ORIGINAL ARTICLE

A STUDY OF NASAL EOSINOPHILIA IN ASTHMA PATIENTS

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ABSTRACT: INTRODUCTION: Asthma is an inflammatory, reversible and progressive disease with episodes of exacerbations. Asthma is now considered as single airway disease and hence comprehensive diagnosis, treatment and follow-up of upper and lower respiratory tract is essential. Both the Nasal and Bronchial mucosa are the elements of united airway disease and Eosinophils measured from this mucosa can be an indirect marker of Airway inflammation in asthma. Eosinophil infiltration is hallmark feature of pathogenesis of asthma which is the trigger for the chronic airway inflammation and these are raised in acute exacerbations. Hence assessing Eosinophil is evidence of serological marker for Airway inflammation (AI). Nasal Smear Eosinophils (NSE) is used in asthma with Allergic Rhinitis (AR). This study Using Definition of GINA explores the utility of NSE as marker for management of Asthma. **METHODS:** In this study 100 patients diagnosed with bronchial asthma attending the outpatient department in KIMS were studied. The severity of asthma was assessed as per FEV1 classification- Adapted from 2007 NHLBI Guidelines for the diagnosis and treatment of Asthma Expert panel Report 3. The Statistical analysis was done with SAS 9.2, SPSS 15.0, Stata 10.1, Med Calc 9.0.1, Systat 12.0 and R environment ver.2.11.1.software. **RESULTS:** Asthma was more common in Females in this study with M: F ratio of 1.04 and Allergic Rhinitis was found in 55% of Asthmatics. Nasal Eosinophilia was seen in 44% in asthmatic group and 49% in Asthma with Allergic Rhinitis. Absolute Eosinophil count (AEC) and Differential count (DC) for Eosinophils was almost same in both groups. The Sensitivity of NSE with respect to DC is 70% and in AR group with Asthma and in Asthma group with/without AR it was 38%. Similarly the NSE with respect to AEC was 47% in AR group with Asthma whereas in Asthma group with/without AR it was 38%. **CONCLUSIONS**: The finding confirms that symptoms, Variable airflow Obstruction and Airway inflammation are interlinked in asthma. The result of this study does not favor tailored approach in diagnosis of asthma by selecting test such as NSE. There was no Uniform Correlation of Eosinophil measure with severity of asthma in the study but the NSE identifies different aspects of Airway Inflammation having different Phenotypes and is more severe in AR with Asthma, hence it is a simple method and tool for assessing Airway inflammation and Management of Asthma. If assessment of Airway inflammation is required percentage of NSE is recommended initially given its ease of use.
KEYWORDS: Asthma: Asthma, Nasal Eosinophilia, Eosinophilia, allergic rhinitis.

INTRODUCTION: Asthma is an inflammatory, reversible, reactive & progressive disease, notable for episodes of exacerbations and remissions. As Asthma represents an airway disease a reciprocal diagnosis, follow up and treatment of both upper and lower respiratory disease is essential. Both nasal and bronchial mucosa are elements of a united airway. Eosinophils are an indirect marker of airway inflammation in asthma. Total eosinophils count reflects asthmatic activity and is useful for regulating steroid dosage and for early detection of exacerbations. The cellular infiltrates in asthma is incriminated as an important trigger for chronic airway inflammation.

ORIGINAL ARTICLE

The current reviews indicate that the relationship of eosinophils and asthma is very important for diagnosis and management of asthma. There is a need to work on all the aspects of this relationship that includes finding correlation of symptoms of asthma with markers of eosinophil activity, identifying the substances attracting, activating or developing eosinophils and developing drugs to neutralize these substances.

Research Works are now underway to develop asthma therapy leading to inhibition of eosinophil priming of cytotoxic mechanisms in vivo. For the development of more effective anti-asthma drugs it, seems relevant to unravel and interfere with the steps of eosinophil activation. There are now attempts to inhibit eosinophil differentiation at bone marrow level.

Markers of airway inflammation are needed for prediction of asthma deterioration and evaluation of disease severity. It is important to find a marker of disease activity, ideally one that is simple to measure, reliable and inexpensive. As yet no such marker has been found for asthma. Therefore, there is a need for assessing different eosinophil products to develop a serological marker of airway inflammatory activity in asthma.

Treatment decisions in asthma are based on assessments of symptoms and simple measures of lung function, which do not relate closely to underlying eosinophilic airway inflammation. Thus the need for this study to evaluate the usefulness of nasal eosinophilia.

