ORIGINAL ARTICLES

Vascular Endothelial Growth Factor in Benign and Malignant Pleural Effusions

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OBJECTIVE: Vascular endothelial growth factor (VEGF) is a potent inducer of capillary permeability and its role as a crucial mediator in pleural fluid formation has been established. This study was conducted to assess the usefulness of VEGF for diagnosing malignant and non-malignant pleural effusions of various causes.

PATIENTS AND METHODS: VEGF levels in pleural effusions collected from 52 patients (20 with malignant effusion, 12 with tuberculous effusion, 10 with transudative effusion, and 10 with parapneumonic effusion) were assessed by enzyme-linked immunosorbent assay.

RESULTS: The median level of VEGF was significantly higher (P=.001) in exudative effusions (10.16 pg/mL) than in the transudative effusions (3.82 pg/mL). Although malignant pleural fluids tended to have higher median and mean levels of VEGF compared to tuberculous effusions, the difference was not statistically significant. Pleural VEGF levels in subtypes of lung cancer and in malignant effusions of different origins were not significantly different.

CONCLUSIONS: In conclusion, although VEGF levels in pleural effusions of different origins vary, they were only able to discriminate exudates from transudates significantly in this study. Further studies in larger groups of patients are needed to establish the role of VEGF in diagnosing malignant and/or tuberculous effusions.

Key words: *Vascular endothelial growth factor (VEGF). Pleural effusion. Lung cancer. Tuberculosis.*

El factor de crecimiento endotelial vascular en los derrames pleurales benignos y malignos

OBJETIVO: El factor de crecimiento endotelial vascular (VEGF) es un potente inductor de la permeabilidad capilar y desempeña un papel clave como mediador en la formación del derrame pleural. Este estudio se ha realizado para evaluar la utilidad del VEGF en el diagnóstico de los derrames pleurales malignos y no malignos de diversas causas.

PACIENTES Y MÉTODOS: Mediante la técnica de inmunoabsorción ligada a enzimas se determinaron las concentraciones de VEGF en los derrames pleurales correspondientes a 52 pacientes (20 con derrame pleural maligno, 12 con derrame de origen tuberculoso, 10 con derrame de tipo trasudado y 10 con derrame paraneumónico).

RESULTADOS: La concentración media del VEGF fue significativamente mayor (p = 0,001) en los derrames pleurales (10,16 pg/ml) de tipo exudado que en los derrames de tipo trasudado (3,82 pg/ml). Aunque los derrames pleurales malignos mostraron una tendencia a las concentraciones medianas y medias mayores de VEGF, en comparación con los derrames de origen tuberculoso, la diferencia no fue estadísticamente significativa. Tampoco fueron significativamente diferentes las concentraciones pleurales de VEGF en los distintos subtipos de cáncer pulmonar, y tampoco en los derrames malignos de distintos orígenes.

CONCLUSIONES: Aunque las concentraciones de VEGF son distintas en los derrames pleurales de orígenes diferentes, en nuestro estudio no han permitido discriminar los exudados de los trasudados. Son necesarios nuevos estudios de investigación sobre grupos más numerosos de pacientes con objeto de establecer el papel que puede desempeñar la concentración de VEGF en el diagnóstico de los derrames malignos, tuberculosos o ambos.

Palabras clave: *Factor de crecimiento endotelial vascular (VEGF). Derrame pleural. Cáncer pulmonar. Tuberculosis.*

Introduction

Pleural effusion, reported in over 3000 cases per million population each year, is a common clinical problem[1] that may develop as a result of increased fluid production and/or reduced drainage, although increased production is the underlying cause in most exudative effusions.[2] In spite of the significance of this health problem, none of the current treatment approaches, such as pleurectomy or pleurodesis, seem to be sufficiently efficient.[3] Our lack of knowledge of basic mechanisms by which fluid accumulates within the pleural space limits further attempts to prevent recurrent effusions.

TABLE
Vascular Endothelial Growth Factor Concentrations and Characteristics of Pleural Fluid by Effusion Type*

	Malignant (n=20)	**Tuberculous (n=12)**	**Parapneumonic (n=10)**	**Transudate (n=10)**
Edad, years	63.86 (8.63)	41.20 (22.57)	59.10 (20.21)	67.54 (9.63)
VEGF, pg/mL	19.56 (9.05)	13.17 (5.83)	10.69 (6.70)	4.81 (3.38)
Proteines, g/dL	4.90 (0.56)	5.41 (0.77)	4.47 (1.45)	2.40 (0.79)
Glucosa, mg/dL	102.26 (46.90)	68.77 (46.05)	82.75 (53.53)	184.90 (122.45)
LDH, IU/L	1212.52 (294.90)	1429.88 (976.62)	1190.44 (875.29)	204.45 (102.70)

*Data are means (SD). LDH indicates lactate dehydrogenase.

