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The diagnostic significance and the assessment of the value of vascular endothelial growth factor as a marker for success of chemical pleurodesis in malignant pleural effusion

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ABSTRACT

Differential diagnosis of pleural effusion is an important issue, since the treatment modalities and prognosis strictly depend on early and correct diagnosis of the underlying etiology. We assessed the efficacy of vascular endothelial growth factor (VEGF) in the differential diagnosis of patients with malignant and non-malignant pleural diseases. And also is assessed of the VEGF as a marker for success of chemical pleurodesis in malignant pleural effusion. Pleural effusions of 40 patients with a mean age of 55 (range, 26 to 78 years) were examined. A total of 20 patients had malignant pleural effusion; malignant meso- thelioma (n = 7), lung cancer (n = 5) and metastatic malignancies (n = 8). Twenty patients had benign pleural effusion; fibrinous pleuritis (n = 6), tubercu- losis (n = 3) empyema (n = 5), congestive heart failure (n = 3), and acute pancreatitis (n = 3). Definitive di- agnosis was obtained in all cases with blind or open pleural biopsy, and cytological examination. VEGF levels were determined by enzyme-linked immu- nosorbent assay. The VEGF level of pleural effusion was comparably higher in the malignant group. The mean level of VEGF in patients with malignant pleu- ral effusions (21.7 ± 1.8 ng/ml) was significantly (P < 0.001) higher than that of (13.2 ± 1.5 ng/ml) non-malignant effusions. No significant difference was found regarding the VEGF levels and histological types in malignant pleural effusions. Negative correlation was observed between success rate of pleurodesis and VEGF level of pleural effusion (p = 0.015). The measurement of VEGF levels in pleural effusion may be useful to differentiate malignant from nonmalignant pleural effusions. VEGF level may also be an important prognostic marker for ef- fective treatment of the patients who had malignant pleural effusions with pleurodesis. It is important issue in here whether VEGF could be useful in prog- nostication of outcome of chemical pleurodesis or not.

Keywords: Malignant Pleural Effusion; Pleural Effusion; Chemical Pleurodesis; Vascularendothelial Growth Factor

1. INTRODUCTION

Pleural effusion is an important problem in malignant or non-malignant pleural disease, causing severe symptoms such as dyspnea and chest pain. Management of the pleural effusion (PE) depends on the underlying etiology. Inflammatory PE can be treated easily with success, contrary to malignant PE, in which the main goal is to decrease symptoms and increase the quality of life as much as possible. This “mandatory” differential diagnosis is still difficult, time-consuming and expensive. Pleural fluid accumulation in malignancy is generally believed to be secondary to lymphatic obstruction by malignant cells [1]. However recent studies pointed to vascular endothelial growth factor (VEGF) as a key agent in this entity [2,3]. VEGF, produced by malignant pleural tissue, is thought to both enhance tumor angiogenesis leading to local growth, and increase vascular permeability leading to PE [2,3].

We conducted a study to investigate the role of VEGF in differentiating between malignant and non-malignant pleural effusions in a series of 40 patients.

2. MATERIAL AND METHODS

2.1. Materials

We measured the VEGF levels of pleural effusions in 40 patients consisting of 24 (60%) male and 16 (40%) female patients with a mean age of 54.5 years (range, 26 to

78 years) by enzyme-linked immunosorbent assay (ELISA). Twenty patients had malignant pleural effusion including malignant mesothelioma (n = 7), lung cancer (n = 5), metastasis from genitourinary system malignancies (renal cell Ca, endometrium Ca, ovarian Ca) (n = 3), metastasis from breast carcinoma (n = 3), adenocarcinoma metastasis from gastrointestinal system (n = 1), Non Hodgkin-lymphoma (n = 1). Twenty patients had benign pleural effusion associated with fibrinous pleuritis (n = 6), tuberculosis (n = 3) empyema (n = 5), congestive heart failure (n = 3), and acute pancreatitis (n = 3). Definitive diagnosis was obtained in all patients with either open or blind pleural biopsies and cytological examination (**Table 1**).

