).

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CHAPTER ONE

INTRODUCTION

CHAPTER ONE

INTODUCTION

Cancer of the colon is the disease characterized by the development of malignant cells in the lining or epithelium of the first and longest portion of the large intestine. Malignant cells have lost normal control mechanisms governing growth. These cells may invade surrounding local tissue, or they may spread throughout the body and invade other organ systems (Abeloff, *et al*. 2013).

Colorectal (including anal) cancer is the third most common cancer in the world. An estimated 1.24 million people worldwide were diagnosed with colorectal cancer in 2008, accounting for 10% of the total (Ferlay, *et al*. 2012).

In sudan according to cancer records colorectal adenocarcinoma is common causes of death in males than females in 2011 which represents163 cases of males 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 (Rim, *et al*. 2009).

The risk of developing colorectal cancer increases with advancing age. More than 90% of cases occur in people aged 50 or older (Ries, *et al*. 2002). Other risk factors include having Inflammatory bowel disease, personal or family history of colorectal cancer, genetic syndrome, lack of regular physical activity, low fruit and vegetable intake, a low-fiber and high-fat diet, Overweight and obesity, alkohol consumption and tobacco use (Curry, *et al*. 2003).

Diagnosis of colorectal cancer is via sampling of areas of the colon suspicious for possible tumor development typically done during colonoscopy or sigmoidoscopy, depending on the location of the lesion. The extent of the disease is then usually determined by a CT scan of the chest, abdomen and pelvis. There are other potential

imaging test such as MRI, which may be used in certain cases. Colon cancer staging is done next and based on the TNM system which is determined by how much the initial tumor has spread, if and where lymph nodes are involved, and the extent of metastatic disease (Cunningham, *et al*. 2010).

Treatment depends on many things, including stage of the cancer, treatments may include, surgery (most often a colectomy) to remove cancer cells, chemotherapy to kill cancer cells and Radiation therapy to destroy cancerous tissue (Atkin, *et al*. 2010).

HER2 is a member of the human epidermal growth factor receptor (HER/EGFR/ERBB) family (Mitri Z, *et al*. 2012). HER2 is encoded by ERBB2, a known proto-oncogene located at the long arm of human chromosome 17 (17q12). HER2 is named because it has a similar structure to human epidermal growth factor receptor, or HER1. Neu is so named because it was derived from a rodent glioblastoma cell line, a type of neural tumor. ErbB-2 was named for its similarity to ERBB (avian erythroblastosis oncogene B), the oncogene later found to code for EGFR. Gene cloning showed that HER2, Neu, and ErbB-2 are all encoded by the same gene (Coussens, *et al*. 1985). The c-erbB2 protien expression in a large cohort of colorectal tumors and lymph node metastases. C-erbB-2 was expressed in 81.8% of tumors. They did not find any correlation between c-erbB2 staining and lymph node metastases (McKay , *et al*. 2002)

1.2 OBJECTIVES

1.2.1 General objective:

To detect the expression of HER2 among colorectal tumors patients using immunohistochemistry.

1.2.2 Specific objectives:

1- To correlate between HER2 immunohistochemistry expression and histological diagnosis.

2- To correlate between HER2 immunohistochemistry expression and grade.

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO

LITERATURE REVIEW

2.1 Anatomy and function of colon and rectam:

2.1.1 Colon:

The parts of the colon is either in the abdominal cavity (intraperitoneal) or behind it in the retroperitoneum. Retroperitoneal organs in general do not have a complete covering of peritoneum, so they are fixed in location. Intraperitoneal organs are completely surrounded by peritoneum and are therefore mobile"Peritoneum". (Schünke, *et al*. 2009).

In the colon, the ascending colon, descending colon and rectum are retroperitoneal, while the caecum, appendix, transverse colon and sigmoid colon are intraperitoneal (Ackermann, *et al*. 2006)

The colon has the typical histological structure as the digestive tube mucosa, submucosa, muscularis and serosa/adventitia. The mucosa is lined by simple columnar enterocytes (lamina epithelialis) with long microvilli. It is covered by a layer of mucus which aids the transport of the feces. The mucosa does not contain villi but many crypts of Lieberkuhn in which numerous goblet cells and enteroendocrine cells are found. The connective tissue layer (lamina propriae mucosae) is filled with macrophages, plasma cells and other immune cells. The submucosa comprises blood vessels, lymph nodes and particularly fat tissue. The inner circular musculature of the muscularis is strongly pronounced whereas the outer longitudinal musculature is practically only found in the taeniae (Drenckhahn, *et al*. 2008).

