Original Article

Usefulness of induced sputum eosinophil count to assess severity and treatment outcome in asthma patients

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ABSTRACT

Context: Currently treatment decisions in asthma are governed by clinical assessment and spirometry. Sputum eosinophil, being a marker of airway inflammation, can serve as a tool for assessing severity and response to treatment in asthma patients. **Aims:** To establish correlation between change in sputum eosinophil count and forced expiratory volume in one second (FEV_1) % predicted value of asthma patients in response to treatment. In this study, we also predicted prognosis and treatment outcome of asthma patients from baseline sputum eosinophil count. **Settings and Design:** A longitudinal study was conducted to determine the treatment outcome among newly diagnosed asthma patients who were classified into A ($n = 80$) and B ($n = 80$) groups on the basis of initial sputum eosinophil count (A $\geq$ 3% and B < 3%). **Materials and Methods:** After starting treatment according to Global Initiative for Asthma Guideline, both A and B groups were evaluated every 15 days interval for the 1st month and monthly thereafter for a total duration of 12 months. In each follow-up visit detailed history, induced sputum eosinophil count and spirometry were done to evaluate severity and treatment outcome. **Results:** FEV_1% predicted of group A asthma patients gradually increased and sputum eosinophil count gradually decreased on treatment. Longer time was required to achieve satisfactory improvement (FEV_1% predicted) in asthma patients with sputum eosinophil count $\geq$3%. There was statistically significant negative correlation between FEV_1 % predicted and sputum eosinophil count (%) in of group A patients in each follow-up visit, with most significant negative correlation found in 8th visit ($r = -0.9237$ and $P = < 0.001$). Change in mean FEV_1% (predicted) from baseline showed strong positive correlation ($r = 0.976$) with change in reduction of mean sputum eosinophil count at each follow-up visits in group A patients. **Conclusions:** Sputum eosinophil count, being an excellent biomarker of airway inflammation, can serve as a useful marker to assess disease severity, treatment outcome, and prognosis in asthma patients.

KEY WORDS: Asthma, forced expiratory volume in one second, induced sputum, sputum eosinophil count, treatment outcome

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INTRODUCTION

Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play an important role, resulting in hyper responsiveness of the airway which explains most of the symptomatology of asthma.[1] The severity and control of asthma in both children and adults are judged predominantly by subjective or objective measures. Subjective measures usually consist of a series of questions based on clinical assessment and quality of life questionnaires.[1] Spirometry, peak flow measurement, and bronchoprovocation testing constitute the traditional objective means of measuring asthma.[1] Evaluation of symptoms and lung function measurement currently governs treatment decisions in asthma. Nowadays, many markers of airway inflammation such as sputum eosinophil and exhaled nitric oxide have been advocated for asthma monitoring.[2] These are supposed to be more sensitive markers than subjective and traditional objective measures, as they provide direct measurement of airway inflammation. However, correlation of clinical findings with the biological markers of airway inflammation is not well established. Measurement of sputum eosinophil count may be helpful in this purpose. Analysis of results available from induced

sputum is almost identical to results of secretions obtained through bronchial wash and bronchoalveolar lavage.[3,4] Bronchoscopy is invasive, potentially hazardous, expensive, involving risk to life of patient. Although analysis of sputum in asthma had many advantages, it was hampered as an investigative tool by lack of standardization and the inability to obtain sample in all cases.[5] This problem was overcome by inducing sputum with hypertonic saline, a technique which is safe, easy to perform and all spectrum of asthma severity in terms of symptoms and lung function can be studied to evaluate relationship between eosinophilic airways inflammation and asthma.

With this perspective in mind, we wanted to establish correlation between change in sputum eosinophil count and forced expiratory volume (FEV_1) % predicted value of asthma patients in response to treatment. In this study, we also predicted prognosis and treatment outcome of asthma from baseline sputum eosinophil count.

MATERIALS AND METHODS

160 asthma patients were selected on the basis of clinical parameters and spirometry with following inclusion and exclusion criteria:

Inclusion criteria were (i) 15-45 years of both male and female who never received steroid previously or received inhaled steroids previously but not in last three months before observation; (ii) normal chest X-ray (CXR); (iii) clinical features suggestive of asthma; and (iv) spirometry finding $FEV_1/FVC < 70\%$ and significant bronchodilator reversibility (12% and > 200 ml increase in FEV_1 after 4 puffs of short-acting beta2-agonist).

