

Original Article

Serum Levels of IL-17A Increase in Asthma But Don't Correlate with Serum Level of IgE and Asthma Severity

Masouma Mowahedi¹ M.Sc., Mohammad Samet² M.D., Fateme Zare³ M.Sc., Morteza Samadi^{3,4*} Ph.D.¹Department of Immunology, International Campus, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.²Department of Pulmonology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.³Department of Immunology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.⁴Reproductive Immunology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

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Background and Aims: Recent evidence suggests that T helper 17 (Th17) cells are involved in the emergence of asthma. Th17 cells have a key role in inducing inflammation in asthmatic airways. Thus interleukin (IL)-17A, the main cytokine of Th17, contributes to airways inflammation.

Materials and Methods: We evaluated the level of IL-17A and total immunoglobulin E (IgE) by ELISA method in sera of 100 asthmatic patients and 81 healthy controls, to determine if serum concentration of IL-17A is associated with asthma severity. We classified patients into three groups; mild (n=28), moderate (n=33) and severe asthma (n=39).

Results: Serum IL-17A and IgE concentrations were significantly higher in the asthmatic patients than the control group ($p=0.026$ and $p<0.01$, respectively). Mean serum IL-17A and IgE values were 37.73 pg/ml and 39.02 IU/ml in the control group, but 68.55 pg/ml and 295.87 IU/ml in the patients group, respectively. Nevertheless, there were no significant differences between the three groups of asthmatic patients. Mean serum IL-17A and IgE values were 94.17 pg/ml and 255.07 IU/ml in the mild group, 71.29 pg/ml and 271.27 IU/ml in the moderate group, and 47.85 pg/ml and 345.97 IU/ml in the severe group, respectively. Moreover, there was no correlation between serum levels of IL-17A and IgE.

Conclusion: It was found that IL-17A, like IgE, rises in sera of asthmatic patients though in a different manner. IgE increases in serum consistent with disease severity though the increase of IL-17A in serum has an inverse relationship with IgE elevation.

* Corresponding Author: Department of Immunology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. **Tel:** +989126901505, **E-mail address:** samadi.for@gmail.com

Introduction

Asthma is a heterogeneous disease that is characterized by chronic inflammation in the airways, wheezing, cough and shortness of breath. Reversible airway obstruction and remodeling in airway may occur as well [1]. Asthma is thought to be a Th2 lymphocyte inflammatory disease, with infiltration of eosinophils, Th2-associated cytokine production and airway hyper-responsiveness (AHR). However, recent data suggest that asthma cannot be taken only as an allergic inflammatory disorder. The clinical heterogeneity of asthma might reflect the contribution of other T cell subsets. Asthma is more than just a Th2 type disease [1, 2] and Th17 may also be involved in the pathogenesis of allergic asthma. An increase in Th17 cells and more production of Th17 cytokines in allergic asthmatics suggests that Th17 immunity is possibly involved in the systemic immune responses of allergic asthma [3]. There are some clinical data showing that Th17 inflammation has a major role in those with more severe asthma and neutrophilic inflammation, including steroid-resistant patients [4]. The main cytokine of Th17 cells is IL-17 which has an important role in providing protection against infection and in inducing and maintaining chronic inflammatory diseases [5]. The IL-17 family of cytokines consists of IL-17A and IL-17F [1]. Recent data indicate that IL-17A expression in bronchial submucosa is mildly to moderately increased [6] and IL-17A produced by Th17 cells may contribute to pathogenesis

of asthma [7]. In this study, we compared the serum concentrations of IL-17A and IgE first in asthmatic and healthy groups and then in three distinct groups of mild, moderate and severe asthmatics to investigate the presence of any relationship between IL-17A, IgE and severity of asthma.

