

A Prospective Study of Sputum Eosinophils in Acute Exacerbation of Asthma”

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Abstract: Acute airway inflammation is considered to characterize asthma exacerbations. Eosinophils play a major role in airway inflammation in asthma. Monitoring the airway inflammation by measuring sputum eosinophils can reduce the airway inflammation and thus the exacerbations. The study aimed to find the levels of sputum eosinophils during well controlled and acute exacerbation of asthma and to find the association of sputum eosinophils with acute exacerbation of asthma. This study was conducted on 46 subjects with exacerbation of asthma. They were treated on a combination of inhaled long acting beta 2 agonists with an inhaled corticosteroid and short acting beta 2 agonists as and when required. These subjects were later followed up twice. Follow up visit 1 being one month after the initial visit and the follow up visit 2 being one month after the follow up visit 1 and their sputum eosinophil count and level of asthma control were assessed by asthma control questionnaire and spirometry in the initial and the follow up visits. Descriptive statistics, frequencies, chi-square and independent sample t-test were used. Results revealed that in out of the forty six subjects who came initially with exacerbation twenty eight (61%) had eosinophilia and eighteen (39%) had normal counts In follow up visit 1, eight subjects who had exacerbation, seven (87.5%) subjects had eosinophilia and the remaining one patient (12.5%) had normal eosinophil count in the sputum. There is a significant association (p value = 0.001) between sputum eosinophilia and exacerbation of symptoms. In the follow up visit 2, out of four subjects who came with exacerbation one (25%) patient had elevated sputum eosinophil count. In CONCLUSIONS: The sputum eosinophils are significantly increased in most asthmatic subjects during exacerbation. Monitoring the sputum eosinophils can serve as an indicator for predicting the exacerbation and thus tailoring the treatment when sputum eosinophils are increased can reduce the airway inflammation and thus prevent exacerbations. Measurement of sputum eosinophils is simple and an inexpensive tool in monitoring asthma. This makes it a practical tool of potentially widespread applicability.

Key words: Asthma • Exacerbation • Sputum • Eosinophils • Airway Inflammation • Eosinophilic Airway

INTRODUCTION

Asthma is a syndrome characterized by recurrent episodic airway obstruction, airway inflammation and bronchial hyperresponsiveness. It is a syndrome with a variety of phenotypes, in which various precipitating factors result in clinical, physiological and pathological manifestations. World Health Organization (WHO) in its factsheet on asthma quoted that worldwide asthma cases are increasing at a rate of 50 per cent every decade.

The symptoms of asthma are due to the underlying airway obstruction brought about by the various inflammatory mediators. Eosinophils are one of the major

inflammatory mediators in the asthmatic airway and controlling this eosinophilic inflammation forms one of the basis of asthma management. In the last decade interest has been shown to monitor airway inflammation using non-invasive markers as it would be convenient and less morbid for the patient. These inflammatory markers should correlate closely with the severity of the disease. Inflammatory changes in sputum have been reported to correlate closely with disease severity in asthma [1]. All of these features make the non-invasive methods an interesting method monitoring for airway inflammation of asthma. Therefore a biomarker that reflects the underlying pathology rather than its physiological effects and which

changes with specific interventions should be used to define the treatment options and the response to treatment.

Among various newer methods being developed for monitoring airway inflammation, sputum eosinophils and measurement of exhaled nitric oxide (NO) seem to be the most promising, as they are reproducible and less invasive and therefore applicable to sequential measurements. Measuring sputum eosinophils uses simple staining methods and is cost effective method. Both methods have been found useful in evaluating the anti-inflammatory effects of drugs [2-5].

This study was done to analyze the level of sputum eosinophils during well controlled and acute exacerbation of asthma and to find its association with exacerbation of asthma.

MATERIALS AND METHODS

This is a prospective observational study that involves humans. All the subjects who satisfy the inclusion criteria were included in this study. The inclusion criteria being the subjects with primary diagnosis of asthma who come with acute exacerbation, 18 years of age or older, reversible bronchospasm (> 12% increase in FEV1), exacerbation is defined as episodes of progressive increase in shortness of breath, cough, wheezing, or chest tightness or combination of these symptoms, respiratory distress is common. The episode is characterized by decrease in expiratory airflow that can be documented and quantified by measurement of lung function (PEFR or spirometry) [6]. Subjects with non-reversible airway obstruction in spirometry (<12% change in FEV1), asthmatic subjects unable to do spirometry like history of myocardial infarction, congestive heart failure, coronary artery disease and sepsis, subjects with contraindications for spirometry and subjects who have a history of smoking were excluded from the study.

