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Original article

Significance of CXCL9 and Vascular Endothelial Growth Factor-A (VEGF-A) Expression in Colorectal Adenocarcinoma :(Immunohistochemical Study)

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Abstract:

Background: Colorectal carcinoma (CRC) is the third most common cancer in the world. The chemokine CXCL9 is a small molecule belongs to (CXC family) which secreted by (IFN-γ) stimulated macrophages. VEGF-α plays central role in the regulation of angiogenesis and lymphangiogenesis. **Aim :** This work aimed to evaluate the role of CXCL9 and VEGF α in colorectal adenocarcinoma. **Material and method:** This retrospective study was carried upon 50 cases of colorectal adenocarcinoma. CXCL9 and VEGFα immunostaining was done and assessed for each case**. Results:** There was an insignificant statistical correlation between both CXCL9 and VEGF -α expression and patient`s age, sex, tumor size and site (P value >0.05). But there was a highly significant inverse statistical correlation between CXCL9 expression and tumor grade, depth of tumor invasion, lymph node metastasis, distant metastasis lymphovascular invasion and tumor stage (P value <0.01).There was a significant direct statistical correlation between VEGF expression and tumor grade, depth of tumor invasion, lymph node metastasis, distant metastasis lymphovascular invasion and tumor stage (P value <.0.01)**.**There was a highly significant inverse statistical correlation between CXCL9 and VEGF-α immunoexpression in studied cases of colorectal carcinoma (P value <0.01). **Conclusion:** The expression of CXCL9 and VEGF-αin CRC cases correlate with the most important clinicopathological variables, which are currently used for risk evaluation in colorectal cancer and, thus they may represent a useful predictors of prognosis in CRC.

Keywords: Colorectal adenocarcinoma, CXCL9, VEGF-α, Angiogenesis.

Abbreviations: Interferon-gamma (IFN- γ), Vascular endothelial growth factor- α (VEGF- α), Colorectal carcinoma (CRC).

Introduction:

Colorectal cancer (CRC) is one of the most prevalent cancers worldwide and the third most common cause of death of all forms of cancer, responsible for approximately 600, 000 deaths yearly **(1).**

In Egypt according to National cancer institute registry, Cairo University, colorectal carcinoma represent the 3rd most common cancer in males (6.5%) and the 4th in females (6.4%).It constitutes 15.3% of total gastro intestinal tract malignancies(GIT) and 6.48% of all malignancies **(*2*).**

The main prognostic factors in CRC are lymph node involvement, size of the tumor and stage of disease. However, these factors do not fully predict individual clinical outcome **(*3*)**.

Clinicopathological parameters play a major role in determining the management of CRC, but are usually not reliable predictors of prognosis. Therefore, studying novel biomarkers of colorectal cancers, which have the potential value of serving as prognostic markers and new therapeutic targets, are still a clinical problem to be solved **(1).**

Emerging studies are focusing on the role of chemokines and chemokine receptors in tumorigenesis and progression .It has been shown that CXC chemokines and their receptors can modulate tumor behavior by several important mechanisms as regulation of angiogenesis, activation of a tumor-specific immune response by attracting leukocytes, stimulation of tumor cell proliferation and metastasis **(4)**.

The C-X-C motif chemokine ligand 9 (CXCL9) has been identified to play an important role in the pathogenesis of many cancers **(5).** Little is known about the relationship of CXCL9 expression to clinical characteristics of CRC patients and whether CXCL9 can be used as an independent molecular marker for predicting the prognosis of CRC patients so it will be a matter of interest.

Vascular endothelial growth factor -α (VEGF α) is a member of the PDGF/VEGF growth factor family. It encodes a heparin-binding protein which exists as a disulfide-linked homodimer. This growth factor induces proliferation and migration of vascular endothelial cells, and is essential for both physiological and pathological angiogenesis **(6)**.

