ORIGINAL ARTICLE

The Diagnostic Value of Sputum Eosinophil Counts in Patients with Cough Variant Asthma

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Abstract:

Background: Cough Variant Asthma is a variant form of asthma presenting chronic persistent cough without wheezing or dyspnoea, near normal pulmonary function but increased airway responsiveness. Many cases are missed due to lack of proper evaluation.

Objective & Method: To find out the role of eosinophil counts in sputum for the diagnosis of Cough Variant Asthma, Observational study was carried out in department of Respiratory Medicine of National Institute of Diseases of the Chest and Hospital (NIDCH), Mohakhali, Dhaka, Bangladesh, during the period of September 2014 to September 2015. 50 Patients with chronic cough suspected as cough variant asthma attending in NIDCH were selected. Methacholine challenge test was performed to diagnosis of Cough Variant Asthma. After Methacholine test, patient was encouraged to produced sputum, Sputum was collected and was sent to microbiology department of NIDCH. Report collected from laboratory was put in the data collection sheet.

Result: 50 patients were included in the study. The mean age was found 19.1 ± 7.6 years with range from 8 to 30 years and Male to female ratio was found 1.4.:1.

Among the study patients 39% had positive family history of asthma, 58% had associated allergic rhinitis, 16% patients had associated eczema, 4% patients had associate conjunctivitis, 20 patients had leucocytosis. 96% patients had sputum Eosinophil, 90% patients had >3% sputum eosinophil and 10% patients had d"3% sputum eosinophil count.All patients had Trigger factor with negative CFT for Filaria and increased S IgE.

Conclusion: Increased Eosinophil count in sputum could be a diagnostic tool in Cough Variant Asthma.

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Inroduction:

Asthma is a chronic inflammatory disorder of the airways, which is associated with airway hyper-

responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing, particularly at night and in the early

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morning. These episodes are usually associated with widespread but variable airflow obstruction within the lung that is often reversible either spontaneously or with treatment²³. According to the first national asthma prevalence study (NAPS) in Bangladesh about 7 million people (5.2%) suffering from current asthma, more than 90% of whom do not take modern treatment. So poorly controlled asthma remains a major problem in Bangladesh¹⁹. In asthma many cells and cellular elements play a role, in particular, mast cells, eosinophil, T lymphocyte, macrophage, neutrophils and epithelial cells. Eosinophil infiltration is a characteristic feature of allergic inflammation. Sputum eosinophilia is a hallmark of asthma and is probably the major effecter cell in asthma⁸. Thus eosinophil counts and measurement of their products in sputum have a potential role in acting as objective markers of bronchial inflammation in asthma⁷.

Asthma is a heterogeneous disease, with different underlying disease processes. Many phenotypes have been identified. Cough-variant asthma is a type of asthma in which the main symptom is a dry, non-productive cough. People with coughvariant asthma or CVA often have no other "classic" asthma symptoms, such as wheezing or shortness of breath¹⁹. CVA is somewhat difficult to diagnose because the cough may be the only symptom, with normal physical examination, chest X- rays, and spirometry. There are three recommended ways to diagnose the cough variant asthma of variability in lung function or of air way hyper-responsiveness and search for sputum $eosinophils^{10}$. Positive methacholine challenge test will indicate asthma, But Methacholine test is also positive in a wide variety of other diseases . This test also causing a false negative response. Methacholine challenge testing is more useful in excluding a diagnosis of asthma than in establishing one because its negative predictive power is greater than its positive predictive power². Another way to diagnose CVA can be done with standard asthma treatment. If the cough responds to these treatments, a diagnosis of cough variant asthma can be made¹⁹.

Materials & Methods:

Study design: Observational study.

Place of study: This study was carried out from September 2014 to September 2015 in the Department of Respiratory Medicine in NIDCH, Mohakhali, Dhaka. sputum examination was performed in the Microbiology department of NIDCH

Study population: Patients having chronic cough attending Outpatient Department of NIDCH, Mohakhali, Dhaka. A total of 50 patients were enrolled in this study.

Sampling method: Purposive sampling

Selection criteria of patients: Cough variant asthma patients fulfilling inclusion and exclusion criteria.

Inclusion criteria; 1) Age d" 30. 2) years.Patients suffering from chronic cough >8 weeks. 3) Positive methacholine challenge test.

Exclusion criteria: 1) Smokers. 2) Patients having COPD. 3) Patients having other causes of chronic cough e.g. Post nasal drip, GERD, ILD, Heart failure, Use of ACE inhibitors.

