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Original Research Article

A study on sputum cytological phenotypes among bronchial asthma patients during exacerbations in a tertiary care center

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Abstract

Background: Asthma is a chronic reversible airway disease which is emerging as an important public health problem in era of raising air pollution. There is diversified population of patients from children, adults to advanced ages who are victims of bronchial asthma. It has been a regular practice in modern day medicine to perform a spirometry to assess the severity of asthma. In the recent times, more interest has been drawn towards the sputum phenotypes in better understanding of pathogenesis of asthma. Our study aims at looking into spirometric evaluation in correlation with sputum cytological phenotypes.

Methodology: Patients attending the chest clinic with clinical features of exacerbation of bronchial asthma above 18 years of age are taken into the study. Chest X-ray is taken rule out pneumonia, tuberculosis and pulmonary infiltrate with eosinophilia syndrome. They are subjected to perform spirometry with post bronchodilator response to confirm the diagnosis of asthma and severity of asthma classified as per GINA guidelines. Sputum samples of these patients are taken to determine their phenotypes.

Results: A total of 135 patients participated in the study. Out of which 22 patients were not taken into the study considering inability to perform a spirometry and some of them were excluded as they submitted salivary sample as they were found to be salivary samples and some of them could not perform a satisfactory spirometry test. A total of 113 of sputum samples were accepted for cytological examination. Among these patients, 49% were male patients and 50% were female patients. The common cellular phenotypes identified was Eosinophlic with 44.2%, neutrophilic occupying43.4% and lymphocytic with 2.7% and no specific cell types in some samples (9.7%). Among the severe persistent type of asthma, most of patients had neutrophils in their sputum cytology (59%). Milder forms of asthma patients mostly recovered with eosinophil in their sputum cytology reports (40%).

Conclusions: Even though asthma was thought to be a disease which invites eosinophil in the airway, there is a switch over to neutrophilic type of asthma in severe forms of asthma. Sputum cytology among patients with asthma exacerbations may a add on and guiding tool in addition to spirometry in the management of asthma exacerbations.

Introduction

Asthma is a characterised by chronic airway inflammation and defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary in time and intensity together with variable airflow limitation. Asthma is identified as one airway disease where there is concurrent occurrence of allergic rhinitis and even asthma-COPD overlap syndrome [1]. Asthma-COPD overlap syndrome characterised by persistent airflow limitation with the features of both asthma and COPD [2]. Spirometry is a part of routine investigation carried out in outpatient department in the management of asthma. To assess the type of exacerbation, sputum cytology is a simple non-invasive test. Understanding the type of exacerbation and population of cells in sputum cytology may guide in the treatment of bronchial asthma. The GINA guidelines also emphasises the need of sputum cytology as a parameter in the management of asthma –COPD overlap syndrome [3]. The pathogenetic pathway of asthma travels through Th2 driven pathway which recruits eosinophil in the airway, but there prevails some varied pathogenesis occurring in the airway of chronic persistent form of asthma inviting neutrophils and other granulocytes in the airway [4]. Recent trend in the management of asthma involve not only clinically but questions the role of the sputum endotypes in the management of asthma. This study tries to correlate the

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sputum cytological phenotypes with severity of airflow obstruction with spirometry analysis.

Materials and Methods

This study was conducted at the chest clinic in Saveetha Medical College Hospital outpatient department.

Inclusion criteria

Those adult patients (>18 yrs.) presenting to the chest clinic with clinical features of asthma presented during exacerbation with more sputum production and wheeze were included into study after obtaining an informed consent.

Exclusion criteria

1. Smokers were excluded from the study as neutrophils may be present normally in their sputum leading to bias.
2. Patients with active pulmonary tuberculosis, underlying pneumonia, pneumothorax, recent history of myocardial infarction.
3. Sputum samples which are non eosinophilic type where sputum count (<2%eosinophils and 60%neutrophils) as it may lead to bias.

A chest X-ray was done to rule out underlying tuberculosis, pneumonia, and pneumothorax.

