Sudan University of Sciences and Technology

College of Graduate Studies

Immunohistochemical Detection of Vascular Endothelial Growth Factor Expression in Colorectal Tumors among Sudanese Patients

الكشف بالطرق المناعية الكيميائية عن ظهور عامل نمو بطانة الاوعية الدموية في أورام القولون والمستقيم لدى المرضى السودانيين

A Dissertation Submitted in Partial Fulfillment of the Requirements of M.Sc. Degree in Medical Laboratory Sciences (Histopathology and Cytology)

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2020

الآية

قال تعالى:

(قَالَ رَبِّ اشْرَحْ لِي صَدْرِي (25) وَيَسِّرْ لِي أَمْرِي (26) وَاحْلُلْ عُقْدَةً مِنْ لِسَانِي (27) يَفْقَهُوا قَوْلِي(28))

سورة طه الايات 25-28

DEDICATION

I dedicate this dissertation to:

My Self
My Family
My Friends
And all people who supported me

Acknowledgement

I 'm grateful to Allah for his care, insight, mercy and peace in my life.

I 'm deeply indebted to my supervisor Dr. Abu Elgasim Abass for his help; guidance and patience.

Deep thank to Ustaz Abubakr Mahboob and Ustaz Shahinaz Shaban for their great help and support.

Deep thank for all my friends and colleagues for their support and encouragements.

Abstract

This is a hospital based descriptive retrospective case study aimed to detect the expression of vascular endothelial growth factor in benign and malignant colorectal tumors and colorectal carcinoma features (grade). The study was conducted in Khartoum-Sudan in Al Safwa laboratory during the period from November 2019 to January 2020. Seventy eight archival formalin fixed paraffin embedded tissue blocks were used. They have been selected as thirty nine benign colorectal tumors and thirty nine malignant colorectal tumors. The targeted area from tissue blocks was determined and collected for processing by tissue microarray (TMA). Sections were stained by immunohistochemistry for VEGF expression. The data obtained were analyzed by SPSS computer program.

The mean age of the patients was 43 years and ages of them ranged between 3 years to 81 years and divided as less than or equal 40 years old has frequency of 29(37%) and more than 40 years old has frequency of 49(63%). In this study male were 52 (67%) while female were 26 (33%).

All the 39 tissues that constituted benign histopathological diagnosis expressed negative VEGF staining, and from the 39 cancerous tissues 7 (18%) showed positive cytoplasmic staining, so the relation between the expression pattern of VEGF and the histopathological diagnosis was found to be statistically significant (P value = 0.006).

The results shows that out of 7 positive samples for VEGF expression, 2 (29%) samples were positive from low grade adenocarcinoma, 2 (29%) samples were positive from moderate grade adenocarcinoma, 3 (42%) samples were positive from high grade adenocarcinoma, so the relation between VEGF expression pattern and histological grading one of the clinicopathological features of CRC was insignificant (P value = 0.562).

The study conclude that the expression of VEGF has a significant correlation with malignant form of CRC tumors, with no association with grade of CRC.

المستخلص

هذه دراسة وصفية تراجعية مستشفوية تهدف إلى الكشف عن عامل نمو بطانة الاوعية الدموية في اورام القولون والمستقيم الحميدة والخبيثة وخواص السرطان القولوني الشرجي (درجة). تم اجراء الدراسة في الخرطوم– السودان في معمل الصفوة خلال الفترة من نوفمبر 2019 وحتى يناير 2020. ثمانية وسبعون قالبا شمعيا مؤرشفة مثبتة بالفورمالين من انسجة مأخوذة جراحيا تم استخدامها. لقد تم اختيارها كتسعة وثلاثون ورم حميد قولوني شرجي و تسعة وثلاثون ورم سرطاني قولوني شرجي. المنطقة المستهدفة من قوالب الانسجة تم تحديدها وجمعها لمعالجتها بطريقة (TMA) كتل برافين نسيجية متناهية الصغر. تم صبغ الانسجة بالطرق المناعية الكيميائية للكشف عن عامل نمو بطانة الاوعية الدموية. تم تحليل البيانات التي تم الحصول عليها بواسطة برنامج الحزمة الإحصائية للعلوم الاجتماعية(SPSS).

