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Original Research Article

Role of Eosinophils in Sputum and Its Correlation with Spirometry in Acute Exacerbation and Remission in Bronchial Asthma and Chronic Obstructive Pulmonary Disease

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Abstract: According to GOLD guidelines 2013, COPD is the 4th leading cause of death. Dyspnea is an important and debilitating symptom for which most patients with COPD seek medical attention. The aim of the present study is to compare the eosinophils in sputum of patients with asthma and COPD. The presence of eosinophilia has been considered typical asthmatic inflammation whereas neutrophils, macrophages and lymphocytes are the most significant inflammatory cells found in the airways of patients with COPD. Thirty patients presenting with airway disorders were prospectively studied in the year of 2012 to 2014. They were evaluated with spirometry and sputum for eosinophils count. Out of the thirty patients 20 were COPD and 10 patients were found to be asthma. In the bronchial asthma group 9 out of 10 have (90%) had elevated sputum eosinophils (>3%) and one patient have not elevated eosinophils levels (10%) mean level in exacerbation of asthma (13.2%), in asthma group during remission 8 patients have elevated sputum eosinophils levels, 2 patients did not have. Mean level is (3.8%). In the COPD group out of 20 patients, 18 patients had elevated sputum eosinophils and two patients have not eosinophils mean level in COPD – (9.8%) exacerbation. In COPD (remission) group 3 patients have sputum eosinophils levels, 17 patients did not have. Mean level is (1.5%).There was no difference in the occurrence of sputum eosinophils levels during exacerbation in asthma than COPD. But mean level of eosinophils were more in asthma than COPD. During remission sputum eosinophils levels was more common in asthma than COPD.
Keywords: Eosinophilia, Bronchospasm, Asthma , SerumIGE, Sputum eosinophil counts, Allergic bronchitis, Interluekin3,Tumour necrosis factor.

INTRODUCTION:

Asthma is an inflammatory, reversible, reactive and progressive disease, notable for episodes of exacerbations and remissions [1]. Eosinophils are an indirect marker of airway inflammation in asthma [2]. Total eosinophils count reflects asthmatic activity and is useful for regulating steroid dosage and for early detection of exacerbations [3]. Eosinophils are currently regarded as the effector cells responsible for much of the pathology of asthma. Eosinophils-mediated damage to the respiratory epithelium is a major pathogenic mechanism in asthma.

The current reviews indicate that the relationship of eosinophils and asthma is very important for diagnosis and management of asthma. There is a need to work on all the aspects of this relationship that includes finding correlation of symptoms of asthma with markers of eosinophil activity, identifying the substances attracting, activating or developing eosinophils and developing drugs to neutralize these substances.

Research Works are now underway to develop asthma therapy leading to inhibition of eosinophil priming of cytotoxic mechanisms in vivo. For the development of more effective anti-asthma drugs it, seems relevant to unravel and interfere with the steps of eosinophil activation. There are now attempts to inhibit eosinophil differentiation at bone marrow level.

It is important to find a marker of disease activity, ideally one that is simple to measure, reliable and inexpensive. As yet no such marker has been found

for asthma. Therefore, there is a need for assessing different eosinophil products to develop a serological marker of airway inflammatory activity in asthma.

Chronic obstructive pulmonary disease is a common condition and a major cause of mortality. COPD is characterized by irreversible airflow obstruction. The physiological abnormalities observed in COPD are due to a combination of emphysema and obliteration of the small airways in association with airway inflammation. The predominant cells involved in this inflammatory response are CD8+ lymphocytes, neutrophils, and macrophages. Although eosinophilic airway inflammation is usually considered a feature of asthma, it has been demonstrated in large and small airway tissue samples and in 20%–40% of induced sputum samples from patients with stable COPD [4]. This airway eosinophilia is increased in exacerbations. Thus, modifying eosinophilic inflammation may be a potential therapeutic target in COPD. Eosinophilic airway inflammation is resistant to inhaled corticosteroid therapy, but does respond to systemic corticosteroid therapy, and the degree of response is related to the intensity of the eosinophilic inflammation. In COPD, targeting treatment to normalize the sputum eosinophilia reduced the number of hospital admissions. Whether controlling eosinophilic inflammation in COPD patients with an airway eosinophilia will modify disease progression and possibly alter mortality is unknown, but warrants further investigation. The aim of the present study is to compare eosinophil levels in COPD and asthma to help in guiding further treatment.

