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Role of Serum Interleukin 6, Albumin and C-Reactive Protein in COPD Patients

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Background: Chronic obstructive pulmonary disease **(**COPD) is a non-specific inflammation, which involves the airways, lung parenchyma and pulmonary vessels. The inflammation causes the activation of inflammatory cells and the release of various inflammatory mediators such as interleukin-8 (IL-8), IL-6 and tumor necoris factor alpha (TNF-a). The purpose of the present study was to measure serum IL-6, C-reactive protein (CRP) (as a positive phase reactant) and albumin level (as a negative phase reactant) in COPD patients (only due to cigarette smoking not bio-mass), non COPD smokers and healthy subjects using enzyme-linked immunosorbent assay (ELISA); we compared the differences in inflammatory factors among groups.

Materials and Methods: A total of 180 males were enrolled in this study and divided into three equal groups. The first group was 60 smokers who had COPD. The second group included 60 smokers without COPD and the third group consisted of people who were not smokers and did not have COPD; 5 mL of venous blood was taken from all participants and it was collected in a test tube containing anticoagulant and then centrifuged at 3000 rpm for 10 minutes. Serum was separated and used to measure the amount of IL-6, CRP and albumin. Spirometry was performed according to the criteria set by the American Thoracic Society.

Results: The mean serum level of IL-6 was 83.2±7.5 pg/mL in group I, 54.9±24.3 pg/mL in group II and 46.9±10.4 pg/mL in group III. There was a significant difference among the three groups (P<0.001). The mean serum level of CRP was 28.9±14.9 mg/dL in the first group, 19.9±8.5 mg/dL in the second group and 4.2±2.3 mg/dL in the third group (P=0.02). But by controlling the confounding effects of age, this difference was not significant (P=0.49). The mean serum level of albumin was I 4.1±0.57 mg/dL in group I, 4.3±0.56 mg/dL in group II and 4.1±0.53 mg/dL in group III. There was no significant difference among the three groups in this regard (P=0.099).There was a significant inverse relationship between serum levels of IL-6 and FEV 1 (r=-0.341, P<0.001). Moreover, there was a significant inverse relationship between serum levels of IL-6 and FEV1/FVC (r=-0.309, P<0.001). Serum albumin level was not different among various stages. Level of CRP and IL6 increased as the stage of COPD got worse in smokers.

Conclusion: Our study showed that serum level of IL-6 predicts development of COPD in smokers with a high sensitivity among all inflammatory factors namely CRP, IL-6, and albumin.

Key words: Interleukin-6, C-Reactive Protein, Albumin, Chronic Obstructive Pulmonary Disease

INTRODUCTION

Chronic obstructive pulmonary disease is the most prevalent cause of morbidity and mortality due to lung diseases in both developing and developed countries (1). The global initiative for lung disease (GOLD) has estimated that this disease is probably going to be the third

cause of death worldwide by the year 2020 (2). The cause of death in COPD is not only respiratory failure, but also cardiovascular complications, lung cancer or other causes which often remain unrecognized (3). Risk factors of COPD include: 1) Cigarette smoking, 2) Occupational exposures (dust and fumes, coal mining, gold mining, cotton textile dust) and 3) α_1 antitrypsin deficiency etc. Cigarette smoking is a major risk factor for mortality from chronic bronchitis and emphysema. Chronic exposure to cigarette smoke may lead to inflammatory cell recruitment within the terminal air spaces of the lung (4).

Chronic obstructive pulmonary disease is a non-specific inflammation, which occurs in the airways, lung parenchyma and pulmonary vessels. The process leading to COPD development is heterogeneous (5). Several mechanisms such as apoptosis, cell proliferation and the release of metalloproteinases and fibrosis of the small airways are the contributing factors in advanced diseases, development of autoimmunity and activation of dendritic cells and T-helper cells. During the exacerbation period, macrophages are unable to ingest apoptotic cells and bacteria. The inflammation causes activation of inflammatory cells and release of various inflammatory mediators such as IL-8, IL-6 and TNF-a. These mediators can destroy lung structure and promote the inflammatory response of neutrophils (5,6). One of the main steps in the treatment of COPD is suppression of inflammation to prevent these complications.

Many recent studies indicated that CRP levels are related to important clinical outcomes, including exercise tolerance, health status and COPD exacerbation (7, 8-12).

Serum proteins are affected by inflammation. Albumin is a negative acute phase reactant and albumin levels decrease during the acute phase response due to increase in catabolism of albumin (13).

The purpose of this study was to investigate the plasma level of IL-6 as a main inflammatory factor, CRP as a positive acute phase reactant and albumin as a negative phase reactant in smoker COPD patients and compare it with smoker non-COPD individuals and healthy controls.

