

Role of Induced Sputum Differential Cell Count for Assessment of Severity in Persistent Asthma

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Abstract

Sputum may provide an alternative source of bronchial cells to investigate characteristics of airway inflammation and its functional correlates in patients with asthma. This study analyse the cellular profile of induced sputum and differentiate from other chronic disease like COPD and assess severity of the disease which will help to take decision to manage the patient properly. This study was carried out at Asthma Center, Department of Medicine, IDCH, Dhaka, during the period from July 1999 to October, 2000. 48 asthma patients were taken in group A. The age ranged from 15-50 years with mean age was 31.44 ± 10.45 . There was male and female with male female ratio 1.8:1. In group B total number of 24 subjects having no history of asthma were taken as control. The age range from 15-50 years, male female ratio 1:1. Sputum induction was done with inhaled hypertonic saline for five minutes period for upto 20 minutes. Plugs from the lower respiratory tract were selected for differential counting in cytocentrifugation preparations. Cellular profile shows eosinophils count are significantly higher and macrophage count lower in persistent asthma, then in normal healthy person. The eosinophil count correlate with the severity of asthma.

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Introduction

Bronchial asthma is a major respiratory problem and is a common disease of both children and adults. There is however marked variation in its prevalence and severity part of the variation in prevalence may be due to difficulty in separating other wheezing disorders, especially bronchitis in the very young and nonspecific bronchitis in the elderly. A survey in Bangladesh was done by Asthma Association, Bangladesh in 1999 and showed the prevalence of bronchial asthma about 5% (NAPS) similar to international report. In the vast majority of cases the disease is mild and often

not recognised. There is evidence that the prevalence of asthma is increasing¹.

Asthma is a chronic inflammatory disorder of the airways in which many cells play a role particularly mast cells, eosinophils, T-lymphocytes, and neutrophil². In susceptible individuals this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness and cough particularly at night and/or in the early morning. The inflammation also causes an associated increase in airway responsiveness to a variety of stimuli³.

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Activated eosinophils release toxic proteins capable of causing airway tissue damage, including MBP, eosinophils derived neutrotoxin, eosinophil cotaionic protein (ECP) and eosinophils peroxide until relatively recently, eosinophils were considered to be effector cells in Asthma without the capacity to generate cytokines⁴. Direct examination of the inflammatory response should help to improve understanding of the pathogenesis and treatment of asthma, but is relatively inaccessible to study. Noninvasive methods of obtaining sputum from patients should provide a useful investigational tool in asthma⁵.

In patients with stable asthma bronchial biopsy and bronchoalveolar lavage have been used to study airway inflammation but discontent, inconvenience and risk limit their use⁶, use of induced sputum cell count has been recently been proposed as a noninvasive alternative to bronchoalveolar lavage (BAL) to investigate airway inflammation in asthma⁷. Sputum induction also yield sample more concentrated and richer in airway secretions than those obtained by bronchoscopy and various chemical substance have been detected in the supernatant of sputum. Number of eosinophils in sputum have been reported to correlate with disease severity in asthma⁸.

Materials and Methods

This was a prospective case control study. A total number of 48 patients of persistent asthma were taken as cases and 24 healthy persons were taken as controls. Cases are in group A and controls are in group B. The patients were selected on the basis of criteria of inclusion like

1. History of episodic chest tightness, wheezing, dyspnoea, coughing at night or early morning in the previous year at least more than two occasions in a month, usually patient have almost daily attack of dyspnoea or usually patient have dyspnoea to some extent continuously for more than 6m or more.
2. FEV₁ is <80 prediced value.
3. Informed consent of the patient taken

Criteria of exclusion of the patients from the study:

1. Age below 15 and above 50 years
2. Smoker patients

3. Patients taking oral or inhaled corticosteroids for last 6 weeks.
4. Recent myocardial infarction, LVF.
5. Other causes of chronic cough and respiratory distress e.g. P.T. Bronchiectasis, COPD, ILD.
6. Severe acute asthma

The controls were selected so as to match the case in respect of age, sex and occupation. Confirmation of the diagnosis of persistent asthma all the subjects were examined clinically and were identified according to the predetermined criteria as well as inclusion and exclusion criteria. Investigations were done to rule out exclusion criteria. Inhaled bronchodilator was withheld for six hours before the induction. Spirometry was done and FEV₁ was measured. The hypertonic saline was nebulized with an ultrasonic nebulizer and was inhaled for five minutes periods for upto 30 minutes. The concentration of saline was increased at intervals of 10 minutes from 3% to 5%.

Ten minutes after the start of nebulization and every five minutes thereafter, subjects were asked to rinse the their mouth and throat carefully and try to cough sputum into a container. Plugs from salivary contamination was then suspended in DTT (Dithiotriol) solution (0.1%) and incubated for 30 minutes at 37°C. The cells were centrifuged at 1500g for 10 minutes and resuspended in saline. Three sputum slides were then prepared for cytological examination. The cells were air dried, fixed with methanol and stained with may-Grun Wald-Giemsa. Differential counts were determined by counting 200 nonsquamous cells on each sputum slide.

Results

48 patients of both sexes were selected randomly on the basis of inclusion and exclusion criteria. The patients were of different ages ranging from 15 to 50 years with a mean age of 31.44±10.45 in group A and 29.42±8.75 in group B. Male-female ratio in group A was 1.08:1 and in group B 1:1.

The age and sex distribution of the patients of the series is shown in Table-I.

