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ORIGINAL ARTICLE

Role of vascular endothelial growth factor (VEGF) in diagnosis of pleural effusion of different origins



M. Fathy ^a, M. Al Ansary ^b, M. Zakaria ^a, H. Abdel-Hafiz ^{a,*}, M. Said ^a

^a Department of Chest Diseases, Faculty of Medicine, Cairo University, Egypt

^b Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, Egypt

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KEYWORDS

Vascular endothelial growth factor (VEGF);
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Abstract *Background:* The undiagnosed pleural effusions are an important clinical problem so scientists spent much effort and time in searching for a new parameter to help in the diagnosis of pleural effusions. Vascular endothelial growth factor (VEGF) helps in differentiation between malignant and inflammatory pleural effusions and might play an important role in tumor progression and the formation of malignant effusions.

Aim of the work: To determine the level of pleural fluid VEGF in order to evaluate its value as a marker for differentiation between different types of pleural effusions.

Subjects and methods: The present study was conducted on 73 patients with pleural effusion, admitted to Kasr Elaini Hospital (Chest Department) during the period from August 2011 to October 2012, after having their written consent. All patients were subjected basically to full history taking, thorough clinical examination, routine laboratory investigations, plain chest radiography thoracentesis. Medical thoracoscopy was carried out for cases with undiagnosed exudative pleural effusion ($n = 46$).

Results: Pleural fluid VEGF, and pleural fluid VEGF/serum VEGF ratio both are highest in malignant pleural effusion with statistically high significance ($p < 0.001$), followed by infectious effusion then tuberculous one. Using ROC curve analysis, the cut-off used for VEGF in pleural fluid in discriminating malignant from other groups is 1800 pg/ml with sensitivity of 95% and specificity of 96%.

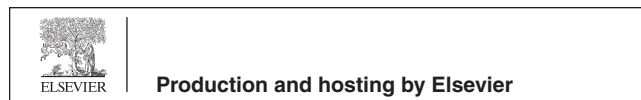
Conclusion: VEGF is highest in malignant pleural effusion. The differential diagnosis of effusions might be further improved by including vascular endothelial growth factor concentrations into the diagnostic armamentarium available to the clinician.

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* Corresponding author. Tel.: +20 01001649587.

E-mail address: hamed-md@hotmail.com (H. Abdel-Hafiz).

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Introduction

Pleural effusion is an accumulation of fluid in the pleural space, as a result of excessive transudation or exudation from the pleural surface. It is a sign for many diseases and not a diagnosis, so correct diagnosis remains a major challenge to

clinicians [1]. The undiagnosed pleural effusions are an important clinical problem so scientists spent much effort and time in searching for a new parameter to help in the diagnosis of etiology of different types of pleural effusions [2].

Vascular endothelial growth factor (VEGF) is a multifunctional cytokine that increases microvascular permeability and induces endothelial cell growth and angiogenesis. It was reported that it enhances the activation and migration of monocytes through the Fms-like tyrosine kinase (FLT) receptor of VEGF. This cytokine also helps in differentiation between malignant and inflammatory pleural effusions [3]. Maximum VEGF concentrations in malignant effusions indicate abundant local release of VEGF within the pleural cavity, which suggests that VEGF might play an important role in tumor progression and the formation of malignant effusions [4].

The aim of the present study is to determine the level of serum and pleural fluid vascular endothelial growth factor in order to evaluate its value as a marker for differentiation between different types of pleural effusions.

Subjects and methods

The present study was conducted on 73 patients with pleural effusion of different etiologies, admitted to Kasr El-Aini Hospital (Chest Department) during the period from August 2011 to October 2012, after having their written consent prior to participation in the study.

These patients were 41 males and 32 females with age ranged from 18 to 70 years. The etiology of exudative pleural effusion was established by definite pathological diagnosis of pleural fluid and/or biopsy. The patients were classified into two groups, according to the type of effusion whether transudate or exudate.

All patients were subjected basically to full history taking, thorough clinical examination, routine laboratory investigations, plain chest radiography (postero-anterior and lateral views) and thoracentesis. Medical thoracoscopy was carried out for cases with undiagnosed exudative pleural effusion ($n = 46$). The pleural fluid obtained was examined as regards: Gross appearance and nature of the fluid, total protein in gm/dl was measured on synchron CX5 auto analyzer, Lactate dehydrogenase enzyme (LDH) was measured in IU/L, adenosine deaminase enzyme (ADA) was measured in IU/L, total and differential cell count of the pleural fluid, bacteriological examination by: culture and sensitivity & Zeil-Neelsen stain for acid fast bacilli (AFB), cytological examination for malignant cells. Levels of pleural fluid vascular endothelial growth factor (VEGF) in pg/ml using ELISA for human VEGF, using the commercially available Orgenium Laboratories' vascular endothelial growth factor (VEGF) ELISA. Venous blood samples were obtained simultaneously to measure protein, glucose, LDH, ADA & VEGF using the commercially available Orgenium Laboratories' vascular endothelial growth factor (VEGF) ELISA.

