TANAFFOS

Comparative Study of Vascular Endothelial Growth Factor in Exudative and Transudative Pleural Effusion

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Background: Increased vascular permeability is one of the main mechanisms in the production of pleural effusion (PE) and vascular endothelial growth factor (VEGF) has a significant role in its pathogenesis. This study aimed to compare pleural levels of VEGF in transudative and exudative PEs besides the other pleural markers.

Materials and Methods: In this prospective cross-sectional study, 80 patients with PE were divided into 4 groups as transudative (N=15), parapneumonic (N=15), tuberculosis (N=25), and malignant (N=25) PE. Biochemical tests measured the pleural protein, LDH, cholesterol, glucose, polymorphonuclear cell (PMN), and lymphocyte. ELISA measured the pleural VEGF level.

Results: Out of 80 patients, 51 were male, and the total mean age was 55.34±18.53. There were significant differences in pleural VEGF between exudative and transudative effusion (P<0.001) and between malignant and benign effusion (P=0.014). The highest mean difference in pleural VEGF levels was seen in the comparison of transudative and malignant groups (Mean difference=-136.56; P<0.002). The VEGF level in 3 groups was not significantly different; transudative vs tuberculous, parapneumonic vs tuberculous, and parapneumonic vs malignant. Furthermore, VEGF higher than 73.09 pg/ml had a 64% sensitivity and 82% specificity for the diagnosis of malignancy. Among pleural markers (VEGF, protein, LDH, and glucose), VEGF had the highest area under curve (AUC=0.734). Moreover, pleural protein, LDH, and glucose levels significantly correlated with pleural VEGF; however, pleural cholesterol, PMN, and lymphocyte were not correlated.

Conclusion: VEGF is assumed as an important factor in the pathogenesis of exudative PE, especially malignant effusion. It can distinguish between lymphocytic exudative PEs.

Key words: Pleural Effusion, Exudate, Transudate, Vascular Endothelial Growth Factor (VEGF)

INTRODUCTION

Pleural effusion (PE) is a common medical complication and a significant provenance of morbidity (1), and is described as excessive fluid retention in the pleural cavity (the mean normal amount of pleural fluid is 8.4±4.3 ml) (2). It's estimated more than 400 people per 100,000 are affected by PE (3). PE results from an impaired balance between pleural fluid production and absorption; arising from increased vascular permeability, resulting in plasma leakage, and lack of drainage of the pleural space due to obstruction of vessels and lymphatics of the lung and pleura (4). About 30-40% of PE is idiopathic or

indeterminate, but in 60-70% of cases, PE can be caused by more than *50* diseases, including lung disease, systemic disease, organ dysfunction, drug-induced PE, and tumor metastasis (5). PEs are classically categorized into "transudates" and "exudates" based on the mechanism of fluid formation. Transudative effusions (TEs) result from an imbalance in oncotic and hydrostatic pressure in pleural capillaries (e.g. Hypoproteinemia), increased negative intrathoracic pleural pressure (e.g. Atelectasis), or the progress of ascitic fluid from the peritoneal cavity to the pleural cavity, through defects in the diaphragm or lymph vessels (e.g. Hepatic hydrothorax) (2-5). Systemic diseases such as heart failure, renal failure, and liver cirrhosis can cause transudative PEs as well (6). In contrast, exudative effusions (EEs) are the result of inflammation of the pleura, increased capillary permeability, decreased lymphatic drainage, or other local conditions of the pleural surface, like tuberculosis, pneumonia, pancreatitis, malignancy, pulmonary infarction, collagen diseases, or systemic lupus erythematosus (7).

Vascular endothelial growth factor (VEGF) is a pluripotent, cell-specific, and multi-functional cytokine that plays an important role in angiogenesis and vascular permeability. VEGF is 10,000 times more effective than histamine as a permeability-enhancer. VEGF causes proliferation, migration, and differentiation of endothelial cells, as well as the formation of malignant pleural and peritoneal fluid. Increased VEGF level is also seen in chronic respiratory diseases such as asthma and cystic fibrosis (8).