Exclusion criteria were (i) clinical features and spirometry suggestive of chronic obstructive pulmonary disease, congestive heart failure, and bronchiectasis; (ii) smoker; (iii) mixed and restrictive pattern of lung function in spirometry; (iv) pregnant; (v) not giving consent; and (vi) could not perform spirometry correctly.

After that they were divided into two groups (group A and group B) consisting of 80 patients each [Figure 1]. They were enrolled for the study on the basis of induced sputum eosinophil count. Asthma patients with sputum eosinophil count ≥ 3% were in group A and < 3% in group B. It was a longitudinal comparative study to assess the treatment outcome.

Sputum induction and processing
After baseline FEV_1 and FVC measurements, subjects were pretreated with inhaled salbutamol (200 µg by metered-dose inhaler), and 10 min later nebulization was done with hypertonic (3%) sterile saline solution for three periods of 5 min at most by means of an ultrasonic nebulizer. The subjects were instructed to cough sputum into containers. If any symptom occurred, nebulization

was discontinued. The volume of the selected sputum was measured and 0.1% dithiothreitol (Sigma Chemicals, Poole, United Kingdom) added to the sputum in a 4:1 ratio to break up the disulphide bonds and disperse the cells. The cell suspension was aspirated until homogenized and filtered to remove any remaining debris. Phosphate-buffered saline was then added to the cell suspension.[6,7]

Smear preparation
After separation from supernatant by centrifugation, thick particles of sputum were transferred to the slide from sputum collection bottle. Then they were gently crushed between the two slides and material was distributed thinly and evenly over the surface of the slide. For fixation purpose, prepared slides were immediately immersed in a Copplings jar with 95% ethyl alcohol as fixative. Staining was done by hematoxylin and eosin (H and E) stain. The quality of induced sputum was assessed based on the presence of an adequate number of cells for enumeration, the presence of pulmonary macrophages on the slide and the proportion of squamous epithelial cell (less than 50% squamous cells). The eosinophil count was then expressed as a percentage of the total cell count as it is more accurate than absolute count.[8]

Spirometry
Spirometry was performed before sputum induction using the Jaeger Masterscope® spirometry system (Jaeger, Wuerzburg, Germany) according to the American Thoracic Society (ATS) guidelines. Before the recordings were taken, all the subjects were well motivated to ensure that the recordings were done at optimum levels. The spirometric measurements were made with the subjects in a comfortable sitting position. The body height and body weight were measured by using a standard scale without wearing footwear. All the measured lung volumes, which were obtained, were expressed in terms of body temperature pressure that was saturated with water vapor. The body surface area was calculated by using the Du-Bois and Du-Bois formula.

Study design
Both groups of asthma patients were classified according to the severity of clinical features before giving treatment and were prescribed step wise approach of asthma management according to Global Initiative for Asthma (GINA) Guideline, starting with the recommended dose of ICS and LABAs.[1] Level of asthma control was determined according to GINA guideline and management approach was prescribed based on control status. For controlled asthma patients, short-acting beta2- agonist was prescribed as needed basis; for partly controlled asthma patients, budesonide (100 µg) metered dose inhaler (MDI) was advised as one puff twice daily basis and for uncontrolled asthma patients formoterol (6 µg) and budesonide (100 µg) combination in MDI form was prescribed one puff twice daily. Both A and B groups were evaluated every 15 days interval for the 1st month and monthly thereafter for a total duration of 12 months. In each follow-up visit, evaluation
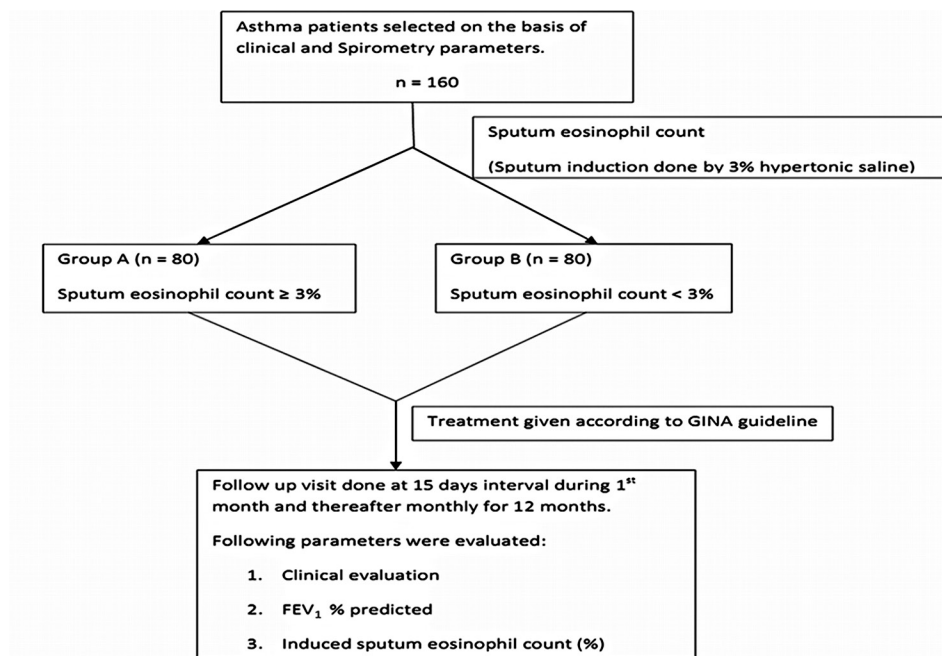
GINA = Global Initiative for Asthma. FEV$_1$ = Forced expiratory volume in one second.