For determination of the etiology of pleural effusion further different relevant investigations and procedures were performed whenever needed: e.g. complete blood picture (CBC), erythrocyte sedimentation rate (ESR), tuberculin test by the Mantoux method, liver and kidney function tests, sputum examination for AFB (ZN stain), sputum culture and

sensitivity, abdominal ultrasonography, CT-chest, Echo-heart and fiberoptic bronchoscopy.

The patients were classified into two groups, according to the type of effusion whether transudate or exudate according to Lights criteria [5].

Group I

This group included 10 cases with transudative pleural effusion. They were 6 males (60%) and 4 females (40%), with mean age of 60 ± 7.1 years.

Group II

This group included 63 cases with exudative pleural effusions, 35 males (55.6%) and 28 females (44.4), with mean age of 49.1 ± 15 years. Group II is further subdivided into malignant pleural effusion, tuberculous pleural effusion and infectious pleural effusion.

Results

The present study was conducted on 73 patients with pleural effusion of different etiologies. The etiology of exudative pleural effusion was established by definite pathological diagnosis of pleural fluid and/or biopsy (Tables 1 and 2).

Table 2 shows that cases with exudative pleural effusion have higher mean fluid VEGF (2109 ± 1338) than that of cases with transudative pleural effusion (142 ± 28) and the difference is statistically highly significant ($P < 0.01$). And it also shows that cases with exudative pleural effusion have higher mean serum VEGF (325 ± 89) than that of cases with transudative pleural effusion (170 ± 55) and the difference is statistically highly significant ($P < 0.01$) (Table 3).

Table 3 shows that the highest mean value of pleural fluid VEGF is among the malignant effusion group (3208 ± 609.52), followed by the infectious effusion group (974.12 ± 217.98) followed by the tuberculous effusion group (364.09 ± 27.73) (Table 4).

Table 4 shows that the difference in the mean value of fluid VEGF between the malignant effusion group and the tuberculous group was highly significant ($p < 0.001$) and difference between the malignant group and the infectious effusion group was also statistically highly significant ($p < 0.001$). But the difference in the mean value of fluid VEGF between the infectious effusion group and the tuberculous group was statistically insignificant (Fig. 1 & Table 5).

Table 5 shows that Group II patients (with exudative pleural effusion) have higher serum VEGF mean

Table 1 The Study groups.

Patients	Number	Percent %
Group I (Transudative pleural effusion)	10	13.7
Group IIa (Malignant pleural effusion)	35	47.9
Group IIb (Tuberculous pleural effusion)	11	15.1
Group IIc (Infectious pleural effusion)	17	23.3
Total	73	100.0
Controls	13	100.0

Table 2 Comparison between the exudative group collectively and the transudative group.

	Groups				p-Value
	Group II exudate		Group I Transudate		
	Mean	Std. Deviation	Mean	Std. Deviation	
VEGF. fluid	2109.12	1338.78	142.00	28.98	< 0.001
VEGF. serum	325.11	89.004	170.90	55.90	< 0.001

Table 3 Comparison between the levels of VEGF in pleural fluid in the different exudative groups.

	Group IIa (Malignant pl effusion)		Group IIb (Tuberculous pl effusion)		Group IIc (Infectious pl effusion)	
	Mean	±SD	Mean	±SD	Mean	±SD
VEGF in pl fluid pg/ml	3208.86	609.52	364.09	27.73	974.12	217.98

Table 4 Significance of pleural fluid VEGF in exudative groups.

VEGF.fluids			p-Value
	Group IIA (Malignant pl effusion)	Group IIB (Tuberculous pl effusion)	
	Group IIA (Malignant pl effusion)	Group IIC (Infectious pl effusion)	< 0.001
	Group IIB (Tuberculous pl effusion)	Group IIC (Infectious pl effusion)	0.08

value than Group I patients (with transudative effusion), and the differences are statistically highly significant (Table 6).

Table 6 shows that the patients with malignant pleural effusion have the highest ratio (mean value 8.75 ± 1.54), followed by the infectious effusion group (mean value 3.45 ± 0.77), while lowest values are observed in the group

of tuberculous pleural effusion (mean value 1.66 ± 0.29) (Table 7).

