Original Article

Determination of sputum eosinophil count and serum absolute eosinophil count in patients with bronchial asthma and its correlation with disease severity and response to treatment

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Abstract

Background: Sputum eosinophils can be used to assess severity of disease and response to treatment in bronchial asthma. Eosinophilic inflammation in the airways can also be marked by blood eosinophilia. In this study, we tried to determine the sputum eosinophil count and serum absolute eosinophil count in patients with asthma and correlate them with disease severity and treatment response. **Materials and Methods:** It was a cross-sectional intervention study including all consecutive cases with a diagnosis of bronchial asthma based on spirometry and clinical history. An induced sputum sample and blood were sent for eosinophil count to the laboratory. All the patients were started on inhaled corticosteroids and followed up at the end of 1 month with spirometry, sputum eosinophil count and AEC. Statistical Package for the Social Sciences for Windows v20.0 (IBM SPSS Corp.; Armonk, NY, USA) was used for statistical analysis. **Results:** There was no significant difference in the mean sputum eosinophil count (%) in mild, moderate and severe disease (f = 0.24; *P* = 0.79) or in AEC (f = 1.48; *P* = 0.24). At follow-up, all patients with moderate and severe disease showed significant improvement in FEV1 (*P* = 0.0001). The mean sputum eosinophil count and AEC (%) in the three subgroups was also seen to decrease at the end of the follow-up period (f = 0.08; *P* = 0.9 and f = 2.75; *P* = 0.07, respectively). **Conclusion:** Sputum eosinophils and AEC are important markers of airway inflammation. All our patients showed improvement in FEV1, sputum eosinophil count and AEC after 1 month of treatment thus confirming the role of ICS in the treatment of eosinophilic asthma.

Keywords: Bronchial asthma, eosinophils, induced sputum, spirometry

Introduction

Asthma is a chronic disorder involving the airways. The Global initiative for Asthma (GINA) in 2022 has defined asthma as: "a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms, such as wheeze, shortness of breath, chest tightness and cough, that vary over time and in intensity, together with variable expiratory airflow limitation."[1]

Asthma is the most common chronic respiratory disease with a rising prevalence in the last two decades.[2] In 2019, nearly 262 million people were affected with asthma worldwide with

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about 4.55 lakh deaths.[3] The mortality is still higher in patients presenting with exacerbation.[4] Asthma is also a chronic disease in children less than 18 years of age.[5] The INSEARCH (Indian Study on Epidemiology of Asthma, Respiratory Symptoms and Chronic Bronchitis in Adults) reported 17.23 million cases of asthma in the country with a prevalence of 2.05%.[6] Asthma accounted for nearly 13.2 thousand deaths and 27.9% of the disability-adjusted life years (DALYs) among Indians.[3] A recent study has determined the prevalence of asthma among children to be 7.9% with a higher prevalence among boys.[7]

Currently, asthma severity and control are being assessed by subjective methods including clinical history and quality-of-life questionnaires and objective measures like spirometry and peak expiratory flow rate.[8]

Eosinophilic inflammation in the airways may be marked by blood eosinophilia as has been evidenced by few previous studies.[9,10] This investigation may be very useful in places where induced sputum or bronchoscopy are unavailable. The correlation of eosinophils in blood and airways is driven by multiple inflammatory stimuli, which recruit eosinophils from bone marrow to the blood and finally to the tissues. However, in all previous studies, the threshold level of eosinophil count is variable, thus increasing the confusion.

Sputum eosinophils are also excellent direct markers of airway inflammation. The sputum eosinophil count can be used for assessment of disease severity and treatment response.[11] Sputum eosinophil-guided treatment has shown reduction in exacerbations and improvement in quality of life.[12,13] The recommendation to utilize sputum eosinophil count along with other clinical criteria has already been placed by the European Respiratory Society (ERS)/American thoracic Society (ATS) guidelines.[14] However, unavailability of sputum induction procedure and delay in results restricts its use to certain specific centres.[15]

Aims and Objectives

1. To determine eosinophil count in sputum and absolute eosinophil count (AEC) in serum in patients with bronchial asthma.
2. To correlate the sputum eosinophil count, serum AEC and sputum eosinophil count/serum AEC ratio with severity of asthma.
3. To determine the response of treatment with inhaled corticosteroids in patients and correlate with the spirometry (follow-up), sputum eosinophil count, serum AEC and sputum eosinophil count/serum AEC ratio.

