Original Research Article

Pattern of inflammatory phenotypes using sputum cytology among patients with asthma in a tertiary care centre, Kerala –A prospective cohort study

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Abstract

Background: Asthma is a chronic inflammatory airway disease in which various inflammatory cells play a pivotal role in pathogenesis. Based on the inflammatory cells count in the sputum, various inflammatory phenotypes are identified. The identification of asthma phenotypes has potential clinical significance, as natural history and treatment response differ according to phenotype. **Objectives:** To find out pattern of inflammatory phenotypes of asthma patients and to compare the level of asthma control after 3 months of optimized treatment in different phenotypes. **Methods:** We did a hospital based prospective cohort study in the Department of Pulmonary Medicine, Medical College, Thiruvananthapuram, Kerala, over a period of one year. 138 consecutive non-smoker, asthma patients were included. Sputum was examined for differential count. The phenotype was considered eosinophilic if eosinophil count was ≥3% and noneosinophilic if eosinophil count < 3%. Each patient was reassessed after 3 months of optimized treatment and level of asthma control was assessed according to GINA guidelines. **Results:** 53.6% of study population were in the eosinophilic and 46.4% were in the noneosinophilic phenotype. Mean age of eosinophilic phenotype was 38.4 ± 11.7 years and that of noneosinophilic was 48.8 ± 10.6 years ($p<0.001$). Mean BMI of eosinophilic asthma patient was 23.3 ± 2.4 and that of noneosinophilic was 24.9 ± 2.3 ($p <0.001$). Atopic symptoms were present in 73% of the eosinophilic phenotype and 25% of the noneosinophilic phenotype ($p<0.001$). There was no statistically significant difference between the two phenotypes with regard to the gender, duration of illness, family history of asthma, or initial severity of the disease. After 3 months of optimized treatment, in eosinophilic phenotype 73% of patients became controlled or partially controlled. In noneosinophilic group only 46.9% became controlled or partially controlled ($p= 0.002$).**Conclusion:** Noneosinophilic asthma occured in older, obese patients, were usually non atopic and had poor asthma control after three months optimized treatment, compared to eosinophilic phenotype. Thus phenotypic classification could help to guide clinical decisions in personalized medicine approach.

Keywords: Asthma, Eosinophilic, Noneosinophilic, Phenotypes

Introduction

Asthma is a chronic inflammatory disease of the airways, characterised by airway hyper -responsiveness, airflow limitation and respiratory symptoms such as dyspnoea, wheeze and cough [1]. Chronic inflammatiory process in the airway leads to tissue remodelling of the airway structure. Although originally thought to be a Th2-driven inflammatory response, the immune response in asthma is now considered highly heterogeneous [2]. The pattern of granulocyte infiltration can be used to identify different inflammatory phenotypes in asthma. Recognized granulocyte phenotypes using induced sputum are eosinophilic (EA), neutrophilic, mixed granulocytic and paucigranulocytic asthma [3]. Knowledge of inflammatory phenotype is useful because it relates to

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treatment response, mechanistic pathways involved in disease pathogenesis and future disease risk [3]. The population attributable risk of asthma because of eosinophilic inflammation is about 50%, and conversely, this means that up to 50% of asthma cannot be attributed to eosinophilic inflammation, and represents asthma associated with non-eosinophilic processes [3]. In these patients, bronchial biopsy shows significantly fewer eosinophils in the bronchial mucosa than subjects with EA [3]. This confirms that non-eosinophilic asthma is a consistent pattern/phenotype in the airway lumen and the airway mucosa. A key aspect of asthma inflammatory phenotype analysis is that it can be applied to individual patients [3]. The underlying principle relates to the association between a clinical response to corticosteroids and the presence of a selective sputum eosinophilia. Clinically useful applications of induced sputum analysis are the detection of non-adherence to corticosteroid therapy, assessment of adequacy of inhaled corticosteroid therapy, long-term therapy management in asthma, oral corticosteroid dose adjustment in refractory asthma [3].

Use of sputum induction as noninvasive measurements of airway inflammation in the diagnosis and management of asthma is very important for every patient diagnosed with bronchial asthma before starting asthma management and for asthmatic patients who were not controlled by full asthma management to understand the type of airway inflammation [4]. Sputum analysis is a simple, noninvasive method to study the airway inflammation [4]. Based on sputum eosinophil count, patients may be divided into eosinophilic (eosinophils≥3%) and non eosinophilic (eosinophils<3%) phenotypes [5].

