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Il-6 Levels in Induced Sputum and Serum in Acute Attack and Stable Period of Chronic Obstructive Pulmonary Disease

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Abstract: COPD is a disease characterized by chronic inflammation in the airways and increased systemic inflammation. Interleukin-6 is a major immune and inflammatory mediator. To examine the level of IL-6, an inflammatory mediator, in induced sputum and serum of patients with COPD in the acute attack and stable period. Thirty consecutive patients diagnosed with COPD were included in the study. In the attack and stable period, IL-6 levels were studied in the induced sputum and blood taken simultaneously. Twenty-one patients who did not have any pathological findings in terms of chest diseases were selected as the control group. ELISA kit (Bender MedSystems, Vienna, Austria) was used for IL-6 measurements and values were measured as pg/ml. Mann-Whitney U test was used for intergroup comparisons and Wilcoxon Signed Ranks test was used for intragroup comparisons. The serum IL-6 level in the patient group was 6.66±7.49 pg/ml in the acute attack, while it was 3.08±4.07 pg/ml in the stable period. This decrease was found to be statistically significant ($p<0.005$). Sputum IL-6 level did not show a statistically significant change in acute attack (3.54±2.75 pg/ml) and stable period (3.44±6.03 pg/ml). In our study, there was a negative correlation between sputum IL-6 level in acute attack and COPD year (p: 0.02, r: -0.42). A positive correlation was found between acute attack serum IL-6 level (p:0.007,r:0.479) and stable period sputum IL-6 levels (p:0.017, r:0.429) and the number of acute attacks. The high serum IL-6 level in COPD attack shows that the immune response is not only in the respiratory system, but also systemically, and supports that COPD is a systemic inflammatory disease. In our study, we could not detect any contribution of IL-6 level in induced sputum. Studies involving larger numbers of cases and evaluating multiple markers are needed.
Keywords: Chronic Obstructive Pulmonary Disease; Induced Sputum; IL-6.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is an irreversible progressive lung disease and is among the leading causes of death and disability worldwide (1-3). COPD is a disease characterized by chronic inflammation in the airways and increased systemic inflammation. Although the cause of inflammation is not known exactly, it is thought that the toxic effects of smoking and inhalation of other harmful particles and

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gases and autoimmunity play a role in the development of inflammation (4-8). It has been reported that the number of exacerbations is high, especially in patients with moderate to severe COPD (9). The reason why exacerbations are more common in some patients with COPD is not yet known. The diagnosis of acute exacerbation in chronic obstructive pulmonary disease is made when clinically specific symptoms of acute attack occur and lung functions decrease (9,10). Several studies have shown that cytokines (such as IL-1, IL-6, γ-interferon) that control the acute phase response are elevated in serum and sputum during an acute attack of COPD (11,12). There are many studies showing that IL-6 is a major immune and inflammatory mediator (13-15).Induced sputum is a simple and non-invasive method that allows the material obtained from the lower respiratory tract to be examined for various purposes. Since Gibson et al. demonstrated that sputum induction is a reliable and valid method in 1989, it has been used to demonstrate inflammation in asthma and COPD (16). The aim of our study is to examine the level of IL-6, a major immune and inflammatory mediator, in induced sputum and serum of patients with COPD during acute attacks and in the stable period.

2. Material and Methods

2.1. Case selection

Thirty patients were selected consecutively from the outpatient or hospitalized patients who were diagnosed with COPD acute attack and started treatment at Atatürk Chest Diseases and Thoracic Surgery Training and Research Hospital between December 2003 and March 2004.Patients with COPD who recently had at least two of the complaints of increased shortness of breath, change in the amount and color of sputum, exacerbation of cough, and high fever were evaluated as COPD acute attack. The time elapsed sınce being diagnosed with COPD was defined as years of COPD. The PA chest radiographs of the patients included in the study did not have any radiological appearance suggestive of pneumonia and/or another disease. The patients were not given any antibiotic and steroid (inhaled or systemic) treatment until sputum induction was performed. After sputum induction, acute attack treatments were arranged according to the COPD stage of the patients. The patients were called again 6-8 weeks after the acute attack to collect stable period sputum; It was evaluated by physical examination, anamnesis and pulmonary function test. Sputum induction was performed again in patients who were accepted to be in a stable period and their acute attacks had passed. Twenty-one patients who did not have any pathological findings in terms of chest diseases were selected as the control group.