Materials and Methods

The study was conducted in the Dept. of Respiratory Medicine, Kalinga Institute of Medical Sciences, Bhubaneswar, in the period October 2020 to April 2022. It was a cross-sectional interventional study. All consecutive cases with a diagnosis of bronchial asthma based on history and spirometry attending the Department of Pulmonary Medicine and General Medicine were included in the study. The study was approved by the Institutional Ethics Committee (KIIT/KIMS/IEC/429/2020).

Inclusion criteria

Patients with a diagnosis of bronchial asthma based on clinical history and spirometry with the following criteria were included:

(i) >15 years age who never received inhaled or oral steroids previously.
(ii) Normal chest X-ray (CXR).

Exclusion criteria

(i) Clinical features and spirometry suggestive of chronic obstructive pulmonary disease.
(ii) Patients with congestive heart failure.
(iii Patients with other obstructive lung diseases like bronchiectasis.
(iv) Patients with a history of smoking.
(v) Mixed and restrictive pattern of lung function in spirometry
(vi) Pregnancy
(vii) Patient unwilling to give consent; and
(viii)Patient unable to perform spirometry correctly.

Procedure

All consecutive patients admitted to or attending the OPD of the Department of Respiratory Medicine and General Medicine with a diagnosis of bronchial asthma were included in the study. A written informed consent was obtained from all the participants. All patients were evaluated through a detailed history and routine blood investigations. An induced sputum sample was collected from all patients at the time of out-patient visit or before the initiation of any medications in case of admitted patients.

The sample was immediately sent to the laboratory at KIMS for processing. Determination of sputum eosinophil count was performed by H and E (haematoxylin and eosin) stain. The eosinophil count was presented as percentage of total count as it has better accuracy than absolute count. Blood eosinophil count in the laboratory was determined using an automated analyser. The absolute eosinophil count was determined by multiplying the percentage of eosinophils with the total leucocyte count.

All the patients were started on inhaled corticosteroid therapy and followed up at the end of 1 month with a spirometry, sputum eosinophil count and AEC.

Statistical analysis

Continuous variables were presented as mean ± standard deviation (SD). Categorical variables were presented as numbers and percentages. The Chi-square test was used to compare proportions in different groups. Student's *t* test was used to

compare two independent groups. Statistical Package for the Social Sciences for Windows v20.0 (IBM SPSS Corp.; Armonk, NY, USA) was used for all statistical analysis. A *P*-value less than 0.05 was considered statistically significant.

Results

There were 16 male and 35 female patients with a female to male ratio of 2.19:1. The mean age of all the patients was 42.47 ± 18.93 years. Majority of the patients belonged to the age group of 40-60 yrs (20;39.2%) [Table 1]. Cough was the most common complaint at presentation seen in all the patients (100%) [Table 2].

In our study, 28 patients (54.9%) had a family history of bronchial asthma and 21 patients (41.18%) had an associated allergic rhinitis. A total of 24 patients (47.06%) had food allergy.

Based on clinical history and spirometry, 16 patients (31.3%) were found to have mild disease, 13 (25.4%) had moderate disease, and 22 (43.13%) had severe disease. There was no significant difference in the presenting history and clinical presentation in mild, moderate and severe disease [Table 2].

The mean FEV1 (%) in patients with mild disease was 90.25 ± 7.84; 66.46 ± 4.65 and 43.21 ± 14.3 were the mean FEV1 (%) in moderate and severe disease, respectively (f = 89.01; $P < 0.00001$).

There was no significant difference in the mean sputum eosinophil count (%) in mild, moderate and severe disease (f = 0.24; $P = 0.79$). Similar was the scenario in case of the AEC (f = 1.48; $P = 0.24$) [Table 2]. The sputum eosinophil count and AEC showed a significant correlation in the linear regression model [Figure 1].

All the patients were followed up for 1 month. The mean FEV1 (%) in the three subgroup of patients were determined (f = 5.6; $P = 0.0067$) [Table 3]. The improvement in FEV1 was statistically significant in patients with moderate and severe disease ($P = 0.0001$), but not in patients with mild disease ($P = 0.3$) [Table 4].

