

## ORIGINAL RESEARCH

### Association of bronchial asthma severity with sputum eosinophil count and serum IgE levels

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#### ABSTRACT

**Aim:** To see if there was a link between sputum eosinophil counts, serum immunoglobulin E (IgE), and asthma severity.

**Material and methods:** It was a prospective observational study conducted in the department of pulmonary medicine at TMMC & RC, Moradabad for one and half year among 62 patients diagnosed as asthma on the basis of spirometry. All the study participants were instructed to cough sputum into sterile plastic containers. Total IgE levels more than 100 IU/mL was taken as abnormal. The degree of reversibility in forced expiratory volume 1 s (FEV1) of 12% and 200 ml from the prebronchodilator value was considered as diagnostic for asthma as per American Thoracic Society standards and GINA 2019 guidelines.

**Results:** Mild, moderate and severe bronchial asthma was revealed in 35.4%, 29.2% and 35.4% of the subjects respectively. S.E.C. and Sr.IgE level increase along with severity of asthma with statistically significant difference. According to Pearson correlation analysis, significant positive correlation was found between S.E.C and Sr.IgE.

**Conclusion:** According to the data, both SEC & IgE are connected to asthma severity. Asthma is associated with eosinophilic inflammation, which is a common symptom. As SEC evaluation is a simple, inexpensive & noninvasive diagnostic approach of airway inflammation, it could be the preferable approach for monitoring airway inflammation and guiding therapy in day-to-day practice.

**Keywords:** Asthma, IgE, SEC

#### INTRODUCTION

Asthma is a disorder characterized by severe chronic airway inflammation. It is the most common respiratory ailment in both developed and developing countries, with evidence suggesting that its prevalence has increased globally over the previous two decades<sup>1</sup>. Asthma is a main source of morbidity and mortality around the biosphere, particularly in poor nations, where it affects more than 300,000 people each year<sup>2</sup>.

<sup>4</sup>. Asthma ruthlessness has traditionally been determined using a combination of subjective and objective assessments<sup>5</sup>. In a

population of 1.31 billion persons, over 6% of Indian children and 2% of adults have asthma symptoms each year<sup>6</sup>. Asthma is a diverse condition with intermittent symptoms such as, cough, chest tightness and wheeze, all of which are caused by flexible airflow blockage.

It is critical to identify relevant biomarkers in order to predict disease progression and

medication response. Numerous non-invasive indicators for measuring airway inflammation have been employed in asthma monitoring throughout the last decade.<sup>7</sup> When stimulated, eosinophils degranulate and produce granules containing cytotoxic cationic proteins that damage asthmatic patients' airways. Furthermore, these compounds affect the concentration and duration of eosinophilic responses at the inflammatory site. IgE has been shown to have a role in asthmatic bronchial hyperresponsiveness, and ongoing research is focusing on the clinical feasibility and plausibility of employing monoclonal anti-IgE antibodies to treat IgE-mediated illnesses.<sup>8</sup>

Eosinophils have been identified as key pro-inflammatory and epithelial-damaging cells in both allergic and non-allergic asthma<sup>9</sup>, but alternative inflammatory cells such as neutrophils may also be tangled<sup>10</sup>. Allergic asthma is the most common endotype of asthma, accounting for more than 60% of cases, while non-atopic eosinophilic asthma accounts for approximately 25%–30% of cases<sup>11</sup>.

High levels of IgE identify type 1 hypersensitivity, yet the mechanisms that control IgE production are unknown. IgE is produced via a variety of biosynthetic pathways, including "direct" category recombination (CSR) from IgM in germinal centre B-cells, or "sequence", which can occur outside of germinal centres. Type 3 ILCs appear to be important in IL-22-mediated defence against fungus and bacteria.<sup>12</sup>

We sought to see if there was a link amid asthma ruthlessness and other inflammatory markers in our study. The relationship amid clinical signs and functional characteristics and biomarkers of inflammation is ailing understood. As a result, the goal of this study was to see if there was a link between sputum and peripheral eosinophil counts, as well as serum immune globulin E (IgE), and asthma severity.

## MATERIAL AND METHOD

It was a prospective observational study conducted in the department of pulmonary medicine at TMMC & RC, Moradabad for one and half year after the college research committee and the ethical committee gives the approval. Patients who are diagnosed as asthma on the basis of spirometry and who provide consent were included in the study. Patients of allergic bronchopulmonary aspergillosis, pregnant women with eclampsia or pre-eclampsia, haemoptysis of unknown origin, thoracic, abdominal or cerebral aneurysms, unstable cardiovascular status or recent myocardial infarction or pulmonary embolus and who underwent recent eye, thoracic or abdominal surgery were excluded from the study.

