International Journal of Pulmonary and Respiratory Sciences
www.pulmonologyjournals.com
Online ISSN: 2664-8512, Print ISSN: 2664-8504
Received: 01-11-2021, Accepted: 17-11-2021, Published: 02-12-2021
Volume 3, Issue 1, 2021, Page No. 12-15

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. (as per GINA guidelines). After assessing the severity of Bronchial asthma all patient underwent Sputum examination for sputum eosinophil counts, blood sampling for Absolue 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[1]. 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 [1]. 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 [2].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 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

breathe is left then inspire rapidly to a maximum capacity. Spirometry was repeated again 15-30 minutes after administration of short acting beta agonist, 200-400mcg of salbutamol, to check for bronchodilator reversibility. Spirometry repeated again 15-30 minutes after administration of short acting beta agonist, 200-400mcg of salbutamol, to check for bronchodilator reversibility before and twenty minutes post nebulisation with salbutamol. 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 GINA guidelines. The severity of asthma was assessed according to the GINA criteria. This includes frequency of diurnal and nocturnal symptoms, frequency of short-acting beta 2-agonist used, interference with daily activity and spirometry (FEV1).

Sputum examination for induced sputum eosinophil count

All the study participants was 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 aspirate and filter 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 considerd 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

Severity	N	%	Mean FEV1
Mild	16	22.9	82.93
Moderate	35	50	67.57
Severe	19	27.1	36.89

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

	AEC (/cu.mm)	
	Mean	SD
Mild (N=16)	167.5	46.8
Moderate (N=35)	365.1	78.2
Severity (N=19)	484.7	65.5
P-value	0.0001	

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

Table 3: Mean serum IgE according to severity of asthma

	S. IgE (IU/ml)	
	Mean	SD
Mild (N=16)	245.8	225.5
Moderate (N=35)	992.2	528.8
Severity (N=19)	1725.6	614.0
P-value	0.0001	

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

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

	Sputum Eosinophil %	
	Mean	SD
Mild (N=16)	0.4	0.5
Moderate (N=35)	1.1	0.8
Severity (N=19)	2.5	1.3
P-value	0.00001	

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.

r value	AEC (/cu. mm)	S. IgE (IU/ml)	Sputum Eosinophil %
FEV1%	-0.7007615	-0.6196451	-0.6707932
P-value	0.0001	0.0001	0.0001

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 was 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 5090/mm3); only 31% of the children had less than 400 eosinophils/mm3. Eosinophilia were not related to age but showed a significant, although not high, correlation with each other ($p < 0.05$). Trivedi and Patel [10] found that AEC was raised in 61(56 %) patients. 57(52.3%) patients had 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 was 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 each of the groups ($p <0.0001$ between intermittent and mild persistent cases 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

1. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2020. Available from: www.ginasthma.org
2. Kumar RM, Pajanivel R, Koteeswaran G, Menon SK, Charles PM. Correlation of total serum immunoglobulin E level, sputum, and peripheral eosinophil count in assessing the clinical severity in bronchial asthma. Lung India,2017:34(3):256-261
3. Bandyopadhyay A, Roy PP, Saha K, Chakraborty S, Jash D, Saha D. Usefulness of induced sputum eosinophil count to assess severity and treatment outcome in asthma patients. Lung India,2013:30(2):117-23.
4. Razi E, Moosavi GA. Serum total IgE levels and total eosinophil counts: relationship with treatment response in patients with acute asthma. J Bras Pneumol,2010:36(1):23-8.

5. Bartoli ML, Bacci E, Carnevali S, Cianchetti S, Dente FL, Di Franco A *et al*. Clinical assessment of asthma severity partially corresponds to sputum eosinophilic airway inflammation. Respir Med,2004:98(2):184-93
6. Merghani TH. Mild respiratory symptoms in asthmatic patients might not be due to bronchoconstriction. J Family Community Med,2017:24(2):102-105.
7. Zein JG, Dweik RA, Comhair SA, Bleecker ER, Moore WC, Peters SP *et al*. Severe Asthma Research Program. Asthma Is More Severe in Older Adults. PLoS One,2015:10(7):e0133490.
8. Manise M, Holtappels G, Van Crombruggen K, Schleich F, Bachert C, Louis R. Sputum IgE and cytokines in asthma: relationship with sputum cellular profile. PLoS One,2013:8(3):e58388.
9. Khadadah M, Onadeko BO, Ezeamuzie CI, Mustafa HT, Marouf R, Sugathan TN. The association of skin test reactivity, total serum IgE levels, and peripheral blood eosinophilia with asthma in Kuwait. J Asthma,2000:37(6):481-8.
10. Trivedi PP, Patel AH. Serum immunoglobulin E and absolute eosinophil count as markers of severity in childhood asthma. Int J Contemp Pediatr,2020:7:413-8.
11. Kovač K, Dodig S, Tješić-Drinković D, Raos M. Correlation between asthma severity and serum IgE in asthmatic children sensitized to Dermatophagoides pteronyssinus. Archiv Med Res,2007:38(1):99-105.
12. Sciuca S. Prognostic importance of IgE for the evolution of recurrent wheezing into the bronchial asthma in children. World Allerg Organizat J,2007:11:119-20.
13. Chaudhary GS, Kumar A, Shashtri M. Comparison of total serum immunoglobulin E and absolute eosinophil count levels among asthmatic and non- asthmatic children. Ind J Child Health,2017:4(3):345-7.
14. Duncan CJA, Lawrie A, Blaylock MG, Douglas JG, Walsh GM. Reduced eosinophil apoptosis in induced sputum correlates with asthma severity. Eur Respir J,2003:22:484-490
15. Ronchi MC, Piragino C, Rosi E, Stendardi L, Tanini A, Galli G *et al*. Do sputum eosinophils and ECP relate to the severity of asthma? Eur Respir J,1997:10:1809-1813
16. Kartasamita CB, Rosmayudi O, Demedts M. Total serum IgE and eosinophil count in children with and without a history of asthma, wheezing, or atopy in an urban community in Indonesia. The Respiratory Disease Working Group. J Allergy Clin Immunol,1994:94(6-1):981-8.