The mean sputum eosinophil count (%) in the 3 subgroups was also seen to decrease at the end of the follow-up period (f = 0.08; $P = 0.9$). The reduction in eosinophil count was significant in patients with mild and moderate disease ($P = 0.018$ and 0.03 respectively) but not in those with severe disease ($P = 0.076$).

The absolute eosinophil count (cells/μl) also showed decrease in three groups at the end of follow-up period (f = 2.75; $P = 0.07$). The reduction was significant in patients with mild ($P = 0.002$) and moderate ($P = 0.036$) disease but not in patients with severe disease ($P = 0.056$).

Table 1: Age and sex distribution

Age Group (years)	Male	Female	Total	%
≤20	4	6	10	19.6
21-40	6	8	14	27.5
41-60	3	17	20	39.2
>60	3	4	7	13.7

Table 2: Clinical features based on severity of disease

			Severity of asthma			*P*
			Mild	Moderate	Severe	
Cough	Y	*n*	16	13	22	
		%	100.0%	100.0%	100.0%	
SOB	N	*n*	2	3	3	0.694
		%	12.5%	23.1%	13.6%	
	Y	*n*	14	10	19	
		%	87.5%	76.9%	86.4%	
Chest pain	N	*n*	1	3	7	0.165
		%	6.3%	23.1%	31.8%	
	Y	*n*	15	10	15	
		%	93.8%	76.9%	68.2%	
Wheeze	N	*n*	1	1	2	0.949
		%	6.3%	7.7%	9.1%	
	Y	*n*	15	12	20	
		%	93.8%	92.3%	90.9%	

Discussion

This study involved 51 consecutive patients of bronchial asthma to determine their sputum eosinophil count and absolute eosinophil count of blood and correlate them with disease severity and outcome at the end of 1 month.

Our study had a female to male ratio of 2.19:1. Females have a higher prevalence of asthma than males up to the 5th decade; the gender gap may narrow after this.[16] The role of sex hormones, genetic and epigenetic variations, environmental, and social factors have been implicated in this gender variation.[17] A female preponderance of the disease has been reported previously.[18]

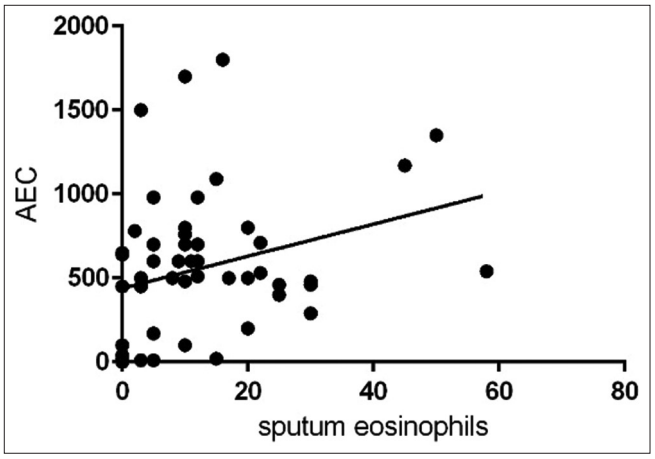

Figure 1: Linear regression graph showing correlation between AEC and sputum eosinophils at presentation ($P = 0.03$)

Table 3: Mean FEV1, AEC and sputum eosinophils at the time of presentation

	Mild (*n*=16)	Moderate (*n*=13)	Severe (*n*=22)	*P*
Mean FEV1 (%)	90.25±7.84	66.46±4.65	43.21±14.3	<0.0001
Mean AEC (cells/µl)	425±244.24	551.54±376.18	664.55±537.1	0.24
Mean Sputum eosinophil count (%)	12.3±9.9	14.84±16.28	11.68±13.45	0.79
Sputum eosinophil count/AEC	0.287±0.028	0.1224±0.23	0.0319±0.06	0.08