The main task of the colon is the temporary storage and transport of the feces. Thereby it daily absorbs about 1 liter of water which leads to a thickening of the stool. Furthermore it absorbs sodium, potassium and chloride but can also secret potassium into the lumen itself. The physiological intestinal flora is rich in anaerobic bacteria (approx. 10^{11}/g) which live in symbiosis with the human body. They fulfill essential functions such as decomposing indigestible food ingredients (e.g. cellulose), producing vitamin K, promoting the intestinal peristalsis and supporting the immune system (Drenckhahn, *et al*. 2008).

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 (Guyton 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:

2.2.1 Benign colorectal diseases:

2.2.1.1 Ulcerative Colitis:

Ulcerative colitis is a form of colitis, a disease of the colon (the largest portion of the large intestine), that includes characteristic ulcers, or open sores (Danese and Fiocci, 2011).

2.2.1.2 Crohn's Disease:

Crohn's disease, also known as Crohn syndrome and regional enteritis, is a type of inflammatory bowel disease (IBD) that may affect any part of the gastrointestinal tract from mouth to anus (Cho, *et al*. 2011).

2.2.1.3 Diverticular Disease:

Diverticulosis, also known as "diverticular disease", is the condition of having diverticula in the colon, which are outpocketings of the colonic mucosa and submucosa through weaknesses of muscle layers in the colon wall (Comparato, *et al*.2007).

2.2.1.4 Irritable Bowel Syndrome (IBS):

Irritable bowel syndrome (IBS) or spastic colon is a symptom-based diagnosis as a functional gastrointestinal disorder (FGID), IBS has no known organic cause. On set of IBS is more likely to occur after an infection (postinfectious IBS-PI), or a stressful life event, but varies little with age (Saito, *et al*. 2002).

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.

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 Types of colorectal cancer:

2.3.2 .1 Adenocarcinoma:

Adenocarcinomas are tumors that start in the lining of internal organs. Adeno means gland. These tumors start in cells with glandular properties, or cells that secrete. They can form in many different organs, such as the lung or the breast. In colorectal cancer, early tumors start as small adenomatous polyps that continue to grow and can then turn into malignant tumors. The vast majority of colorectal cancers are adenocarcinomas (Varmus, 2010).

2.3.2 .2 Gastrointestinal stromal tumors (GIST):

These are tumors that start in specialized cells in the wall of the digestive tract called the interstitial cells of Cajal. These tumors may be found anywhere in the digestive tract, although they rarely appear in the colon. They can be benign (noncancerous) at first, but many do turn into cancer. When this happens, they are called sarcomas. Surgery is the usual treatment if the tumor has not spread (Varmus, 2010).

2.3.2 .3 Lymphoma:

A lymphoma is a cancer that typically starts in a lymph node, which is part of the immune system. However, it can also start in the colon, rectum, or other organs (Varmus, 2010).

2.3.2.4 Carcinoids:

Carcinoids are tumors that start in special hormone-producing cells in the intestine. Often they cause no symptoms at first. Surgery is the usual treatment (Varmus, 2010).

2.3.2.4 Sarcoma:

Sarcoma. Tumors that start in blood vessels, muscle, or connective tissue in the the colon and rectum wall (Varmus, 2010).

2.3.3 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 (Lynch and Chappele, 2003).

2.3.4 Risk factor of colorectal cancer:

Greater than 75-95% of colon cancer occurs in people with little or no genetic risk (Atkin *et al.* 2010).

2.3.4.1Genetic Factors:

Heredity is perhaps the strongest risk factor for developing colorectal cancer. It is estimated that approximately 20% of all cases of colorectal cancer are hereditary. This risk increases if you have a primary relative, such as a parent, sibling, or child who develops colorectal cancer **(**Thorat, *et al*. 2013).

