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ORIGINAL ARTICLE
EFFECT OF SMOKING CESSATION ON AIRWAY INFLAMMATION IN COPD

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ABSTRACT

Smoking cessation is the only treatment in patients with chronic obstructive pulmonary disease (COPD) effective in slowing down disease progression. Its effect on airway inflammation in COPD is unknown, although cross-sectional studies suggest ongoing inflammation in ex-smoker.

The aim of this study is to evaluate the role of shisha cessation on airway inflammation of different COPD stages and asymptomatic smokers.
Forty eight patients were included, 19 COPD current smokers, 19 COPD ex-smokers and 10 asymptomatic smokers.

Sputum and total leucocytic count was significantly increased in COPD smoker group. Neutrophils percentage was increased in COPD ex-smoker group compared to COPD smoker , where asymptomatic-smokers showed the least percentage. The most elevation of macrophages was recorded for asymptomatic-smokers groups.

Key words: *smoking cessation, COPD, airway inflammation.*

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD), a common preventable and treatable disease, is characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lungs to noxious particles or gases. Exacerbations and co morbidities contribute to the overall severity in individual patients. [(1)]

Smoking is recognized as the cause of COPD in vast majority of patients although it remains a central question in respiratory-medicine why a minority (15-20%) of smokers develops clinical-COPD [(1)]. Smoking induced airway inflammation thought to play an important pathogenic role in COPD.

Until now, smoking cessation is the only treatment effective in slowing down the accelerated decline in forced expiratory volume in one second (FEV1) and thus progression of the disease. However, the exact role of smoking cessation on airway inflammation in COPD is still unknown. [(2)]

In asymptomatic smokers, longitudinal studies investigating sputum and bronchoalveolar lavage (BAL) fluid showed that airway inflammation decreased after cessation of smoking, e.g. the number of macrophages and neutrophils [(3)]. This suggests that the inflammatory changes in the airways due to smoking are reversible in smokers without COPD.

Studies have investigated the effects of smoking cessation on airway inflammation of COPD patients by comparing smokers and ex-smokers with COPD. Airway inflammation in induced sputum did not differ between COPD smokers and COPD ex-smokers [(4)]. Furthermore, Rutgers et al. showed that airway inflammation was more excessive in ex-smokers with COPD than in healthy asymptomatic smokers.[(5)]
Together, these cross-sectional studies suggest that there is ongoing inflammation in COPD after smoking cessation.

AIM OF THE WORK

The aim of this study is to evaluate the role of shisha cessation on airway inflammation of different COPD stages and asymptomatic smokers.

SUBJECTS AND METHODS

- ***Subjects:***

In this prospective study, a total of 45 subjects were included in the study.

The subjects were categorized into three groups:

1. Seventeen subjects are COPD current Shisha smokers for at least 20 years.
2. Eighteen subjects are COPD ex-shisha smokers for at least 1 year with past history of Shisha, smoking more than 20 years.
3. Ten subjects are asymptomatic shisha smokers they didn't have chronic respiratory symptoms or airway obstruction, and an FEV1 > 85%.

The subjects were categorized in the COPD group when FEV1/FVC post bronchodilator was <0.7. The severity of COPD was based on FEV1 % predicted post-bronchodilator; e.g. > 80% was mild COPD, 50–80% was moderate COPD, 30–50% was severe COPD.

All COPD patients included had chronic respiratory symptoms, i.e. chronic cough and sputum production for at least 3 months for 2 successive years.

All subjects included in the study are more than 45 years old. They all underwent lung function tests, sputum induction and analysis.

Subjects were asked not to use long or short acting b2-agonists and/or ipratropium at least 12 h before the test. They did not suffer from a respiratory tract infection nor used oral corticosteroids in the month prior to the study.

- ***Sputum induction:***

After pretreatment with inhaled 200 μg sulbutamol, sputum was induced by inhalation of 3.5% sterile hypertonic saline solution at room temperature. The saline was inhaled via a pneumatic nebulizer and inhaled up to 20 min. After 5 min and at subsequent 3.5 min, intervals the subjects were asked to rinse their mouths, blow their noses and cough sputum into a sterile cup, kept on ice for future analysis. The induction was stopped after expectoration of an adequate amount of sputum (i.e. 2 ml). Inhalation was discontinued if troublesome symptoms occurred. [6]