Figure 1: Flow chart describing the patient flow and study method

of sputum eosinophil count and spirometry were done and clinical history of night waking due to breathlessness during last 1 month, number of exacerbations in between visits, and impairment of quality of life were asked. In each follow-up visits, patient's asthma control status was assessed and treatment was step up or step down according to the asthma control status of patients. Finally, the severity of the disease among A and B groups were compared. Change of sputum eosinophil count and FEV$_1$% predicted in response to therapy in group A and group B were evaluated and inference was drawn.

Study outcome variables
Change in mean FEV$_1$% (predicted) and mean sputum eosinophil count was the main outcome variables. The incidence of asthma exacerbations, sleep disturbances, performance of daily activity, and change in status of asthma control were the other outcome variables of the study. Exacerbations were defined as a worsening of symptoms requiring increased use of short-acting β$_2$ agonists by four extra puffs a day for at least 48 h, or by nocturnal awakening or early morning symptoms two or more times in 1 week, with or without a reduction in FEV$_1$ of at least 20%.

Statistical analysis
By unpaired "*t*" test we compared mean values of FEV$_1$% predicted and sputum eosinophil count (%) of A and B groups of asthma patients to determine level of significance (*P* value). Correlation between FEV$_1$ (% predicted) and sputum eosinophil count (%) of group A patients was evaluated in each follow-up visit. Statistical calculation was done by SPSS 12 software.

RESULTS

Demography and baseline data
Mean age of groups A and B were 28.57 ± 9.55 years and 27.45 ± 5.70 years, respectively. Males and females constitute 52.5% and 47.5% of group A, respectively. Among group B, males and females constitute 60% and 40%, respectively. There was no statistically significant difference between A and B groups in respect to age, sex, duration of cough, breathlessness, wheeze, family history of asthma, allergy to house dust, gastro esophageal reflux disease, allergic rhinitis, food allergy, and allergy to pets [Table 1].

It was observed that in initial visit, around 60% and 40% patients of group A belonged to severe persistent and moderate persistent group of asthma category, respectively. On the contrary, in group B, 12.5% were suffering from moderate persistent asthma, whereas 87.5% of patients were suffering from mild persistent asthma at the initial visit.