We have limited data from India about the prevalence of inflammatory phenotypes of asthma in adults. The aim of this study was to find out the pattern of the inflammatory phenotypes among patients with asthma using sputum cytology [6], attending the outpatient clinic in a tertiary care centre of South India, and to compare the effectiveness of treatment among eosinophilic and noneosinophilic phenotypes after 3 months of optimized treatment as per GINA guideline 2015[1].

Materials and Methods

Study design- Prospective cohort study for a period of one year.

Study setting- Department of Pulmonary Medicine, Government Medical College, Thiruvanathapuram, Kerala.

Inclusion criteria- All consecutive, non-smoker patients diagnosed with asthma as per GINA [1], attending the outpatient clinic of our hospital.

Exclusion criteria- Clinical or radiological evidence of concurrent respiratory illness, use of systemic or inhaled steroid therapy in the preceding one month and those patients who were unable to bring up sputum for pathological examination.

Participants- We included 138 consecutive Asthma patients after getting informed written consent.

Data source- The demographic details, symptoms of the patient, and spirometry results were collected using a duly filled proforma. Sputum samples were taken from these patients and sputum cell count was done by microscopy of Leishman stained smears. Leishman stain uses a methanol solution of staining dyes. 7-10 drops are applied to the slide, with the specimen. After 20 seconds, 10-15 drops of a buffer solution is added and mixed with the stain. The specimen is left staying for 20-30 minutes, and then washed off with the buffer solution. At least 200 non-squamous cells were counted on satisfactory slides, under oil immersion. All cell percentages were averaged to give the final values. Eosinophil percentage was expressed as a percentage of total inflammatory cells, excluding squamous cells.

Asthma patients with eosinophil count 3% or more were considered as eosinophilic asthma and those with eosinophil count less than 3% were considered as noneosinophilic asthma. All the patients were started on treatment according to GINA guideline and after three months of optimized treatment [1], the level of asthma control assessed according to GINA guideline [1].

Statistical analysis- Data were entered in Microsoft Excel and analyzed using Epi Info version 3.5.3. For descriptive statistics, quantitative variables were described by mean and standard deviation. Qualitative variables were described by percentage distribution. For inferential statistics between groups, comparison of qualitative variables were analysed by chi-square test and quantitative variables were compared by student t test. P value of less than 0.05 was considered as level of significance.

Results

The mean age of the patients in the study group was 43+12 years.Majority of the patients (68%) belonged to the age group of less than50 years. Nearly 60% of the patients were females. Mean duration of illness was 11.2 ± 4.6 years. 16.7% of study population had mild, 50% had moderate and 33.3% had severe persistent asthma before starting treatment.

50.7% of patients had atopic symptoms. 50% of patients had family history of asthma. Mean BMI of study population was 24 ± 2.4. 53.6% of study population were eosinophilic and 46.4% were noneosinophilic phenotype.

Table-1: Mean age of phenotypes.

PHENOTYPE	N	Age in years		t	P
		Mean	SD		
Eosinophilic	74	38.4	11.7	-5.458	< .001
Noneosinophilic	64	48.8	10.6		

Mean age of eosinophilic phenotype was 38.4 ± 11.7 and that of noneosinophilic was 48.8 ± 10.6 (p<0.001).

Table-2: Percentage distribution of the phenotype according to atopy.

Atopy	Phenotype				Total	
	Eosinophilic		Noneosinophilic			
	N	%	N	%	N	%
NO	20	27.0	48	75.0	68	49.3
YES	54	73.0	16	25.0	70	50.7
Total	**74**	**100.0**	**64**	**100.0**	**138**	**100.0**

P<0.001

Atopic symptoms were present in 73 % eosinophilic and 25 % noneosinophilic phenotypes (p< 0.001).

Table-3: Mean BMI of the phenotype.

Phenotype	N	BMI		t	p
		Mean	sd		
Eosinophilic	74	23.3	2.4	-3.996	<0.001
Noneosinophilic	64	24.9	2.3		

Mean BMI of eosinophilic asthma patient was 23.3 ± 2.4 and that of noneosinophilic was 24.9 ± 2.3 (p <0.001).

There was no statistically significant difference between the two phenotypes with regard to the gender, duration of illness, family history of asthma, or initial severity of the illness.

Table-4: Phenotype and level of control with treatment.