Sample Size: Any number of patients during 1.5 year of study or minimum 62 patients.

Where  $Z_{\alpha/2}$  : Standard normal variate

p: Prevalence rate

E: Error

We take  $Z_{\alpha/2} = 2.58$  at 5% type 1 error

$$p = 2.38 \%^{13}$$

$$E = 5\%$$

$$n = \frac{Z^2 \alpha / 2 P (100 - P)}{(E^2)}$$

$$= \frac{(2.58)^2 \times 2.38 \times (100 - 2.38)}{(5)^2}$$

$$= 62$$

## Investigations

- Haemoglobin
- Differential count

- Sputum Eosinophilcount
- SerumIgE
- Spirometry
- Chest X-ray PAView
- ECG
- If needed: CTscan

### SPECIMEN COLLECTION

All the study participants were instructed to cough sputum into sterile plastic containers. The sputum was homogenized by adding phosphate-buffered saline (PBS), vortexed for 30 s, and centrifuged for 10 min. Then 0.1% dithiothreitol was added to the cells in a ratio of 4:1, which was agitated for 20 min to break up the disulfide bonds and disperse the cells. Cells were washed once more with PBS and resuspended. The cell suspension was aspirated and filtered to remove any remaining debris. Supernatant was separated from cell pellet. Sputum sample was transferred to the slide and was distributed thinly and evenly over the slide. Staining was done by hematoxylin and eosin stain and analyzed using microscopy to determine the count for eosinophils. Under aseptic precaution, 5 ml of blood into vacutainers from each patient and measured for serum IgE. The total IgE levels was measured using FluoroenzymeImmunoassay method. Total IgE levels more than 100 IU/mL was taken as abnormal. Patients were subjected to PFT and were assessed for postbronchodilator reversibility after administering 200 µg of inhaled salbutamol by repeating the test after 15 min from the baseline. The degree of reversibility in forced expiratory volume 1 s (FEV<sub>1</sub>) of 12% and 200 ml from the prebronchodilator value was considered as diagnostic for asthma as per American Thoracic Society standards<sup>14</sup> and GINA 2019 guidelines<sup>15</sup>. Severity was calculated according to asthma severity scoring system as mentioned in table 1:

**Table 1: Asthma severity scoring system**

Variables	Score levels*		
	1	2	3
FEV <sub>1</sub> (% predicted)	>80	70-80	<70
Asthma attacks in the preceding year	2	3-6	>6
Hospitalizations in the preceding year	0	1-2	>2
Inhaled or oral corticosteroids taken in the preceding year	Inhaled corticosteroids	Oral corticosteroids	–

\*Each criterion was scored as 1, 2, or 3 with the exception of corticosteroid use, with a cumulative asthma severity score ranging from 4-11. Severity levels were defined as mild (score 4-5), moderate [6], or severe (>7). FEV<sub>1</sub>, forced expiratory volume in one second.

Data was collected and subjected to statistical analysis.

### Statistical Analysis

Data was collected and analysed using SPSS software version 24. Tests used to compare significant difference was Anova and level of significance was set at <0.05.

Pearson correlation was used to correlate sputum eosinophil count and serumIgE.

## RESULTS

Out of 65 subjects, 56.9% and 43.1% of the subjects were male and female respectively. Maximum subjects were from age group of 21-30 years (33.85%) followed by 41-50 years (27.69%). Mean age among the study subjects was  $36.51 \pm 15.40$  years (table 2).

**Table 2: Gender distribution among the study subjects**

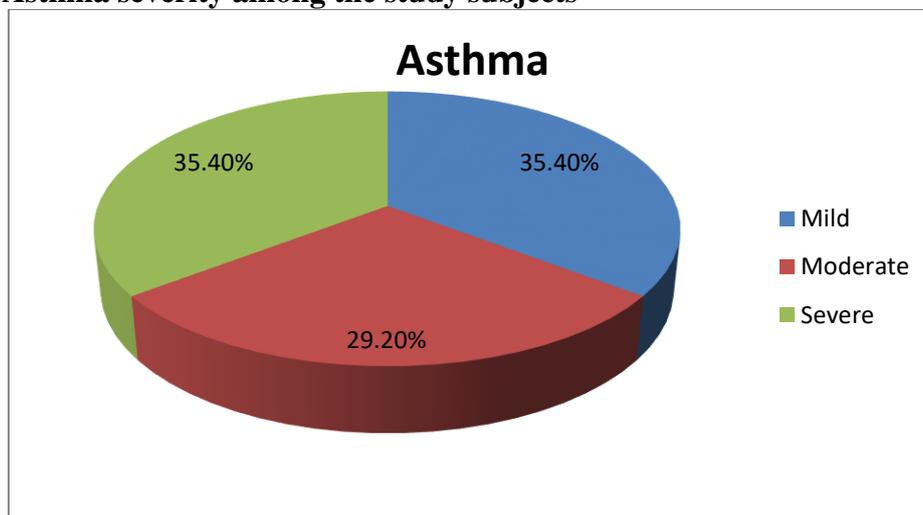
Gender	N	%
Male	37	56.9
Female	28	43.1
Age Group (in years)		
≤20	7	10.77
21-30	22	33.85
31-40	8	12.31
41-50	18	27.69
51-60	4	6.15
>60	6	9.23
Total	65	100
Mean±SD	36.51±15.40	