Materials and Methods

Subjects

One hundred patients aged 11-77 years (51 males and 49 females) who had referred to Khatam-olania Clinic (affiliated with Shahid Sadoughi University of Medical Sciences, Yazd, Iran) suffering from asthma from September 2013 to March 2014, were included in the study. All patients were visited by a specialist, and their asthma was confirmed. Clinical history and spirometry test were performed for all the subjects. Then they were categorized in three distinct levels of mild, moderate and severe asthma according to the current Global Initiative for Asthma (GINA) guidelines [8]. Eighty one healthy sex-matched subjects, aged 13-75 years (40 males and 41 females) as non-asthmatic and non-allergic persons were considered as the control group. All subjects including cases, controls and the parents of children gave signed informed consent prior to sampling. The study was approved by the ethics committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran. Whole blood samples were obtained from all of the subjects. The serum was separated by centrifugation. Serum

samples were isolated immediately and stored at -80°C until analysis.

IL-17 and IgE Assay

Serum levels of IL-17A and IgE were measured by two commercial ELISA assay kits (MABTECH AB, Sweden, Cat No. 3520-1H-6 and Monobind Inc. USA, Cat No. 2525-300, respectively) according to the manufacturer's instructions. The optical density was read by Stat Fax 3200 microplate reader (Awareness, USA). The IL-17A and IgE ELISA test systems have sensitivity of 0.01 pg/ml and 0.125 IU/ml, respectively.

Statistical Analysis

Statistical analysis was performed by SPSS software (version 16), using student's t-test or one-way ANOVA. P values less than 0.05 were considered as indicator of statistically-significant difference. Correlations were assessed by Pearson (r) correlation coefficients. No corrections were made for multiple comparisons.

Results

To identify whether IL-17A correlates with IgE and how IL-17A rises with the severity of disease in asthmatic patients, 100 patients with

an established diagnosis of asthma who were receiving asthma therapy as well as 81 healthy subjects were selected. The analysis of results showed a significant difference between the content of IL-17A and IgE in the group of asthmatic patients compared to healthy control group ($p=0.026$ and $p<0.01$, respectively) (Table 1, Fig 1.A and 1.B). Moreover, there was non-significant difference between the content of IL-17A and IgE in three groups of asthmatic patients conversely or in accordance with severity of disease ($p=0.345$ and $p=0.549$, respectively) (Table 2 and 3 and Fig 2.C and 1.D).

Assessment of serum IL-17A and IgE content in the asthmatic group and control group indicated a significant increase in serum levels of them in asthmatic patients compared to the other group ($p=0.026$ and $p<0.01$, respectively) (Table 1, Fig 3.A and 1.B). Mean of serum IL-17A values were 37.73 pg/ml (min=9.5, max=309.17) in the control group but 68.55 pg/ml (min=16.08, max=1180.79) in the patients. Also, mean of serum IgE values was 39.02 IU/ml (min=1, max=163) in the control group but 295.87 IU/ml (min=5, max=1674) in the asthmatics.

Table 1. The content of serum IL-17A and IgE in asthmatic and healthy group

| | Case/ Control | Number | Mean | Std. Deviation | Minimum | Maximum | P value |
|-----------------------|---------------|--------|--------|----------------|---------|---------|---------|
| IL-17A (pg/ml) | Case | 100 | 68.55 | 128.26 | 16.08 | 1180.79 | 0.026 |
| | Control | 81 | 37.73 | 41.95 | 9.5 | 309.17 | |
| | Total | 181 | | | 9.5 | 1180.79 | |
| IgE (IU/ml) | Case | 100 | 295.87 | 367.890 | 5 | 1674 | < 00.1 |
| | Control | 53 | 39.02 | 41.540 | 1 | 163 | |
| | Total | 153 | | | 1 | 1674 | |

Assessment of serum IL-17A content in the three studied groups of asthmatic patients indicated a non-significant decrease in the serum levels of patients conversely correlated with asthma severity ($p=0.345$; Table 2 and

Fig 1.D). Mean of serum IL-17A value was 94.17 pg/ml in the group of patients with mild disease, 71.29 pg/ml in those patients with moderately-severe disease, and 47.85 pg/ml in those with a severe disease.

