EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 6
257-264
Year: 2019

# ROLE OF SPUTUM EOSINOPHIL COUNT IN ASSESSING THE SEVERITY AND TREATMENT OUTCOME IN ASTHMA PATIENTS

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**Article Received on 09/04/2019** **Article Revised on 30/04/2019** **Article Accepted on 21/05/2019**

## ABSTRACT

**Introduction:** Most clinicians treat asthma based on clinical assessment and PFT. Markers of airway inflammation like sputum eosinophil count and FENO serve as good markers of asthma severity as they are more sensitive than other subjective and objective methods of assessment of airway inflammation. **Methods:** After baseline FEV1 and FVC measurements, asthma patients were pretreated with inhaled salbutamol and then nebulized with hypertonic saline (3% NaCl). Induced sputum was examined for sputum eosinophil count(SEC) and patients were grouped as those with SEC>3% (groupA)and SEC<3% (groupB) For groupB, budesonide (100 µg) MDI was advised one puff twice daily and for groupA formoterol (6 µg) and budesonide (100 µg) combination in MDI form was prescribed two puffs twice daily. Asthma severity was assessed in both the groups. **Results:** Group A had mean SEC of 6.04±2.18 before treatment, and 3.28±0.67 during the last follow-up visit and Group B patients had a mean SEC 1.62±0.49 prior to treatment and 1.52±0.50 during the last follow-up visit.The mean FEV**1** values in group A prior treatment was 58.12±12.60 and during the last follow-up visit was 82.98±7.00(p value=0.000). In group B the mean FEV1 was 78.56±12.68 prior treatment and during the last follow-up visit the mean value was 90.50±5.84.(p value=0.000). **Conclusion:** A negative correlation was observed between sputum eosinophil count and FEV1 suggesting that the lung functions were lower and hence the disease was more severe in patients with a higher sputum eosinophil count.

**KEYWORDS:** Most clinicians groupA formoterol count.

## 1.0 INTRODUCTION

Asthma is a common chronic disease of the airway characterized by intermittent airflow obstruction, bronchial inflammation and airway hyper-responsiveness that varies markedly, both spontaneously and with treatment.[1]

This disorder affects an estimated 1- 18% of population and as such is a major health care issue in most countries.[2]

Asthmatics harbor a special type of inflammation in the airways that makes them more responsive than non-asthmatics to a wide range of triggers, leading to excessive narrowing with consequent reduced airflow and symptomatic wheezing and dyspnoea. Narrowing of the airways is usually reversible, but in some patients with chronic asthma there may be an element of irreversible airflow obstruction.

Asthma is a world-wide problem with an estimated 300 million affected individuals. The prevalence of asthma has risen in the affluent countries over the last 30 years but now appears to have stabilized with approximately 10-12% of adults and 15% of children affected by the disease. The prevalence of atopy and other allergic diseases has also increased over the same time, suggesting that the reasons for the increase are likely to be systemic rather than confined to the lungs.

The severity of asthma does not vary significantly within a given patient; those with mild asthma rarely progress to more severe disease, whereas those with severe asthma usually have severe disease at the onset.[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.

Spirometry, peak flow measurement, and bronchoprovocation testing constitute the traditional objective means of measuring asthma.

Clinicians usually use history, clinical examination and spirometry to assess the patient and treat the asthma patients Markers of airway inflammation like sputum eosinophil count and FENO would be of more help in the era of phenotype based treatment of asthma.

Hence we chose sputum eosinophil count to guide the assessment of severity and treatment outcomes in these patients.

## 2.0 MATERIALS AND METHODS

**2.1 Study Design**: The present study was conducted in the Department of TB and respiratory diseases, Institute Of Medical Sciences, Banaras Hindu University, Varanasi during the period of July 2016 to June 2018 was a longitudinal comparative study.

**2.2 Study population:** Patients coming from eastern part of Uttar Pradesh, adjoining area of Bihar, Jharkhand, Madhya Pradesh and Chhattisgarh. Patients were selected from the OPD as well as those admitted in the Department of Tuberculosis and Respiratory Diseases Institute Of Medical Sciences, Banaras Hindu University, Varanasi.

**2.3 Study procedure:** First of all the Institutional Ethics Committee was requested to approve the protocol. Once protocol was approved, then prior to enrollment in the study the patient was evaluated to determine eligibility and was explained about the study purpose, procedures and patients responsibilities as the potential participant.

