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Page: 31-36

Interleukin 16 (IL-16) in Asthma and Allergic Rhinitis. A Comparison between Upper and Lower Airways

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Asthma is a chronic inflammatory disease of bronchial mucosa, in which mast cells, eosinophils and activated T cells are of considerable importance. The increased chemotactic activity for T cells in patients with asthma is mainly attributable to IL-16. A strong association between asthma and allergic rhinitis exists from a clinical and epidemiologic standpoint of view. Although it is clear that the condition of the upper airways has impact on the lower airway physiology, the precise mechanisms underlying this relation are far from being resolved. This work was assessed the role of interleukin 16 (IL-16) in bronchoalveolar lavage (BAL) fluid in both diseases using quantitative sandwich enzyme immuno-assay, and the effect on ventilatory function. The results showed abnormally increased levels of IL-16 (294.4 ± 15.24 pg/ml), serum eosinophils with absolute count (510.0 ± 93.57, P>0.05), and total serum IgE (287.9 ± 61.22 IU/ml) using ELFA in patients of combined asthma and rhinitis, than in each of them alone. There was reduction in FEV_1 (forced expiratory volume in the first second) in the same group (81.6 ± 2.01%). There was a negative correlation between BAL IL-16 and FEV_1. In conclusion IL-16 may be considered as a marker of severity of airway inflammation.

Allergic rhinitis and bronchial asthma are the most frequently encountered abnormalities of nasal and the bronchial mucosa (Person et al., 1992). There is an increasing awareness that in many cases the nasal disease may precede the development of asthma and, hence, that patients with allergic rhinitis may exhibit early signs of asthma (Kapsali et al., 1997). Practicing physicians and clinical investigators have long noted a relation between allergic rhinitis and bronchial asthma. Dysfunction of the upper and lower airways frequently occurs together and appears to share key elements of pathogenesis.

Despite the strong clinical association between these diseases, it remains controversial whether rhinitis serves as a risk factor for the development of asthma and whether active nasal disease can affect lower airway symptoms and function (Corren, 1997). As research progresses, the exact nature of this relationship is becoming clearer. Many researchers are beginning to adhere to the precept of one airway, one disease, postulating that asthma and rhino sinusitis are merely upper and lower airway manifestation of mucosal inflammation (Grossman, 1997). Various studies report the prevalence of asthma in patients with chronic sinusitis as ranging between 40% and 75% (Slavin, et al., 1980). There have also been extensive attempts to characterize inflammatory mechanisms of both disorders.

Various studies have pointed to a variety of inflammatory mediators such as histamine, thromboxane, IL-3, IL-4 and specific attention has focused on the eosinophil, as playing a central in the manifestation of both diseases, because it has been shown to produce these mediators when activated. Activated cells such as eosinophils, mast cells, neutrophils have been shown to synthesize an important group of inflammatory mediators known as the cysteinyl leukotrienes (Arango and Kountakis, 2002). Although a variety of cytokines and chemokines including IL-1, IL-2, IL-8, RANTES, macrophage inflammatory protein-1α [M1P-1α], M1P–1B, monocyte chemotactic protein (MCP-1), MCP-2, MCP-3, and MCP- 4 are chemo-attractants for T cells, the T-cell cytokine IL-16 seems to be of particular interest (Center et al., 1996). Because IL-16 is a natural ligand of the CD_4 molecule, it selectively induces migration of CD_4^+ T cells and eosinophils. IL-16 also leads to an induction of IL-2 receptor and MHC class II molecules on CD_4^+ T cells and primes them for responsiveness to IL-2 (Taub et al., 1993). On this basis, IL-16 may play a crucial role in the allergic inflammation in patients with asthma and may also be found in the nasal sinus mucosa, providing an etiology for the manifestation of the chronic sinusitis.

IL-16 (previously known as lymphocyte chemo-attractive factor) is produced in airway mainly by CD_8^+ T cells. It is synthesized by both "T cells, eosinophils and non-immune Fibroblasts", and act as a potent chemo-attractant for various CD_4^+ cells such as T cells, monocytes and eosinophils. IL-16 plays a key role in the initiation and /or maintenance of an inflammatory response (Center et al., 1996).