2.2. Sputum Induction

After measuring the initial FEV1 value of the cases, 3% hypertonic saline was inhaled with an ultrasonic nebulizer for 7 minutes. HicoUltrasonat 806 E (Germany) with an output of 0.5 ml/min and a particle size of 5 micrometers was used as ultrasonic nebulizer. Afterwards, the FEV1 value was measured again. The nose was closed, the mouth was rinsed with water, and sterile coarse sputum was extracted. FEV1 value; The process was repeated with an interval of 7 minutes until it fell more than 20%. Sputum obtained in this way was kept in the refrigerator for half an hour or for a maximum of two hours and examined in the microbiology laboratory of our hospital (17). The sputum was separated from the salivary part as macroscopically as possible and 0.1% Dithiothreitol equal to the measured amount was added to it (opens the disulfide bonds that bind the glycoprotein fibers and provide the gel form of the sputum, and mucolysis. Thus, homogenization of the sputum sample for cellular analysis) was added. It was incubated at 37 °C for 20 minutes and vortexed every 5 minutes. Then it was centrifuged for 10 minutes at 2000 rpm (ring perminute). A 3 cc sample was taken from the supernatant for biochemical analysis. After two preparations of the sediment were prepared for evaluation in the pathology laboratory, the remaining sediment was mixed with Dulbecco's phosphate buffersalin solution (D-PBS). Total cells were counted by Neubauerhemocytometry. Viability was evaluated by the trypanblueexclusion (0.4%) method. Blue-stained cells were considered dead, and unstained cells were considered live. If cell viability was less than 50% and squamous (epithelial) contamination was more than 20%, sputum was not included in the examination. If not; The cell suspension is prepared as 1x106/ml, 75 ml of cell suspension is placed in the centrifuge dish and left for 6 minutes. After centrifugation at 450 rpm, two smears were prepared. It was air-dried and stained with Wright's dye. Cell distribution was evaluated (400 cells) (18, 19). Cell counting, viability evaluation and evaluation of cell distribution were performed by a microbiologist. The supernatant portion of the sputum was stored at -70 °C for IL-6 to be studied until measurement

2.3. Study of IL-6 in Serum

Simultaneously with sputum induction, IL-6 in 5 cc blood serum taken from patients and control group was stored at -70 °C until measurement was made. ELISA kit (Bender MedSystems, Vienna, Austria) was used for IL-6 measurements and values were measured as pg/ml.

2.4. Statistical Analysis

SPSS 9.0 for Windows package program was used for statistical analysis. Mann-Whitney U test was used for intergroup comparisons and WilcoxonSignedRanks test was used for intragroup comparisons. Pearson correlation analysis was used for correlations. A P value of <0.005 was considered significant.

3. Results

Twelve (40%) of the 30 COPD patients included in the study were female, 18 (60%) were male, and of the 21

subjects in the control group, 10 (47.6%) were female and 11 (52.4%) were male. Biomass exposure was the only factor that varied between the patient and control groups (<0.005). No statistically significant difference was found between the patient and control groups in other parameters. The general characteristics of the groups are shown in Table 1.The mean duration of COPD of the patients was 8.83 ± 5.62 years. The mean annual number of acute attacks in the patient group was 3.53±1.61. When the patient group was compared within the acute and stable periods, it was found that the sedimentation rate was significantly higher in the acute attack. Apart from that, FEV1 in liter and percentage, total cell number in sputum, neutrophil and macrophage percentage were found to be statistically significantly higher in acute attack. In the patient group, serum IL-6 level was 6.66±7.49 pg/ml in acute attack and 3.08±4.07 pg/ml in stable period. This decrease was found to be statistically significant (p<0.005). Sputum IL-6 level did not show a statistically significant change in acute attack (3.54±2.75 pg/ml) and stable period (3.44±6.03 pg/ml) (Table3).When the patient and control groups are compared; induced sputum IL-6 levels in acute attack and stable period and serum IL-6 level in stable period did not show a significant difference with the control group. The only parameter that was significant for IL-6 level was that it was measured as high in the serum in acute attack.