The aim of this work is to evaluate the expression of CXCL-9 and VEGF-α in colorectal carcinoma and correlate their expression with different clinicopathological

variables and with each other to clarify their actions hoping to find new prognostic and therapeutic options of this disease.

Material and methods:

Study Groups:

This is a retrospective study performed on formalin fixed paraffin embedded biopsy specimens of 50 cases of colorectal adenocarcinoma and 7 cases of colitis taken as a control group, collected from Pathology department, and Early cancer detection unit (ECDU), Faculty of Medicine, Benha University, during the period of 2012 to 2017. Cases were selected on basis of availability of demographic data and clinicopathological data. The study was approved by the Ethical Committee of faculty of medicine, Benha University.

A-Histopathological Examination:

Formalin fixed /Paraffin embedded blocks were cut at 5 μm thickness, stained using hematoxylin and eosin stain and examined microscopically to assess tumor grade, depth of invasion (T), and Lymphovascular invasion .

Colorectal adenocarcinoma cases were graded into well differentiated (G I), moderately differentiated (G II) and poorly differentiated tumors (G III) **(7)**. Pathological tumor-node-metastasis (TNM) staging was determined according to the criteria of the American Joint Committee on Cancer (**AJCC) 8th edition (8).**

B-Immunohistochemical Procedure:

According to manufacture instructions, the sections were deparaffinized then hydrated through a series of descending alcohols. Then put in 10mm citrate buffer (PH=6) and were twice pretreated by microwaving oven for antigen retrieval. The endogenous peroxidase activity was inactivated by incubation in 3% hydrogen peroxide (H2O2). One to two drops of the primary monoclonal antibody, CXCL9 and VEGF-α at a dilution of 1:100 ***(0.1mg/ml concentration, Chongqing, YPA1536, China and 0.1mg/ml concentration, Chongqing, China YPA1071 respectively***) was applied to each section. Slides were incubated in humid chamber overnight at 4c.The sections incubated with Avidin-biotin-peroxidase system (DAKO, Glostrup, Denmark) for 30 minutes. Two or three drops of streptavidin enzyme label were put on each slide for 30 minutes at room temperature. Peroxidase reaction was detected by addition of dia-monobenziase tetrahydrochloride. Slides were rinsed well in tap water for 5 minutes then slightly counterstained with Mayer's Hematoxylin for 1-2 minutes and dehydrated in ascending alcohol. The slides were cleared

in xylene for 3 changes and cover slides were applied.

- **Negative & positive controls:**

According to manufacture instructions, sections from normal human spleen were used as a positive control for CXCL9, and sections from liver from cases with hepatitis C were used as a positive control For VEGF-α.

For negative controls, samples were treated as described above, except that the primary antibody was replaced with a solution of BSA in phosphate-buffered saline (PBS).

- **Immunohistochemical assessment:**

CXCL9 and VEGF-α expression were detected as cytoplasmic brown stain. Five random fields x 400 were selected and analyzed. The percentage of cells stained was estimated and the intensity of immunostaining was graded based on a visual assessment of the intensity of brown reaction product within the cell cytoplasm. The final immunostaining score reported was the average of two observers.

The immunoreactivity of CXCL9 protein was classified as follows: No expression or < 5% of cells stained as negative (0); Weak staining, 5-25% of cells stained as (1+); Moderate staining , 26–50% of cells stained as(2+) and Strong staining , >50% of cells stained as(3+) **(1).**

The immunoreactivity of VEGF-α protein was classified as follows: No expression or < 10% of cells stained as (0); weak, 10-25% of cells stained as (1+), moderate or intense staining that was between 26% -75% of cells stained as (2+) and staining that was >75% of cells stained as(3+)**(9).**

Statistical analysis:

Results were analyzed using SPSS (version 16) statistical package for

Microsoft windows as follow: P value >0.05 is non-significant (N)

P<0.05 is significant (S) and P≤ 0.001 is highly significant (HS)

Results:

This study was carried upon 50 cases of colorectal adenocarcinoma, 28 cases (56%) were males and 22 cases (44%) were females. The age of studied cases ranged from 23 to 70 years old, they were distributed into two age groups as follow: 13 cases (26%) were <50 years old and 37cases (74%) were >=50 years. Studied cases ranged in size from 3 to 20 cm in the largest dimension, with mean size 5 cm, 21 cases (42%) were <5 cm, while 29 cases(58%) were >=5 cm. According to tumor site 29 cases (58%) were

located in right colon while 21 (42%) cases were located in left colon.

