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Research Article

Association of High Peripheral Neutrophil Count in Healthy Young Smokers with Impaired Pulmonary Function Test

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Abstract: Young adults in developing countries are attracted and addicted to the habit of smoking due to various reasons. The inhaled smoke from cigarette produces inflammatory reaction in the airways leading to impairment in lung function. Our present study was designed to assess the relationship between the pulmonary function parameters like forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), percentage of FEV1 and forced expiratory flow during 25-75% of expiration (FEF 25-75%) and peripheral differential leukocyte count in healthy young smokers. The study was done in 50 healthy smokers and their results compared with 50 healthy non smokers of the same age group. Pulmonary function test (PFT) was assessed using computer spirometry and the peripheral differential leukocyte count (DLC) was done using spread technique. The variables of PFT and DLC were statistically correlated using Pearson`s correlation test. Forced expiratory lung volumes like FVC, FEV1, FEF 25-75% were decreased in smokers when compared to non smokers and their blood picture showed increased neutrophil count. Pearson`s correlation test showed a negative / inverse relationship between neutrophil and lung function parameters in smokers. The results of our study suggest that the impaired lung function test in smokers is associated with high peripheral neutrophil count.
Keywords: Healthy young smokers, PFT, Computer spirometry, DLC, Spread technique, Negative correlation

INTRODUCTION

Tobacco use is a major preventable cause of premature death and diseases worldwide. Nearly one million people die in India every year due to tobacco use. In India, 5.7% of adults are cigarette smokers and their average age of initiation of tobacco use was 17.8 years [1].

Tobacco smoke contains more than 2000 potential noxious constituents and most of which are potential carcinogens [2]. These compounds cause direct inflammatory response and cell mediated oxidative damage to the lungs by alveolar macrophages and neutrophils [3, 4].

Also, smoking has been implicated as a potential cause of chronic idiopathic neutrophilia in smokers. However it gets reverted back after cessation with use of electronic cigarette [5]. The neutrophils play a crucial role in chronic obstructive pulmonary disease (COPD) pathogenesis and are considered as the primary effector cells involved in the airway and systemic inflammatory process [6].

The peripheral blood neutrophils are activated by different inflammatory mediators and this step precedes their extravasations to the lung tissue [7]. The activated neutrophils migrate to the lung tissue and release various proteolytic enzymes like elastase and matrix metallo-proteases which strip the bronchial epithelium, reduce ciliary beating and stimulate excess mucus secretion leading to mucus retention, bacterial proliferation and recurrent infections. Neutrophil elastase also stimulates pro-inflammatory mediators like Interleukin 8 (IL-8), Interleukin-1β (IL-1 β) and TNF-α from epithelial cell which produces chemo attractant cleavage products that lead to lung inflammation and further neutrophil recruitment to the lungs. It also impairs the host defences by damaging the major opsonophagocytic receptors on the neutrophil and by weakening the efficacy of immunoglobulins [8].

In this study, we have studied and correlated the blood changes in young healthy smoking adults with impaired lung function test.

MATERIALS AND METHODS

The present study was carried out in 50 healthy male smokers between the age group of 18-30 years and their data was compared with 50 healthy male non smokers of the same age group. The aim, methodology and implication of the study were explained prior to the commencement of the procedure. Written informed consent was obtained from all the subjects.

The lung function parameters like FVC, FEV1, FEV1%, FEF 25-75% were assessed by computerised spirometer (MEDIKRO – WINDOWS SPIROMETER / MODEL – M 9831 –1.8-0.4) after satisfactory demonstration regarding the procedures. The guidelines of American Thoracic society (ATS) and European Respiratory society (ERS) were strictly followed [9]. A

minimum of three forced expiratory maneuvers were performed and the best of the readings was accepted for statistical analysis.

Peripheral differential leukocyte count was performed by spread technique following all aseptic precautions. The first 200 cells were counted under oil immersion objective in the microscope by two independent observers to ensure adequate precision [10].

The data was analysed for statistical significance using Wilcoxaon Signed Rank test and the results of PFT and DLC were correlated using Pearson`s correlation test.

