Al-Mukhtar Journal of Sciences **38 (3): 288-302, 2023**
ISSIN: online 2617-2186 print 2617-2178
Journal Homepage https://omu.edu.ly/journals/index.php/mjsc/index
Doi: https://doi.org/10.54172/mjsc.1375

Expression and Significance of Vascular Endothelial Growth Factor Receptors 2 and 3 in Endometrial Carcinoma

Nada Y. Ibrahim
Obstetrics and Gynecology, Faculty of Medicine, Omar Al Mukhtar University, Libya.

ARTICLE HISTORY

Received:
21 May 2023

Accepted:
20 September 2023

Key words;
Endometrial carcinoma, VEGF receptor, VEGF receptors2-3.

Abstract: The aim of this work is to evaluate the expression and significance of Vascular Endothelial (VEGFR2,3) in endometrim. The study was applied to 70 females selected from El-Shatby University Hospital. Group I: 35 patients with dysfunctional uterine bleeding. Group II: 35 patients diagnosed with endometrial carcinoma. In the present work, VEGFRs 2 and 3 expression was detected by quantitative real-time PCR. The results revealed that: regarding relative VEGFR gene quantitation, there were no significant differences in the rates of VEGFR 2 and 3 expression between controls and cases. Regarding VEGFR 2 and 3 with type I & II, low and high histological grade, early and late stage, and lymphovascular invasion of endometrial cancer cases, there was no statistically significant relation. In this particular study, it was concluded that the expression levels of VEGFR2 and VEGFR3 do not exhibit any significant increase in endometrial cancers compared to dysfunctional uterine bleeding. Furthermore, we have observed that there exists no discernible correlation between VEGFR2 and VEGFR3 with regard to the histological type, grade, stage, or lymphovascular invasion of the endometrial cancer case.

دراسة مستقبلات عامل نمو بطانة الأوعية الدموية 2 و3 و دلالتها في سرطان بطانة الرحم

الكلمات المفتاحية :
سرطان الرحم، مستقبلات عامل نمو بطانة الأوعية الدموية 2و3.

المستخلص : الهدف من هذا العمل هو تقييم تعبير مستقبلات عامل النمو البطاني الوعائي من النوع 2 و3 (VEGFR2،3) في بطانة الرحم، وأهميته. طبقت الدراسة على 70 أنثى تم اختيارهن من مستشفى الشاطبي الجامعي. المجموعة الأولى: 35 مريضة تعاني من نزيف رحمي مختل. المجموعة الثانية: تم تشخيص 35 مريضة على أنه سرطان بطانة الرحم. في العمل الحالي ، تم الكشف عن تعبير VEGFRs 2 و3 بواسطة تفاعل البوليميراز المتسلسل في الوقت الحقيقي الكمي. أظهرت النتائج ما يلي: فيما يتعلق بالكمية النسبية للجينات VEGFRs، لا توجد فروق ذات دلالة إحصائية في معدلات التعبير VEGFR2 & 3 بين الضوابط والحالات. بخصوص VEGFR 2 & 3 مع النوع الأول والثاني، الدرجة النسيجية المنخفضة والعالية، المرحلة المبكرة والمتأخرة، الغزو اللمفاوي لحالة سرطان بطانة الرحم، لم تكن هناك علاقة ذات دلالة إحصائية. في هذه الدراسة، خلصنا إلى: لا يزيد التعبير عن VEGFR2 و VEGFR3 في سرطانات بطانة الرحم بالمقارنة مع نزيف الرحم المختل. لم يلاحظ أي ارتباط بين VEGFR2 و VEGFR3 مع النوع النسيجي، أو الدرجة، أو المرحلة، أو الغزو اللمفاوي لحالة سرطان بطانة الرحم.

INTRODUCTION

Endometrial cancer represents the most prevalent invasive gynaecological malignancy in Europe and North America. This particular malignancy's occurrence is on the rise, with a yearly diagnosis rate of 150,000 cases across the globe. It stands as the fifth most prevalent type of cancer and the seventh most frequent cause of mortality among women (Amant et al., 2005; Plataniotis & Castiglione, 2010).

The racial difference in the occurrence of uterine corpus cancer can be related to the distribution of identified risk factors such as socioeconomic status (Elwood et al., 1977),

***Corresponding author:** Nada Y. Ibrahim: nada.elsafi1610@gmail.com, Obstetrics and Gynecology, Faculty of Medicine, Omar Al Mukhtar University, Libya.

reproductive history (Brinton, 1992), and use of exogenous estrogens (McDonald et al., 1977). The bases for racial variances in cancer survival are not as clearly described (Hill et al., 1996).

Angiogenesis is a pivotal process in the advancement of tumors. Folkman in 1971, studied the significance of angiogenesis in cancer biology. In 1990, (Folkman, 1990) collated supporting data on this matter. It is currently recognized by a vast majority of scholars and researchers that the process of angiogenesis, which refers to the formation of new blood vessels, plays a critical role not only in facilitating the growth and development of tumors, but also in the initial advancement from a pre-cancerous tumor state to a full-blown cancerous condition. (Hanahan & Folkman, 1996).

In 1995, (Folkman, 1995) conducted a review of emerging clinical applications of angiogenesis research, which have since focused primarily on two aspects. The quantification of angiogenesis in cancer patients is employed for the purpose of diagnosis and prognosis, as well as for impeding tumor growth by means of angiogenesis inhibition. Notably, significant advancements have been made in both directions in recent years (McNamara et al., 1998; Thompson et al., 1999).

Angiogenic factors are produced by both malignant and infiltrating cells. The angiogenic switch is associated with the activation, manifestation, and release of angiogenic factors by malignant cells during the development of tumors. Moreover, tumors possess the ability to generate inhibitors of angiogenesis. (Takahashi et al., 1996).

