Original article

Positive correlation of serum interleukin-13 and total immunoglobulin E in bronchial asthma patients

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Abstract

Background: Asthma is a chronic inflammation of airways, associated with hyper-responsiveness of the bronchial tree, resulting in reiterated attacks of cough, wheezing, chest tightness, and breathlessness. Aapproximately 4 Pakistani adults out of 100 persons have been reported to suffer from asthma in year 2002-03.This study focuses on determining interleukin-13 (IL-13) and immunoglobulin E (IgE) in asthma patients and healthy subjects. ***Settings and Design: Methods and Material:*** The study assessed the level and correlation of IL-13 and IgE in bronchial asthma patients. Fifty bronchial asthma patients with allergy history and thirty healthy individuals were included. The patients were selected from Gulab Devi Chest Hospital, Lahore, Pakistan. Total serum IgE and IL-13 were determined by ELISA technique.SPSS (Version 20.0) software was used for statistical analysis. Kolmogorov-Smirnov, Shapiro-Wilk test, Student t-test and Mann-Whitney test have been used in analysis. ***Results:*** The mean (±SD) values of IL-13 concentration (pg/ml) in asthmatics and controls (healthy) were 1574 (± 409) and 390 (± 23). Moreover, median (IQR) value for both groups were 1415.8 (1398-1554) and 394.3 (384.3-404.3), respectively. ***Conclusions:*** To sum up, we have found that bronchial asthma patients had significantly increased level of total serum IgE and IL-13 and we found a positive correlation between these parameters.

Keywords: Allergy; bronchial asthma; IgE; IL-13.

Introduction

Bronchial asthma is characterized by chronic inflammation of lungs in which there is an increased bronchial hyper-responsiveness resulting in cough, wheezing, breathlessness and chest tightness[1]. It mostly affects children as well as young adults and is one of the leading causes of disability, financial burden, and poor life quality in these patients by affecting them emotionally and psychologically[2-4]. Asthma can be fatal if no appropriate treatment[5].

The numbers of asthma sufferers vary greatly in different parts of the world[6]. Overall, more than 3 billion people are suffering from asthma globally[7], and this burden is expected to exceed 100 million patients by 2025[8]. Aapproximately, 4 Pakistani adult out of 100 persons had asthma in year 2002-03[9]. Asthma is a complex disease and has been linked with multiple genes and wide spectrum of predisposing factors. For the sake of simplicity, asthma can be categorized into extrinsic and intrinsic asthma[1].

Extrinsic asthma is induced by type I hypersensitivity

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reaction against common aeroallergens[10]. Dendritic cells (DCs), acting as antigen presenting cells (APCs), present aeroallergens to CD4$^+$ T cells which leads to induction of Th2 cells[11]. Th2 cells secrete cytokines which include IL-13, IL-9, IL-4 and IL-5[12]. Allergic asthma is characterized by Th2 cytokines` predominance and increased release of immunoglobulin E (IgE)[13]. The gene controlling production of IL-13 is located on chromosome 5q31. The product of this gene is a 33 amino acid long peptide i.e. IL-13[14]. The physiological functions of IL-13 include eliminating helminthic parasites from the intestine as well as promoting isotype switching of B cell to produce IgE[15]. It also mediates airway hyperactivity and excessive mucus secretion[16]. If IL-13 is produced in high amount, it can be pathological and had already been labeled as basic interceder of allergic asthma[17]. Currently, there is a huge research effort to test anti-IL-13 in the clinics because of its increasingly established role not only in allergic asthma but also in other diseases like mucosal inflammation, ulcerative colitis and diseases in which fibrosis is an important component[18,19]. Normally when compared to other immunoglobulins, IgE is lowest in concentration in serum[20] and it binds to eosinophils, mast cells and basophils by high-affinity receptors, called FCεRI[21]. Upon mast cell de-granulation, vasoactive amines (e.g. histamine) are released which induce type I hypersensitivity or allergic reaction[22]. The physiological functions of IgE are protective in nature and include initiation of acute inflammation, recruitment of neutrophils and eosinophils to act against attacking parasitic worms as these cannot be removed by phagocytes[23].