متوسط اعمار المرضى هو 43 عاما وتراوحت اعمارهم بين 3 اعوام حتى 81 عاما وتم تقسيمها كأقل من او يساوي 40 عاما وعددهم 29(37%) واكبر من 40 عاما وعددهم 49(63%) . في هذه الدراسة عدد الذكور كان 52(67%) وعدد الاناث كان 26(33%).

جميع الانسجة البالغ عددها 39 ذات التشخيص التشريحي المرضي الحميد اظهرت دلالة صبغية سالبة لعامل نمو بطانة الاوعية الدموية, ومن 39 نسيجا سرطانيا 7(18%) عينات اظهرت دلالة صبغية عصارية ايجابية, اذن العلاقة بين نمط ظهور عامل نمو بطانة الاوعية الدموية والتشخيص التشريحي المرضي وجد بانها ذات فروق ودلالة احصائية (P value=0.006).

اظهرت النتائج انه من 7 عينات ايجابية لظهور عامل نمو بطانة الاوعية الدموية, 2(29%) عينات كانت ايجابية من الورم السرطاني الغدي المنخفض الدرجة, 2(29%) عينات كانت ايجابية من الورم السرطاني الغدي متوسط الدرجة, 3(42%) عينات كانت ايجابية من الورم السرطاني الغدي مرتفع الدرجة, مع عدم وجود علاقة بين نمط ظهور عامل نمو بطانة الاوعية الدموية ودرجة الورم (احد المعالم المرضية للسرطان القولوني الشرجي) حيث وجد بانها غير ذات دلالة احصائية (P value = 0.562).

هذه الدراسة خلصت بان نمط ظهور عامل نمو بطانة الاوعية الدموية لديه علاقة ذات دلالة احصائية مع الاورام السرطانية القولونية الشرجية, وانه لاتوجد علاقة ذات دلالة احصائية بين عامل نمو بطاقة الاوعية الدموية ودرجة الورم القولوني الشرجي.

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Abbreviations

Abbreviation	Meaning
CRC	Colorectal Cancer
MDCT	Multidetector Helical Computed Tomography
CT	Computed Tomography
MRI	Magnetic Resonance Imaging
PET-CT	Positron Emission Tomography-Computed Tomograph
CEA	Carcinoembryonic Antigen
CA 19-9	Cancer Antigen 19-9
TAG-72	Tumor Associated Glycoprotein -72
TPS	Tissue Polypeptide Specific antigen
VEGF	Vascular Endothelial Growth Factor
VEGFRs	Vascular Endothelial Growth Factor Receptors
PIGF	Placental Growth Factor
DNES	Diffuse Neuroendocrine System
TNM	Tumor Node Metastasis
PDGF	Platelets Derived Growth Factors
MVD	Microvascular Density
TMA	Tissue Microarray
IHC	Immunohistochemistry

CHAPTER ONE

INTRODUCTION

Chapter One

Introduction

1.1 Introduction

Colorectal cancers are cancers of colon and rectum. More than 1.2 million patients are diagnosed with colorectal cancer every year, and more than 600,000 die from the disease (Brenner, *et al*. 2014). Colorectal cancer (CRC) is a major cause of morbidity and mortality throughout the world. Colorectal cancer is the third most commonly occurring cancer in men and the second most commonly occurring cancer in women. There were over 1.8 million new cases in 2018 (WHO, 2018).

Most colorectal cancers develops from growths called colorectal polyps that form in the lining of the colon or rectum, but not all polyps become cancerous, the malignant potential and subsequent screening intervals are dependent on polyp type, all adenomas have variable degrees of dysplasia ranging from low-grade to high-grade. Classically, it is believed that the malignant potential of adenomas correlates with type of polyp, size, and degree of dysplasia. Higher grades of dysplasia, increasing percentage of villous tissue within the polyp, and polyps greater than 1 cm in diameter are associated with increased risk of malignancy (Colucci, *et al*. 2003).

There are several risk factors for colorectal cancer, the most significant factors include obesity, smoking, high dietary intake of fat and red meat, alcohol use, lack of physical exercise, older age and a family history of inflammatory bowel disease (Nasaif and Mahmoud, 2018).