Histopathological results:

There was insignificant statistical correlation between tumor grade and age, sex, tumor site, tumor size and depth of tumor invasion of studied case (P value >0.05).

There was a highly significant statistical correlation between tumor grade and, presence of lymph node metastasis, distant metastasis, lymphovascular invasion and tumor stage (P value <0.01) (fig. 1).

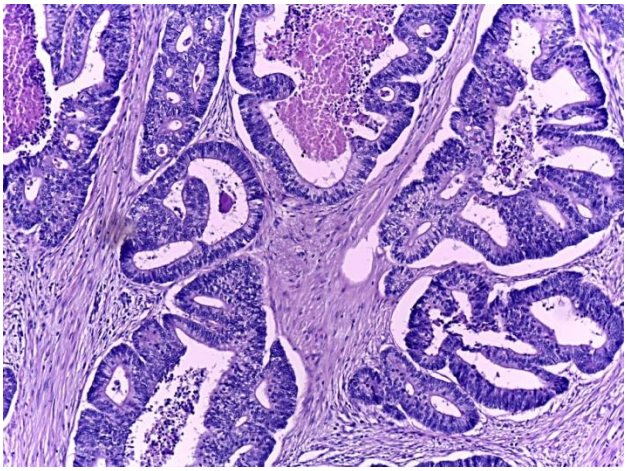

Fig. (1): Grade II cribriform adenocarcinoma of colon composed of fused glands (H&Ex200)

❖ ***CXCL9 expression in studied cases***:

CXCL9 expression was negative in normal colonic mucosa of the control group of colitis cases.

Out of 50 cases colorectal adenocarcinoma ;15 cases (30%) were negative for CXCL9 expression score (0) ; 13 cases (26%) showed score (1+) ; 16 cases (32%) showed score (2+) and 6 cases (12%) showed score (3+).There was insignificant statistical correlation between extent of CXCL9 immunohistochemical expression and age, sex, tumor site tumor size of studied cases (P value >0.05).

- **Correlation between CXCL9 expression and clinicopathological features of studied cases:**

There was a highly significant inverse statistical correlation between CXCL9 expression and tumor grade (P value <0.01), depth of tumor invasion (T) (P value <0.01), lymph node metastasis (N) (P value <0.01), distant metastasis (M) (P value <0.05), lymphovascular invasion (P value <0.05) and tumor stage (P value <0.01). As illustrated in table(1), (fig. 2).

Table(1): Correlation between CXCL9 expression and clinicopathological features of studied cases:

Clinicopathological features		**CXCL9 expression**					**P value**
		0	(1+)	(2+)	(3+)	Total	
Tumor site	Rt colon	9 (60%)	6 (46%)	10(62.5%)	4(66.5%)	29(58%)	>0.05
	Lt colon	6 (40%)	7 (54%)	6 (37.5%)	2(33.5%)	21(42%)	
Tumor size	<5 cm	7(46.5%)	4(30.8%)	9 (56.2%)	1(16.7%)	21(42%)	>0.05
	>=5cm	8 (53.4%)	9(69.3%)	7 (43.8%)	5(83.3%)	29(58%)	
Tumor grade	Grade I	1 (6.5%)	1 (7.5%)	2(12.5%)	(66.5%)	8 (16%)	<0.01
	Grade II	6 (40%)	5(38.5%)	13(81%)	2(33.5%)	26 (52%)	
	Grade III	8(53.5%)	7 (54%)	1 (6.5%)	0 (0%)	16 (32%)	
Depth of tumor	T1	1 (6.6%)	0 (0%)	2 (12.5%)	3 (50%)	6 (12%)	
invasion	T2	1 (6.6%)	7(53.8%)	7(43.75%)	2(33.3%)	17 (34%)	
	T3	5(33.3%)	4(30.7%)	7(43.75%)	0(0%)	16 (32%)	<0.01
	T4	8(53.2%)	2(15.5%)	0 (0%)	0(0%)	10 (20%)	
LN metastasis	N0	4(26.5%)	6(46.1%)	9 (56.3%)	5(83.5%)	24 (48%)	
	N1	2(13.5%)	6(46.1%)	7(43.7%)	1(16.5%)	16(32%)	<0.01
	N2	9(60%)	1(7.8%)	0(0%)	0(0%)	10(20%)	
Distant metastasis	M0	9 (60%)	9(69.2%)	15(93.7%)	5(83.3%)	38 (76%)	
	M1	6 (40%)	4(30.8%)	1 (0.3%)	1(16.7%)	12(24%)	<0.05
LV invasion	Absent	4(26.5%)	4(30%)	8(50%)	5(83.5%)	21(42%)	
	Present	11(73.5%)	9(70%)	8 (50%)	1(16.5%)	29(58%)	<0.05
Tumor stage	Stage I	1(6.7%)	2(15.3%)	4 (25%)	4(66.6%)	11(22%)	
	Stage II	3(20%)	4(30.7%)	5(31.3%)	1(16.7%)	13(26%)	<0.01
	Stage III	5(33.3%)	3(23.3%)	6(37.5%)	0(0%)	14(28%)	
	Stage IV	6(40%)	4(30.7%)	1(6.2%)	1(16.7%)	12(24%)	

LN: lymph node **LV**: lymphovascular

VEGF- α expression in studied cases:

VEGF- α expression was negative in normal colonic mucosa of the control group of colitis cases. Out of 50 cases colorectal adenocarcinoma, 7 cases (14%) were negative for VEGF- α expression score (0), 6 cases (12%) showed score (1+), 18 cases (36%) showed score (2+) and 19 cases (38%) showed score(3+) for VEGF- α expression.There was insignificant statistical correlation between extent of VEGF-α immunohistochemical expression and age, sex, tumor site tumor size of studied cases (P value>0.05)

Correlation between VEGF-α expression and clinicopathological features of studied cases :

There was a highly significant direct statistical correlation between VEGF-α expression and tumor grade (P value <0.01), depth of tumor invasion (T) (P value <0.01), lymph node metastasis (N) (P value <0.01), distant metastasis (M) (P value <0.01), lymphovascular invasion (P value <0.01) and tumor stage (P value <0.01) **(Table 2)** (fig.3,4).

Table(2): Correlation between VEGF-α expression and clinicopathological features of studied cases:

