J Korean Med Sci 2000; 15: 279-83
ISSN 1011-8934

Vascular Endothelial Growth Factor in Malignant and Tuberculous Pleural Effusions

The purpose of this study is to assess the usefulness of soluble vascular endothelial growth factor (VEGF) in the effusions of patients with malignant and tuberculous diseases. Using a sandwich enzyme-linked immunoadsorbent assay, VEGF concentration was measured in malignant (n=17) and tuberculous (n=11) pleural effusions. Pleural biopsy, cytology or microbiological methods were used to make final diagnoses. Adenosine deaminase (ADA) levels in tuberculous pleural effusions were significantly higher than those in malignant pleural effusions. The median level of VEGF in patients with malignant effusions (median, 2418 pg/mL; range, 97-62103 pg/mL) was significantly higher than tuberculous effusions (median, 994 pg/mL; range, 44-3552 pg/mL). There were no significant differences in pleural VEGF levels in patients with different histological types of lung cancer. The VEGF level was not correlated with ADA, lactate dehydrogenase and total protein levels of pleural fluid. In conclusion, pleural VEGF levels in patients with malignant effusions were significantly higher than tuberculous effusions, and the measurement of pleural VEGF is helpful in discriminating between malignant and tuberculous effusions. Further studies are needed to determine the clinical value of VEGF as a tumor marker and a prognostic factor.

Key Words: *Vascular Endothelial Growth Factor; Pleural Effusion; Endothelium, Vascular*

Sung-Chul Lim, Sook-In Jung, Young-Chul Kim, Kyung-Ok Park

Departments of Internal Medicine, Chonnam National University Medical School, Kwangju, Korea

Received: 24 January 2000
Accepted: 21 February 2000

Address for correspondence
Sung-Chul Lim, M.D.
Department of Internal Medicine, Chonnam National University Hospital, 8 Hak-dong, Dong-gu, Kwangju 501-757, Korea
Tel: +82.62-220-6585, Fax: +82.62-225-8578
E-mail: lscmd@chonnam.chonnam.ac.kr

INTRODUCTION

The development of a vascular supply is a fundamental requirement for organ development and differentiation during embryogenesis as well as for wound healing and reproductive functions in adults (1, 2). Angiogenesis is also implicated in the pathogenesis of a variety of disorders including proliferative-retinopathies, age-related macular degeneration, tumors, rheumatoid arthritis and psoriasis (1, 2).

Tumor angiogenesis is also closely associated with prognosis (3-4) and may be regulated by angiogenic factors, which are produced by the tumor cells (1, 4-5). The search for potential regulators of angiogenesis has yielded numerous candidates including fibroblast growth factor, transforming growth factor, hepatocyte growth factor, tumor necrosis factor, angiogenin and interleukin-8 (6).

Vascular endothelial growth factor (VEGF), also known as vascular permeability factor, is a homodimeric 34 to 42 kDa, heparin-binding glycoprotein with angiogenic, mitogenic and vascular permeability-enhancing activities specific for endothelial cells (7-9). VEGF has been identified in some malignant tumors, such as ovarian cancer (10), melanoma (11), gastric cancer (12), and lung cancer (13). However, little is known about VEGF's relevance to circulation and malignant effusions. Recently, several investigators (14-17) demonstrated that pleural VEGF concentrations were significantly higher in malignant than in nonmalignant effusions.

This study aimed to assess the diagnostic usefulness of soluble VEGF in the pleural effusions of patients with malignant and tuberculous diseases and to determine whether VEGF has a potential pathogenic role in the development of pleural effusion.

MATERIALS AND METHODS

Study population

The study population for this report consisted of 28 patients diagnosed with malignant (n=17) and tuberculous (n=11) pleural effusion between January 1993 and

October 1998 at Chonnam National University Hospital, Kwangju, Korea. Tuberculous pleural effusion was diagnosed either by positive acid fast bacilli, growth of *Mycobacterium tuberculosis* by culture and/or demonstration of granulomatous lesions in pleural biopsy specimens. The diagnosis of malignant effusion was made by cytopathologic detection of malignant cells in the aspirated fluids or pleural biopsy specimens.

Samples

Aliquots of pleural fluids obtained by thoracentesis were examined for differential cell count, bacterial and mycobacterial stain and cytology. Samples were processed for bacteriologic culture as well as to determine pH, protein, glucose, cholesterol, triglyceride, amylase and lactate dehydrogenase (LDH). About 20 mL of pleural fluid was centrifuged at 2,500 rpm for 10 min to pellet the cellular element, and the supernatants were stored at -70℃. Pleural biopsy was performed for feasible subjects with Cope biopsy needles.

