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Comparative study of eosinophil count in peripheral blood, sputum and BAL fluid in patients of bronchial asthma

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ABSTRACT

Introduction: Bronchial Asthma 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 over the course of disease.
Objective: To compare the findings of eosinophil counts in peripheral blood, induced sputum and bronchoalveolar lavage fluid in asthmatic patients and healthy individuals.
Materials and Methods: This Case Control Cross sectional study was conducted among patients attending respiratory OPD at Sir Sunder Lal Hospital, BHU, Varanasi, with diagnosis of bronchial asthma (100) and healthy controls.
Results: Significant association was found between eosinophilic bronchial asthma and absolute eosinophil count (W = 1168.000, p = 0.020), total serum IgE (W = 1338.000, p = <0.001), BAL eosinophil count ($\chi 2$ = 94.589, p = <0.001), sputum eosinophil count ($\chi 2$ = 14.057, p = <0.001).

1. 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.

Absolute eosinophil count and total serum IgE, are useful markers for eosinophilic bronchial asthma. Inducing sputum with hypertonic saline is a non-invasive technique that can be used to study the relationship between eosinophilic airway inflammation and asthma. BAL eosinophil count also helps in assessment of the severity of asthma. Patients not responding to the conventional treatment, should undergo phenotypic assessment for targeted therapy using biologic therapy.

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2. Materials and Methods

This Case Control Cross sectional study was conducted among patients attending respiratory OPD at Sir Sunder Lal Hospital, BHU, Varanasi, with diagnosis of Bronchial Asthma who were chosen on the basis of history, physical examination, Chest X-Ray and Computed Tomography of chest, Spirometry, from October 2020 – July 2022. Adults more than 18 year without any sex specifications with diagnosed bronchial asthma were included in the study.

2.1. Inclusion criteria

1. Adults aged 18- 60 years with diagnosis of Bronchial Asthma as per GINA guidelines.
2. Healthy individuals (non-asthmatic) willing to participate as control.
3. Patient receiving treatment for bronchial asthma.

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4. Adult patient with diagnosis of Bronchial Asthma based on PFT (pulmonary function test) and clinical diagnostic feature.

   (a) FEV1/FVC >0.70 or greater but with worsening disease less than 60% of predicted.
   (b) Post bronchodilator reversibility FEV1>12% and 200ml.

2.2. Exclusion criteria

1. Patient not giving consent.
2. Hemodynamically unstable patient.
3. Patient not meeting clinical and PFT criteria for Bronchial Asthma.
4. Other obstructive lung disease.

Under aseptic conditions, 5 ml of blood was drawn from each patient's medial cubital vein into vacutainers and measured for peripheral eosinophil count. Under aseptic precaution, 5 ml of blood was taken from medial cubital vein into vacutainers from each patient and the total IgE levels were determined. The sandwich principle is used to conduct the test. Total IgE levels greater than 100 IU/mL were considered abnormal. [1,2]

2.3. Sputum eosinophil count

Following baseline FEV1 and FVC measurements, subjects were pretreated with inhaled salbutamol (200 g via metered-dose inhaler), followed by 10 minutes of nebulization with hypertonic (3%) sterile saline solution via nebulizer. [3] After staining with hematoxylin and eosin, the count of eosinophils was determined using microscopy and expressed as a percentage. [4] A ≥3% eosinophil count in the sputum was considered abnormal.

2.4. Bronchoscopy

The instrument used was fibre optic bronchoscope, OLYMPUS BF TYPE 1T150. A thorough history and clinical evaluation were performed.

Investigations included routine blood examination, random blood sugar, blood urea, serum creatinine, screening for HIV and HbsAg, ECG, X- Ray chest and CT thorax and COVID-19 RTPCR. Patients were taken up for the procedure after fasting overnight. The procedure was thoroughly explained to the patient and/or the patient's attendant.

Following approval by the Institutional Ethical Committee and taking Informed consent from the patient, the procedure was performed. BAL fluid was sent for eosinophil count.

2.5. Statistical analysis

Descriptive statistics (mean, median, and standard deviation) were used to describe the data. The mean differences with paired observation were tested by Kruskal Wallis Test, Wilcoxon-Mann-Whitney U Test, depending on whether the data were skewed or not. To test the association between two variables, Spearman correlation, Chi square, and Fischer's exact test were used. The statistical significance level $p < 0.05$ is considered the significance cut-off point.