Outcome variables
Improvement of asthma status of group A was demonstrated in 13 subsequent follow-up visits. It was seen that mean number of sleep disturbances in first follow-up visit in group A and group B are 21 and 3, respectively. After that, it came down gradually and at the last follow-up visit it became 2 (group A) and 1 (group B). Control status of group A patients also improved in successive follow-up visits with treatment. In group A patients in the first follow-up visit, only 10 patients out of 80 achieved control of asthma, whereas in group B 64 patients out of 80 achieved control of asthma. At the end of 1 year 72 patients in group A and 76 patients of group B achieved control.
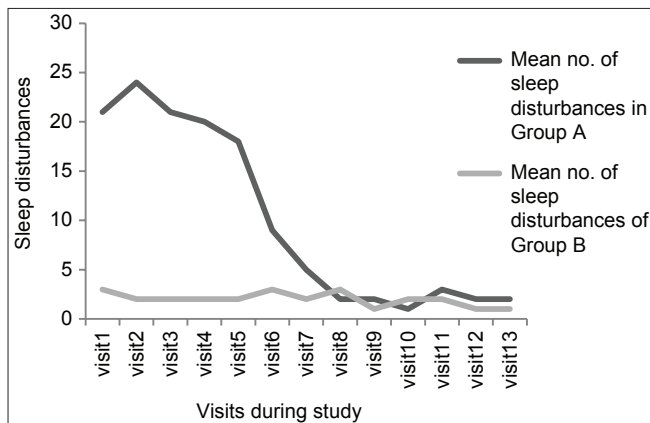
At the first follow-up visit, mean number of patients who could perform daily activities was 67.5% in group A and 88.4% in group B. At the end of one year it became equal in both the groups. Episodes of exacerbation in between two visits diminished in successive follow-up visits. In the first follow-up visit, there were 37 exacerbations in group A, whereas single exacerbation occurred in group B. Thereafter, number of exacerbations diminished gradually and at the end of one year, there were 5 exacerbations in group A and that of group B became nil. There were statistically significant difference ($P < 0.001$) between two groups in respect to number of sleep disturbances, ability to perform daily activity, control status, and number of exacerbations at the first follow-up visit [Figure 2].

Mean sputum eosinophil count (%) of group A was always at a higher level than group B. At the initial visit, mean sputum eosinophil count (%) of group A was around 9% which gradually came down with treatment in subsequent

follow-up visits and at 9th visit (after 8 months) it became 3%. After that, in subsequent follow-up visits mean sputum eosinophil count (%) did not change significantly. On the other hand, mean sputum eosinophil count (%) of group B patients remained more or less same around 2% [Figure 3a].

Mean FEV_1 (% predicted) of group A patients gradually increased starting from 1st visit up to 9th visit (8th follow-up visit at 7th month) and mean sputum eosinophil count (%) gradually decreased [Table 2]. Thereafter, mean FEV_1 (% predicted) maintained a satisfactory level ($\geq 80\%$) in subsequent follow-up visits. In group B, satisfactory level of FEV_1 (% predicted), i.e., $\geq 80\%$ was achieved after 15 days, whereas in group A patients time required to achieve the same satisfactory level of FEV_1 (% predicted) was 6 months [Figure 3b]. There was statistically significant negative correlation between FEV_1 (% predicted) and sputum eosinophil count (%) in of group A patients in each follow-up visit with most significant negative correlation found in 8th visit ($r = -0.9237$ and $P \leq 0.001$) [Table 3]. Change in mean FEV_1 (% predicted) from baseline showed strong positive correlation ($r = 0.976$) with change reduction in mean sputum eosinophil count at each follow-up visits in group A patients indicated by scatter plot [Figure 4].

Table 1: Demographic and baseline characteristics

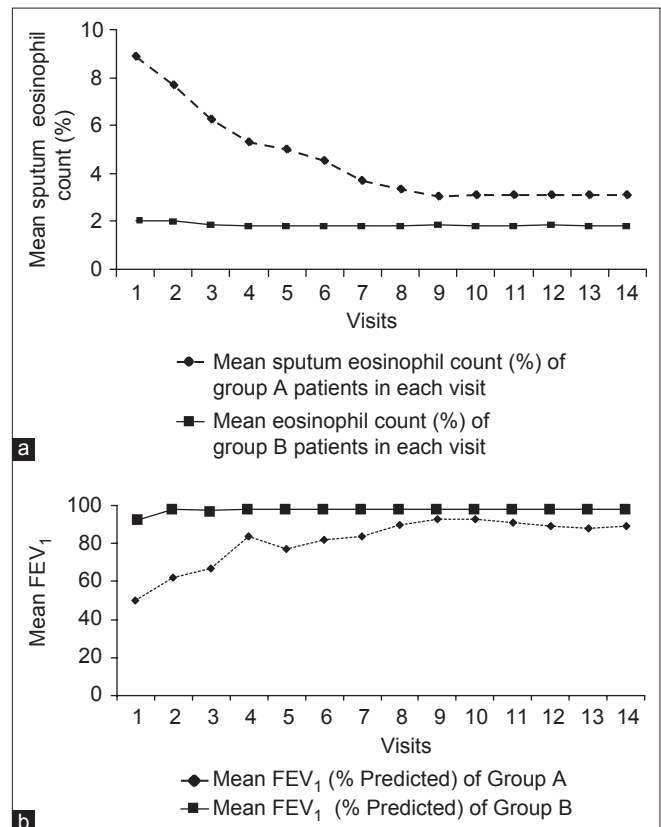| Baseline data	Group A (n=40)	Group B (n=40)	P value
Demographic data			
Age (Year) Mean±SD	28.575±9.55	27.45±5.706	0.7390
Sex			
Male	52.5%	60%	
Female	47.5%	40%	
Symptoms of patients			
Duration of cough (days) Mean±SD	31.5±10.96	27.46±20.98	0.417
Duration of SOB (days) Mean±SD	33.625±23.97	27.9±21.17	0.261
Duration of wheeze (days) Mean±SD	32±24.023	29.025±21.92	0.498
Other associated factors			
Family history of asthma	47.5%	42.5%	0.904
Gastroesophageal reflux disease	55%	65%	0.659
History suggestive of allergic rhinitis	70%	65%	0.892
Food allergy	25%	30%	0.8820
Allergy to pets	25%	22.5%	0.9660
Treatment dose (budesonide equivalent)			
At the beginning of the study Mean±SD	400±0	225±66.14	<0.0001
At the end of the study Mean±SD	250±86.6	163.75±48.07	<0.0001