2.3.4.2Age:

Colorectal cancer most commonly occurs after age 50, though certain forms of this cancer may develop earlier. However, colorectal cancer can occur at any age **(**Thorat, *et al*. 2013).

2.3.4.3 Gender:

Although both men and women develop colorectal cancers, men are at a higher risk **(**Thorat, *et al*. 2013).

2.3.4.4 Lifestyle Factors:

2.3.4.4 .1 Diet:

Diets high in fat, particularly fat from animal sources, and low in fiber have been associated with increased risk of colorectal cancer. Eating a diet that is high in fruits and vegetables may help lower the risk **(**Thorat, *et al*. 2013).

2.3.4.4 .2 Lack of exercise:

Regular exercise has been shown to decrease the risk of developing colorectal cancer. Even moderate exercise of 30 minutes per day is beneficial **(**Thorat, *et al*. 2013).

2.3.4.4 .3 Obesity:

Obesity increases the risk of colorectal cancer, particularly when weight is distributed in the waist, rather than on the hips and thighs **(**Thorat, *et al*. 2013).

2.3.4.4 .4 Smoking:

Smokers are at increased risk of getting colorectal cancer and dying due to colorectal cancer than nonsmokers **(**Thorat, *et al*. 2013).

2.3.4.4 .5 Alcohol:

Three or more alcoholic beverages a day increases risk of colorectal cancer. Drinking alcohol is also associated with a higher risk of forming large benign tumors called colorectal adenomas **(**Thorat, *et al*. 2013).

2.3.4.5 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).

2.3.5 Diagnosing of colorectal cancer:

2.3.5.1 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).

2.3.5.2 Microsatellite instability (MSI) testing:

Sometimes the tumor tissue will be tested to see if it shows changes called Microsatellite instability (MSI) (Varmus, 2010).

2.3.5.3 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).

2.3.5.4 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 (Varmus, 2010).

2.3.6 Treatment of colon cancer:

The treatment of colorectal cancer can be aimed at cure or palliation. The decision on which aim to adopt depends on various factors, including the person's health and preferences, as well as the stage of the tumor (Cunningham, *et al*. 2010).

2.3.6.1 Surgery:

For people with localized cancer, the preferred treatment is complete surgical removal with adequate margins, with the attempt of achieving a cure (Cunningham, *et al*. 2010).

2.3.6.2 Chemotherapy:

In both cancer of the colon and rectum, chemotherapy may be used in addition to surgery in certain cases. The decision to add chemotherapy in management of colon and rectal cancer depends on the stage of the disease (Cunningham, *et al*. 2010).

2.3.6.3 Radiation therapy:

While a combination of radiation and chemotherapy may be useful for rectal cancer, (Cunningham, *et al*. 2010). Its use in colon cancer is not routine due to the sensitivity of the bowels to radiation (Vincent, *et al*. 2008).

2.3.7The 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 marker:

Tumor markers are measurable biochemicals that are associated with a malignancy. They are either produced by tumor cells (tumor-derived) or by the body in response to tumor cells (tumor-associated). They are typically substances that are released into the circulation and thus measured in the blood. There are a few exceptions to this, such as tissue-bound receptors that must be measured in a biopsy from the solid tumor or proteins that are secreted into the urine (Nordenson, *et al*. 2002).

2.4.1 HER2 Marker:

HER2 is so named because it has a similar structure to human epidermal growth factor receptor, or HER1. Neu is so named because it was derived from a rodent glioblastoma cell line, a type of neural tumor. ErbB-2 was named for its similarity to ErbB (avian erythroblastosis oncogene B), the oncogene later found to code for

EGFR. Gene cloning showed that HER2, Neu, and ErbB-2 are all encoded by the same orthologs ERBB2, a known proto-oncogene, is located at the long arm of human chromosome 17 (17q12) (Coussens**,** *et al*. 1985).

HER2/neu in Gastrointestinal tumors. They stated that either HER-2/neu protein overexpression or gene amplification is associated with approximately one-fourth of all gastrointestinal tract malignancies (Ross and McKenna, 2001). HER2 gene amplification was more frequently observed in CRCs located in the rectum than in the right and left colon (Conradi, *et al*. 2013).