Vascular endothelial growth factor (VEGF), initially known as vascular permeability factor, is a 34-42 kD homodimeric protein.[4] VEGF acts mainly at the vascular endothelium, where it can induce morphologic changes, alter gene expression, stimulate cell proliferation and migration, and inhibit apoptosis.[5-9] The ability of VEGF to induce vascular permeability accounts for its crucial role in pleural fluid formation, and it is also a key angiogenic factor for neoplasms.[10]

The purpose of this study was to determine the level of VEGF in pleural fluids of different origins. We especially aimed to see if pleural VEGF levels were significantly different in benign and malignant effusions.

Materials and Methods

We prospectively studied 52 patients who underwent thoracocentesis between August 2001 and June 2002. Standard definitions were used for identifying the cause of pleural effusion, as follows: *a)* malignant effusion (n=20), an effusion with positive cytology for a malignant process; *b)* parapneumonic effusion (n=10), in patients with a clinical history suggesting a recent infection with no features of empyema and no recurrence at 6 weeks of outpatient follow up; *c)* transudate (n=10), an effusion secondary to left ventricular impairment or hypoalbuminemia with a protein content of less than 3 g/dL and no identifiable cause; and *d)* tuberculous effusion (n=12), diagnosed either by a positive acid fast bacilli test, growth of *Mycobacterium tuberculosis* in culture, demonstration of granulomatous lesions in pleural biopsy specimens, or any combination of these findings.

Pleural fluid cytology and microbiological studies were routine for all patients. Pleural fluid specimens were cultured for *M tuberculosis*, and protein, glucose, and lactate dehydrogenase (LDH) levels were assessed, along with adenosine deaminase activity. About 20 mL of pleural fluid was centrifuged at 3000 rpm for 10 minutes to pellet the cellular elements, and the supernatant was stored at –70°C for subsequent VEGF measurement.

A quantitative sandwich enzyme-linked immunoabsorbent assay technique with a VEGF kit (Cytelisa Human VEGF, Cytimmune Sciences Inc, Rockville, MD, USA) was used following the manufacturer's guidelines to measure VEGF in pleural effusions.

Statistical Analysis

The results were analyzed using the SPSS computer software package, version 10.0 (Chicago, IL, USA). For correlations between variables, we used the Pearson correlation coefficient. Significance was set at $P=.01$. Differences among 4 groups for VEGF were evaluated by Kruskal-Wallis analysis of variance: when differences were significant at $P<.05$ according to the Kruskal-Wallis test, a multiple comparison test was used to find out which groups differed from the others.

Results

A total of 52 patients (9 women and 43 men) were included in the study. Among the 20 patients with malignant tumors, 13 had primary lung cancer (8 adenocarcinomas, 4 small cell carcinomas, and 1 squamous cell carcinoma), and 7 had malignant mesothelioma. The mean (SD) ages, VEGF levels, and biochemical parameters are shown in the table.

Patients with transudative effusions had the lowest mean VEGF levels and those with malignant effusions had the highest. The median level of VEGF in the exudative effusions (10.16 pg/mL) was significantly higher than the median level in the transudates (3.82 pg/mL) ($P=.001$). Although malignant pleural effusions tended to have higher median and mean levels of VEGF compared to tuberculous effusions, the differences were not statistically significant. The mean VEGF levels were likewise similar in malignant and parapneumonic effusions. The mean VEGF levels in all 4 effusion types are shown in the figure.

We then analyzed the data for all patients to detect relationships between pleural fluid VEGF levels as the dependent variable and pleural fluid LDH, total protein, albumin, and glucose levels. The strongest correlation was between the pleural VEGF levels and LDH levels ($r=0.75$, $P<.001$).

Among patients with malignant pleural effusions, VEGF levels were similar, regardless of the primary tumor site or histological subtypes of lung cancer.

