Bronchoalveolar Lavage Findings in Patients With Mild to Moderate COPD Who do not Currently Smoke

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Abstract

In this study, we aimed to examine the effect of smoking cessation on bronchoalveolar lavage (BAL) findings in COPD patients who were heavy or less heavy smokers. Twenty five ambulatory patients with stable mild to moderate COPD according to the GOLD classification were included the study. Sixteen patients had a history of more than 40 pack-years cigarette (Group 1) and 9 had a history of less than 40 pack-years cigarette (Group 2) were compared for the relationship of smoking history and spirometric tests with BAL cell count and interleukin-8 (IL-8) levels.

Mean forced expiratory volume in one second (FEV_1) value in Group 1 was significantly lower as compared to Group 2 (1412±106.8 ml and 1488±239.4 ml respectively) (p=0.009). In the total group of patients, a positive correlation was found between intensity of smoking history and increased neutrophils. (p=0.002, r=0.583) and a negative correlation between neutrophils and FEV_1 (p=0.003, r=- 0.435). No association was observed between intensity of cigarette smoking history and IL-8, FEV_1.

Patients with COPD who had a history of heavy smoking had higher neutrophil counts in the airways as compared to less heavy smokers and increased neutrophil counts were associated with altered lung functions. Despite quitting smoking, neutrophilic inflammation was found to continue in patients with COPD

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Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by a chronic inflammatory process in the large and small airways, as well as in the lung parenchyma. COPD is the only leading cause of death that is increasing in prevalence and it is expected to be the fourth leading cause of death worldwide in 2020 (1). The pathogenesis of COPD is multifactorial, involving airway inflammation, protease-antiprotease imbalance, oxidative stress and recurrent infection (2). As a result of these factors, a persistent airflow obstruction with reduction in the maximum expiratory flow develops in COPD.

Techniques allowing precise cell phenotyping and determination of cytokine production have allowed for a much better description of the inflammatory profiles in the lungs of smokers, ex-smokers and patients with COPD. There are different techniques to investigate airway inflammation like spontaneous or induced sputum examination, bronchial biopsy and bronchial or bronchoal-

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veolar lavage (BAL). While induced sputum seems to be a combination of resident mucus and mostly samples the large airways, BAL samples mainly the alveolar compartment (3).
In COPD patients, the inflammatory process which contributes to narrowing in the small airways, leads to an increased number of CD8 lymphocytes, neutrophils, increased connective tissue deposition and epithelial metaplasia (4). Interleukin-8 (IL-8) that is produced by epithelial cells, macrophages and neutrophils is a cytokine with potent neutrophil chemotactic and activation properties (5). In normal persons who do not smoke, BAL cellularity consists predominantly of macrophages and neutrophils are usually under 4 percent. Sybill et al showed that, in cigarette smokers, there is an increase in polymorphonuclear neutrophils in peripheral blood as well as in BAL fluid in comparison with nonsmokers (6). The effect of smoking cessation to the BAL cellularity is controversial. Kulawik reported no significant differences in cell count and macrophage phenotypes between active smokers and ex-smokers in induced sputum in patients with COPD (7). In contrast, Rutgers showed increased numbers of inflammatory cells in the airways of patients with COPD who were ex-smokers, suggesting that inflammation may persist in the airway once established (8).
Also in smokers, the relationship between the severity of airflow limitation and the severity of airway inflammation is well documented (9-11), while little is known about the relationship between the severity of airflow limitation and the ongoing inflammation in ex- smokers.
Survival in COPD directly correlates with the level of forced expiratory volume in one second (FEV_1) (12). As neutrophils and IL-8 may lead to progressive lung damage, the aim of this study was firstly to assess the type of inflammatory process in the BAL of patients with COPD who do not currently smoke and secondly to find out if there is any correlation between smoking history and inflammatory indices in BAL with lung functions, especially with FEV_1.

