Correlation of induced sputum eosinophil, absolute eosinophil count and serum IgE with clinical severity of bronchial asthma

Gopal Purohit, Vijay K Sharma*, C R Choudhary, Hemant Borana, Balveer Choudhary
Department of Pulmonary Medicine, Kamla Nehru Chest Hospital, Jodhpur, Rajasthan, India

Abstract

**Background:** Asthma is a chronic inflammatory disorder of the airway. Eosinophil and Serum IgE being a marker of airway inflammation, can serve as a tool for assessing severity and response to treatment in asthma patients. The correlation of clinical assessment with various markers of airway inflammation in asthma is not well established in the Indian population. Present study correlates induced sputum eosinophil, blood eosinophil and Serum IgE count in assessing the clinical severity in bronchial asthma.

**Aims:** To study the correlation of sputum eosinophil, absolute eosinophil count and Serum IgE in assessing the clinical severity of asthma.

**Methods:** It was a cross sectional quantitative study, which was carried out between Feb 2020 to Feb 2021. Total 70 asthmatics was selected which underwent history, examinations and spirometry, after written consent. Severity of asthma was assessed by clinical features and by FEV1 in spirometry. After assessing the severity of Bronchial asthma all patient underwent Sputum examination for sputum eosinophil counts, blood sampling for Absolute eosinophil counts and Serum IgE levels. After that sputum eosinophil counts, absolute eosinophil counts and Serum IgE levels were correlated with severity of Bronchial Asthma.

**Conclusion:** Assessment of eosinophil count in sputum and blood, and serum IgE in blood are simple and inexpensive method that can show a direct measurement of airway inflammation. Thus it can help to identify specific phenotypes in asthmatic patients who are more responsive to steroids, which needs to be demonstrated in future studies. It could be the preferred method in routine practice in monitoring airway inflammation and guiding management.

**Keywords:** asthma, sputum eosinophil count, absolute eosinophil count, serum immunoglobulin E, severity of asthma

Introduction

Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation. Airflow limitation may later become persistent. Symptoms and airflow limitation may resolve spontaneously or in response to medication, and may sometimes be absent for weeks or months at a time. On the other hand, patients can experience episodic flare-ups (exacerbations) of asthma that may be life-threatening and carry a significant burden to patients and the community. The correlation between clinical, functional, and various biological markers of airway inflammation in asthma severity is not well established. Over the last decade, various non-invasive markers for measurement of airways inflammation such as exhaled nitric oxide, sputum differential cytology, and serum proteins such as eosinophilic cationic protein have been used in monitoring of asthma. There is not much data on the relation between clinical symptoms and functional parameters to biomarkers of airway inflammation. Therefore, this study will be done with the intention to find the correlation of sputum and peripheral eosinophil count and serum immunoglobulin E (IgE) with severity of asthma.

Aims and Objectives

To study the correlation of induced sputum eosinophil, absolute eosinophil count and serum IgE with clinical severity of Bronchial Asthma. (as per GINA guideline)

**Material and Methods**

This was an analytical study was conducted in Department of Respiratory medicine, Kamla Nehru Chest Hospital, Jodhpur over one year from Feb 2020 to Feb 2021 after approval by ethical committee after volunteer written consent. All patients of Bronchial asthma defined by GINA guideline of age 18-60 year were enrolled. Patient with Acute exacerbation of Bronchial Asthma as per GINA guideline. Clinical features and spirometry suggestive of chronic obstructive pulmonary disease (as per GOLD criteria), Not willing to give consent, Not able to perform spirometry correctly, History of recent myocardial infarction (upto one month as per documentation), On chronic corticosteroid therapy (as per documentation) were excluded from study. After apply exclusion and inclusion criteria total 70 patients were enrolled in study.

**Diagnosis and assessment of severity of asthma**

**Spirometry**

All defined Bronchial Asthma patients underwent Spirometry for Confirmation of diagnosis and assessment of severity of bronchial asthma by FEV1 (as per GINA guideline) by Flow sensing spirometer. Patients were asked to hold the mouth piece between the lips to get a good seal, breathe in and out for 2-3 tidal breaths then expire as fast and as hard as possible for as long as possible until no
The sputum was homogenized by adding bonds and dispersing the cells. The cell suspension was 4:1, which was agitated for 20 min to break up the disulfide bonds. We added 0.1% dithiothreitol to the cells in a ratio of 4:1 and distributed evenly over the slide. Staining was done by hematoxylin and eosin stain and analyzed using microscopy to determine absolute count. Sputum eosinophil count ≥3% was expressed as a percentage as it is more accurate than absolute count. Sputum eosinophil count ≥3% was considered abnormal [2].

