

# The Immunohistochemical Expression of Vascular Endothelial Growth Factor in Gastrointestinal Stromal Tumor. A Clinicopathological Study.

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**Summary:**

**Back ground:** Gastrointestinal Stromal Tumors (GISTs), although are rare, are the most common mesenchymal tumors of the digestive system. Vascular endothelial growth factor(VEGF) is associated with the malignant potential of several types of carcinomas.

**Objectives:** to evaluate the expression of VEGF in GIST and its correlation with different clinic- pathologic parameters and risk categories.

**Patients, material, and methods:** This is a retrospective study including thirty two randomly selected cases of GISTs collected from the pathology laboratory of the gastroenterology and hepatology teaching hospital and from private laboratories from the period January 2010 to December 2013. VEGF immunohistochemical marker was applied to the tumor tissue sections to evaluate its expression and to correlate it with other parameters.

**Results:** The mean age of the patient was 42.5 years with a female to male ratio of 1.3:1. The stomach was the most common site for the tumor. VEGF was expressed in 12(37.5%) cases. There was a significant statistical correlation between overexpression of VEGF and the patients' gender and the primary tumor site. There was no significant statistical correlation between VEGF overexpression and age of the patients, size of the tumor, mitotic index, and risk of malignant potential categories.

**Conclusion:** There is a significant statistical correlation between VEGF expression and both gender of the patients, and the primary site of GIST.

**Key Word:** GIST, VEGF.

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**Introduction:**

Gastrointestinal Stromal Tumor(GIST) is the most mesenchymal tumor of the gastrointestinal tract. GIST may arise anywhere in the alimentary tract and rarely in extraintestinal sites (1). GISTs are distinct from smooth muscle tumors, and share features with interstitial cells of Cajal, the putative progenitor of GIST (2). Angiogenesis plays a central role in the process of tumor growth and metastatic dissemination. The vascular endothelial growth factor (VEGF) family of peptide factors and receptors are key regulatory of this process (3). In general, angiogenesis is thought to be initiated by paracrine release of angiogenic factor by tumor cells, such as VEGF, basic fibroblast growth factor and platelet derived growth factor(4,5). VEGF is one of the well studied angiogenic factors, it is produced and secreted by tumor cells and is associated with tumor neovascularization in various kind of malignant tumors. (6,7,8). The well established role of VEGF in promoting tumor angiogenesis and pathogenesis of human cancer has led to rational designed development of agents that selectively target this pathway (9,10,11). Microvessel density was correlated with both VEGF overexpression and worse prognosis in GIST. Their results

suggest that angiogenesis associated with VEGF may play an important role, at least in part, in progression of GIST (12). The Aim of This study is to evaluate the expression of VEGF in gastrointestinal stromal tumors and its correlation with risk categories.

**Patients and Methods:**

This is a retrospective study that included 32 cases diagnosed as GISTs, they are selected randomly from the pathology laboratory of the gastroenterology and hepatology teaching hospital and private laboratories in Baghdad, from the period January 2010 to December 2013. The specimens represent total excision of the lesion for the studied cases. All the clinical information including age, gender, site and size of the tumor had been taken from the patient's files. The paraffin embedded sections were stained with hematoxylline and eosin (H&E) stain and reviewed. The risk category was assigned to each tumor referred to the grading criteria adopted by Fletcher CDM and others in their consensus approach at 2006 which depends mainly on the tumor size, number of mitoses/50HPF and tumor location (13) as shown in table 1.

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**Table 1: Rates of metastases or tumor-related death in GISTs grouped by tumor location, tumor size and mitotic rate**

Tumor Parameters		Percent Of Patients With Progressive Disease During Long-Term Follow-Up And Characterization Of Risk (In Parentheses) For Metastasis			
Tumor size	Mitotic rate	Gastric GISTs	Jejunal and ileal GISTs	Duodenal GISTs	Rectal GISTs
=2 cm	=5/50-HPFs	0% (none)	0% (none)	0% (none)	0% (none)
>2 cm to =5 cm	=5/50-HPFs	1.9% (very low)	4.3% (low)	8.3% (low)	8.5% (low)
>5 cm to =10 cm	=5/50-HPFs	3.6% (low)	24% (moderate)	34% (high)[b]	57% (high)[b]
>10 cm	=5/50-HPFs	12% (moderate)	52% (high)		
=2 cm	>5/50 HPFs	0%[a]	50%[a]	[c]	54% (high)
>2 cm to =5 cm	>5/50 HPFs	16% (moderate)	73% (high)	50% (high)	52% (high)
>5 cm to =10 cm	>5/50 HPFs	55% (high)	85% (high)	86% (high)	71% (high)[b]
>10 cm	>5/50 HPFs	86% (high)	90% (high)		