P<0.05 is statistically significant, SD: Standard deviation

Figure 2: Line diagrams showing outcome variables (mean no. of sleep disturbances, performance of daily activity, control status, and no. of exacerbations) of group A and group B during initial and follow-up visits

Figure 3: (a) Line diagram showing sputum eosinophil count (%) of group A and group B during initial and follow-up visits. (b) Line diagram showing FEV_1 % predicted of group A and group B during initial and follow-up visits

Table 2: Comparison of FEV$_1$% predicted and sputum eosinophil count between two treatment groups

Visit	% FEV$_1$ predicted (mean±SD)			Sputum eosinophil count % (mean±SD)		
	Group A n=80	Group B n=80	Difference between two groups (P value)	Group A n=80	Group B n=80	Difference between two groups (P value)
1	50±14	80.17±4	<0.0001	8.87±5.05	2±0	<0.0001
2	62±18.36	94.6±3.48	<0.0001	7.65±4.69	2±0	<0.0001
3	67±17.85	94±3.43	<0.0001	6.24±3.74	1.17±0.42	<0.0001
4	71.6±10.91	95±1.87	<0.0001	5.32±3.22	1.75±0.44	<0.0001
5	77.85±12.72	95.35±1.8	<0.0001	5.02±3.02	1.75±0.44	<0.0001
6	82.6±11.38	95.77±1.29	<0.0001	4.55±2.52	1.75±0.44	<0.0001
7	84.65±16.99	95.97±1.27	<0.0001	3.7±1.88	1.75±0.44	<0.0001
8	90.82±8.37	95.92±1.16	<0.0001	3.35±1.29	1.75±0.44	<0.0001
9	93.02±5.97	95.87±1.11	<0.0050	3.02±0.53	1.8±0.40	<0.0001
10	93.02±5.24	95.9±1.15	<0.0020	3.07±0.61	1.75±0.44	<0.0001
11	91.47±6.46	95.87±1.11	<0.0001	3.1±0.59	1.75±0.44	<0.0001
12	89.05±8.09	95.87±1.11	<0.0001	3.1±0.59	1.77±0.42	<0.0001
13	88.22±6.87	95.87±1.11	<0.0001	3.1±0.59	1.75±0.44	<0.0001
14	89.1±6.29	95.87±1.11	<0.0001	3.2±0.59	1.75±0.44	<0.0001

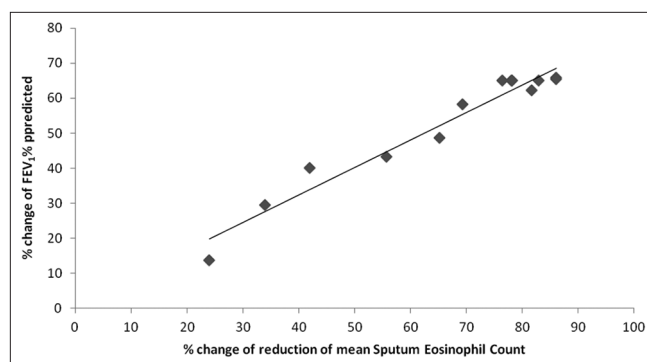FEV$_1$: Forced expiratory volume in one second, SD: Standard deviation

