Indian J Allergy Asthma Immunol 2011; 25(2): 91-96

An Immunological Study of Bronchial Asthma with Special Reference to Interleukin-4, Interferon-ã and Immunoglobulin E

J K Mishra,[1] Rashid Parvez,[1] Usha,[2] Girish Singh[3]

[1]Department of T.B. & Respiratory Diseases, [2]Department of Pathology, [3]Division of Biostatistics, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Abstract

Asthma is a disorder defined by its clinical, physiological, and pathological characteristics. The predominant feature of the clinical history is episodic shortness of breath, particularly at night, often accompanied by cough. The main physiological feature of asthma is episodic airway obstruction characterized by expiratory airflow limitation. The dominant pathological feature is airway inflammation, sometimes associated with airway structural changes. This immunological study on bronchial asthma was conducted at Sir Sunderlal Hospital, Institute of Medical Sciences, Banaras Hindu University, Varanasi in the year 2010. Based on clinical history one group of 70 cases with history of asthma and another group of 36 controls with no history of asthma were made. The mean serum level of IL-4, IgE, and AEC was significantly higher in cases as compared to controls whereas; mean IFN- ã as well as FEV_1 were significantly lower in cases as compared to controls. The cut-off values at appropriate sensitivity and specificity using ROC curve for IL-4, IFN- ã, IgE, AEC and FEV_1 were 7.25, 0.35, 225, 345 and 79.50 respectively. The imbalance in the serum levels of IL-4 and IFN- ã is predicted to drive the asthma pathogenesis while reducing IgE levels may now be considered as a treatment strategy.

Keywords: IL-4, IFN-ã, serum IgE, bronchial hyper-responsiveness, AEC, FEV_1

INTRODUCTION

Asthma is a common, chronic disease of airway inflammation that manifests with recurrent episodes of coughing, breathlessness, wheezing, and chest tightness. These episodes are associated with airflow obstruction that is at least partially reversible. Making the diagnosis of asthma requires a clinical evaluation of the patient's symptom, medical history, physical examination and diagnostic tests.

Asthma is a problem worldwide, with an estimated 300 million affected individuals. Despite hundreds of reports on the prevalence of asthma in widely differing populations, the lack of a precise and universally accepted definition of asthma makes reliable comparison of reported prevalence from different parts of the world problematic. Nonetheless, based on the application of standardized methods to measure the prevalence of asthma and wheezing illness in children and adults it appears that the global prevalence of asthma ranges from 1% to 18% in different countries. There is limited data on asthma epidemiology from the developing world, including India. Although some attempts have been made, studies suffer from several scientific drawbacks including lack of uniformity of methodology and analysis of data. Asthma rates are officially low in India, though there is some recent evidence that the true prevalence is higher than previously thought. To date, the total estimated burden

Address for Correspondence: Prof. J. K. Mishra, Professor, Department of T.B. & Respiratory Diseases, Room No.1352, Institute of Medical Sciences, Banaras Hindu University, Varanasi, 221005 UP, India, e-mail: jkmishra.imsbhu@gmail.com

of Asthma is an overall prevalence of 3% (30 million patients), and among adults over the age of 15, a median prevalence of 2.4%.[1]

The factors influencing the development and expression of asthma may be classified into two categories *viz.*, host factors (genetic, obesity, sex) and environmental factors (allergens, infections, occupational sensitizers, tobacco smoke, diet). Asthma being an inflammatory disorder of the airways, involves several inflammatory cells and multiple mediators that result in characteristic pathophysiological changes. In ways that are still not well understood, this pattern of inflammation is strongly associated with airway hyper-responsiveness and asthma symptoms. Over 100 different mediators are now recognized to be involved in asthma and mediate the complex inflammatory response in the airways.[2] The mucosal mast cells activated by allergens through high-affinity Immunoglobulin E (IgE) receptors, as well as by osmotic stimuli resulting to increased in numbers in airway smooth muscle may be linked to airway hyper-responsiveness.[3] Eosinophils and T-lymphocytes present in increased numbers in the airways, release specific cytokines, including Interleukin-4 (IL-4) that orchestrate eosinophilic inflammation and IgE production by B-lymphocytes.[4,5] Interactions of dendritic cells with regulatory T cells ultimately stimulate production of Th-2 cells from naive T cells.[6,7]

