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Assessment of the Relationship Between Induced Sputum Eosinophil Count and Absolute Eosinophil Count as Indicators of the Severity of Bronchial Asthma

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ABSTRACT

Asthma stands as a prevalent significant non-communicable ailment, profoundly affecting the quality of life for numerous individuals. On a global scale, asthma holds the 16th position among the primary contributors to years lived with disability and ranks 28th among the leading factors contributing to the overall burden of disease, as assessed through disability-adjusted life years. This study aimed to establish a correlation between sputum and absolute eosinophil counts with asthma severity, FEV1 and other factors related to asthma. This prospective study was carried out in the department of respiratory medicine, Belagavi institute of medical sciences, Belagavi, Karnataka. Evaluation of asthma severity. The degree of asthma severity was evaluated following the guidelines set forth by GINA. This encompassed the following aspects asthma control questionnaire. This questionnaire encompassed the frequency of both daytime and nighttime symptoms the frequency of using short-acting beta-agonists and the extent to which daily activities were disrupted over the past 4 weeks. The relationship between absolute eosinophil count (AEC) and FEV1 was investigated in this study. When the AEC was between 301-500 or greater than 500, there was a statistically significant relationship between the two variables. This suggests that higher AEC levels are associated with lower FEV1 values. For a sputum eosinophil count of less than 3%, there was no statistically significant relationship between the count and FEV1. However, when the count was between 3-5%, there was some correlation but it was not statistically significant. For a sputum eosinophil count greater than 5%, there was a statistically significant relationship between the count and FEV1. Evaluating eosinophil levels in sputum and blood represents a straightforward and economical approach that directly quantifies airway inflammation. Consequently, this method holds the potential to pinpoint distinct phenotypes within asthmatic individuals who may exhibit heightened steroid responsiveness.

INTRODUCTION

The global initiative for asthma GINA defines asthma as a persistent inflammatory condition of the air passages, encompassing various types of inflammatory cells such as eosinophils[1]. It is characterized by recurring symptoms such as wheezing, breathlessness, chest tightness and cough, which fluctuate in intensity and are accompanied by varying limitations in the outflow of breath[2]. Globally, asthma is positioned as the 16th major contributor to years lived with disability and holds the 28th rank among the primary sources of disease burden, quantified through disability-adjusted life years. Approximately 300 million individuals across the globe are afflicted by asthma and there's a projected likelihood that an additional 100 million could be impacted by the condition by the year 2025[1]. Asthma stemming from intricate interactions among numerous cells and cellular components generates airway hyper-responsiveness, largely accountable for the array of asthma symptoms[3]. The diagnostic criteria included a history of erratic respiratory symptoms and variable restrictions on expiratory airflow on spirometry. Evaluating asthma control and seriousness typically involves subjective approaches, such as clinical evaluation and quality of life surveys, as well as objective assessments including spirometry, peak expiratory flow rate, and bronchoprovocation testing[1]. However, according to the current GINA guidelines, asthma severity is evaluated based on the level of treatment necessary for symptom and exacerbation management. In the past decade, several non-invasive markers have emerged for gauging airway inflammation to monitor asthma severity, including exhaled nitric oxide, different types of cells in sputum, and serum eosinophilic cationic protein[4]. The use of biological markers to gauge asthma severity has been explored to a limited extent and the relationship remains poorly defined. Eosinophils, which are recognized as agents of inflammation, play a pivotal role in the development of asthma. Eosinophils and their related substances have been consistently detected in individuals with asthma[5]. Both sputum and blood eosinophilia serve as biomarkers for eosinophilic airway inflammation.

A high number of eosinophils in the sputum (mucus coughed up from the lungs) is a sign of eosinophilic asthma, a type of asthma that is often more severe than other types of asthma[6]. Studies have shown that there is a relationship between the number of eosinophils in the sputum and the risk of asthma exacerbations (sudden worsening of asthma symptoms)[7]. People with eosinophilic asthma who have a high number of eosinophils in their sputum are more likely to have asthma exacerbations than people with eosinophilic asthma who have a lower number of eosinophils in their sputum. Treatment strategies that aim to reduce the number of eosinophils in the sputum are more effective in preventing asthma exacerbations than treatment based on usual care[1]. This is important because asthma exacerbations can be dangerous and can lead to hospitalization. However, data linking clinical symptoms and functional parameters to these biomarkers of airway inflammation are relatively scarce. Therefore, this study aimed to establish a correlation between sputum and absolute eosinophil counts with asthma severity, FEV1 and other factors related to asthma.