Table 7 shows that the difference in the ratio of VEGF (pleural fluid VEGF/serum VEGF) between the malignant effusion group and the tuberculous group was highly significant ($p < 0.001$) and difference between the malignant group and the infectious effusion group was also statistically highly

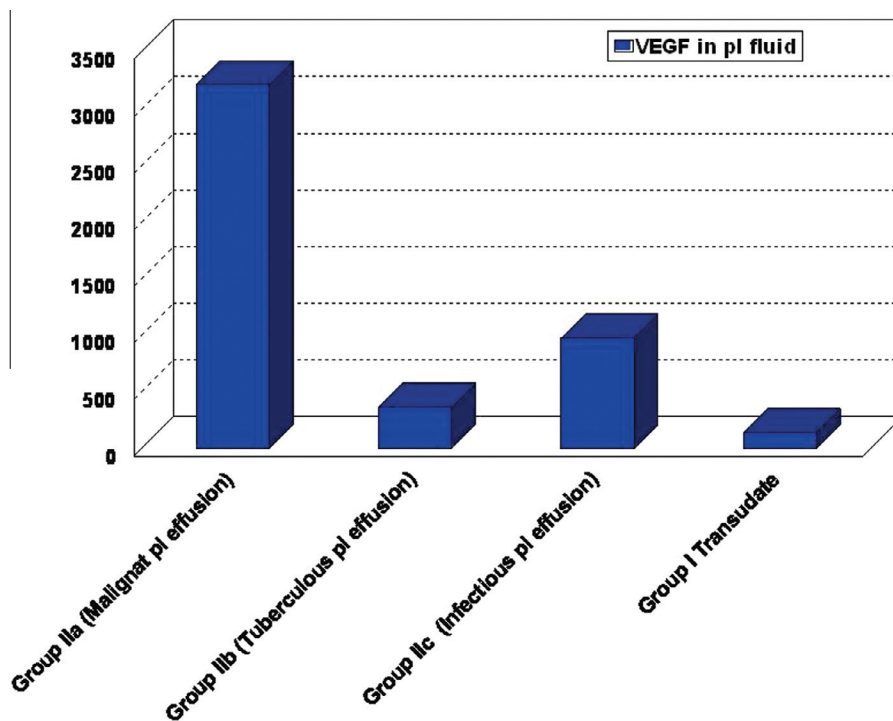


Figure 1 Comparison between the levels of VEGF in pleural fluid in the different groups.

Table 5 Comparison between the serum levels of VEGF in the different groups.

	Groups				<i>p</i> -Value
	Group II (Exudative effusion)		Group I (Transudative effusion)		
	Mean	±SD	Mean	±SD	
VEGF serum pg/ml	325.11	89.00	170.90	55.90	< 0.01*

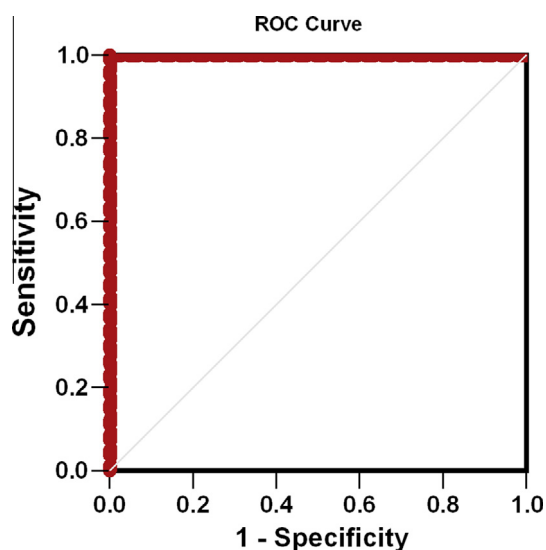
* Highly significant.

Table 6 Comparison between the ratio of VEGF (pleural fluid VEGF/serum VEGF) among the exudative group of effusion.

	Study groups					
	Group IIa (Malignant pl effusion)		Group IIb (Tuberculous pl effusion)		Group IIc (Infectious pl effusion)	
	Mean	±SD	Mean	±SD	Mean	±SD
VEGF fluid/serum ratio	8.75	1.54	1.66	0.29	3.45	0.77

Table 7 Significance of the ratio of VEGF (pleural fluid VEGF/serum VEGF) among the exudative group of effusion.