Table 3: Correlation between mean FEV$_1$% and mean sputum eosinophil count (%) in group A patients in all follow-up visits

Visit	FEV$_1$ (%) of group A (Mean±SD)	Eosinophil count (%) of group A (Mean±SD)	Correlation coefficient (r)	P value
1	50±14	8.87±5.05	−0.7527	<0.001
2	62±18.36	7.65±4.09	−0.78442	<0.001
3	67±17.85	6.25±3.74	−0.8009	<0.001
4	71±10.90	5.32±3.22	−0.8585	<0.001
5	77.85±127	5.02±3.025	−0.8863	<0.001
6	82.6±11.38	4.55±2.52	−0.8986	<0.001
7	84.65±16.99	3.7±1.88	−0.4966	<0.001
8	90.82±8.37	3.35±1.29	−0.9237	<0.001
9	93.02±5.24	3.02±0.53	−0.6724	<0.001
10	93.02±5.24	3.07±0.61	−0.8194	<0.001
11	91.47±6.46	3.1±0.59	−0.6441	<0.001
12	89.05±8.03	3.1±0.59	−0.6615	<0.001
13	88.22±6.87	3.1±0.59	−0.6566	<0.001
14	89.1±6.29	3.1±0.59	−0.7683	<0.001

FEV$_1$:Forced expiratory volume in one second; SD: Standard deviation

Figure 4: Scatter plot showing correlation between changes in mean FEV$_1$ (% predicted) and change in reduction of mean sputum eosinophil count (%) during follow-up visits in group A

DISCUSSION

Asthma is a chronic airway disorder characterized by an ongoing inflammatory process in which eosinophils play a major role. Three inflammatory phenotypes of asthma are (i) eosinophilic (> 2.75% of sputum cells), (ii) neutrophilic (> 51-65% of sputum cells), (iii) mixed inflammatory, and (iv) paucigranulocytic.[9] Eosinophilic asthma is common, accounting for 25-55% of patients with corticosteroid-naive asthma, and is repeatable. This asthma phenotype is not reserved to patients with severe asthma, nor is it a consequence of asthma therapy, but it is present across the range of asthma severity. Such phenotyping has therapeutic implications as patients with noneosinophilic inflammation respond poorly, if at all, to treatment with inhaled corticosteroids. In our study, we divided asthma patients into two groups according to sputum eosinophil count, eosinophilic (sputum eosinophil

count >3% - group A) and noneosinophilic (sputum eosinophil count <3% - group B), which is similar to the study of eosinophilic airway inflammation and prognosis of childhood asthma by Lovett, *et al.*[10]

In our study, at initial visit 90% of control group (group B) of patient had mild persistent asthma and their mean sputum eosinophil count was 2%. On the other hand, 60% of cases (group A) were suffering from severe persistent asthma and 40% of cases (group A) were suffering moderate persistent asthma and their mean baseline sputum eosinophil count was 8.87%. So, it could be said that higher sputum eosinophil count was associated with increased severity of asthma and vice-versa. Our study was similar to that of CJA Duncan's study as higher sputum eosinophil count was associated with increased severity of asthma.[11] According to Bartoli, *et al.*, the assessment of asthma severity according to clinical and functional findings only partially corresponds to the severity of eosinophilic airway inflammation as assessed by induced sputum analysis.[12] Asthma symptoms and severity score like number of exacerbations, sleep disturbances, and inability to perform daily activity gradually decreased in

successive follow-up visits starting from first follow-up visit. Side by side sputum eosinophil count also gradually came down in each successive follow-up visit. So, it can be said that asthmatic subjects' symptoms and severity scores are related with sputum eosinophil count that is higher the sputum eosinophil count more is the severity. There was a number of studies suggesting that induced sputum eosinophil count can be used as a tool for monitoring response of asthma patients to corticosteroid therapy.[13,14] Control status of both the group improved with treatment. But despite of high dose of ICS+LABA four cases of group A and 2 cases of group B did not achieve control despite of their sputum eosinophil count became low with treatment.

