

Original Article

Vascular Endothelial Growth Factor in Pleural Effusions and Correlation with Radiologic and Biochemical Parameters

N Bayram, Y Karakan, M Uyar, B Ozyurt¹, A Filiz

Departments of Respiratory Medicine and ¹Biochemistry, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey

ABSTRACT

Introduction: Pleural effusion is a common clinical problem with management difficulties. The aim of this study is to evaluate vascular endothelial growth factor (VEGF) in differential diagnosis of pleural effusions and the presence of correlation between radiological features and biochemical properties.

Materials and Methods: The study included patients with pleural effusion. VEGF levels in the pleural fluid were measured by enzyme-linked immunosorbent assay.

Results: A total of 97 patients who had exudative pleural effusion related to lung cancer ($n = 17$), nonpulmonary malignancies ($n = 25$), mesothelioma ($n = 9$), pneumonia ($n = 14$), tuberculosis ($n = 8$), miscellaneous causes ($n = 6$), and transudative effusion ($n = 18$) were included. Pleural VEGF levels were higher in exudative effusions with respect to transudative effusions ($P < 0.001$) and in effusions related to malignancies versus benign causes ($P < 0.001$). Pleural VEGF was inversely correlated with pleural fluid glucose and pH levels and had positive correlation with lactate dehydrogenase, protein levels ($P < 0.001$), hematocrit, and eosinophil values in the pleura ($P < 0.05$). Pleural VEGF levels were also higher in patients with massive effusions and pleural thickening (both $P < 0.001$).

Conclusions: The overlap of pleural VEGF levels between the groups may limit the value of VEGF in discriminating between malignant versus benign and exudative versus transudative effusions; however, it may be a useful adjunct to various methods. The VEGF levels in pleural fluid seem to be related to the degree of inflammation and pleural invasion.

KEYWORDS: *Malignant pleural effusion, pleural fluid amount, pleural thickening, vascular endothelial growth factor*

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INTRODUCTION

Disorders of the pleural fluid formation through absorption lead to excessive accumulation of fluid in the pleural space.^[1] Excessive fluid retention in the lung interstitium is the most common cause of pleural fluid formation.^[2] Increased level of vascular endothelial growth factor (VEGF) may cause increased capillary permeability and therefore cause pleural fluid accumulation.^[3,4]

VEGF is a multifunctional growth factor family, especially those with specific activity for endothelial cells.^[5] VEGF is also known as vascular permeability factor, and permeability-enhancing effect is 10,000 times more potent than histamine. VEGF causes proliferation,

migration, and differentiation of endothelial cells.^[6] Low glucose levels, oxidative stress, and hypoxia-inducible transcription factor-1 also play an effective role in the release of VEGF.^[7] Nitric oxide contributes to the permeability and vasodilation by increasing VEGF levels. Acidosis, hypoglycemia, and inflammation also enhance the release of VEGF expression.^[8,9] It facilitates invasion and metastasis besides migration of endothelial cells and therefore stimulates the release of

Address for correspondence: Dr. N Bayram,
Department of Respiratory Medicine, Faculty of Medicine,
Gaziantep University, Gaziantep, Turkey.
E-mail: nbayram@gantep.edu.tr

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matrix metalloproteinases, urokinase, and tissue-type plasminogen activator.^[9] The increase in vascular permeability and fluid leakage is the most important mechanism in the formation of exudative pleural effusion, and VEGF plays a critical role here.^[8] VEGF was also found to increase permeability of the mesothelial layer.^[10] Local increase in production of VEGF is more likely rather than by diffusion from systemic circulation in exudative effusions.^[11] In this study, we aimed to evaluate the possible impact of pleural VEGF on the differential diagnosis of pleural effusions, especially on differentiation of malignant and benign pleural effusions, and whether there is a correlation between radiological and biochemical parameters.

MATERIALS AND METHODS

Patients with pleural effusion admitted to Gaziantep University Pulmonary Medicine Clinic from June 2005 to June 2006 were prospectively enrolled in the study. Ethical committee approval was obtained (2005-06-06). The study was supported by the university research fund (TF.06.04).

A volume of 20 cc of the pleural fluid and simultaneous blood samples was obtained from the patients enrolled in the study. Routine biochemical tests in the pleural effusion and blood samples were performed. 5 cc of pleural fluid samples was separated for VEGF measurement and then centrifuged for 10 min at 4000 rpm, and supernatants were stored at -70° . Quantikine R Kit (R & D System GmbH in Wiesbaden, Germany) was used applying quantitative sandwich enzyme immunoassay techniques for determination of VEGF in the pleural fluid. VEGF concentration <9 pg/ml was accounted as under limit values. If VEGF levels were >2000 pg/ml, the procedure was repeated as described in the kit package with 1/10 dilution with calibrator diluent. Results were specified as $>20,000$ pg/ml if the re-analysis showed concentration as >2000 pg/ml.