MATERIALS AND METHODS

Study Design

This was a comparative-descriptive study that was done from 2013 to 2014 at Al-Zahra Hospital in Isfahan, Iran. The institutional review board approved the study. The COPD patients were selected among male patients referred to the Pulmonary Clinic of Al-Zahra Hospital in Isfahan. Participants were divided into three groups. The first group was 60 smokers who had stable COPD. This group had a history of chronic cough, sputum, persistent dyspnea and cigarette smoking (no inhalation of bio-mass). They had FEV1/FVC<0.7 and FEV1<80% predicted in their spirometry. In order to rule out asthma and confirm irreversible airway obstruction, they were assessed based on their clinical history and response to pre- and-post bronchodilator (less than 12% increase in FEV1 and 200cc in the FEV1 volume after inhaling 400mcg Salbutamol). In this group patients were classified to four subgroups (mild, moderate, severe and very severe) based on the "Global Initiative for Chronic Obstructive Lung Disease" (GOLD) criteria (14, 15).

The second group included 60 of smokers with a history of at least 10 packs/ year, but they had not been diagnosed with COPD. The third group consisted of 60 healthy people who were not smokers and did not have heart disease, chronic lung disease or other inflammatory conditions.

The exclusion criteria included recent pulmonary infections, primary diagnosis of other respiratory or chronic inflammatory diseases, recent (<four months) myocardial infarction, unstable angina or congestive heart failure (New York Heart Association class III or IV). From all participants, 5 mL venous blood was obtained and injected into a test tube containing anticoagulant and then centrifuged at 3000 rpm for 10 minutes. Serum was separated and used to measure the amount of IL-6, CRP, and albumin. Spirometry was performed (Ferrari KOKO

Louisville, CO, USA) according to the criteria set by the American Thoracic Society (15).

A questionnaire was filled-out containing demographic characteristics, history and amount of cigarette smoking, spirometry results, severity of the disease based on the GOLD criteria, demographic characteristics and history of cigarette smoking in healthy subjects.

Statistical analysis

The quantitative data among three groups were analyzed by one-way ANOVA. To assess the relationship between laboratory findings associated with inflammation (IL6) and spirometric parameters, we used the Pearson's correlation, ANCOVA and regression analysis. A value of P< 0.05 was taken to indicate statistical significance. All data were reported as mean ± standard deviation (SD). Analysis was done using SPSS software version 22.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The 180 male candidates enrolled in this study were divided into three equal groups. The first group was a number of smokers with COPD, the second group included a number of smokers without COPD and the third group consisted of people who were not smokers, and did not have COPD. The mean age of the participants was 59.1±13.6 years in the first group, 48.2±12.3 years in group II, and 39.9 ± 11.8 years in the third group. The mean serum level of IL-6 was 83.2±7.5 pg/mL in group I, 54.9±24.3 pg/mL in group II and 46.9 ± 10.4 pg/mL in the third group. There was a significant difference among the three groups in this regard (P<0.001). The mean serum CRP level was 28.9 ± 14.9 mg/dL in the first group, 19.9±8.5 mg/dL in the second group, and 4.2±2.3 mg/dL in the third group (P = 0.02). But by controlling the confounding effects of age, this difference was not significant (P= 0.49); the reason for this was probably due to the large SD in the participants. The mean serum level of albumin was 4.1±0.57 mg/dL in group I, 4.3±0.56 mg/dL in group II and 4.1±0.53 mg/dL in group III. There was no significant difference among the three groups (P=0.099) and by controlling the confounding effect of age this result did not change (P=0.099, Table 1).

Table 1. Data analysis among the three groups

Variables	Groups	N	Mean	SD	Min	Max	P1	P2
	Smoker &COPD	60	59.1	13.6	30	91	<0.001	-
Age	***Smoker***	60	48.2	12.3	27	73		
	Control	60	39.1	11.8	22	72		
	Smoker &COPD	60	45.0	36.5	10	150	<0.001	-
Pack/Year	***Smoker***	60	21.5	12.9	10	70		
	Control	60	0	0	0	0		
	Smoker &COPD	60	47.5%	17.1	18%	83%	<0.001	<0.001
FEV1	***Smoker***	60	76.3%	14	32%	97%		
	Control	60	82.1%	10	46%	98%		
	Smoker &COPD	60	0.60	0.07	0.34	0.70	<0.001	<0.001
FEV1/FVC	***Smoker***	60	0.81	0.05	0.71	0.96		
	Control	60	0.81	0.04	0.71	0.90		
	Smoker &COPD	60	83.2	75	26.3	459.4	<0.001	0.02
IL-6	***Smoker***	60	54.9	24.3	17	170.9		
	Control	60	46.9	10.4	26.8	68.5		
	Smoker &COPD	60	4.1	0.57	2.2	5.1	0.099	0.099
Albumin	***Smoker***	60	4.3	0.56	2.8	5.1		
	Control	60	4.1	0.55	2.9	5.1		
	Smoker &COPD	60	14.9	28.9	1.0	131	0.02	0.49
CRP	***Smoker***	60	8.5	19.9	1.0	112		
	Control	60	4.2	2.3	1.0	17		