All the cases' tissue sections were stained with immunohistochemical marker VEGF. As for the evaluation of the VEGF expression, the cytoplasmic staining intensity in the tumor cells graded as: 0 (no staining), 1 (weak staining), and 2 (strong staining). When the tumor cells were stained for VEGF, the staining pattern was essentially diffuse and homogenous throughout the section. Therefore, the expression of VEGF was evaluated by staining intensity, low expression (score 0 and 1) and high (score 2). Expressed VEGF as analyzed statistically in relation to several criteria. P value was considered significant when it was less than 0.05.

**Results:**

Thirty two cases of GIST were studied (14 males and 18 females) with a female to male ratio of 1.3:1. The age range of the patients was 15-70 years with a mean of 42.5 years. Nine (28.1%) patients were equal and below 40 years old while 23(71.9%) were above forty. The stomach was the most common tumor site 18(56.25%) cases, followed by the small intestine 8(25%) cases, and rectum 2(6.25%)cases. There were also two cases of mesenteric and two cases of retroperitoneal GISTs. Twelve (37.5%) cases out of 32 expressed VEGF and these cases were analyzed statistically in relation to several criteria. Regarding the age of the patients, nine (28.1%) cases were positive for VEGF expression in the above forty years age group and three (9.4%) cases were positive for VEGF in the age group equal and less than 40 years. There was no significant statistical relation between the age of the patients and VEGF expression, as shown in table 2.

**Table :2 The patient's age distribution in relation to VEGF expression.**

Age	Negative and weak VEGF expression	Strong VEGF expression	Total
≤40 years	6 (18.75%)	3 (9.375%)	9 (28.1%)
>40 years	14 (43.75%)	9 (28.125%)	23 (71.9%)
Total	20 (62.5%)	12 (37.5%)	32 (100%)

P value >0.05 NS

Out of the eighteen female cases, four (12.5%) showed strong positivity for VEGF, and out of the 14 male cases, eight (25%) showed strong positivity for VEGF. There was a significant statistical relation between gender and VEGF expression (P value=0.020) as shown in table 3.

**Table3: The Patient's sex distribution in relation VEGF expression.**

Sex	Negative and weak VEGF expression	Strong VEGF expression	Total
Female	14 (43.75%)	4 (12.5%)	18 (56.25%)
Male	6 (18.75%)	8 (25.0%)	14 (43.75%)
Total	20 (62.5%)	12 (37.5%)	32 (100%)

VEGF was expressed strongly in seven (21.9%) cases of gastric GISTs, two (6.25%) cases of small intestinal GISTs and one (3.1%) of each rectal, retroperitoneal and mesenteric GISTs. VEGF was significantly correlated with the primary tumor site, P value 0.0189. Table 4.

**Table :4 The tumor site distribution in relation to VEGF expression.**

Site	Negative and weak VEGF expression	Strong VEGF expression	Total
Stomach	11 (34.375%)	7 (21.875%)	18 (56.25%)
Small intestine	6 (18.75%)	2 (6.25%)	8 (25%)
Rectum	1 (3.125%)	1 (3.125%)	2 (6.25%)
Retroperitonium	1 (3.125%)	1 (3.125%)	2 (6.25%)
Mesentery	1 (3.125%)	1 (3.125%)	2 (6.25%)
Total	20 (62.5%)	12 (37.5%)	32 (100%)

P value 0.0189



In this study the size of the tumor was ranging from 4cm to 26cm. according to the tumor size cases were divided into three groups: those with tumor size equal and less than 5cm in largest dimension, 5.1-10cm, and more than 10cm. None of the 5cm and less tumor size cases expresses VEGF, five (15.625%) cases measured 5.1-10cm express VEGF and seven (21.875%) cases measured more than 10cm expressed VEGF. Although there was positive correlation between tumor size and VEGF expression, this did not reach a statistical significance, table 5.

