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Serum levels of vascular endothelial growth factor in chronic obstructive pulmonary disease

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Abstract

Background: Chronic obstructive pulmonary disease (COPD) is a third leading cause of death.

Methods: In this case control study, we prepared 5 cc bloods from the antecubital vein of 100 COPD patients and 40 healthy individuals as control group. Vascular endothelial growth factor (VEGF) expression protein level was measured by ELISA in both groups.

Results: We found that concentration of VEGF in blood serum of patients with COPD (189.9±16pg/ml) was significantly higher than the control group (16.4±3.48pg/ml) ($p<0.001$). While VEGF serum level in emphysematous patients wasn't significantly different with control group ($p=0.07$). Furthermore VEGF serum level in COPD patients was proportionally increased with severity of disease ($p<0.001$). Besides all COPD patients, regardless of their smoking status, were experienced significantly higher levels of VEGF than healthy ones ($p=0.001$; $z=4.3$).

Conclusion: Our results suggest VEGF serum concentration as the sensitive index for severity and activity of COPD and its prognosis.

Keywords: Chronic obstructive pulmonary disease, Vascular Endothelial Growth Factor, Chronic bronchitis, Emphysema.

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Introduction

In recent decades, the prevalence and mortality of chronic obstructive pulmonary disease (COPD) as a major worldwide health problem is increasing (1) and it is expected to be as third leading cause of death in the world by the year 2020 (2, 3). The formation of oxidants in pulmonary epithelial due to the production reaction of local free radicals, intracellular reactive oxygen species and the extensive cellular injuries, plays an important role in the pathophysiology of COPD (4). The pulmonary emphysema is an important phenotype of COPD, which is characterized by destruction and enlargement of the pulmonary alveoli (5). Besides, emphysema over the time, leads to chronic bronchitis due to the alveolar destruction and the chronic airway inflammation. Chronic bronchitis is a clinical syndrome defined by the chronic cough and the sputum production. Increasing the

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amount of smooth muscle and the connective tissue in the airway walls are occurred in chronic bronchitis (6). Cigarette smoking (CS) is the major risk factor for COPD.

Molecular and cellular mechanisms that are responsible for the development of COPD, emphysema and bronchitis are not fully understood. There are pathogenetic theories for the development of COPD including an imbalance between the protease and antiprotease system, dysregulation of oxidant-antioxidant activity and chronic airway inflammation (7). Recent studies have suggested that increased apoptosis in the alveolar wall accounts in part for the loss of lung tissue and characterizes emphysema (8,9).

Recently it is found that vascular endothelial growth factor (VEGF) is produced by several cell types, which is thought to be important for maintaining structural homeostasis in the adult lung (10,11). In another report, based on previous in-vitro animal study, on BEAS-2B, VEGF has been implicated in the pathogenesis of acute exposure to cigarette smoke extract for up-regulating VEGF mRNA in a human bronchial epithelial cell line (12); however, there is little known about the regulation of VEGF in the airway epithelium in response to cigarette smoking exposure in-vivo specially the terminal bronchioles and (13), the major sites of airflow limitation in COPD (14, 15).

We hypothesized that the development of COPD and its severity would be associated with serum level of VEGF in the airway epithelium. So in present study, we evaluated the VEGF expression protein level using an enzyme-linked immunosorbent assay (ELISA) in COPD patients.

Methods

Patients

This is a case-control study comprised 140 participants (63 women and 77 men with mean age of 63.460 ± 11.7 years old). Among them 100 patients had a chronic obstructive pulmonary disease (COPD) and 40 were healthy individuals as a control group. The patients were enrolled in this study from May 2010 to May 2012. The study was approved by the local ethics committee of Mashhad University of Medical Science and the written informed consent form was obtained from all patients. The diagnosis was based on the NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria (16) and by Spirometry test. The pulmonary function was performed by Spirometry using MIR00155; TUK/MIR 009 (Rome, Italy) by the same technician, at the same time before breakfast. Pulmonary function values were expressed as the percentage of predicted values (17). Measured parameters included: FEV1- forced expiratory volume in the first second (% predicted) and FEV1/FVC-forced expiratory volume in the first second to forced vital capacity (%). The patients whom presented a predicted FEV1< 80%, and FEV1/FVC< 70% or mild COPD were entered to this study and whom with FEV1/FVC> 70% were excluded. Besides, subjects with a history of asthma or atopy, and a systemic infection or an inflammatory process that could be associated with the abnormal biomarker profile were excluded. Chronic bronchitis was defined as the presence of chronic productive cough for three months in each of two successive years; other causes of chronic cough were excluded. Emphysema was defined as the abnormal permanent enlargement of the airspaces distal to the terminal bronchioles, accompanied by destruction of their walls. Thus, bronchitis was defined in the clinical terms, and emphysema in the terms of anatomic pathology, based on the results of high-resolution computed tomographic (HRCT) scans of the lungs.

