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STUDY OF SPUTUM CYTOLOGY IN COPD PATIENTS

General Medicine

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ABSTRACT

Introduction: COPD are forms of obstructive airway diseases with associated chronic inflammation of the airways. Historically, COPD represent distinct ends of the spectrum of airways disease separated by multiple features such as smoking exposure, bronchodilator reversibility and airway inflammation with remodeling. Moreover, a negative correlation has been reported between the percentage of neutrophils and FEV_1. The neutrophilic inflammation partly explains the resistance of most COPD patients to steroid treatment, though some investigators report a decrease in the neutrophilic counts after steroid treatment. **Aims and Objectives:** To study the sputum cytology profile in chronic obstructive pulmonary disease (COPD). **Material and Methods:** This study was carried out in COPD patients admitted in TB and Chest Hospital, Badi, Department of Respiratory Medicine, RNT medical college, Udaipur, Rajasthan, India. After diagnosis and assessment of COPD which includes pre and post bronchodilator assessment, Morning induced sputum was collected and haematoxylin - and eosin - stained specimen underwent cytology. **Results:** Majority of the patients (82%) were having moderate to severe COPD, only 8% and 10% had mild & very severe COPD respectively. Neutrophilic (58%) phenotype is the commonest among COPD patients and eosinophilic phenotype (20%) also found in significant number of cases. Mean blood eosinophilic count among COPD patients in this study were 2.79±1.93. **Conclusion:** Neutrophilic phenotype is predominant phenotype in most of the COPD patients but significant proportion of patients had eosinophilic phenotype. Blood eosinophil counts enabled identification of presence or absence of sputum eosinophilia with reliable accuracy. There is positive correlation between sputum eosinophil and severity of disease (FEV_1) COPD. Specific phenotyping of COPD inflammatory profiles are worthwhile and of clinical importance.

KEYWORDS

Neutrophilic phenotype, sputum eosinophilia, COPD

INTRODUCTION

COPD are forms of obstructive airway diseases with associated chronic inflammation of the airways. Historically, COPD represent distinct ends of the spectrum of airways disease separated by multiple features such as smoking exposure, bronchodilator reversibility and airway inflammation with remodeling.

Moreover, a negative correlation has been reported between the percentage of neutrophils and FEV1. The neutrophilic inflammation partly explains the resistance of most COPD patients to steroid treatment, though some investigators report a decrease in the neutrophilic counts after steroid treatment[1].

Sputum and blood eosinophil levels can be used as a biomarker in severe COPD exacerbations for predicting higher readmission rates and addition of steroid in treatment of COPD patient and is recently recommended by GOLD 2020 report.

AIMS AND OBJECTIVES

1. To study the sputum cytology profile in chronic obstructive pulmonary disease (COPD).
2. To correlate it with severity of disease (FEV_1).

MATERIALS AND METHODS

This study was carried out in COPD patients admitted in TB and Chest Hospital, Badi, Department of Respiratory Medicine, RNT medical college, Udaipur, Rajasthan, India.

After diagnosis and assessment of COPD which includes pre and post bronchodilator assessment, Morning induced sputum was collected and haematoxylin - and eosin - stained specimen underwent cytology.

Cellular components were counted in 20 fields. 300 cells were counted and a cytogram created, which displayed the percentage composition of every type of cell such as bronchial epithelium, macrophages and leucocytes (neutrophils, eosinophils, lymphocytes, monocytes). The cytology analysis was done using the mean percentage composition of a particular cell.

Sputum was induced after spirometric evaluation (with pre and post bronchodilator assessment) using nebulization with 4.5% saline in participants whose FEV1 was >1 L, using our previously described methods. In those with FEV1,1 L, 0.9% saline was used.[2,3]

Sputum adequacy is determined by less than 10 squamous cell (10x), more than 25 neutrophil per 10x low power field. The sputum differential cell count has been defined in large normal populations (Beldaet al 2000)[4] In their study they found following mean for sputum inflammatory cells. Eosinophil: 0.4 0±0.9% Neutrophil :37.5 ±20.1% Macrophage: 58.8±21.0% Lymphocyte: 1.0±1.1% Bronchial epithelial cells 1.6±0.9%.

Venous blood samples were sent for differential leukocyte count to be determined by Sysmax CBC analyzer.

Diagnosis and assessment of severity of COPD was done by spirometry as per GOLD (COPD) guidelines 2017.[5,6] The Spirometric evaluation of the patients was performed using a Computerised Spirometer Helios 702 as per ATS guidelines. Patients were classified according to severity of disease as under:

Classification of Severity of Airflow Limitation in COPD (Based on Post Bronchodilator)
In patients with FEV_1 / FVC < 0.7
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.

Shortness of Breath is graded as per Modified Medical Research Council Dyspnea Scale (MMRC Scale).[7]

Modified Medical Research Council Dyspnea Scale (MMRC Scale)
Grade Description
0 - Not troubled with breathlessness except with sternous exercise
1 - Troubled by shortness of breath when hurrying on the level or walking up a slight hill
2 - Walks slower than people of the same age on the level because of breathlessness or has to stop for breath when walking at own pace on the level
3 - Stops for breath after walking about 100 yards or after a few minutes on the level
4 - Too breathless to leave the house or breathless when dressing or undressing.