AIM OF THE STUDY:

1. The aim of the present study is to compare the eosinophil levels in the sputum of patients during acute exacerbation and remission of Asthma and COPD, in correlation with spirometry.
2. To compare sputum eosinophils in COPD and Asthma.
3. To know the levels of sputum eosinophils in acute exacerbation and remission of COPD and Asthma.
4. Sputum eosinophil levels in correlation with spirometry

MATERIALS AND METHODS

This was a prospective study conducted on 30 patients who were recruited from outpatient & in patients department of Pulmonary Medicine, Govt. CD & TB Hospital. This study was done at" Government CD & TB Hospital, Hanamkonda between 15-11-2012 to 15-10-2014.

INCLUSION CRITERIA:

1. All age groups above 15 years
2. Asthma & COPD cases based on GOLD, GINA guidelines.
3. Mild to moderate COPD and asthma.

EXCLUSION CRITERIA:

4. H/o Pulmonary Tuberculosis
5. Bronchiectasis
6. Comatose patients
7. PLHA
8. Patient with well documented chronic history of heart failure.

Bronchial Asthma, and/or a History of exposure to risk factors for the disease, such as smoking, fumes, irritants, and dust exposure will be noted. Detailed occupation was enquired to check for any exposure. Patients with prior h/o of tuberculosis will be excluded from this study to avoid effects of post infective bronchitis and bronchiectasis. Routine investigation which included Hb, TLC, DLC, ESR, absolute eosinophils count, Urea, sugar, urine examination will be done. Specific investigation like sputum eosinophils in acute Exacerbation and remission in bronchial asthma and COPD. Sputum sample sent to pathological examination for Eosinophil levels during acute Exacerbation and remission.

Spirometry meeting ATS criteria used – Spirometry will be done in sitting position by the patient. Best of the 3 man oeuvres will be defined as the highest FEV1 and highest PEF regardless from which man oeuvres they come from same or different effort. If medication has been taken within 6 hours PFT was not performed and the visit will be rescheduled.

Reversibility Testing – At the screening visit after completion of three acceptable pre-bronchodilator forced expiratory man oeuvres, all patients will be asked to inhale salbutamol (100µg) so as to document the degree of reversibility. Within 10 minutes of the pre-bronchodilator forced expiratory man oeuvres, two separate doses approximately 30 seconds apart of 100µg of salbutamol (albuterol) will be administered.

Total dose 200µg of Salbutamol will be delivered. The patient will be encouraged to hold his breath for 10 seconds after each inhalation. Three additional acceptable post-bronchodilator forced expiratory man oeuvre tests will be recorded within 10–30mins after the last dose of salbutamol is inhaled. The severity of Asthma as assessed by FEV1 and grouped into Severe, moderate and mild group based on FEV1 classification – Adapted from 2007 NHLBI Guidelines for the diagnosis and treatment of Asthma Expert panel Report 3.

Sputum Smear Cytology:

After demonstration of sputum production Sputum is collected (at least 1ml) in a cup filled with

10-15ml of 50% alcohol and allowed for Fixation for 2-4 hours. Gross physical examination on Petri dish (Gross o/e) is undertaken before Slide is made with 0.1ml material taken with Forceps, Compress & spread after crushing cells (BCZ cells entrapped with mucus). The slide is Fixed with 95% alcohol (for 10-20min) and 2 slides are made and stained with Pap and H & E stain, Examined with 40 X (high Power) for DC and smear graded by differential count.