Table 2. The content of serum IL-17A in three groups of asthmatic patients

| Asthmatic patients | | Number | Mean | Std. Deviation | P value |
|--------------------|----------|--------|-------|----------------|---------|
| IL-17A (pg/ml) | Mild | 28 | 94.17 | 216.66 | 0.345 |
| | Moderate | 33 | 71.29 | 88.45 | |
| | Severe | 39 | 47.85 | 44.59 | |
| | Total | 100 | 68.55 | 128.26 | |

Assessment of serum IgE content in the three studied groups of asthmatic patients indicated a non-significant increase in serum levels of patients on the basis of the severity of asthma ($p=0.549$; Table 3 and Fig 1.C). Mean of

serum IgE value was 255.07 IU/ml in the patients with mild disease, 271.27 IU/ml in the group with moderate asthma, and 345.97 IU/ml in the group with severe disease.

Table 3. The content of serum total IgE in three groups of asthmatic patients

| Asthma severity | | Number | Mean | Std. Deviation | P value |
|-----------------|----------|--------|--------|----------------|---------|
| IgE (IU/ml) | Mild | 28 | 255.07 | 328.99 | 0.549 |
| | Moderate | 33 | 271.27 | 305.62 | |
| | Severe | 39 | 345.97 | 438.99 | |
| | Total | 100 | 295.87 | 367.89 | |