Materials and Methods

A total of 25 ambulatory patients with a medical history and clinical and radiological findings consistent with stable mild to moderate COPD, according to GOLD classification (13), were included in the study. Eight subjects whose postbronchodilator FEV_1 value was greater or equal than 80% of the predicted, were considered as mild COPD and 17 subjects whose FEV_1 value was in the range between 50-80% of the predicted were considered as moderate (2a) COPD. The patients had all ceased smoking at least one year prior to the enrollment. Cumulative cigarette consumption was measured in pack-years. All subjects had a smoking history of at least 20 pack-years and 16 had a smoking history of more than 40 pack-years.
Inclusion criteria were: (1) FEV_1 value in the range of more than 50% of the predicted value, (2) reversibility with inhaled-beta 2 agonists of less than 15% of predicted FEV_1, (3) stable COPD defined as no acute exacerbation within the preceding three months, (4) no history of systemic disease or other pulmonary disease, (5) no therapy with inhaled or systemic corticosteroids within 3 months prior to entry into the study, (6) no history of asthma or atopy.
All patients were on therapy with inhaled salbutamol and ipratropium bromide. In 9 patients, sustained-released theophyline was also being given.
Informed consent was obtained from all patients. The study was approved by the Ethics Committee of Ege University.
Spirometric tests and BAL analysis with bronchoscopy were ascertained on entry into the study. Pulmonary function tests were performed by the standard method using a dry rolling-seal spirometer. Three technically adequate maneuvers were required and the best values for FVC and FEV_1 was accepted.
Transnasal fiberoptic bronchoscopy was performed using an Olympus flexible fiberoptic bronchoscope, following the guidelines of the National Institutes of Health. Premedication included atropine (0.5 mg-IM) administered 30 minutes before the procedure, with local upper airways anaesthesia with 5 ml of 2% lidocaine. To perform lavage, the bronchoscope was wedged into the segmental bronchus of the middle lobe and 20 ml x 5, a total 100 ml of sterile warmed saline solution was infused. Fluid was gently aspirated immediately after the infusion had been completed and was collected in a sterile container. The fluid was immediately centrifuged at 500xg for 10 minutes. Supernatants were removed and frozen in 1 ml sterile polystyrene tubes at –80°C.
IL-8 concentrations were determined by two-site sandwich IL-8 specific enzyme-linked immunosorbent assay (ELISA-Chemikline). The concentration of IL-8 in the samples was calculated by comparison to the curve obtained with different concentrations of standards included in each kit. Tests were done twice for validation.
For BAL cell counts, the cell pellet was washed with phosphate-buffered saline solution. Cells were resuspended in Hank's balanced salt solution and counted using a haemochromocytometer chamber. Cytocentrifuges were stained by the May-Grünwald Giemsa method. The differential cell counts of macrophages, lymphocytes, neutrophils and eosinophils were made under light microscopy at x400 magnifications, counting approximately 300 cells. All cell data are expressed as percentages.

Statistical analysis

Parametric data are expressed as the mean ±SEM. Parametric data were compared using Student's t test. Nonparametric data comparisons were made using Mann-Whitney U test between the two groups. The relationship between the proportion of cells and smoking history, expressed as the number of pack years and the results of pulmonary function tests were tested by the Spearman's correlation test. In each case, a p value of <0.05 was considered significant.

Results

Sixteen patients with a history of more than 40 pack-years cigarette smoking (Group 1) and 9 patients with a history of

Table 1. Clinical and demographic findings			
	Ex-smokers ≥40 pack-years	*Ex- smokers <40 pack-years*	*Total n*
Number (male/female)	16 (13/3)	9 (7/2)	25 (20/5)
Age (years)	66.1±2.3	63.7±1.4	64.8±1.2
Smoking history			
Pack-years	55.3±3.4	24.7±2.02*	43.6±3.7
Mean time period after	3.6±0.8	2.4±1.2	2.8±0.6
Smoking cessation (years)			
Spirometry			
FVC (ml)	2337.5±137.5	2177.7±283	2280±132.2
FEV_1 (ml)	1412±106.8	1488.8±239.4*	1440±107.2
FEV_1/FVC	%61.4±3.3	%67.9±2.1	%64.1±1.9
Values are expressed as means ±SEM; * = p <0.05.			

less than 40 pack-years cigarette (Group 2), with stable COPD were enrolled for the study. Bronchoscopy was performed without any complications. Clinical and demographic findings of the patients are shown in Table 1. Both patient groups were similar with regard to age, sex and mean time period after smoking cessation. Mean FEV_1 value and percentage of predicted FEV_1 in Group 1 were 1412±106.8 ml (% 60.1±2.2). In Group 2 these values were 1488.8±239.4 ml (% 66.3±1.9) respectively (p=0.009).