MATERIALS AND METHODS

This prospective study was carried out in the department of respiratory medicine, Belagavi institute of medical sciences, Belagavi, Karnataka. Institutional ethical committee approval was obtained for the study based on the helsinki declaration for human research protocol. Written consent was obtained from all the participants of the study after explaining the nature of the study in the vernacular language.

Inclusion criteria:

- All stable asthmatic patients report to our Institute
- Aged 18 and above
- Males and females
- Voluntarily willing to participate in the study

Exclusion criteria:

- Acute Asthmatic exacerbation
- COPD
- Patients who were unable to perform spirometry correctly
- History of MI

Following the acquisition of the informed written consent, data regarding patient demographics, medical history and pertinent examinations were documented:

- Patient demographics encompassed details such as age, gender, BMI and occupation
- Information pertaining to smoking history, clinical indications, observable symptoms and concurrent medical conditions was collected

Evaluation of asthma severity: The degree of asthma severity was evaluated following the guidelines set forth by GINA[1]. This encompassed the following aspects:

- **Asthma control questionnaire:** This questionnaire encompassed the frequency of both daytime and nighttime symptoms the frequency of using short-acting beta-agonists and the extent to which daily activities were disrupted over the past 4 weeks

- Number of exacerbations per year
- **Spirometry:** Patients underwent pulmonary function testing (PFT) utilizing the flow-sensitive Winspiro PRO 5.7 spirometer. The severity of obstruction was categorized based on the criteria established by the european respiratory society and the american thoracic society, which are reliant on the FEV1 values. After administering 200µg of inhaled salbutamol, patients were evaluated for post-bronchodilator reversibility. This evaluation was repeated 15 mint after the baseline test. A change in forced expiratory volume in 1 second (FEV1) of at least 12%-200 mL from the pre-bronchodilator value was indicative of asthma diagnosis in accordance with GINA guidelines[1]

Participants were selected and categorized according to asthma severity. Blood samples and induced sputum samples were collected. Sputum was processed by selecting the viscid or denser portion, adding DTT solution, PBS solution and filtering through a 48 µm nylon mesh. The total cell count (TCC) was estimated, and the cell pellet was diluted to an adjusted concentration of 1.0×106 cells mL^{-1}. Cytospin smears were prepared and stained with giemsa and May-Grunwald stains to determine the differential cell count (DCC). To perform an absolute eosinophil count, draw blood from a finger prick and mix it with eosinophil diluting fluid. Shake the pipette for 10 sec and load the chamber with the diluted blood sample. Allow the cells to settle for 3 min, then count the number of eosinophils in the 4 corner squares under the microscope. Multiply the number of eosinophils by 25 to get the absolute eosinophil count. Clean the counting chamber after completing the count.

Statistical analysis: Continuous variables were summarized as mean and standard deviation (SD) if they were normally distributed, or as median and interquartile range (IQR) if they were not normally distributed. Categorical variables were summarized as frequencies and percentages. Paired tests were used to compare the first and last visits of patients. For continuous variables that were normally distributed, the paired t-test was used. For continuous variables between different groups, ANOVA values were calculated along with the Pearson coefficient correlation (R) values.

RESULTS

A total of n = 50 consecutive cases were included in the study based on the inclusion and exclusion criteria. N = 26 were males and n = 24 were females. The male-to-female ratio was approximately 1:1. The maximum number of cases 56% were included in 21-40 years. The age range spanned from 20-60 years, exhibiting variability. The average age of the study participants was 39.5±5.2 years (Table 1).

Gender was examined in relation to the severity of bronchial asthma, revealing that a higher proportion of females exhibited severe asthma compared to males. This observation demonstrated a noteworthy correlation, as indicated by a p-value of 0.041 as given in Table 2.

Table 3 presents the correlation between sex and eosinophil counts. Among the male group, there were 26 participants, with a mean sputum eosinophil count of 4.50 and a standard deviation of 1.0. The corresponding p-value for the correlation between gender and sputum eosinophil count was 0.478. In the female group, 24 participants had a mean sputum eosinophil count of 1.63. Similarly the category of absolute eosinophil count was presented for both the male and female participants. Among males, 26 participants had a mean absolute eosinophil count of 316.92, with a standard deviation of 89.57. The p-value for the correlation between gender and absolute eosinophil count in this case was 0.812. Among females, there were 24 participants and the mean absolute eosinophil count was slightly higher at 348.64. The p-values provided in the table represent the statistical significance of the correlation between gender and eosinophil count.