P1: One-way ANOVA, P2: ANCOVA, CRP: C-reactive protein, COPD: Chronic obstructive pulmonary disease, FEV1: Forced expiratory volume in 1 second, FVC: Forced vital capacity, IL-6: Interleukine-6, Max: Maximum, Min: Minimum, N: Number of patients in each group, SD: Standard deviation

After spirometry and determining the mean FEV1 and ratio of FEV1/ FVC, it was demonstrated that the mean FEV1 among the three groups was significantly different (P<0.001). The difference in the mean FEV1 measured between the first and the second and between the first and third groups was statistically significant (P<0.001). The analysis showed that there was a significant correlation between serum levels of IL-6 and age (P=0.038), serum CRP level and age (P=0.022), FEV1 and age (P=0.019), and also FEV1/FVC and age (P=0.019). On the other hand, there was no significant correlation between serum albumin level and age (P=0.506).

There was a significant inverse relationship between serum levels of IL-6 and FEV 1 (r=-0.341, P<0.001); also there was a significant inverse relationship between serum levels of IL-6 and FEV1/FVC (r=-0.309, P<0.001). Based on simple linear regression analysis, FEV1 and FEV1/FVC were predictable from serum level IL-6 (FEV1=0.777 – (0.001 IL-6), FEV1/FVC=0.793 – (0.001 IL-6).

As it can be seen, we sorted our COPD patients based on the GOLD criteria. Serum albumin level was not different in various stages. The mean CRP and IL-6 levels increased as the stage of COPD got worse in smokers (except for the mean IL-6 level in group FEV1>=0.8 that can be due to small number of participants in this group) (Table 2).

Table 2. Variables in COPD patients based on GOLD criteria.

Variables	FEV1	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
IL6	>=0.8	4	88.3250	18.50826	9.25413	72.00	108.40
	0.50-0.79	22	59.1818	22.70357	4.84042	26.30	127.80
	0.30-0.49	24	86.8542	79.10189	16.14661	39.40	432.00
	<0.30	10	125.5400	127.01766	40.16651	48.50	459.40
	Total	60	83.2533	75.01529	9.68443	26.30	459.40
Alb	>=0.8	4	4.4000	0.43205	0.21602	4.00	5.00
	0.50-.079	22	4.1091	0.70637	0.15060	2.20	5.10
	0.30-0.49	24	4.1958	0.44573	0.09098	3.10	5.00
	<0.30	10	3.9000	0.54160	0.17127	2.80	5.00
	Total	60	4.1283	0.57019	0.07361	2.20	5.10
CRP	>=0.8	4	3.5000	2.38048	1.19024	2.00	7.00
	0.50-.079	22	10.9955	26.45698	5.64065	1.00	127.00
	0.30-0.49	24	16.3333	27.82033	5.67880	2.00	126.00
	<0.30	10	24.9000	40.79611	12.90086	3.00	131.00
	Total	60	14.9483	28.93624	3.73565	1.00	131.00
Age	>=0.8	4	62.2500	14.63728	7.31864	49.00	79.00
	0.50-0.79	22	51.4545	13.95478	2.97517	30.00	91.00
	0.30-0.49	24	63.2083	11.95273	2.43984	40.00	87.00
	<0.30	10	65.1000	10.20294	3.22645	49.00	83.00
	Total	60	59.1500	13.68263	1.76642	30.00	91.00

Alb: Albumin, CRP: C-reactive protein, FEV1: Forced expiratory volume in 1 second, FVC: Forced vital capacity, IL-6: Interleukine-6, GOLD: Global Initiative for Lung Disease, N: Number of patients in the first group.

DISCUSSION

Inflammation of the airways is the main pathology in COPD. A large number of inflammatory cells accumulate in the airways, including neutrophils and macrophages. These cells release various inflammatory mediators, causing pulmonary damage (16-18). Chronic obstructive pulmonary disease is a systemic inflammatory disease, characterized by abnormal activation of inflammatory cells and abnormal increase of circulating cytokines such as CRP, IL-8, TNF, IL-6, and leptin (19, 20). Cigarette smoking is a major risk factor for mortality from chronic bronchitis and emphysema. Chronic exposure to cigarette smoke may lead to inflammatory cell recruitment within the terminal air spaces of the lung (4).