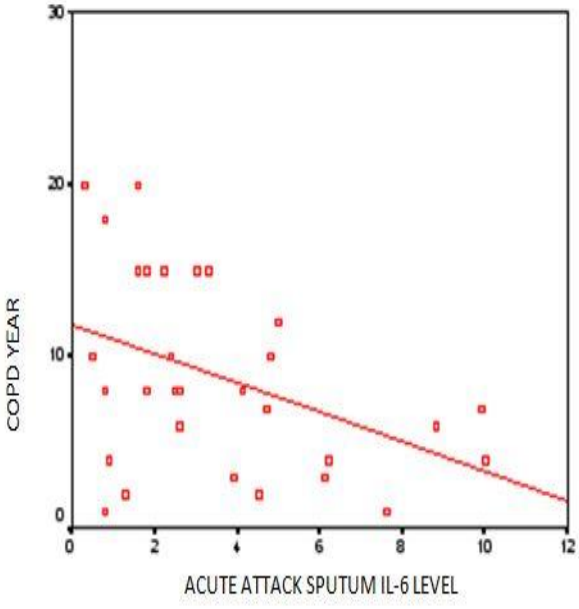

Figure 1: Relationship between acute attack sputum IL-6 level and COPD year.

These values are shown in Table 3.When the patients who had 2 or less attacks per year (10 cases-group1) and those who had 3 or more attacks (20 cases-group2) in the patient group, interestingly, the amount of sputum during the acute attack was found to be statistically significantly higher in group1 compared to group 2. Sputum IL-6 levels were found to be statistically significantly higher in group 2 compared to group 1. These values are shown in Table 4.

In our study, A positive correlation was observed between acute attack serum IL-6 level and number of attacks (p=0.007, r = 0.479). Similarly, a positive correlation was found between stable period sputum IL-6 level and number of attacks (p=0.017, r =0.429). İnterestingly, a negative correlation was observed between acute attack sputum IL-6 level and the year of COPD (p= 0.02, r= -0.42) (Figure 1-2).

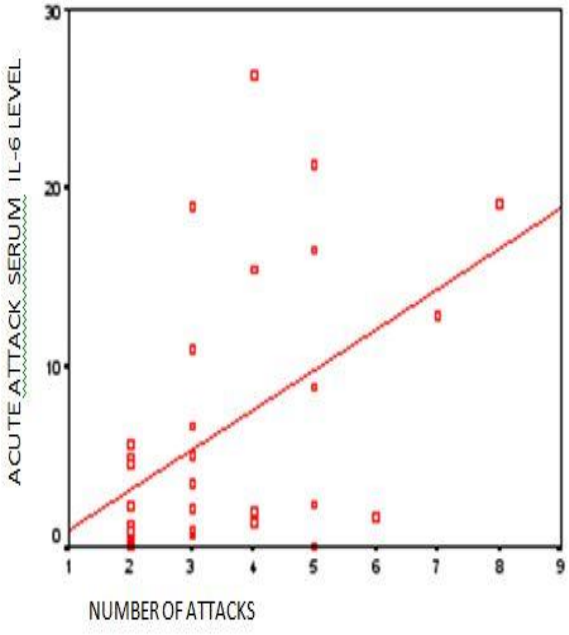

Figure 2: The relationship of serum IL-6 level with the number of attacks in acute attack.

4. Discussion

Induced sputum is a method used to show inflammation in the airways in COPD patients, allowing the study of mediators involved in the pathogenesis of COPD acute attack (20). In our study, induced sputum IL-6 level did not show a significant change in COPD attack and

stable period, while serum IL-6 level was found to be statistically significantly higher in acute attack compared to stable period. The attack serum IL-6 level and the sputum IL-6 level in the stable period were found to be statistically significantly higher.

Table 1: General characteristics of groups.