Spirometric evaluation is done to confirm the diagnosis of asthma with post bronchodilator reversibility testing and their severity of obstruction is estimated as per the recommendation of GINA guidelines. The patients are requested to submit sputum for cytological analysis. If they cannot bring out sputum, induction of sputum is done to collect a sufficient amount of mucus from the lower airways in order to study the features of inflammation in asthma.

The sputum was gently mixed and placed in an incubator at 37.0 c for 2 hours and was centrifuged at 2000 rpm for 10 minutes. The slides were prepared and fixed with ethanol and stained with Haematoxylin, eosin and pap stain. The slides were subjected to microscopic examination and Cellular components were counted in 20 fields. The percentage composition of every type of cell: bronchial epithelium, macrophages and leucocytes were analysed for Differential count. Sputum studied were grouped under the following categories.

1. Neutrophilic predominant (>61%)
2. Eosinophilic (>2%)
3. Non-eosinophilic (<2%eosinophils and 60%neutrophils)

The study was conducted after obtaining ethical clearance from the institution's ethical committee.

Results

A total of 135 patients were eligible to take part in the study. Out of which 10 sputum samples were non eosinophilic type were excluded, 6 patients were not able to perform spirometry and withdrawn from the study and 6 patients had submitted salivary samples and not representative from lower respiratory tract and hence discarded. A total of 113 patients were taken up into the study with informed consent and satisfied the inclusion criteria for the study. Among the study groups, there were equal representations from both sexes (male-56, female-57) (Table 1). On comparison of age with asthma severity, it is realized that mean average age among asthma patients were in the range of 35- 40 years of age spread among various groups based on severity of asthma ($p<0.005$). Among these asthma patients, most of them (66%) (Table 1) came up with a positive history of atopy in the form of allergic rhinitis, sinusitis and eczema which is much anticipated association among these patients. On comparing the severity of asthma with atopy (table 2), it is seen that atopy is commonly distributed across various forms of asthma based on the severity.

Among the sputum cytological phenotypes, 3 different cellular population were identified during asthma exacerbations. Common cell type recovered from the sputum during asthma exacerbations were eosinophilic predominant (44%), neutrophilic (43%), and lymphocytes (2.7%) (Table 1). (Fig. 1-4) All patients who underwent spirometric analysis to grade the severity of their asthma were distributed almost equally among all groups based on the severity in spirometry. But most of our patients were falling under mild persistent type of asthma (41%). In comparison of sputum cytological phenotypes with severity of asthma, (Table 2), it is realized that eosinophils were the predominant cell among mild persistent asthma. Neutrophils were the predominant cell type among patients with severe forms of asthma.

One-way ANOVA test is done to find if there is significant difference among severity of asthma with various age groups of patients. It is realized that P value shows statistical significance among various group with the ages.

Table 1: Frequency table showing the demographic profile among asthma patients during exacerbations

Variables	**Gender**			**Atopy**		
Answers	**Male**	**Female**	**Total**	**Atopy +**	**No atopy**	**Total**
N	56	57	113	75	38	113
%	49.60%	50.40%	100.00%	66.40%	33.60%	100.00%

Cell type					**Asthma Grading**			
Eosinophilic	**Lymphocytic**	**Neutrophilic**	**No. cells**	**Total**	**Mild**	**Moderate**	**Severe**	**Total**
50	3	49	11	113	41	37	35	113
44.20%	2.70%	43.40%	9.70%	100.00 %	36.30%	32.70%	31.00 %	100.00%

Gender	Asthma Grading					
	Mild		Moderate		Severe	
	N	%	N	%	N	%
Male	21	37.5%	18	32.1%	17	30.4%
Female	20	35.1%	19	33.3%	18	31.6%
Total	41	36.3%	37	32.7%	35	31.0%

Table 2: Comparison of atopy among patients with severity of asthma

Atopy	Asthma Grading					
	Mild		Moderate		Severe	
	N	%	N	%	N	%
Atopy +	24	32.0%	29	38.7%	22	29.3%
No atopy	17	44.7%	8	21.1%	13	34.2%
Total	41	36.3%	37	32.7%	35	31.0%

