# Relation of Spirometry and Cytomorphological Changes Secondary to Cigarette Smoking

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## Abstract

**Relation of spirometry and cytomorphological changes secondary to cigarette smoking**

**Objectives:** The purpose of this study was to evaluate the relation between the changes of lung function parameters and sputum cytology among smokers and non-smokers with or without chronic obstructive pulmonary (COPD) and non-smoker healthy controls.

**Patients:** Sixty-nine male cases. Thirty-two smokers group (n=22 COPD, mean age ± SD, 59 ± 9 year-old, n=10 without COPD, mean age SD, 41 ± 5 year-old), twenty-nine ex smokers group (n=23 with COPD, mean age SD ± 61 ± 11 year-old, n=6 without COPD, mean age SD ± 41 ± 5 year-old), 8non-smokers healthy group (mean age SD ± 40 ± 7).

**Intervention:** Sputum processing and spirometry.
**Measurement and Results:** Spirometric measurements FEV1 and FEV1/FVC ratio were noted. Sputum processing and differential cell counts were performed by a blinded observer. We found that there was a negative correlation between FEV1/FVC ratio and increasing age (r= - 0.72, p< 0.001) so as the duration of the cigarette smoking (r= -0.66, p<0.001). In addition there was a negative correlation between FEV1/FVC ratio and pack-year cigarette smoking (r= - 0.66). In histological assessment, neutrophil counts were significantly increased in smokers and ex-smokers groups compared with the nonsmokers group (p<0.005). All groups revealed that there were significant differences in the number of macrophages, pigmented macrophages, neutrophils, mucous, mucous spirals, columnar epitheial cells (p< 0.001).

**Conclusion:** Cigarette smoking causes cellular changes after deterioration of lung function test as in natural causes of COPD. This clearly shows that smoking or its cessation is a powerful factor in determining the subjects' outcome

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**Key words:** *Sputum analysis, Spirometry, Smokers, COPD*
**Abbrevations:** *COPD = Chronic obstructive pulmonary disease*

## Introduction

Cigarette smoking is major preventable cause of morbidity and mortality in developed countries (1). In this regard, cigarette smoking is a major etiological factor in a large number of diseases, including atherosclerotic vascular disease, chronic obstructive pulmonary disease (COPD) and many types of cancer, including lung cancer (1-4).

COPD is characterized by progressive airflow limitation, and to date, cessation of smoking is the only intervention that has been shown to slow disease progression (3-5). The pathogenesis of COPD is poorly understood. Currently accepted concepts include a progressive inflammatory response possibly associated with unrestrained protoelytic enzyme release and toxic oxygen

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radical production induced by cigarette smoke and other inhaled pollutants (5).
Cigarette smoking causes cytomorphological changes in sputum before causing any change in lung function test (6). Inflammation, hypersecretion and desquamation with hyperplasia in basal cells, metaplasia and increasing number of atypical cells are observed in the sputum of chronic smokers (7). All these changes lead to an increased number of brown pigmented macrophages and neutrophils that are characteristic changes in the beginning of COPD-termed as respiratory bronchiolitis (8).

This article reports further analysis of the effect of smoking on the lung function and sputum cytology subjects with smokers and ex smokers including those with COPD and without COPD.

## Methods

Sixty-nine male subjects were recruited from outpatient clinics. All cases were divided into three groups, according to cigarette smoking. Thirty-two smokers group (n= 22 with COPD, mean age SD,59 9 year-old ; n= 10 without COPD, mean age SD, 44 9 year-old), 22 ex smokers group (n= 23 with COPD, mean age SD,61 11 year-old; n=6 without COPD, mean age SD, 41 5 year-old), 8 non smokers group (mean age SD, 40 7year-old) were enrolled. Diagnosis of COPD was based on the following criteria: FEV1/FVC ratio < 0.7, post bronchodilator FEV1 < 85 % predicted value, reversibility with inhaled 2 agonist of < 15 % of predicted FEV1: all criterias were required. Subjects had no history of asthma and atopy or variability of symptoms. All subjects gave written informed consent, and this study was approved by the ethics committee of our center.