Patients who had a history of environmental exposure (n = 20) showed no significant correlation with the severity of asthma (p = 0.231) (Fig. 1). Smoking status and its correlation with sputum and absolute eosinophil counts were analyzed. The average (mean) sputum eosinophil count for non-smokers was 1.85, with a standard deviation of 1.36. The p-value indicating the statistical significance of the relationship between smoking status and sputum eosinophil

Table 1: Age-wise distribution of cases in the study

Age	Frequency	Percentage
18-20	2	4
21-30	18	36
31-40	10	20
41-50	14	28
50-60	6	12
Total	50	100

Table 2: Showing the severity of asthma distribution in males and females

	Severity						
Gender	Mild	Percentage	Moderate	Percentage	Severe	Percentage	p-value
Male	6	12	9	18	11	22	0.041
Female	3	6	3	6	18	36	
Total	9	18	12	24	29	58	

Table 3: Correlation of gender and eosinophil count

	No.	Mean	Standard deviation	p-value
Sputum eosinophil count				
Male	26	4.50	1.00	0.478
Female	24	5.63	1.00	
Absolute eosinophil count				
Male	26	316.92	89.57	0.812
Female	24	348.64	95.55	

Table 4: Showing the relationship between different allergens and the severity of asthma symptoms

	FEV1				
	80% and above	70-79%	60-69%	50-59%	p-value
Absolute eosinophil count					
<300	0	0	0	0	-
301-500	0	8	6	12	0.012
>500	0	5	9	10	0.017
Sputum eosinophil count (%)					
<3	0	1	0	0	-
3-5	0	5	8	4	0.284
>5	0	10	10	12	0.011

Table 5: Showing the Pearson Correlation coefficient values with variables

Pearson correlation (R) values	Dyspnoea duration	Wheeze duration	Expectoration duration	Cough duration
Severity of asthma	+0.754	+0.771	+0.278	+0.534
FEV1	-0.657	-0.514	-0.158	-0.473
Sputum eosinophil count	+0.412	+0.503	+0.617	+0.221
Absolute eosinophil count	+0.571	+0.439	+0.387	+0.482

count was 0.218. Among the smokers, there were 5 participants. Smokers had an average sputum eosinophil count of 2.25, with a standard deviation of 1.0. The comparison between smokers and non-smokers by p-value was found to be 0.325; hence, it was not significant. For the absolute eosinophil counts the mean absolute eosinophil count for nonsmokers was 295.64±125.21 and the mean absolute eosinophil count for smokers was 410.18±210.36 the p values were (0.015) hence it was significant. There was no significant correlation between family history and eosinophil count. Of the 50 patients, 22 had a family history of asthma and most of them had severe asthma.

Table 4 shows the absolute eosinophil count, FEV1 (forced expiratory volume in 1 sec) and corresponding p-values for different ranges of FEV1 percentages. When the absolute eosinophil count was between 301-500, there were varying FEV1 values corresponding to different FEV1 percentage ranges. When the AEC is greater than 500, similar to the previous range, there are FEV1 values associated with different FEV1 percentage ranges. This suggests further interaction between the two variables in this range. The p-values for the different absolute eosinophil count ranges and FEV1 percentages were 0.012- 0.017, respectively, suggesting a statistically significant relationship between these variables within those ranges. for a sputum eosinophil count of less than 3%, the FEV1 values in the different FEV1 percentage ranges were generally low, with some variation. This indicates a weak or negligible relationship between the sputum eosinophil count and FEV1 in this range. When the sputum eosinophil count was between 3-5%, FEV1 values showed more variability across the FEV1 percentage ranges. The differences between FEV1 values in the different ranges might suggest some correlation but the p-value of 0.284 indicates that this correlation is not statistically significant at the commonly used significance level of 0.05. For a sputum eosinophil count greater than 5%, there appeared to be higher FEV1 values in different ranges. This finding suggests a potential relationship between higher sputum eosinophil counts and improved FEV1 values. The low value of 0.011 indicates that this correlation is statistically significant at the 0.05 level.

