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Vascular endothelial growth factor in diagnosis of pleural effusion

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ABSTRACT

Background: Vascular endothelial growth factor (VEGF) is a glycoprotein which is an important mediator of angiogenesis and vascular permeability. Different VEGF levels were found in carcinomatous, inflammatory and tuberculous pleural effusions, implying a varying degree of influence on the process of fluid accumulation in the pleural space in different disease states. Aim of the work: to assess the role of serum and pleural fluid VEGF levels to evaluate its value as a marker in diagnosis of pleural effusion of different etiologies.

Methods: Forty patients with pleural effusion, 19 males and 21 females, with a mean age of 55.960 ± 6.835 years, were classified into transudative and exudative groups. All patients were subjected to thorough history, clinical examination, radiological examination, laboratory investigations, sputum for acid fast bacilli, tuberculin test, thoracocentesis and occasionally pleural biopsy or thoracoscopy and both serum and pleural fluid VEGF were measured in all patients.

Results: There was a significant difference between exudate and transudate regarding serum and pleural fluid VEGF levels that were higher in exudates than transudate. In comparison between the subtypes of exudative effusion, there was higher concentration of VEGF in pleural of malignant than tuberculous, parapneumonic, and collagen exudative effusion.

Conclusion: VEGF pleural fluid level could differentiate between malignant and non malignant effusion, while could not differentiate between tuberculous and nontuberculous, or between parapneumonic and nonparapneumonic exudative effusions.

Introduction

Diagnosing the aetiology of pleural effusion is an important issue, since the treatment and prognosis strictly depend on early and correct diagnosis of the underlying aetiology [1]. Vascular endothelial growth factor (VEGF) is a glycoprotein considered an important mediator of angiogenesis and vascular permeability [2]. VEGF is expressed by a number of different tumor cell types, such as adenocarcinoma, urinary bladder carcinoma, fibrosarcoma and lymphoma cells [3]. Different VEGF levels were found in carcinomatous, inflammatory and tuberculous pleural effusions, implying a varying degree of influence on the process of fluid accumulation in the pleural space in different disease states [4]. VEGF increases microvascular permeability 20,000 times more potently than histamine [5]. Targets for VEGF bioactivity outside the vascular endothelium include macrophages, type II pneumocytes, monocytes for which it may be chemotactic [6]. It also has a vasodilatory function [7].

Aim of the work

The aim of the present study is to assess the role of serum and pleural fluid VEGF levels as a marker in diagnosis of different aetiologies of pleural effusion.

Patients and methods

This study was a prospective study carried out on 40 patients with pleural effusion. They were 19 female and 21 male, their ages ranged from 45 to 75 years with a mean of standard deviation (SD) of (55.965 ± 6.835) and an informed written consent was taken from all patients. All patients were subjected to detailed history taking, thorough clinical examination, investigations (chest X-ray, CT chest, complete blood picture, erythrocyte sedimentation rate, fasting and postprandial blood sugar, kidney function tests, liver function tests, ECG, abdominal ultrasound, serum and pleural fluid

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LDH, protein and albumin, sputum for acid fast bacilli for three successive days, tuberculin test and thoracocentesis was done and pleural fluid was subjected to biochemical (LDH, protein, albumin, glucose, ADA and ph), microbiological (gram stain, Z-N and culture) and cytological examination (cell count and cytological examination for malignant cells).

- Invasive investigations (pleural biopsy, and/or thoracoscopy) were done if needed.
- Both serum and pleural fluid VEGF levels were measured in all patients.

The patients were classified according to their final diagnosis into transudative and exudative groups according to Light's criteria [8].

Samples preparation and storage

1. Pleural fluid samples were centrifugated and stored at −20 °C until analysis.
2. Serum samples were allowed to clot in a serum separator tube at room temperature, centrifugated at approximately 1000×*g* for 15 min and stored at −20 °C until analysis.

Reagents

Lyophilized recombinant human VEGF standard:10 ng/-tubex2), One 96-well plate precoated with anti-human VEGF antibody, Sampled diluents buffer: 30 ml, Biotinylated antihuman VEGF antibody: 130 ml (dilution 1:100), Antibody diluents buffer: 12 ml, Avidin-Biotin-Peroxidase Complex (ABC): 130 ml (dilution 1:100), ABC diluents buffer: 12 ml, Tetramethylbenzidine (TMB) color developing agent: 10 ml and TMP stop solution: 10 ml.

Summary of assay procedure

1. Samples and standards were added and the plate was incubated at 37 °C for 90 min without washing.
2. Biotinylated antibodies were added and the plate was incubated at 37 °C for 60 min. The plate was washed 3 times with 0.01 MTBS.
3. ABC working solution was added and the plate was incubated at 37 °C for 30 min. The plate was washed 5 times with 0.01 MTBS.
4. TMB color developing agent was added and was incubated at 37 °C in dark for 25–30 min.
5. TMB stop solution was added and results were ready to be read. Statistical presentation and analysis of the present study was conducted using the mean, standard deviation and Student test, Linear Correlation Coefficient (r), Chi-square, ANOVA and ROC curve by SPSSV17.

Results

Table 1
Demographic data of transudative and exudative groups according to sex and age.

		Groups						Test	
		Transudate		Exudate		Total		t/X^2	P-Value
		N	%	N	%	N	%		
Sex	Female	10	66.67	11	44.00	21	52.50	1.960	0.162
	Male	5	33.33	14	56.00	19	47.50		
Age	Range	52.000–75.0000		45.000–70.000				1.536	0.132
	Mean ± SD	59.000 ± 6.835		55.960 ± 5.560					

Table 2
Classification of transudative and exudative groups according to diagnosis.

	N	%
Diagnosis (Transudate)		
Hepatic	6	40.00
Cardiac	6	40.00
Renal hydronephrosis	3	20.00
Total	15	100.00
Diagnosis (Exudate)		
Malignant	10	40.00
Tuberculous	6	24.00
Parapneumonia effusion	7	28.00
Collagen	2	8.00
Total	25	100.00

Table 3
Criteria of pleural fluid and serum levels of protein and LDH in transudates and exudates.

		Groups		*T*-test	
		Transudate	Exudate	T	P-Value
Serum Protein	Range	5.900–8.100	6.000–7.000	0.851	0.400
	Mean ± SD	6.665 ± 0.661	6.528 ± 0.358		
Serum LDH	Range	145.000–596.000	163.000–625.000	1.714	0.094
	Mean ± SD	388.267 ± 139.933	461.840 ± 126.142		
Pleural fluid Protein	Range	1.650–2.9	3.45–4.700	−11.740	<0.000**
	Mean ± SD	1.98 ± 0.503	3.806 ± 0.450		
Pleural fluid LDH	Range	87.000–197.000	315.000–1405.000	10.471	<0.000**
	Mean ± SD	129.333 ± 37.116	884.120 ± 276.75		

** Highly significant ($P \leqslant 0.001$).