Procedure for staining and fixing of sputum- Sputum sample is first shaken on a petridish and placed against a dark background. With a curved forceps bits of sputum (e.g. white flakes) are picked up and spread evenly on a clean background. While it is still moist, the slide is immersed into a jar containing fixative solution and fixed for 30 minutes (50% ether + 50% alcohol). Slide is washed under tap water for 5 minutes. Hematoxylin stain is then evenly spread over the slide and kept for 5 minutes. Slide is again rinsed under water. 1% acid alcohol is placed over the slide for differentiation for approximately 8 seconds. Slide is washed under water. Eosin stain is evenly spread over the slide and kept for 15 seconds. Finally slide is washed in alcohol, dehydrated, cleared and mounted under low power and 40 X microscopes. Sputum Eosinophils Seen By Hematoxylin & Eosin Stain.

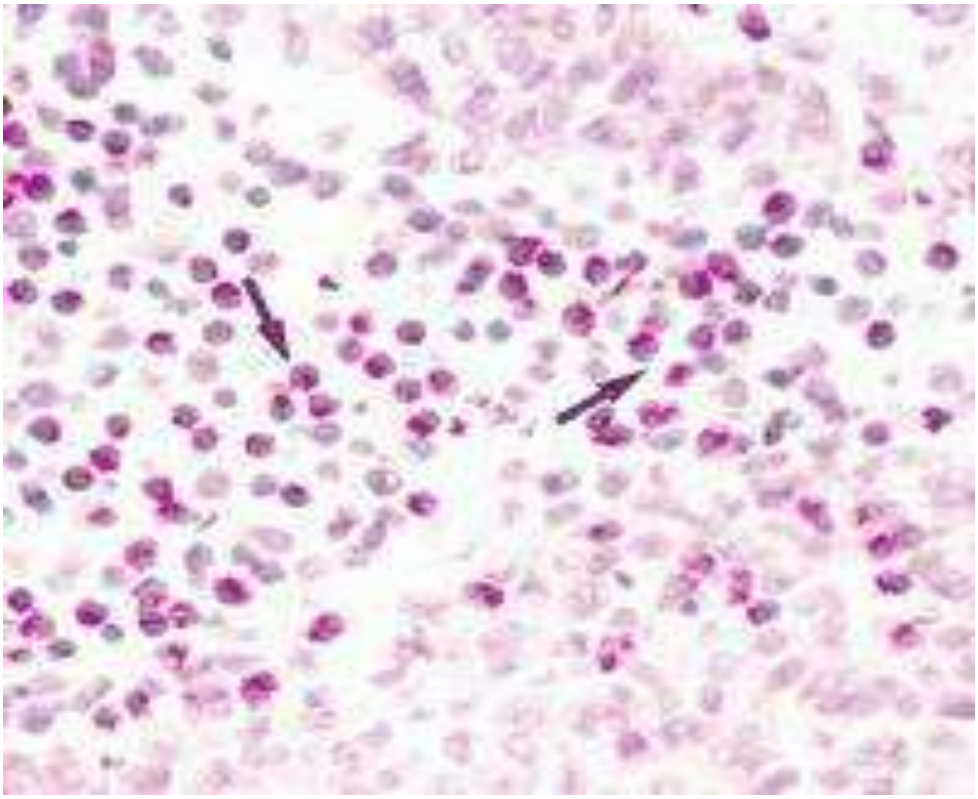

Fig 1: Eosinophilis seen under Light Microscopy

Normal cut off value in Sputum Smear Cytology - Sputum Esonophilis - Cut off value- ≥3%, eosinophilia-positive and <3%, eosinophilia-negative groups.