2.2. Analysis of Material Properties and Invitro Study

VEGF concentrations were measured using an enzyme-linked immunosorbent assay (ACCUCYTE®, assay system, Human VEGF ELISA kit, Cytimmune Sciences, Inc. Maryland USA). Tecnique of sampling and storage of PE: Pleural effusion was collected in a sterile centrifuge tube and centifugated at 3000 rpm for 10 min at 4˚C. The cell-free supernatant was then separated and stored immediately at –70˚C until assayed for VEGF. VEGF level in PE measured in duplicate for each sample with an ELISA kit that recognizes the soluble isoform VEGF 165. This assay is sensitive to 0.195 ng/ml. The kit used for the detection of total (bound and unbound) VEGF is designated to capture a specific VEGF complex consisting of VEGF antibody, biotinyl-ated VEGF and sample/standard. The optical density was measured at 492 nm. The glucose and lactate dehydrogenase (LDH) measurements were obtained from fluid collection in a sterile tube using an automated analyzer.

Table 1. Definitive diagnosis and VEGF levels in patients with pleural effusions.

Malignant effusion	n/Mean VEGF level (ng/ml)	Nonmalignant effusion	n/Mean VEGF level (ng/ml)
Malignant mesothelioma	7 (20.7 ± 3.8)	Fibrinous Pleurit	6 (12.8 ± 2.1)
Lung Cancer (ADC*)	5 (24.2 ± 2.8)	Empyema	5 (20.8 ± 1.8)
Metastatic Malignancies	8 (20.9 ± 3.6)	Congestive Heart Failure	3 (7.3 ± 1.2)
Breast Cancer	3	Acute Pancreatitis	3 (12.1 ± 4.4)
Metastasis of GUS** Cancer	3	Tuberculosis	3 (8.8 ± 2.7)
Metastasis of GIS*** ADC	1	-	-
Non-Hodgkin's Lymphoma	1	-	-
Total	20 (21.7 ± 1.8)	-	20 (13.2 ± 1.5)

ADC*. Adenocarcinoma; GUS**. Genitourinary system; GIS***. Gastrointestinal system

2.3. Statistical Analysis

Statistical comparisons of baseline data between groups were performed by the Mann-Whitney *U* test as appropriate. Data were considered statistically significant if *P* values were less than 0.05. Data were expressed as mean ± the standard deviation (SD). Correlations were analyzed with the Spearman rank order correlation. All statistical analyses were performed with the Statistical Package for the Social Sciences (version 11.0; SPSS, Inc., Chicago, Illinois, USA).

3. RESULTS

3.1. Definitive Diagnosis and VEGF Levels in Patients with Pleural Effusions

Twenty patients had malignant pleural effusion associated with malignant mesothelioma (20.7 ± 3.8 ng/ml), lung cancer (24.2 ± 2.8 ng/ml), metastasis from genitourinary system carcinomas (renal cell Ca, endometrium Ca, ovarian Ca) (21.3 ± 6.9 ng/ml) metastasis from breast carcinoma (19.7 ± 4.3 ng/ml), adenocarcinoma metastasis from gastrointestinal system (23.8 ng/ml), and Non-Hodgkin's Lymphoma (21.1 ng/ml). Twenty patients had benign pleural effusion associated with fibrinous pleuritis (12.8 ± 2.1 ng/ml), tuberculosis (8.8 ± 2.9 ng/ml) empyema (20.8 ± 1.8 ng/ml), congestive heart failure (7.3 ± 1.2 ng/ml), and acute pancreatitis (12.1 ± 4.4 ng/ml) (**Table 1**).

3.2. Laboratory Results

The mean level of VEGF in patients with malignant pleural effusions (21.7 ± 1.8 ng/ml) was significantly higher than that of non-malignant (13.2 ± 1.5 ng/ml) pleural effusions ($p < 0.001$) (**Figure 1**). No significant differences were observed in concentration of pleural VEGF in different histological types of malignant pleural effusion (malignant mesothelioma, lung cancer ($p = 0.482$) and malignant mesothelioma, other metastatic carcinomas (excluding lung cancer) ($p = 0.354$). Mean LDH level of PE was 614.32 ± 288.62 IU/L and mean glucose level of PE was 77.0 ± 30.6 mg/dL. The closest correlation was between the pleural effusion VEGF level and the LDH level ($r = 0.894$, $p < 0.001$) (**Figure 2**). No significant correlation existed between the pleural effusion VEGF level and the glucose level ($r = 0.079$, $p = 0.628$).