Vascular endothelial growth factor (VEGF) is categorized among the most influential angiogenic factors. It exhibits a discernible mitogenic effect on endothelial cells and seems to be devoid of mitogenic effect on other cell types.(Ferrara et al., 1992). This peptide that binds to heparin generates five distinct molecular isoforms, which arise through the process of alternative splicing of mRNA (Neufeld et al., 1999).

Most types of tumor cells generate multiple isoforms of VEGF concurrently, although the prevailing variants are typically VEGF121 and VEGF (Neufeld et al., 1999). VEGF appears to have a pivotal function in the regulation of tumor angiogenesis. The induction of VEGF release by tumor cells is prompted by hypoxia (Shweiki et al., 1992). As solid neoplasms enlarge, the cells within the enlarging conglomeration often experience hypoxia due to the progressive separation from adjacent blood vessels. The upregulation of vascular endothelial growth factor (VEGF) by neoplastic cells is also enhanced by the activation of oncogenes, such as ras. (Rak et al., 1995) or inactivation of tumor suppressor genes, such as p53, (Kieser et al., 1994), and by other cytokines, such as transforming growth factor beta (TGF-b) (Pertovaara et al., 1994) and nitric oxide (Chin et al., 1997). Suppression of tumor growth has been demonstrated in vivo through the inhibition of Vascular Endothelial Growth Factor (VEGF). The VEGF family, comprising VEGF – A to D, is a multifunctional cytokine that is an important regulator of tumor angiogenesis (Berchuck et al., 1989; Boocock et al., 1995).

VEGF-A elicits angiogenic consequences through its interactions with the unique receptors VEGFR-1 and VEGFR-2, predominantly eliciting effects within vascular endothelial cells (Brown et al., 1993; Jeltsch et al., 1997). The localization of Flt-4 is significantly confined to the cells of the lymphatic endothelium, thereby implying that Flt-4 serves as a distinctive identifier for the cells of the lymphatic endothelium (Guidi et al., 1995; Smith, 1998). The investigation was executed to evaluate the manifestation and importance of Vascular Endothelial Growth Factor Receptors type 2 and 3 in the context of endometrial cancer.

MATERIALS AND METHODS

This investigation was conducted on a cohort of seventy individuals, 35 diagnosed with endometrial carcinoma and 35 with endometrial hyperplasia, presented to the Shatby Maternity University Hospital. Laboratory work was done in the Clinical Pathology Department, University of Alexandria, in the period from 1/2015 to 8/2015.

Group I: Thirty-five patients presented with endometrial carcinoma attended the gyne-oncology clinic of Shatby Maternity University Hospital, Alexandria University.

Group II: Thirty-five subjects matched for age with the study group presented with abnormal uterine bleeding served as a control group.

All patients in group I were diagnosed according to: pathologically proven endometrial carcinoma by endometrial biopsy.

The study was subjected to the following: medical examination and routine investigations: D&C biopsy for preliminary histopathological examination and imaging investigations.

Total abdominal hysterectomy with bilateral salpingo-oophorectomy: The biopsies were histopathologically tested, diagnosed, and graded using the criteria of the modified (FIGO) surgical staging and grading system for uterine corpus carcinoma (Beddy et al., 2012; Holland, 2010; McCluggage et al., 2010).

(VEGFRs) 2 and 3 mRNA expression analysis by quantitative real-time PCR**:**

Quantitation of VEGFR2 and VEGFR 3 mRNA expression by quantitative real-time polymerase chain reaction (qRT- PCR), which was done for all patients and controls included in this study (van't Veer et al., 2006).

RNA isolation and cDNA preparation followed by quantitative real-time qRT-PCR were done to assess VEGFRs 2 and 3 mRNA expression in all cases and controls.

Reverse transcription, commonly referred to as RT, followed by polymerase chain reaction or PCR, represents the most preferred technique for analyzing mRNA expression originating from diverse sources. Real-time PCR, renowned for its high sensitivity, enables researchers to quantify even the slightest changes in gene expression (van't Veer et al., 2006).

a) Sample collection two milligrams (mg) of fresh endometrial tissue were collected in tubes containing RNA latter QIAGEN Inc. 2006

b) RNA isolation purification of mRNA from human tissues was done using QIAamp® RNA blood mini kit (Qiagen, Germany, catalog # 52304

c) Quantification and storage of total RNA. The concentration of RNA was determined by measuring the absorbance at 260 nm (A 260) using Nanodrop® 2000 spectrophotometer

d) One-step reverse transcription quantitative real-time polymerase chain reaction (qRT-PCR) and QuantiTect Probe RT-PCR assays were used for quantitative real-time one-step RT-PCR using sequence-specific probes.

Data Analysis

Data processing was performed using Rotor Gene Q software.

Relative quantitation of VEGFR 2 and 3 mRNA expression: relative quantitation was expressed by a comparative Ct method where the amount of target, normalized to an endogenous reference; GAPDH and relative to the average ΔCt of normal controls, Livak

KJ and Schmittgen TD 2001 was given by: $2^{-\Delta\Delta Ct}$

Statistical analysis: Data were analyzed by using SPSS software version 20.0. Significance of the obtained results was judged at the 5% level.

RESULTS

Characteristics of Patients Included in the Study

A. Age.
B. Obstetrics and menstrual histories.

Clinical Characteristics of Tumors According to:

- Pathological types.
- Histopathological grade (Low and High).
- Stage: early (I and II) and late (III and IV).
- Lymphovascular invasion.

Relative VEGFRs Gene Expression

- Analysis of studied cases according to relative gene quantitation.
- Correlation between relative VEGFR2 and VEGFR3 genes quantitation.
- Correlation between VEGFR2 and age.
- Correlation between VEGFR3 and age.
- Relation between relative VEGFR2 quantitation and clinical characteristics of tumors (pathological type, histopathological grade, FIGO stage, lymphovascular-invasion and uterine enlargement).
- Relation between relative VEGFR3 quantitation and clinical characteristics of tumors (pathological type, histopathological grade, FIGO stage, lymphovascular invasion, and uterine enlargement).