Sputum Eosinophilis:

Sputum eosinophilia was first described as a feature of asthma by GOLLASCH. There is now strong evidence supporting the association of airway eosinophilic inflammation and symptomatic asthma. Although central, sputum eosinophils and their pro inflammatory mediators are only a part of the heterogeneous inflammatory response that distinguishes asthma from other airway diseases. There is increasing evidence that other inflammatory mechanisms may be involved in producing the two prominent clinical features of asthma: increased bronchial responsiveness and reversible airflow limitation.

The prevalence of non eosinophilic inflammation in asthma is variable and this may be due, in part, to differences in subject characteristics such as the severity and control of asthma, smoking, and dose of corticosteroid treatment, concurrent infection and recent exposure to aggravating environmental allergens or pollutants. Nevertheless, studies highlighted the heterogeneity of airway inflammation in asthma and this cannot always be anticipated from clinical parameters.

Hence knowing the characteristics of airway inflammation of symptomatic or uncontrolled asthmatics would be of interest in accessing the effects of different asthma treatments. This is particularly relevant in relation to corticosteroid treatment, as not all asthmatics respond similarly to these drugs.

Statistical analysis was performed using statistical package for social sciences (SPSS Version 17). Numerical data was entered as such. Categorical data was appropriately coded. Descriptive measures obtained included frequencies, proportions, mean and standard deviation.

Inferential statistics obtained included Chi square test ($\chi 2$), student's t test**,** P- Values. Epidemiological indices obtained included were sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy (DA). It is important to find a marker of disease activity, ideally one that is simple to measure, reliable and inexpensive. As yet no such marker has been found for asthma. Therefore, there is a need for assessing different eosinophil products to develop a serological marker of airway inflammatory activity in asthma.

STATISTICAL ANALYSIS AND DESCRIPTIVE VALUES:

In the present study we evaluated a total of 30 patients, 15 belonged to asthma group and 15 belonged to the COPD group according to their history and spirometry results.

Age Distribution:

This prospective observational study was conducted on 30 patients in Government CD and TB Hospital, Hanamkonda.

Table 1: Age of the study group was ranged from 21-75 years.

Age Group	No. of Patients	Percentage
< 30 years	1	3.333%
31 – 40 years	1	3.333%
41 – 50 years	7	23.333%
51 – 60 years	9	30.000%
61 – 70 years	10	33.333%
> 71 years	2	6.666%
Total:	**30**	**100%**

Out of the total 30 patients evaluated, 3% of cases were less than 30 years of age. Same percentage between 30 and 40 years of age, 23% of cases were in between 41 and 50 years age, 30% between 51 and 60 years, 33% were between 61 and 70 and 6% more than 70 years.86% cases were in between the age group of 41 and 70 years.

From the table-2, of the total 30 patients studied, males constituted 57% of the patients and 43% were females.

Table2: Sex Distribution.

Sex	Total	Percentage
Males	17	56.66%
Females	13	43.33%
Total:	**30**	**100%**

Table 3: Smoking Status.

	Bronchial Asthma	COPD
Present	0	11
Absent	15	4
Total:	**15**	**15**

From the table-3, of the total 30 patients studied, among the 15 COPD patients, 11 gave a positive smoking history, i.e. smoking was an associated risk factor in 73% of COPD patients. Whereas none of the asthmatic patients were smokers. Thus from the present study we infer that smoking is a risk factor in COPD patients and not in asthmatics.

Table 4: Sputum Eosinophils during Exacerbation:

	Bronchial Asthma	**COPD**
Present	15	15
Absent	0	0
Total:	**15**	**15**

From the table-4, of the total 30 patients studied, all the patients included in the study demonstrated eosinophilia in sputum during exacerbation. However the mean eosinophil levels in asthmatics was 14.6% whereas in COPD patients it was much lower (9.86%).Statistical significance P = 1.