100 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 FEV1/FVC < 70% and significant bronchodilator reversibility (12% and > 200 ml increase in FEV1after 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 50 patients each 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.

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. 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) metereddose 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 two puffs 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 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 stepped up or stepped 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 FEV1% predicted in response to therapy in group A and group B were evaluated and inference was drawn.

### 2.4 Aims and objectives of the study

**PRIMARY OBJECTIVE**

- To establish correlation between change in sputum eosinophil count and forced expiratory volume (FEV 1) % predicted value of asthma patients in response to treatment.

- **SECONDARY OBJECTIVE**:

To look for the prognosis and treatment outcome of asthma from baseline sputum eosinophil count.

**STATISTICAL ANALYSIS**

Unpaired "*t*" test was used to compare mean values of FEV1% predicted and sputum eosinophil count (%) of A and B groups of asthma patients to determine level of significance (*P* value). Correlation between FEV1 (% predicted) and sputum eosinophil count was evaluated in each follow-up visit. Statistical calculation was done by SPSS 16 software.

## 3. OBSERVATION AND RESULTS

A total of 100 age and sex matched patients were included in the study of whom 50 had a sputum eosinophil count >3% (group A) and 50 patients had a sputum eosinophil count of <3% (group B).

### 3.1 DESCRIPTIVE STATISTICS

**Table 1: Age Comparison Between The Groups.**

| | Group A (n=50) | Group B (n=50) | Remarks t-value p-value |
|---|---|---|---|
| Age (years) | 28.32±8.055 | 26.06±7.04 | t=1.493 p=0.139 |

The above table shows age comparison between the groups. The mean age in group A was 28.32±8.055 and in group B was 26.6±7.04.

**Table 2: Sex Comparison Between The Groups.**

| Sex | Group A (n=50) | | Group B (n=50) | | Total (n=100) | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| F | 22 | 44.0% | 29 | 58.0% | 51 | 51.0% |
| M | 28 | 56.0% | 21 | 42.0% | 49 | 49.0% |
| otal | 50 | 100.0% | 50 | 100.0% | 100 | 100.0% |

The above table shows comparison of sexes in both the groups. There were 22 females and 28 males in group A and 29 females and 21 males in group B.

**Table 3: Family History Of Asthma.**

| Family history | Group A (n=50) | | Group B (n=50) | | Total (n=100) | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| Present | 23 | 46.0% | 27 | 54.0% | 50 | 50.0% |
| Absent | 27 | 58.0% | 23 | 46.0% | 50 | 50.0% |
| Total | 50 | 100.0% | 50 | 100.0% | 100 | 100.0% |

The above table shows the family history of asthma in both the groups. There was a positive family history in 23 patients (46)% of group A and 27 patients (54)% of group B.

**Table 4: History Of Allergic Rhinitis.**

| | Group A (n=50) | | Group B (n=50) | | Total (n=100) | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| Absent | 20 | 40.0% | 28 | 56.0% | 48 | 48.0% |
| Present | 30 | 60.0% | 22 | 44.0% | 52 | 52.0% |
| Total | 50 | 100.0% | 50 | 100.0% | 100 | 100.0% |

The above table shows history of allergic rhinitis in between the groups. There was a positive history of allergic rhinitis in 30 people (60)% of group A and 22 people (44)% in group B.

**Table.5: History of Food Allergy.**

| | Group A (n=50) | | Group B (n=50) | | Total (n=100) | |
|---|---|---|---|---|---|---|
| | % | N | % | N | % | % |
| Absent | 20 | 40.0% | 28 | 56.0% | 48 | 48.0% |
| Present | 30 | 60.0% | 22 | 44.0% | 52 | 52.0% |
| Total | 50 | 100.0% | 50 | 100.0% | 100 | 100.0% |

The above table shows the history of food allergy in between the groups. A positive history for food allergy was obtained in 30 patients (60) % in group A and in 22 patients (44)% of group B.

**Table 6: History of Pet Allergy.**

| | Group A (n=50) | | Group B (n=50) | | Total (n=100) | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| Absent | 43 | 86.0% | 43 | 86.0% | 86 | 86.0% |
| Present | 7 | 14.0% | 7 | 14.0% | 14 | 14.0% |
| Total | 50 | 100.0% | 50 | 100.0% | 100 | 100.0% |

The above table shows history of pet allergy in both the groups. There were 7 patients (14)% in each group who had a positive history for allergy to pets.