Clinicopathological features		**VEGF- α expression**					**P**
		0	(1+)	(2+)	(3+)	**Total**	**value**
Tumor site	**Rt colon**	4 (57%)	3 (50%)	10(52.6%)	12 (63%)	29(58%)	>0.05
	Lt colon	3 (43%)	3(50%)	8(47.4%)	7 (37%)	21(42%)	
Tumor size	**<5cm**	3 (43%)	1(16.7%)	9 (50%)	8 (42.2%)	21(42%)	>0.05
	>=5cm	4 (57%)	5(83.3%)	9(50%)	11(57.8%)	29(58%)	
Tumor grade	**Grade I**	4 (57%)	2(33.3%)	1 (5.5%)	1 (5.2%)	8 (16%)	<0.01
	Grade II	3 (43%)	4(66.7%)	11(61.1%)	8(42.2%)	26 (52%)	
	Grade III	0 (0%)	0 (0%)	6 (33.4%)	10 (52.6%)	16 (32%)	
Depth of tumor	**T1**	5(71.6%)	0 (0%)	0 (0%)	1 (5.2%)	6 (12%)	
invasion	**T2**	1(14.2%)	4(66.6%)	9(50%)	3(15.7%)	17(34%)	
	T3	1(14.2%)	2(33.3%)	6(33.3%)	8(42.1%)	17(34%)	<0.01
	T4	0(0%)	0(0%)	3(16.7%)	7(37%)	10(20%)	
LN metastasis	**N0**	7(100%)	5(83.3%)	7(38.8%)	5(26.3%)	24(48%)	
	N1	0(0%)	1(16.7%)	10(55.5%)	5(26.3%)	16(32%)	<0.01
	N2	0(0%)	0(0%)	1(5.7%)	9(47.4%)	10(20%)	
Distant metastasis	**M0**	7(100%)	5(83.3%)	14(77.8%)	12(63.1%)	38(76%)	
	M1	0(0%)	1(16.7%)	4(22.2%)	7(36.9%)	12(24%)	<0.01
LV invasion	**Absent**	7(100%)	5(83.5%)	7(38.8%)	2(10.5%)	21(42%)	
	Present	0(0%)	1(16.5%)	11(61.2%)	17(89.5%)	29(58%)	<0.01
Tumor stage	**Stage I**	6(85.5%)	2(33.3%)	1(5.5%)	2(10.5%)	11(22%)	
	Stage II	1(14.5%)	3(50%)	6(33.3%)	3(15.7%)	13(26%)	<0.01
	Stage III	0(0%)	0(0%)	7(38.8%)	7(36.9%)	14(28%)	
	Stage IV	0(0%)	1(16.7%)	4(22.4%)	7(36.9%)	12(24%)	

LN: lymph node **LV**: lymphovascular

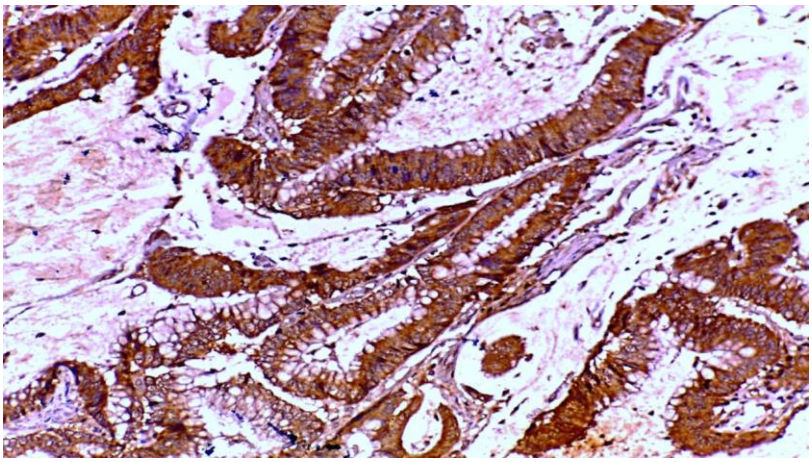

Fig.2: Well differentiated adenocarcinoma showing strong cytoplasmic staining, score (3+) for CXCL9 (ABC X400).

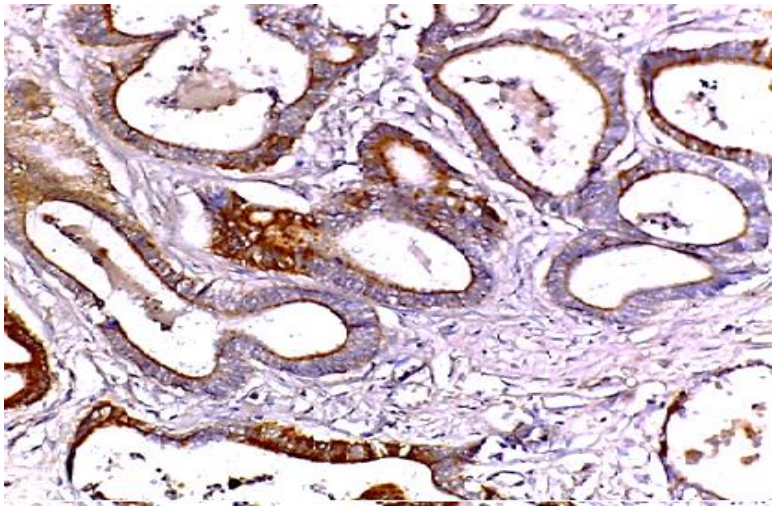

Fig. 3: Well differentiated adenocarcinoma showing weak cytoplasmic staining, , score (1+) for VEGFα (ABCX200).