METHODOLOGY: The study was conducted on 100 patients diagnosed with bronchial asthma attending the outpatient department of Kempegowda Institute of Medical Sciences Subjects underwent following procedures- Clinical assessment, Questionnaire, Spirometry, Nasal Sputum smear and sputum smear and Blood for Eosinophils. Reversibility of airway obstruction was defined as FEV1 > 12% and 200ml after Salbutamol inhalation. All asthmatic patients were diagnosed by Spirometry and classified based on FEV1 (FEV1classification- Adapted from 2007 NHLBI Guidelines for the diagnosis and treatment of Asthma Expert panel Report 3. A total of 100 patients were selected after meeting the inclusion criteria and exclusion criteria of the study. Meticulous efforts were taken to analyze Nasal smear Eosinophilis. This was an Observation Clinical Study.

INCLUSION CRITERIA:
1. Age ≥18 years
2. Bronchial Asthma Cases proven by Spirometric evidence of airflow reversibility.

EXCLUSION CRITERIA:
1. Patients having on-going or past history of tuberculosis and COPD, ILD.
2. Any history of hemoptysis.
3. Those on oral corticosteroids prior to the study.
4. Cases with IHD.

Initial assessment included detailed clinical history and examination and later Proformas were filled. History of exposure to risk factors for the disease, such as smoking, fumes, irritants, and dust exposure were noted.

Spirometer meeting ATS was criteria used. Spirometer was done in sitting position by the patient. Best of the 3 manoeuvres will be defined as the highest FEV1 and highest PEF regardless from which manoeuvres they come from same or different effort. A maximum of 8 manoeuvres are

ORIGINAL ARTICLE

allowed. If medication has been taken within 6 hours PFT was not performed and the visit will be rescheduled.

Reversibility Testing: At the screening visit after completion of three acceptable pre-bronchodilator forced expiratory manoeuvres, all patients will be asked to inhale salbutamol (100µg) so as to document the degree of reversibility. Within 10 minutes of the pre-bronchodilator forced expiratory manoeuvres, two separate doses approximately 30seconds apart of 100µg of salbutamol will be administered.

Total dose 200 µg of Salbutamol will be delivered. The patient will be encouraged to hold his breath for 10 seconds after each inhalation. Three additional acceptable post-bronchodilator forced expiratory manoeuvre tests will be recorded within 10–30mins after the last dose of salbutamol is inhaled.

Nasal Smear Cytology: Nasal scrapping made form nasal bud scrap in inferior meatus. The Material were spread on slide, Fixed with 95% alcohol and Stained- Pap and H& E stain. The slides were Examined under 40X and DC performed and graded for Eosinophilis. Nasal Smears were collected by scraping mucus membrane of inferior meatus with cotton scab, air dried, stained with May Grundwald & Giemsa stain and rinsed in tap water. The slides were decoloured with Alcohol and again rinsed with water, dried and examined with oil immersion(X 1000).

STATISTICS: Descriptive statistics were used to summarize clinical characteristics of participants. Comparisons in this group were made using t test for normally distributed variables – Sensitivity, Specificity and Positive Predictive Value was calculated. All statistical tests were two sided and significant was accepted at 95% level. Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean $\pm$ SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5% level of significance.

Statistical software: The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, Med Calc 9.0.1, Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

RESULTS & OBSERVATIONS: A strong relation between history and symptoms and bronchodilatory [BDR] response was noted. The Base line characteristics are illustrated in table 1 with mean age of 46 years. The percentage of patients with Bronchial Asthma with Respiratory allergy in the age group of < 20 & 70-80 years is 9%, while in age group of 21-40 had 31%. The highest Percentage of asthmatics [24%] was in the age group of 31-40 years. The Incidence of Asthma was more common in females constituting about 51% with F: M ratio = 1.04 as is shown in table 2.

ORIGINAL ARTICLE

Age in years	No. of patients	%
18-20	7	7.0
21-30	10	10.0
31-40	21	24.0
41-50	22	22.0
51-60	24	21.0
61-70	14	14.0
71-80	2	2.0
Total	100	100.0
Table 1: Age Distribution Mean ± SD: 46.65±14.77		

Gender	No. of patients	%
Male	49	49.0
Female	51	51.0
Total	**100**	**100.0**
Table 2: Gender distribution of patients studied		

In this study most common symptom and sign in Allergic Rhinitis with Asthma was Rhinorrhea accounting 70% for Nasal obstruction accounting for 67% of all symptoms. The Most common symptom in all asthmatic patients with or without Allergic Rhinitis is Wheeze and Episodic breathlessness.

The severity of Asthma as assessed by FEV1 in Severe, moderate and mild group constituted 46%, 47 % and 7% as shown in table 3.