Thus determination of serum levels of IL-4 and IFN-ã may be useful for understanding and monitoring the inflammatory response in asthma. Keeping in view these facts, the aim of the present work was to evaluate serum level of Interleukin-4, Interferon gamma and Immunoglobulin E in Bronchial asthma and to study their interrelationship. Further, also to determine the cut-off value of IL-4, IFN- ã and IgE, through ROC curve and find sensitivity and specificity for the chosen cut-off.

MATERIAL AND METHOD

The present study based on total 106 subjects was conducted at Sir Sunderlal Hospital, Institute of Medical Sciences, Banaras Hindu University, Varanasi in the year 2010. Based on the clinical history of investigations two group were made. Group-1 consisted of 70 cases who had history of asthma (history of allergy and spirometry with post bronchodialation reversibility e”12% and e”200ml (FEV_1). Group-2 had 36 controls that had no history of asthma.

Patient selection

Inclusion Criteria:

1. Cases of bronchial asthma

   Cases taken fulfilled the criteria of bronchial asthma (history of allergy and spirometry with post-bronchodialator reversibility e”12% and e”200ml (FEV_1).

Exclusion Criteria:

1. No history of bronchial asthma, allergy, viral infection, HIV, alcohol intake parasitic, infestation severe burn cases Churg-Strauss syndrome and Wiskott -Aldrich syndrome.
2. Any other respiratory co-morbidity like pneumonia, pulmonary Koch’s disease.
3. Any other systemic co-morbitity like hypertension, diabetes.
4. Patients not consenting.

The Diagnosis and clinical evaluation

A detailed medical history of patients known or thought to have asthma was taken. Clinical examination was done including examination of the respiratory, cardiovascular and central nervous system. Also, routine haematological investigations and X-ray chest PA view were done.

The diagnostic test for absolute eosinophil count (AEC), pulse oxymetry, serum level of IgE, arterial blood gas (ABG) analysis, sputum for fungal mycelia, stool examination for parasites, pulmonary function test (PFT) with reversibility. Serum was separated from the blood, which was aseptically drawn by venipuncture. After clotting and centrifugation the sample was immediately transferred in plastic tubes and stored at $<-18^0$C for a maximum of 2 months or preferably at $<-70^0$C for longer time. Serum IL-4, serum IFN- ã and serum IgE were estimated by ELISA technique. These investigations were done in Immuno-

pathlogy Department, IMS-BHU. AEC was done in CCI lab, S.S.Hospital BHU. Pulmonary Function Test (PFT) was done in TB and Respiratory Department with Medisoft PFT machine.

Statistical Analysis

Analysis of data was done using SPSS ver.16 statistical software package. Cross tables, mean and standard deviation (SD) for both groups were obtained and intergroup comparison was done. ROC curve with area under the curve was used to determine the cut-off value of IL-4, IFN- ã and IgE. Sensitivity and specificity for the chosen cut-off were also determined. To determine the degree of linear interrelationship between these variables, coefficient of correlation was also determined.

RESULT

The present study was based on 106 subjects. The number of cases in Group-1 (cases with history of bronchial asthma) was 70 whereas; in Group-2 (control group with no history of bronchial asthma) the number was 36. The percentage of males in Group-1 was 54.3 while of females it was 45.7. Similarly the percentage of males and females in Group-2 was 63.9 and 36.1 respectively (Table-1). In this study, age-range for asthmatic cases was 10-70 yrs (mean age 30.78±11.27) and for control group it was 10-60 yrs (mean age 29.23±12.94). The difference between mean ages of the two groups was statistically not significant (Table-2).