CHAPTER THREE

MATERIALS AND METHODS

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 HER2 marker in colorectal tumor using immunohistochemical method.

3.2.2 Study samples:

Thirty colorectal tissue blocks were obtained from tissues previously diagnosed 20 colorectal cancer and 10 colorectal hyperplasia at Wad Madani hospital during the period from May to September 2013.

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:

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

Sections were dewaxed in hot air 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 HER2 (Dako code A0485) 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

CHAPTER FOUR

RESULTS

A total of 30 samples from patients with colorectal tumor were investigated by immunhistochemistry method, 20 of them were malignant colorectal tumors representing (66.7%), and the remaining 10 (33.3%), were benign as indicated in table (4.1).

The age of the study population ranged 17 to 80 years old with mean age of 50 years. Most patients were older than 40 years representing 22 (73.3%) and the remaining 8 (26.7%) 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 17 (56.7%) and the remaining 13 (43.3%) were female.

The description of tumor grade revealed that well differentiated tumor in 7 (35%) patients and moderately differentiated tumor in 13 (65%) patients.

As mentioned in table (4.5), malignant colorectal tumors revealed positive expression of HER2 in 9 (30.0%) patients and negative expansion of HER2 in 11 (36.7%) patients, while all benign colorectal tumor showed negative expansion of HER2, this result show significant statistical association (P value 0.009).

Correlation between HER2 expression and tumor grade revealed that well differentiated tumor showed positive expression in 2 (10%) patients and negative expression of HER2 in 5 (25%) patients and moderately differentiated tumor showed positive expression of HER2 in 7 (35%) patients and negative expression of HER2 in 6 (30%) patients, this result showed insignificant statistical association (P value 0.125).

Table (4.1) Distribution of sample among the study population

Sample	**Frequency**	**Percent**
Malignant	20	66.7%
Benign	10	33.3%
Total	30	100%

Table (4.2) Distribution of age among the study population

Age group (year)	**Frequency**	**Percent**
Less than 40 year	8	26.7%
40_60 year	8	26.7%
61_80 year	14	46.6%
Total	30	100%