FEV1%	No. of patients	%
<60.0	46	46.0
60-80	47	47.0
>80	7	7.0
Total	**100**	**100.0**
Table 3: Distribution of FEV1 percentage Mean ± SD: 60.67±13.48		

The Eosinophils in Blood reported as Differential Count in asthmatics was raised in 43% and Absolute Eosinophil Count (AEC) was increased in 32% of asthmatics is shown in Table 4.

DC of Eosinophilis in blood	No. of patients (n=100)	%
<5.0	57	57.0
>5.0	43	43.0
Table 4: Distribution of Eosinophils as Differential count in asthmatics		

ORIGINAL ARTICLE

AEC	No. of patients	%
< 440	68	68.0
> 440	32	32.0
Total	**100**	**100.0**
Table 5: AEC distribution shown in asthmatics		

58% of Asthmatics had less than 5 % of Eosinophilis in Nasal smear cytology while 42% of them had Sputum smear Eosinophilis > 5%.

Nasal Smear Cytology Eosinophilis	No. of patients (n=100)	%
<5.0%	58	58.0
5.0-50.0%	39	39.0
>50.0%	3	3.0
Table 6: Eosinophils Expressed as DC in Nasal Smear Cytology		

There is almost uniform distribution of eosinophils in Nasal and Sputum smear across all age groups. The incidence of Allergic rhinitis in Asthma group was 55%.

In this study group, all 100 asthma patients were investigated for eosinophils in Blood, Nasal Eosinophilia. Nasal Eosinophilia is Positive in 42%, and Eosinophils in DC were 43% and AEC> 440 being 32% in 100 patients with Asthma.

In 55 asthmatic patients who had allergic rhinitis in addition, Nasal Eosinophilia was positive in 54% and Eosinophilis DC was 49% with AEC> 440 in 31%.

	Asthma group	Asthma with AR
Nasal Eosinophilia	42%	54%
Blood Eosinophilis as DC	43%	49%
AEC> 440 cells	32%	31%
Table 7: Showing percentage of Nasal Eosinophilia in asthma patients (without Allergic Rhinitis) and Asthma patients with Allergic Rhinitis		

NSE	Asthma Group with AR	Asthma group without AR	Total
< 5 %	28 (51 %)	30 (66 %)	58
> 5%	27 (49 %)	15 (44 %)	42
Table 8: Showing percentage of Nasal eosinophilia in Asthma group with AR and asthma Patients without AR			

AEC	Asthma with AR	Asthma group without AR	Total
> 440	17 (31 %)	15 (33 %)	32
< 440	38 (69%)	30 (67%)	68
Table 9: showing the percentage of AEC in asthma patients and Asthma with AR group			

ORIGINAL ARTICLE

| DC	Asthma with AR	Asthma group without AR	Total
> 5%	3 (60 %)	25 (60 %)	43
< 5%	22 (40%)	20 (40 %)	57
Table 10: showing the percentage of Eosinophils expressed As DC in Asthma group and Asthma with AR group			

Nasal Eosinophilia is slightly more common with allergic rhinitis with asthma (49 % v/s 44%).AEC is more common in asthma group of patients without with AR (31% v/s 33%) DC is same in both groups (60% v/s 60%).

Variables	Grouping	Severity of Asthma			P value
		Severe (n=46)	Moderate (n=47)	Mild (n=7)	
Eosinophil % in DC	< 5.0%	25(54.3%)	30(63.8%)	2(28.6%)	0.170
	> 5.0%	21(45.7%)	17(36.2%)	5(71.4%)	
AEC	<440	31(67.4%)	33(70.2%)	4(57.1%)	0.797
	>440	15(32.6%)	14(29.8%)	3(42.9%)	
Nasal Smear Cytology E	<5%	26(56.5%)	28(59.6%)	3(42.9%)	0.654
	5.0-50.0%	19(41.3%)	16(34%)	4(57.1%)	
	>50.0%	1(2.2%)	3(6.4%)	0 (0%)	
Table 11: Correlation of Eosinophils in DC, AEC, and Nasal Smear Cytology (NSE) with Severity of asthma					

- The Eosinophilis in Blood measured as Differential Count are positively correlated with mild form of asthma while negatively correlated in moderate and severe form of the asthma; however the p value is not significant and hence statistically insignificant.
- The Eosinophilis in AEC were negatively correlated with all forms of Asthma. However the p value is not significant and hence statistically insignificant.
- The Eosinophilis in Nasal Smear were positively correlated with mild form of Asthma and negatively correlated in Moderate and severe form. However the p value is not significant and hence statistically insignificant.