Characteristics of Patients Included in the Study

Age: Table (1) shows a comparison between the two studied groups according to age. There were no statistically significant differences between the two studied cases regarding age (P=0.178).

Table: (1). Comparison between the two studied groups according to age

Age (years)	Cases (n= 35)		Controls (n= 35)		Test of sig.	p
	n	%	n	%		
<50	5	14.3	8	22.9	□□□ □□□ □□	0.459
50 - <60	6	17.1	8	22.9		
≥60	24	68.6	19	54.3		
Min. – Max.	43.0 – 74.0		44.0 – 77.0			
Mean ± SD.	61.46 ± 9.28		58.49 ± 8.98		t= 1.361	0.178
Median	65.0		60.0			

χ^2: Chi square test
t: Student t-test

Obstetrics and Menopausal histories: Table (2) shows a comparison between cases and controls according to obstetric and menstrual histories. There were no statistically significant differences between cases and controls regarding parity (p=0.380), abortion (0.809), menopausal history (p=0.673), and duration since menopause (p=0.704).

Table: (2). Shows a comparison between cases & controls according to obstetric and menstrual histories

Gravidity	Cases (n= 35)	Controls (n= 35)	Test of sig.	p
Min. – Max.	0.0 – 14.0	0.0 – 9.0		
Mean ± SD.	5.31 ± 3.66	5.54 ± 2.39	Z=0.668	0.504
Median	5.0	5.0		
Parity				
Min. – Max	0.0 – 11.0	0.0 – 9.0		
Mean ± SD.	4.31 ± 3.22	4.69 ± 2.29	Z=0.877	0.380
Median	4.0	5.0		
Abortion				
Min. – Max.	0.0 – 10.0	0.0 – 5.0		
Mean ± SD.	± 1.94	0.83 ± 1.42	Z=0.242	0.809
Median	0.0	0.0		
Menopausal history	N %	n %		
Premenopausal	2 5.7	4 11.4	χ^2= 0.729	p= 0.673
Postmenopausal	33 94.3	31 88.6		
Duration since menopause	(n= 33)	(n= 31)		
Min. – Max.	1.0 – 27.0	2.0 – 25.0		
Mean ± SD.	13.61 ± 7.24	14.39 ± 8.12	Z= 0.379	0.704
Median	12.0	15.0		

Z: Z for Mann Whitney test
χ^2: Chi square test
FE: Fisher Exact test

Clinical Characteristics of Tumors According to:

Pathological Types: As shown in Table (3), 32 patients (91.4%) had endometriod adenocarcinoma (type I), while 2 patients (5.7%) with papillary and 1 patients (2.9%) with clear cell carcinoma (type II).

Histopathological Grade: Grade I (well differentiated): included 13 patients (37.1%).

Grade II (Moderately differentiated): included 15 patients (42.9%).

Grade III (Poorly differentiated): included 7 patients (20%).

FIGO Stage

Stage I: was present in 20 patients (57.1%)

Stage II: 8 patients (22.9%)

Stage III: 7 patients (20%)

Stage IV: no patients

Table: (3). Distribution of the studied cases (n=35) according to pathological type, histological grade, and FIGO stage

Type	N	%
Type I (Endometriod)	32	91.4
Type II (papillary & clear cell)	3	8.6
Histological Grade		
Low	28	80.0
Grade I (well differentiated)	13	37.1
Grade II (moderately differentiated)	15	42.9
High	7	20.0
Grade III (poorly differentiated)	7	20.0
Stage		
Early	28	80.0
I	20	57.1
II	8	22.9
Late	7	20.0
III	7	20.0
IV	0	0.0

Lymphovascular Invasion: Table (4) shows the distribution of the studied cases according to lymphovascular infiltration. Lymphovascular invasion was present in 7 patients (20%).

Table: (4). Distribution of the studied cases (n=35) according to lymphovascular infiltration

	n	%
Lymphovascular infiltration		
Negative	28	80.0
Positive	7	20.0

Relative VEGFRS Gene expression

Analysis of Studied Cases according to Relative Gene Quantitation: A descriptive analysis of the studied cases according to relative gene quantitation is shown in Table (5) In endometrial adenocarcinomas, gene expression was scored as normal expression (1), under expression (<1), over expression (>1).

VEGFR2 normal expression was seen in 1 (2.9%) tumor, under expression in 31 (88.6%), and over expression in 3 (8.6%).

VEGFR3 normal expression was seen in 0 tumors, under expression in 32 (91.4%), and over expression in 3 (8.6%).

Table: (5). Descriptive analysis of the studied cases (n = 35) according to relative gene quantitation

Relative VEGFR2 Gene Quantitation	N	%
Normal "1"	1	2.9
Under expression "<1"	31	88.6
Over expression ">1"	3	8.6
Min. – Max.	0.01 – 2.48	
Mean ± SD.	0.40 ± 0.57	
Median	0.12	
Relative VEGFR3 Gene Quantitation	n	%
Normal "1"	0	0.0
Under expression "<1"	32	91.4
Over expression ">1"	3	8.6
Min. – Max.	0.01 – 3.39	
Mean ± SD.	0.30 ± 0.69	
Median	0.06	

Correlation between Relative VEGFR2 and VEGFR3 Genes Quantitation: Correlation between the relative VEGFR 2 and 3 genes quantitation is shown in Figure (1). A statistically significant positive correlation was evident between the relative VEGFR2 and VEGFR3 genes.

