Quantitative assessment of leukocyte count in different grades of functional lung impairment in obstructive airway disease patients

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***Abstract*- Systemic inflammation is associated with impaired lung function and inflammation is a part of Obstructive Airway Disease (OAD). COPD and bronchial asthma are the two most common OAD. This study was done to grade the severity of functional lung impairment and measure the circulating levels of leukocytes in different grades of severity. and to determine the possible relation between the severity of functional lung impairment and the leukocytes levels. A cross-sectional study enrolling 40 COPD & 25 asthmatic patients of both sexes. Lung function was studied, then absolute neutrophil count, absolute eosinophil count, absolute basophil count, absolute lymphocyte count and absolute monocyte count were determined using suitable diluting fluids. Out of 40 COPD patients, 12.5 % of patients had mild, 20% had moderate, 40% had severe and 27.5% had very severe COPD. Out of 25 asthma patients, 48% had mild, 32% had moderate and 20% had severe asthma. Significant increase in neutrophil count with the increase in COPD severity and with the increase in asthma severity, eosinophil count increases significantly. Significant inverse correlation between neutrophil count and FEV_1 in COPD patients, and eosinophil count and FEV_1 in asthma patients was seen.**

***Index Terms*: Asthma, COPD, OAD, FEV1% predicted, leukocyte counts.**

INTRODUCTION

Obstructive diseases of the airways are a diverse group of respiratory diseases. In general, they result from narrowing of any portion of the airways (from upper airway to bronchioles less than 2mm in diameter) and finally resulting in a reduction of maximal airflow, with the consequent increase in the work of breathing. (1) Included in the obstructive pulmonary disorders are chronic obstructive pulmonary disease of the airways (chronic bronchitis and emphysema), asthma, bronchiectasis, small airway disease, and upper airway obstruction. (2)

The 2011 revision of the Global Initiative for Asthma (GINA) guidelines defines asthma as a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role, in particular, mast cells, eosinophil, T-lymphocytes, macrophages, neutrophils and epithelial cells(3).The 2011 revision of the GOLD guidelines defines COPD (Chronic Obstructive Pulmonary Disease) as a preventable and treatable disease which is characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. (4) Neutrophils, eosinophil, alveolar macrophages, and lymphocytes all appear to participate in the inflammatory process associated with COPD. (5)

The gold standard definition of obstructive airway disease is Forced Expiratory Volume in 1 sec (FEV1) and ratio of FEV1 to Forced Vital Capacity (FEV1/FVC) less than fifth percentile.(6) The level of FEV1 is also used to grade the severity of airway obstruction.(7)

Assessment of ongoing inflammation is often determined by evidence of inflammatory cells in respiratory tract. The present study was undertaken to explore whether circulating levels of leukocytes in blood bear any relationship with the severity of the disease as reflected in the functional lung impairment assessed by spirometry.

MATERIALS AND METHODS

Study design:

This was a cross-sectional study carried out in the Department of Physiology and Department of Respiratory Medicine, Regional Institute of Medical Sciences (RIMS), Manipur, India, during the period from March 2013 to July

2014. A written informed consent was taken from all the patients and approval from the Institutional Ethics Committee was also taken.

Inclusion criteria:
OAD patients of both sexes (male and female) between the ages of 18 to 65 years who attended Respiratory Medicine OPD and those admitted in the Respiratory Medicine Ward of Regional Institute of Medical Sciences (RIMS), Imphal were included in the study.

Exclusion criteria:
Patients with associated diseases like worm infestations, atopic skin disease and other allergic diseases, cardiac problems, renal failure, diabetes mellitus, hypertension, pulmonary fibrosis, neuromuscular diseases, and ascites were excluded.

Data collection procedure:
A) Spirometric studies were conducted by means of a Computerized Spirometer- Helios 401/701 (Recorders and Medicare system, Chandigarh, India). The study variables recorded by spirometry include Forced Vital Capacity (FVC), Forced Expiratory Volume in one second (FEV_1), FEV_1/FVC, Peak Expiratory Flow Rate (PEFR), Forced Expiratory Flow ($FEF_{25-75\%}$).