Brief Procedure: In all the subjects detailed history was taken with special reference to smoking history, history of other comorbidities and history of previous treatments. This was followed by systemic clinical examination. All routine investigations and other special investigations like spirometry to check for reversibility and echocardiography to rule out cardiac causes for breathlessness was done. The Asthma Control Questionnaire (ACQ) was used to measure the symptoms of exacerbations. The Asthma Control Questionnaire has

been validated to measure the adequacy of asthma management as identified by international guidelines. Responses are given on a 7 point scale and the overall score is the mean of responses. The scale consists of 5 symptoms (shortness of breath, wheezing, waking dyspnoea, nocturnal dyspnoea, activity limitations) bronchodilator use and measurement of FEV1 were completed by the clinician. The Asthma Control Questionnaire has strong evaluative and discriminative properties and can be used with confidence to measure asthma control [7].

All the subjects who satisfied the inclusion criteria were later told to give sputum sample after deep coughing in a sterile container and the sputum was stained with eosin and haematoxylin and analyzed using microscopy to determine the count for eosinophils and was expressed in percentage. Sputum eosinophil count = 3 % was considered abnormal [8].

Subjects were treated with inhalation therapy consisting of a long acting beta2agonist with an inhaled corticosteroid and a short acting beta 2 agonist as and when required. These subjects were later followed up twice.

- Follow up visit 1 - One month after the initial visit and
- Follow up visit 2 - One month after the follow up visit 1.

The sputum eosinophil count and spirometry were measured in both the follow up visits. The subjects in both the follow up visits were classified as well controlled and not well controlled which fell under the category of exacerbation, based on the symptoms and spirometry.

The sputum eosinophil count during the initial visit of exacerbation and the subsequent two follow up visits were compared and tabulated. Also the sputum eosinophil count during exacerbation and well controlled phase were compared. Descriptive statistics, frequencies, chi-square and independent sample t-test were used to analyze the results.

RESULTS

Fifty subjects were included in this study after fulfilling the inclusion criteria and their written consent, out of which 46 subjects came for follow up. The group included 33 males and 13 females. All the subjects included in this study were nonsmokers.

Table 1: Comparison Of Selected Baseline And Follow up

	Baseline (n=)		1 month after treatment for asthma* (n=)		2 months after treatment for asthma** (n=)	
	Well controlled	Exacerbation	Well controlled	Exacerbation	Well controlled	Exacerbation
Eosinophils						
Normal	0	18 (39.1)	29 (76.3)	1 (12.5)	37 (88.1)	3 (7.5)
Abnormal	0	28 (60.9)	9 (23.7)	7 (87.5)	5 (11.9)	1 (25.0)

*chi-square = 11.86; p value <0.05; **chi-square=0.552; p=0.457

Table 2: Comparison Of Asthma Levels Of Baseline And After Treatment For Asthma

Eosinophils	1 month after treatment for asthma* (n=)		2 months after treatment for asthma** (n=)	
	Normal	Abnormal	Normal	Abnormal
Normal	16 (88.9)	2 (11.1)	18 (100)	0 (0)
Abnormal	14 (50)	14 (50)	22 (78.6)	6 (21.4)
Relative risk	0.222		-	
95% CI	0.057-0.864		-	
P value	0.001		0.011	

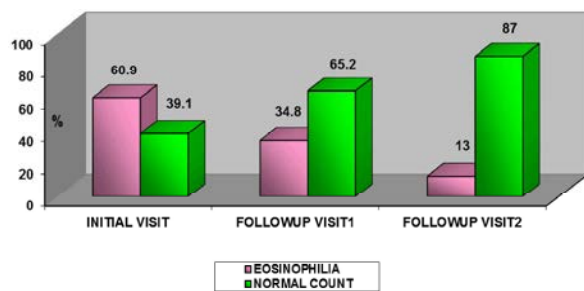


Fig. 1: Eosinophil Count During Each Visit

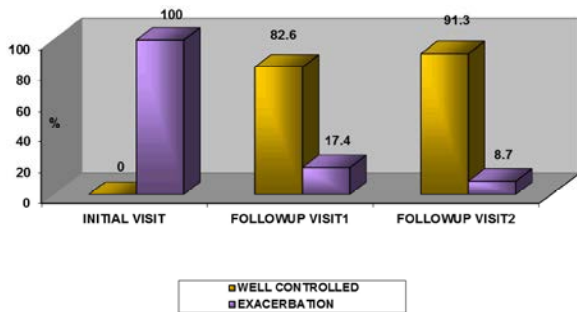


Fig. 2: Representation Based On Asthma Symptoms during Each Visit

The mean levels of eosinophils for different visits were presented in Figure 1. It was observed that the mean levels of eosinophils were decreased significantly as the treatment increases. The mean was decreased to more than half of the times. Thus treatment was effective in order to control the eosinophil levels.

The above Figure 1 and Table 1 showed that the subjects with abnormal levels of eosinophils were associated significantly with exacerbation of asthma.

However after treatment for asthma (1 month), the percentage of subjects who are well controlled (76.3%) was increased significantly and this percent was higher (88.1%) when the follow up was increased to 2nd month period. In follow up visit 1, out of thirty eight well controlled asthmatics, twenty nine (76%) subjects had normal eosinophil count and nine (24%) subjects had increased eosinophils levels in their sputum. Out of the remaining eight subjects who had exacerbation, seven (87.5%) subjects had eosinophilia and the remaining one patient (12.5%) had normal eosinophil count in the sputum. Hence in the above data there is significant association (p value = 0.001) between sputum eosinophilia and exacerbation of symptoms. In the follow up visit 2, out of four subjects who came with exacerbation three (75%) subjects had normal eosinophil count.