The mean IL4 value for cases (10.16±2.69) was found higher than those of control group (8.06±4.01) and this difference between means was statistically significant ($p<0.01$). Similarly mean IgE value for cases (236.70±73.21) was found higher than those of control group (209.33±42.60) and this difference was statistically significant ($p<0.05$). The mean value of AEC for case group was 464.31 (±121.58) while it was 223.53 (±83.37) for control group and the intergroup comparison resulted statistically significance ($p<0.001$). The mean IFN-ã for cases (0.390±0.266) was observed to be significantly lower ($p<0.02$) than that of control group (0.518±0.314). Similarly, mean FEV_1 value for cases (75.63±4.68) was found to be lower than that of control group (82.36±1.92) and this difference was statistically significant ($p<0.001$) (Table-2).

To measure the degree of linear relationship between IL-4, IFN- ã, IgE, FEV_1 and AEC, Karl-

Table 1. Age and sex distribution according to groups

Variables		Group-1: Case	Group-2: Control
Number		70	36
Sex	Male	38 (54.3%)	23 (63.9%)
	Female	32 (45.7%)	13 (36.1%)
Age (yrs)	10 - 20	07	10
	21 – 30	33	12
	31 – 40	21	07
	41 - 50	04	05
	51 – 60	04	02
	61 - 70	01	00

Table 2. Mean, sd and statistical significance

Variables	Group-1: Case mean ± sd	Group-2: Control mean ± sd	*Between the group comparison (p-value)
Age (yrs.)	30.78±11.27	29.23±12.94	0.471 (not sig.)
Interleukin-4(pg/ml)	10.16±2.69	8.06±4.01	0.001
IFN-ã (iu/ml)	0.390±0.266	0.518±0.314	0.011#
IgE(iu/ml)	236.70±73.21	209.33±42.60	0.041
AEC(cells/cumm)	464.31±121.58	223.53±83.37	0.000
FEV_1	75.63±4.68	82.36±1.92	0.000

* Independent Sample t-test, # Wilcoxon-Mann-Whitney test

Table 3. ROC area under the curve, cut-off value, sensitivity and specificity

Variable	ROC Area under the curve	Std. Error	Cut-off value	Sensitivity	Specificity
IL-4 (pg/ml)	0.709	0.061	7.25	84.3%	58.3%
IFN- ã(iu/ml)	0.644	0.060	0.350	74.3%	64.1%
IgE (IU/ml)	0.676	0.055	225	62.9%	66.7%
AEC (Cells/cumm)	0.958	0.016	345	84.3%	97.2%
FEV_1 (%)	0.962	0.022	79.50	95.7%	100%

Pearson's coefficient of correlation and its statistical significance were also determined. A negative correlation coefficient though, not statistically significant ($p>0.05$) was observed between IL4 and IgE, IL4 and AEC, IFN- ã and IgE, IFN- ã and AEC, IgE and FEV_1. Whereas, a positive correlation coefficient though, not statistically significant ($p>0.05$) was observed between IL4 and IFN- ã, IL4 and FEV_1, IgE and AEC. It is to be noted that correlation coefficient between IFN- ã and FEV_1 was positive and statistically significant ($p<0.05$) whereas, correlation coefficient between AEC and FEV_1 was negative and statistically significant ($p<0.05$).

ROC curve was plotted and area under the curve, cut-off value of the tests and its sensitivity, specificity at that cut-off were determined and shown in Table-3. The cut-off values for IL-4, IFN- ã, IgE, AEC and FEV_1 were 7.25, 0.35, 225, 345 and 79.50 respectively.