Considering the importance of IL-13 and IgE, present study assessed the levels and correlation of IL-13 and IgE in bronchial asthma patients.

Subjects and methods

This case-control research assessed 50 asthmatics and 30 non-asthmatics (control group). The study was conducted by the immunology department, university of health sciences (UHS), Lahore, Pakistan after ethical clearance from UHS. Asthmatic patients between ages 18 to 40 years, from both genders with allergy history (checked by using modified questionnaire of European Community Respiratory Health Survey) were included in this study. These patients were selected from Gulab Devi Chest Hospital, Lahore. Patients suffering from diabetes and autoimmune disorders were excluded from the study. Serum level of IL-13 and IgE were determined by

ELISA (KomaBioTech, Korea). SPSS 20.0 software was used for statistical analysis. Kolmogorov-Smirnov, Shapiro-Wilk test, Student t-test and Mann-Whitney test have been used in analysis. The statistical significance of the p-value was set at $\leq$ 0.05. This IL-13 analysis is continuation of the work which focused on measuring total leukocyte count, its percentage, absolute eosinophil count, level of total serum IgE and frequency of HLA-DR B1*12 in these patients and healthy subjects. Figure 1 below presents the standard curve of IL-13 ELISA using ELISA reader's software version 2.2 (BioRad USA).
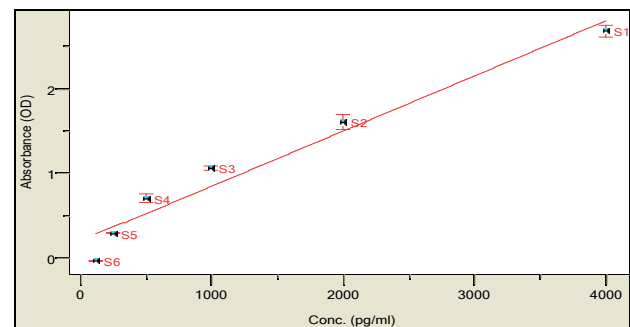

Figure 1: Standard Curve of IL-13 ELISA. Concentration values are plotted against x axis, absorbance values against y axis. S1 to S5 are standards and concentrations are 4000, 2000, 1000, 500, 250 and 125 pg/ml respectively.

Results

The mean concentration of IL-13 in asthmatics was higher than controls. Similarly median value of IL-13 concentration was high in asthma patients in comparison to controls and the discrepancy was significant statistically (p= 0.000). Table 1 below provides details of this analysis.

Table 1: Comparison of IL-13 Concentration (pg/ml) in asthmatics and healthy controls

Statistical measures	Asthmatics (n=50)	Controls (n=30)	p Value*
Mean ± SD	1574.6 ± 409	390.7 ± 23	0.000
Median (IQR)	1415.8 (1398.9-1554.8)	394.3 (384.3-404.3)	0.000
* Mann-Whitney Test, SD = Standard deviation, IQR = Inter quartile range.			

As mentioned earlier, this IL-13 measurement is part of the project in which there are other research activities on samples and data from same patients and healthy subjects. The demographic data, total leukocyte count, its percentage, absolute eosinophil count, level of total serum IgE and frequency of HLA-

DR *B1*12* have already been published[24]. Therefore, further analysis is performed on correlating these IL-13 related results with other parameters. The mean IL-13 concentration was higher in asthmatics with HLA-DRβ1*12 allele compared to asthmatics without this allele. However, mean concentration of IL-13 was high in controls with HLA-DRβ1*12 allele compared to control group without this allele and on comparison the difference was not significant statistically. Table 2 below presents the results.