Table 3: Comparison of severity of asthma with cell type

Cell type	Asthma Grading					
	Mild		Moderate		Severe	
	N	**%**	**N**	**%**	**N**	**%**
Eosinophilic	20	40.0%	25	50.0%	5	10.0%
Lymphocytic	1	33.3%	1	33.3%	1	33.3%
Neutrophilic	13	26.5%	7	14.3%	29	59.2%
No cells	7	63.6%	4	36.4%	0	0.0%
Total	41	36.3%	37	32.7%	35	31.0%

One way Anova test

Descriptives								
Age								
	N	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**		**Minimum**	**Maximum**
					Lower Bound	**Upper Bound**		
Mild	41	39.78	15.501	2.421	34.89	44.67	20	75
Moderate	37	38.46	14.017	2.304	33.79	43.13	18	68
Severe	35	48.80	13.702	2.316	44.09	53.51	18	70
Total	113	42.14	15.044	1.415	39.34	44.95	18	75

Anova					
Age					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	2281.921	2	1140.960	5.441	.006
Within Groups	23067.814	110	209.707		
Total	25349.735	112			

Post Hoc Tests

This test is used to test if there is significant difference in the mean age of three different group. It is to correlate the age and the disease severity.

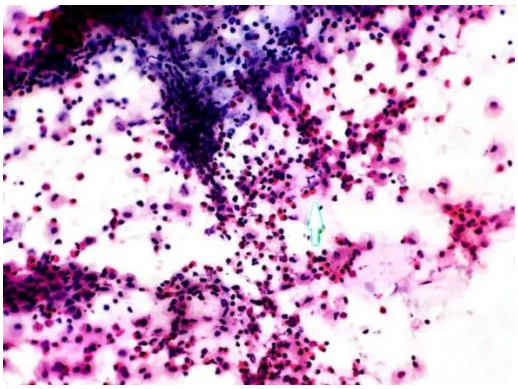

Fig. 1: Showing eosinophil in blue arrow mark in sputum sample

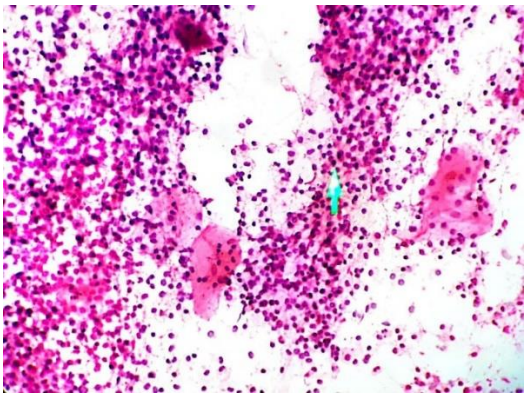

Fig. 2: Showing lymphocytes in sputum sample

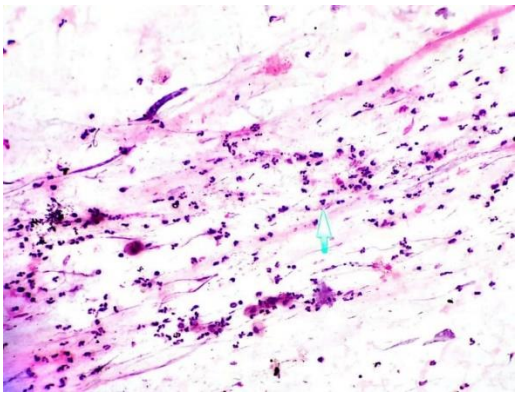

Fig. 3: Showing neutrophils in sputum sample

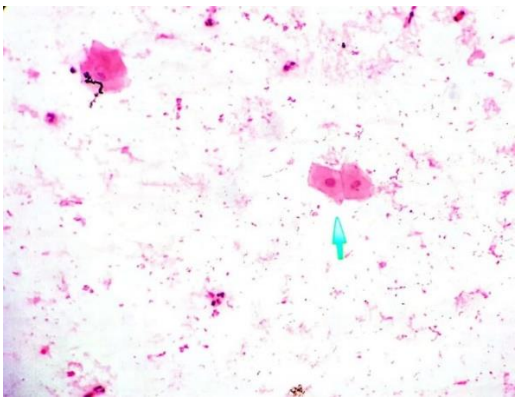

Fig. 4: Showing squamous cells in salivary sample

Discussion

Asthma was described though as reversible airway disease which is eosinophil predominant. This study was aimed at looking into the sputum cytological phenotypes among asthma exacerbations so that better understanding of pathogenesis can be established. Our study which has been conducted in rural area had equal representation of patients among both sexes. This fact is in correlation with a large multicenter study by Jindal et al., in which there is a most of the asthma patients were residents of rural area [5]. This explains that excluding smokers and the intrinsic factors responsible for asthma, it is also revealed in this study that rising air pollution in the form of environmental tobacco smoke, biomass exposure in rural area contributes largely to asthma exacerbation [5]. A hospitalization-based survey carried out in India showed that 20–30% of the population suffers from allergic rhinitis and 15% of them developed atopic asthma [6]. As in our study where atopy was coexisting factor among most of our patients, multicentric study by Bhattacharya et al is also in cohesion with the fact that atopy was most commonly seen in Indian subcontinent among asthma patients [6]. Owing to our tropical climatic condition, most common allergen is found to be pollen, fungal allergen, insects and dust mites.