In this study we assessed the level of IL-16 in bronchoalveolar lavage, blood eosinophils and total serum IgE in bronchial asthma with or without allergic rhinitis.

Subjects and Methods

This study included 30 patients (19 male and 11 female), whose age ranged from 18 – 42 years old (mean 29.5 ± 9.33) they were from Al-Zahraa University Hospital, and all of them were non smoker. They were divided into three groups: -

Group 1 asthmatic group, included 10 patients.

Group 2 included 10 patients suffering from allergic rhinitis.

Group 3 included 10 patients suffering from combined asthma and allergic rhinitis.

All subjects gave their written consent after being fully informed about the purpose and nature of the studies, which were approved by the Ethics Committee of Al-Zahraa University Hospital.

All patients were subjected to the following:

- Full medical history and clinical examination including general local chest and ENT examination. Patients with bronchial asthma were defined according to the criteria of the American Thoracic Society 1987. They all have history of recurrent attacks of dyspnea, wheeze and cough more than 15% reversibility in FEV_1. The diagnosis of allergic rhinitis was based on the patient's history, CT scan appearance, and physical examination. Nasal endoscopy was carried out on all patients and symptoms were recorded for stuffy nose (nasal obstruction), rhinorhea, sneezing, wheezing and coexisting symptoms such as watering of the eyes cough ear block, sore throat and postnasal drip were noted. Attention was placed on the presence of associated bronchial asthma in these patients.
- Lung function test using spirometry, with special attention to FEV_1. Ventilatory lung function (FEV_1 % predicted) was measured with a Vitalograph Alpha (Vitalograph Ltd, Buckingham, UK) in a standardized manner (Quanjer, 1983)
- Laboratory investigation including, liver function, kidney function, and blood sugar. Patients with diabetes, liver, or kidney disease were excluded.
- Fiberoptic bronchoscopy and BAL. Broncho-alveolar lavage (BAL) was performed using Fiberoptic bronchoscopy (Olympus). Patients were premedicated with atropine. Xylocaine was used for topical anesthesia, the tip of the bronchoscope was wedged in to the medial segment of the middle lobe (for better fluid recovery), and then 20 ml saline were injected and pulled back by negative suction pressure. The recovered fluid was centrifuged for 15 minutes at 4 ^{o} C and stored frozen at -70^o C for assay of IL-16, using the ELISA technique.
- Absolute count of blood eosinophils, and estimation of total serum IgE. Eosinophils were purified from peripheral blood obtained from patients as described previously (Ide Weiler et al., 1994).

Immunological Studies

1- Total serum IgE was assessed by automated quantitative Enzyme Linked Fluorescent Assay technique (ELFA), using the assay principle with the indirect immunoassay method with final fluorescent detection. The reaction steps were performed using the VIDAS apparatus "Bio Merieux France". Quantification of serum IgE were determined according to the manufacturer's instructions. The minimum detectable concentration of IgE by the kit was 5.0 IU/ml. Twenty µl of serum was pipetted in each well, in duplicate. The optical density was read in a microplate reader (BIO-RAD 550) at 450 nm. The IgE values in the samples were calculated using a standard plot and expressed as IU/ml of serum.