The initial step in the management of PE is recognizing the origin of pleural fluid, which is necessary to ensure optimum treatment by determining the pathophysiological process and clinical features (5,9). As a result, misclassification of transudates as exudates can lead to inappropriate patient handling or possibly unnecessary and invasive diagnostic investigations that increase health care costs and morbidity (10). The second step of PE management is analyzing the fluid using biochemical, pathological, and clinical findings. Biochemical analysis of the liquid acquired by a thoracentesis (including differential leukocyte counts, glucose, total proteins, albumin, lactate dehydrogenase (LDH), cholesterol, pH, amylase, adenosine deaminase, and tumor markers for malignancy) is mostly the first procedure to separate TEs from EEs (2). Cytological examination, thoracoscopic and different radiological procedures are other methods (with its own benefits and concerns) used to differentiate TEs from EEs (11-13). The criteria most frequently used to distinguish transudates from exudates is related to the measurement of LDH and protein in both pleural fluid and serum, namely Light's criteria (7).

Studies indicated that VEGF level is consistently higher in exudative PEs than in transudative PEs (14). It is reported that pleural fluid VEGF levels in patients with PE due to malignancies are higher than those due to benign diseases (15, 16). However, studies assessing various types of PE and comparing VEGF with other pleural markers are required.

Overall, clinicians need more accurate and sensitive tests to diagnose the underlying cause of PE among suspected patients. Regarding the weakness and limitations of current methods and the importance of differentially diagnosing the type of PEs, we designed this study aiming to compare the pleural VEGF levels in all types of PEs in an effort to improve the accuracy of pleural fluid categorization.

MATERIALS AND METHODS

In this prospective cross-sectional study, we studied inpatients and outpatients admitted to Ghaem Hospital (Mashhad, Iran). We included those with PE until the required sample for each group was completed. The sample size was considered according to recent studies. We excluded patients with coagulation disorder (INR more than 2 and platelets less than 100,000 per microliter) and/or patients with malignancy undergoing chemotherapy with anti-VEGF drugs.

Our study was approved by the Mashhad University of Medical Science Ethics Committee. Demographic information, medical history, and medication history were obtained. Before thoracentesis, all cases signed the informed consent. Pleural fluid was tapped and prepared for biochemical markers analysis. Also, 5 ml of the samples were heparinized, centrifuged, and frozen at -20 ° C for subsequent VEGF testing. Biochemical tests measured the serum and pleural protein (g/dl), LDH (U/l), cholesterol (mg/dl), glucose (mg/dl), polymorphonuclear cell (PMN), and lymphocytes.

We categorized PEs as exudative (65 cases) and transudative (15 cases) according to Light's criteria (7). We separated parapneumonic (15 cases) pleural effusion, related to pneumonia, according to biochemical tests, clinical signs, and predominance of neutrophils. A malignant (25 cases) pleural effusion was diagnosed if the pleural biopsy or pleural fluid cytology found malignant cells. A tuberculous (25 cases) pleural effusion was described as one when granuloma was found on the pleural biopsy specimen.

Laboratory analysis

The level of pleural VEGF (pg/ml) was measured using the enzyme-linked immunosorbent assay (ELISA) (AviBion Human VEGF ELISA Kit, Finland). The assays were done according to the manufacturer's instructions at Bu Ali Research Institute. The detection limit was between 0.148 and 12 (pg/ml).

Statistical analysis

Continuous data are reported as mean±standard deviation (SD). We used Spearman/Pearson's correlation coefficients to calculate the correlation of VEGF with other variables (Pleural protein, Pleural LDH, Pleural cholesterol, Pleural glucose, Pleural PMN, Pleural lymph). Chi-square, independent t-test, ANOVA, and Kruskal–Wallis tests were used as appropriate (each test mentioned below the tables). POST-HOC analysis of ANOVA test was employed for comparison of pleural VEGF levels of four groups. The level of significance was set at *P=0.05*. Data were analyzed using SPSS, version *15*. Moreover, the ROC (Receiver Operating Characteristic) curve was used to assess the sensitivity and specificity of VEGF, protein, LDH, and glucose in the diagnosis of malignancy.

RESULTS

In this survey, 80 patients with the diagnosis of PE were included: 15 patients with transudative PE, 15 patients with parapneumonic PE, and 50 patients with lymphocytic exudative PE including tuberculosis (TB) (n=25), and malignancy (n=25). Fifty-one of these participants were male (63.75%) and 29 were female (36.25%) with a mean age of 55.34 ± 18.53 years (range, 21-86 years). It is described in Table 1.