$y = 0.1326x + 59.702$
$R^2 = 0.0272$
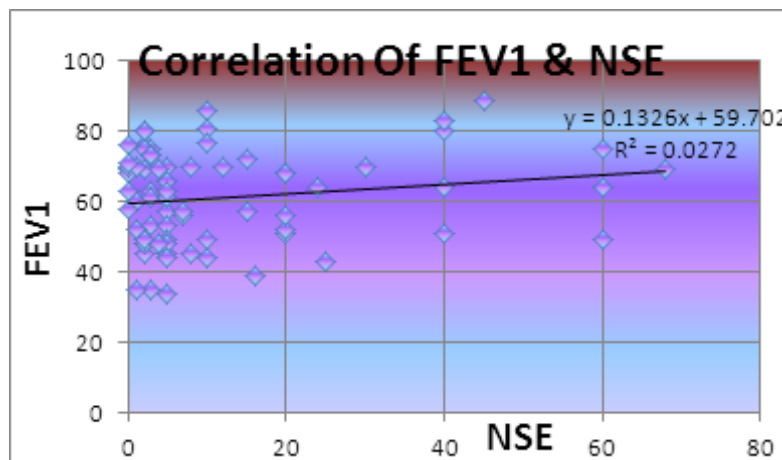
ORIGINAL ARTICLE

Variables	Grouping	Severity of Asthma in Allergic Rhinitis			P value
		Severe (n=23)	Moderate (n=25)	Mild (n=7)	
Eosinophil % in DC	<5.0%	13(56.5%)	17(68%)	3(42.9%)	0.440
	>5.0%	10(43.5%)	8(32%)	4(57.1%)	
AEC	<440	16(69.6%)	10(40%)	5(71.4%)	0.096+
	>440	7(30.4%)	15(60%)	2(28.6%)	
Nasal Smear Cytology E(NSE)	<50%	13(56.5%)	17(68%)	3(42.9%)	0.440
	5.0-50.0%	10(43.5%)	8(32%)	4(57.1%)	
	>50.0%	-	-	-	

Table: 12: Correlation of Eosinophils in DC, AEC, Nasal Smear Cytology (NSE) with Severity of asthma with Allergic rhinitis.[55] PATIENTS

- The Eosinophilis in DC were positively correlated with some positive trend in mild form of Asthma and negatively correlated in Moderate and severe form. However the p value is not significant and hence statistically insignificant.
- The Eosinophilis in AEC were negatively correlated with all forms of Asthma. However the p value is insignificantly and hence statistically not significant.
- The Eosinophilis in Nasal Smear were positively correlated with mild form of Asthma and negatively correlated in Moderate and severe form. However the p value is not significant and hence statistically insignificant.

DISCUSSION: Asthma is a heterogeneous disorder with varying intensity of symptoms, varying treatment response, variable prognosis and pathogenesis. Patients presenting with Symptoms of Asthma with and without Allergic Rhinitis are more common in Adult outpatient department and with detailed history & examination, diagnosis is usually not a problematic and routine investigations may not contribute much to the diagnosis but helps in ruling out various diseases.

To confirm the allergic nature of the diseases complicated tests such as IgE, Skin tests, RAST-Radio Allergo Sorbent Tests, ELISA- Enzyme linked Immune Absorbent test may not be possible in many hospitals setting. Hence simple noninvasive tests- nasals smear Cytology and Sputum Smear Cytology for Eosinophilis used as a simple Economical test for evaluating Allergy and Infiltrations in Asthma patients.

No precise data has been produced on Nasal cellular infiltrates Cut off values to Diagnosis and discriminate asthma phenotypes using Nasal Smear Cytology. One of the Aim and Need of this study was to study if that cellular infiltrates in nasal mucosa of Asthmatics could differentiate asthma severity and asthma phenotypes.

Asthma was more common in females constituting about 51% with Female to Male ratio of 1.04. Allergic Rhinitis with Asthma constituted about 55% [with Male to Female ratio constituting about 31:24]. The incidence in Chowdary et al [1] study Allergic rhinitis was 50%. In the study by Naveen kumar et al.[2] In children incidence of AR with asthma was 64.8% and the disease was more common in males-62%

In this study most common symptom and sign in Allergic Rhinitis with Asthma was

ORIGINAL ARTICLE

Rhinorrhea, Nasal obstruction was 70% & 67%. The Most common symptom in all asthmatic patients with or without Allergic Rhinitis is Wheeze and Episodic breathlessness.