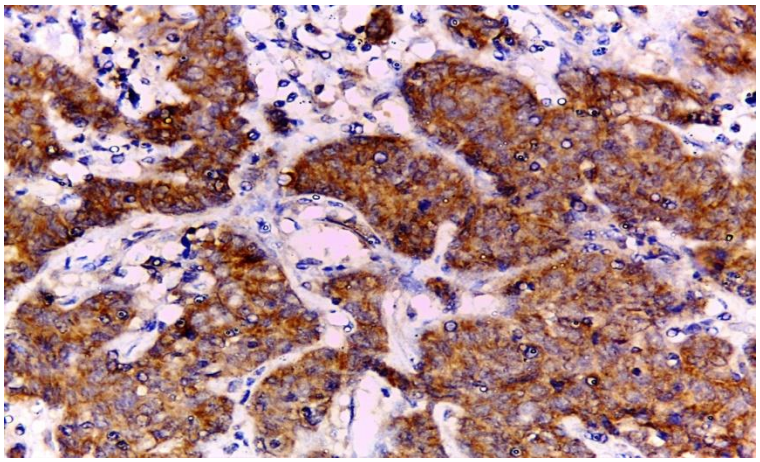

Fig.4: Poorly differentiated adenocarcinoma showed strong cytoplasmic staining, (Score3+), for VEGF α (ABCx400)

Correlation between CXCL9 expression and VEGF - α expression in studied cases:

There was a highly significant inverse statistical correlation between CXCL9 and VEGF- α expression in studied cases of CRC (P value <0.01), **(Table 3)**.

Table (3): Correlation between CXCL9 and VEGF- α immuno expression in studied cases

CXCL9 score	VEGF-α score 0		+1		+2		+3			P value
	No	%	No	%	No	%	No	%	Total	
0	0	0%	0	0%	2	13.3%	13	86.7%	15	
1+	0	0%	1	7.6%	8	61.5%	4	30.9%	13	
2+	3	18.7%	3	18.7%	8	50%	2	12.6%	16	<0.01
3+	4	66.6%	2	33.4%	0	0%	0	0%	6	
Total	7	14%	6	12%	18	36%	19	38%	50	

Discussion:

Colorectal cancer (CRC) is one of the most prevalent cancers worldwide.

It is the third most commonly diagnosed cancer and the third leading cause of cancer death in both men and women in the USA **(10).**

According to Egyptian cancer institute registry, colorectal carcinoma represents the 3rd most common cancer In males (6.5%) and the 4th in females (6.4%) **(2).**

This retrospective study was done on 50 cases of different grades and stages of colorectal adenocarcinoma. Each case was immunohistochemically stained and evaluated for both CXCL9 and VEGF-α. Then the expression of both markers was correlated with different histopathological variables and with each other.

Statistical analysis was performed on the relation between tumor grade, and clinicopathological variables, it revealed a positive statistical correlation between tumor grade and presence of lymph node metastasis, distant metastasis, presence of lymphovascular invasion and tumor stage.

However, no significant statistical correlation was detected between tumor grade and age, sex, tumor site or tumor size of studied cases.