Sputum examination for induced sputum eosinophil count
All the study participants were instructed to cough sputum into plastic containers after taking consent and after nebulizing with 3% hypertonic saline. The sputum was homogenized by adding phosphate-buffered saline (PBS) and was centrifuged for 10 min. We added 0.1% dithiothreitol 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. The cell suspension was aspirated and filtered to remove any remaining debris. Supernatant was separated from cell pellet. Sputum sample 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. The eosinophil count was expressed as a percentage as it is more accurate than absolute count. Sputum eosinophil count ≥3% was considered abnormal [2].

Blood investigation for absolute eosinophil count and serum IgE
All patients underwent blood sampling after taking consent under aseptic precaution, 5 ml of blood was taken from medial cubital vein into vacutainers from each patient and was measured for peripheral eosinophil count and serum IgE and estimation of eosinophil percentage was done by automated analyser. Blood eosinophil counts more than 300 was considered abnormal [2]. Total IgE levels more than 100 IU/mL was taken as abnormal [2].

Results and Observations

Table 1: Distribution according to severity of bronchial asthma

<table>
<thead>
<tr>
<th>Severity</th>
<th>N</th>
<th>%</th>
<th>Mean FEV1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>16</td>
<td>22.9</td>
<td>82.93</td>
</tr>
<tr>
<td>Moderate</td>
<td>35</td>
<td>50</td>
<td>67.57</td>
</tr>
<tr>
<td>Severe</td>
<td>19</td>
<td>27.1</td>
<td>36.89</td>
</tr>
</tbody>
</table>

Table 2: Mean absolute eosinophil counts according to severity of asthma

<table>
<thead>
<tr>
<th></th>
<th>AEC (/cu.mm)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Mild</td>
<td>167.5</td>
<td>46.8</td>
<td>365.1</td>
<td>78.2</td>
<td>484.7</td>
</tr>
<tr>
<td>Moderate</td>
<td>365.1</td>
<td>78.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>484.7</td>
<td>65.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean AEC significantly correlated with severity of asthma (P-value=0.0001)

Table 3: Mean serum IgE according to severity of asthma

<table>
<thead>
<tr>
<th>Severity</th>
<th>IgE (IU/ml)</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>245.8</td>
<td>225.5</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>992.2</td>
<td>528.8</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>1725.6</td>
<td>614.0</td>
<td></td>
</tr>
</tbody>
</table>

Mean serum IgE significantly correlated with severity of asthma. (P-value=0.0001)

Table 4: Mean sputum eosinophil % according to severity of asthma

<table>
<thead>
<tr>
<th>Sputum Eosinophil %</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild (N=16)</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Moderate (N=35)</td>
<td>1.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Severe (N=19)</td>
<td>2.5</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Mean sputum eosinophil % significantly correlated with severity of asthma. (P-value=0.0001)

Table 5: Pearson correlation between FEV1% and induced sputum eosinophil count, blood eosinophil count and serum IgE.

<table>
<thead>
<tr>
<th>r value</th>
<th>AEC (/cu.mm)</th>
<th>S. IgE (IU/ml)</th>
<th>Sputum Eosinophil %</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1%</td>
<td>-0.7007615</td>
<td>-0.6196451</td>
<td>-0.6707932</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

In our study, we found a strong significant inverse correlation between FEV1% and induced sputum eosinophil count, blood eosinophil count and serum IgE.

Discussion
In our study, we found that mean age of patients was 31.3 years with median age 31 years. Kumar et al [2] found mean age was 37.42 years. Merghani et al [6] reported ages ranged from 16 to 86 years with a mean (SD) of 43.2 (16.1)

In our study, we found that 58.6% patients of asthma were female Raji and moosavi [4] found mean age of 36.4 years with 55.6% males and 44. 4% females. Merghani et al [6] reported total of 428 patients were included in the study; 57.5% were females.

Correlation of absolute eosinophil count and severity of asthma
In our study, we found that Mean absolute eosinophil count in severe asthmatic patients was 484.7/cu.mm followed by 365.1/cu. Mm in moderate and 167.5/cumm in mild asthmatic patients. Mean AEC increases significantly according to severity (FEV1%), shows strong positive correlation which was statistically significant (P-value=0.0001). Kartasamita et al [16] reported that median value for eosinophils was 580/mm3 (range, 70 to 5909/mm3); only 31% of the children had less than 400 eosinophils/mm3. Eosinophilia were not related to age but increased levels of both serum IgE and AEC. 53(48.6%). Mean serum AEC (eosinophils/µl) was 411.3±299.5 in intermittent, 438.6±232.9 in mild persistent, 582.9±377.4 in moderate persistent and 1147.7±893.1 in severe persistent cases. Mean AEC increased with increasing severity of asthma and this was statistically significant (P <0.0001).

Chaudhary et al [13], have noted that the AEC levels were
higher in patients with asthma compared to normal children. In this study, AEC was found to be raised in 56% cases of asthma. Also, AEC increased with increasing severity of asthma.