Screening for colorectal cancer can detect cancers at early stages. Colonoscopy, flexible sigmoidoscopy, and fecal occult blood tests are established tools for screening (Kolligs, 2016).

Different imaging procedures are used in patients suspected or known to suffer from colorectal cancer. These techniques thus play an important role in primary diagnostics, staging, evaluation of treatment response, follow-up, and even for minimally invasive interventions, including the rapid technological evolution of Multidetector Helical Computed Tomography (MDCT), the novel application of CT colonography, improvements in Magnetic Resonance Imaging (MRI) with development of novel sequence techniques, an increasing use of Positron Emission Tomography (PET)-CT, and novel imaging-guided interventional procedures (Baebler, *et al*. 2016).

Diagnostics and monitoring of CRC can be done by: Carcinoembryonic Antigen (CEA), Cancer Antigen (CA) 19-9, tumor antigen of colorectal cancer such as Tumor Associated Glycoprotein (TAG-72), Tissue Polypeptide Specific antigen (TPS). Increased values of tumor markers evaluate recurrences or metastases, especially to the liver (Ławicki, *et al*. 2002).

Tumor growth and metastasis are dependent on angiogenesis. Vascular Endothelial Growth Factor (VEGF) plays an important role in the angiogenesis of numerous solid malignancies including colon cancer. Evidence from preclinical and clinical studies indicates VEGF is the predominant angiogenic factor in human colon cancer and is associated with formation of metastases and poor prognosis (Lee, *et al*. 2000).

VEGF, also known as VEGF-A, is a protein with vascular permeability activity that was originally purified from a fluid secreted by a tumor. A few years later, a protein with angiogenic activity was independently purified and named VEGF. Molecular cloning, however, revealed that these 2 proteins were identical and encoded by a single gene. The VEGF family includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, PlGF (Placental Growth Factor), VEGF-E (Orf-VEGF), and Trimeresurus flavoviridis svVEGF. With the exception of the latter 2

members, 5 genes of the VEGF family exist in mammalian genomes, including humans (Shibuya, 2011).

VEGF is expressed in approximately 50% of CRCs, with minimal to no expression in normal colonic mucosa and adenomas (Bendardaf, *et al*. 2008).

1.2 Rationale

Colorectal cancer is a major cause of morbidity and mortality throughout the world, and it is the third most commonly occurring cancer in men and the second most commonly occurring cancer in women (WHO, 2018).

Vascular endothelial growth factor (VEGF) is a potent angiogenic factor and was first described as an essential growth factor for vascular endothelial cells; it will be used in this study to test its diagnostic value in colorectal cancer. The significance of this marker will lead to better understanding of colorectal tumors.

1.3 Objectives

1.3.1 General objective:

To detect VEGF expression in different types of colorectal tumors among Sudanese patients.

1.3.2 Specific objectives:

1- To detect VEGF in colorectal tissues using immunohistochemistry.

2-To correlate the expression pattern of VEGF to the histopathological diagnosis of tumors and grade of CRC.

CHAPTER TWO

LITERATURE REVIEW

Chapter Two
Literature Review

2.1 Anatomy of colon:

The colon, is part of the digestive tract, it includes the cecum and ascending colon, transverse colon, descending colon, and sigmoid colon. The colon accounts for almost the entire length of the large intestine. It receives chime from the ileum at the ileocecal valve, an anatomical as well as physiological sphincter that prevents reflux of the cecal content into the ileum. The colon has no villi but is richly endowed with crypts of lieberkuhn that are similar in composition to those of the small intestine, except for the absence of Paneth cells. The number of goblet cells increases from the cecum to the sigmoid colon, but the surface absorptive cells are the most numerous cell type. DNES (Diffuse Neuroendocrine System) cells are also present, although they are few in number (Leslie and James, 2001).

2.2 Colorectal cancer epidemiology:

Colorectal cancer is the third most deadly and fourth most commonly diagnosed cancer in the world. Nearly two million new cases and about one million deaths was detected in 2018. CRC incidence has been steadily rising worldwide, especially in developing countries that are adopting the “western” way of life. The CRC is more incident among men than women and 3–4 times more common in developed than in developing nations (Rawla, *et al*. 2019).