Table (4.3) Distribution of sex among the study population

Sex	**Frequency**	**Percent**
Male	17	56.7%
Female	13	43.3%
Total	30	100%

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

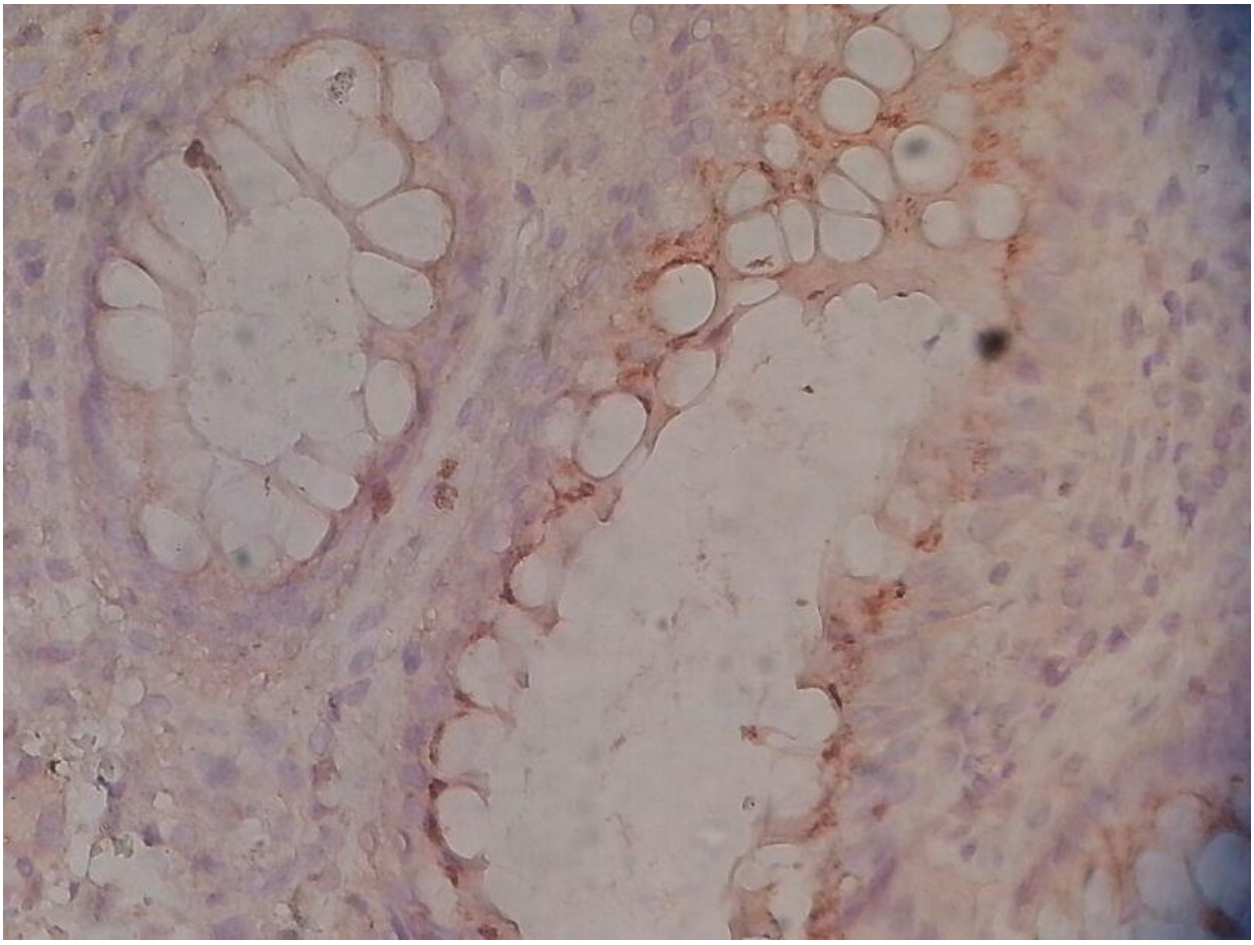
Tumor grade	**Frequency**	**Percent**
Well differentiated tumor	7	35%
Moderate differentiated tumor	13	65%
Total	20	100%

Table (4.5) Immunohistochemical expression of HER2 among the study samples

Sample	**HER2**				**Total**		**P Value**
	Positive		Negative				
	N	%	N	%	N	%	
Malignant	9	30%	11	36.7%	20	66.7%	0.009
Benign	0	0.0%	10	33.3%	10	33.3%	
Total	9	30%	21	70%	30	100%	