Table 5: Sputum Eosinophilia during Remission:

	Bronchial Asthma	**COPD**
Present	14	2
Absent	1	13
Total:	**15**	**15**

From the table-5, of the total 30 patients studied, during remission, 14 patients (93%) out of the 15 asthmatics had sputum eosinophilia whereas among COPD group sputum eosinophilia was present in a 13% cases. The mean eosinophil levels in asthmatic group was 5.6% and in COPD as 1.8%.Statistical significance P = 0.0001.

Table 6: Blood AEC Count:

	Bronchial Asthma	**COPD**
	(Cells / cumm)	**(Cells / cumm)**
Normal	11	15
Elevated	4	0
Total:	**15**	**15**

From the table-6, of the total 30 patients studied, the AEC was elevated in 4 of the 15 asthmatics (26.66%). None of the COPD patients showed an elevated eosinophil count. The mean AEC levels in asthma group were 381 cells/cumm and in COPD group, it was lower at 336 cells/cumm.

Table 7: Pre Bronchodilator FEV1:

Pre FEV1 (in litres)	**Asthma**	**COPD**
< 1 litre	10	9
1.01 – 1.5 litres	2	4
1.51 – 2 litres	0	2
2.01 – 2.5 litres	1	0
> 2.5 litres	2	0

Out of the 30 patients in this study, the minimum prebronchodilator FEV1 was 295ml, the maximum FEV1 was of 2.968 out of the 30 patients in the study. Among the asthmatic group, 10 patients had FEV1 values below 1 litre, 2 patients had between 1-1.5 litre, 1 patient had between 2-2.5 litres and 2 patients had FEV1 values more than 2.5 litres.

Among the COPD group, 9 patients had FEV1 values below 1 litre, 4 patients between 1-1.5 litres, 2 patients between 1.5-2 litres and none of the patients had FEV1 values above 2 litres. The mean pre bronchodilator FEV1 in asthma group was 1.076 litres and in COPD group, it was 0.92 litres. The mean values in smokers among COPD group were 0.89 litres.

Table 8: Post Bronchodilator FEV1

Post FEV1 (in litres)	Asthma	COPD
< 1 litre	8	8
1.01 – 1.5 litres	2	5
1.51 – 2 litres	2	2
2.01 – 2.5 litres	1	0
> 2.5 litres	2	0

The minimum post bronchodilator FEV1 was of 314ml and the maximum FEV1 was of 3.054 litres. Out of the 30 patients in this study, among the asthmatic group, 8 patients had FEV1 values below 1 litre, 2 patients had between 1-1.5 litres, 2 patients had between 1.5-2 litres, 1 patient had between 2-2.5 and 2 patients had FEV1 values more than 2.5 litres.

Among the COPD group, 8 patients had FEV1 values below 1 litre, 5 patients between 1-1.5 litres, 2 patients between 1.5-2 litres and none of the patients had FEV1 values above 2 litres. The mean pre bronchodilator FEV1 in asthma group was 1.372 litres and in COPD group, it was 0.97 litres. The mean difference in FEV1 values pre and post bronchodilator were 296ml in asthma group and 52ml in COPD group.

Table 9: Post Bronchodilator Reversibility:

	Reversibility	Total
Asthma	Present	15
COPD	Absent	15

From the above table, of the total 30 patients studied, reversibility was seen in 15 patients of asthma with bronchodilator therapy, whereas in COPD group 15 patients were not reversible with bronchodilator therapy.

Table 10: Sputum Eosinophilia Comparison with Severity of Airway Obstruction in Stable COPD and Asthma

	Bronchial Asthma		COPD	
	Mild	Moderate	Mild	Moderate
	Persistent	Persistent	(Stage I)	(Stage II)
No. of Patients	7	8	5	10
Mean Sputum Eosinophils	4.5	6.6	0.4	2.5
Mean FEV1% Predicted	82	65	85	71

In our study mean FEV1% predicted in mild bronchial asthma patients were 82, in moderate asthma patient 65 whereas in mild COPD group it is 85 and in moderate COPD are 71. Sputum eosinophilia as a positive correlation with severity of asthma as well as COPD however correlation was more with bronchial asthma than COPD (4.5 versus 6.6 and 0.4 versus to 0.5).