3. Observation and Results

The study group consisted of 100 cases, and 20 controls, in which all cases are diagnosed as bronchial asthma, clinically and/ or spirometry over a study period of 2 years.

Majority of the patients were in the age group of 18-30 years (52.5). Mean age of the patient with bronchial asthma was 30 years. Out of the total 100 patients 63 (52.5%) of the participants were male and 57 (47.5%) of the participants were female.

In our study it was found that, the mean AEC (/mm^3) was 845.00 ± 692.06. 42 (35.0%) of the participants had AEC: ≤500 /mm^3. 78 (65.0%) of the participants had AEC: >500 /mm^3. In our study, the mean Total Serum Ig E (U/mL) was 1389.82 ± 1389.88. (16.7%) of the participants had Total Serum Ig E: ≤100 U/mL, which was seen in controls, all cases had (83.3%) had Total Serum Ig E: >100 U/mL.

In our study, the mean Sputum Eosinophil Count (%) was 1.45 ± 1.40. 86 (71.7%) of the participants had Sputum Eosinophil Count: <3% and 34 (28.3%) of the participants had Sputum Eosinophil Count: ≥3%. In our study, 33.3% of the participants had BAL Eosinophil Count: <1%. 66.7% of the participants had BAL: Eosinophil Count: ≥1%.

Significant association (p<0.05), was found between AEC (/mm^3), Total Serum IgE (U/mL), BAL Eosinophil Count (%), Sputum Eosinophil Count (%) with Eosinophilic Bronchial Asthma.

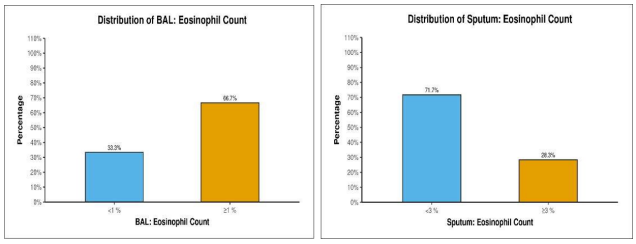

Fig. 1: Graphical representation of frequency distribution of BAL eosinophil count and sputum eosinophilcount in participants

4. Discussion

In all cases of bronchial asthma and controls, we performed the following tests after taking informed consent from the

Table 1: Distribution of participants in terms of AEC

AEC	Frequency	Percentage	95% CI
≤500 /mm^3	42	35.0%	26.7% - 44.3%
>500 /mm^3	78	65.0%	55.7% - 73.3%

Table 2: Association between AEC (/mm^3) and parameters

Total Serum IgE (U/mL)* **	**Correlation Coefficient (rho) = 0.42**	**<0.001**
Total Serum IgE		-
≤100 U/mL	-	
>100 U/mL	973.50 ± 689.15	
BAL: Eosinophil Count (%)* **	Correlation Coefficient (rho) = 0.28	0.004[1]
BAL: Eosinophil Count* **		0.013[3]
<1%	679.17 ± 299.61	
≥1%	1066.45 ± 750.27	
Sputum: Eosinophil Count (%)* **	Correlation Coefficient (rho) = 0.24	0.017[1]
FEV1		0.003

Table 3: Association between Total Serum IgE (U/mL) and parameters

BAL: Eosinophil Count (%)* **	Correlation Coefficient (rho) = 0.53	<0.001[1]
BAL: Eosinophil Count* **		
<1%	909.58 ± 394.33	<0.001[3]
≥1%	1886.97 ± 1494.46	
Sputum: Eosinophil Count (%)* **	Correlation Coefficient (rho) = 0.39	<0.001[1]
AEC (/mm^3)* **	Correlation Coefficient (rho) = 0.42	<0.001[1]
Eosinophilic Bronchial Asthma* **		
Yes	1866.36 ± 1495.57	<0.001[3]
No	936.09 ± 380.71	
FEV1	-	0.001

Table 4: Comparison between our study and other studies with different variables

Correlation	Our study	Lex C et al.	Zhang XY et al.
AEC and BAL Eosinophil	p=0.004 (significant)	-	-
AEC with Sputum Eosinophil	p=0.017 (significant)	-	p <0.001 (significant)
Sputum Eosinophil with BAL Eosinophil	p =<0.001 (significant)	p=0.045 (significant)	-

patient and Institutional Ethical Committee.