2- IL-16 assay: BAL IL-16 was measured with commercially available quantitative sandwich enzyme immuno-assay kit (Bio Source International Inc, Camarillo, USA). The assay was performed according to the manufacturer's instructions with the reagents provided. Results were expressed in pg/ ml relative to a set of standard IL- 16 protein provided with the test kit. Quantitation of IL-16 protein in the BAL fluid was accomplished by ELISA as previously described by (Cruikshank et al, 1995). Briefly, anti-IL-16 monoclonal antibody was coated directly onto 96-well ELISA plates at a concentration of 1 µg/ml in coating buffer (0.1 M sodium bicarbonate, pH 8.8) and incubated overnight at 4° C. To eliminate nonspecific binding by the primary antibody, the plates were blocked with 300 µl of blocking buffer (phosphate-buffered saline [PBS] containing 10% fetal bovine serum [FBS] and 0.05% NaN_3) for 2 h at ambient temperature. The plates were then washed twice with PBS Tween (PBS containing 0.05% Tween 20). A standard curve was generated using serial dilutions of recombinant interleukin 16 (rIL-16). BAL fluid samples (100 µl) were incubated in duplicate in the 96-well plates (Nunc, Naperville, IL) at 37°C for 1h. Following the 1-h incubation, the protein was removed and the wells were washed with PBS containing 0.1% Tween 20. Nonspecific binding was reduced by blocking with 1% bovine serum albumin for 1 h. After washing, 100 l of rabbit polyclonal anti-IL-16 antibody (10 µg/ml) diluted in PBS containing 0.05% Tween 20 was added to each well. The presence of IL-16 was then detected by incubating for 1 h with biotinylated goat anti-rabbit IgG diluted 1:500 in PBS. The lower limit of detection for the ELISA is routinely 10-15 pg/ml. Linearity was in the range of 12-500 pg/ml (coefficient of 0.993 in that range).

Statistical Analysis

Data were expressed as mean ±SEM. The significance difference between groups was calculated using two tailed student's t-test. Pearson's with correlation coefficient between BAL IL- 16 and FEV_1. P values < 0.05 were considered statistically significant.

Results

In this study the levels of eosinophils was elevated in the three groups of patients, but significantly higher in group 3 of asthma and allergic rhinitis than in asthmatic patients alone (group 1) and rhinitis patients alone (group 2) (Table 1).

Asthmatic patients had higher IgE levels than patients with pure rhinitis. Serum IgE were significantly elevated in rhinitis patients with asthma (group 3) in relation to both groups1, 2 (Table 1).

There was highly significant increase in the BAL IL-16 in rhinitis patients with asthma when compared to asthmatic patients alone, and rhinitis alone (Table 1) (Figure 2). Also there was negative correlation between BAL IL 16 and FEV_1 among the three groups (Figure 1a, b, and c). FEV_1 was significantly reduced in patients with asthma and rhinitis (group 3) than the other groups.

Table 1. Comparison between bronchoalveolar lavage (BAL) IL-16 and serum eosinophils, IgE, and forced expiratory volume in first second (FEV_1) in patients with asthma, allergic rhinitis and combined asthma and rhinitis.

Parameter		Mean ± SEM	P value
BAL IL-16 (pg/ ml)	(group 1)	258.1 ± 16.37	
	(group 2)	201.5 ± 14.46	<0.05
	(group 3)	294.4 ± 15.24	NS
Eosinophils (Absolute no.)	(group 1)	342.0 ± 96.31	
	(group 2)	171.1± 29.07	NS
	(group 3)	510.0 ± 93.57	<0.05
IgE (IU/ml)	(group 1)	249.1 ± 12.5	
	(group 2)	127.7 ± 10.42	<0.05
	(group 3)	287.9 ± 61.22	NS
FEV_1 (% predicted)	(group 1)	88.5 ± 3.14	
	(group 2)	102.3 ± 1.28	<0.01
	(group 3)	81.6 ± 2.01	NS

Group 1= asthma; Group 2= allergic rhinitis; Group 3= asthma plus allergic rhinitis; P<0.05=significant; NS= non significant