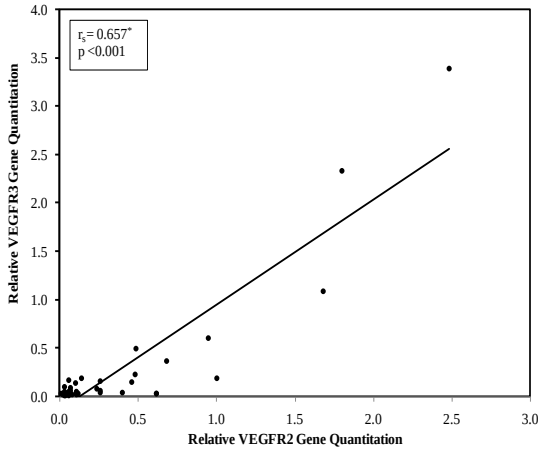

Figure: (1). Correlation between relative VEGFR2 gene quantitation and relative VEGFR3 gene quantitation

Correlation between VEGFR2 and Age: Figure (2) shows a correlation between relative VEGFR2 gene quantitation and age. It illustrated that there was no significant correlation between VEGFR2 and age (p=0.920).

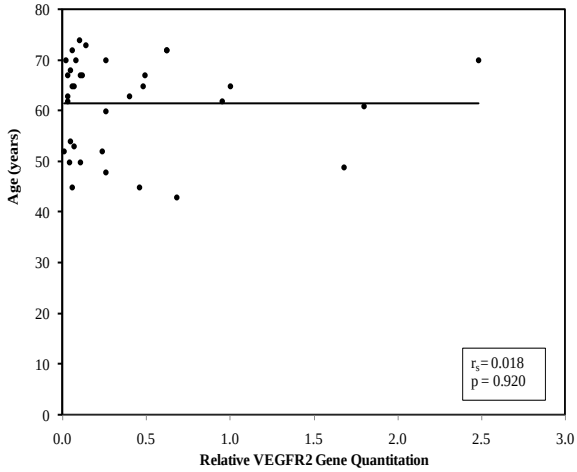

Figure: (2). Correlation between relative VEGFR2 gene quantitation and age

Correlation between VEGFR3 and Age: Correlation between relative VEGFR3 gene quantitation and age. It illustrated that there

was no significant relation between VEGFR3 and age (p=0.182).

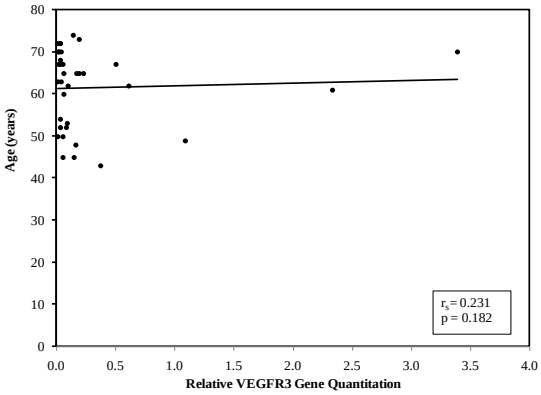

Figure: (3). Correlation between relative VEGFR2 gene quantitation and age

Relation between Relative VEGFR2 Quantitation and Clinical Characteristics of Tumors (Pathological Type, Histopathological Grade, FIGO Stage, and Lymphovascular-Invasion: There was no statistically significant relationship between the VEGFR2 gene and pathological type (p=0.443), histopathological grade (p=0.741), stage (p=0.132), and lymphovascular infiltration (p=0.079).

Relation between relative VEGFR3 gene quantitation and clinical characteristic of the tumor (pathological type, histological grade, FIGO stage, and lymphovascular infiltration

There was no statistically significant relationship between the VEGFR3 gene and pathological type (p=0.193), histopathological grade (p=0.535), stage (p=0.172), and lymphovascular infiltration (p=0.222).

Table: (6). Relation between Relative VEGFR2 Quantitation and Clinical Characteristics of Tumors

	n	Relative VEGFR2 Gene Quantitation			Z	p
		Min. – Max.	Mean ± SD.	Median		
Type I (Endometriod)	32	0.01 – 1.80	0.35 ± 0.46	0.12	0.767	0.443
Type II	3	0.06 – 0.26	0.16 ± 0.14	0.16		
Histological Grade						
Low	28	0.01 – 1.80	0.38 ± 0.48	0.12	0.330	0.741
High	7	0.03 – 2.48	0.47 ± 0.89	0.14		
r_s(p)			0.039(0.823)			
Stage						
Early	28	0.01 – 2.48	0.47 ± 0.62	0.25	1.507	0.132
Late	7	0.03 – 0.46	0.13 ± 0.15	0.07		
r_s(p)			0.306(0.073)			
Lymphovascular Infiltration						
Negative	28	0.01 – 2.48	0.48 ± 0.61	0.26	1.755	0.079
Positive	7	0.03 – 0.14	0.08 ± 0.04	0.07		

Z: Z for Mann Whitney test
r_s: Spearman coefficient

Table: (7). Relation between relative VEGFR3 the tumor gene quantitation and clinical characteristics

	n	Relative VEGFR3 Gene Quantitation			Z	p
		Min. – Max.	Mean ± SD.	Median		
Type I (Endometriod)	32	0.01 – 2.33	0.21 ± 0.45	0.06	1.301	0.193
TypeII	3	0.05 – 0.16	0.11 ± 0.08	0.11		
Histological Grade						
Low	28	0.01 – 2.33	0.23 ± 0.47	0.06	0.621	0.535
High	7	0.01 – 3.39	0.56 ± 1.25	0.10		
r_s(p)			0.061(0.730)			
Stage						
Early	28	0.01 – 3.39	0.36 ± 0.76	0.06	1.366	0.172
Late	7	0.01 – 0.15	0.06 ± 0.05	0.03		
r_s(p)			0.162(0.353)			
Lymphovascular Infiltration						
Negative	28	0.01 – 3.39	0.36 ± 0.76	0.06	1.221	0.222
Positive	7	0.01 – 0.19	0.07 ± 0.06	0.03		

DISCUSSION

Endometrial carcinoma ranks as the most prevalent intrusive gynecological neoplasm in Europe and North America (Chan et al., 2007; Papanikolaou et al., 2006).