Analyses of cells recovered in BAL confirmed that bronchial inflammation in COPD patients who do not currently smoke is associated with an increase in polymorphonuclear leukocytes. Since the percentages of eosinophils were very low, these values are not reported. Although there was a tendency of higher mean neutrophil count in Group 1, this was not statistically significant (p=0.52). Other BAL cell findings and IL-8 levels were similar in both groups and there was no significant statistical difference (Table 2, Figure 1).

Considering all 25 patients, we found a negative correlation between increased neutrophils and low FEV_1 value (Figure 2) and also a positive correlation between increased neutrophils and smoking history (r=0.583, p=0.002) (Figure 3). No association was found between IL-8 and smoking history (r=-0.143, p=0.496).

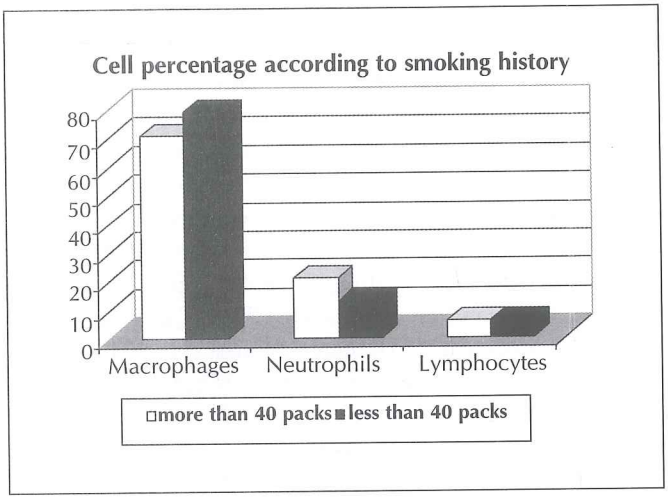

Figure 1. *Cell percentage according to smoking history.*

Discussion

COPD patients who smoked more than 40 pack years had lower FEV_1 values and a tendency to have higher neutrophils in their BAL than the patients who smoked less than 40 pack-years. Despite smoking cessation, neutrophils persisted in the airways and increased neutrophils were associated with altered lung functions. We found a weak correlation

Table 2. Bronchoalveolar lavage findings				
	Ex-smokers ≥40 pack-years	Ex- smokers <40 pack-years	Total	p
Macrophages (%)	71.8±2.3	79.3±2.8	74.6±1.9	0.850
Neutrophils (%)	21.6±2.1	13.6±2.4	18.8±1.8	0.522
Lymphocytes (%)	6.5±1.0	6.3±1.3	6.4±0.9	0.916
IL8 (pg/ml)	0.77±0.94	0.74±0.75	0.8±0.2	0.554
Values are expressed as means ±SEM.				

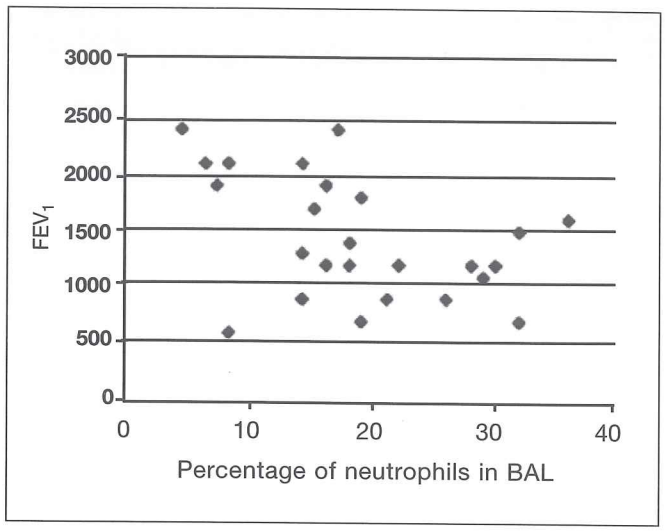

Figure 2. *Relationship between neutrophil percentage in BAL and lung functions.*