Discussion

VEGF, initially known as vascular permeability factor, is a 34-42 kD homodimeric protein which induces vasodilatation in a dose dependent manner in vivo.[11] It is 50 000 times more potent than histamine in inducing vascular permeability[8] and is a potent mitogen for micro- and macrovascular endothelial cells derived from arteries, veins, and lymphatic vessels.[12-15] VEGF is
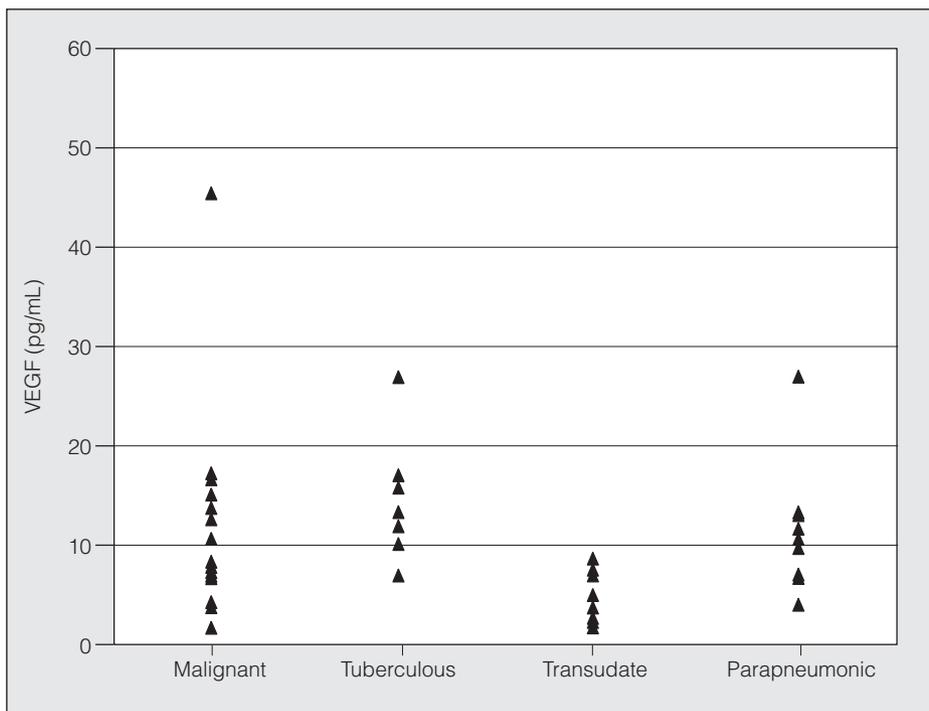
Figure. Distribution of vascular endothelial growth factor (VEGF) levels in the 4 patient groups. Data for 1 patient with a VEGF level of 190 pg/mL in the malignant group has been left out of the graph.

also believed to function as a tumor angiogenesis factor. It functions through 3 signaling tyrosine kinase receptors, and the receptors are primarily expressed on endothelial cells as well as on pleural tissue and most tumor cells.[7] Therefore much attention has been focused on its contribution to pleural fluid accumulation and tumor progression.

VEGF has been shown to be present in significant quantities in pleural and peritoneal effusions of different etiologies. In experimental models of malignant ascites, fluid accumulation has been seen to correlate directly with VEGF production.[16] VEGF has also been identified in various malignant tumors, including several histological types of lung cancer.[17-19] The mechanism of the pleural fluid accumulation in patients with malignancy is not definitely known, but increased capillary permeability in the visceral pleura is thought to play an important role in the formation of exudative effusions.[20] The role of VEGF in pleural fluid accumulation through increasing vascular permeability has been reported in an animal model.[21]

The results of our study demonstrate that pleural fluid VEGF levels differ in function of etiology. Our findings are consistent with previous reports that VEGF levels of transudative and exudative effusions are significantly different. Thickett et al[4] showed that 90% of exudative pleural effusions had VEGF levels that were greater than levels found in transudates. Low levels of VEGF in transudative effusions are unsurprising, as they occur due to changes in osmotic or hydrostatic pressure gradients whereas VEGF is believed to act on endothelial cell permeability.

Two previous studies found that VEGF levels in malignant effusions were significantly higher than those in nonmalignant effusions and suggested that this difference may be helpful in differentiating malignant and nonmalignant effusions.[22,23] However Cheng et al,[24] while finding that VEGF levels in patients with malignant tumors were higher than those in patients with effusion related to coronary artery bypass grafts, detected no significant VEGF difference between malignant and parapneumonic effusions. They suggested that VEGF levels are unlikely to be useful diagnostically. Consistent with their results, we found that the mean levels of VEGF were indeed higher in malignant effusions than in the exudates arising from other causes, but the difference was not statistically significant. However, we believe that large controlled studies are needed before a definitive conclusion about diagnostic utility can be reached.

Although some authors reported finding that pleural fluid VEGF levels in patients with lung cancer were significantly higher than those in patients with breast cancer,[16,24] the explanation for this difference is not clear and there is no evidence that breast carcinoma cells produce less VEGF than do lung carcinoma cells. In contrast, we found no significant differences when we compared VEGF levels in malignant pleural effusions of various origins. Nor were there significant differences in pleural VEGF levels for different histological subtypes of lung cancer, consistent with former investigations.[22]

When we sought a possible correlation between VEGF levels and other biochemical pleural fluid

markers, only the correlation with pleural fluid LDH level was significant, as reported previously by Cheng et al.[24] Since LDH is a crude marker of inflammation, this finding was not surprising.

In conclusion, although there are variations in VEGF levels in pleural effusions of different origins, this marker was only able to discriminate exudates from transudates. More studies in larger groups of patients are needed to establish the role of VEGF in diagnosing malignant and/or tuberculous effusions.

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