On comparison between normal and raised AEC, significant difference was found (W = 0.000, p = <0.001), with the median AEC (/mm^3) being highest in the AEC: >500 /mm^3 group. Roshan M Kumar et al. 2017 had a similar observation, with increased peripheral eosinophil count in 57.9% of the study population.[5] In our study the controls had Total Serum Ig E: ≤100 U/mL. While cases (83.3%) had Total Serum Ig E: >100 U/mL which matched with study by Roshan M Kumar et al. 2017 where 96.1% of study population had levels of total serum IgE of >100 IU/ml.[5] A similar observation by Ahmad Al Obaidi et al. 2008 indicated that the mean serum IgE level was 554 ± 447 IU/mL in asthmatics, and in control subjects was 69 ± 33 IU/mL being statistically, highly significant (p < 0.0001). In addition, the median was very high in asthmatics (448 IU/mL) as compared to control subjects (56 IU/ml), which resembled findings in our observation, with median being 76.90 IU/ml in controls and 1389.82 IU/ml in cases.[6]

In our study, the mean Sputum Eosinophil Count (%) was 1.45 ± 1.40. 86 (71.7%) of the participants had Sputum Eosinophil Count: <3% and 34 (28.3%) of the participants had Sputum Eosinophil Count: ≥3%, which was stastiscally significant with severity of asthma(p <0.001), this was identical to study by Roshan M Kumar et al. 2017 where, 20 (26.3%) patients had abnormal sputum eosinophil count an abnormal sputum eosinophil count and severity of asthma were statistically significant (P = 0.004).[5]

In another study by Louis R, et al. 2000 asthmatics had a higher sputum eosinophilia than control subjects, with a progressive increase which was related to asthma severity, absolute neutrophil counts were significantly raised in severe asthmatics compared with intermittent asthmatic, unlike our study which had increase in sputum

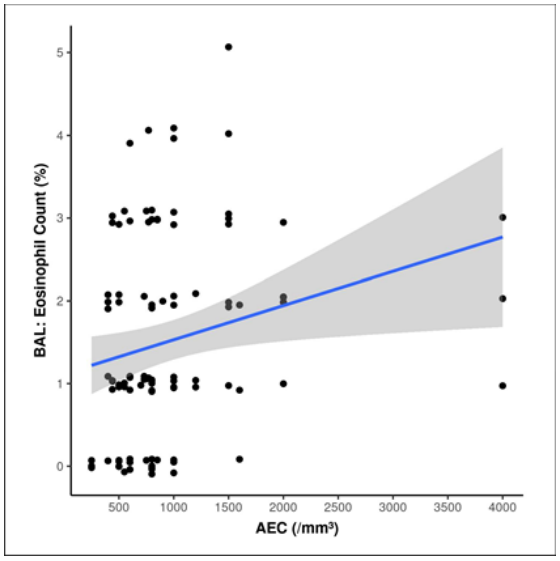

Fig. 2: Correlation between AEC (/mm^3) and BAL: Eosinophil Count (%)

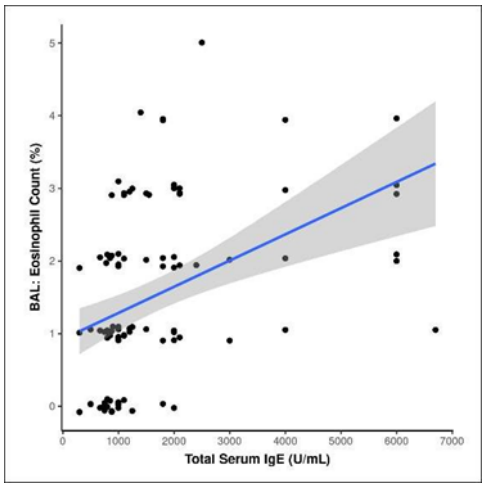

Fig. 3: Correlation between Total Serum IgE (U/mL) and BAL: Eosinophil Count (%)

eosinophil count with severity of asthma.[7] In our study significant association between sputum eosinophil count and eosinophilic bronchial asthma was found, p value <0.001, that is in resemblance with the findings of a study by Park, S.-W et.al 2005, where the percentages of eosinophils in sputum were significantly higher in the Eosinophilic Bronchitis and asthma groups than in the healthy control group ($p < 0.01$).[8]