In our study, control status of group A gradually improved as well as sputum eosinophil count (%) gradually decreased in subsequent follow-up visits. Moreover, patients with baseline higher level of sputum eosinophil count (%) were more difficult to control with treatment than that of other group of asthma patients with low baseline sputum eosinophil count (%). So, we can predict the control status of asthma patients from baseline sputum eosinophil count (%). We also showed that higher sputum eosinophil count was associated with a greater number of exacerbations both of which were gradually diminished in subsequent follow-up visits. Among group A patients, at lower range of sputum eosinophil count (%) maximum number of patients had higher FEV_1 (% predicted) values at the initial visit. In the contrary, most of the patients with higher initial sputum eosinophil count (%) had lower FEV_1 (% predicted). Moreover, higher the baseline sputum eosinophil count (%) more time is required to achieve satisfactory FEV_1 (% predicted) level ($\geq$80%) with treatment. Mean FEV_1 (% predicted) of group A at initial visit was 50% and that of group B was 80%. On the other hand, sputum eosinophil count (%) of groups A and B were 8.87% and 2%, respectively, at the initial visit. By applying correlation coefficient, it was shown that FEV_1 (% predicted) was inversely correlated with sputum eosinophil count (%) in all successive follow-up visits in group A patients. Change in FEV_1 (% predicted), expressed as percentage change from baseline, also correlated ($r = -0.976$) with change reduction in sputum eosinophil count (%) in follow-up visits in group A patients. So, instead of FEV_1 (% predicted) sputum eosinophil count can be hypothesized as a marker of disease severity.

In the last few years, induced sputum has been increasingly advocated at this purpose as a noninvasive, safe, and reproducible method. CJA Duncan and their colleagues in their study investigated the relationship between induced sputum eosinophil apoptosis and clinical severity score, airway obstruction and symptoms score in patients with chronic stable asthma and concluded that asthmatic subjects' symptoms score, severity score, age, etc., positively correlated with sputum eosinophil count (%); asthmatic subjects' baseline FEV_1 (% predicted) inversely correlated with sputum eosinophil and mild asthmatics

have significant lower percentage of sputum eosinophil count than moderate and severe asthma groups.[11] Our study also showed similar results. Inhalational budesonide is effective in controlling eosinophilic airway inflammation. Measuring airway inflammation by quantitative sputum cell counts gives the most comprehensive information and is of most clinical value in initiating early effective treatment.[5,15]

Our study had some limitations like small sample size, exclusion of childhood asthma cases from the study, sputum neutrophil count was not seen in uncontrolled asthma patients of noneosinophilic and eosinophilic group, eosinophil based management was not given to cases and control groups. Recently, a Cochrane review and a meta-analysis study stated that a treatment strategy based on the sputum eosinophil count reduction, in contrast to a strategy using international guidelines, leads to a greater decrease in the overall risk of exacerbations and reduces the number of severe asthma exacerbations. This is particularly true in patients with moderate-to-severe asthma.[16,17] Malerba *et al*. in their study of Usefulness of "Exhaled Nitric Oxide and Sputum Eosinophils in the Long-term Control of Eosinophilic Asthma" observed that asthma interventions on sputum eosinophil count (with a cut off 3 to 4%), in addition to clinical or functional data, reduces the frequency of asthma exacerbations.[13]

Our study conclusively proved that sputum eosinophil count is a simple, inexpensive, easy, and noninvasive tool to assess asthma control in day to day practice. So, modern guidelines of asthma management should include measurement of sputum eosinophil count in all asthmatics at initial and if possible in all successive follow-up visits. Asthmatics with eosinophilic phenotype should be monitored with more frequent follow-up visits as they are more difficult to control. Sputum eosinophil count (%) can be helpful to define the disease severity at presentation, predict the time required in controlling the disease status and guide the treatment protocol and follow up of asthma patients.

CONCLUSION

Asthmatic patients with higher sputum eosinophil count ($\geq$ 3%) were difficult to control with standard therapy and number of exacerbations, episodes of sleep disturbance, and disability to perform daily activity were higher in these group of patients than other group of asthma patients whose sputum eosinophil count was low (< 3%). More the initial sputum eosinophil count, more time was required to achieve satisfactory FEV_1 (% predicted) level of $\geq$ 80%. In conclusion, on the basis of presented data it can be hypothesized that sputum eosinophil count serve as an excellent biomarker of airway inflammation as well as marker of disease severity. Sputum eosinophil count (%) can also be used for predicting control status of asthma.

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