3.3 Comparison of Success of Chemical Pleurodesis and VEGF Level

Seventeen patients who had malignant pleural effusion underwent chemical pleurodesis. Five of the patients

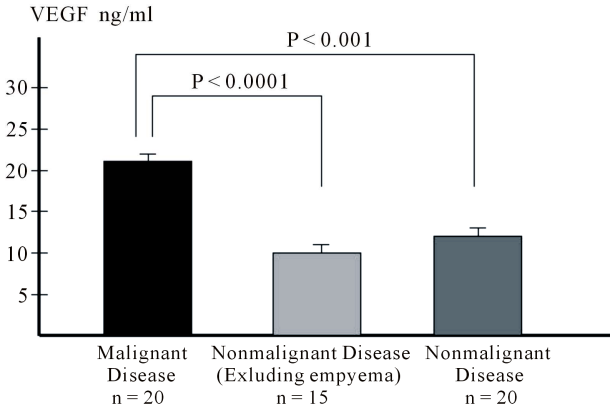

Figure 1. Comparison of VEGF levels of pleural effusion between the patients with malignant and nonmalignant disease.

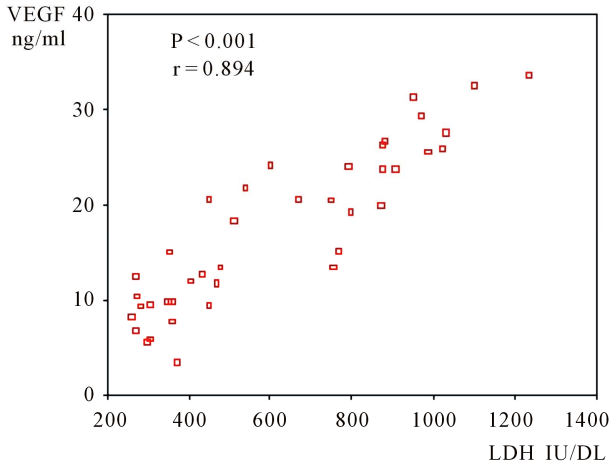

Figure 2. Relationship between pleural fluids LDH levels and VEGF levels are shown.

(29%) were readmitted with reaccumulation in 60 days. The mean VEGF level of PE (28.3 ± 3.7 ng/ml) of these re- admitted patients were higher than that of (17.4 ± 6.7 ng/ml) other patients (p = 0.015). Negative correlation was observed between success rate of pleurodesis and VEGF level of PE (**Figure 3**).

4. DISCUSSION

Pleural fluid accumulation is a common clinical problem in pleural diseases. Treatment of PE in inflammatory diseases is much easier than in malignant PE. The primary target in malignant PE, except for early stages of malignant mesothelioma is to prevent further accumulation of the effusion and to increase the quality of life, which means that "cure" is no more expected. Surgical treatment including decortication or pleuropneumonectomy has been proven to increase the survival rates in early stages of malign mesothelioma [4]. This difference among treatment approaches in benign and malignant

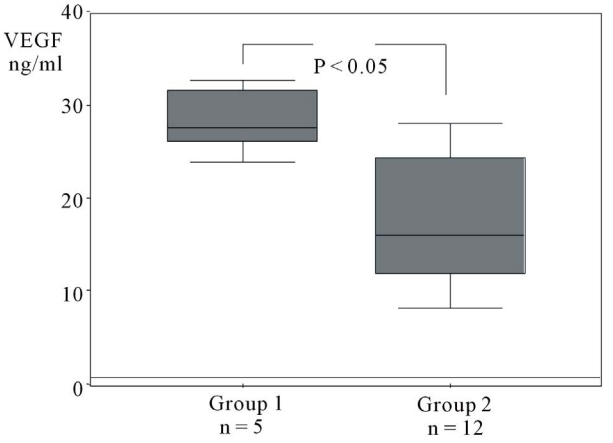

Figure 3. Negative correlation between VEGF levels of PE and success rate of pleurodesis in patients with "group 1" or without "group 2" recurrent pleural effusion.

diseases makes the accurate and early diagnosis mandatory, in order not to let medicine harm the patient.