**Table 7: Percentage Of Sputum Eosinophils Pre.**

| | Pre | Post | Intra group comparison Wilcoxon Signed Rank's Test Z-value p-value |
|---|---|---|---|
| **Group A (n=50)** | 6.04±2.18 | 3.28±0.67 | Z=-5.709 p=0.000 (HS) |
| **Group B (n=50)** | 1.62±0.49 | 1.52±0.50 | Z=-1.091 p=0.275 |
| **Student's t-test (Inter group comparison)** | t=13.957 p=0.000 | t=14.819 p=0.000 | |

The above table shows the percentage of sputum eosinophils in both the groups, prior to and after treatment. The group A patients had a mean sputum eosinophil percentage of 6.04±2.18 before treatment, and 3.28±0.67 during the last follow-up visit.

The group B patients had a mean sputum eosinophil percentage of 1.62±0.49 prior to treatment and 1.52±0.50 during the last follow-up visit.

**Table 8: Treatment Dose ( Budesonide Equivalent).**

| | Initial dose | During last follow up | Intra group comparison Wilcoxon Signed Rank's Test Z-value p-value |
|---|---|---|---|
| **Group A (n=50)** | 396.00±28.28 | 328.00±161.67 | Z=-2.863 p=0.004 (HS) |
| **Group B (n=50)** | 200.00±0.00 | 172.00±119.59 | Z=-2.722 p=0.006 (HS) |
| **Student's t-test (Inter group comparison)** | t=49.000 p=0.000 | t=5.485 p=0.000 | |

The above table shows the dose of inhaled corticosteroids (ICS) in both the groups in terms of budesonide equivalent. The mean initial dose of ICS in the group A patients was 396.0±28.28 and the dose required during the last visit was 328.00±161.67, (p value=0.004) which is statistically significant.

Group B patients were put on an initial dose of 200µg of budesonide equivalent ICS, and during the last follow-up the mean dose of ICS required was 172.00±119.59.,(p alue=0.006) which was statistically highly significant.
From the above table it can also be concluded that the dose of ICS required in group A patients was much higher than group B suggesting the presence of a more severe disease in group A.

**Table 9: Mean FEV1 (percentage predicted).**

| | Pre | Post | Intra group comparison Paired t-test t-value p-value |
|---|---|---|---|
| **Group A (n=50)** | 58.12±12.60 | 82.98±7.00 | t=-13.123 p=0.000 |
| **Group B (n=50)** | 78.56±12.68 | 90.50±5.84 | t=-8.696 p=0.000 |
| **Student's t-test (Inter group comparison)** | t=-8.085 p=0.000 | t=-5.828 p=0.000 | |

The above table shows mean FEV1 in both the groups. The mean values in group A prior to treatment was 58.12±12.60 and during the last follow-up visit was 82.98±7.00.,(p value=0.000) which was statistically highly significant.

In group B the mean FEV1 was 78.56±12.68 prior to treatment and during the last follow-up visit the mean value was 90.50±5.84 (p value=0.000) which was statistically highly significant.

The group A patients' mean FEV1 never approached those of group B, suggesting that the severity of disease was more in this group of people.

**Table 10: Sleep Disturbances (Night Awakenings).**

| | Pre | Post | Intra group comparison Wilcoxon Signed Rank's Test Z-value p-value |
|---|---|---|---|
| **Group 1(n=50)** | 9.98±3.15 | 2.60±2.42 | Z=-13.115 p=0.000 (HS) |
| **Group 2(n=50)** | 3.40±2.90 | 0.64±0.92 | Z=-6.961 p=0.000 (HS) |
| **Student's t-test (Inter group comparison)** | t=10.851 p=0.000 | t=5.344 p=0.000 | |

The above table describes the incidence of nocturnal awakenings in both the groups of asthma patients. The group A patients had an average of 9.98±3.15 awakenings during the first fifteen days after starting treatment, which later fell to 2.60±2.42 during the last visit,(p value=0.000) which was statistically highly significant.

In contrast the average sleep disturbance in group B patients during the first fifteen days of initiating therapy was 3.40±2.90 which later became 0.64±0.92 during the last visit, (p value=0.000) which was statistically highly significant.

From the above table we understand that the disease severity is much higher in group A patients, but even they gradually improved with treatment over a time period of one year.