Table 5 reports the pearson correlation (R) values between various variables related to asthma. The positive or negative sign indicates the direction of the correlation, where "+" indicates a positive correlation and "-" indicates a negative correlation. Dyspnoea duration has a strong positive correlation with wheeze duration expectoration duration, cough duration and severity of asthma, suggesting that as the duration of dyspnoea increases, these other variables tend to increase as well. FEV1 has negative correlations with all other variables, indicating that as the forced expiratory volume in 1-second decreases, the other variables tend to increase (Table 5).

DISCUSSIONS

The current research aimed to establish a correlation between induced sputum eosinophil levels and absolute eosinophil counts in the evaluation of the clinical severity of bronchial asthma. In our investigation involving 50 cases of asthma, it was observed that 58% of the patients exhibited severe asthma, 24% had moderate asthma and 18% had mild asthma. The prevalence of asthma was found to be higher in the middle age group, with an even

distribution between genders. Dust and seasonal allergens emerged as the most prevalent triggers. Notably, a greater proportion of beedi workers were identified compared to individuals in other occupations. Furthermore, our study identified several risk factors associated with severe asthma. These factors included being female, exposure to environmental dust, smoking, having a positive family history of asthma and experiencing long-duration symptoms. Notably, there was a substantial correlation between induced sputum eosinophil levels and absolute eosinophil counts with the presence of severe persistent asthma.

In this study, we found out of 50 cases 52% were males and 48% were females. While the ratio of females to males experiences a shift throughout development, childhood typically sees a lower occurrence of asthma in females compared to males. However, as individuals transition to adulthood, asthma becomes more prevalent in females than males due to the influence of hormonal factors[8]. A comparison of gender in relation to the severity of bronchial asthma revealed that a majority of females exhibited a greater prevalence of severe asthma compared to males, demonstrating a noteworthy and statistically significant correlation ($p = 0.041$). Various sources in the literature have highlighted that asthma tends to be more severe in women and is linked with heightened healthcare utilization[9]. Following the onset of puberty, a shift in gender-related patterns occurs, leading to an increased prevalence and severity of asthma in women[10-12]. Girls who experience early maturation and pregnant women often encounter elevated estrogen levels, resulting in a higher cumulative exposure to sex hormones, which places them at an elevated risk of developing asthma later in life. Conversely, the use of oral contraceptives might offer a protective effect, potentially reducing the risk of asthma exacerbations in women with asthma[13]. We identified that 28% of cases were exposed to risk factors associated with their occupation, with 16% of these cases being individuals employed in the beedi industry. Literature indicates that a systematic analysis of population-based risk factors indicates that approximately 16% of all instances of adult-onset asthma can be attributed to exposure in occupational settings[14]. Notably, a disparity exists between the rates of asthma diagnosed by healthcare professionals as being work-related and rates that encompass self-reported instances of work-related[15]. This study found no substantial correlation existed between the population group exposed to environmental factors, the severity of the condition, and eosinophil count. The literature suggests that occupational asthma can be categorized into two pathophysiological variants: Eosinophilic and non-eosinophilic. Among these, the non-eosinophilic variant was prevalent. Both groups exhibited indications of sputum neutrophilia. While sputum eosinophilia was linked to more severe disease and greater reversibility with bronchodilators, there was no discrepancy in peak expiratory flow response to workplace exposures. Consequently, our study's findings were in alignment with previous literature.

Within our study, cases with a family history of asthma and individuals who smoked were predominantly those with severe persistent asthma. However, no significant correlation was observed with eosinophil count. Literature also highlights tobacco smoking as a notable contributor to heightened asthma morbidity across both children and adults[16-18]. Typically, among smokers with the non-eosinophilic variant, asthma tended to be severe, and there was limited response to steroid treatments. In our study, dust and seasonal allergens emerged as the prevailing triggers. In nations with elevated levels of air pollution like India, particulate matter in inhaled substances stands out as a potent risk or triggering factor in the development of asthma.