**Correlation of serum immunoglobulin E and severity of asthma**

Here, in our study, Mean serum IgE in severe asthmatic patients was 1725.6 IU/ml followed by 992.2 IU/ml in moderate and 245.8 IU/ml in mild asthmatic patients. Mean serum IgE increases significantly according to severity, shows strong positive correlation which was statistically significant (P-value=0.0001). Kartasamita et al [16] reported overall median value of IgE was 436 IU/ml (range, 18 to 9707 IU/ml); almost 94% of the children showed an IgE value of more than 100 IU/ml, and 29% showed a value of more than 1000 IU/ml. Trivedi and Patel [10] found that Serum IgE levels were raised above the normal limits for age in 94 (86.2%). They found that Mean serum IgE levels were 231.9±182.6 IU/ml in intermittent, 557.9±318.2 IU/ml in mild persistent, 1399±693.1 IU/ml in moderate persistent and 2031±553.9 IU/ml in severe persistent cases. Serum IgE levels increased with increasing severity of asthma and this was statistically significant (p<0.0001) when the values were compared within the groups. The increasing level of serum IgE was found to be statistically significant when compared between the groups (p <0.0001 between intermittent and mild persistent and also between mild persistent and moderate persistent cases and p=0.05 between moderate persistent and severe persistent cases). Kovac et al [11] observed that the serum IgE concentration was much higher in severe persistent asthma compared to other grades of asthma. A study done by Sciuca et al [12] in children with recurrent wheeze, showed that raised levels of IgE was directly proportional to the increased risk of bronchial asthma in them. Manies et al [8] reported that Total IgE was detectable in the sputum supernatant from the majority of subjects (70%). Sputum IgE were increased in eosinophilic asthmatics when compared to healthy subjects (p<0.001).

**Correlation of sputum eosinophil count and severity of asthma**

Here, we found that Mean sputum eosinophil % in severe asthmatic patients was 2.5% followed by 1.1% was in moderate and 0.4% in mild asthmatic patients. Mean sputum eosinophil % increases significantly according to severity, shows strong positive correlation which was statistically significant. (P-value=0.0001). In concordance with our results Kumar et al [2] found that 26.3% patients had abnormal sputum eosinophil count. Sputum eosinophil count and severity of asthma had statistically significant (P = 0.004). More than half of the patients with severe asthma had normal sputum eosinophil counts suggesting that there could be specific phenotypes of asthma with elevated sputum eosinophils, which may not be a common feature among all asthmatics. The importance of identifying this phenotype of asthma with elevated sputum eosinophilia could be related to steroid responsiveness, which future studies should demonstrate in the Indian population. Dunchan et al [14] found Sputum eosinophil counts significantly higher in moderate (p<0.05) and severe asthma patients (p<0.05) and there was strong Positive correlation between sputum eosinophil counts and severity of asthma. (p<0.0001). Bartoli ML et al [5] found Sputum eosinophil counts were significantly higher in moderate persistent and mild persistent asthma compare to mild intermittent asthma.(p<0.05) Khadadah et al [9] found that sputum eosinophilia has been shown to be the best predictor of a short-term response to corticosteroids.

**Correlation of sputum eosinophil count, absolute eosinophil count, and serum immunoglobulin E with FEV1**

In our study, we found a strong significant inverse correlation between FEV1% and induced sputum eosinophil count, blood eosinophil count and serum IgE. There was a statistically significant correlation between these parameters. (p<0.05). Kumar et al [2] found observed a statistically significant inverse correlation between sputum eosinophil count and FEV1% (p<0.011), significant inverse correlation between total serum IgE levels and predicted FEV1 (P = 0.04), which was, however, a weak correlation (r = −0.23) but no significant correlation between AEC and FEV1%. Ronchi et al [15] found significant Inverse correlation between sputum eosinophil counts and FEV1. (p<0.05) but Blood eosinophil counts were not related to severity of asthma or FEV1. Dunchan et al [15] found Inverse correlation between blood eosinophil counts and FEV1 (p=0.01) Bandyopadhyay A et al [3] found that There was statistically significant negative correlation between FEV1 % predicted and sputum eosinophil count (%). Change in mean FEV1 % (predicted) from baseline showed strong positive correlation (r = 0.976) with change in reduction of mean sputum eosinophil. Bartoli ML et al [5] found significant Inverse correlation between sputum eosinophil counts and FEV1. (p<0.03)

**Conclusion**

Assessment of sputum eosinophil count, AEC and serum IgE are simple and inexpensive method that can show a direct measurement of airway inflammation and can suggest severity of disease and allergic etiology of disease. Thus it can help to identify specific phenotypes in asthmatic patients who are more responsive to steroids, which needs to be demonstrated in future studies. It could be the preferred method in routine practice in monitoring airway inflammation and guiding management, predictor of the clinical response to corticosteroids and predictor of asthma control.

**References**