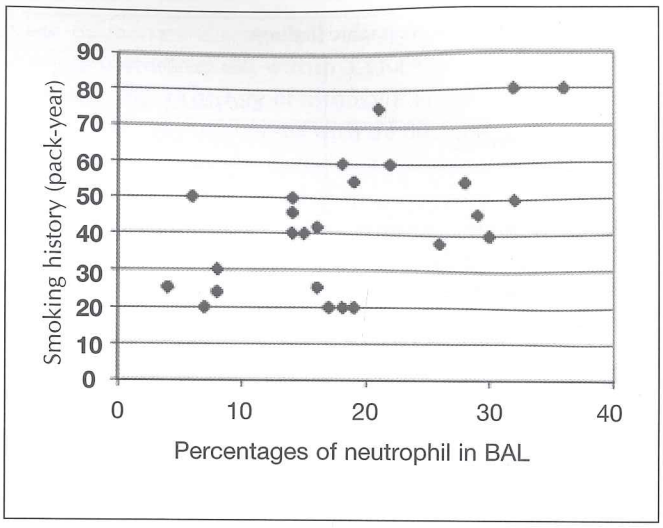

Figure 3. *Relationship between neutrophil percentage and smoking intensity.*

between a heavy smoking history and low FEV_1 and no association was observed between IL-8 and. FEV_1

Cigarette smoking represents a major risk factor for COPD. The components of cigarette smoke such as nicotine lead to retention of the neutrophils within the pulmonary microvasculature (14). The first inflammatory cell responding to smoking is most likely the neutrophils. The pathological findings of the airways in COPD patients revealed increased numbers of macrophages and predominantly CD8+ T cells in bronchial biopsy specimens (15), increased mast cells in bronchial glands (16), predominance of neutrophils in BAL and in induced sputum (17, 18). In our study we focused on neutrophil percentage and IL-8 concentration as a measure of distal airway inflammation and we examined the inflammatory process in BAL which strengths our study as BAL gave a better assessment of small airways and alveolar inflammation. One of the limitations of our study is that we were not able to ascertain total cell counts and T lymphocytes subgroups in the lavage specimens.

Turato et al examined the number of inflammatory cells, the markers of mononuclear cell activation and the expression of endothelial adhesion molecules and cytokines in the subepithelium by bronchial biopsies and found that there were no differences between current smokers and ex-smokers for any parameter examined (19). Wright et al found increased numbers of total inflammatory cells and neutrophils in the walls of the membranous bronchioles in current and ex-smokers when compared to nonsmokers. Current and ex-smokers had similar numbers and types of inflammatory cells in the airways, indicating that this inflammatory response does not abate after smoking cessation (20). COPD patients who were ex-smokers were shown to have increased numbers of inflammatory cells and especially eosinophils in the airways, suggesting the ongoing inflammation once established (8). We found that despite quitting smoking, neutrophilic inflammation continues in patients with COPD.

The higher number of neutrophils in the airways could increase the antimicrobial defense but could also impair bronchial cell function. Neutrophils can cause tissue damage through the release of proteases and formation of oxygen radicals. Thompson et al found that in COPD patients with higher bronchial sample neutrophils had significantly more sputum production and lower FEV_1, FEV_1/FVC and FEF_{25-75} than did the subjects with lower bronchial sample neutrophils (3). The superoxide anion release and elastase activity from the neutrophils appears to be correlated with the annual decrease in FEV_1 over the course of several years (21). Correlated with the degree of airflow limitation, there is a causal relationship between the high smoking history and the numbers of neutrophils and lung function decline.

Interleukin-8, one of the mediators that have a role in the pathogenesis of COPD, along with leucotrien-B4 (LTB4) and tumor necrosing factor-alfa (TNF-α), is a cytokine with potent neutrophil chemotactic and activation properties. Elastase produced by resident neutrophils in airways induces the bronchial epithelium to secrete IL-8, which in turn recruits additional neurophils, which self perpetuates the inflammatory process. Oxidant stress was also found to stimulate IL-8 production in a variety of cell lines (22). There are other factors affecting neutrophil accumulation in the airways, like oxidant stress, nitric oxide, TNF-α, IL-1α and IL-1β. Also, the medication which is taken by the patient such as inhaled steroids, may also decrease the percentage of neutrophils in sputum (23) and in BAL fluid (24).

Compared to current smokers, the ex-smokers had a lower concentration of the chemoattractant IL-8 concentration (25,26). Another limitation of our study was that we did not have a control group of patients with COPD who currently smoke. By smoking cessation, IL-8 level may have been decreased and that may explain why we could not find a relationship between IL-8 and investigated parameters.

Longitudinal studies investigating clinical parameters with macrophage activity, neutrophils and CD8+ lymphocytes in the airways before and after smoking cessation is needed for more information about the ongoing inflammation in COPD and this information may contribute to our further understanding of the pathophysiology of COPD.

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