**Sputum processing:** Morning sputum of all subjects was taken spontaneously on three consecutive days and sent to the pathology laboratory. Fresh samples were kept at 4 C for no more than 2 h before further processing. The whole sputum sample was diluted with Hanks' balanced salt solution (HBSS) containing dithiothreitol (DTT) (Sigma Chemicals, Poole, UK), with a final DTT concentration of 0.2 %, and was vortexed at room temperature. When homogeneous, the volume was recorded and the sample was further diluted with HBSS and centrifuged at 300x g for 10 min. The supernatant separated. Slides were prepared and stained with papanicolau method. Differential cell counts were performed by a blinded observer with 300 non-squamous cells counted on each of two slides for each sample. These slides were examined in light microscopy to count cellular and acellular sputum components. The cellular component of sputum were alveolar macrophages, pigmented macrophages, neutrophils, columnar cells, metaplastic and displastic cells. Acellular and cellular components were counted and scored from zero to ten according to number of count, meaning that the least counted cells scored as zero and the most counted cells scored as ten. Sputum slides that had at least 300 macrophages in smokers and 150 macrophages in non smokers were enrolled only in the study.

**Statistical analysis:** The statistical package SPSS for windows 5.0 was used for all statistical analysis. The differences between the two groups and controls were analyzed using Mann-Whitney-U test and to assess correlation with variables, correlation analysis was used. The Kruskal-Wallis test was used in more than two groups and if $p < 0.05$ then multiple compression analysis was performed.

## Results

**Spirometry:** FEV1/ FVC rations in COPD (n =22) and smokers without COPD (n= 10) were 50 1, 80 4 respectively ($p < 0.001$). In the ex-smokers' group

**Table 1: Subjects' characteristics**

| | | Case number | Age | Pack-year | Duration ex smoke | Duration COPD | FEV1/ FVC |
|---|---|---|---|---|---|---|---|
| Smokers | COPD (+) | 22 | 59 9 | 51 4 | _ | 6 3 | 50 12 |
| | COPD (-) | 10 | 44 9 | 19 7 | _ | _ | 80 4 |
| Ex smokers | COPD (+) | 23 | 61 11 | 46 4 | 10 9 | 7 6 | 52 1 |
| | COPD (-) | 6 | 41 5 | 13 6 | 7 5 | _ | 82 3 |
| Non smokers | | 8 | 40 7 | _ | _ | _ | 85 7 |

**Table 2: Comparison of FEV1/FVC and cytological changes in subjects according to smoking status**

| Characteristics | Smokers ( n= 32) | Ex smokers ( n= 29) |
|---|---|---|
| Age | 11 | 57 13 |
| Pack-year | 41 3 | 39 3 |
| FEV1/FVC | 59 17 | 58 1 |
| Macrophages | 5.8 1.7 | 4.5 1.9** |
| Pig. Macrophages | 5.4 1.7 | 3.6 2*** |
| Neutrophils | 6.5 2.2 | 5.5 2.4 |
| Mucous spirals | 3.8 2.5 | 3.8 2.5 |
| Columnar cells | 2.2 1.1 | 1.9 1.3* |
| Mucous | 5.7 2.1 | 4.9 2.4 |
| Metaplasia | 0.8 1.4 | 0.4 0.8 |
| Displasia | 0.7 1.4 | 0.2 0.4 |

*p <0.05, **p <0.01, ***p <0.001

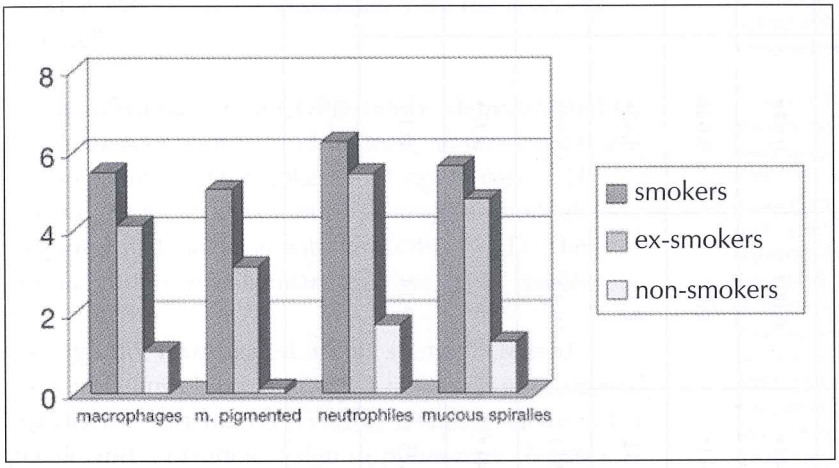


***Figure 1a.*** *Cytological scores of subjects (macrophages, macrophages pigmented, neutrophils, mucous spirals) (p<0.001)*