Study procedure: In the first phase a standard questionnaire was designed with a view to collect data. Informed written consent was taken from each patient. Initial evaluation of the patient by history and clinical examination was performed and recorded in the preformed data sheet. Subjects were explained the procedure. Pulse, blood pressure, base line laboratory investigation like CBC, CXR & ECG test were done. Baseline spirometry was performed before methacholine used. Prepared 10 doubling concentrations of methacholine are followed 0.03, 0.06, 0.125, 0.25, 0.50, 1, 2, 4, 8, 16, mg/dl².Methacholine challenge test was performed. Concentration of methacholine started from minimum concentration 0.03mg/dl and gradual increased the dose up to the level at which 20% fall in FEV_1 is observed from base line or the highest concentration (16mg/dl) of the drug has been delivered. A total of 10 doses are given if the entire procedure is finished without a positive response. Another spirometry was performed and result was recorded. Patients were resuscitated by nebulized bronchodilator. All the procedure were performed in the respiratory laboratory of NIDCH. After full recovery, patient was encouraged to produce sputum, when failed, hypertonic saline was used with nebulizer for induction of sputum. Sputum was collected in a plastic container and labeled accordingly and was sent to microbiology department of NIDCH for sputum eosinophil counts. Report was collected from laboratory and put in the data collection sheet. In cough variant asthma sputum eosinophil countis $>3.2\%^{20}$.

Sputum assays: The volume was assessed by the size and number of plugs: a cumulative size of 4.5x 9 mm was estimated to be necessary to perform all investigation¹⁵. Differential counts were determined by counting 200 non-squamous cells on each sputum slide²².

Data analysis: Statistical analyses were carried out by using the Statistical Package for Social Sciences version 16.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Continuous variables were expressed as mean, standard deviation, and categorical variables as frequencies and percentages.

Ethical issue: Informed written consent was taken from all patients.

Results and Observations:

A total 50 patients were included in the study. The mean age was found 19.1±7.6 years with range from 8 to 30 years and Male to female ratio was found 1.4.:1.

Table-I	
Age distribution in the study patients (r	ı=50)

Age (in year)	No. of Patients	Percentage
≤10	9	18.0
11-20	19	38.0
21-30	22	44.0
$Mean \pm SD$	18.9	± 7.2
Range (min-max)	8	-30

Among 50 patients majority (44.0%) patients age 21-30 years, the mean age was found 19.1 ± 7.6 years with range from 8 to 30 years.



Fig.-1: *Pie chart showing sex distribution of the study patients* (n=50)

Among 50 patients 29 (58.0%) were male and 21(42.0%) patients were female. Male to female ratio were found 1.4:1.



Family history of Asthma

Fig.-2: Pie chart showing family history of asthma of the study patients (n=50) It was observed that 39(78.0%) patients had family history of asthma.

Table-II Associated allergic rhinitis in the study patients (n=50)

Associated allergic	No. of patients	Percentage
Rhinitis		
Yes	29	58.0
No	21	42.0

Table II shows that 29(58.0%) patients had associated allergic rhinitis.

Table-IIIAssociated eczema in the study patients (n=50)

Associated Eczema	No. of patients	Percentage
Yes	8	16.0
No	42	84.0

Table III shows that 8(16.0%) patients had associated eczema.

Table-IV Associated allergic conjunctivitis in the study patients (n=50)

Associated	No. of patients	Percentage
conjunctivitis		
Yes	2	4.0
No	48	96.0

Table IV shows that only 2(4.0%) patients had associate allergic conjunctivitis.

	Table-V	
Associated	trigger factor in the patients (n=50)	e study
Associated	No. of patients	Percentage

trigger factor	Ŧ	0
Yes	50	100.0
No	0	0.0

Trigger factors include: cold, dust, exercise, smoke, strong smells, allergen exposure. Table V shows that all (100.0%) patients had trigger fact

Table-VI					
Leucocytos is	in	the	study	patients	(n=50)

Leucocytosis	No. of patients	Percentage
(>11000/cmm)		
Yes	10	20.0
No	40	80.0

Table VI shows that 10(20.0%) patients had leucocytosis.

Table-VII	
Eosinophilia in the study patients ((n=50)

Eosinophilia (>6%)	No. of patients	Percentage
Yes	16	32.0
No	34	68.0

Table VII shows that 16(32.0%) patients had eosinophilia.



Fig.-3: Bar diagram showing total circulating eosinophil in the study patients. It was observed that majority 28(56.0%) patients had normal total circulating eosinophil (<400/cmm) and 22(44.0%) had increased (>400/cmm) total circulating eosinophil.

Table-VIIICFT for filaria in the study patients (n=50)

CFT for filaria	No. of patients	Percentage
Positive	0	0.0
Negative	50	100.0

Table VIII shows that all (100.0%) patients had negative CFT for filaria.

Table-IXS. IgE level in the study patients (n=50)

S. IgE level	No. of patients	Percentage
Normal (< 200 IU/ml)	0	0.0
Increased (> 200 IU/ml)	50	100.0

Table IX shows that all (100.0%) patients had increased S. IgE level



Fig.-4: Pie chart showing sputum eosinophil in the study patients .It was observed that 48(96.0%) patients had sputum Eosinophil.