Mild, moderate and severe FEV1 was revealed in 18.5%, 38.5% and 43.1% of the subjects respectively which was according to the severity criteria. Mild, moderate and severe acute exacerbation/year was reported among 32.3%, 47.7% and 20% of the subjects respectively. Mild, moderate and severe hospitalization/year was shown in 67.7%, 29.2% and 3.3% of the subjects respectively (table 3). Mild, moderate and severe bronchial asthma was revealed in 35.4%, 29.2% and 35.4% of the subjects respectively as shown in graph 1.

**Table 3: Distribution of components of bronchial asthma**

Components	Mild		Moderate		Severe	
	N	%	N	%	N	%
FEV1	12	18.5	25	38.5	28	43.1
AAPY	21	32.3	31	47.7	13	20
HPY	44	67.7	19	29.2	2	3.1
I/O.C.S	54	83.1	11	16.9	0	0

**Graph 1: Asthma severity among the study subjects**



It can be well appreciated from table 4 that S.E.C. level increase along with severity of asthma i.e. 0.74%, 1.79% and 6.044% in mild, moderate and severe bronchial asthma respectively. Also Sr.IgE level increase along with severity of asthma i.e. 86.70, 703.84 and 2065.43 in mild, moderate and severe bronchial asthma respectively. When S.E.C. and Sr.IgE level was compared according to bronchial asthma, significant difference was found as  $p < 0.01$ .

**Table 4: S.E.C and Sr. IgE comparison according to category of bronchial asthma**

Bronchial Asthma		S.E.C	Sr. IgE
Mild	Mean	0.74%	86.70
	SD	0.75%	63.480
Moderate	Mean	1.79%	703.84
	SD	0.976%	1270.259
Severe	Mean	6.044%	2065.43
	SD	2.402%	3320.769
Anova Test		70.91	5.35
p value		<0.01*	0.007*

\*: statistically significant

According to Pearson correlation analysis, significant positive correlation was found between S.E.C and Sr.IgE i.e. S.E.C increase along with increase in Sr.IgE ( $r=0.54$ ,  $p < 0.01$ ) as shown in table 5.

**Table 5: Correlation between S.E.C and Sr. IgE**

Parameters	Value
r value	0.54
p value	<0.01*

\*: statistically significant

## DISCUSSION

Noninvasive procedures of airway inflammation measurement are safer, easier, and more convenient for checking in patients, especially those with severe asthma. In the Indian population, the relationship between clinical assessment and numerous markers of airway inflammation in asthma is not well recognized. As a result, the current prospective observational study was undertaken among 65 cases of bronchial asthma in the dept. of Pulmonary Medicine, TMMC & RC, to determine the relationship between bronchial asthma severity and sputum eosinophil count and serum IgE levels.

Out of 65 subjects, 56.9% and 43.1% of the subjects were male and female respectively. Hence there were more males as compared to females in our study. Similarly Roshan M Kumar et al<sup>16</sup> in their study too found more males as compared the females.

Maximum subjects were from age group of 21-30 years (33.85%) followed by 41-50 years (27.69%). Mean age among the study subjects was  $36.51 \pm 15.40$  years in our study. Similar age distribution was shown by Issa HY et al<sup>17</sup>, Anupama N<sup>18</sup> and Bapna A et al<sup>19</sup> in their study. In a study by Roshan M Kumar et al<sup>16</sup>, the mean age among the study subjects was 37.42 years.

Mild, moderate and severe FEV1 was revealed in 18.5%, 38.5% and 43.1% of the subjects respectively. Mild, moderate and severe acute exacerbation/year was reported among 32.3%, 47.7% and 20% of the subjects respectively. Mild, moderate and severe hospitalization/year was shown in 67.7%, 29.2% and 3.3% of the subjects respectively.