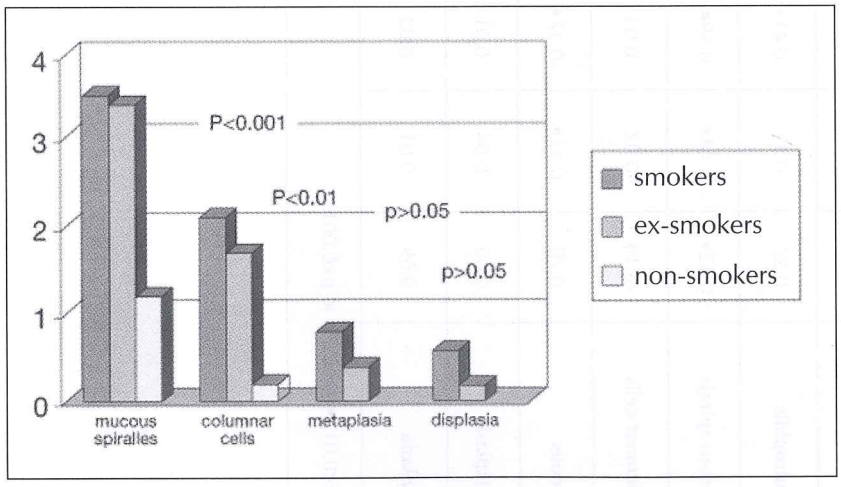


***Figure 1b.*** *Cytological scores of subjects (mucous spirals, columnar cells, metaplasia, displasia).*

FEV1 /FVC ratios in subjects with COPD and without COPD were 52 2, 82 3 respectively (p <0.001). In the non-smoker healthy control group FEV1 / FVC ratio was 85 7 (Table 1.).

**Cells:** Thirty-two smokers and 29 ex-smokers were compared with cytological parameters. Macrophages (p <0.01), pigmented macrophages (p < 0.001), columnar cells (p< 0.05) were significantly increased in smokers compared with ex-smokers group (Table 2).

Among the three groups, smokers had a significantly increased number of macrophages, pigmented macrophages, neutrophils, mucous spirals (Figure 1A), but there was no difference with respect to metaplasia and displasia among the groups (Figure 1B). In the non-smokers' group, mucous spirals and columnar cells were significantly lower than in the other two groups (p<0.001, p<0.01) (Figure 1B).

There was a positive correlation between FEV1 / FVC ratio and age (r= -0.72 ; p <0.001) and also duration of smoking (r= - 0.45; p< 0.01). Positive correlation was observed between duration of cigarette smoking and neutrophils (r=0.43), macrophages (r=0.45), mucous spirals (r= 0.44) and mucous components (r=0. 42) (table 3)

In ex-smokers (n=29), there was negative correlation between FEV1/ FVC ratio and duration of smoking and numbers of pigmented macrophages (r=0.57 ; p <0.001), neutrophils (r=0.38; p< 0.05), columnar cells (r=0.37; p<0.05).

## Discussion

This study revealed that cessation of cigarette smoking could reduce the number of neutrophils, macrophages, pigmented macrophages in the bronchial airway. Pack-year history could be a prognostic factor for estimating reduction of the FEV1 /FVC ratio.

Cigarette smoking alters cellular structures of the bronchial airway and lung function test (2, 3, 4). A number of published studies have shown decreasing cellular changes with cessation of cigarette smoking (3,5,6,9). We found normal lung function test and less