Effusions occupying $>2/3$ of the hemithorax were defined as massive pleural effusions. Lesser amount of effusion was defined as nonmassive pleural effusions. If the pleural fluid was just blunting the costodiaphragmatic angle, then

it was recorded as minimal effusion. Bilateral effusions were classified according to the larger side. Thorax computed tomography (CT) was evaluated in terms of the presence and differential diagnosis of pleural thickening.

Statistical analysis

SPSS 13.0 for Windows program (Chicago, SPSS Inc.) was used for evaluating data. Descriptive data were presented as mean and standard deviation for normally distributed data and median and lower and upper limit for data without normal distribution. Spearman's correlation test, Mann-Whitney U, Kolmogorov-Smirnov, Chi-square, Kruskal-Wallis analysis of variance (ANOVA), Bonferroni, one-way ANOVA, and *t*-tests were used for group correlation and comparisons. $P < 0.05$ was necessary for statistical significance.

RESULTS

The study enrolled 97 patients including 51 male and 46 female patients. Forty-six of these patients had benign, and 51 had malignant causes for pleural effusion. Malignant cases were primary lung cancer ($n = 17$), extrapulmonary metastasis ($n = 25$), and mesothelioma ($n = 9$). Other exudates included pneumonia ($n = 4$), tuberculosis ($n = 8$), and miscellaneous diseases ($n = 6$). Transudates were caused by heart failure ($n = 15$), renal failure ($n = 2$), and hypoalbuminemia ($n = 1$).

There was no difference in terms of smoking history and asbestos exposure between groups, but the mean age of patients with tuberculous pleurisy was statistically lower. Pleural VEGF levels and demographic characteristics of the patients according to the etiology are presented in Table 1.

Pleural VEGF levels were not correlated with age, gender, or associated with smoking and asbestos exposure. Pleural VEGF levels in transudative effusions were statistically significant ($P = 0.000$) [Table 1] and significantly higher in the malignant group compared to the benign group ($P < 0.001$) [Table 2].

Pleural VEGF was negatively correlated with glucose levels and pH, while there was a positive correlation with protein, lactate dehydrogenase (LDH), cholesterol,

Table 1: Patient groups and pleural fluid vascular endothelial growth factor levels

Patient groups	Age, year (mn) \pm SD	VEGF (pg/ml) Median	VEGF (pg/ml) Upper limit-lower limits
Lung cancer ($n=17$)	65.53 \pm 9.10	2222.77	284.47-20,000
Other malignancy ($n=25$)	49.20 \pm 16.21	3414.19	232.85-20,000
Mesothelioma ($n=9$)	56.44 \pm 8.29	3056.59	488.05-20,000
Parapneumonic effusion ($n=14$)	50.07 \pm 14.94	4117.35	230.34-20,000
Tuberculous effusion ($n=8$)	30.37 \pm 15.50*	809.96	47.94-4064.14
Other benign exudates ($n=6$)	36.33 \pm 15.05	829.03	217.71-4979.79
Transudative effusion ($n=18$)	63.55 \pm 14.88	141.94**	9-873.35

* $P < 0.05$; ** $P < 0.001$. VEGF=Vascular endothelial growth factor; SD=Standard deviation

hematocrit, and number of eosinophils in the pleura [Table 3].

Radiological characteristics with respect to the amount of pleural fluid on chest X-ray and the presence of pleural thickening on CT of patient groups are given in Tables 4 and 5. Pleural thickening was detected more frequently in pleural effusions due to malignancy. Nonmassive effusions were common in benign causes, while massive effusions were mainly malignant. Pleural VEGF levels were significantly higher in the patients with pleural thickening [Table 5].

Pleural VEGF levels were highest in patients with massive effusion, both in malignant and benign

effusions. In the malignant group, the difference between nonmassive and minimal effusion was also statistically significant ($P < 0.05$) [Table 4].

DISCUSSION

We found higher VEGF levels in exudative and malignant pleural effusions. Many studies have demonstrated that VEGF levels were lower in transudative pleural effusions.^[3,4,12,13] A study postulated that lower VEGF levels in transudative effusions reflect levels in systemic circulation, whereas higher levels in exudates result from increased local production.^[12] The presence of bacterial pathogens in the pleural cavity stimulates the release of VEGF from mesothelial cells and increases mesothelial permeability.^[10] We have also found elevated levels of pleural VEGF in parapneumonic effusions in our study. High levels of VEGF is detected in pleural effusions secondary to malignancy.^[4,12,14,15]

Difference has been detected between effusions due to malignancy and parapneumonic, tuberculous, and heart failure.^[14,15] VEGF may play an important role in tumor progression and formation of pleural fluid. Thickett *et al.* stated that levels >1000 pg/ml are detected in patients with malignant effusion excluding empyema.^[4] A study showed that VEGF 2000 pg/ml cutoff value had a sensitivity and specificity of 100% and 84%, respectively, in malignant pleural effusions.^[16]