Then 40 healthy control individuals were matched to COPD patients and they were checked about COPD by Spirometry test. Moreover the cigarette smoking was considered in all studied individuals.

Blood sampling and analysis

Blood samples (5cc) were taken from the

antecubital vein in the morning between 9:00 and 10:00am after an overnight fasting. Blood was processed within one hour of collection, and serum was aliquotted and stored at -70 °C until analysis. The concentrations of VEGF was measured using commercial, enzyme linked immunoassay kits R&D SYSTEMS (R&D SYSTEMS Inc., 614 McKinley Place NE, Minneapolis, MN55413, USA) following the manufacturer's instructions. VEGF could be measured in the range 62 to 707pg/ml, and the sensitivity of assay was less than 9.0pg/ml.

Statistical analysis

The data were analyzed using SPSS for windows version 11.05 (SPSS Inc., Chicago, IL, USA). All data were checked for normality by Kolmogorov–Smirnov test (K–S test). Numerical data are expressed as mean ± standard deviation (SD) or as proportions of the sample size. Correlation between severity of COPD and the other variables was investigated by spearman's correlation coefficient. Mann-Whitney U-test was used to compare the continuous data collected as VEGF levels in different age range, cigarette and non-cigarette patients and control individuals, emphysema and bronchitis patients with each other. Kruskal-Wallis test was used to compare VEGF level in different severity of COPD and in emphysema and bronchitis patients with each other. A p-value less than 0.05 were considered significant.

Results

In this case- control study, a total of 100 COPD patients (60 female and 40 male) matched with 40 control healthy individuals (17 female and 23 male) were enrolled. Demographic information about studied individuals is presented in Table 1. There were statistical significant difference between age of COPD patients (63.4 ± 11.7yrs) and that of control healthy ones (40.4 ± 12.9yrs) (p=0.001). There were not any difference between age of 63 (45%) studied females (53.2 ± 14.4yrs) and 77 (55%) studied males (59.9 ± 14.9yrs) (p>0.05). Therefore, we categorized studied individuals into different age categories as follow; lower or equal to 40 (30 ones), between 41 to 60 years old (41 ones) and equal or more than 60 (69 ones). Most of COPD patients were cigarette smokers; besides, there was significant difference in VEGF level of cigarette smoking individuals (133.7 ± 15.9pg/ml) and non-cigarette smoking ones (61.1 ± 10.5pg/ml) (p<0.001; z=3.6).

Comparison of VEGF level of studied subjects with age, kind of COPD and cigarette smoking is presented in Table 2. This table shows that there are significant difference between VEGF levels of studied

Table 1. Demographic information of studied individuals

Variable		COPD patients	Control	p
Gender	Male	40 (40)	23 (57.5)	0.06
	Female	60 (60)	17 (42.5)	
Age	≤40	4 (4)	26 (65)	0.001
(years)	40-60	32 (32)	9 (22.5)	
	≥60	64 (64)	5 (12.5)	
Positive smoking		71 (71)	11 (27.5)	0.001
Negative Smoking		29 (29)	29 (72.5)	

Data in the parenthesis shows the percentages in each group.

Table 2. Comparison of VEGF level (pg/ml) of studied subjects with different variables

Variable		COPD patients	Healthy individuals	p
Age	≤40	70± 6.85	14.8±3.40	0.02
(years)	40-60	105.8±15.43	26.8±4.48	0.04
	≥60	159.2±15.80	5.6±1.25	0.002
VEG level		148.5±15.59	16.4±3.48	0.07
Positive smoking		151±16.4	20±2.1	0.001
Negative Smoking		107±12.8	15±3.8	0.001

Data presented as Mean± standard deviation

Table 3. VEGF level (pg/ml) in different severity of COPD

Severity	COPD patients	Chronic bronchitis
Mild	40.2±4.53	45.7±4.86
Moderate	94.03±11.80	117±12.69
Slight	177±17.29	257.6±15.94
Sever	150.2±16.12	228.6±16.15
p	0.025	0.001

COPD patients and healthy individuals as COPD ones experienced significantly higher VEGF levels ($p<0.001$). Besides, this difference was prominent in two age categories (between 40 to 60 and equal to 60 or more), and the older patients considered to have higher levels of VEGF than younger individuals. Also COPD patients had considerably higher amounts of VEGF compared to control ones ($p=0.07$). Cigarette smoker individuals had higher levels of VEGF in both groups, COPD and healthy ones; however, all of COPD patients, regardless of their smoking status, had significantly higher levels of VEGF than healthy ones ($p=0.001$; $z=4.3$).