The phenomenon of angiogenesis exhibits a paramount significance in the advancement of diverse tumors, such as endometrial carcinomas. Several cytokines, along with their corresponding receptors, have been demonstrated to be implicated, specifically in relation to VEGFR1, -2, and -3 (Guidi et al., 1995).

The scrutiny of the expression of various factors that promote the growth of new blood vessels and the receptors that bind to them has been extensively studied and confirmed to be apparent in a wide range of cancerous conditions, including breast, pancreatic, and colorectal tumors (Kim et al., 2011; Lozano-Leon et al., 2011).

The examination of their expressions in cases of endometrial cancers has been the subject of prior investigation, as evidenced by the works of (Brys et al., 2007; Donoghue et al., 2007). However, several of these studies have produced conflicting data, with certain instances indicating an increase in expression relative to normal endometrium, while others fail to demonstrate such an effect. Similarly, some studies have established a correlation between the expression of VEGFs or their receptors and prognostic factors, while others have not observed this association. (Guidi et al., 1995).

In the present work, VEGFRs 2 and 3 expression, with the occurrence of a particular event, specifically the detection of a specific element, was identified and established through the utilization of a highly precise and quantitative technique known as quantitative real-time polymerase chain reaction (PCR) in all patients with a wide range of expression between the highest and the lowest values.

In the current study, relative VEGFR2 gene quantitation ranged from 0.01 to 2.48 with a mean of 0.40 ± 0.57, and between 0.01 and 3.39 with a mean of 0.30 ± 0.69 in the VEGFR3 gene. VEGFR2 normal expression was seen in 1 (2.9%) tumor, under expression in 31 (88.6%), and over expression in 3 (8.6%). VEGFR3 normal expression was seen in 0 tumors, under expression in 32 (91.4%), and over expression in 3 (8.6%). There were no notable disparities observed in the frequencies of VEGFR 2 and 3 manifestation among the control group and the affected individuals.

Our study revealed that the relative VEGFR2 gene ranged from 0.01 to 2.48 with a mean of 0.40±0.57, and between 0.01 and 3.39 with a

mean of 0.30±0.69 in the VEGFR3 gene. VEGFR2 normal expression was seen in 1 (2.9%) tumor, under expression in 31 (88.6%), and over expression in 3 (8.6%). VEGFR3 normal expression was seen in 0 tumors, under expression in 32 (91.4%), and over expression in 3 (8.6%). There were no notable variations found in the rates of expression of Vascular Endothelial Growth Factor Receptor 2 and 3 (VEGFR2&3) between the control group and the group of individuals with the condition under investigation.

In agreement with our study, Erdem et al. (2007) conducted a comparative analysis of various markers of angiogenesis, including vascular endothelial growth factor (VEGFRs), CD34, and endoglin, in proliferative endometrium (PE), endometrial hyperplasia (EH), and endometrial carcinoma (EC). The aim was to assess the potential impact of angiogenesis on the process of malignant transformation.

The present study comprised a cohort of 66 individuals, out of which 12 exhibited proliferative endometrium, 23 showed endometrial hyperplasia (11 with simple hyperplasia and 12 with complex hyperplasia exhibiting atypia), and 31 manifested endometrial carcinoma, and were all incorporated in this investigation.

Histological specimens of both proliferative endometrium (PE) and endometrial hyperplasia (EH) were extracted via (D&C) and (TAH) procedures. Meanwhile, histological specimens of endometrial adenocarcinoma (endometrioid type) were procured from surgically treated patients. In cases of endometrial cancer (EC), tumors were categorized according to the (FIGO) staging. The cohort consisted of 16 patients with stage I disease, 7 with stage II disease, and 8 with stage III disease. Histologically, 15 patients were diagnosed with (grade [G1]), while 16 patients had (G2) and (G3) adenocarcinomas. Furthermore, five out of eight patients with stage III disease were found to have metastases to the pelvic lymph nodes. It was observed that Vascular Endothelial Growth Factor Receptors (VEGFRs) expression was significantly higher in EC and EH specimens than in PE specimens, but no difference in expression was detected between EC and EH samples (Erdem et al., 2007).

(Wang et al., 2014) the investigation delved into the examination of VEGF-A, VEGFR2, and VEGFR3 expression in endometrial tumors in comparison to the normative endometrium. The investigation consisted of a collective of 76 individuals who had received a medical diagnosis of endometrial adenocarcinomas. The average age of these individuals was determined to be 64 years, with a minimum age of 39 years and a maximum of 88 years. Among the 76 cases, there were 43 endometrioid adenocarcinomas, 22 serous carcinomas, 7 clear cell carcinomas, and 4 carcinosarcomas. It was observed that a total of 10 tumors were categorized as grade I, while 25 tumors were classified as grade II, and 41 tumors were designated as grade III. These classifications encompassed all types of tumors including serous, clear cell, and carcinosarcomas, which are specifically labeled as high-grade.

The reasons that have led to the different results between the current and all other existing studies, such as that of (Wang et al., 2014), is that the majority of studies about VEGFRs expression in endometrial cancer have compared normal endometrium in controls group with endometrial cancer, whereas in our study, the control group had dysfunctional uterine bleeding. This is due to the fact that the ethics committee refused to collect samples from normal cases. In addition, the number of cases in our study is small in comparison with other studies.