RESULTS

The results of pulmonary function test parameters and differential leukocyte count are discussed below.

Table 1: Pulmonary function test parameters and Differential leukocyte count in smokers and non-smokers.

Variables	Smokers (Mean ± SD)	Non-Smokers (Mean ± SD)	Wilcoxon Signed Rank Test For 'P' – Value
Number	50	50	
Age (years)	23.34 ± 6.5	23.34 ± 6.5	
FVC (L)	3.5± 1.3	4 ± 0.5	0.005*
FEV1 (L)	3.3 ± 1.2	3.9 ± 0.5	< 0.001*
FEV1 %	94.2 ± 1.5	98 ± 3.3	0.875
FEF 25-75% (L/Sec)	4.7 ± 5.2	6.2 ± 1.1	< 0.001*
% of Neutrophil	70.1 ± 5.2	59.7 ± 4.4	< 0.001*
% of Eosinophil	2.1 ± 0.8	2.7 ± 1.2	0.003*
% of Basophil	0.4 ± 0.6	0.5 ± 0.5	0.555
% of Lymphocyte	23.7 ± 3.8	32 ± 4.6	0.001*
% of Monocyte	3.8 ± 1.8	5.1 ± 1.5	0.001*

'p'- value of < 0.05 was considered statistically significant *

The lung function test parameters like FVC, FEV1, FEV1 %, FEF 25-75% were 3.5± 1.3 L, 3.3 ± 1.2L, 94.2 ± 1.5 , 4.7 ± 5.2 L/sec respectively. For non-smokers it was FVC = 4 ± 0.5 L, FEV1 3.9 ± 0.5= L, FEV1 % = 98 ± 3.3, FEF 25-75% = 6.2 ± 1.1 L/sec. This result indicates that all the lung function parameters were reduced in smokers when compared to non-smokers. The 'p' value analysed by Wilcoxon Signed rank test was significant for FVC (0.005), FEV1 (<0.001) and FEF 25-75% (< 0.001) in smokers.

In differential leukocyte count, the percentage of neutrophil count showed a significant increase than the normal range in smokers. For smokers, the percentage of was 70.1 ± 5.2 and for non smokers it was 59.7 ± 4.4. The 'p' valve < 0.001 was of high statistical significance.

Though the other leukocyte subset like Eosinophil, Basophil, Lymphocyte and Monocyte showed a statistically significant change between the smoking and non-smoking group, they were still under the normal range. For smokers the percentage of Eosinophil, Basophil, lymphocyte and Monocyte was 2.1 ± 0.8, 0.4 ± 0.6, 23.7 ± 3.8, and 3.8 ± 1.8 respectively whereas for non-smokers it was Eosinophil 2.7 ± 1.2 %, Basophil 0.5 ± 0.5 %, Lymphocyte 32 ± 4.6% and Monocyte 5.1 ± 1.5 %.

Table 2: Correlation between elevated neutrophil count & impaired lung function parameters in smokers

Variable	Pearson Correlation	P value
FVC	-57.0 %	P <0.01*
FEV1	-71.7 %	P <0.01*
FEF 25 to 75	-74.1 %	P <0.01*
FEV1 / FVC	-13.8 %	P = 0.3

*statistically significant

When the elevated neutrophil count in smokers were correlated with the lung function parameters like FVC, FEV1, FEV1 %, FEF 25-75% we found that there exist a negative correlation between them.

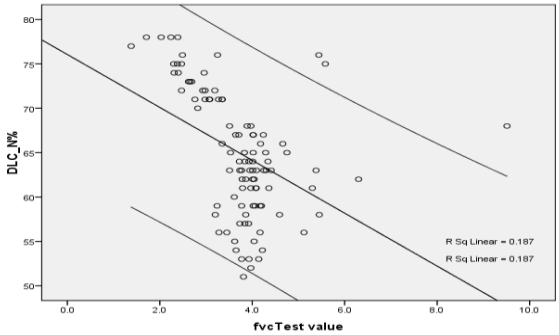

Fig.1: correlation between neutrophil and FVC value in smokers

Fig.1 shows that there is negative correlation between FVC value and neutrophil percentage (r = -57%, p <0.01) in smokers. i.e. as neutrophil count increases, FVC value decreases