Mild, moderate and severe bronchial asthma was revealed in 35.4%, 29.2% and 35.4% of the

subjects respectively in this study. Similar distribution of asthma was reported by Manise et al<sup>20</sup>, Khadadah et al<sup>21</sup> and Roshan M Kumar et al<sup>16</sup>. Issa HY et al<sup>17</sup> in their study found least subjects in severe asthma category.

Mean S.E.C. among the study subjects was  $2.92\% \pm 2.84\%$  with minimum and maximum of 0% and 10% respectively. S.E.C. level increase along with severity of asthma i.e. 0.74%, 1.79% and 6.044% in mild, moderate and severe bronchial asthma respectively. When S.E.C. was compared according to category of bronchial asthma, significant difference was found as  $p < 0.01$  in our study. Issa HY et al<sup>17</sup>, Manise et al<sup>20</sup> and Roshan M Kumar et al<sup>16</sup> in their studies too found positive correlation between S.E.C. and severity of bronchial asthma. Khadadah et al<sup>21</sup> in their study showed that significant difference was found when S.E.C. was compared according to category of bronchial asthma. However, dissimilar results were reported by Gibson et al<sup>22</sup> and Palomino et al<sup>23</sup>; i.e. they did not observe any relation between S.E.C. and bronchial asthma.

Eosinophilic asthma is a kind of severe asthma marked by high amounts of eosinophils. Eosinophils have a part in airway inflammation, and higher eosinophil counts have been linked to more frequent exacerbations and poor. The significance of sputum eosinophilia detection is linked to steroid response. Without the essential for further anti-inflammatory treatment, a therapeutic plan that normalises tempted sputum eosinophil count lowers asthma exacerbations and hospitalizations.

Mean Sr. IgE among the study subjects was  $967.26 \pm 2231.27$  with minimum and maximum of 12 and 16038 respectively. Sr. IgE level increase along with severity of asthma i.e. 86.70, 703.84 and 2065.43 in mild, moderate and severe bronchial asthma respectively. When Sr. IgE level was compared according to category of bronchial asthma, significant difference was found as  $p < 0.01$  in the present study. The mean total serum IgE in the overall population of South India was found to be substantially greater than in the Western people, at 522.19 IU/ml. In our asthmatic group, overall serum IgE levels were extremely high, with average of 967.24 IU/ml. To determine the mean total IgE levels in the general population, we did not include controls from the general population.

Certain peoples in Asia (particularly India) and Africa produce a large amount of IgE. These high levels of IgE are thought to guard against a variety of assaults, including venom detoxification and neutralisation, ectoparasite expulsion, and xenobiotic breakdown.<sup>24</sup> Issa HY et al<sup>17</sup> in their study too found positive correlation amid IgE and severity of bronchial asthma. Borish L et al<sup>25</sup> and de Marco R et al<sup>26</sup> in their study reported similar results as mentioned in our study. Khadadah et al<sup>21</sup> in their study showed that significant difference was found when IgE was compared according to category of bronchial asthma. In a study by Roshan M Kumar et al<sup>16</sup>, Kartasamita et al<sup>27</sup>, no relation was found between Sr. IgE level and category of bronchial asthma. These findings are in contrast to our study.

The severity of the sickness and the degree of exposure are associated to total IgE levels. You're more likely to be exposed to allergens and develop asthma if you don't have control over your environment. Total IgE levels can be used as a biomarker to determine whether or not actions to manage the environment of probable allergens were successful, or whether they need to be reevaluated. The use of inhaled beclomethasone dipropionate at 800 mg per day for three months significantly lowered total IgE levels and improves asthma symptoms, according to a double-blind trial. Furthermore, total IgE levels reduce two weeks after corticosteroid treatment.<sup>28</sup>

## LIMITATIONS

1. Patients with high blood IgE levels were not evaluated for allergic bronchopulmonary aspergillosis.

2. Patients with asthma who did not have an eosinophilic pattern were not studied.
3. Patients with a high eosinophil count were not tested for parasite infection.
4. There is no control group.
5. There was no follow-up inquiry.
6. No follow up was done.

## CONCLUSION

According to the data, both SEC & IgE are connected to asthma severity. Asthma is associated with eosinophilic inflammation, which is a common symptom. As shown in subsequent research, SEC evaluation and Serum IgE is a simple, inexpensive & noninvasive diagnostic approach of airway inflammation that may help to identify unique phenotypes of asthma that are more steroid responsive. In day-to-day practice, it could be the preferable approach for monitoring airway inflammation and guiding therapy.

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