The duration of symptoms displayed a direct correlation with asthma severity and eosinophil count, while an inverse correlation was observed with FEV1. This finding indicated that patients who experienced prolonged symptom durations were not adherent to treatment, had poor compliance, and continuous exposure to triggers were more likely to present with severe persistent asthma characterized by FEV1 levels below 60, frequent exacerbations, and an elevation in eosinophil count. In our study, we found that most of the cases had a higher percentage of sputum eosinophil count (Table 5). This group consisted mostly of people with moderate to severe persistent asthma. Our findings are consistent with those of other studies. We also found that people with severe persistent asthma were more likely to have a high sputum eosinophil count (26%), but more than half of them had normal sputum eosinophil levels[19-20]. This is also consistent with the findings of other studies. We did not find a significant difference in sputum eosinophil levels between people with mild, moderate, and severe persistent asthma. We also did not find a dose-response relationship between asthma severity and the proportion of people with higher sputum eosinophilia. This suggests that there is an asthma phenotype with sputum eosinophilia that can be seen in any asthma severity. This is supported by the findings of other studies. The study also found that there was no significant difference in sputum eosinophil levels between people with mild, moderate, and severe persistent asthma[3]. This suggests that there is an asthma phenotype with sputum eosinophilia that can be seen in any asthma severity.

Recognizing the significance of identifying this particular asthma phenotype characterized by heightened sputum eosinophilia could potentially be linked to its responsiveness to steroids, a relationship that future studies might elucidate within the Indian population. In our current study, despite observing a statistically significant inverse correlation between sputum eosinophil count and the predicted forced expiratory volume in 1 sec (FEV1), as well as the predicted, forced vital capacity (FVC), the strength of this correlation has been modest. Several other studies have similarly reported a significant correlation between sputum eosinophil count and predicted FEV1 ($p<0.05$)[22-24].

In our investigation, we noted an elevated absolute eosinophil count in 30.5% of the study's participants, mainly encompassing individuals with moderate and severe persistent asthma, mirroring the pattern observed in sputum eosinophil levels. We found that a notably high absolute eosinophil count (>350) was considerably more prevalent in patients diagnosed with severe persistent asthma. Comparable findings have been reported in several other studies[3,20,25]. In our study, no substantial correlation was observed between absolute eosinophil levels in cases of mild and moderate persistent asthma. Conversely, we identified a significant inverse correlation between FEV1 and absolute eosinophil count. We identified a noteworthy connection between absolute eosinophil count and sputum eosinophil count. Although only a limited number of studies delve into the correlation between sputum and absolute eosinophil counts, among them, the study conducted by Khadadah et al. [26] revealed a positive correlation between total blood eosinophil counts and eosinophilic cationic protein.

CONCLUSION

Evaluating eosinophil levels in sputum and blood represents a straightforward and cost-effective approach that directly quantifies airway inflammation. Consequently, this method holds the potential to pinpoint distinct phenotypes within asthmatic individuals who may exhibit heightened steroid responsiveness. This aspect warrants validation through more research. As a result of its potential advantages, this method may emerge as the favored choice for routine clinical practice, aiding in the continuous monitoring of airway inflammation and guiding patient management.

REFERENCES

1. Dharmage, S.C., J.L. Perret and A. Custovic, 2019. Epidemiology of asthma in children and adults. Front. Pediatr., Vol. 7, No. 246. 10.3389/ fped.2019.00246
2. GINA, 2022. Global strategy for asthma management and prevention., https://gin asthma. org/gina-reports/
3. Saha, K., A. Bandyopadhyay, P. Roy, S. Chakraborty, D. Jash and D. Saha, 2013. Usefulness of induced sputum eosinophil count to assess severity and treatment outcome in asthma patients. Lung India, 30: 117-123.
4. Cianchetti, S., E. Bacci, L. Ruocco, M.L. Bartoli and M. Ricci *et al*., 2004. Granulocyte markers in hypertonic and isotonic saline-induced sputum of asthmatic subjects. Eur. Respir. J., 24: 1018-1024.
5. Green, R.H., C .E. Brightling, S. McKenna, B. Hargadon and D. Parker *et al*., 2002. Asthma exacerbations and sputum eosinophil counts: A randomised controlled trial. Lancet, 360: 1715-1721.
6. Tillie-Leblond, I., D. Montani, B. Crestani, J. de Blic and M. Humbert et al., 2009. Relation between inflammation and symptoms in asthma. Allergy, 64: 354-367.
7. Petsky, H.L., C.J. Cates, T.J. Lasserson, A.M. Li, C. Turner, J.A. Kynaston and A.B. Chang, 2010. A systematic review and meta-analysis: Tailoring asthma treatment on eosinophilic markers (exhaled nitric oxide or sputum eosinophils). Thorax, 67: 199-208.
8. Morosco, G. and J. Kiley, 2007. Expert panel report 3 (EPR-3): Guidelines for the diagnosis and management of asthma-summary report 2007. J. Allergy Clin. Immunol., 120:
9. ENFUMOSA., 2003. The ENFUMOSA cross-sectional European multicentre study of the clinical phenotype of chronic severe asthma. European network for understanding mechanisms of severe asthma. Europ. Respir. J., 22: 470-477.
10. Tantisira, K.G., R. Colvin, J. Tonascia, R.C. Strunk, S.T. Weiss and A.L. Fuhlbrigge, 2008. Airway responsiveness in mild to moderate childhood asthma. Am. J. Respir. Crit. Care Med., 178: 325-331.
11. Schatz, M. and C.A. Camargo, 2003. The relationship of sex to asthma prevalence, health care utilization, and medications in a large managed care organization. Ann. Allergy, Asthma Immunol., 91: 553-558.
12. Venn, A., S. Lewis, M. Cooper, J. Hill and J. Britton, 1998. Questionnaire study of effect of sex and age on the prevalence of wheeze and asthma in adolescence. BMJ, 316: 1945-1946.
13. Salam, M.T., M. Wenten and F.D. Gilliland, 2006. Endogenous and exogenous sex steroid hormones and asthma and wheeze in young women. J. Allergy Clin. Immunol., 117: 1001-1007.