Table 4: Mean FEV1, AEC and sputum eosinophils at the time of follow-up

	Mild (*n*=16)	Moderate (*n*=13)	Severe (*n*=22)	*P*
Mean FEV1 (%)	94.23±13.46	79.63±8.27	67.27±19.1	0.0067
Mean AEC (cells/µl)	178.3±167.56	286.54±206.4	394.09±356.54	0.07
Mean sputum eosinophil count (%)	4.87±5.05	4.15±4.45	5.27±10.15	0.9
Sputum eosinophil count/AEC	0.099±0.254	0.03±0.0678	0.0093±0.135	0.177

Asthma can present in any age group. The mean age of the patients in our study was 42.47 ± 18.93 years. However, the age of presentation is observed to be similar in several studies.[19] Patients, particularly women in the age group of 35–55 years report poor physical and social functioning and thus access healthcare facilities.[20]

A diagnosis of asthma is supported by the presence of a positive family history and a history or family history of allergic rhinitis or eczema. However, they may not be found in all patients of asthma.[11] Allergic rhinitis (AR) may co-exist with asthma. The prevalence of AR in asthma in the USA and Europe was greater than 50% and nearly 100% in patients with allergic asthma. Patients with concomitant disease also had higher number of visits to the doctor or emergency department and absence from work and lower productivity.[21] Our study had a positive family history in 50.98% patients, and AR was present in 41.18% patients.

Eosinophilic asthma has been recognized as an important subphenotype of asthma associated with increased asthma severity, atopy and late onset of disease.[22] Induced sputum differential count is considered as the gold standard in diagnosing eosinophilic airway inflammation, though other noninvasive biomarkers have now been made available.[22] Adult-onset eosinophilic asthma may develop in the absence of any allergen that points at some novel pathways involved in the activation of TH2 response. The role of innate lymphoid cells has been documented.[23] Among the 51 patients in our study, 41 (80.4%) were detected to have eosinophilic asthma based on their sputum eosinophil count (>3%).

The mean sputum eosinophil count was seen to increase with the severity of disease though this trend was not statistically significant ($P = 0.79$). This was supported by previous studies.[11,24]

Blood eosinophilia lacks sensitivity and specificity in the diagnosis of eosinophilic asthma though it can be obtained easily. A direct correlation between blood eosinophil count and symptom severity and a negative correlation with FEV1 have been seen in previous study.[25] Our study did not show significant correlation between AEC and disease severity.

The correlation between blood eosinophils and sputum eosinophil counts has provided varied results. Pignatti *et al.* showed that blood eosinophil count correlated with sputum eosinophil count in bronchial asthma and COPD, whereas Hastie *et al.* did not see any significant correlation.[26,27] Our study has shown a significant correlation between the two variables in the linear regression model ($P = 0.038$).

Usually, patients with eosinophilic asthma respond well to inhaled corticosteroids although some may be 'steroid refractory'.[28] Corticosteroids enter the nuclear DNA of eosinophils after binding to the glucocorticoid receptors on the cytoplasm. They alter the transcription of target genes for certain cytokines (IL-3,5 and GM-CSF) thus increasing eosinophil apoptosis. Hence, it decreases eosinophilic bronchial inflammation and eosinophils in sputum.[29] Our patients were followed up for a period of 1 month after being advised inhaled corticosteroids (ICS). Other supportive medications were added as needed. The FEV1, sputum eosinophils and AEC showed decline at follow-up. This was supported by previous studies.[11,29,30]

Our study showed that mean AEC increased with severity of disease although no such trend was seen with sputum eosinophil counts. After initiation of ICS for 1 month, there was a decrease in both AEC and sputum eosinophil counts along with improvement in spirometry values.

Spirometry is unavailable at many peripheral centres. It is subjective and the interpretation requires trained staff. These simple tests like sputum eosinophil count and absolute eosinophil count will not only help in diagnosis of eosinophilic asthma but also help in the follow-up of these patients.

Conclusion

Thus, sputum eosinophils may be important markers of airway inflammation, but they are not good markers of disease severity.

Similar is the result in case of AEC. All our patients showed improvement in FEV1, sputum eosinophil count and AEC after 1 month of treatment, thus confirming the role of ICS in treatment of eosinophilic asthma. Our study was carried out over a limited time and on a restricted number of patients. We recommend more comprehensive prospective studies involving larger population size to determine the correlation between sputum eosinophils, AEC and severity of disease in cases of asthma.

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Conflicts of interest

There are no conflicts of interest.

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