**Table 11: Number of Exacerbations.**

| Exacerbation | | Pre | | Post | | Wilcoxon Signed Rank test Z-value p-value |
|---|---|---|---|---|---|---|
| | | N | % | | | |
| Group 1 (n=50) | 0 | 30 | 60.0% | 43 | 86.0% | Z=-3.035 P=0.002 (HS) |
| | 1 | 10 | 20.0% | 6 | 12.0% | |
| | 2 | 8 | 16.0% | 1 | 2.0% | |
| | 3 | 2 | 4.0% | 0 | 0.0% | |
| Group 1 (n=50) | 0 | 47 | 94.0% | 48 | 96.0% | Z=-0.577 P=0.564 |
| | 1 | 3 | 6.0% | 2 | 4.0% | |
| | 2 | 0 | 0.0% | 0 | 0.0% | |
| | 3 | 0 | 0.0% | 0 | 0.0% | |
| Inter group comparison (Chi square test) | | $\chi 2$=17.522 p=0.001 | | $\chi 2$=3.275 p=0.194 | | |

The above table shows the number of exacerbations in both the groups. It was observed that among 50 people in group A patients 10 patients had 1 exacerbations, 8 people had 2 and 2 patients had 3 exacerbations during the first fifteen days (p=0.001), and after treatment, during the last follow-up visit, 6 patients in group A had 1 exacerbation, 1 patient had 2 exacerbations, (p=0.002) which was statistically highly significant.

In contrast to this, among the 50 patients in group B, only 3 patients had a single exacerbation during the first fifteen days of initiating therapy and 2 patients had a single exacerbation during the last follow up visit.

The number of exacerbations gradually reduced in both the groups after treatment.

Even from the above table we can conclude that the disease severity was more in the group.

**4.0 DISCUSSION**

In our study a total of 100 patients were selected among whom 50 patients belonged to group A (sputum eosinophil count >3%) and another 50 patients to group B (sputum eosinophil count <3%).

They were age and sex matched.

The mean age in group A was 28.32±8.055 and in group B was 26.6±7.04. (p value=0.139) There were 22 females and 28 males in group A and 29 females and 21 males in group B. Age and sex difference between groups were not statistically significant.

There was a positive family history of Asthma in 23 patients (46)% of group A and 27 patients (54)% of group B. The differences in the groups were not very significant.

There was a positive history of allergic rhinitis in 30 people (60)% of group A and 22 people (44)% in group B. The differences in the groups were not statistically significant.

A positive history for food allergy was obtained in 30 patients (60)% in group A and in 22 patients (44)% of group B. The results were not statistically significant.

**Sputum Eosinophil and FEV1**

The group A patients had a mean sputum eosinophil percentage of 6.04±2.18 before treatment, and 3.28±0.67 during the last follow-up visit. The group B patients had a mean sputum eosinophil percentage of 1.62±0.49 prior to treatment and 1.52±0.50 during the last follow-up visit.

The mean values FEV**1** in group A prior to treatment was 58.12±12.60 and during the last follow-up visit was 82.98±7.00.,(p value=0.000). In group B the mean FEV1 was 78.56±12.68 prior to treatment and during the last follow-up visit the mean value was 90.50±5.84 (p value=0.000).

We observed a negative correlation between sputum eosinophil count and FEV1 suggesting that the lung functions were lower and hence the disease is more severe in patients with a higher sputum eosinophil count.

We also noted that the levels of sputum eosinophils in group A patients never came below the cut-off values of 3%.

In a study by Bandyopadhyay et al.(2013)- Usefulness of induced sputum eosinophil count to assess severity and treatment outcome in asthma patients, which included 160 patients among whom 80 had sputum eosinophil count >3% and another 80 had a count of <3%.

Mean FEV1 (% predicted) of patients with high eosinophil count gradually increased starting from 1st visit up to 9th visit (8thfollow-up visit at 7th month) and mean sputum eosinophil count (%) gradually decreased. Thereafter, mean FEV1 (% predicted) maintained a satisfactory level (≥ 80%) in subsequent follow-up visits.