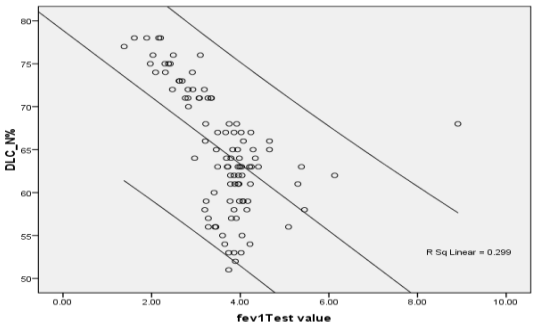

Fig. 2: correlation between neutrophil and FEV1 value in smokers

Fig. 2 shows that there is negative correlation between FEV1 value and neutrophil percentage (r = -71.7%, p <0.01) in smokers. i.e. as neutrophil count increases, FEV1 value decreases

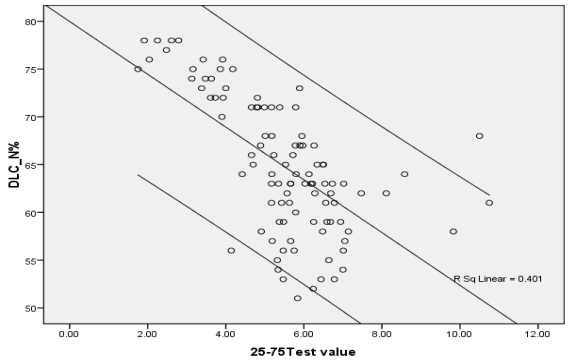

Fig. 3: correlation between neutrophil and FEF 25-75% value in smokers

Fig. 3 show that there is negative correlation between FEF 25-75% value and neutrophil percentage (r = -74.1%, p <0.01) in smokers. i.e. as neutrophil count increases, FEF 25-75% value decreases.

DISCUSSION

Tobacco smoking is the main cause of obstructive pulmonary disease [11]. The noxious constituents in the cigarette smoke increases the number of pulmonary alveolar macrophages which release chemical substance that attract leukocytes to the lungs. These leukocytes in turn release proteases including elastase, which attacks the elastic tissue in the lungs. At the same time, α1- anti trypsin, a plasma protein that normally inactivates elastase and other protease is inhibited by oxygen radicals released by leukocytes. The final result is protease – anti protease imbalance with increased destruction of lung tissue impairing the lung function [12].

In our present study, we observed that FVC and FEV1 were reduced in smokers when compared to non-smokers. This indicates that there is a generalised airway obstruction in the smoking group. Similar results were obtained in the study done by Prieto F *et al* in 271 healthy smoking men [13]. FEF 25-75% is assessed to find the patency of smaller airways less than 2 mm diameter. Inflammatory response to cigarette smoke mainly affects the smaller airways. This is speculated to be the initial step of COPD [14]. In our study, FEF 25-75% values were significantly reduced in smokers when compared to non smokers. This was in agreement with the study done by Santos S *et al.* [15].

The blood leukocyte changes in our study showed a raise in neutrophil count than the normal range in smokers. Similar increase in neutrophil number and neutrophil chemo attractant IL-8 have been demonstrated in bronchoalveolar lavage by Ohbayashi O and Takizawa H [16], in sputum by Vander Vaart H *et al* [17], and in peripheral blood by Schwartz J and Weiss ST [18].

When we compared this elevated neutrophil count with the impaired lung function test parameters using Pearson`s correlation test, we observed that there was a negative correlation between the raise in neutrophil count and impairment in lung function. That is, less the lung function more will be the neutrophils indicating active inflammatory process in the airway limiting the flow of air. Similar results were also observed by Franchin M *et al* [19] and O`Donnell RA *et al* [20].

CONCLUSION

This study suggest that increase in peripheral neutrophil count secondary to the inflammatory response in the lung caused by cigarette smoke is closely associated with the airway dysfunction in healthy smokers

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