Significance of assessment of sputum eosinophilia in bronchial asthma

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ABSTRACT

Introduction:

Eosinophilic airway inflammation is an important distinguishing characteristic feature of bronchial asthma in adults. To assess this type of airway inflammation, sputum eosinophilic counts are generally considered to be the gold standard. Treatment guided by sputum eosinophilic count reduces the frequency of asthma exacerbation.

Aims and objectives:

The main aims are to know the percentage of Bronchial asthma subjects who have increased eosinophilic count and to compare the eosinophilic count in different severity groups of bronchial asthma, in smokers and non-smokers with asthma.

Materials & Methods:

This is cross sectional observative study conducted from January to june 2016 in Prathima Institute of Medical Sciences on 45 patients who have symptoms suggestive of bronchial asthma. A blood eosinophil count, absolute eosinophil count, sputum neutrophil and eosinophil count and spirometry was done on each case in the Department of Pathology in collaboration with Department of Pulmonology of Prathima Institute of Medical Sciences. More than 18yrs of age are taken up in this study.

Results and Conclusion: Cases of moderate, severe and acute exacerbation of asthma showed significantly high eosinophil count (p-value <0.05) than controls. Cases of mild asthma and smokers showed no significant rise in eosinophil count (p-value >0.05) than controls. Smokers had a low mean sputum eosinophil count and a high mean neutrophil count than controls.

Keywords: Bronchial asthma, Sputum , Eosinophil count

INTRODUCTION

Asthma is now recognized as one of the most important chronic conditions in the world, resulting in considerable morbidity and, in some cases, mortality, and posing a high level of burden on Health services and economies worldwide[1]. While high-income countries are acknowledged to have the highest prevalence, the rate of asthma is also increasing in other countries, possibly as a result of adopting westernized lifestyles[2]. The World Health Organization(WHO) has estimated that 15 million disability-adjusted life years are lost annually due to asthma, representing 1% of the global disease burden[3]. The definition of asthma provided by the current guidelines has not changed significantly in the last two decades and indicates that it is a chronic inflammatory disorder of the airways characterized by variable airway obstruction and airway hyper responsiveness, which manifest in the form of various respiratory symptoms - dyspnea, wheezing, cough, chest tightness and phlegm production. According to unidimensional classification Asthma can be classified as Eosinophilic Asthma and Non-Eosinophilic asthma. Eosinophilic asthma is defined by the demonstration of greater than normal eosinophil numbers in the lower airways. This is quantified in population-based studies using the noninvasive technique of sputum induction as >2% of the viable sputum cell count[4].

Asthma is a chronic inflammatory disorder of the airways in which many cells & cellular elements play a role. The chronic inflammation is associated with airway hyper responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness & coughing particularly at night or in the early morning. These episodes are usually associated with airway obstruction that is often reversible either spontaneously or with treatment. Asthma affects an estimated 300 million individuals worldwide. It is a serious global health problem affecting all age groups, with increasing prevalence, rising treatment costs & rising burden for patients and community. Asthma is characterized by variable airflow limitation, which is validated by spirometry or measurements of airway inflammation[5]. Airway inflammation, which is traditionally considered to be eosinophilic treated by anti-inflammatory medications of which corticosteroids are the most effective[6,7].

At present, the airway inflammation is only objectively measured in clinical practice in a few academic centres. The most comprehensive measurement of airway inflammation is spontaneous or induced sputum cell counts this measurement has become established worldwide in research. The sputum cell count is noninvasive and has excellent reliability, validity

and responsiveness. Its application in research has emphasised the heterogeneity of airway inflammation in each of the common airway conditions of asthma, chronic obstructive pulmonary disease (COPD) and chronic cough. Recent European Respiratory Society/American Thoracic Society guidelines on severe asthma recommend sputum eosinophilic counts combined with clinical criteria to guide asthma therapy[8].