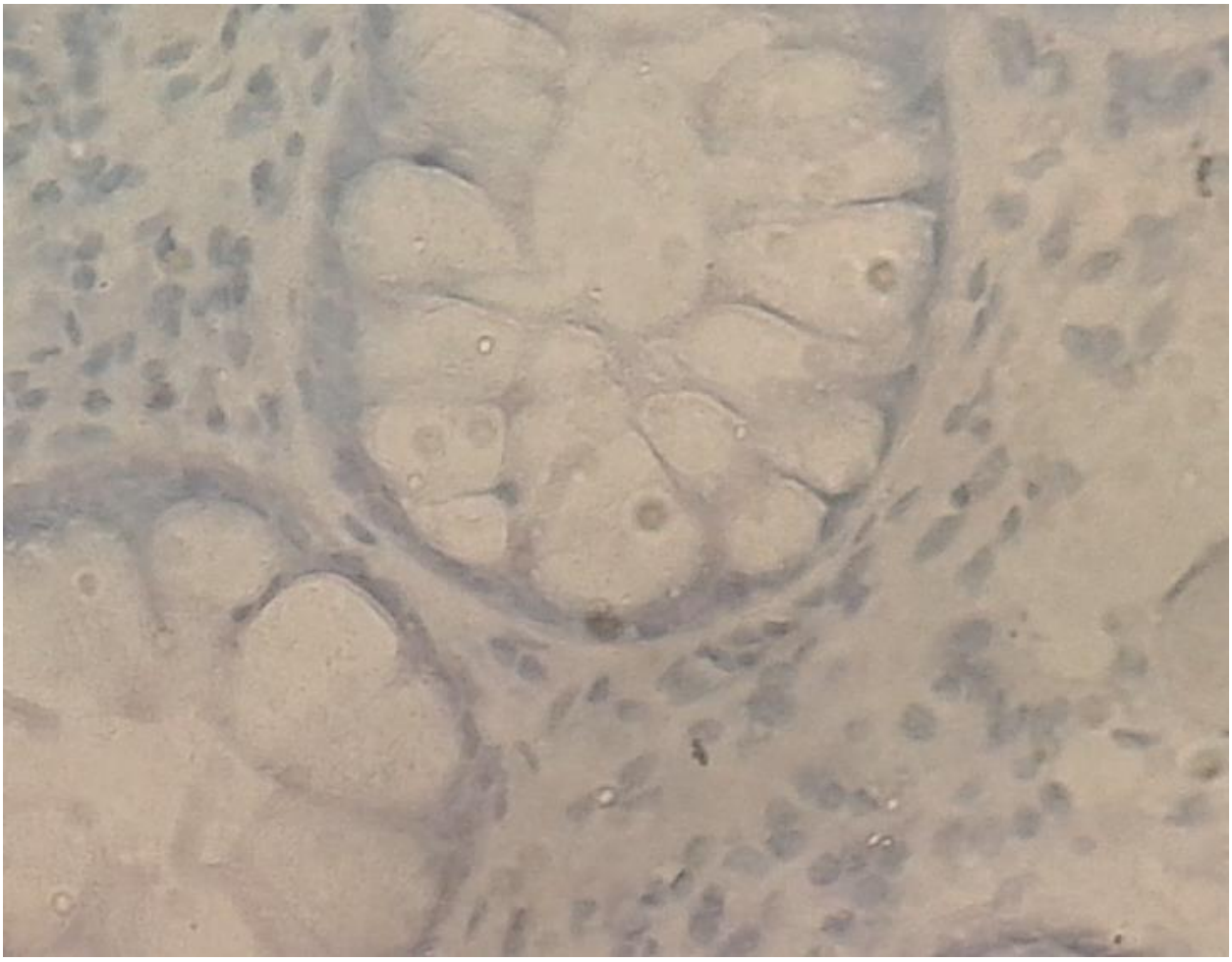
Table (4.6) Correlation between HER2 expression with cancer grade

Grade	HER2				Total		P Value
	Positive		Negative				
	N	%	N	%	N	%	
Well differentiated tumor	2	10%	5	25%	7	35%	0.125
Moderate differentiated tumor	7	35%	6	30%	13	65%	
Total	9	45%	11	55%	20	100%	



Microphotography (4.1):

Colon adenocarcinoma moderately differentiated showed membranous and cytoplasmic positive expression of HER2 (40X).



Microphotograghy (4.2):

Rectal adenocarcinoma moderately differentiated showed cytoplasmic negative expression of HER2 (40X).

CHAPTER FIVE

DISCUSSION

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 (Boyle, *et al*. 2008).

In this study out of thirty samples of patients with colorectal tumor were investigated by immunhistochemical method, 20 of them were malignant colorectal tumors representing (66.7%), and the remaining 10 (33.3%), were benign. The age of the study population ranged between 17 to 80 years old with mean age of 49 years. Most patients were older than 40 years representing 22 (73.3%) and the remaining 8 (26.7%) were younger than 40 years. This means older than 40 years are more susceptible for colorectal tumor due to acidosis (low degree of PH). 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 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. This 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 the most colorectal cancer patients are moderately differentiated tumor patients then in second place well differentiated tumor patients and poor differentiated tumor was not observed, 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 20% and 10%, respectively.

HER2 gene amplification was more frequently observed in CRCs (Conradi, *et al*. 2013).

Malignant colorectal tumors revealed positive expression of HER2 in patients and negative expression, while all benign colorectal tumor showed negative expression of HER2 patients, this result show significant statistical association (P value 0.009). This result supported by Nathanson (2003), studied HER2/neu expression and gene amplification in colon cancer.

HER2/neu overexpression in different grades of colorectal adenocarcinomas (Schuell, *et al* 2006).