Colon cancer is most prevalent in Western countries (Carethers, 2018). For colon cancer, Southern Europe, Australia/New Zealand, and Northern Europe are the regions of highest incidence. In Japan, South Korea, Saudi Arabia, Oman, Yemen, UAE, Bahrain, Qatar, Kuwait, and Slovakia CRC is the most diagnosed cancer among men (Rawla, *et al*. 2019).

In Sudan, colorectal cancer is the fourth most common cancer, accounting for 5.4% of all new cases in 2018, but it has received less attention. Previously, a study from National Cancer Institute, University of Gezira, Sudan, assessed the clinical presentation in colorectal cancer patients between 2006 and 2011 and found that 58% of the patients presented at a late stage of the disease, and about 97% had rectal bleeding (Khougali, 2019).

2.3 Risk factors of CRC:

There are several risk factors measured for colon cancer include:

2.3.1 Alcohol consumption:

Alcohol intake, even in small amounts, has been proposed to be associated with an increased risk of CRC. The risk is particularly remarkable with heavy drinking (Rossi, *et al*. 2018).

2.3.2 Cigarette smoking:

Cigarette smoking is an established risk factor for colorectal cancer (Limsui, *et al*. 2010).

2.3.3 Diabetes:

The risk of CRC mortality is significantly increased in both sexes and women with diabetes (Tan, *et al*. 2016).

2.3.4 Age:

Significantly elevated morbidity and mortality rates were found with increasing age. The distribution of tumor stages revealed a significantly higher percentage of locally advanced tumors in the older age group (Marusch, *et al.* 2005).

2.3.5 Familial history:

Up to 30% of CRCs have evidence of a familial component and about 5% are thought to be due to well-characterized inherited mutations. Those with a family history of Lynch Syndrome, Familial Colorectal Cancer Type X, Familial Adenomatous Polyposis, *MutYH* associated polyposis, Peutz-Jeghers Syndrome, Juvenile Polyposis Syndrome, PTEN Hamartomatous Syndrome, and Serrated Polyposis Syndrome are at an increased risk of developing colorectal cancer (Patel and Ahnen, 2012).

2.4 Diagnosis of colon cancer:

Diagnosis of colorectal cancer is via tumor biopsy typically done during sigmoidoscopy or colonoscopy. There is another method called **Barium enema,** this method examines the entire colon and rectum (Hamzehzadeh, *et al*. 2017).

For local staging of colon cancer, abdominal CT is widely used and recommended for evaluation of local tumor infiltration. MRI is recommended as the examination of first choice for locoregional staging of rectal cancer (Baeßler, *et al*. 2016).

Different imaging tests provide different information for assessing TNM stage. For example, endoscopic ultrasound can provide information on the "local stage" (i.e., the depth of invasion of the cancer into the bowel wall), but not on the presence of distant metastases. In contrast, whole-body CT or PET/CT may not be useful for assessing depth of invasion into the bowel wall, but can provide information on metastatic lesions (Bruening, *et al*. 2014).

The tumor, node, metastases (TNM) staging system is widely used to predict the prognosis for patients with colorectal cancer (Li, *et al*. 2016).

2.5 Management of colon cancer:

Progress in the development of imaging modalities has enabled more accurate staging based on the TNM classification. The therapeutic management of CRC should involve a multi-modal approach, including high-quality surgery and an optimal choice of chemotherapy and radiotherapy regimens according to disease characteristics and patient preferences (Nakayama, *et al*. 2013).

2.5.1 Surgery:

Surgery remains as the cornerstone curative treatment for rectal cancer. A surgeon may remove the cancer using one of those types of surgery , local excision, resection of the colon with anastomosis, resection of the colon with colostomy **(**Damin and Lazzaron, 2014).

2.5.2 Chemotherapy:

It is recommended to take adjuvant chemotherapy following surgery for persons with stage III colon cancer, and stages II and III rectal cancer (Benson, *et al*. 2000). FOLFOX (FOLinic acid [leucovorin], Fluorouracil, OXaliplatin) is the preferred regimen (Rabeneck, *et al*. 2015).