Pleural VEGF level was not correlated with age, gender, and positive cytology rate (P=0.402, P=0.109, and P=0.146, respectively). Pleural VEGF levels in patients with exudative effusions were significantly higher than transudative (P<0.001), and also in effusions related to malignancies than benign groups (P=0.014). The comparison of the studied PE groups based on VEGF is presented in Table 2. Pleural fluid markers and VEGF levels differed significantly among studied groups with P<0.001, as these levels based on the types of PE, are shown in Table 3. Moreover, pleural VEGF was significantly correlated with pleural protein, LDH, and glucose levels, while there was no significant correlation with pleural cholesterol, PMN, and lymphocytes (Table 4). In the calculation of sensitivity and specificity of pleural markers, VEGF≥7309 had a sensitivity of 64% and a specificity of 82% for the diagnosis of malignancy (Table 5, Figure 1). Also, the area under the curve (AUC) was higher for VEGF than protein, LDH, and glucose levels (AUC = 0.734, 95% CI [0.617-0.851]) (Table 5).

Table 1. Age and gender distribution of patients according to the type of pleural effusion

| | Gender | | | | Age |
| | Male | | Female | | Mean ± SD* |
	No.	Percent (%)	No.	Percent (%)	
Transudate	8	53.3	7	46.7	65.27 ± 12.30
Parapneumonic	13	86.7	2	13.3	52.40 ± 21.94
Tuberculosis	15	60	10	40	44.64 ± 18.37
Malignancy	15	60	10	40	61.84 ± 13.90
p-value in result of test	0.220 C				0.001 A

*SD: Standard deviation

C=Based on Chi-squared test

A=Based on Anova test

Table 2. Comparison of studied groups according to VEGF

Comparison of groups in terms of VEGF	Mean difference (pg/ml)	p-value*
Transudate and parapneumonic group	-121.1013	0.018
Transudate and tuberculosis group	-29.8938	0.860
Transudate and malignancy group	-136.5618	0.002
Parapneumonic and tuberculosis group	91.2074	0.063
Parapneumonic and malignancy group	-15.4605	0.973
Tuberculosis and malignancy group	-106.6680	0.005

*Based on POST-HOC analysis of ANOVA test

Table 3. Evaluation of pleural fluid markers and VEGF in the studied patients based on the type of pleural effusion

| Pleural fluid markers | Study groups* | | | | p-value |
	Transudate	Parapneumonic	Tuberculosis	Malignancy	
VEGF (pg/ml)	7.1773± 7.6582	128.2786±136.8250	37.0712±33.4691	143.7392±162.5413	0.000A
Protein (g/d1)	1.38 ± 0.37	4.26 ± 0.85	4.5 ± 0.74	4.10 ± 0.61	0.000A
LDH (u/l)**	120 ± 54.70	2174.26 ± 4751.34	4.5 ± 0.74	641.8 ± 924.34	0.000K
Cholesterol (mg/dl)	39.40 ± 27.99	64.86 ± 18.63	422.44 ±172.12	99.28 ± 13. 74	0.000A
Glucose (mg/dl)	157.93 ± 64.04	66 ± 34.66	83.08 ± 13.71	86.64 ± 22.74	0.000A
PMN* (%)**	18.66 ± 11.87	79.66 ± 12.8	84.12 ±18.62	17.0 ± 11.27	0.000A
Lymphocyte (%)	81.33 ± 11.87	20.33 ± 12.88	22.60 ± 18.77	83.0 ± 11.27	0.000A

*Data based on mean ± Standard deviation

** Lactate dehydrogenase

*** Polymorphonuclear

K: Based on Kruskal–wallis

A= Based on ANOVA test

Table 4. Comparison of the relationship between pleural fluid markers and VEGF

Correlation of the studied variables with the level of pleural fluid VEGF	r*	p-value
Pleural protein	0.248	0.027P
Pleural LDH	0.461	0.000SP
Pleural cholesterol	0.151	0.180P
Pleural glucose	-0.251	0.025P
Pleural PMN (%)	0.147	0.193P
Pleural lymph (%)	-0.147	0.193P

*r: Correlation coefficient

SP: Based Spearman

P= Based on Pearson test

Table 5. Evaluation of sensitivity and specificity of VEGF and other pleural markers in the diagnosis of malignancy