Based on this study the statistical association between HER2 expression and tumor grade showed insignificant association (P value 0.125). It revealed that well differentiated tumor showed positive and negative expression of HER2 in patients also moderately differentiated tumor showed positive and negative expression of HER2 in patients , this study compatible with Delektorskaia, *et al* (2003), who reported that no correlation between HER-2/neu staining and Grades of tumors.

CONCLUSION

AND

RECOMMENDATIONS

Conclusion and Recommendations

Conclusion:

On the basis of this study we concluded that:

HER2 expression association with malignant tumor was 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.

Mayer's haematoxylin.

Citrate buffer (PH 6.8).

Phosphate buffer (PH 7.4).

Primary antibody (HER2).

Secondary antibody.

Substrate-Chromogen.

Polyclonal Rabbit Anti-Human c-erbB-2 Oncoprotein

Code A0485

ENGLISH

Intended use

For in vitro diagnostic use.

Polyclonal Rabbit Anti-Human c-erbB-2 Oncoprotein is intended for use in immunohistochemistry. The antibody labels normal epithelial cells, which generally express c-erbB-2 protein at a very low level. It is a useful tool for the identification of overexpression of c-erbB-2 oncoprotein in a variety of epithelial neoplasms, for example subsets of breast carcinomas, pulmonary adenocarcinomas, colorectal adenocarcinomas, pulmonary squamous and gastric adenocarcinomas (1), transitional cell carcinomas of the urinary bladder (2), and endometrial adenocarcinomas (3). The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Synonym for antigen

HER2 (human epidermal growth factor receptor 2) (4), HER2/neu (5), ErbB2 (4, 5) and p185^{HER2} (6).

Summary and explanation

c-erbB-2 oncoprotein is a 185 kDa transmembrane tyrosine kinase belonging to the epidermal growth factor receptor (EGFR) family. This family comprises four homologous receptors ErbB-1 (EGFR, HER1), ErbB-2 (HER2/neu), ErbB-3 (HER3), and ErbB-4 (HER4) (4). The c-*erb*B-2 proto-oncogene is located on chromosome 17 at q21 (7). Activation of c-erbB-2 oncoprotein, either by homo- or heterodimerization, triggers intracellular signalling events, which are crucial for cell growth, differentiation and survival. Mechanisms promoting receptor dimerizations include ligand binding and high receptor density (overexpression). An overexpression of c-erbB-2 oncoprotein is often a result of gene amplification (4).

c-erbB-2 oncoprotein is frequently overexpressed in human carcinomas, thus 25-30% of human breast carcinomas overexpress this receptor (6, 8), whereas overexpression has not been found in benign breast disease (4).

In the last two decades, monoclonal antibodies which block activation of c-erbB-2 oncoprotein have been developed and clinical activity of a humanized monoclonal antibody, Herceptin™, has been documented (6).

Reagent provided

Affinity-isolated rabbit antibody purified by using immobilized c-erbB-2 oncoprotein peptide and provided in liquid form in 0.05 mol/L Tris/HCl, 0.1 mol/L NaCl, 15 mmol/L NaN_3.

Protein concentration g/L: See label on vial.

Immunogen

Synthetic human c-erbB-2 oncoprotein peptide from the intracytoplasmic part of the c-erbB-2 oncoprotein. The peptide was coupled to keyhole limpet hemocyanin (KLH).

Specificity

The antibody labels an intracellular domain of c-erbB2 oncoprotein.

Precautions

1. For professional users.
2. This product contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
3. As with any product derived from biological sources, proper handling procedures should be used.
4. The product may be used in different techniques and in combination with different sample types and materials, therefore each individual laboratory should validate the test system applied.
5. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
6. Unused solution should be disposed of according to local, State and Federal regulations.

Storage

Store at 2–8 °C. Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the user must verify the conditions. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Dako Technical Support.

Specimen preparation

Paraffin sections: The antibody can be used for labelling paraffin-embedded tissue sections fixed in formalin. Pre-treatment of tissues with 20 minutes of heat-induced epitope retrieval is required. Optimal results are obtained with EnVision™ FLEX Target Retrieval Solution, Low pH, Code K8005 or Dako Target Retrieval Solution, Low pH, Code S1699/S1700. Alternatively, EnVision™ FLEX Target Retrieval Solution, High pH, Code K8000/K8010, may be used at a different antibody dilution (see Staining Procedure). The tissue sections should not dry out during the treatment or during the following immunohistochemical staining procedure.