	Patient (N=30)	Control (N=21)	p
Female/male	12/18	10/11	>0.005
Mean age	61.13 ± 8.15	58.66 ± 10.01	>0.005
Smoker/Non-smoker	18/12	12/9	>0.005
Smoking (pack/year)	19.2 ± 19.92	15.47± 16.57	>0.005

Table 2: Sedimentation rate, FEV1, induced sputum cell count and neutrophil, macrophage percentages in the acute and stable period in the patient group.

	Attack	Stable Period	P
Sedimentation rate (mm/h)	35.4±24.71	16.83±16.16	<0.005
FEV_1 (lt)	1.33±0.28	1.56±0.32	<0.005
% FEV_1	52.9±11.24	61.21±9.33	<0.005
Induced sputum cell count (ml)	$30x10^6$±$35x10^6$	$13x10^6$±$25x10^6$	<0.005
Neutrophil(%)	84.53±16.60	70.47±25.71	<0.005
Macrophage (%)	15.47±16.60	29.53±25.71	<0.005

Table 3: Comparison of serum and induced sputum IL-6 levels of COPD (attack, stable period) cases and control group.

	Attack	Stable Period	Control Group	P (ANOVA)
Serum IL-6 (pg/ml)	6.66±7.49*&	3.08±4.07	1.24±1.41	< 0.005
Induced sputum IL-6 (pg/ml)	3.54±2.75	3.44±6.03	3.61±3.44	>0.005

*p< 0.005 Attack vs Stable, & p< 0.005Attack vs control.

Table 4: Comparison of induced sputum amount and IL-6 (serum/sputum) level according to the number of acute attacks.

	Group1 (n=10)	Group2 (n=20)	p
Sputum amount (cc) (during attack)	6.3±2.21	1.43±0.33	<0.005
Serum IL-6 level (pg/ml) (during attack)	2.2±2.18	7.97±10.14	<0.005
Sputum IL-6 level (pg/ml) (in stable period)	0.87±1.73	3.35±4.12	<0.005

In the study of Pizzichi et al. (21) on the total cell number and distribution in induced sputum in healthy adults, the total cell number in sputum was measured as 3.1x106/ml, and in the cell distribution, 62.9% macrophages, 24.1% neutrophils, 1.3% lymphocytes, 0.5% eosinophils were found. In the study of Peleman et al. (22) including 16 healthy adults and 21 patients with COPD, the total number of cells in induced sputum in patients with COPD was found to be statistically significantly higher than in the healthy adult group. Cell distribution in induced sputum in COPD was 74.9% neutrophils, 20.9% macrophages, 2.4% eosinophils, 0.6% lymphocytes, while in the control group it was measured as 22.5% neutrophils

and 74.0% macrophages. There was no significant change in the percentages of lymphocytes, eosinophils and epithelial cells in either group. As a result, they reported that there was a high number of neutrophils in the sputum of COPD patients, and macrophage dominance in the sputum in healthy adults. In the study of Bhowmik et al. (11) on the relationship between inflammatory markers in sputum in sputum in COPD acute attack and changes in lung functions, the total number of cells in the sputum of patients with COPD in acute attack was 2.86x106 cells/ml, while the cell distribution was 84% neutrophils, 14% macrophages, and 1% eosinophil was determined as 2.8% lymphocyte. In the stable periods of the same patients, the cell number decreased to 1.99x106 cells/ml, and the cell distributions were 81% neutrophils, 13% macrophages, 1% eosinophils, and 2% lymphocytes. According to this result, they reported that the total number of cells in the sputum was high in acute attack and that neutrophils were dominant in the sputum. In our study, when acute attack and stable periods were compared in COPD cases, the number of cells in induced sputum was 30×106±35×106 in the attack period, while it was 13×106±25×106 in the stable period, and the difference was statistically significant ($p<0.005$). When the acute attack and the stable period were compared, there was a statistically significant increase in the percentage of neutrophils in the attack (84.53±16.60% in the attack, 70.47±25.71% in the stable period) and a statistically significant decrease in the percentage of macrophages (15.47±16.60% in the attack, 29.53%±25.71% in the stable period) detected (Table 2).