Several studies have demonstrated correlation between pleural VEGF and LDH,^[3,15-17] protein,^[3] leucocyte,^[14] monocytes, as well as macrophages.^[18] LDH is a marker for inflammation that is thought to be correlated with VEGF. Low glucose level and acidosis are also known to increase the secretion of VEGF.^[7,8] We observed that pleural VEGF was correlated with the number of eosinophils and pleural fluid cholesterol level, high LDH, low glucose, and low pH, presumably due to high VEGF levels in inflammatory and malignant processes. Hemorrhagic effusions tend to occur during malignant invasion of the pleura and are reportedly contain elevated levels of VEGF.^[19] Overproduction of VEGF by tumor cells in hemorrhagic effusion is suggested to be the cause of fluid accumulation and hemorrhagic in this setting.^[19]

The relationship between pleural VEGF and pleural thickening has been identified for the first time in our study. VEGF levels were higher in patients with

Table 2: Vascular endothelial growth factor levels in benign and malignant groups

Group	VEGF median level (pg/ml)	Upper limit-lower limit (pg/ml)
Benign effusion (n=46)	737.89	9-20,000
Malignant effusion (n=51)	2954.52*	232.85-20,000

* $P < 0.001$. VEGF=Vascular endothelial growth factor

Table 3: Correlation of vascular endothelial growth factor with biochemical and cellular properties of pleural fluid

Parameters	VEGF level (pg/ml)	
	r	P
Glucose (mg/dl)**	-0.472	0.000
Protein (g/dl)**	0.372	0.000
LDH (U/l)**	0.619	0.000
Cholesterol (mg/dl)*	0.332	0.001
pH**	-0.568	0.000
Hct (%)*	0.319	0.001
Eosinophil count (/μl)*	0.296	0.021

* $P < 0.05$; ** $P < 0.001$. LDH=Lactate dehydrogenase; Hct=Hematocrit; VEGF=Vascular endothelial growth factor

Table 4: Vascular endothelial growth factor levels with respect to pleural thickening

	VEGF (pg/ml)	
	No thickening	Pleural thickening present
Benign group*	252.55 (9-873.68)	1887.66 (47.94-20,000)
Malignant group**	790.66 (232.85-20,000)	3414.19 (284.47-20,000)

* $P < 0.001$; ** $P = 0.05$. VEGF=Vascular endothelial growth factor

Table 5: Vascular endothelial growth factor levels with respect to the amount of pleural fluid

	VEGF (pg/ml)		
	Minimal	Nonmassive	Massive*
Benign group	332.16 (9-881.22)	738.70 (9-20,000)	7745.25 (929.45-20,000)
Malignant group	790.66 (232.85-841.24)	1856.17 (284.47-20,000)	6231.04 (325.15-20,000)

* $P < 0.05$. VEGF=Vascular endothelial growth factor

pleural thickening in either benign or malignant pleural effusions. VEGF levels may be related to the intensity of the inflammation and fibrosis due to inflammation and degree of pleural invasion. Pleural fibrosis due to pleural inflammation is often associated with exudative pleural effusion. Transforming growth factor- β (TGF- β), basic fibroblast growth factor, and platelet-derived growth factor play a key role in the development of pleural fibrosis. Other cytokines such as tumor necrosis factor- α , interleukin (IL)-1, IL-6, IL-8, and VEGF may also have a role in this process.^[20] TGF- β also leads the release of VEGF from the mesothelial cells.^[11,21] Anti-VEGF antibodies have been shown to reduce angiogenesis and pleurodesis in rabbits, and angiogenesis is necessary for pleurodesis.^[22] These findings appraise the role of VEGF in the development of pleural fibrosis.

There was a significant correlation between pleural VEGF levels and the amount of pleural fluid detected on chest X-ray in our study. The significance was also apparent in the benign group which underlines the role of VEGF in the formation of excessive pleural fluid. Gary Lee *et al.*^[11] demonstrated a strong correlation between the volume of pleural fluid and VEGF levels in their experimental study; however, our study is the first clinical study of this relationship.

CONCLUSIONS

High VEGF, LDH levels, lower pH and glucose levels, the presence of pleural thickening, and massive pleural effusion are clues for malignancy. Pleural VEGF levels may be an adjunctive parameter in differential diagnosis of pleural effusions due to various etiologies. Values >5000 pg/ml are strongly suggestive of malignant pleural effusion if infection can be excluded. Further clinical studies regarding the effect of VEGF inhibition on treatment of malignant pleural effusions and intractable massive effusions are warranted.

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Conflicts of interest

There are no conflicts of interest.

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