1) Classification of severity of airflow limitation in COPD patients according to GOLD guidelines -
(In patients with $FEV_1/FVC < 0.70$)

GOLD 1	Mild	$FEV_1 \geq 80\%$ predicted
GOLD 2	Moderate	$50\% \leq FEV_1 < 80\%$predicted
GOLD 3	Severe	$30\% \leq FEV_1 < 50\%$predicted
GOLD 4	Very severe	$FEV_1 < 30\%$predicted

2) Classification of severity of airflow limitation in Asthma patients according to GINA guidelines -

Mild	$FEV_1 \geq 80\%$predicted
Moderate	$60\% \leq FEV_1 < 80\%$predicted
Severe	$FEV_1 < 60\%$predicted

B) Assessment of blood leukocyte counts was done by using 1ml of blood collected by venipuncture from the subjects under aseptic condition using a plastic sterile disposable syringe. The sample was collected in a vial containing EDTA as anticoagulant. Under a compound microscope in an Improved Neubauer's counting chamber, Total leukocyte count (TLC) and Differential leukocyte count (DLC) were determined using Turk's fluid and Leishman's stain, respectively. After finding out TLC and DLC, different absolute leukocyte counts were determined by using the formula-
Absolute leukocyte count= DLC/100×TLC
E.g. Absolute neutrophil count= Neutrophil % / 100× TLC

Statistical analysis:
Appropriate statistical analysis like One way ANOVA and Pearson's correlation were used wherever necessary using standard software like Microsoft Excel 2010 and SPSS version 16.0. Data are presented in the form of Mean ± Standard Deviation (SD) and Mean ± Standard Error Mean (SEM). Level of statistical significance was set at $p < 0.05$, two-tailed.

RESULTS

Table 1: Socio-demographic profile of the patients of COPD and asthma groups

Parameters	**COPD**	**Bronchial asthma**
No. of subjects	40	25
Age (mean) in years	54.69±16.65	32.38±18.0925
Male: Female	22:18	11:14
Type of residence, n (%)	Urban=32(80) Rural=8(20)	Urban= 18(72) Rural= 7(28)
Smoking status, n (%)	Non-smoker=10(25) Smoker=18(45) Ex-smoker=12(30)	Non-smoker=12(48) Smoker= 5(20) Ex-smoker=8(32)

Table 1 shows that male cases are more than female cases in COPD group whereas more female cases in asthma group. The patients in the asthma group are younger than the COPD groups. Maximum of the study population resides in the urban areas in both the groups. With regards to smoking habit, maximum of the COPD cases are smoker whereas non-smoker in asthma cases.

Table 2**:** FVC, FEV_1, FEV_1/FVC % predicted, PEFR, FEF_{25-75} values according to severity of lung impairment in COPD patients

Stage	**N (%)**	**FVC**	**FEV_1**	**FEV_1/FVC % pred.**	**PEFR**	**FEF_{25-75}**
1(Mild)	5(12.5)	2.90±0.76	2.77±0.55	97.00±6.06	6.34±1.17	3.43±1.61
2(Moderate)	8(20)	1.46±0.45	1.32±0.32	87.69±13.75	2.76±1.44	1.76±1.18
3(Severe)	16(40)	1.09±0.26	0.84±0.23	70.52±24.21	2.07±1.58	1.28±1.31
4(Very severe)	11(27.5)	0.55±0.20	0.34±0.12	66.16±14.55	0.59±0.37	0.28±0.08
p-value		0.000*	0.021*	0.000*	0.040*	0.004*

Table 2 shows that there is a progressive decline of lung function through the different stages and this finding is statistically significant (*)

Table 3: Mean±SEM of absolute leukocyte counts with respect to severity of lung impairment in COPD patients

Parameters	**Mild**	**Moderate**	**Severe**	**Very severe**	**p-value**
ANC	4617±331.8	6339±690.2	6512.2±198.9	8667.3±174.1	0.042*
AEC	182.6±63.5	274.9±62.04	200.8±55.7	215.4±58.8	0.741
ABC	0	0	0	0	
ALC	2276.6±170.1	2245±96.16	1801.8±62.5	2003.5±79.8	0.209
AMC	202.4±67.34	291.7±21.10	308.1±72.60	384.1±48.21	0.192

(ANC= absolute neutrophil count, AEC= absolute eosinophil count, ABC= absolute basophil count, ALC= absolute lymphocyte count, AMC= absolute monocyte count)

Table 3 shows that there is a statistically significant (*) increase in absolute neutrophil count with the increase in COPD severity. Absolute monocyte count also increases with the increase in severity but not statistically significant.