Level of control	Phenotype				Total	
	Eosinophilic		Noneosinophilic			
	N	%	N	%	N	%
Controlled or Partialy controlled	54	73.0	30	46.9	84	60.9
Uncontrolled	20	27.0	34	53.1	54	39.1
Total	**74**	**100.0**	**64**	**100.0 (P<0.002).**	**138**	**100.0**

After 3 months of optimized treatment according to GINA guidelines, among the eosinophilic phenotype 73% of patients became controlled or partly controlled and 27% remained uncontrolled. In the noneosinophilic phenotype 46.9% became controlled or partly controlled and 53.1% remained uncontrolled. These changes were statistically significant (p= 0.002).

Discussion

Despite its prevalence and cost to the healthcare system, the pathogenesis of Asthma remains poorly understood. It is clear that asthma is a heterogeneous disease and recent approaches have attempted to define asthma subgroups based on inflammatory phenotypes. Characteristics of the cellular makeup of sputum, blood, bronchoalveolar lavage fluid, and endobronchial biopsies have been examined in asthmatic subjects, with a primary focus on eosinophils and neutrophils, and, more recently, mast cells.

The inflammatory phenotypes identified to date include eosinophilic asthma, neutrophilic asthma, pauci-granulocytic asthma, and T helper 2-associated asthma. Defining these phenotypes has already led to more personalized and successful targeted therapies, with new developments on the horizon [7]. While exposure to allergen in a sensitised individual can elicit an eosinophilic response, the triggers of the noneosinophilic phenotypes have been less well studied. Chronic infection is a possible cause, since infections typically elicit a neutrophil response similar to that observed in neutrophilic asthma (NA) [8]. It is not clear whether the distinct inflammatory phenotypes occur as a result of different aetiological pathways. It is possible that this heterogeneity in inflammatory response could lead to different levels of repair and remodeling between phenotypes. This suggestion is supported by the finding of a negative correlation between sputum neutrophil count and FEV/FVC ratio, and the absence of collagen deposition in the subreticular basement membrane in neutrophilic patients [9]. About 41% of asthmatics have eosinophilic inflammation. Neutro-phillic constitute 20%, combined eosinophilic and neutrophilic form 8% and the remaining 31% is paucigranulocytic [9].

The advantage of distinguishing between inflammatory phenotypes in asthma is to identify subgroups of patients who are more likely to respond to individually tailored treatment. Studies have proved that eosinophilic airway inflammation predicts good response to inhaled corticosteroids (ICS), whereas noneosinophilic asthma is less responsive to ICS [11,12]. Inflammation-based asthma management promises improved disease control, providing that inflammatory phenotypes are reproducible and reliable. Under such a condition asthmatics' sputum inflammatory profiles could help guide clinical decisions in personalized medicine approach [10]. Our study shows that 53.6% of study population were eosinophilic and 46.4% were non eosinophilic phenotypes, which correlate with result of previous study by Gibson PG, where the population attributable risk of asthma because of eosinophilic inflammation is about 50%, [3]. According to some literature about 41% of asthmatics have eosinophilic inflammation [9].

In this study,73% of eosinophilic asthmatics had atopic symptoms whereas in non eosinophilic group only 25% were atopic. Pranab Haldar et al repoted atopy in 95% early onset eosinophilic asthma and 51.9% of non eosinophilic asthma [16]. According to Amelink M etal adult-onset asthma were predominately nonatopic (55%) [13].Non eosinophilic asthma was found to occur in older age group compared to eosinophilic asthma in the study. Pranab Haldar et al repoted that eosinophilc asthma asthma usually occurs at younger age [16].

In our study non –eosinophilic phenotypes were having higher BMI compared to eosinophilic phenotype, correlating with finding that obesity in patients with difficult-to-treat asthma is inversely related with sputum eosinophils of van Veen IH et al [14]. No statistically significant difference between the two phenotypes regarding gender, family history of asthma, duration of symptoms, or intial asthma severity was observed in this study, Similar to the finding of cluster analysis [16].

In our study, with optimised treatment, asthma control in eosinophilic and noneosinophilic phenotype are 73% and 46.9% respectively, a result that is comparable to previous literature. John V etal also reported that Eosinophilic asthma is corticosteroid responsive.and Noneosinophilic asthma is a sizeable subgroup of asthma, which includes patients with severe disease, and it may be relatively corticosteroid resistant [15].

Conclusion

Sputum cytology is a simple, non invasive tool to identify inflammatory phenotypes of asthma. As

response to treatment vary in eosinophilic and non eosinophilic asthmatics, phenotyping help to understand the underlying pathobiology and to provide clinicians with directions for personalized management

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