Table 2: Comparison of IL-13 concentration (pg/ml) in individuals with and without HLA-DRβ1*12 positivity

Statistical measures	Asthma Patients (n=50)			Controls (n=30)		
	HLA-DR*β1*12* +ve (n = 21)	HLA-DR*β1*12* -ve (n = 29)	p Value*	HLA-DR*β1*12* +ve (n = 06)	HLA-DR*β1*12* -ve (n = 24)	p Value*
Mean ± SD	1624.5 ± 367	1538.4 ± 440.2	0.188	85.3 ± 26.2	292.1 ± 22.6	0.406
Median (IQR)	1430.4 (1402.7-1421.2)	1407.4 (1394.3-1483.5)		389.6 (365.4-404.3)	396.6 (386.2-404.3)	
* SD = Standard deviation, Mann-Whitney Test, IQR = Inter quartile range						

As published earlier, the asthma patients had statically significant high levels of total serum IgE compared to healthy individuals[24], therefore Spearman's correlation test was applied and a positive correlation was observed (r value 0.674 and *p*= 0.000) between IL-13 and IgE levels. In addition to this, asthma patients had increased percentage of peripheral blood eosinophils therefore second correlation was applied between serum IL-13 concentration and percentages of eosinophil, however it was not significant statistically, with r value 0.113 and *p*=0.574. Table 3 presents the results of these correlation studies.

Table 3: Correlations of serum IL-13 with total serum IgE and percentages of eosinophils in peripheral blood of asthma patients

Parameters	Statistical measures	
	r Value*	p Value*
IL-13 and IgE	0.674	0.000
IL-13 and eosinophil percentage	0.113	0.574
*Spearman's correlations, IL-13 = Interleukin 13, IgE = Immunoglobulin E		

Discussion

The current study has found an elevated serum levels of IL-13 in bronchial asthmatics with known allergy history compared to healthy individuals (*p*=0.000). Asthma is characterized by high levels of cytokines, particularly secreted by Th2 cells. Out of these Th2 cytokines, IL-13 is the one that mediate many important aspects of airway inflammation[19]. High level of IL-13 is responsible for increased IgE production[15] and eosinophil survival[25]. All patients of the current study had significantly increased total serum IgE and percentages of eosinophils in peripheral blood, as compared to controls[24]. Therefore, it is confirmed that total serum IgE is reciprocally in directly linked with the development of allergic disorders like asthma. This study also observed positive correlation of IL-13 with total serum IgE concentration (r= 0.674 and p= 0.000). There was a positive correlation of IL-13 with percentages and absolute count of eosinophils in peripheral blood of asthma patients but this correlation is not significant. This study is in accordance with Akiki*et al*'s findings of high serum level of IL-13 (along with other cytokines) in adult asthmatic patients (with documented sensitivity to a number of allergens) but they could not report correlation of serum IL-13 and total IgE[26]. An Indian study also reported significantly increased IL-13 level in pediatric allergic asthma patients with concomitant documented food allergy[27]. They also observed positive correlation of serum IL-13 and total IgE. However in the present study patient population was adult with known allergy (checked by modified questionnaire of European Community Respiratory Health Survey) and was diagnosed clinically. Some other studies have reported increased level of IL-13 and its correlation with total IgE in sputum specimens of asthmatics[28-30].

Allergic diseases like asthma are characterized by skewing of Th2 cell response[14]. Many factors have been blamed for this skewing such as major histocompatibility (MHC)[31]. MHC is another reason of increased level of IgE in asthma patients and many studies had documented this increased IgE

production due to inheritance of certain HLA class II alleles[32,33]. To see if increased IL-13 level in asthma patients is because of HLA-DR*B1*12*, odds ratio with 95% confidence interval was calculated using regression analysis, and it was observed that HLA-DR*B1*12* isn`t a risk factor for increased IL-13 level in asthmatic patients.

It is concluded that IL-13 concentration is higher in the serum of bronchial asthma patients with allergy history and there is positive correlation between serum levels of IL-13 with total serum IgE level.

Conclusion

We have detected significantly increased level of total serum IgE and IL-13 in bronchial asthma patients compared to healthy subjects included in the study.

We also found a positive correlation between these two parameters. These chemicals and cells involved in their production can serve as valuable targets for further research, diagnostic purposes as well as for treatment targets and monitoring.

Conflict of Interest: We have no conflicting interests to declare.

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