- ***Sputum processing:***

Sputum was collected in sterile containers and analyzed immediately on receipt. The volume of the sputum was measured, and the plugs were selected and weighted. A freshly prepared 0.1% solution of dithiotheritol was added in a volume that was equal to the double weight of the sputum, and the mixture was vortexed for 15 mm. Then a double volume of the phosphate-buffered saline solution was added, and the mixture was briefly vortexed. After filtration through two layers of a sterile gauze to remove debris and mucous, the sputum was centrifuged for 10 mm at 800. The cell pellet was resuspended in a phosphate-buffered saline solution. Cell viability was determined by a trypan blue exclusion test, cells were counted using a Bürker chamber. A differential cell count was performed using slides stained with may-Grünwald-Giemsa method. Three hundred cells were counted. Smears with <50% of squamous cells and >200 non-squamous cells were qualified as adequate. [6]

- ***Lung function:***

Lung functions (FEV1, FEV1/vital capacity) were measured using dry wedge spirometry (Se) according to standardized guidelines [7] using Sensor Medics V max 229. Bronchodilator responsiveness to 400 mg salbutamol was measured and post-bronchodilator values were used.

No complications were observed in the study group after performing spirometry.

RESULTS

The study was conducted on three groups of subjects: Seventeen COPD shisha smokers (37.78%), Eighteen COPD shisha ex-smokers (40%) and ten asymptomatic smokers.

The patients underwent spirometry were the mean value of FEV1 % predicted for COPD smoker group was 62.34%, COPD ex-smoker group was 58.27%

Table 1. shows the mean value of TLC and the mean value of differential leucocyte percentage of the three groups.

Table 1. *Mean value of TLC and mean value of differential leucocyte percentage*

Grouping	TLC (106/ml)	Differential leucocytic percentage (%)			
		Neutrophils	Macrophages	Eosinophils	Lymphocytes
Smokers	1.892	62.86	32.5	3	2.23
Ex-smokers	1.268	65.47	30.13	3.13	2.8
Asymptomatic-smokers	0.979	55.1	41.3	2.0	1.6

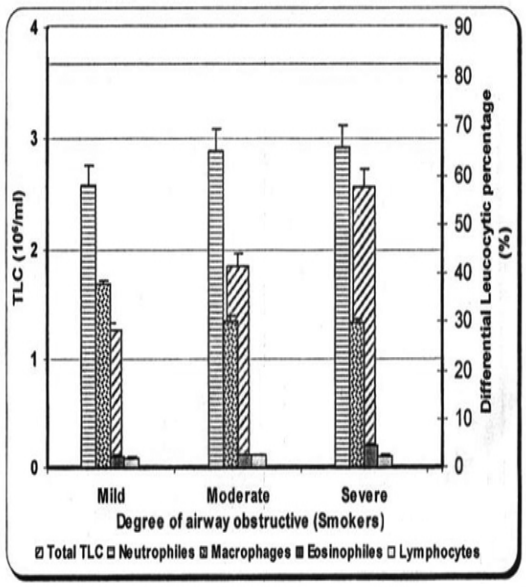

Fig 1. Airway inflammation (total leucocytic count and differential leucocytic percentage) in COPD smokers of different grades.

TLC count was increased with decreasing FEV1 values. The eosinophil percentage was doubled in severe obstruction in comparison to mild and moderate COPD.

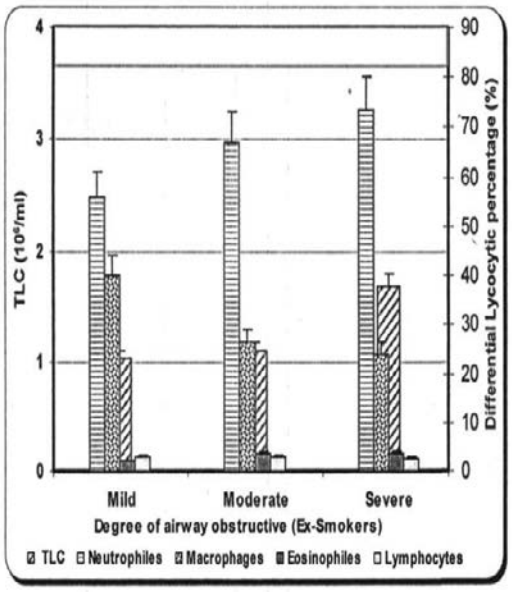

Fig 2. Airway inflammation (total leucocytic count and differential leucocytic percentage) for COPD ex-smoker group of different grades.

The total leucocytic count was significantly higher in severe obstruction than the moderate one. However, it was more in severe obstruction. The highest neutrophil count was found in severe airway obstruction.