Figure 2 shows that during follow up visit 1, 83% of the subjects were well controlled and the rest 17% (n=8) of the subjects came with exacerbation. Out of these 8 subjects who came with exacerbation, 7 of them had sputum eosinophilia. In the follow up visit 2, 91% of the subjects were well controlled and the rest 9% of subjects (n=4) came with exacerbation. Out of these 4 persons who came with exacerbation, 3 subjects had normal sputum eosinophil count.

The above Table 2 shows the subjects with abnormal levels of eosinophils after treatment. It shows that after treatment, subjects with abnormal were turned to be normal after the treatment with RR of 0.222 (95% CI: 0.057-0.864). The treatment was effective almost to 20percent. However after follow up for the period of 2 months, 78.6% of the subjects turned to be normal.

DISCUSSION

Through this study, an attempt was made to study the level of sputum eosinophils in acute exacerbation and the level of sputum eosinophils during acute exacerbation was compared to that of well controlled asthma. This study suggests that there is a significant association between acute exacerbation of asthma and sputum eosinophilia.

Out of the forty six subjects who came initially with exacerbation twenty eight (61%) had eosinophilia and eighteen (39%) had normal counts. After the treatment for asthma (1 month), the percentage of subjects who are well controlled (76.3%) was increased significantly and this percent was higher (88.1%) when the follow up was increased to 2nd month period. Interestingly, in the follow up visit 1, out of thirty eight well controlled asthmatics, twenty nine (76%) subjects had normal eosinophil count and nine (24%) subjects had increased eosinophils levels in their sputum. This might suggest an underlying airway inflammation but were free of symptoms of exacerbations. These well controlled nine subjects who have increased sputum eosinophil count probably need increase in their dose of inhaled corticosteroid or an addition of oral corticosteroid. Out of the remaining eight subjects who had exacerbation, seven (87.5%) subjects had eosinophilia and the remaining one patient (12.5%) had normal eosinophil count in the sputum. Hence in the above data there is significant association (p value = 0.001) between sputum eosinophilia and exacerbation of symptoms.

In the follow up visit 2, out of four subjects who came with exacerbation three (75%) subjects had normal eosinophil count. This could be because of the effect of the inhaled corticosteroid, which might have decreased the eosinophil levels in the sputum after 2 months of treatment and also reduced the subjects with exacerbation. The subjects who came with exacerbations (mean BMI -25) fell in overweight category and this might suggest that obesity being a possible risk factor for developing asthma [9,10]/

Preventing asthma exacerbation is one of the important goals in management of asthma. Inflammation has been assumed to be an important factor in underlying asthma exacerbation. Exacerbations of asthma are characterized by increasing airway obstruction. In this study it has been shown that the sputum eosinophils is significantly increased in exacerbation of asthma. According to Jatakanon *et al* [11] in 2000 showed that the changes in sputum eosinophils predict loss of asthma control. Higher eosinophil percentages in induced sputum

from asthmatic subjects are associated with lower FEV₁ and heightened sensitivity to methacholine [12,13]. When the treatment strategy is aimed at keeping sputum eosinophils low, subjects have fewer asthma exacerbations [14]. As Yeung *et al* [14] in 1996 stated that the degree of symptoms may however be a more sensitive measure of the onset of an exacerbation because the increase in symptoms usually precedes the deterioration in peak flow rate. Similarly in this present study the asthma was assessed based on symptoms as well as the spirometry.

In 2004 Miranda *et al.* [15] showed that eosinophilic airway inflammation was associated with increased symptoms and near fatal events. A study by Anees *et al.* [16]. asthmatic subjects with high sputum eosinophils were strikingly worse than those with low sputum eosinophils, since they had much poorer lung function and greater bronchial hyperresponsiveness. Thus all these study state about the underlying airway inflammation and its effects. Hence monitoring of the underlying airway inflammation is more scientific than measuring the physiological effects caused by it. Measurement of sputum eosinophil is therefore simple, safe, non-invasive and repeatable method of directly obtaining repeated samples of airway secretions and it correlates with the degree of underlying airway inflammation.

This study takes the various time interval in to consideration as the subjects are being followed up after 1month of the initial visit and again 1 month after the follow up visit 1. This time frame helps to assess the level of sputum eosinophils in relation to exacerbation and thus aid in finding the correlation between them as well as to monitor the treatment response. If the sputum eosinophil was found to be increased in any of the follow up visits, it might possibly suggest underlying airway inflammation not controlled adequately and thus might warrant an addition of an oral corticosteroid or increase in dose of inhaled corticosteroid. The study though evaluated the eosinophilic airway inflammation, the non-eosinophilic inflammation of airway was not evaluated.

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