	Area under the curve	Confidence Interval 95%	Sensitivity	Specificity	Cut off point
VEGF (pg/ml)	0.734	0.617-0.851	0.64	0.82	7309≤
Protein (g/dl)	0.532	0.407-0.657	0.96	0.31	3.2≤
LDH (u/l)	0.698	0.585-0.811	0.96	0.42	330≤
Glucose (mg/dl)	0.457	0.332-0.582	0.80	0.40	80.5≤

DISCUSSION

Undiagnosed PE is a major clinical concern. The available methods can be inefficient in up to 40% of cases (17). These put forward the need for more diagnostic approaches. Regarding deficiency of assessment of four PE types and comparison of sensitivity and specificity of VEGF with common pleural markers, we explored the diagnostic potential of VEGF in four PE types and compared it with other pleural markers.

The results of this prospective cross-sectional study on 80 patients with PE indicated that VEGF had the highest potential in the diagnosis of pleural effusions compared with protein, LDH, and glucose solely. Moreover, the level of pleural protein, pleural LDH, and pleural glucose was correlated with the level of pleural fluid VEGF. Also, the mean value of pleural fluid VEGF in malignant patients was 143.73 pg/ml, which was significantly higher than tuberculous PE (37.07 pg/ml), and transudative PE (7.17 pg/ml); but the difference with parapneumonic PE was insignificant (128.27 pg/ml). The mean value of pleural fluid VEGF in malignant patients in previous studies vary between 19.56 to 3208 pg/ml (18, 19). Consistent with previous studies, this study found higher pleural VEGF levels in exudative effusions compared to transudates. Fathy et al. (18) reported the mean value of pleural fluid VEGF in malignant patients (median 3208 pg/mL) was significantly higher than tuberculous PE (median 364 pg/ml) and infectious PE (median 974 pg/ml). Economidou et al found that VEGF level was significantly higher in exudates than in transudates. However, reported no significant differences of pleural VEGF levels between malignant and inflammatory effusions(20). Khalil et al. (21) reported higher pleural fluid VEGF levels in exudates than transudate effusions. Also,

comparison of various subtypes of exudative effusion, found a higher concentration of pleural VEGF in malignant than tuberculous, parapneumonic, and collagen exudative effusions. The exact underlying mechanism for this association is not known. However, it is proposed that VEGF functions through tyrosine kinase receptors primarily expressed on endothelial cells, pleural tissue, and most tumor cells (22). Moreover, it is suggested that VEGF plays an important role in increasing vascular permeability thus resulting in PE in malignant patients (23).

In this study ROC analysis showed that by considering the cut-off value of 73.09 pg/ml, pleural fluid VEGF had a sensitivity of 64% and specificity of *82%* in the diagnosis of malignant PE. Hariyanto et al. reported a sensitivity and specificity of 85.29% and 84.22% at cut-off value of 416.60 pg/ml for VEGF-A level in pleural fluid for the diagnosis of malignant PE (24). Gad et al. found sensitivity and specificity of pleural fluid VEGF (cut-off value 1590 pg/ml) of 96.2% and 98.7%, respectively, in differentiating malignant exudative from benign exudative PE (25). Fathy et al. found a sensitivity of 95% and specificity of 96% in the diagnosis of malignant PE (cut-off value of 1800 pg/ml)(18). Khalil et al. (21) reported specificity of 53.3% and sensitivity of 100.0 % at cut-off value of 720 pg/ml. A possible explanation for the inconsistency of these numbers is the different cut-off points assessed in each study. According to high VEGF levels in parapneumonic PE, probably the specificity and sensitivity of the VEGF test are higher among lymphocyte-predominant PEs; future studies should assess this point.

Limitations

This study had two limitations. First, the high cost of this procedure makes it only proper for suspected cases.

Second, this study should be performed on a larger population.

CONCLUSION

In conclusion, our results show that pleural fluid VEGF is an important biomarker in the differential diagnosis of effusions, especially in differentiating malignant effusions. Regarding the cut-off value of 73.09 pg/ml, pleural fluid VEGF has a sensitivity of 64% and specificity of 82% in the diagnosis of malignant PE. Considerably, further research is required to investigate the implications of anti-VEGF therapies' on the management of pleural fluid accumulation.

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