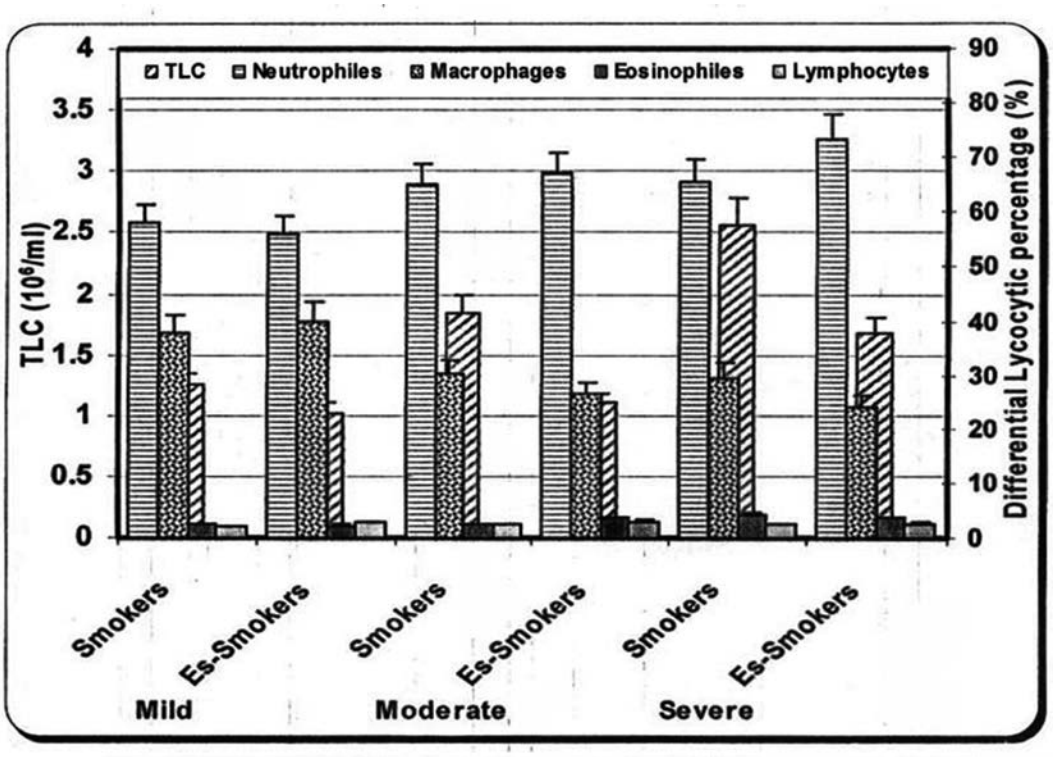

Fig 3. Comparison of total leucocytic count and differential leucocytic percentage between COPD smoker and COPD ex-smokers groups according to FEV1 values (mild, moderate, severe)

In mild degree of any obstruction, there was no statistically difference in neutrophil percentage between smokers and ex-smokers. However, in severe airway obstruction, neutrophil percentage was significantly higher in ex-smoker group than smoker group. In moderate airway obstruction, eosinophils percentage was increased in ex-smokers group in comparison to smoker one. In addition, lymphocytes percentage was not changed in all studied subjects.

Table 3. *Comparison of total leucocytic count and differential leucocytic percentage between different groups*

Grouping	TLC (106/ml)	Differential leucocytic percentage (%)			
		Neutrophils	Macrophages	Eosinophils	Lymphocytes
Smokers	1.892	62.86	32.5	3.2	2.23
Ex-smokers	1.268	65.47	30.13	3	2.8
Asymptomatic-smokers	0.979	55.1	41.3	2.0	1.6
Pr>	0.0001	0.0001	0.001	0.05	0.001

Total leucocytic count was significantly increased in COPD smoker group (1.892x106) in comparison to COPD ex-smoker group (1.268x106) and in asymptomatic-smokers (0.979x106). Neutrophils percentage was increased in COPD ex-smoker group (65.47) compared to COPD smoker one (62.86) where asymptomatic-smokers showed the least percentage (55.1). The most elevation of macrophages was recorded for asymptomatic-smokers group (41.3).

DISCUSSION

Smoking cessation is the only effective treatment in the patients with chronic obstructive pulmonary disease (COPD). It slows disease progression by decreasing decline in FEV1 but its effect on airway inflammation in COPD patients is still unknown. Therefore, the present study was conducted to analyze the patterns of airway inflammation in COPD patient depending on their smoking status, and compare it to the smokers without airway obstruction (Asymptomatic smokers). Induced sputum was collected from the three study groups and the cellular composition of the sputum was analyzed. Each COPD group (Smoker and Ex-smoker) was categorized into three subgroups according to their FEV1 measured by spirometry. A comparison study between total leucocytic count and FEV1 in smokers groups (mild, moderate and severe) was done and it showed that there is inverse relationship between total leucocytic count and FEV1, so that the decrease in FEV1 was associated with increase of the total leucocytic count.