The severity of Asthma as assessed by FEV1 in Severe, moderate and mild group constituted 46%, 47 % and 7%. As Adapted from 2007 NHLBI Guidelines for the diagnosis and treatment of Asthma Expert panel Report 3.[3]

Nasal Smear Cytology for Eosinophilis (NSE), Absolute Eosinophil Count & Differential count in Blood was done in all 100 patients recruited for the study. Nasal Smear Eosinophilis was Positive in 42%, and Eosinophils in blood reported as Differential Count were 43% and Absolute Eosinophil Count being 32% in 100 patients with Asthma.

The incidence of Allergic rhinitis in Asthma group was 55%. Based on FEV1 Allergic Rhinitis with Asthma group is divided in to Severe, moderate and mild which constituted 42%, 45% and 13% respectively. In 55 asthmatic patients with allergic rhinitis, NSE was positive in 54%, Eosinophilia in DC in 49% and AEC>440 in 31%.

Chowdary et al[1] studies showed AEC >440 cells/cu mm was significant. In Comparison with Naveen Kumar study nasal Eosinophilia was seen in 64% of asthma with AR group.

More than 5% in NSE were considered positive as reported in Nasal Eosinophilis and its diagnostic significance as per Abbey Sood[4] in IG government Medical College, Shimla. But Sanil et al[5] & Crobach et al[6] used more than 10 cells as Significant Nasal Smears. The IAP text book recommended more than 10% as Significant in children. Sanil et al study measured Nasal Eosinophils (NSE) as Absolute value- Mean values of Nasal eosinophils (Cell/HPF).

Malak Shaheen et al study[7] showed 20%, 53% & 27% in Severe, Moderate & Mild forms of Asthma. In the same reference study the Nasal smear Cytology for Eosinophilis was measured 56 Eosinophilis per High Power Field and was significant, while in the present study the NSE are positive in 23, 45 & 13 percentages in severe, moderate and Mild form of asthma with Allergic Rhinitis.

Nasal Smear Eosinophils is more common with allergic rhinitis with asthma (49% v/s 44%), but AEC is more common in non-AR (31% v/s 33%) and Differential count of Eosinophils in Blood is same in both groups (60% v/s 60%)

The Eosinophilis in Blood measured as Differential count are positively correlated with mild form of asthma while negatively correlated in moderate and severe form of the asthma; however the p value is not significant and hence statistically insignificant. The Eosinophilis in AEC were negatively correlated with all forms of Asthma however the p value is not significant and hence statistically insignificant. The Eosinophilis in Nasal Smear were positively correlated with mild form of Asthma and negatively correlated in Moderate and severe form, however the p value is not significant and hence statistically insignificant.

The Eosinophilis in DC were positively correlated with some positive trend in mild form of Asthma and negatively correlated in Moderate and severe form, however the p value is not significant and hence statistically insignificant. The Eosinophilis in AEC were negatively correlated with all forms of Asthma; however the p value is not significant and hence statistically not significant.

Peripheral Eosinophils contributes equally in Diagnosis In asthma and with or without Allergic Rhinitis. But Nasal smear Cytology contributes more in diagnosis.

The finding confirms that symptoms, Variable airflow Obstruction and AHR are interlinked in asthma. Bronchodilatory reversibility used traditionally as most specific tests for the diagnosis of

ORIGINAL ARTICLE

asthma. The result favors tailored approach in diagnosis of asthma using BDR initially, and then selecting test- NSE depending on facilities available in the centers.

So NSE identifies different aspects of Airway Inflammation having different Phenotypes and are more severe in Allergic rhinitis with Asthma, hence it is an simple method and tool for assessing AI and Management of Asthma.

There was no Uniform Correlations of Eosinophils measure with severity of asthma in the study but the Nasal smear is a simple Economical and non- invasive procedure and can be used as alternative to AEC and DC as better efficient in diagnosis and Management of Asthma. If assessment of AI is required percentage of SSE/NSE is recommended initially given its ease of use.

CONCLUSION: NSE identifies different aspects of Airway Inflammation which have different causes and can result in different clinical effects. Nasal Eosinophils are characteristic of Atopic Asthma and may be steroid responsive. A significant association was not demonstrated between symptoms of Asthma and Underlying Airway Inflammation as Measured by NSE. It therefore cannot be used as surrogate markers of Airway Inflammation in these settings. Accurate Tools and methods which measure each of these components are required to optimize management. Research targeted at simplifying these techniques and methods are to be addressed in further studies.

There was no Uniform Correlations of Eosinophilis measure with severity of asthma in the study but the Nasal smear cytology is a simple Economical and non- invasive procedure and can be used as alternative to AEC and DC as they are better efficient in diagnosis and Management of Asthma. If assessment of AI is required percentage of NSE is recommended initially given its ease of use. Thus NSE may be useful in delineating a subtype of AI.

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ORIGINAL ARTICLE

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