Differential (DC%) Count of Induced Sputum

Differential Count of induced sputum shown in Table I and Table II. Eosinophils cells are more in severe patients. But in mild and moderate grade patients eosinophils count do not vary more. In both patients and controls lymphocytes cells count are near to similar. Results was statistically highly significant.

Different cell count of induced sputum shown in Table-II and Table-III

Eosinophils are more in severe patients. But in mild and moderate grade patients eosinophils count do not vary much. In both patients and controls lymphocytes cells and more to similar. Results are statistically highly significant.

Figure 1 shows the relationship of sputum eosinophil with control group and different grades of asthmatic.

Table-I
Comparison of age and sex distribution of group A and group B (n=48)

		Group A	Group B	P value
	Age (years)	15-50 yrs.	15-50 yrs.	
	Range (mean)	(31.44 ± 10.45)	(29.42 ± 8.75)	
	15-20 years	7 (14.6%)	4 (16.7%)	
	21-30 years	13 (27.1 %)	9 (37.5%)	<.001
	31-40 years	14 (29.2%)	9 (37.5%)	
	41-50 years	14 (29.2%)	2 (8.3%)	
Sex	Mean	21.76±20.38	(.75±.74)	
	Range	(3-70)	(0-2)	
	Male	25 (52.1%)	12 (50%)	
		21.76±20	.67±.11	
	Female	23(47.9%)	12(50%)	
		31.82±27	.78±12	
	Ratio	1.08:1	1:1	

Table -II
Differential (DC%) count of induced sputum eosinophils macrophage cell in group A.

	Eosinophil (%) (mean % ± SD)	Macrophage (%) (mean % ± SD)	P-value
Control (n=24)	0.75 ± 0.74	84.17 ± 5.24	P<0.001
Mild-(n=19)	5.58 ± 1.74	73.32 ± 4.61	
Moderate (n=14)	18.43 ± 2.71	53.57 ± 2.95	
Severe (n=15)	610.87 ± 4.63	28.07 ± 4.43	

Table-III
Differential (DC%) count of induced sputum lymphocyte neutrophil cell in group B.

	Lymphocyte (%) (mean % $\pm$ SD)	Neutrophil (%) (mean % $\pm$ SD)	P-value
Control (n=24)	2.08 $\pm$ 0.83	12.25 $\pm$ 3.98	P<0.001
Mild (n=19)	2.68 $\pm$ 0.75	18.58 $\pm$ 4.79	
Moderate (n=14)	4.0 $\pm$ 0.55	24.0 $\pm$ 2.91	
Severe (n=15)	3.73 $\pm$ 0.96	7.33 $\pm$ 0.82	

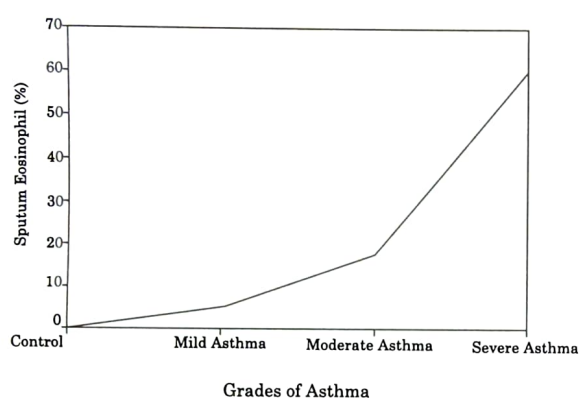


Fig.-I : Relationship of sputum eosinophil with control group and different grades of asthmatics.

Discussion

In this study we observed that most of the patients were in second and third decade of life. Bronchial asthma cases were selected from Asthma Centre, IDCH, by convenience selection criteria though asthma afflicts men and women equally after the age 30. Analysis of these patients in respect to sex showed the overall male female ratio in this study was 1.08:1.

Though persistent asthma is a disease of long duration, so lack of proper treatment is a crucial factor for disease persistence. In this study most of the patients 31 (64.6%) were getting bronchodilator only in irregular basis. In this present study, we try to exclude the patients having allergic rhinitis eczema or atopic dermatitis by history taking. Because there is an association between eczema and both the development and persistent of asthma.

Several examinations of sputum induced by hypertonic saline solution nebulization with a

pretreatment of inhaled β_2 -agonists have been reported and the repeatability responsiveness and validity of this method have been demonstrated. Sputum induction has been accepted as a noninvasive and useful method of evaluating airway inflammation in bronchial asthma. The results show that such samples differed between asthmatic and normal subjects. The alternative are BAL, biopsy which are invasive with discomfort and risk inhalation of hypertonic saline causes airway constriction in patients and try to prevent this we gave on inhaled α_2 -agonists before the procedure and increased these concentration of hypertonic saline gradually. The total number of cells in the sputum was similar in asthma and central group. The differential count showed significantly more eosinophils in the asthmatic patients than in normal subjects. The number of macrophages was lower in patients with persistent asthma and no difference was seen in the lymphocyte count in the two groups. In persistent asthma patients the eosinophils count was directly related to clinical score and related to the percentage predicted FEV₁.

Conclusion

Concerning the management of persistent asthma, it is very important to monitor the airway inflammation as well as the severity of symptoms. Our findings indicate that the noninvasive measurement of airway inflammation by induced sputum method serve as a useful marker for evaluating the severity of asthma and monitoring airway inflammation to achieve the optimal control of asthma.

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