In contrast to our study, (Yokoyama et al., 2000) in the course of their research, the researcher acquired newly obtained surgical

samples of endometrial carcinoma from a total of 86 patients. The (FIGO) criteria was followed for the surgical staging of all patients. The surgical procedure included radical or modified radical (TAHSOP), pelvic and para aortic lymphadenectomy. The analysis of endometrial carcinoma staging demonstrated that there were 9 patients classified as being at stage Ia, 34 patients classified as being at stage Ib, 7 patients classified as being at stage Ic, 1 patient classified as being at stage IIa, 4 patients classified as being at stage IIb, 9 patients classified as being at stage IIIa, and 22 patients classified as being at stage IIIc.

The histological types were categorized into 80 instances, along with an additional occurrence of endometrioid adenocarcinoma, three instances of adenosquamous carcinoma, one instance of adenoacanthoma, and two instances of clear cell adenocarcinoma. Furthermore, surgical specimens were procured from 14 women who had complex atypical endometrial hyperplasia (AEH) and from 15 women who underwent surgical treatment for uterine cervical neoplasia or ovarian tumor and had histologically confirmed normal endometrium. There was a noteworthy dissimilarity in the occurrence of VEGFR-3 identification between the conventional endometrium and CAH. The frequency of VEGFR-3 identification in stage I/II carcinoma was notably more elevated than that in the conventional endometrium, even though there was no substantial contrast in the frequency of VEGFR-3 detection between CAH and stage I/II carcinoma. The frequency of VEGFR-3 detection in stage III/IV carcinoma was notably higher than in the conventional endometrium, CAH, and stage I/II carcinoma.

(Guidi et al., 1995) the focal strong expression of VEGFr mRNA by endometrial tumor cells was initially described, accompanied by an observation that flt-1 and KDR mRNAs were strongly expressed by the endothelial cells surrounding microvessel density MVs.

(Holland, 2010) the mRNA of VEGF-A was verified to be present in the epithelial cells of the EC, yet not within the normal endometrium and atypical complex hyperplasia.

The observed discrepancies in results may be attributed to the varying methodologies employed, as our investigation utilized qRT-PCR, a modality that demonstrates enhanced sensitivity towards variations in gene expression, in contrast to other studies that made use of immune-histochemical techniques.

In our investigation, no discernible association was observed between Vascular Endothelial Growth Factor Receptor 2 (VEGFR2) and Vascular Endothelial Growth Factor Receptor 3 (VEGFR3) in relation to the histological type grade stage or lymphovascular invasion. Relative VEGFR2 gene expression ranged from 0.01 to 1.80 with a mean of 0.35±0.46 in type I (endometriod type) and ranged between 0.06 to 0.26 with a mean of 0.16±0.14 in type II. There was no statistically significant relationship (p=0.443). The expression of the VEGFR2 gene with low histological grade ranged from 0.01 to 1.80 with a mean value of 0.38±0.48, and from 0.03 to 2.48 with a mean value of 0.47± 0.89 in high grade. There was no statistically significant relationship. (p=0.741), VEGFR2 expression ranged from 0.01 to 2.48 with a mean of 0.47 ± 0.62 in early-stage patients and ranged from 0.03 to 0.46 with a mean of 0.13 ± 0.15 in late stage. There was no statistically significant relationship (p=0.132), and VEGFR2 expression ranged from 0.03 to 0.14 with a mean value of 0.08±0.04. There was no statistically significant relation between relative VEGFR2 gene quantitation and lymphovascular infiltration (p=0.079).

Relative VEGFR3 gene expression ranged from 0.01 to 2.33 with a mean of 0.21±0.45 in

endo metriod type and ranged between 0.05 and 0.16 with a mean of 0.11± 0.08 in type II. There was no statistically significant relation between relative VEGFR3 gene quantitation and type I (p=0.193) or with type II (p=721). The expression of the VEGFR3 gene with low histological grade ranged from 0.01 to 2.33 with a mean value of 0.23±0.47, and from 0.01 to 3.39 with a mean value of 0.56±1.25 in high grade. There was no statistically significant relation between relative VEGFR3 gene quantitation and histological grade (p=0.535). VEGFR3 expression ranged from 0.01 to 3.39 with a mean of 0.36 ± 0.76 in early-stage patients and ranged from 0.01 to 0.15 with a mean of 0.06 ± 0.05 in late stage. There was no statistically significant relation between relative VEGFR3 gene quantitation and stage (p=0.172), and VEGFR3 expression ranged from 0.01 to 0.19 with mean value of 0.07±0.06 in positive lymphovascular invasion patients. There was no statistically significant relation between relative VEGFR3 gene quantitation and lymphovascular infiltration (p=0.222). In agreement with our study, Wang et al. (2014) conducted analysis which also discovered a lack of any connection between VEGFR2 and the histological type, grade, stage, or lymphovascular invasion. However, it was ascertained that the manifestation of VEGFR3 exhibited a significant correlation with the tumor stage, although it did not exhibit a significant association with the histological type, grade, or lymphovascular invasion.

(Giatromanolaki et al., 2001) found that no significant correlation was found between the expression of (VEGFRs) and the histologic type, histologic grade, depth of myometrial invasion, or lymph vascular space invasion. However, it is worth noting a slight connection between the increased expression of VEGF and the advanced International Federation of Gynecology and Obstetrics (FIGO) stage. The diversity of the angiogenic function in distinct areas of a neoplasm poses a difficulty in the precise evaluation of neovascularization in tumor tissue. Specifically, the expression of Vascular Endothelial Growth Factor (VEGF) is known to be highest in hypoxic areas of the tumor near necrotic regions. As such, the specific location within the tumor that is examined may significantly impact the results of the evaluation of VEGF expression in the tumor. These variables are likely to contribute to the variability observed in studies examining the expression of angiogenic factors in tumors (Poon et al., 2001).