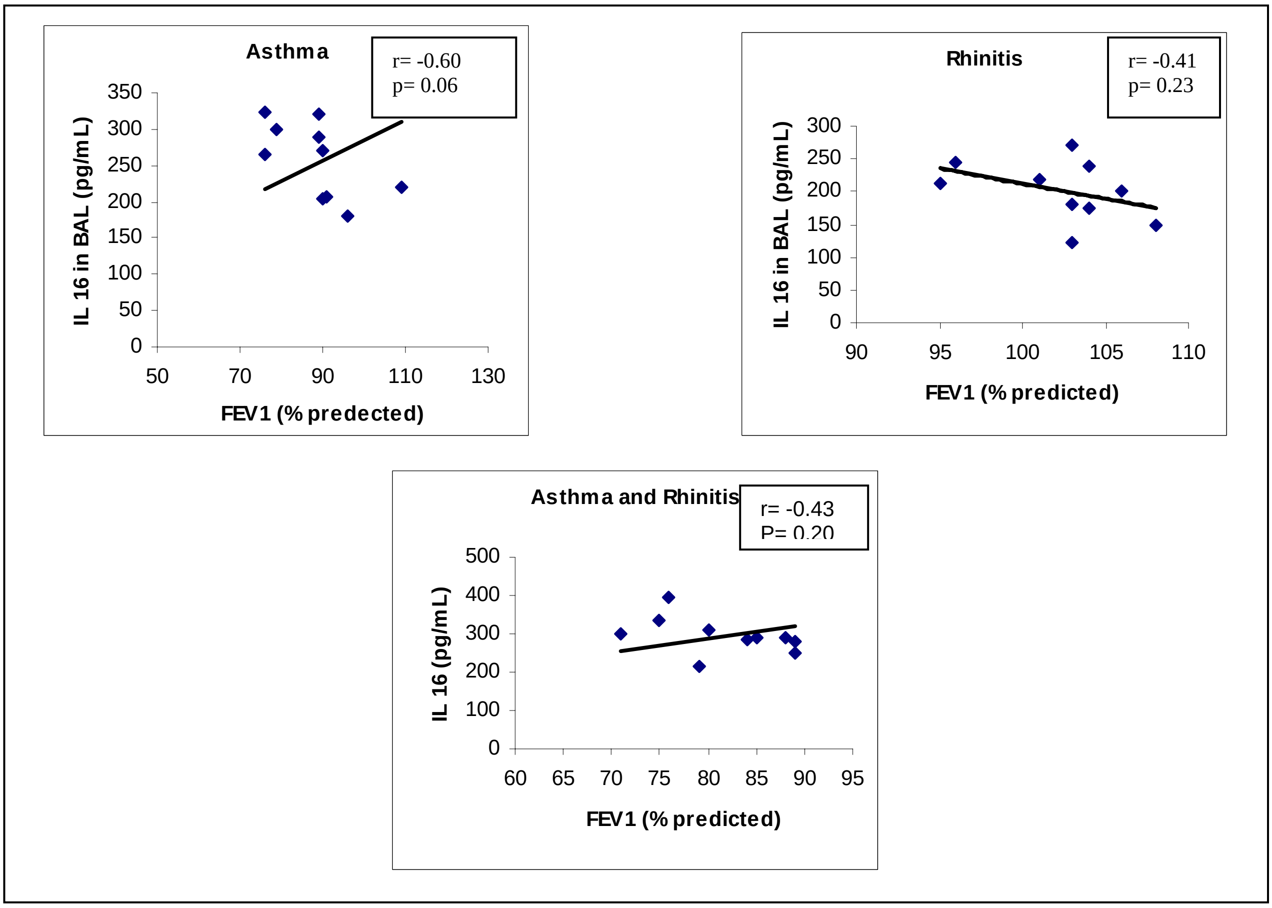

Figure 1. Correlation coefficient data between IL-16 and FEV1 among patients with asthma, rhinitis and asthma plus rhinitis.

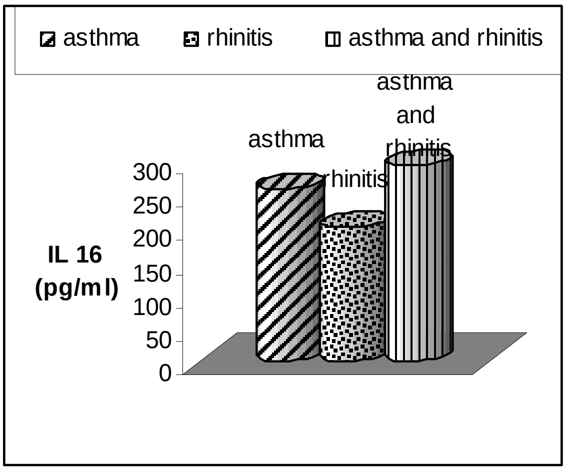

Figure 2. Bronchoalveolar lavage (BAL) IL- 16 level in patients with asthma, rhinitis and asthma plus rhinitis.