2.5.3 Radiation:

The availability of radiation therapy is most relevant for cancers of the rectum, as local recurrence is much more common than in colon cancer, because of the inability to obtain wide margins and the lack of a serosal barrier (Hoffe, *et al*. 2010). Preoperative radiation is associated with improved surgical outcomes and disease free survival (Sebag-Montefiore, *et al*. 2009).

2.6 Angiogenesis in cancer:

New growth in the vascular network is important since the proliferation, as well as metastatic spread, of cancer cells depends on an adequate supply of oxygen and nutrients and the removal of waste products. Angiogenesis is regulated by both activator and inhibitor molecules. More than a dozen different proteins have been identified as angiogenic activators and inhibitors. Levels of expression of angiogenic factors reflect the aggressiveness of tumor cells (Nishida, *et al*. 2006). The key signaling system that regulates proliferation and migration of endothelial cells forming the basis of any vessel are vascular endothelium growth factors (VEGF) and their receptors. The VEGF-dependent signaling system is necessary for formation of the embryonic vascular system (Karamysheva, 2008). In recent decades, a variety of signaling molecules, such as VEGF-VEGFRs, ephrin-Eph receptors, angiopoietin-Tie, and the Delta-Notch system, have been identified as playing important roles in angiogenesis (Shibuya, 2011).

2.7 VEGF:

The vascular endothelial growth factor (VEGF) and its receptor (VEGFR) have been shown to play major roles not only in physiological but also in most pathological angiogenesis, such as cancer. VEGF belongs to the Platelet Derived Growth Factors (PDGF) supergene family characterized by 8 conserved cysteines and functions as a homodimer structure. The VEGF family of genes contains at least 7 members, including the viral genome–derived VEGF-E, whereas the VEGFR family of genes has 3 to 4 members depending on the vertebrate species (Shibuya, 2011).

VEGF-A regulates angiogenesis and vascular permeability by activating 2 receptors, VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk1 in mice). On the other

hand, VEGF-C/VEGF-D and their receptor, VEGFR-3 (Flt-4), mainly regulate lymphangiogenesis. On the other hand, VEGFs have pro-angiogenic potential for the maintenance of various tissues at physiological levels and for the formation of new blood vessels to overcome ischemic diseases (Shibuya, 2011).

2.7.1 Structure and biochemical functions:

Genes encoding novel tyrosine kinase receptors were isolated in the early 1990s, and the tyrosine kinase receptors that positively and negatively regulate the formation of blood and lymph vessels were denoted VEGFRs. Three genes are encoding three full-length receptors (VEGFR-1, -2, and -3) and one soluble molecule (sVEGFR-1), and most VEGFRs show similar overall structures that comprise of three primary domains. The kinase domains of VEGFRs are the most conserved region, with high sequence identities 78–80% (Park, *et al*. 2018).

There are five VEGF family members (VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor) encoded from the mammalian genome (Park, *et al*. 2018).

Among them, VEGF-A (known as VEGF) is one of the most critical factors for blood vessel formation during early embryogenesis. VEGF-A binds to Ig domains 2 and 3 localized in the ECD of VEGFR-1 and VEGFR-2 (Park, *et al.* 2018). VEGF-B and placenta growth factor (PIGF) bind to VEGFR-1, but their mechanisms that activate the receptor are different (Roskoski, 2008). VEGF-C and VEGF-D are specific ligands for VEGFR-3, which plays a critical role in angiogenesis and lymphangiogenesis in adults (Roskoski, 2007).

VEGFR-1 is expressed in vascular endothelial cells and non-endothelial cells, including haematopoietic stem cells, macrophages, and monocytes. The VEGFR-2

expression is detected in not only vascular endothelial and lymphatic endothelial cells, but also megakaryocytes and haematopoietic stem cells (Park, *et al*. 2018).

2.7.2 Anti angiogenic therapy in colorectal cancer:

VEGF plays an important role in colorectal cancer (CRC) biology, and its inhibition by using bevacizumab, an anti-VEGF antibody, proved for the first time to be effective and became indispensable for the treatment of metastatic CRC (mCRC). Several large phase III studies showed also relevant responses and tolerability of other anti-angiogenic drugs such as ramucirumab, aflibercept, and regorafenib, and led to the approval of these therapeutics. Nevertheless, the efficacy of anti-angiogenic therapies is rather limited and the high expectations raised by preclinical studies were not fulfilled in the clinics. Furthermore, to date, no predictive biomarkers for anti-angiogenic agents could be identified and validated (Seeber, *et al*. 2018).