**Table 5: Distribution of GIST cases according to the tumor size and VEGF expression.**

Tumor size	Negative and weak VEGF expression	Strong VEGF expression	Total
≤5cm	5 (15.625%)	0	5 (15.625%)
5.1-10cm	9(28.125%)	5 (15.05%)	14 (43.75%)
>10cm	6(18.75%)	7 (21.875%)	13 (40.625%)
<b>Total</b>	<b>20(62.5%)</b>	<b>12 (37.5%)</b>	<b>32 (100%)</b>

P value >0.05 NS

Twenty two (68.75%) cases had mitotic count less than or equal to 5/50HPF. And 10 (31.25%) cases had more than 5/50HPF mitotic figures. Seven (21.875%) of the cases with mitotic count less or equal to 5/50HPF expressed VEGF, 5 (15.625%) cases had mitotic count more than 5/50HPF expressed VEGF. There was no statistical relation between the mitotic count and VEGF expression, table 6.

**Table 6: Distribution of GIST cases according to the tumor mitotic figures and VEGF expression.**

Mitotic Count /50HPF	Negative and weak VEGF expression	Strong VEGF expression	Total
≤5	15 (46.875%)	7 (21.875%)	22 (68.75%)
>5	5(15.625%)	5 (15.625%)	10 (31.25%)
<b>Total</b>	<b>20(62.5%)</b>	<b>12 (37.5%)</b>	<b>32 (100%)</b>

P value >0.05 NS

The risk category of primary GISTs depends on the mitotic index, tumor size and location as explained in table 1. In this study there were 22 (68.75%) cases categorized as high risk of malignant potential, eight (25%) cases as intermediate risk, and two (6.25%) as low risk group. Four (12.5%) cases of the intermediate risk group expressed VEGF and eight (25%) cases of high risk GISTs expressed VEGF. There was positive correlation between tumor size and VEGF expression; this did not reach a statistical significance, table 7.

**Table 7: Distribution of GIST cases according to risk groups and VEGF expression.**

Risk category	Negative and weak VEGF expression	Strong VEGF expression	Total
Very low	0	0	0
Low	2 (6.25%)	0	6 (6.25%)
Intermediate	4 (12.5%)	4 (12.5%)	8 (25%)
High	14 (43.75%)	8 (25%)	22 (68.75%)
<b>Total</b>	<b>20 (62.5%)</b>	<b>12 (37.5%)</b>	<b>32 (100%)</b>

P value >0.05 NS

**Discussion:**

Gastrointestinal stromal tumors are the most common mesenchymal tumors of the GI tract with an estimated incidence of 6.8 cases per million worldwide (1,14,15). In this study the mean age was 42.5 years which is comparable with other Iraqi studies(16,17,18). Kon Y et al (19) found a mean age 55.1 years. In this study 18(56.25%) cases were females and 14(43.75%) cases were males with a female to male ration 1.3:1. These findings were comparable with Iraqi and abroad studies (16,17,18,19). In the present study the stomach was the most common site involved by GIST, 18 (56.25%) cases followed by small intestine 8 (25%) cases. This topographic distribution is in agreement with several other studies (17,18,19). Regarding the expression of VEGF, this study showed VEGF expression in 12 (37.5%) cases of GIST. Masakazu Imamura et al (12) studied 95 cases and found 45 (47.36%) cases express VEGF strongly. Takahashi R et al (20) detected VEGF expression in 14 (26.4%) of 53 lesions and their findings correlated significantly with tumor size, liver metastases, ki67 labeling index and microvessel density. In the current study, no significant statistical correlations were found between the age of the patient, tumor size, mitotic index, risk category, and VEGF expression by the tumor cells; however VEGF expression was significantly correlated with the patient's gender p value 0.020 and the primary site of the tumor (P value 0.0189). These findings were comparable to Imamura M et al (12) findings, who found that VEGF expression was significantly correlated with gender and primary site of the tumor. However McAuliff Jc et al (21) found that only 9 (17%) cases out of 53 showed strong positivity for VEGF, most tumors in their studies whether of the stomach, intestine, and colon were stained negative or weak positive for VEGF. VEGF expression seemed to have an equal distribution among primary and metastatic tumors, therefore high VEGF expression did not associated with metastatic disease compared with primary disease (3). Takahashi R. et al (20) concluded that angiogenesis associated with VEGF may play an important role in the progression of GIST. VEGF expression may serve as an indicator of a poor prognosis. McAuliff Jc et al (21) presents a rational to consider exploration of a front-line therapy of GIST with a regimen targeting both kit and VEGF based on the presence of tumor VEGF levels.

**Conclusion:**

There is a significant statistical correlation between VEGF expression and both gender of the patients, and the primary site of GIST.

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