VEGF fluids				<i>p</i> -Value
	Group IIA (Malignant pl effusion)	Group IIB (Tuberculous pl effusion)	Group IIC (Infectious pl effusion)	
	Group IIA (Malignant pl effusion)	Group IIB (Tuberculous pl effusion)	Group IIC (Infectious pl effusion)	< 0.001
	Group IIA (Malignant pl effusion)	Group IIC (Infectious pl effusion)	Group IIB (Tuberculous pl effusion)	< 0.001
	Group IIB (Tuberculous pl effusion)	Group IIC (Infectious pl effusion)	Group IIA (Malignant pl effusion)	0.03

**Figure 2** Results of ROC curve analysis for fluid VEGF in diagnosis of malignant pleural effusion.

significant ($p < 0.001$). But the difference between the infectious effusion group and the tuberculous group was statistically insignificant.

ROC curve analysis

ROC (Receiver Operating Characteristic) curve was constructed to establish the optimal cut-off points and the likelihood ratios (LRs) of VEGF between malignant and non-malignant effusions (Fig. 2).

The cut-off used for VEGF in pleural fluid in discriminating malignant from other groups is 1800 pg/ml with sensitivity of 95% and specificity of 96%.

Discussion

In some cases of pleural effusions, the cause might be obvious, such as pleural effusions associated with Congestive heart failure or Liver cell failure. In other cases the cause of pleural effusions might not be obvious necessitating extensive diagnostic procedures in an attempt to identify the cause of effusion [6]. Pleural fluid cytology and blind pleural biopsy are the methods most commonly used but are inadequate procedures for the diagnosis. In some studies, blind pleural biopsy has been reported to be inadequate in up to 40% of the patients [7].

This situation puts forward the need for a different method directed to pleural fluid. Certain molecular markers, if proven to be sensitive and specific enough, can help the physician decide whether the patient should have further investigation or not to diagnose a suspected malignancy, *i.e.* open pleural biopsy (VATS, mini-thoracotomy). Among these biomarkers, insulin growth factor, hepatocyte growth factor and Simian Virus-40 have been proven to play an important role in the development and progression of malignant mesothelioma [8].

VEGF is another molecule that has been expressed, and has been taking an important role in the development of malignant pleural effusion. Although some authors have claimed that lymphatic blockage resulting from tumor cells is the main contributing factor for the development of malignant pleural effusion, others have accused VEGF at the first place [9].

In our study Mean value of pleural fluid VEGF for the malignant group was 3208 pg/mL. Lim et al. [10] found that the mean value of pleural fluid VEGF for the malignant group was 2418 pg/mL. Cheng et al. [11] found that the median level

of pleural fluid VEGF for the malignant group was 4097 pg/mL. Ziara et al. [12], found the median VEGF level in malignant pleural effusion 2450 pg/ml and this was significantly higher than tuberculous pleural effusion (median 994 pg/ml), and transudative pleural effusion.

These results are also in agreement with Yanagawa et al. [13] who reported that exudative pleural effusions contained significantly higher amounts of VEGF than transudative pleural effusions, and that among exudative pleural effusions, levels of VEGF in malignant pleural effusion 2292 ± 782 pg/mL were significantly higher than those of benign exudative pleural effusions 522 ± 245 pg/mL. Dalokay et al. [14], reported that the mean level of VEGF in patients with malignant pleural effusions (2117 ± 118 pg/ml) was significantly higher than that of non-malignant (732 ± 305 pg/ml) pleural effusions ($p < 0.001$).

Sack et al. [15] found that the ratio for pleural fluid VEGF/serum VEGF in malignant pleural effusion was mean value 7.6 ± 1.8 , for empyema was mean value 4.46 ± 11.38 , for tuberculous effusion was mean value 2.93 ± 3.46 and the transudative group showed mean value of 0.849 ± 1.32 and this is consistent with Table 6.

By using the ROC (Receiver Operating Characteristic) analysis, Fig. 2 this study shows that at a cut-off value of 1800 pg/ml, pleural fluid VEGF has a sensitivity of 95% and specificity of 96% in diagnosis of malignant pleural effusion. Momi et al. [16], found that the sensitivity and specificity of pleural fluid VEGF (at cut-off value 2000 pg/ml) in the diagnosis of malignant pleural effusion were 100% and 84% respectively while Liu et al. [17] reported that pleural fluid VEGF in diagnosis of malignant pleural effusion has 91.4% sensitivity and 90% specificity. Shu et al. [18] show sensitivity and specificity of VEGF in PE of 47% and of 96% (cut-off of 959 pg/ml). In the literature the data are variable and this can be explained by different cut-off points considered.

In conclusion, VEGF is highest in malignant pleural effusion. The differential diagnosis of effusions might be further improved by including vascular endothelial growth factor concentrations into the diagnostic armamentarium available to the clinician. If VEGF is important for pleural fluid accumulation then novel anti-VEGF therapies could directly influence the rapidity of pleural fluid accumulation in addition to possessing an anti-tumor effect.

Conflict of interest

None.

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