Table-XSputum eosinophil count in the study patients(n=50)				

count		
Eosinophil count > 3%	45	90.0
Eosinophil count $\leq 3\%$	5	10.0

Table X shows that 45(90.0%) patients had >3% sputum eosinophil count and 5(10.0%) patients hadd" 3% sputum eosinophil count.

Discussion:

This observational study was carried out with an aim to find out the easy technique for diagnosis of

cough variant asthma and to determine the eosinophil count in patients with cough variant asthma. A total of 50 patients were enrolled in this study.

In this study it was observed that 42.0% patients belong to age 21-30 years and the mean age was found 19.1±7.6 years with range from 8 to 30 years. Yoo *et al.* $(2004)^{25}$ showed the mean (\pm SD) age was found 11.4±2.2 years, which is lesser with the current study. On the other hand Mohamed et al. $(2014)^{18}$ found the mean age was 32.05 ± 10.87 years. The higher mean age and age range obtained by the above authors maybe due to geographical variation, racial influences. In this study it was observed that Cough Variant Asthma is predominant in male subject, where 58.0% patients were male and 42.0% populations were female. Male to female ratio was found 1.4.:1. Similarly, Harish and Suryanarayana (2014)¹⁴ showed 71.7% male and 28.3% female. Mohamed *et al.* $(2014)^{18}$ and Yoo et al. (2004)²⁵ also observed male predominant in their studies, where they found 53.8% and 53.7% were male respectively. In this series it was observed that majority (78.0%)patients had positive family history of asthma. Similarly, Bandyopadhyay et al. $(2013)^4$ and Khakzad et al. (2009)¹⁶ showed family history of Asthma were 47.5% and 43.0% respectively, which are comparable with the current study. In this present study it was observed that 58.0% populations associated with allergic rhinitis. Similarly, Alvarez et al. $(2000)^1$ have et al. found airway eosinophilic infiltration in rhinitic patients. In this current study it was observed that 16.0% and 4.0% patients had associated eczema and conjunctivitis respectively. In this study all patients had trigger factor. Matsumoto et al. $(2012)^{17}$ developed a closed questionnaire listing 18 triggers that were reported by e"1% of 213 patients in a retrospective survey. Hannaway & Hopper (1982)¹³ reported that among 32 children with CVA, 25 reported exercise-induced cough and 14 reported cold air-induced cough, and a majority of these patients experienced worsening of cough during specific seasons. In this study 10(20.0%) patients had increased leucocyte count. Matsuoka et al. $(2011)^{17}$ showed a total leukocyte count of 8200 cells /mm³ with 65.4% neutrophils and 3.0% eosinophils. In this series it was observed that nearly one third (32.0%) patients had Eosinophilia.

Harish and Suryanarayana $(2014)^{14}\,\rm observed\,\,61\%$ had eosinophilia and 39.0% had normal counts. In this present study 56.0% patients had normal total circulating eosinophil and 44.0% had increased total circulating eosinophil. Increased eosinophil counts have also been reported in a large proportion of patients with asthma treated with inhaled corticosteroids observed by Richter et al. (1999)²¹. In this current study it was observed that all (100.0%) patients had negative CFT for filaria and all (100.0%) patients had increase S. IgE level. Surprisingly, there does not appear to be a correlation with total serum IgE, as dem-onstrated in a study by Good *et al.* $(2011)^{12}$ in which bronchoscopy was used to assess asthma phenotypes. Therefore, total serum IgE is not useful as a diagnostic marker for eosinophilic asthma. In this present study it was observed that 96.0% patients had sputum Eosinophil. Harish and Survanarayana (2014)¹⁴ obtained in their study that 87.5% subjects had sputum eosinophilia. In this current study, study patients is divided into two category on the besis of the eosinophil count in sputum as follows, sputum eosinophil count >3%, and d" 3% category, it was observed that 90.0% populations had sputum eosinophil count >3% and 10% had d" 3% sputum eosinophil count. Carney et al. $(1997)^5$ have reported that an increase of eosinophil count (>3.2%) in induced sputum was observed in three out of six patients with chronic cough and bronchial hyperresponsiveness, who might be given a diagnosis of CVA (Corrao et al., $(1979)^6$. Ayik *et al.* $(2003)^3$ mentioned that sputum eosinophilia greater than 3.0% was present in 33.3% patients and they were diagnosed as eosinophilic bronchitis. Niimi et al. (1998)²⁰ showed 5 out of 6 patients a marked increase of eosinophils in sputum (more than 80% of the nucleated cells). Godon et al. (2002)¹¹ studies have revealed that more than 50.0% of asthmatic patients who received no anti-inflammatory treatment have an increased induced sputum eosinophil count. All this studies revealed that asthmatic patients had higher sputum eosinophil count.

Conclusion:

The result of this study showed that Sputum eosinophil count becomes elevated in cough variant asthma (CVA). In majority of the cases, sputum eosinophil count is > 3%. In presence of appropriate background i.e. unproductive cough for more than 8 weeks, and exclusion of other causes, sputum eosinophil count >3% may be considered for the diagnosis of CVA.

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