The present study showed that there is an increase in the total leucocytic count in the smoker COPD group and ex-smoker one and both of them were higher than asymptomatic smokers. Differential leucocytic count among three studied groups shows a significant difference. In details, eosinophils are slightly increased in COPD-smoker group than COPD-ex-smokers; and both of them are increased in percentage than asymptomatic smokers. Hargreave and Leigh conducted that the participation of eosinophils in bronchial constriction in smokers was observed [8]. Similarly, Lebowitz et al concluded that eosinophilia is an important aspect of bronchial constriction in smokers [9]. Cosio and Guerassimov divided cases of COPD in smokers into two types, one with eosinophilia and the other without eosinophilia [10]. The increase of eosinophils may be reflecting the presence of inflammation in smokers with COPD who have airway responsiveness to corticosteroids that is an asthmatic pattern of COPD.

Lacoste et al found an increased number of eosinophils in patients with chronic bronchitis and COPD but lower BAL concentrations of ECP than in asthmatics, suggesting that eosinophils are present but are less activated in COPD [11]. However, other studies showed increased sputum ECP concentrations to a greater level than seen with asthma in moderate to severe COPD [12] suggesting that eosinophils are activated in more severe disease.

The origin of eosinophilic airway inflammation in COPD is unclear, although it is widely assumed that it indicates an asthmatic component to the fixed airways obstruction [13]. This is unlikely to be the case, as most studies on patients with COPD rigorously exclude subjects with variable airflow obstruction and those with clinical features suggesting asthma. It is more likely that smoking and other mechanisms that recruit neutrophils into the airway mucosa in COPD may in turn cause a minor degree of eosinophil influx. [14]

For pulmonary macrophages, their percentage is higher in asymptomatic smokers group than other studied groups (COPD smokers and ex-smokers). However, the pulmonary macrophages are heterogeneous population of cells. Domagala-Kulawik et al found that the population of macrophages expressing CD 116, CD 14, CD54 and CD7L were not significantly different between smokers and ex-smokers with COPD [15]. In addition, CD116 seems to be an important agent in eosinophils activation [16].

In the present study, neutrophil percentage was high in all studied groups. There is no significant difference in the neutrophils between the mild, moderate and severe COPD smoker group. This study revealed there was no correlation between sputum neutrophil percentage and FEV1, so that sputum neutrophil percentage does not predict change in FEV1 after one year.

Similarly, Singh et al found that there were very weak association between sputum neutrophils percentage and FEV1 [17].

On the other hand, Donnell et al recorded a statistically significant relationship between FEV1 and neutrophils percentage. A study for thirty-eight smokers showed that lung function decline over 15 years was associated with sputum neutrophils percentage [18].

It was known that the number of neutrophils in walls of small airway is related to the severity of airflow obstruction. It has previously been reported in limited number of COPD patients (n=45) that the total neutrophils (number/gram) in sputum is related to the subsequent decline in pulmonary function over 7 years [19]. These observations explain our findings; the neutrophils percentage is higher in severe airway obstruction of COPD ex-smokers group.

In the present study, neutrophil percentage of ex-smoker group was higher than the same percentage for smoker COPD group and both of them are higher than asymptomatic smoker group. Richter et al showed that the percentage of neutrophils is similar in both COPD smokers and ex-smokers groups [8,20]. In the other hand, Keatings and Barnes showed that

neutrophil percentage was higher in smokers group than ex-smoker group. [21]

From all previous results and reports that were showing that the higher total leucocytic count in COPD ex-smoker group and the relative increase in some differential leucocytic count such as neutrophils percentage in comparison to the smoker COPD group and role of macrophages, lymophocytes and eosinophils in developing COPD disease.

These findings suggest the ongoing airway inflammation of COPD patient uncoupling with smoker cessation. These results explain why airway inflammation is not resolved in COPD patient after 1 year of smoking cessation.

One explanation is that bacterial colonization was persisting in the lower airway of COPD patients normally not found in healthy individuals [22].

Other explanation is that the observed persistent inflammation in chronic obstructive pulmonary disease may be, at least partly, related to repair of the smoke-induced tissue damage in the airways.

Recently, a suggested hypothesis is that COPD have an autoimmune component, which contributes to the airway inflammation even after smoking cessation [23]. These autoantibodies may be directed against antigen in tobacco or from endogenous auto-antigens as a result of smoking-induced inflammatory and oxidative lung injury. [24]

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