Exclusion Criteria

Tuberculosis, bronchiectasis, malignancy or other confounding inflammatory diseases.

1. Clinical or radiological evidence of concurrent respiratory illness,
2. Use of systemic or inhaled steroid therapy in the preceding one month.

OBSERVATIONS

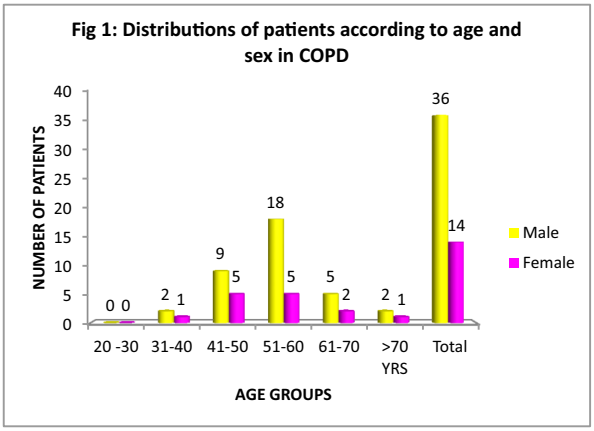


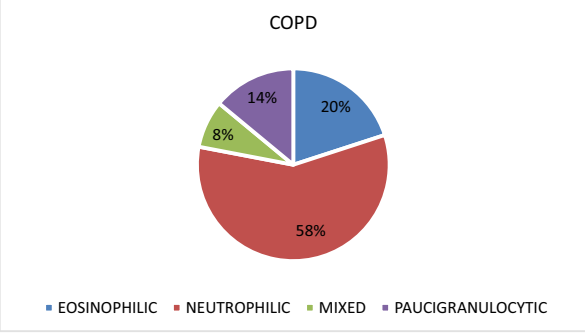
TABLE NO.: 1 Distributions of patients according to COPD severity

COPD SEVERITY	MALE		FEMALE		TOTAL	
	NO	%	NO	%	NO	%
MILD	2	5.5	2	14	4	8
MODERATE	16	44	9	64	25	50
SEVERE	12	33	2	14	16	32
VERY SEVERE	4	11	1	7.1	5	10
TOTAL	36		14		50	100

p=0.37 (NS)

This table shows that majority of the patients (82%) were having moderate to severe COPD, only 8% and 10% had mild & very severe COPD respectively.

Fig.2: Distributions of patients according to sputum cytological phenotypes.



Neutrophilic (58%) phenotype is commonest among COPD patients and eosinophilic phenotype (20%) also found in significant number of cases.

TABLE No: 2 Distributions of patients in phenotypes according to COPD Severity.

COPD SEVERITY	EOSINOPHILIC		NEUTROPHILIC		PAUCIGRANULOCYTIC		MIXED	
	No.	%	No.	%	No.	%	No.	%
MILD	2	20	0	0	2	28.57	0	0
MODERATE	2	20	6	22.22	3	42.85	0	0
SEVERE	3	30	16	59.25	2	28.57	2	50
VERY SEVERE	3	30	7	25.9	0	0	2	50
TOTAL	10		29		7		4	

Above table shows Eosinophilic phenotype had 40% cases of mild and moderate COPD severity whereas 60% cases of severe and very severe COPD severity. Neutrophilic (81.47%) and paucigranulocytic (71.42%) had majority of cases with moderate and severe COPD severity. While 100% cases of mixed phenotype had severe and very severe COPD severity.

TABLE No: 3 Average Sputum Eosinophil and Blood Eosinophil in different phenotypes

PHENOTYPE	SPUTUM		BLOOD	
EOSINOPHILIC	MEAN	SD	MEAN	SD
NEUTROPHILIC	0.59	0.78	5.20	1.99
MIXED	5.00	0.82	2.02	0.82
PAUCIGRANULOCYTIC	1.00	0.82	5.50	0.58
Total	2.12	2.6	1.00	0.29

Mean eosinophilic count among COPD patients in this study were 2.12 whereas in asthmatics patients mean eosinophilic count were 7.12.

Mean blood eosinophilic count among COPD patients in this study were 2.79±1.93 whereas in asthmatics patients mean eosinophilic count were 8.64±3.09.