Discussion

In this study elevation in the absolute blood count of eosinophils was found in the three groups of patients. This elevation was statistically significant in cases of combined asthma and rhinitis when compared to asthma or rhinitis cases. These findings agree with Staikuniene and Sakalauskas, 2003, whom founds that there was no statistical deference between asthmatics and patients with allergic rhinitis in blood and nasal eosinophil count, while patients with combined rhinitis and asthma had higher levels of serum 1gE. They also noted that patients with combined allergic rhinitis and asthma had higher serum IgE and eosinophils than patients with allergic rhinitis alone.

In this study, we found statistically significant elevation of IgE in asthma patients while there was none statistically significant elevation with combined disease asthma and rhinitis than in each of them alone. These findings were compatible with Alvarez et al., 2003, who found that IgE is an important mediator of allergic airway disease including asthma and rhinitis. Asthma patients had higher total and specific IgE levels than in rhinitis patients. On the other hand, Marcuucci, et al., 2001, found that both specific IgE and eosinophils in the nasal mucosa showed a better correlation with allergen exposure than serum evaluations. An important feature of asthma is an exaggerated bronchoconstrictor response to a wide variety of stimuli. Research indicates that airway hyperresponsiveness is important in the pathogenesis of asthma and that the level of airway responsiveness usually correlates with the clinical severity of asthma (Sugita et al., 2003). Gonzalez, et al., 2003, found non-specific bronchial hyper-responsiveness is present in patients with allergic rhinitis, and that the presence of nasal eosinophils is a persistent inflammation parameter suggesting the allergic origin in these patients.

In a study done by Malakauskas, and Bajoriuniene, on 2003, they found no significant differences in bronchial hyperresponsiveness between asthma patients with or without allergic rhinitis. They also found that eosinophil count in nasal secretion did not correlate with non-specific bronchial hyperresponsiveness. These results agree with the results of our study, we found non significant reduction in FEV_1 between asthma patients with rhinitis when compared to asthma patients, but the reduction is significant between asthma patients and patients with rhinitis alone.

Although mast cells and eosinophils are the effector cells inducing acute broncho-constriction and chronic epithelial damage by the release of mediators. The bronchial inflammation in bronchial asthma is characterized by the presence of $CD4^+$ T cells which orchestrate the inflammatory possess by releasing an array of cytokines (American Thoracic Society, 1987). A characteristic feature of inflammation in allergic rhinitis is the local accumulation of inflammatory cells, including $CD4^+$ T lymphocytes, eosinophils, basophils, and neutrophils. Because IL16 is a cytokine that uses the $CD4^+$ molecule as its receptor leading to chemotactic responses and activation of $CD4^+$ T cells it could play an important role in recruitment and activation of $CD4^+$ cells. Krug, et al in 2000 found that the IL-16 concentrations in BAL fluid were significantly elevated in patients with asthma after allergen challenge (median, 97 pg/ml; range, 38-362 pg/ml).

In the present study we found statistically significant elevation in BAL IL-16 in asthmatic patients and in patients with combined disease asthma and rhinitis, while there was statistically insignificant elevation in BAL IL-16 in rhinitis patient when compared to asthmatics with or without rhinitis. There was also negative correlation between BAL IL-16 and FEV_1 among the three groups of patients. This could be explained by Krug, et al, in 2000 who stated that, the importance of the airway inflammatory response to airway hyper-responsiveness is substantiated by several observations. First, airway markers of inflammation correlate with bronchial hyperresponsiveness. Second, treatment of asthma and modification of airway inflammatory markers not only reduce symptoms but also diminish airway responsiveness.

It is concluded that IL-16 could serve as a useful marker of disease activity in cases of asthma plus allergic rhinitis.

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