14. Torén, K. and P.D. Blanc, 2009. Asthma caused by occupational exposures is common: A systematic analysis of estimates of the population-attributable fraction. BMC Pulm. Med., Vol. 9, No. 7. 10.1186/1471-2466-9-7.
15. Mazurek, J.M., G.E. Knoeller, J.E. Moorman and E. Storey, 2013. Occupational asthma incidence: Findings from the behavioral risk factor surveillance system asthma call-back survey United States, 2006-2009. J. Asthma, 50: 390-394.
16. de Koning, H.W., K.R. Smith and J.M. Last, 1985. Biomass fuel combustion and health. Bull. World Health Organ., 63: 11-26.
17. ATS., 2000. American thoracic society: What constitutes an adverse health effect of air pollution? Official statement of the American thoracic society. Am. J. Respir. Crit. Care Med., 161: 665-673.
18. Jindal, S.K. and D. Gupta, 2004. The relationship between tobacco smoke and bronchial asthma. Indian J. Med. Res., 120: 443-453.
19. Fujimoto, K., K. Kubo, Y. Matsuzawa and M. Sekiguchi, 1997. Eosinophil cationic protein levels in induced sputum correlate with the severity of bronchial asthma. Chest, 112: 1241-1247.
20. Duncan, C.J.A., A. Lawrie, M.G. Blaylock, J.G. Douglas and G.M. Walsh, 2003. Reduced eosinophil apoptosis in induced sputum correlates with asthma severity. Eur. Respir. J., 22: 484-490.
21. Ronchi, M., C. Piragino, E. Rosi, L. Stendardi and A. Tanini et al., 1997. Do sputum eosinophils and ecp relate to the severity of asthma? Eur. Respir. J., 10: 1809-1813.
22. Louis, R., L.C.K. Lau, A.O. Bron, A.C. Roldaan, M. Radermecker and R. Djukanovic, 2000. The relationship between airways inflammation and asthma severity. Am. J. Respir. Crit. Care Med., 161: 9-16.
23. Green, R.H., C.E. Brightling, S. McKenna, B. Hargadon and D. Parker *et al*., 2002. Asthma exacerbations and sputum eosinophil counts: A randomised controlled trial. Lancant, 360: 1715-1721.
24. Ige, O., A. Falade and O. Arinola, 2012. Atopy is a risk factor for adult asthma in urban community of Southwestern Nigeria. Lung India, 29: 114-119.
25. Lemiere, C., P. Ernst, R. Olivenstein, Y. Yamauchi and K. Govindaraju *et al*., 2006. Airway inflammation assessed by invasive and noninvasive means in severe asthma: Eosinophilic and noneosinophilic phenotypes. J. Allergy Clin. Immunol., 118: 1033-1039.
26. Khadadah, M., B.O. Onadeko, C.I. Ezeamuzie, H.T. Mustafa, R. Marouf and T.N. Sugathan, 2000. The association of skin test reactivity, total serum ige levels, and peripheral blood eosinophilia with asthma in Kuwait. J. Asthma, 37: 481-488.