MATERIALS & METHODS

Inclusion criteria for cases are age > 18 years, symptoms suggestive of bronchial asthma, acute exacerbation of asthma, smokers and nonsmokers. Exclusion criteria for cases are clinical features & spirometry suggestive of COPD, patients who were unsuccessful in giving sputum either normally or by induction. Inclusion criteria for controls are no nasal or chest symptoms, no past history of asthma or other chronic respiratory diseases, not a previous or current smoker, normal spirometry (FEV1 > 80% predicted, ratio of FEV1 to vital capacity [FEV1/ FVC] > 80%).

Severity groups :

Table 1: Severity groups of asthma.

	MILD	MODERATE	SEVERE
Daytime Symptoms	Twice a week but < once a day	Daily	Daily and often
Effect on activity	None	Difficult to sleep	Disrupt sleep
Nocturnal symptoms	Twice a month but < once a week	Once a week	Daily
Spirometry (FEV_1)	60-80%	40 – 60%	< 40%

Assessment of cases :

Assessment was done based on following :

- Presence of respiratory symptoms
- Severity of symptoms
- Spirometry (FEV1)
- Exposure to allergens/ allergic history
- Family history
- Treatment history
- Smoking history

Acute Exacerbation :

Episodes of progressive increase in shortness of breath, cough, wheezing or chest tightness.

Sputum induction in cases and controls :

Hypertonic saline solution was nebulized with an ultrasonic nebulizer (Sirius; Technomed; Firenze, Italy) with a 2.8 mL/min output, and was inhaled for 5 min periods for up to 30 min. NaCl solution was increased at intervals of 10 min from 3 to 4 to 5%. Every 5 min after the start of nebulization, subjects were asked to rinse their mouth, discard saliva, and cough sputum into a clean container.

Sputum processing in cases :

Spontaneous or induced sputum was collected into a wide mouthed container. In the labaratory sputum was transferred to the petridesh. Whitish dense threads or streaks representing mucus was choosen. Sputum were finely disturbed over two microscopic slides using metal spatulas. The smears were air dried and stained with leishman or May-Grunwald-Giemsa methods. Two hundred cells were counted under oil immersion objective from randomly selected fields. Macrophage, lymphocyte, neutrophil, and eosinophil were expressed as percentages of total inflammatory cells excluding squamous cells.

RESULTS

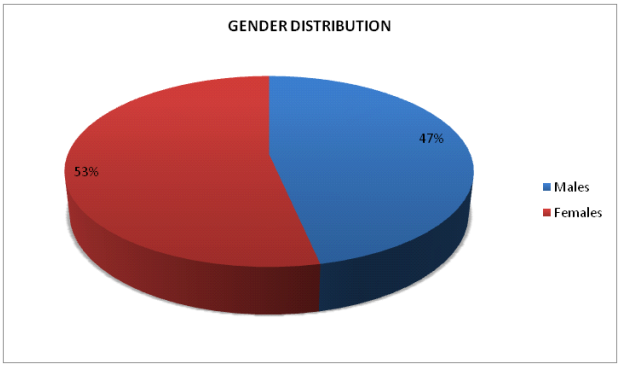

Fig 1 : Pie chart showing distribution of cases in males and females.

Table 2: Age distribution of cases.

AGE GROUP OF STUDY	NO.OF CASES
18-28 years	09 (20%)
29-38 years	08 (18%)
39-48 years	13 (29%)
49-58 years	06 (13%)
59-68 years	05 (11%)
69-78 years	04 (09%)
Mean age in the study : 44 years	Total cases : 45

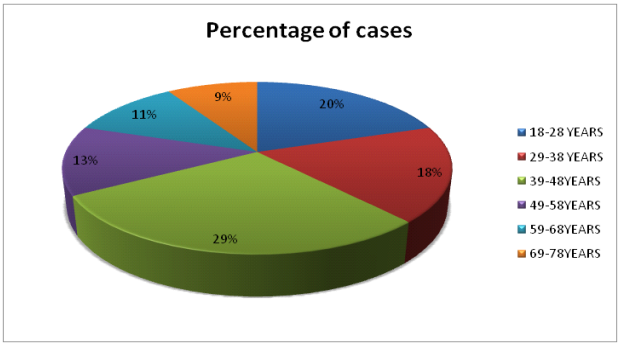

Fig 2: Pie chart showing age distribution of cases.