In our study, 33.3% of the participants had BAL Eosinophil Count <1%, in which 66.7% of the participants had BAL Eosinophil Count ≥1%. There was a significant difference between the BAL eosinophil in terms of AEC (/mm^3) (W = 603.500, p = 0.013) in terms of Sputum Eosinophil Count (%) (W = 240.000, p = <0.001), also

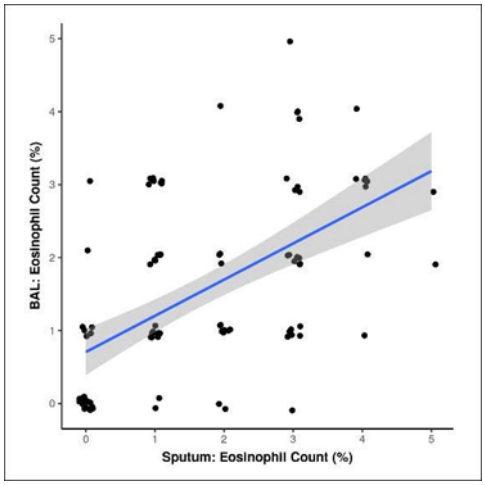

Fig. 4: Correlation between Sputum: Eosinophil Count (%) and BAL: Eosinophil Count (%)

in terms of Total serum IgE (p value<0.001). In a study by Wenzel SE et al. 1997, a significant difference existed among the BAL eosinophil concentrations in the three groups with severity of asthma (p = 0.007), with eosinophils greater in the mild asthmatics than in both the normal controls and two times greater than the numbers in severe asthmatics.[9] Another study by S Sur et al. 1995 found that the BAL fluid differential cell count in patients with symptomatic asthma (eosinophils, 4.4% ± 3.0% neutrophils) revealed a statistically significant increase in BAL eosinophils (p = 0.0001) compared with BAL cells recovered from patients with asymptomatic asthma (0.6% ± 1.0% eosinophils).[10] Lex C, et al. 2006 showed that there was a significant relationship between sputum and BAL eosinophils (n 20, r 0.45, p 0.045), which was similar to the results of our study. There was also a positive relationship between FeNO and BAL eosinophils and the correlation between both markers was even stronger (n 24, r 0.54, p 0.006), contrast to our study, where we didn't use FeNo as a biomarker in our study to analyse eosinophilic asthma.[11]

We in our study found significant association of peripheral blood eosinophil count, sputum eosinophil count and BAL fluid eosinophil count with severity of bronchial asthma, and with eosinophilic asthma. Zhang XY et al. 2014 showed in their study that there was a significant positive relationship between blood eosinophil parameters (Spearman correlation 5 0.493, P <.001) and the percentage of sputum eosinophils, which was analogous to our study.[12] Roshan M Kumar et al. 2017 found a significant association between peripheral eosinophil count, sputum eosinophil count, and serum IgE (g100 IU/mL)[6] which is similar to our observations. Another observation by Khadadah et al. 2000 in their study reported positive correlation between total blood eosinophil counts, serum total IgE levels, and eosinophilic cationic protein.[13] This study demonstrated

a correlation between IgE and S-ECP levels which was different compared to our study by the fact that patients were not compared pre and post treatment and eosinophilic cationic protein levels weren't assessed, also skin testing for atopy wasn't done in our study.

5. Limitations

The present study is a cross-sectional study with a small sample size from a single centre and a tertiary hospital. The overlap between asthma and COPD/chronic bronchitis was overlooked. Patients were not followed up for treatment response and assessment of the eosinophil count in blood, sputum and BAL after treatment wasn't done.

6. Conclusion

Asthma is a heterogeneous condition with a wide range of patient populations. Biologic agents offer a significant opportunity to provide individualised treatment for patients who do not respond to standard asthma therapy. We have demonstrated the importance of phenotypic assessment for patients with severe asthma, that will benefit with biologicals.

7. Source of Funding

None.

8. Conflict of Interest

The authors declare that there is no conflict of interest.

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