Table 4: FVC, FEV_1, FEV_1/FVC% predicted, PEFR, FEF_{25-75} values according to severity of lung impairment in asthma patients

Stage	**N (%)**	**FVC**	**FEV_1**	**FEV_1/FVC % pred.**	**PEFR**	**FEF_{25-75}**
Mild	12(48)	2.56±1.02	2.48±0.89	98.06±3.74	5.16±2.35	3.22±0.97
Moderate	8(32)	1.87±1.11	1.29±0.31	86.81±0.24	3.20±1.56	0.93±1.20

Severe	5(20)	1.55±0.57	0.89±1.29	82.64±14.36	3.25±1.15	1.21±0.42
p-value		0.235	0.520	0.081	0.300	0.009*

Table 4 shows that there is a progressive decline of lung function through the different stages of asthma but only the decline in FEF_{25-75} is found to be significant (*)

Table 5: Mean±SEM of absolute leukocyte counts with respect to severity of lung impairment in asthma patients

Parameters	**Mild**	**Moderate**	**Severe**	**p-value**
ANC	3281.5±212.9	4526.6±290.6	4677.5±460.0	0.059
AEC	309.3±43.6	381.2±50.3	441.6±156.12	0.032*
ABC	0	0	0	
ALC	1933.6±230.23	2106.7±186.12	2086.2±615.8	0.847
AMC	313.6±130.4	630.4±128.2	635.3±226.6	0.317

Table 5 shows that there is a statistically significant (*) increase in absolute eosinophil count with the increase in asthma severity. Absolute neutrophil and monocyte counts also increases with the increase in severity but not statistically significant.

DISCUSSION

The present study was done to explore whether the circulating levels of leukocytes in blood bear any relationship with the severity of functional lung impairment assessed by spirometry. The study was based on the primary data collected from 40 COPD and 25 Bronchial Asthma patients.

Based on GOLD and GINA guidelines, grading of severity of functional lung impairment of COPD and bronchial asthma respectively was done based on FEV_1% predicted. In COPD group, maximum patients (40%) presented with severe COPD and maximum asthma patients (48%) presented with mild severity. There was a progressive decline of lung function through the different stages of COPD and asthma (Table 2 and 4).

In the present study, high neutrophil count in COPD patients (Table 3) was observed similar to the findings reported in a study by Rathod VPS et al [8]. It is well known that neutrophils play a crucial role in the pathophysiology of COPD, as they release multiple mediators and tissue degrading enzymes such as elastases that orchestrate tissue destruction and chronic inflammation. In COPD, the neutrophilic inflammatory response dominates.

Cigarette smoking is the major factor in the development of obstructive airways disease. Investigators have studied a variety of potential mechanisms by which cigarette smoke might alter protease-antiprotease balance in the lung. In our study, majority of COPD patients were smoker (45%) which might have contributed to the increased neutrophil count in COPD patients. This was in conformity with the study conducted by Willemse BWM et al [9]. Other reasons of high neutrophil count may be the presence of infection and use of steroids for treatment as steroids causes an increase in neutrophil count by accelerating release from bone marrow and decreasing its migration out of circulation.

Monocytes were predominantly associated with symptoms indicative of obstructive airway disease, in similar relation to neutrophils, but both of these leukocyte counts were also increased in asthma patients. In our study, we also found an increase in monocyte count in both COPD and asthma groups. Similar results were seen in a study conducted by Sarah A. Lewis et al [10].

The present study (Table 5) found a higher eosinophil count in bronchial asthma patients and the count increases as the severity increases. Similar findings were also observed in a study conducted by Horn BR et al [(11)]. Taylor KJ et al [(12)] also documented a similar relationship between asthma severity and peripheral eosinophil count. Asthma was classically considered an allergic disease with an eosinophilic inflammatory picture but in the last decade another phenotype of asthma has been defined, non-allergic asthma characterized by neutrophilic inflammation [(13)].

CONCLUSION

Our study shows that a relationship seems to exist between blood leukocyte counts and severity of functional lung impairment in COPD and asthma patients. COPD patients were found to have high neutrophil and monocyte counts while asthma patients with high eosinophil count in the peripheral blood with the increase in the severity of functional lung impairment. However, smoking status, presence of respiratory infections, use of bronchodilators and inhaled corticosteroids for treatment can influence the results.

It can be concluded from the findings of the present study that spirometry is important for diagnosis of OAD. Determining the peripheral leukocyte counts will be useful as it can serve as an indirect and inexpensive marker of severity of functional lung impairment in chronic obstructive airway patients along with a proper history and physical examination. Further investigations and more advanced studies involving more number of patients in different hospitals are required to broaden our current knowledge to corroborate or disprove our findings.

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