The present study showed a gradual increase in depth of tumor invasion (T) parallel with increase in the tumor grade. However, no

significant statistical correlation was noted (p value > 0.05)

The CXC family of chemokines and their receptors are crucial for

Inflammation and antitumor immunity .These small proteins are secreted not only by tumor cells but also by leukocytes, fibroblasts, endothelial cells and epithelial cells **(11).**

Statistical analysis was performed on the relation between CXCL9 expression in CRC cases and clinicopathological variables, it revealed a highly significant inverse statistical correlation between CXCL9 expression and tumor grade and depth of tumor invasion (T) in studied cases (P value <0.01).

These results were in agreement with **some studies (12, 13, & 14),** which found that CXCL9 expression was inversely correlated with tumor grade and depth of tumor invasion (T).

The key to explain these results is the role of CXCL9 in promoting the immune activities, as it can cause elevation in cytotoxic T lymphocyte (CTLs) responses and increase infiltration of CD4+ or CD8+ lymphocytes and NK cells that express the receptor CXCR3 into tumors and so they are responsive to CXCL9 **(15 & 16).** Consistently with this observation, it was observed that tumor infiltrating lymphocytes (TILs) which express CXCR3 are increased in human gastric and colorectal cancer and identified CXCL9 as T-cell homing factor in CRC (17 & 18).

Homing of T-cells into the tumor, leading to a preferential TH1–type recruitment and tumor growth inhibition through ability of TH 1cells to produce interferon-γ and enhance anti-tumor immunity by activating macrophages and CD8 cytotoxic T lymphocytes, which are crucial effectors for anti-tumor immunity **(17).**

Moreover, antitumor responses of tumor infiltrating lymphocytes (TILs), particularly cytotoxic T lymphocytes (CTLs) may be due to their ability to specifically recognize tumor associated antigens (TAAs) and attack tumor cells in humans (19).

In this study there was a highly significant inverse statistical correlation between CXCL9 expression and presence of lymph node metastasis (N) (P value <0.01), distant metastasis (M) (P value <0.05), lymphovascular invasion (P value <0.05) and tumor stage (P value <0.01).

In agreement with our results, it was found that CXCL9 expression was correlated inversely with presence of tumor metastasis and tumor stage in CRC (20 & 16).

These results can be explained by effect of CXCL9 in tumor microenvironment (TME), as its elaboration recruit activated CXCR3 expressing T lymphocyte effectors with anti-tumor reactivity **(21).** Which is confirmed by the study carried by researcher**s (22),** who observed that tumors from CRC patients containing a high density of tumor-infiltrating T cells, notably TH1 and CD8+ effector T cells, were found to be less likely to disseminate to lymphovascular structures and to regional lymph nodes

Also **in 2018 (23)** it was suggested that the CXCR3/ligand axis regulates tumor associated macrophages (TAMs) and macrophage polarization in the TME that affects tumor growth and progression.

Moreover, a study done before **(24)** it was showen that when CXCL9 coupled with its receptor CXCR3, has antiangiogenic properties through direct interaction with the endothelium and/or the recruitment of T or NK cells, which can destroy the tumor vasculature **.**

The angiostatic activity of CXCL9 may be resulted from not only its effect on the biological functions of endothelial cells but also through its effect on tumor cells through inhibiting their capability to stimulate new vessel formation which lead to tumor regression **(25).**

It was observed that CXCL9 overexpression result in the inhibition of NSCLC tumor growth and metastasis via a decrease in tumor-associated angiogenesis **(26)**

In contradiction with our results high expression of CXCL9 was associated with high tumor grade and stage, as reported by some researchers in their study on glioblastoma **(27),** others in their studies on prostatic carcinoma **(28)** and in hepatocellular carcinoma **(29)**

This discrepancy with our results can be explained by the ability of CXCL9 to recruit not only CTL, inhibiting tumor development, but also other host immune cells, such as regulatory T cells (Tregs) and tumor-associated macrophages, which mediate immune tolerance in tumors **(30).**