Staining procedure

Dilution: Polyclonal Rabbit Anti-Human c-erbB-2 Oncoprotein, Code A0485, may be used at a dilution range of 1:600–1:800 or 1:1000–1:1200 (see Visualization) when applied on pretreated, formalin-fixed, paraffin-embedded sections of human mammary carcinoma overexpressing the c-erbB-2 oncoprotein using a 20 minutes incubation at room temperature.

Visualization (HIER at low pH):
K8000/K8010: EnVision™ FLEX, High pH, Code K8000/K8010, replacing the High pH Target Retrieval Solution from this kit with EnVision™ FLEX Target Retrieval Solution, Low pH, Code K8005, using the antibody at a dilution range of 1:600–1:800,

K5007*: Dako Real™ EnVision™ Detection Kit, Peroxidase/DAB+, Rabbit/Mouse, Code K5007, using the antibody at a dilution range of 1:600–1:800.

Visualization (HIER at high pH):
K8000/K8010: EnVision™ FLEX, High pH, Code K8000/K8010, using the antibody at a dilution range of 1:1000–1:1200.

K5007*: Dako Real™ EnVision™ Detection Kit, Peroxidase/DAB+, Rabbit/Mouse, Code K5007, using the antibody at a dilution range of 1:1000–1:1200.

Follow the procedure enclosed with the selected visualization system(s).

**K5007 is not available in the United States.*

Optimal conditions may vary depending on specimen and preparation method, and should be determined by each individual laboratory. The recommended negative control is Dako Rabbit Immunoglobulin Fraction (Solid-Phase Absorbed), Code X0936, diluted to the same protein concentration as the primary antibody. Unless the stability in the actual test system has been established, it is recommended to dilute the product immediately before use or dilute in EnVision™ FLEX Antibody Diluent, Code K8006 or Dako Antibody Diluent, Code S0809.

Automation: The antibody is well-suited for immunohistochemical staining using automated platforms such as Autostainer Link 48 and Dako Autostainer Classic.

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Product-specific limitations

Occasional cytoplasmic staining may be observed. It should be disregarded and only staining of the cell membrane should be considered specific for c-erbB-2 oncoprotein.

Performance characteristics

Cells labelled specifically by the antibody display a staining confined to the cell membrane.

Normal tissues: c-erbB-2 oncoprotein is a normal tissue component and the antibody may display a weak labelling of normal epithelial cells. Squamous epithelium in the esophagus and tonsil may in some cases show moderate staining. Prostatic gland tissue has also been found moderately positive. A long range of other normal tissues, such as adrenal gland, bone marrow, brain, heart, liver, lung, skeletal muscle, skin, spleen, thymus and thyroid gland, is negative.

Abnormal tissues: In one study (1), 13/59 of breast carcinomas, 8/29 pulmonary adenocarcinomas, 10/58 colorectal adenocarcinomas, and 6/56 pulmonary squamous and 7/62 gastric adenocarcinomas showed c-erbB-2 oncoprotein overexpression (complete membrane staining – weak to strong – in more than 10% of the tumour cells) when tested with the antibody. In another study (2) 101/177 of transitional cell carcinomas of the urinary bladder showed c-erbB-2 oncoprotein overexpression. Of endometrial adenocarcinomas of endometrioid type 15/112 overexpressed c-erbB-2 oncoprotein (3). No overexpression was found in 30 kidney adenocarcinomas, 12 hepatocellular carcinomas, and 17 malignant melanomas (1).

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Explanation of symbols/ Légende des symboles/ Erläuterung der Symbole

REF Catalogue number Référence du catalogue Bestellnummer	2 °C – 8 °C Temperature limitation Limites de température Zulässiger Temperaturbereich	Manufacturer Fabricant Hersteller
IVD In vitro diagnostic medical device Dispositif médical de diagnostic in vitro In-Vitro-Diagnostikum	LOT Batch code Code du Lot Chargenbezeichnung	
Consult instructions for use Consulter les instructions d'utilisation Gebrauchsanweisung beachten	Use by Utiliser jusque Verwendbar bis	

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