In addition to cytokines such as TNF-α, IL-8 and LTB4 responsible for the pathogenesis of COPD, IL-6 is also an inflammatory mediator. Interleukin-6 (IL-6); It is a glycoprotein cytokine consisting of 184 amino acids located on the 7th chromosome. IL-6; It affects B and T cells monocytes, hematopoietic system stem cells and hepatocytes. As a result, induction, differentiation and immunoglobulin production in B cells and T lymphocytes occurs, macrophages are activated, hematopoiesis and thrombopoiesis are induced, regulation of the acute phase response is achieved. Increased airway obstruction during acute exacerbations of the disease is held responsible for submucosal edema and mucus production (13-15, 23). In the study of Bhowmik et al. (11) in which they investigated the relationship between IL-6 and IL-8 levels in induced sputum in the acute and stable period and the frequency of attacks in moderate and severe COPD cases; IL-6 level in induced sputum was found to be significantly higher in acute attack (122.7pg/ml) compared to stable period (64.0pg/ml; $p<0.05$). In the same study, induced sputum IL-6 level was 22pg/ml in those who had 2 attacks per year, while this level was 101pg/ml in those who had 3 or more attacks per year.

In our study, however, no significant difference was found in the induced sputum IL-6 level in patients with COPD during acute attack and stable periods. When the cases were re-evaluated according to the annual number of attacks, no statistically significant difference was found in sputum IL-6 levels between the groups. However, the amount of sputum and serum IL-6 level during the attack were found to be statistically significantly higher in patients who had 3 or more attacks compared to the group that had 2 attacks per year. In our study, we may attribute the lack of difference in sputum IL-6 levels according to the number of attacks, due to the low number of cases, and the lack of equal distribution of patients between the groups. Another hypothesis is that the high annual number of acute attacks of the patients in our study may explain the high level of induced sputum IL-6 in the stable period as well as in the acute attack. This shows us that IL-6 level is increased in the airways of those who have frequent acute attacks and that this increase in cytokine level also plays a role in the continuation of inflammation. Wang et al. (24) found no statistical difference in induced sputum IL-6 levels of all three groups in their study on smokers and nonsmokers with healthy adults and patients with stable COPD. In our study, no statistical difference was found when the induced sputum IL-6 levels of the control group were compared with the induced sputum IL-6 levels of the patients with COPD in the acute attack and stable period (Table 3).

In the study of Wedzicha et al. (25), a total of 120 attacks were detected in 93 patients with COPD who were followed up for 1 year. While the acute attack serum IL-6 levels of the cases were 4.3pg/ml, it was measured as 1.10pg/ml in the stable period ($p=0.008$). In the study, it was found that the increased serum IL-6 level in acute attack also caused an increase in fibrinogen; It has been shown that this increase in fibrinogen level increases the tendency to cardiovascular diseases and cerebrovascular events. Similarly, in our study, while serum IL-6 level was 6.69±7.49 pg/ml in acute attack, it showed a statistically significant decrease in stable period and was measured as 3.08±4.07 pg/ml. We can attribute this result to the fact that IL-6 is a major immunomodulator that rapidly rises in response to infection and initiates the acute phase response. Acute attack serum IL-6 level was also found to be significantly higher when compared to the control group (Table 3). The cause of acute exacerbation in chronic obstructive pulmonary disease is usually intercurrent infections. In our study, we found a statistically significant positive correlation (r= r:0.479) between the number of acute attacks and the serum IL-6 level of attacks in patients with COPD. In the UPLIFT study, it was shown that the clinical course of the disease worsened in patients who had frequent attacks. According to this result, the clinical

course of the patients is adversely affected because the inflammation is continuous in those who have frequent attacks (26).

5. Conclusions

The high serum IL-6 level in COPD attack shows that the immune response is not only in the respiratory system, but also systemically, and supports that COPD is a systemic inflammatory disease. In our study, we could not detect any contribution of IL-6 level in induced sputum. Studies involving larger numbers of cases and evaluating multiple markers are needed.

Conflict of Interests

No potential conflicts of interest relevant to this article were reported.

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Author Contributions

Hypothesis for research; FU, Planning the design, patient follow up: MÖA, Analysisof data, literature revewand final approval of the version; FU and MÖA

Ethical Approval

Informed consent form was obtained from the participants.

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