In patients with sputum eosinophil count <3%, satisfactory level of FEV1 (% predicted), i.e., ≥ 80% was achieved after 15 days, whereas in the first group patients time required to achieve the same satisfactory level of FEV1 (% predicted) was 6 months. There was statistically significant negative correlation between FEV1 (% predicted) and sputum eosinophil count (%) in patients with high eosinophil count (>3%) in each follow-up visit with most significant negative correlation found in 8th visit ($r = -0.9237$ and $P \leq 0.001$).[3]

In another study by Green et al (2002)-Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial, which recruited 74 patients with moderate to severe asthma from hospital clinics and randomly allocated them to management either by standard British Thoracic Society asthma guidelines (BTS management group) or by normalization of the induced sputum eosinophil count and reduction of symptoms (sputum management group). They assessed patients nine times over 12 months. The primary outcomes were the number of severe exacerbations and control of eosinophilic inflammation, measured by induced sputum eosinophil count.

They found that the sputum eosinophil count was 63% (95% CI 24-100) lower over 12 months in the sputum management group than in the BTS management group (p=0.002). Patients in the sputum management group had significantly fewer severe asthma exacerbations than did patients in the BTS management group (35 Vs 109; p=0.01) and significantly fewer patients were admitted to hospital with asthma (one Vs six, p=0.047). The average daily dose of inhaled or oral corticosteroids did not differ between the two groups.[4]

They concluded that a treatment strategy directed at normalization of the induced sputum eosinophil count reduces asthma exacerbations and admissions without the need for additional anti-inflammatory treatment.

From the above studies we conclude that the sputum eosinophil count directly correlates with the disease severity and treatment with ICS leads to significant change in improving airway eosinophilia as well as lung functions.

**Sleep disturbances and exacerbations**

The group A patients had an average of 9.98±3.15 awakenings during the first fifteen days after starting treatment, which later fell to 2.60±2.42 during the last visit,( p value=0.000) In contrast the average sleep disturbance in group B patients during the first fifteen days of initiating therapy was 3.40±2.90 which later became 0.64±0.92 during the last visit, (p value=0.000) which is statistically highly significant.

From this we understand that the disease severity is much higher in group A, but even they gradually improved with treatment over a time period of one year.

It was observed that among 50 people in group A patients 10 patients had 1 exacerbations, 8 people had 2 and 2 patients had 3 exacerbations during the first fifteen days (p=0.001), and after treatment, during the last follow-up visit, 6 patients in group A had 1 exacerbation, 1 patient had 2 exacerbations, (p=0.002) which is statistically highly significant..

In contrast to this, among the 50 patients in group B, only 3 patients had a single exacerbation during the first fifteen days of initiating therapy and 2 patients had a single exacerbation during the last follow up visit.

The number of exacerbations gradually reduced in both the groups after treatment.

Even from the above finding we can conclude that the disease severity was more in group A.

In a study by Bandyopadhyay et al (2013) - Usefulness of induced sputum eosinophil count to assess severity and treatment outcome in asthma patients, which included 160 patients among whom 80 had sputum eosinophil count >3% and another 80 had a count of <3% It was a longitudinal comparative study to assess the treatment outcome.

The incidence of asthma exacerbations, sleep disturbances, performance of daily activity, and change in status of asthma control were the other outcome variables of their study. Exacerbations were defined as a worsening of symptoms requiring increased use of short-acting β2 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 FEV1 of at least 20%.

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 were 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 also improved in successive follow-up visits with treatment. In group A during 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.[3]

The findings of our study was consistent with the above study in which patients with higher sputum eosinophilia had higher number of sleep disturbances, more number of exacerbations and took more time to achieve adequate control of asthma.

**Treatment Response To ICS**

The mean initial dose of ICS in the group A patients was 396.0±28.28 and the dose required during the last visit was 328.00±161.67, (p value=0.004) and Group B patients were put on an initial dose of 200µg of budesonide equivalent ICS, and during the last follow-up the mean dose of ICS required was 172.00±119.59., (p=0.006) which is statistically highly significant.

From the following it can also be concluded that the dose of ICS required in group A patients was much higher than group B suggesting the presence of a more severe disease in group A.

**CONCLUSION**

From this study we conclude that asthma patients with higher initial sputum eosinophil counts had more severe symptoms and lower lung functions and required higher dose of medications for control of asthma. Sputum eosinophil count is a non invasive and good biomarker

for assessment of severity and treatment outcome in asthmatics.

**ACKNOWLEDGEMENT**
The authors wish to thank all the participants of the study for their active participation and precious time. We are grateful to the management of the healthcare facilities use in the study.

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