**Table 3. Bivariate relation of age, smoking status, cytomorphological changes and lung functions in 32 smokers.**

| | Age | Pack/ year | FEV1/ FVC | Macro- phage | M. pigmented | Neutrophils | Mucous spirals | Columnar cells | Mucous | Metaplasia | Displasia |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Age** | | | | | | | | | | | |
| **Pack/year** | 0.45* | | | | | | | | | | |
| **FEV1/FVC** | -072# | -0.66# | | | | | | | | | |
| **Macrophage** | 0.18 | 0.45* | -0.29 | | | | | | | | |
| **M. pigmented** | -0.11 | 0.22 | -0.15 | 0.66# | | | | | | | |
| **Neutrophils** | 0.27 | 0.43 | -0.41• | 0.43• | 0.80# | 0.61# | | | | | |
| **Mucous spirals** | 0.42• | 0.44• | 0.59# | 0.43• | 0.07 | 0.62# | | | | | |
| **Columnar cells** | -0.19 | 0.02 | 0.03 | 0.32 | 0.17 | 0.42• | 0.41• | | | | |
| **Mucous** | 0.20 | 0.42• | -0.37• | 0.55# | 0.33 | 0.70# | 0.77# | 0.47* | | | |
| **Metaplasia** | 0.06 | 0.04 | 0.01 | 0.41• | 0.16 | 0.37• | 0.20 | 0.39• | 0.24 | | |
| **Displasia** | 0.09 | 0.01 | 0.02 | 0.25 | -0.14 | 0.19 | 0.22 | 0.25 | 0.02 | 0.70# | |

*: p<0.01, •: p<0.05, #: p<0.001

pigmented macrophages in subjects which quit smoking earlier, but the figures were too low to identify a difference in response among others.

The increased levels of sputum neutrophils in smokers are associated with airway obstruction, chronic expectoration and rapid decline of FEV1 (9). Present study has reported that the ratios of FEV1 / FVC with the smokers and ex-smokers' groups were negatively correlated with sputum neutrophils and macrophage numbers just as other studies have shown (3,9).

Sputum is an effective tool for assessing airway inflammation (10). Sputum analysis can also distinguish between asthmatic and healthy subjects and subjects with COPD (5,6 11).

The present study showed increased levels of neutrophils and pigmented macrophages in the smokers' group compared to the non-smokers' group. And also the study revealed that the duration of smoking was positively correlated with the levels of these cells.

Airway inflammation in COPD can be demonstrated by examination of sputum and it clearly defers from that seen in asthma (11). Smokers and ex-smokers with COPD have increased sputum neutrophil numbers compared with subjects without COPD (6,11). The present study shows similar results to other studies.

Although not investigated in this study, increased levels of neutrophils can also be observed in interstitial lung diseases. Increased levels of neutrophils are not a specific and a diagnostic sign in pulmonary diseases but reveal the neutrophilic elastase and disease activity (12).

Smokers with airflow obstruction benefit from quitting despite previous heavy smoking, advanced age, poor baseline lung function.

## References


1. Giovino GA, Henningfield JE, Tomar SL, et al. Epidemiology of tobacco use and dependence. Epidemiol Rev 1995; 17: 45-48
2. Rennard SI, Daughton DM. Cigarette smoking and disease. In: Elias JA, Fisman JA, Grippi E, et al. Pulmonary diseases and disorders. 3dr Edn New York, Mc Graw Hill, 1997; pp: 697-708
3. Scanlon PD, Connett JE, Waller LA et al. Smoking cessation and lung function in mild to moderate chronic obstructive pulmonary disease: The Lung Health Study Am J Respir Crit Care Med 2000
4. Rennard SI, Daughton DM. Smoking cessation. Eur Respir Rev 1999; 9:67, 173-179
5. Culpitt SV, Moziak W, Loukidis S, et al. Effects of high dose inhaled steroid on cells, cytokines and proteases in induced sputum in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1999; 160: 1635-39
6. Ronchi MC, Piragino C, Rossi E, et al. Role of sputum differential cell count in detecting airway inflammation in patients with chronic bronchial asthma or COPD. Thorax 1996; 51: 1000-4
7. Jeffery PK. Structural and inflammatory changes in COPD: a comparison with asthma. Thorax 1998; 53: 129-136
8. Yildirim N. KOAH'ta proteaz -antiproteazlarin rolü. Kronik Obstriktif Akciger Hastaligi Seminer Notlari. Küre Basim Istanbul 1997
9. Stanescu D, Sanna A, Veritier C, et al. Airway obstruction, chronic expectoration and rapid decline of FEV1 /FVC in smokers are associated with increased levels of sputum neutrophils. Thorax 1996; 51: 267-71
10. Pizzichinni E, Pizzichinni MM, Efthimiadis A, et al. Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid-phase measurements. Am J Respir Crit Care Med 1996; 154: 308-17
11. Keatings V, Barnes PJ. Granulocyte activation markers in induced sputum: comparison between chronic obstructive pulmonary disease, asthma and normal subjects. Am J Respir Crit Care Med 1997; 155:449-53
12. Idiopathic pulmonary fibrosis: Diagnosis and treatment. International consensus statement. Am J Respir Crit Care Med 2000; 161: 646-64.