TABLE No. 4: CORRELATION OF SPUTUM AND BLOOD EOSINOPHIL IN COPD

Blood Eosinophil Cutoff Value	Sensitivity	Specificity	Pre+	Pre-	P Value
2%	42.42	100	100	47.22	p<0.001
3%	63.64	100	100	77.78	p<0.001
4%	92.31%	94.59	85.71	97.22	p<0.001

TABLE No. 5: Mean Value Of Various Phenotypes

Diagnosis		PHENOTYPE			
		Eosinophilic	Neutrophilic	Mixed	Paucigranulocytic
FEV1	Mean	45.60	54.00	58.50	66.29
	SD	17.17	18.81	21.86	23.76
TDI	Mean	6.60	5.34	5.00	5.29
	SD	2.50	3.10	3.56	2.43
SI	Mean	309.00	277.24	232.50	267.14
	SD	183.75	133.92	78.90	121.48
Sputum Eosinophil	Mean	6.20	0.59	5.00	1.00
	SD	1.87	0.78	0.82	0.82
Sputum Neutrophil	Mean	55.90	73.38	74.25	59.00
	SD	3.00	8.04	6.50	6.08

*P<0.05 (S); **P<0.001 (HS)

DISCUSSION

The present study was carried out on 50 patients of COPD after exclusion of Tuberculosis, malignancy, bronchiectasis or other confounding inflammatory diseases. These patients were admitted to Department of Respiratory Medicine, TB & Chest Hospital (Badi), RNT Medical College, Udaipur (Rajasthan), India to study the sputum cytology profile in COPD and to correlate it with disease related other factors and spirometry (FEV_1).

In the present study majority of COPD patients (94%) had age more than 40 year and 80% of asthma patients were below 50 years this agrees with previous reports from Gorska et al[8] 2008 in which mean age of COPD patients were 56.8±9.2 years.

In the present study neutrophilic (58%) phenotype is commonest among COPD patients and eosinophilic, paucigranulocytic, mixed were found among 20%, 14%, 8% cases. This was a significant result as GAO P et al[8] also from China in 2012 found neutrophilic phenotype (47.5%) commonest followed by paucigranulocytic (39%), eosinophilic (9%), mixed (5%).[9]

Paucigranulocytic phenotype had highest (66.29% Predicted) mean fev1 and eosinophilic phenotype had lowest mean fev1 (45.60% Predicted) in COPD cases. Whereas Gao P et al[8] also from china 2012 found paucigranulocytic phenotype (68±4% Predicted) having highest and mixed phenotype had lowest (22.8±5% Predicted) FEV1.

Mean sputum eosinophilic count among COPD patients in this study were (2.12±1.6%) whereas in asthmatics patients mean eosinophilic count were (7.12±2.38%).

M.G. Krishna Murthy et al[9] February 2016 evaluated with thirty patients (20 were COPD and 10 patients were asthma) and Mean sputum eosinophilic count among COPD patients were 2.8% and 13.2% in asthmatic patients.

In present study there is negative correlation between sputum eosinophil and FEV1% Predicted in both COPD ($r \geq 0.329$; $p < 0.05$) and asthma ($r \geq 0.313$; $p < 0.05$).

Prescott G. Woodruff et al (San Francisco 2001) found eosinophil percentage in induced sputum is independently associated with lower FEV1 in COPD.[10]

Eosinophilic COPD (eosinophilic and mixed phenotype) 10.6 (OR 10.6) times more associated with exacerbation than Noneosinophilic COPD (paucigraulocytic and neutrophilic). Simon Couillard et al studied on total of 167 COPD patients (55 had eosinophilia) and concluded that the 12-month COPD-related readmission differed significantly between groups OR [eosinophilic vs noneosinophilic] 3.59 [95% CI).[11]

In present study among COPD patients at a threshold of 4% (sensitivity =92.31%, Specificity = 94.59% and Pre + 85.71%, Pre -85.71%, Pre - 97.22%) peripheral blood eosinophil counts enabled identification of the presence or absence of sputum eosinophilia (>3) in 94% of the cases. A threshold of 3% had lesser (78%) classifying ability but better specificity (88.24%). In contrast 2% offered a better specificity (100%) but less sensitivity (42.42%) with 62.00% classifying ability.

Netsanet A Negewo et al[12] 2016 (Australia) studied on 141 COPD patients and concluded that blood eosinophil counts were significantly higher in patients with sputum eosinophilia (n=45) compared to those without ($P<0.0001$). At a threshold of 3% (specificity =76%, sensitivity =60%, and positive likelihood ratio =2.5), peripheral blood eosinophil counts enabled identification of the presence or absence of sputum eosinophilia in 71% of the cases. A threshold of 4% had similar classifying ability but better specificity (91.7%) and higher positive likelihood ratio (3.7). In contrast, 2% offered a better sensitivity (91.1%) for ruling out sputum eosinophilia.

CONCLUSION

Neutrophilic phenotype is predominant phenotype in most of the COPD patients but significant proportion of patients had eosinophilic phenotype. Blood eosinophil counts enabled identification of presence or absence of sputum eosinophilia with reliable accuracy. There is positive correlation between sputum eosinophil and severity of disease (FEV_1) COPD. Specific phenotyping of COPD inflammatory profiles are worthwhile and of clinical importance. The mechanistic pathways behind airway inflammation in COPD and are complex, and clearly there is no one inflammatory cell that is responsible for the spectrum of both disease. Eosinophilic airway inflammation is linked to both exacerbations and decline in lung function. Specific targeting of eosinophilic inflammation may be effective in patients who exhibit an airway eosinophilia.

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