2.7.3 VEGF clinical significance:

Angiogenesis is an essential process required for the growth and metastatic ability of solid tumors. Some studies demonstrated that an increase in microvascular density (MVD) was found to be closely associated with the expression of vascular endothelial growth factor, Several studies have noted that the level of VEGF expression, a strong angiogenic factor, correlates with neovascularity and tumor progression (Zheng, *et al*. 2003).

Meta-analysis study, representing a quantified synthesis of all published studies, found a statistically significant inverse relationship between angiogenesis, assessed by VEGF expression, and survival, confirming that, human invasive colorectal cancer is an angiogenesis-dependent malignancy (Des Guetz, *et al*. 2006).

Various studies have demonstrated that the VEGF expression level correlates with angiogenesis and tumor progression in colorectal cancer, Previous studies also have described an association between VEGF expression and tumor aggressiveness in various types of malignant tumor, including colorectal cancer, and a high VEGF expression level was identified to be correlated with a short overall survival of patients exhibiting lymph node metastasis (Kimura, *et al*. 2016), and can be used as a risk biomarker particularly in patients with advanced stage and grade (Hedaya, *et al*. 2015).

CHAPTER THREE

MATERIALS AND METHODS

Chapter Three
Materials and Methods

3.1 Materials:

Archival colorectal samples paraffin tissue blocks were used in this study.

3.2 Methods:

3.2.1 Study design:

This is a descriptive retrospective case study aiming at evaluating expression of VEGF tumor marker in colon and rectum using immunohistochemistry techniques.

3.2.2 Study samples:

Seventy eight archival formalin fixed paraffin embedded tissue biopsies were used. They have been selected as thirty nine benign colorectal tumors and thirty nine malignant colorectal tumors.

3.2.3 Study area and duration:

This study conducted in Khartoum-Sudan in Al Safwa Laboratory during the period from November 2019 to January 2020.

3.2.4 Samples processing:

The targeted area from tissue blocks was determined and collected for processing by tissue microarray (TMA). One sections (3-4µ) was obtained from each block and treated for staining by immunohistochemistry using monoclonal specific antibody directed against VEGF.

3.2.5 Immunohistochemical staining:

The sections for IHC were incubated overnight in an oven at 65ºc then brought to water using two changes of xylene for three minutes each; two changes of absolute

ethanol three minutes each; two minutes in descending grades of ethanol (90%, 70%, 50%) and finally sections re-hydrated in distilled water.

Samples were steamed for antigen retrieval for VEGF using PT link containing sodium citrate buffer (pH 9.0). Endogenous peroxidase activity was blocked with 3% hydrogen peroxide and methanol for 10 minutes. Then slides were incubated with 200 µl of primary antibodies for VEGF (monoclonal mouse antihuman-ThermoFisher) for 20 min at RT, then rinsed in phosphate buffer saline. After washing with PBS for 3 min, binding of antibodies were detected by incubating for 20 minutes with dextran labelled polymer (Dako kit). Finally, the sections were washed in three changes of PBS, followed (DAB) as a chromogen to produce the characteristic brown stain for 5 min. Sections were counterstained with Mayer's Haematoxylin for 1 min, washed in distilled water and left to air dry for 5min. Finally slides were cleared in xylene and mounted with a cover glass using DPX mounting media (Turley, *et al.* 1998). Expression of VEGF was considered positive whenever at least 10% of the cells were stained (Ranjbar, *et al*. 2015).

Slides validated and examined by Olympus light microscope using 10Xand 40X for cytoplasmic expression.

3.2.6 Data analysis:

The data were analyzed using version 23 SPSS computer program; frequencies, mean and Chi-square test were calculated.