Adenosine deaminase (ADA) activity assay

ADA activity was measured with a commercial assay kit (Toyobo Co, Osaka, Japan). The catalytic reaction of ADA results in inosine, which is converted to uric acid and hydrogen peroxide. Peroxidase was added and then absorbance was measured at 555 nm.

Immunoassay for human VEGF

A quantitative sandwich ELISA technique with VEGF ELISA kit (Quantikine Kit, R&D Systems, Inc., Minneapolis, Minn.) following the manufacturer's guidelines was used to measure VEGF in pleural effusions. Two antibodies in this assay reacted mainly to $VEGF_{165}$. The optical density was determined within 30 min after these treatments were performed with a microtiter plate reader set to 450 nm. After averaging the duplication of this treatment for each sample, the concentrations of pleural fluid VEGF were calculated from the linear part of the standard curve using BioLinx software (Microsoft, Redmont, WA).

Statistical analysis

Except for VEGF, group data are shown as the mean ±standard deviation. Because VEGF values were not normally distributed, the 5th and 95th percentile values were chosen for data description. Difference between patient groups were tested by means of Student's t-test or nonparametric Mann-Whitney U test using SPSS 8.0 for Windows (SPSS Inc., Chicago, IL, U.S.A.). The statistical significance was defined as two-tailed P value less than 0.05.

RESULTS

The characteristics of the subjects are listed in Table 1. Pleural effusions collected from a total of 28 patients with 17 malignant effusions and 11 tuberculous effusions. There was no significant difference of age and smoking habit between patients with malignant and tuberculous pleural effusions.

Results of biochemical and cellular analysis of the pleural fluid are summarized in Table 2. Total protein concentration was significantly higher in tuberculous effusion (4.8±0.8 g/dL) compared with malignant pleural effusion (4.2±0.5 g/dL). Other variables, including cell components and lactate dehydrogenases were not significantly different between the two groups.

Tuberculous effusion showed significantly higher levels of adenosine deaminase (ADA) than those of malignant effusion (91.4±43.2 IU/L vs. 21.3±16.1 IU/L, $p<0.05$) (Fig. 1).

The median VEGF levels in malignant pleural effusions (median, 2418 pg/mL; range, 97-62103 pg/mL) were significantly higher than those of tuberculous pleural effusions (median, 994 pg/mL; range, 44-3552 pg/mL) (Fig. 2). There were no significant differences in pleural VEGF in patients with different histological types

Table 1. Characteristics of subjects

	Malignant effusion (n=17)	Tuberculous effusion (n=11)
Age (years)	59.5±15.0	52.2±22.8
Sex (M/F)	8/9	9/2
Smoking (PYS)	23.4±35.8	16.7±22.4

Data were expressed as mean±standard deviation.
M, male; F, female; PYS, pack-years

Table 2. Comparisons of biochemical parameters

	Malignant effusion	Tuberculous effusion
pH	7.35±0.37	7.33±0.12
WBC (/μL)	2,231±1,558	3,311±1,984
Neutrophil (%)	67.8±25.0	60.0±31.1
Lymphocyte (%)	32.2±25.1	40.0±31.1
Glucose (mg/dL)	106.4±39.8	89.2±35.7
Protein (g/dL)	4.2±0.5	4.8±0.8*
Amylase (U/L)	162.4±215.1	44.8±10.9
LDH (U/L)	1,175.9±1,303.8	712.3±404.0

Data were expressed as mean±standard deviation.
*, $p<0.05$; LDH, lactate dehydrogenase

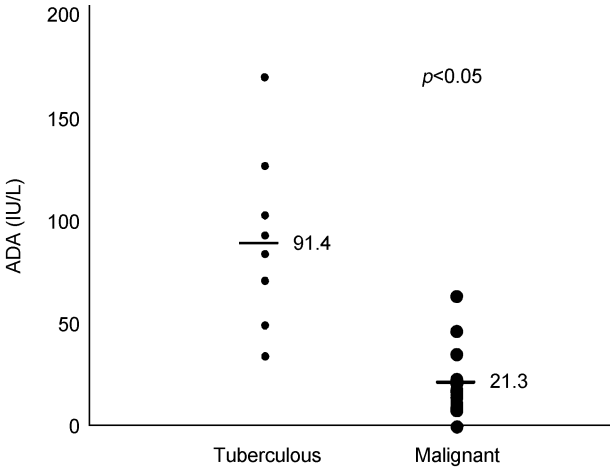

Fig. 1. Pleural fluid adenosine deaminase (ADA) levels between tuberculous and malignant effusions.