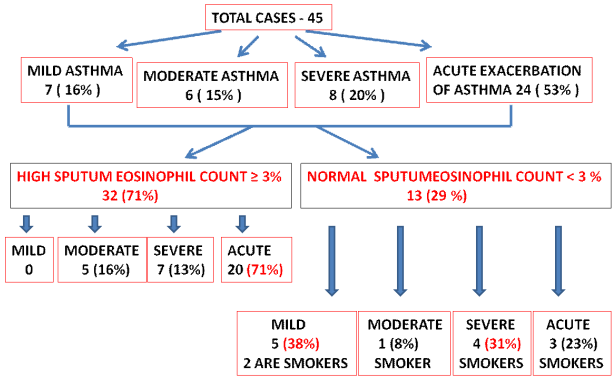

Fig 3: Flow chart of distribution of cases and their results

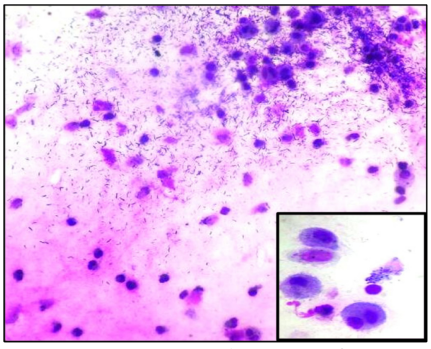

Fig 4: Geimsa stained sputum showing plenty of eosinophils.(100x)

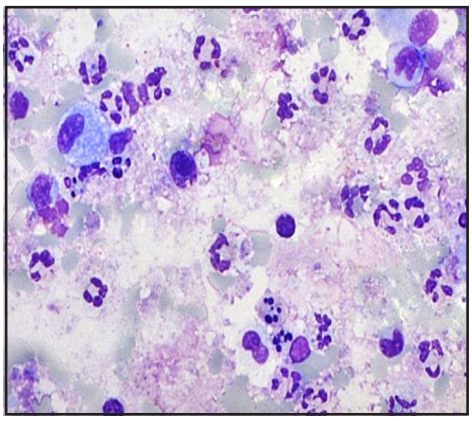

Fig 5: Leishman stained sputum of smoker showing plenty of neutrophils (100x)

Table 3: Mean ± SD for all parameters of sputum in controls, mild, moderate, severe, acute exacerbation of asthma and smokers.

PARAMETERS	CONTROLS	TOTAL CASES - 45				
		MILD	MODERATE	SEVERE	ACUTE EXACEBATION	SMOKERS
NUMBER OF SUBJECTS	20	7	6	8	24	10
		MEAN ± STANDARD DEVIATION				
EOSINOPHIL COUNT	1.8 ± 0.9	2.2 ± 0.6	13.8 ± 2.3	14.5 ± 6.2	20.2 ± 11.6	2 ± 0.9
NEUTROPHIL COUNT	65.2 ± 7.7	76.6 ± 21.1	77.6 ± 3.7	79.4 ± 16.7	72 ± 16.3	94.7 ± 6.2
LYMPHOCYTE COUNT	44.7 ± 11	37 ± 9.4	28.3 ± 3.8	30.6 ± 5.6	34.1 ± 5.6	36.2 ± 9.5
MACROPHAGES	88.3 ± 12.8	79.8 ± 21.2	80.1 ± 2.8	75.5 ± 16.8	75.5 ± 16.8	64.6 ± 9.2

It was observed that mean sputum eosinophil count (%) of control, mild asthma & smokers was at a lower level with a count (%) < 3 % & at a higher level in moderate, severe & acute exacerbation of asthma with a count (%) = 3 %. It was also observed that mean neutrophil count (%) was at a higher level i,e; > 80% in smokers. Mean macrophage count (%) was at higher level i.e; 75 -95 % in controls (Table 1).