DISCUSSION

Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyper-responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing particularly at night or in the early morning. These episodes are usually associated with widespread, but variable airflow obstruction within the lung that is often reversible either spontaneously or with treatment. Recent investigations have demonstrated that allergen-specific CD4+ T helper 2 (Th2)-type lymphocytes and the cytokine, interleukin (IL)-4 play important roles in the development of airway eosinophilic inflammation and bronchial hyper-responsiveness (BHR). Interleukin-4 is thought to be involved in allergic reactions, because it induces the differentiation of naive CD4+ T lymphocytes into Th2-type cells,[8] mast cell proliferation[9] and also IgE synthesis.[10] The present study showed increased mean serum level of IL-4 for cases of bronchial asthma in comparison to healthy control. A number of authors have studied serum cytokines in asthmatic subjects. Bogic´ *et al* [11] have reported significant higher IL-4 serum concentrations in asthmatic group compared to control and these were significantly higher in patients with moderate and severe asthma compared to mild asthmatics. Shahid *et al* [12] had shown an increased concentration of exhaled IL4 in asthamatic and decreased IFN- ã. However, results from recent studies regarding the role of IL-4 in the onset of allergen-induced BHR are still conflicting.[13]

In the present study the mean serum level of IFN-ã in cases was observed to be significantly lower as compared to controls. It had been demonstrated in this study that patient with bronchial asthma (atopic) produce significantly less IFN- ã and more IL4. IFN-ã has been held responsible for regulating the activation, differentiation and recruitment of eosinophil.

In allergic bronchial asthma, IgE is thought to play an important role in the generation of mediators and in the development of BHR.[14] Brusselle *et al*[15] reported that aeroallergen-induced airway inflammation and BHR are dependent on IL-4 in gene disrupted mice. Several other studies have also reported that allergic and asthmatic subjects are more likely to have elevated levels of Th-2 cytokines and reduced levels of Th-1 cytokines. It could be argued that Th1/Th2-imbalance is responsible for the development of IgE-mediated

inflammation. The present study also revealed significantly increased mean serum levels of IgE in comparison to control group. The serum IgE level was observed negatively correlated with the amount of IFN-ã. Further, the mean AEC of cases with bronchial asthma was found significantly higher in comparison to control group. The reason of observing significantly lower mean FEV_1 in cases of bronchial asthma as compared to control group may be due to increased BHR and obstruction in cases. The association between eosinophils and allergic disease has been known for many years. According to Halonen *et al* [16] a significant relationship exists between serum IgE levels and eosinophilia in populations presumed to be free of parasites where IgE levels presumably provide a better clue to atopy than do skin tests. This shows that increased bronchial hyper-responsiveness and obstruction occur if serum level of AEC and IgE are raised. Thus IgE secretion by lymphocytes defines the allergic state and nearly all asthmatics have a higher IgE levels in serum than the normal population, following adjustment for age and sex.

CONCLUSION

Bronchial asthma is characterized by lower respiratory tract inflammation leading to bronchial hyper-responsiveness (BHR) with variable and reversible airflow obstruction. Asthma has significant genetic and environmental components, but since its pathogenesis is not clear, much of its definition is descriptive. Interleukin-4 being the main cytokine involved in the pathogenesis of allergic responses that can also down-regulate acute inflammatory changes, also got additional effects on asthma pathogenesis which include stimulation of mucus producing cells and fibroblasts leading to airway remodeling. It has also been confirmed that the crucial role of IL-4 lies in its effect on Th-2 development, rather than on the induction of IgE synthesis and subsequent mast cell degranulation. On the other hand IFN- ã is a potent inhibitor of IgE synthesis. Thus, this imbalance in the serum levels of IL-4 and IFN- ã is predicted to drive the asthma pathogenesis. The findings of present study support the hypothesis of Th1/Th2 cytokine imbalance and suggest that serum level of IL-4 may be elevated in concert with decreased level of IFN-ã in asthma. Determination of serum levels of IL-4 and IFN-ã may be useful for understanding and monitoring the inflammatory response in asthma. Reducing IgE levels may now be considered as a strategy for the treatment of allergic rhinitis and asthma both.

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