CHAPTER FOUR

RESULTS

Chapter Four

Results

Seventy eight samples were collected and processed as TMA, 39 were benign colorectal samples and 39 were malignant colorectal samples, all samples were tested for VEGF expression by IHC. Out of the 39 benign samples 18 (46%) samples were diagnosed as juvenile rectal polyps, 15 (38%) samples were hyperplastic polyps, 5 (13%) samples were inflammatory polyps and only one (3%) sample was benign rectal polyp. All 39 malignant samples were adenocarcinoma, 14 (36%) samples were low grade, 6 (15%) samples were moderate grade and 19 (49%) samples were high grade as shown in table (4.1).

The mean age of the study population was 43 years, divided into two groups as following: less than or equal 40 years old has frequency of 29(37%) and more than 40 years old has frequency of 49(63%), as shown in table (4.2).

The distribution of gender throught the study population was 52 (67%) for male, while the distribution was 26 (33%) for female, as shown in table (4.3), with male to female ratio equal to 2:1.

The association between the histopathological diagnosis of the tissue to its reaction towards VEGF was investigated. All the 39 tissues that constituted benign histopathological diagnosis expressed negative VEGF staining. From the 39 cancerous tissues 7 (18%) showed positive cytoplasmic staining with significant correlation (P value= 0.006) as shown in table (4.4).

The association between VEGF expression and the grade of the colorectal cancer was investigated. Out of 7 positive samples from 39 colorectal cancer samples, 2 (29%) samples were positive from low grade adenocarcinoma, 2 (29%) samples were positive from moderate grade adenocarcinoma, 3 (42%) samples

were positive from high grade adenocarcinoma, with insignificant correlation (P value=0.562) as shown in table (4.5).

Table (4.1): Histopathological diagnosis of the study samples.

Diagnosis	Type	Frequency	Percentage	Total	Total
Benign	Juvenile rectal polyps	18	23 %	39 50 %	78 100 %
	Hyperplastic polyps	15	19 %		
	Inflammatory polyps	5	7 %		
	Benign rectal ulcer	1	1 %		
Malignant	Adenocarcinoma Low grade	14	18 %	39 50 %	
	Adenocarcinoma Moderate grade	6	8 %		
	Adenocarcinoma High grade	19	24 %		

Table (4.2): Distribution of the age groups among the study population.

Diagnosis	Age groups		Total
	Older than 40 N (%)	Younger than or equal 40 N (%)	
Malignant	35(45%)	4(5%)	39(50%)
Benign	14(18%)	25(32%)	39(50%)
Total	49(63%)	29(37%)	78(100%)

Table (4.3): Distribution of gender among the study population.

Diagnosis	Gender		Total
	Male N (%)	Female N (%)	
Malignant	21(27%)	18(23%)	39(50%)
Benign	31(40%)	8(10%)	39(50%)
Total	52(67%)	26(33%)	78(100%)

Table (4.4): Correlation of VEGF expression pattern with the histopathological diagnosis.

Histopathological diagnosis	VEGF expression pattern		Total	P. value
	Negative N (%)	Positive N (%)		
Benign	39(50%)	0(0%)	39(50%)	.006
Malignant	32(41%)	7(9%)	39(50%)	
Total	71(91%)	7(9%)	78(100%)	

Table (4.5): Association between VEGF and grade of colorectal cancer.

Grade	VEGF expression		Total	P value
	Negative N (%)	Positive N (%)		
Low grade	12(31%)	2(5%)	14(36%)	.562
Moderate grade	4(10%)	2(5%)	6(15%)	
High grade	16(41%)	3(8%)	19(49%)	
Total	32(82%)	7(18%)	39(100%)	