Table 4: By unpaired 't' test, P-value is determined from mean values of sputum eosinophil count in different severity groups, smokers and controls.

EOSINOPHILS	Z - VALUE	P - VALUE	
MILD vs CONTROL	1.55	0.06	NOT SIGNIFICANT
MODERATE vs CONTROL	13	<0.00001	SIGNIFICANT
SEVERE vs CONTROL	6	<0.00001	SIGNIFICANT
ACUTE EXACERBATION vs CONTROL	7	<0.00001	SIGNIFICANT
SMOKERS vs CONTROL	1.51	0.0655	NOT SIGNIFICANT

P-value is significant in moderate, severe and acute exacerbation cases when compared to controls indicating that mean sputum eosinophil count is significantly raised in different severity groups when compared to controls.

Table 5: By unpaired 't' test, P-value is determined from mean values of sputum neutrophil count in different severity groups, smokers and controls.

NEUTROPHILS	Z - VALUE	P - VALUE	
MILD vs CONTROL	1.17	0.121	NOT SIGNIFICANT
MODERATE vs CONTROL	4.33	<0.0121	SIGNIFICANT
SEVERE vs CONTROL	2.22	0.0132	SIGNIFICANT
ACUTE EXACERBATION vs CONTROL	1.58	<0.057	NOT SIGNIFICANT
SMOKERS vs CONTROL	9.24	<0.00001	SIGNIFICANT

P-value is more significant in smokers when compared to controls indicating that mean sputum neutrophil count is significantly raised in smokers when compared to controls.

DISCUSSION

A continuous increase in the number of people with asthma worldwide over the next two decades has been predicted such that there may be an additional 100 million people with asthma by 2025[3]. This will largely result from increases in the proportion of the world's population living in urban areas, in particular in low- to middle-income countries. It is likely, therefore, that without improved access to treatments, hospitalizations and mortality from asthma are likely to increase.

Two biomarkers of eosiniphilic asthma have been widely utilized in both research and clinical settings :

1) The sputum eosinophilic count, obtained with sputum induction; and

2) The fractional exhaled nitric oxide (FeNO).

Of these, the sputum eosinophil count is widely accepted as the gold standard with the strongest evidence base[9]. Only sputum eosinophilic counts have been reliably shown to guide corticosteroid therapy for reducing the frequency of severe asthma exacerbations[10].

In our study 47% cases are males and 53% are females with mean age of 44 years.

Asthma is a chronic airway disorder characterized by an ongoing inflammatory process in which eosinophils play a major role with eosinophilic asthma accounting for 25-55% of patients. According to unidimensional classification Asthma can be classified as Eosinophilic Asthma and Non-Eosinophilic asthma. Such differentiation into Eosinophilic & Non-eosinophilic is important because it has therapeutic implications as patients with non-eosinophilic inflammation respond poorly to treatment with inhaled corticosteroids. In our study, we divided asthma patients into two groups according to sputum eosinophil count, eosinophilic (sputum eosinophil count = 3%) and non-eosinophilic (sputum eosinophil count < 3%). It was observed that mean sputum eosinophil count (%) of control, mild asthma & smokers was at a lower level with a count (%) < 3 % (non-eosinophilic group) & at a higher level in moderate, severe & acute exacerbation of asthma with a count (%) = 3 % (eosinophilic group). which is similar to the study of eosinophilic airway inflammation and prognosis of childhood asthma by Lovett, et al [11].