CONCLUSION

In this particular investigation, we have reached a definitive conclusion.The upregulation of VEGFR2 and VEGFR3 is not observed in endometrial malignancies when compared to dysfunctional uterine bleeding. None correlation was observed between the expression levels of vascular endothelial growth factor receptor 2 (VEGFR2) and vascular endothelial growth factor receptor 3 (VEGFR3) and the histological type, grade, stage, or lymphovascular invasion of endometrial cancer cases. More researches and studies on a larger number of cases, The period should be the longest follow-up period to prove the impact of VEGFR2&3 on endometrial cancer and its prognosis**.**

ACKNOWLEDGEMENT

Thanks to Allah for the accomplishment of this work.

I wish to express my deepest gratitude to those who assisted me in completing this work. Foremost, my thanks are directed to Professor Dr. Ali Bataw, Professor of Biology, University of Omar ALmukhtar, for his unlimited help and continuous insistence on perfection. Without his constant supervision, this work could not have achieved its present form.

ETHICS

All the patients were counseled about the procedure and an informed consent was taken before the beginning of the study.

Duality of interest: No duality of interest is associated with this manuscript.

Funding: There were no specific funding.

Author contributions: My own work.

REFERENCES

Amant, F., Moerman, P., Neven, P., Timmerman, D., Van Limbergen, E., & Vergote, I. (2005). Endometrial cancer. *The Lancet, 366*(9484), 491-505.

Beddy, P., O'Neill, A. C., Yamamoto, A. K., Addley, H. C., Reinhold, C., & Sala, E. (2012). FIGO staging system for endometrial cancer: added benefits of MR imaging. *Radiographics, 32*(1), 241-254.

Berchuck, A., Soisson, A., Olt, G. J., Soper, J. T., Clarke-Pearson, D. L., Bast Jr, R. C., & McCarty Jr, K. (1989). Epidermal growth factor receptor expression in normal and malignant endometrium. *American journal of obstetrics and gynecology, 161*(5), 1247-1252.

Boocock, C. A., Charnock-Jones, D. S., Sharkey, A. M., McLaren, J., Barker, P. J., Wright, K. A., Twentyman, P. R., & Smith, S. K. (1995). Expression of vascular endothelial growt factor and its receptors fit and KDR in ovarian carcinoma. *JNCI: Journal of the National Cancer Institute, 87*(7), 506-516.

Brinton, L. (1992). Epidemiology of gynecologic cancers. *Principles and practice of gynecologic oncology*, 3-26.

Brown, L. F., Berse, B., Jackman, R. W., Tognazzi, K., Manseau, E. J., Senger, D. R., & Dvorak, H. F. (1993). Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in adenocarcinomas of the gastrointestinal tract. *Cancer research, 53*(19), 4727-4735.

Brys, M., Semczuk, A., Rechberger, T., & Krajewska, W. M. (2007). Expression of erbB-1 and erbB-2 genes in normal and pathological human endometrium. *Oncology Reports, 18*(1), 261-265.

Chan, J. K., Urban, R., Cheung, M. K., Shin, J. Y., Husain, A., Teng, N. N., Berek, J. S., Walker, J. L., Kapp, D. S., & Osann, K. (2007). Lymphadenectomy in endometrioid uterine cancer staging: how many lymph nodes are enough? A study of 11,443 patients. *Cancer, 109*(12), 2454-2460.

Chin, K., Kurashima, Y., Ogura, T., Tajiri, H., Yoshida, S., & Esumi, H. (1997). Induction of vascular endothelial growth factor by nitric oxide in human glioblastoma and hepatocellular carcinoma cells. *Oncogene, 15*(4), 437-442.

Donoghue, J. F., Lederman, F. L., Susil, B. J., & Rogers, P. A. (2007). Lymphangiogensis of normal endometrium and endometrial adenocarcinoma. *Human Reproduction, 22*(6), 1705-1713.

Elwood, J. M., Cole, P., Rothman, K. J., & Kaplan, S. D. (1977). Epidemiology of endometrial cancer. *Journal of the National Cancer Institute, 59*(4), 1055-1060.

Erdem, O., Erdem, M., Erdem, A., Memis, L., & Akyol, G. (2007). Expression of vascular endothelial growth factor and assessment of microvascular density with CD 34 and endoglin in

proliferative endometrium, endometrial hyperplasia, and endometrial carcinoma. *International Journal of Gynecologic Cancer, 17*(6).

Ferrara, N., Houck, K., Jakeman, L., & Leung, D. W. (1992). Molecular and biological properties of the vascular endothelial growth factor family of proteins. *Endocrine reviews, 13*(1), 18-32.

Folkman, J. (1990). What is the evidence that tumors are angiogenesis dependent? (Vol. 82, pp. 4-7): Citeseer.

Folkman, J. (1995). Clinical applications of research on angiogenesis. *New England Journal of Medicine, 333*(26), 1757-1763.

Giatromanolaki, A., Sivridis, E., Brekken, R., Thorpe, P. E., Anastasiadis, P., Gatter, K. C., Harris, A. L., & Koukourakis, M. I. (2001). The angiogenic " vascular endothelial growth factor/flk-1 (KDR) receptor" pathway in patients with endometrial carcinoma: prognostic and therapeutic implications. *Cancer: Interdisciplinary International Journal of the American Cancer Society, 92*(10), 2569-2577.

Guidi, A. J., Abu-Jawdeh, G., Berse, B., Jackman, R. W., Tognazzi, K., Dvorak, H. F., & Brown, L. F. (1995). Vascular permeability factor (vascular endothelial growth factor) expression and angiogenesis in cervical neoplasia. *JNCI: Journal of the National Cancer Institute, 87*(16), 1237-1245.

Hanahan, D., & Folkman, J. (1996). Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *cell, 86*(3), 353-364.