DISCUSSION:

This is based on review of literature and actual results obtained in our study. Higher number of patients in our study belonged to age group between 61 and 70 years which is 33.33%.In our study Males are more effected constituting 57% as compared to females i.e., 43%.In our study 73% of COPD groups are smokers and none in asthma group. Exposure to tobacco smoke might be yet another etiologic factor in sputum eosinophilia in patients with COPD. Animal studies have demonstrated increased migration of eosinophils to the respiratory tract following long-term exposure to tobacco smoke; these cells were mainly recruited to the lumen of bronchi and bronchioles, rather than the lumen of pulmonary alveoli. However, no correlation was found between the severity of smoking and the composition of induced sputum.

In our study sputum Eosinophil levels during exacerbation increased more in Asthma as compared to COPD. This is in concurrence with Fusun Yildiz *et al.;* In our study both COPD and asthma patients pronounced elevated sputum eosinophils levels during exacerbation, however sputum eosinophilia is higher in asthma group when compared to COPD group (14.6% versus 9.86%).Explaining the high eosinophil count in induced sputum of COPD patients is not an easy task. During remission of asthma and COPD groups sputum eosinophilia is present majority of stable asthma patients (93%) as compared to stable COPD patients (13%) which is statistically significant. One of the

hypotheses for the similar cellular composition of induced sputum from patients with asthma and COPD could be the so-called Dutch hypothesis, whereby asthma, chronic bronchitis, and emphysema should be considered as various manifestations of chronic non-specific lung disease (CNSLD) rather than separate disease entities. Intrinsic (patient-related) and extrinsic (environmental) factors account for the pathogenesis of this disease. Both inherited predisposition to allergic reactions and bronchial hyper reactivity are considered the main traits determining the development of the disease, and diffuse bronchial obstruction is a common functional abnormality seen in CNSLD.

In our study Absolute Eosinophil count was elevated 26.66% in Asthmatics, whereas none of the COPD patients showed an elevated Eosinophil count. According to Goraska et al most of the COPD patients were diagnosed with mild disease, with mild symptoms and a relatively short mean duration of symptomatic disease (5 years). Eosinophilia in induced sputum seems to be more common in this patient population. Eosinophils may well play a role in triggering the inflammation in early COPD. This has also been previously suggested by other authors. The possibility of an association between COPD and eosinophilic bronchitis or an overlap syndrome between COPD and eosinophilic bronchitis cannot be excluded.

When a comparison was done between severity of obstruction and sputum eosinophilia, it was found that there was no significant association between severity of obstruction and sputum eosinophilia both in COPD and asthma. In our study mean FEV1% predicted in mild bronchial asthma patients was 82, in moderate asthma patient 65 whereas in mild COPD group it is 85 and in moderate COPD is 71.Sputum eosinophilia as a positive correlation with severity of asthma as well as COPD however correlation was more with bronchial asthma than COPD (4.5 versus 6.6 and 0.4 versus to 0.5).

CONCLUSIONS:

- Eosinophils may play an important role in the respiratory tract inflammation not only in asthma patients but also in some subjects of COPD.
- Sputum eosinophilia more pronounced in stable bronchial asthma during exacerbation when compared to COPD patients.
- Sputum eosinophilia is persistent during remission in bronchial asthma when compared to COPD which may help in differentiating both groups.
- Sputum eosinophilia as a positive correlation with severity of asthma as well as COPD and more pronounced as bronchial asthma.
- Eosinophila are the inflammatory marker for asthma and COPD, hence inhaled steroids is the main stay of treatment for asthma and some extent in COPD.
- However asthma with larger samples size is needed to know the precise role of sputum eosinophilia in understanding asthma and COPD.

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