There are varied population of cells recovered from sputum of asthma patients during exacerbations. Most common cell type being eosinophil which falls in place to the fact that most of our patients were atopic individuals. Equal proportion of population also had neutrophils in their sputum. When compared cell type with spirometric evaluation it is explained that where most of the patient with mild to moderate from of asthma arrived from spirometry were shedding eosinophils in their sputum. Whereas, those with severe forms of asthma were most commonly expectorating neutrophils in their sputum [7]. This is concordance with the study by Shaw et al that those who have shown predominant neutrophils in the sputum showed a poor bronchodilator response and fall in FEV1 levels. There is shift in the spectrum of severity of asthma from eosinophils to neutrophils in sputum cytology which is statistically significant. All asthma patients are exposed either to inhalational or oral steroids during their exacerbations. Probably these steroids will increase the life span of neutrophils in asthma patterns with severe form exacerbation which itself explains that there is overuse of steroids to reduce the severity of exacerbation.

The classical model of pathogenesis proposed in understanding of asthma is that allergen and antigen presentation to antigen presenting cells recruits Th2 signalling pathway where the predominant cell will be eosinophil in sputum. There are many mechanism and pathways explained in the development of steroid resistance. As suggested by Silver pill et al, among the patients going for steroid resistance it is identified that antigen presentation is skewed towards Th17 pathway in certain form of severe persistent asthma which are resistant to the management of

steroid [8]. In this subset of population, the predominant cells in the sputum sample are neutrophils. During such episodes of exacerbation, sputum biomarkers and their cytokines may modify the treatment protocol in steroid resistant asthma [9] and will serve as a guide in the management of asthma as sputum cytology is a validated tool, true representative from the respiratory tract in addition to spirometry. With the advent of biomarkers from sputum, serum targeted therapies with monoclonal antibodies and biological agents especially in steroid resistant forms of asthma will be future in the management of asthma [10]. Further studies also quotes the importance of biomarkers in differentiating asthma, asthma-COPD overlap and COPD [11].

Conclusion

Though asthma is diagnosed based on detailed history examination and spirometry to confirm the diagnosis of asthma, there are certain patients who fail to respond to routine treatment protocol. We should effectively perform laboratory test like sputum cytology on a routine which will be a guide in the management of difficult to treat chronic persistent asthma cases.

Future recommendations

Sputum and serum biomarkers should be done to hit the targets in steroid resistant asthma. These biomarker based study may help to identify the mechanism in steroid resistant asthma. The role of monoclonal antibodies in such cases will change the treatment approach in severe persistent asthma.

Conflict of interest

None.

Acknowledgement

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