VEGF level in emphysematous patients (18.76 ± 16.94pg/ml) was significantly lower than that of chronic bronchitis ones (189.9 ± 16pg/ml) ($p= 0.001$; $z= 6.48$). While there was marginal significant difference between VEGF level of emphysema patients (18.76 ± 16.94pg/ml) and that of healthy individuals (16.4 ± 3.48pg/ml) ($p= 0.07$). Hence, we just evaluated VEGF levels in different COPD severity of all studied COPD patients and chronic bronchitis ones (Table 3). There were significant correlations between VEGF serum level and COPD severity ($p= 0.04$; $r_s=0.15$) as the patients with severe COPD had significantly higher level of VEGF than individuals with mild or moderate severity ($p=0.025$, $X^2= 7.3$). Moreover these correlations were found for VEGF level and severity of chronic bronchitis ($p=0.001$, $r_s=0.51$) as severe chronic bronchitis patients had experienced considerably higher level of VEGF than patients with mild or moderate severity ($p=0.001$, $X^2= 21.3$).

Discussion

Chronic obstructive pulmonary disease committed through the structural and functional variations in the pulmonary parenchyma, central and peripheral respiratory track and pulmonary circulation. Moreover, the endothelial and smooth muscle cells proliferation of the vascular wall leading to the pulmonary vessel remodeling and pulmonary hypertension is characteristic of COPD (1,2,18). The contribution of angiogenic growth factors in the pathogenesis of COPD was reported previously (19). In fact, increased expression of VEGF in vascular and airway smooth muscle cells and alveolar epithelium of patients with COPD has been demonstrated in previous works (20,21). It appears that the VEGF which was discovered by Ferrara and Henzel in 1989 (22), has an important effect on the endothelial cells proliferation, apoptosis and vascular wall remodeling (23).

Although VEGF is well known as the survival factor of endothelial cells (24), the function of VEGF in epithelial cells is largely unknown. It was found that bronchial and bronchiolar VEGF was decreased in COPD patients. Some studies have been suggested that VEGF can also be regarded as the survival factor of epithelial cells in an autocrine manner (25,26). It has been reported that the administration of inhibitor of VEGF receptors (SU5416) led to emphysema in adult rat lungs (27,28), while the performance of its specific inhibitors (NVP-AAD777) did not cause the development of emphysema (28). Suzuki et al (30) found that repeated cigarette smoke exposure down-regulated the bronchiolar expressions of VEGF prior to the development of emphysema and the bronchiolar VEGF. VEGF expression was decreased

significantly in COPD smokers compared to lifelong nonsmokers. However, there was no difference in bronchiolar VEGF levels between lifelong nonsmokers and smokers without COPD.

In present study we found that VEGF level in emphysema patients was significantly lower than chronic bronchitis ones. Besides the difference between VEGF level of emphysematous patients and healthy individual participants was not significant. Moreover, our finding demonstrated that the correlation between VEGF serum level and COPD severity was significant, as the patients with severe COPD, especially the chronic bronchitis form, had significantly higher level of VEGF than the patients possessing mild or moderate severity. Contrary to Suzuki et al (30) findings, we demonstrated that smoker COPD individuals had higher levels of VEGF than healthy individuals; however, both nonsmoker and smoker COPD patients, especially patient with chronic bronchitis, were experienced significantly higher levels of VEGF than healthy ones. Our results are in accordance to Kanazawa et al (31) results. Similar to our study they also found that the increased level of VEGF has been observed in bronchitis patients and this increment was little prominent in emphysematous patients compare to control group. Finally they suggested that VEGF may affect the pathogenesis of these two common types of COPD. Besides recently Lee et al (32) found that VEGF expressions were up-regulated in chronic bronchitis and increased expression of VEGF was related to hypoxia inducible factor-1 alpha which regulated VEGF overexpression as a characteristic of chronic bronchitis.

Taken together with the current findings, the cigarette smoking induced increased level of VEGF might cause impaired VEGF signaling in bronchiolar epithelium. Previous studies confirmed the potential role of inhibition of VEGF and its receptors for the treatment of vascular changes in the airway wall (33,34). In present study we strongly suggest that VEGF serum level plays a prominent role in COPD pathogenesis. Accordingly, it can be concluded that the consideration of VEGF serum concentration as a sensitive index for severity and activity of COPD and its prognosis could be probable.

Conclusion

According to our findings, measuring VEGF serum concentration can be recommended as a sensitive index for severity and activity of COPD and its prognosis.

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