It was observed that out of 45 cases 32 cases i.e; 71% were showing high sputum eosinophil count and remaining 13 cases i.e; 29% were showing normal eosinophil count. In 32 cases showing high eosinophil count, 20 cases i.e; 71% belonged to acute exacerbation of asthma & 0 cases i.e; 0% belonged to mild asthma. In 13 cases showing normal eosinophil count, 5 cases i.e; 38% belonged to mild asthma & 4 cases i.e; 31% belonged to severe asthma all the 4 cases being smokers showing high neutrophil count. Our study was similar to that of CJA Duncan's study & Bartoli, et al., who showed higher sputum eosinophil count was associated with increased severity of asthma as assessed by induced sputum analysis [12,13]. According to Bartoli, et al., the assessment of asthma severity according to clinical and functional findings corresponds to the severity of eosinophilic airway inflammation as assessed by induced sputum analysis[14].

In our study, mean macrophage count (%) was at higher level i.e; 75 -95 % in controls. It is similar to study done by Belda et al,. who identified a normal range of cell counts in induced sputum for nonsmoking healthy adults. They show that the majority of the cells are neutrophils and macrophages, whereas eosinophils, lymphocytes, and bronchial epithelial cells are scarce and metachromatic cells (basophils/mast cells) are almost absent[13].

In 13 cases showing normal eosinophil count, 10 were smokers constituting 75% who had a low mean sputum eosinophil count (<3%) and a high mean neutrophil count (> 80%). Smokers had a low mean sputum eosinophil count (< 3%) and a high mean neutrophil count (> 80%). This finding was similar to the study done by Hester vander vaart et al,. smoking induced a greater Interleukin-8 release from stimulated blood cells and a greater increase in sputum neutrophils & lymphocytes having no significant effect on the percentage and number of sputum eosinophils and macrophages[15].

Use of sputum cell counts to improve treatment was provided by a recent report by Green et al. The group performed a single-centre randomized controlled trial with a 1-yr duration in 74 patients with corticosteroid-dependent asthma. They compared the efficacy of treatment to reduce exacerbations when this was monitored by symptoms and spirometry in one arm versus these indices and sputum eosinophils (to be kept < 3%) in the other arm of the study. The sputum eosinophils were used to guide corticosteroid treatment. During the study, if control was maintained for 2 months, a further attempt to reduce corticosteroid treatment was made. There were a large number of severe exacerbations but these were three-fold less in the sputum arm [16,17,18].

These observations support the role of sputum cell counts in the management of moderate-to-severe asthma and confirm different patterns of airway inflammatory response at exacerbations that have different causes and therapeutic implications. However, when treatment strategy is aimed at keeping sputum eosinophils low, patients have fewer asthma exacerbations. Thus, high sputum eosinophils may predict higher exacerbation probability and be a marker of less well-controlled asthma, more responsive to inhaled corticosteroid treatment. First, the use of sputum cell counts greatly reduces the risk for an eosinophilic exacerbation and prolongs the time for patients to be free of an exacerbation. Second, patients who are more likely to benefit from monitoring of sputum cell counts are those with moderate-to-severe asthma and those who require and are maintained on long-acting b2-agonists.[19]

CONCLUSION

Our study observed that out of 45 cases 32 cases i.e; 71% were showing high sputum eosinophil count and remaining 13 cases i.e; 29% were showing normal eosinophil count. In 32 cases showing high eosinophil count, 20 cases i.e; 71% belonged to acute exacerbation of asthma & 0 cases i.e; 0% belonged to mild asthma. In 13 cases showing normal eosinophil count, 5 cases i.e; 38% belonged to mild asthma & 4 cases i.e; 31% belonged to severe asthma all the 4 cases being smokers showing high neutrophil count. By these findings our study conclusively proved that sputum eosinophil count is a simple, inexpensive, easy and noninvasive tool to assess asthma severity in day to day practice. So, modern guidelines of asthma management should include measurement of sputum eosinophil count in all asthmatics at initial and if possible in all successive follow up visits. In conclusion, on the basis of presented data it can be hypothesized that sputum eosinophil count serve as an Excellent Biomarker of airway inflammation as well as marker of disease severity. Sputum eosinophil count (%) can also be used for predicting control status of asthma.

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