Hill, H. A., Eley, J. W., Harlan, L. C., Greenberg, R. S., Barrett II, R. J., & Chen, V. W. (1996). Racial differences in endometrial cancer survival: the black/white cancer survival study. *Obstetrics & Gynecology, 88*(6), 919-926.

Holland, J. F. (2010). *Holland-Frei cancer medicine 8* (Vol. 8). PMPH-USA.

Jeltsch, M., Kaipainen, A., Joukov, V., Meng, X., Lakso, M., Rauvala, H., Swartz, M., Fukumura, D., Jain, R. K., & Alitalo, K. (1997). Hyperplasia of lymphatic vessels in VEGF-C transgenic mice. *Science, 276*(5317), 1423-1425.

Kieser, A., Weich, H. A., Brandner, G., Marmé, D., & Kolch, W. (1994). Mutant p53 potentiates protein kinase C induction of vascular endothelial growth factor expression. *Oncogene, 9*(3), 963-970.

Kim, J. Y., BAE, B. N., Kwon, J. E., KIM, H. J., & Park, K. (2011). Prognostic significance of epidermal growth factor receptor and vascular endothelial growth factor receptor in colorectal adenocarcinoma. *Apmis, 119*(7), 449-459.

Lozano-Leon, A., Perez-Quintela, B. V., Iglesias-García, J., Lariño-Noia, J., Varo, E., Forteza, J., & Domínguez-Muñoz, J. E. (2011). Ductal adenocarcinoma of the pancreas: Expression of growth factor receptors, oncogenes and suppressor genes, and their relationship to pathological features, staging and survival. *Oncology Letters, 2*(1), 161-166.

McCluggage, G., Hirschowitz, L., Ganesan, R., Kehoe, S., & Nordin, A. (2010).

Which staging system to use for gynaecological cancers: a survey with recommendations for practice in the UK. *Journal of clinical pathology*, *63*(9), 768-770.

McDonald, T. W., Annegers, J. F., O'Fallon, W. M., Dockerty, M. B., Malkasian Jr, G. D., & Kurland, L. T. (1977). Exogenous estrogen and endometrial carcinoma: case-control and incidence study. *American journal of obstetrics and gynecology*, *127*(6), 572-580.

McNamara, D., Harmey, J., Walsh, T., Redmond, H., & Bouchier-Hayes, D. (1998). Significance of angiogenesis in cancer therapy. *British journal of surgery*, *85*(8), 1044-1055.

Neufeld, G., Cohen, T., Gengrinovitch, S., & Poltorak, Z. (1999). Vascular endothelial growth factor (VEGF) and its receptors. *The FASEB journal*, *13*(1), 9-22.

Papanikolaou, A., Kalogiannidis, I., Goutzioulis, M., Misailidou, D., Makedos, A., Vergote, I., & Makedos, G. (2006). Pelvic lymphadenectomy as alternative to postoperative radiotherapy in high risk early stage endometrial cancer. *Archives of Gynecology and Obstetrics*, *274*, 91-96.

Pertovaara, L., Kaipainen, A., Mustonen, T., Orpana, A., Ferrara, N., Saksela, O., & Alitalo, K. (1994). Vascular endothelial growth factor is induced in response to transforming growth factor-beta in fibroblastic and epithelial cells. *Journal of biological chemistry*, *269*(9), 6271-6274.

Plataniotis, G., & Castiglione, M. (2010). Endometrial cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of Oncology*, *21*, v41-v45.

Poon, R. T.-P., Fan, S.-T., & Wong, J. (2001). Clinical implications of circulating angiogenic factors in cancer patients. *Journal of Clinical Oncology*, *19*(4), 1207-1225.

Rak, J., Mitsuhashi, Y., Bayko, L., Filmus, J., Shirasawa, S., Sasazuki, T., et al., & Kerbel, R. (1995). Mutant ras oncogenes upregulate VEGF/VPF expression: implications for induction and inhibition of tumor angiogenesis. *Cancer research*, *55*(20), 4575-4580.

Shweiki, D., Itin, A., Soffer, D., & Keshet, E. (1992). Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature*, *359*(6398), 843-845.

Smith, S. (1998). Angiogenesis, vascular endothelial growth factor and the endometrium. *Human Reproduction Update*, *4*(5), 509-519.

Takahashi, Y., Bucana, C. D., Liu, W., Yoneda, J., Kitadai, Y., Cleary, K. R., & Ellis, L. M. (1996). Platelet-derived endothelial cell growth factor in human colon cancer angiogenesis: role of infiltrating cells. *JNCI: Journal of the National Cancer Institute*, *88*(16), 1146-1151.

Thompson, W. D., Li, W. W., & Maragoudakis, M. (1999). The clinical manipulation of angiogenesis: pathology, side-effects, surprises, and opportunities with novel human therapies. *The Journal of pathology*, *187*(5), 503-510.

van't Veer, M. B., Brooijmans, A. M., Langerak, A. W., Verhaaf, B.,

Goudswaard, C. S., Graveland, W. J., van Lom, K., & Valk, P. (2006). The predictive value of lipoprotein lipase for survival in chronic lymphocytic leukemia. *Haematologica, 91*(1), 56-63.

Wang, J., Taylor, A., Showeil, R., Trivedi, P., Horimoto, Y., Bagwan, I., Ewington, L., Lam, E. W.-F., & El-Bahrawy, M. A. (2014). Expression profiling and significance of VEGF-A, VEGFR2, VEGFR3 and related proteins in endometrial carcinoma. *Cytokine, 68*(2), 94-100.

Yokoyama, Y., Sato, S., Futagami, M., Fukushi, Y., Sakamoto, T., Umemoto, M., & Saito, Y. (2000). Prognostic significance of vascular endothelial growth factor and its receptors in endometrial carcinoma. *Gynecologic oncology, 77*(3), 413-418.