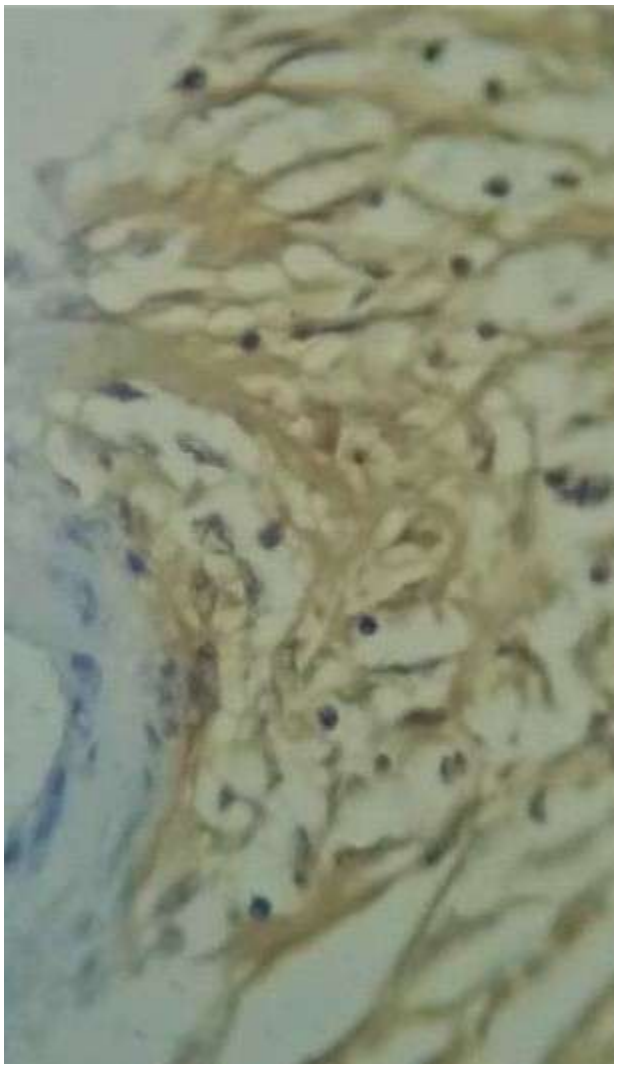

Figure (4.1): Adenocarcinoma of colon showing positive cytoplasmic staining for VEGF expression using (40X).

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

Chapter Five

Discussion, Conclusion and Recommendations

5.1 Discussion

Colorectal cancer is a major cause of morbidity and mortality throughout the world (WHO, 2018).

In this study male patients with CRC were 21(54%) and female patients with CRC were 18(46%), this result indicate that CRC incidence rates is higher in men than women, and this result agreed with Murphy *et al*., (2011), who reported that male rates with CRC were higher than female rates at all subsites for all ethnic groups.

It has been noticed that most of the patients with CRC in this study population were more than 40 years old with frequency of 35(90%) out of 39 patients with CRC, which may indicate that incidence with colon cancer increased with age, this result agreed with Gandomani *et al*., (2017), who reported that the incidence rate of CRC increases with age.

In this study 78 colorectal samples were investigated by immunohistochemistry for VEGF expression. All benign samples were negative for VEGF expression, while 7 (18%) of malignant samples were positive for VEGF expression. The relation between the expression pattern of VEGF and the histopathological diagnosis was found to be statistically significant (P value = 0.006), this result indicates that VEGF is expressed mainly in CRCs with no expression in normal colonic mucosa and benign tumors, this result agreed with Bendardaf *et al*., (2008) and Hashim *et al*., (2010). These findings provide further evidence for the role of *VEGF* in the carcinogenesis and angiogenesis of CRC. Also the study agreed with Lee *et al*., (2000), who found that VEGF expression was positive in colorectal cancer samples while polyps and normal colonic mucosa did not expressed the marker.

The relation between VEGF expression pattern and histological grading one of the clinicopathological features of CRC was not related (P value = 0.562). These findings disagreed with Hashim *et al*., (2010) as there study shows that there was a gradual increase in the frequency of VEGF expression in parallel with the increase in the grade of tumor (in grade I there was 50% VEGF expression, in grade II 52% and in grade III 54.5%). Also it disagreed with Adil *et al*., (2018), who reported that strong positive statistical correlation was found between VEGF expression and grade and stage of the colorectal tumors, and disagreed with Hedaya *et al*., (2015), who reported that VEGF expression in CRC correlated significantly with stage and grade.

This difference can be explained by the small sample size in this study.

5.2 Conclusion

On the base of the data out of this study we conclude that:

- The expression of VEGF is associated with malignant form of colorectal tumors, and there is no association with CRC grade.
- In our study we found that males are more likely to have CRC than females.
- Incidence with CRC in our study increased with age.

5.3 Recommendations

- Further studies should be done on VEGF expression in CRC with large sample size.
- VEGF and MVD expression could be studied in different grades of malignant tumors to reveal the angiogenesis level.

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