The level of IL-6 in plasma is known as a powerful cause of CRP production in the liver (21), and is associated with CRP levels in COPD patients (10,22,23). In this study, the CRP mean values were significantly different among the three groups but by controlling for the confounding effect of age this difference disappeared (P=0.49). Therefore, the effect of age is a main cause for different CRP mean values in the groups. The mean serum level of IL6 was significanltly different among the groups as well. Thus, we can conclude that the mean serum level of IL6 is a more sensitive biomarker to predict inflammation. The plasma level of IL-6 also has shown a relation to malnutrition pathophysiology since it increases in low-weight COPD patients (13). As we know, malnutrition has a corelation to decreased levels of serum albumin. In our study, the mean level of serum albumin did not have significant differences among groups and was in the normal range; thus, the participants did not have malnutrition. We conclude that increase in level of IL6 did not correlate with malnutrition. Pinto-Plata et al. demonstrated that the mean value of CRP remained stable over a 17- month period. Pinto-Plata et al, in another cohort study indicated that the highest level of inflammatory markers was related to the degree of airflow obstruction, functional capacity and health status (25, 26). In this study, the mean CRP mean values and level of IL6 increased proportionally with severity of COPD (Gold criteria). Kolsum et al. has shown that IL-6 did not change over one-year (23). One study showed that chronic systemic inflammation, especially when lasting for at least one year, was associated with a higher incidence of exacerbations and short survival (27). Furthermore, many additional studies showed that the rise in white blood cell counts and plasma level of IL-6, fibrinogen, CRP, chemokine ligand 18, IL-8, and surfactant protein D can predict mortality and morbidity in COPD patients (18, 28, 29).

As a matter of fact, IL-6 regulates many pathways that could contribute to its effect on inflammatory disease progression. During CD4 T-cell differentiation, IL-6 promotes IL-17 and IL-21 production, and suppresses regulatory T cell function. The downstream effect of IL-6 is the deposition of matrix, antibody complexes, proteases in the targeted tissue and consequently tissue destruction (7-12,30).One study showed that the IL-6 mean values did not change significantly during the one-year period, and there was moderate repeatability of IL-6 between the two visits (31). Mehrotra et al. showed that IL-6 played a significant role as a predictor of mortality in COPD patients (32). Celli et al, in a three-year study showed association between mortality and WBC count, IL-6 serum level, fibrinogen, CCL-18, CRP, IL-8, and SP-D in COPD patients. They demonstrated that only IL-6 independently added predictive power to the basic clinical model (12,28). Fibrinogen is another inflammatory factor, which can be used in COPD diagnosis and exacerbation. Also, fibrinogen, an acute phase protein, increases during airway colonization and other comorbidities such as heart failure, diabetes mellitus, and lung cancer (18,29). Brinkley et al, in one study demonstrated that IL-6 level was associated with poor physical function, independent of age, gender, race, and body composition in older adults and multiple comorbidities, including COPD patients (12,33). In our study, the mean serum level of IL6 was significantly different among the three groups (P<0.001), and persisted by controlling the confounding effect of age (P=0.02). Thus, the increase of IL6 level depends on age and inflammation due to smoking.

On the other hand, one study showed that baseline serum CRP did not correlate with mortality in patients with moderate to very severe COPD after a three-year follow up (12, 34). Pinto-Plata et al. reported that the mean level of CRP did not change over a 17-month interval (25). In contrast, epidemiological studies showed an association between baseline levels of systemic inflammatory factors and COPD progression (12, 29, 35, 36). Man et al. showed that in mild to moderate COPD patients, baseline serum levels of CRP were divided into quintiles. After a five-year follow-up, they reported that the highest quintile of CRP was a predictor of mortality compared with the lowest quintile (35). Dahl et al. demonstrated that a baseline serum CRP greater than 3 mg/L was associated with increased risk of hospitalization and death after eight years of follow up in COPD patients (12, 36). In this study, we demonstrated that the CRP mean values did not change significantly in the groups upon controlling the confounding effect of age ($P>0.05$).

We found that between the three groups of participants only serum levels of IL-6 predicted the development of COPD in smokers with a high sensitivity. It seems that a serum level of IL-6 in addition to cigarette smoking is associated with age which should be considered in future studies. Furthermore, the smokers without COPD had increased serum levels of IL-6. This can predict the probability of COPD development in smokers with high serum IL-6 levels which confirms this hypothesis but requires further study.

We demonstrated that an increased serum level of IL-6 was associated with a decrease in FEV1 and FEV1/FVC. We can estimate FEV1, FEV1/FVC from the amount of serum IL-6. Thus, we propose the below mathematical formula for this:

[FEV1=0.777 – (0.001 IL-6), FEV1/FVC=0.793 – (0.001 IL-6)]

In conclusion, our study showed that the serum level of IL-6 predicts the development of COPD in smokers with a high sensitivity among all these inflammatory factors such as CRP, IL-6, and serum albumin.

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