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 [5,6]. 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) or not. 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 [7-9]. Likewise, a recent study has shown that plasma N/CD-13 activity had a strong correlation with tumor load in malignant pleural diseases [10].

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 of VEGF at the first place [11-13]. VEGF is a disulfide-bonded dimeric glycoprotein. Its molecular weight is 34-45 kD, and the most common types are VEGF 165 and VEGF 121 [14]. The suspected molecular mechanisms are increase in local vascular permeability and stimulation of tumor cell growth by angiogenesis, both stimulated by VEGF produced by tumor cells [1-3,11,12]. Increase in capillary permeability functions through binding with fms-like tyrosine kinase receptor (FLT-1). The FLT-1 VEGF receptors have been identi-

fied in pleural mesothelial cells and vascular endothelial cells also high densities in infiltrating malignant tissue [13]. It has been shown to be important regulatory systems for angiogenesis and vasculogenesis [12].

Many studies have shown that several types of tumor cells express VEGF. VEGF is an important cytokine in lung cancer [15,16] Matsuyama et al. reported a positive correlation between serum VEGF and stage progression of the disease [15]. Measurement of serum VEGF levels was suggested to be useful to evaluate lung cancer progression. Similar data are obtained from studies directed to gastric, colorectal and renal cell carcinomas [17-19]. It also seems to be an important determinant in malignant pleural disease. Tickett et al. have reported that median VEGF levels of 2500 pg/ml in malignant PE were significantly higher than of 305pg/ml in the non-malignant group [13]. Our study is in accordance with these previous data, showing an increased level of VEGF in malignant effusion. Lim et al. compared VEGF levels of tuberculous PE (median, 994 pg/ dl) and malignant PE (2418 pg/ dl), reaching to similar results obtained in our study [20]. In our series, VEGF of PE levels in empyema patients were higher than other nonmalignant groups ($p = 0.02$). There were no significant differences between VEGF levels in empyema and in lung cancer ($p = 0.349$). Similar results have been reported by Thickett [13]. The known biological functions of VEGF may promote the accumulation of pleural fluid and increase the loculation and organization of empyema [21]. In our study, when VEGF levels found in empyema cases were excluded, the levels were more significantly higher in malignant than in non-malignant PE ($P < 0.0001$) similar to Thickett's study [13].

If patients having high levels of VEGF in suspicious PE that cannot be diagnosed with blind biopsy of the pleura or cytological techniques, patients should undergo an early open biopsy. Thickett *et al*. reported false negative rate of 50 % in the malignant group after blind biopsy with a median level of VEGF higher than the level in the benign group. However, in the abovementioned study, additional invasive procedures were needed for definitive diagnosis [13]. In our study, the results obtained via pleural fluid cytology in addition to blind biopsy were false negative in 25% ($n = 5$) of the malignant group mean with a mean VEGF level of PE = 21.2 ± 1.4 ng/ml and definitive diagnosis had to be made by VATS and mini-thoracotomy.

VEGF and LDH in pleural effusion correlated because they are both crude markers of the inflammatory response [22].

A new experimental study for the treatment of PE includes VEGF receptor (receptor tyrosine kinase) blockage model for human tumors [23,24]. VEGF receptor blockage model may also provide a new therapeutic approach for pleural malignancies. Further studies are necessary to outline the feasibility of VEGF receptor blockage model as a therapeutic modality.

As a conclusion, malignant pleural effusions show significantly higher VEGF levels compared with non-malignant pleural effusions. Thus, assessment of VEGF levels may be used to differentiate malignant from non-malignant pleural effusions as an adjunct to conventional differential diagnostic techniques. Low level of VEGF in malignant PE patients may be a good prognostic marker for effective treatment of malignant PE with pleurodesis.

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