Moreover, the contradictory role of CXCL9 might be associated with its receptor's splice variants CXCR3-A and CXCR3-B, as they always showed a counteracting role in tumor progression. CXCL9/CXCR3-A could promote tumor migration and invasion via PI3K and MAPK pathways and so on, but CXCL9/CXCR3-B could inhibit endothelial

cells proliferation and tumor angiogenesis **(31).**

Vascular endothelial growth factor-α (VEGF-α) belong to the platelet-derived growth factor supergene family, and plays central roles in the regulation of angiogenesis and lymphangiogenesis. It regulates endothelial cell proliferation, migration, vascular permeability, secretion and other endothelial functions **(32).**

Statistical analysis was performed on the relation between VEGF-α expression in CRC and clinicopathological variables, it revealed a significant direct statistical correlation between VEGF-α expression and tumor grade (P value < 0.01), an observation matching with other studies **(33 & 34)** .

This was supported by the results of a study aimed to determine the role and timing of the VEGFα angiogenic switch during CRC progression, as they found that VEGF α expression was significantly increased from adenoma to carcinoma and with tumor dedifferentiation. These results suggest that VEGFα may play a role early in tumor development at the stage of adenoma formation and in tumor differentiation **(35).**

There was a significant statistical correlation between VEGF-α expression and depth of tumor invasion (P value <0.01), this finding is in agreement with others done on 2016 **(34).**

This finding can be explained by upregulation of proteases and matrix metalloprotinases (MMP) activity by VEGF-α which can facilitate neoplastic progression directly by increasing the local invasiveness of cancer in to the surrounding parenchyma **(36).**

In our study, VEGF-α expression was correlated significantly with LN involvement an observation which is in agreement with other workers **(37 & 38).**

Also a significant correlation was found between the expression of VEGF-α and presence of lymphovascular invasion (P value <0.01) an observation previously noted in other works **(33 & 34).**

These findings are supported by **a study done on 2017** where it was suggested that VEGF may have a role in vascular invasion by increasing the activity of cathepsins in spurting venules and down regulation of the activity of cysteine protease inhibitors, thus it disrupts the normal cathepsin inhibitor balance, leading to degradation of basement membrane of the venules**(39)** .

VEGF expression also correlated significantly with presence of distance

metastases and tumor stage (P value <0.01), This finding indicates equivocally that VEGF is directly proportional to the degree of colorectal tumor spread as has been established before **(40, 41, 42, & 43)**

These results are supported by studies done before **(44 & 45)** as they found increased levels of VEGF-α expression in normal tissues collected from a site distant from the primary tumor as well as the primary tumor, which indicates changes in the surrounding tumor environment that may enhance the subsequent spread of tumor cells, and it can be also explained by upregulation of MMP by VEGF -αas proved previously **(36).**

In contrast to our study it was found that there was no significant correlation between VEGF-α expression and stage of colorectal carcinoma **(46)**.

This discrepancy in results may be attributed to examining lymph node negative cases only in their studies, suggesting that angiogenesis does not play a role in this stage of colorectal tumors.

In our study there was a highly significant inverse statistical correlation between CXCL9 and VEGF-α expression in studied cases of colorectal carcinoma (P value <0.01).

It is supported by a research done in 2012 on liver fibrosis induced angiogenesis in mice, as they present evidence that CXCL9, is a strong counter-regulatory molecule of VEGF-α driven aberrant liver vascularization and perfusion in vitro and in vivo **(47)**.

CXCR3 agonists in humans have been shown to directly interfere with VEGF-α signaling **(48).** These effects appear to be mediated by direct interference of CXCL9 with VEGF/VEGFR2 and its downstream phospholipase C γ, p-JNK, and p-ERK **(1).**

These findings are also supported by earlier study which stated that, interferon-inducible ELR-negative CXC chemokines such as CXCL9, CXCL10, and CXCL11 can inhibit the angiogenesis induced by other angiogenic factors e.g VEGF-α, and bFGF by interacting with a common receptor, CXCR3 **(49)**.

Conclusions:

CXCL9 may have antiangiogenic role in CRC, while VEGF may have angiogenic role in CRC.CXCL9 and VEGF may have role in CRC progression.

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