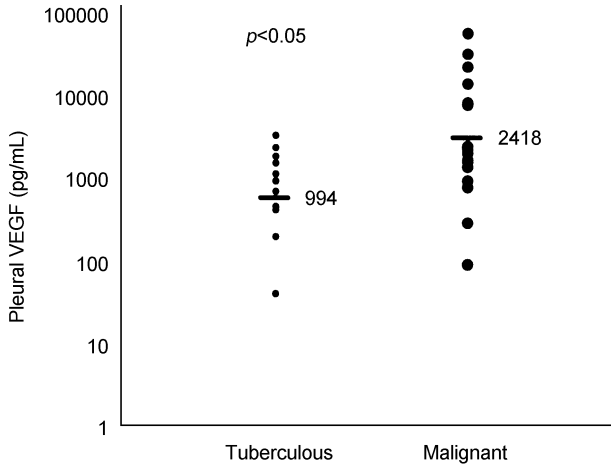

Fig. 2. Vascular endothelial growth factor (VEGF) levels in pleural effusions with tuberculous and malignant disease.

Table 3. Correlation coefficients between pleural ADA, LDH, protein and VEGF

	ADA	LDH	Protein
Correlation coefficient (r)	-0.223	0.217	0.268
Probability (*p* value)	0.295	0.297	0.205

VEGF, vascular endothelial growth factor; ADA, adenosine deaminase; LDH, lactate dehydrogenase

of lung cancer.

The pleural VEGF levels were not correlated with the ADA, lactate dehydrogenase and total protein levels of pleural fluid (Table 3).

DISCUSSION

For the differential diagnosis of tuberculous and malignant pleural effusions, commonly used diagnostic tests, including protein, LDH, glucose, pH and lymphocyte proportions, were reported not to be useful (18). Our findings were consistent with earlier studies except protein. Total protein concentration was significantly higher in tuberculous effusion compared with malignant pleural effusion. Light et al. (19) reported that in many cases with tuberculous pleural effusion, protein contents were high, frequently above 5.0 g/dL.

Adenosine deaminase is an enzyme of purine catabolism which catalyzes the pathway from adenosine to inosine and is found predominantly in T lymphocytes. In an investigation (20), the pleural fluid ADA above 70 IU/L indicated a high probability of tuberculous, whereas ADA below 40 IU/L indicated very low probability of tuberculous. In this study, tuberculous effusion showed significantly higher levels of ADA than those of malignant effusion.

The continuous growth of a tumor beyond a critical size is dependent on the development of new blood vessels, which not only supplies nutrition for further tumor growth, but also serves as a route for systemic metastasis (21). In the process of metastasis, a cancer cell must gain access to the abnormal vasculature, travel through the circulation, settle in the microvasculature of the target organ, escape from the vascular system into the target organ, and induce angiogenesis in the target organ for further growth and additional metastasis (22).

Recently, several angiogenic factors, such as fibroblast factor (7), hepatocyte growth factor (8) and platelet-derived growth factor (23) have been identified. VEGF is also known as a vascular permeability factor due to its ability to induce vascular leakage in the guinea pig skin (24). Dvorak et al. (25) proposed that an increase in microvascular permeability is a crucial step in angiogenesis associated with tumors and wounds.

VEGF has been identified in some malignant tumors, such as ovarian cancer (10), melanoma (11), gastric cancer (12) and lung cancer (13). However, little is known about VEGF's relevance to circulation and malignant effusions.

This study demonstrated that median VEGF levels in malignant pleural effusions were significantly higher than those in tuberculous effusions. These findings are consistent with the data of several investigators (14-17). Kraft et al. (14) reported that elevated levels of VEGF are detectable in the serum of only 0-20% of patients with localized cancer but in 11-65% of patients with metastatic cancer. Yanagawa et al. (15) demonstrated that exudative pleural effusions contained significantly higher amounts of VEGF than transudative pleural effusions. Among exudative pleural effusions, levels of VEGF in

malignant pleural effusions associated with lung cancer were significantly higher than those of benign exudative pleural effusions. There was no significant difference in pleural VEGF in patients with different histological types or clinical stages of lung cancer. Serial measurements of pleural VEGF levels were performed in six lung cancer patients treated with intrapleural instillation of recombinant interferon gamma, and reduction of pleural effusion was associated with decreasing pleural VEGF levels (15). These findings suggest that VEGF plays a role in the accumulation of exudative pleural effusions, especially that of malignant pleural effusion associated with lung cancer.

Unlike other investigators (16), this present study demonstrated that VEGF levels in pleural fluid was not correlated with ADA, LDH, and total protein levels of pleural fluid. This discrepancy certainly cannot be explained by the results of the present study and await further research.

In conclusion, the pleural VEGF levels in patients with malignant effusions were significantly higher than those of tuberculous effusions. We think that the measurement of pleural VEGF is helpful in discriminating between malignant and tuberculous effusions. Further studies are necessary to clarify the significance of pleural fluid VEGF and to evaluate whether this marker will help predict prognosis in malignant pleural effusion.

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