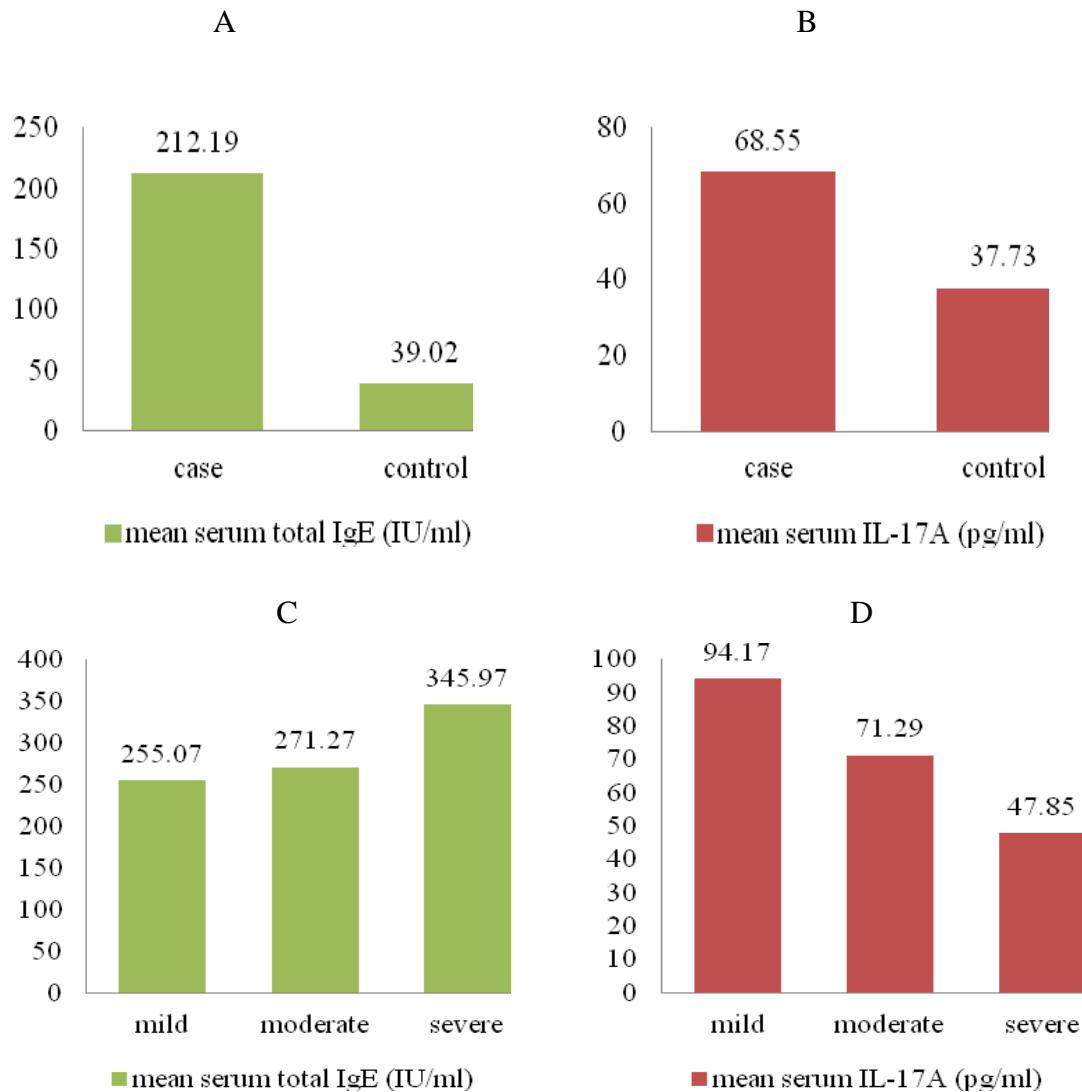


Fig. 1. The concentration of serum IL-17A and total IgE in asthmatic patients and healthy group, with further categorization of three groups of asthmatic patients

Finally, we could not find any relationship between the serum levels of IL-17A and IgE in the patients as well as the healthy group and also in each group of patients with mild, moderate or severe asthma.

Discussion

The importance of Th2 in asthma has lead to classifying asthma into two main subclasses: Th2-high and Th2-low. In Th2-high asthma, amplification in expression of Th2 cytokines

can lead to symptoms of asthma such as airway eosinophilia and bronchial hyperresponsiveness. The increase in serum IgE and development and activation of Th2 cells, eosinophils, and mast cells, are the main features of atopic asthma [9]. It has been accepted that atopic asthma is associated with activation of Th2 cells in the airways and augmented expression of Th2 cytokines that could lead to symptoms of asthma such as airway eosinophilia and bronchial hyper

responsiveness. In other words, the Th2 hypothesis of asthma is accepted, therefore allergen-specific Th2 cells drive many of the immunopathologic features of asthma [10] while non-atopic asthma is characterized by the accumulation of neutrophils and mast cells without elevated serum IgE. However, there are some patients with both eosinophilic and neutrophilic inflammation [11]. On the other hand, expression of Th2 cytokines is also enhanced in patients with non-atopic asthma [10]. Th17 cells are new members of T helper family that produce IL-17A and IL-17F. IL-17A has a key role in allergic responses especially in allergic airway inflammation. IL-17A can initiate inflammatory processes via stimulating production of various proinflammatory cytokines and chemokines, followed by recruiting neutrophils, enhanced antibody production, and activation of T cell subsets [11, 12]. The most reviewed function of IL-17A is to drive inflammation through induction of macrophage and neutrophil chemokines and growth factors such as CXCL2 (MIP-2), CXCL8 (IL-8), CCL-2 (MCP-1), granulocyte colony stimulating factor and granulocyte-monocyte colony stimulating factor so that increased production of IL-17A has been indicated in asthma [4]. The production of IL-17 is increased in peripheral blood mononuclear cells of asthmatic patients. In sputum, it has been shown to correlate with AHR [12]. Previous studies suggest that IL-17A participates in the pathogenesis of non-atopic asthma rather than that of atopic asthma [11]. In line with our results, the content of Th2 and Th17 cells -

related cytokines were higher in allergic asthmatics than healthy controls, though some patients were treated with inhaled glucocorticoid [3].

As indicated before, allergen exposure leads to development of T cell subsets in the airways including Th2 and Th17; however Th2 is prominent. Although Th2 is the key player in allergic inflammation nevertheless Th17 has a crucial role in this process [13]. In mice, it is shown that Th17 cells augment Th2 cell-mediated eosinophil recruitment into the airways, but it is unlikely that IL-17A is important in this process [14]. Mild and moderate asthmatic patients show eosinophilic inflammation, while severe asthmatic patients bear neutrophilic or both neutrophilic and eosinophilic inflammation [11]. As a result, recent studies have revealed the occurrence of neutrophilic inflammation in addition to eosinophilic inflammation in asthmatic airways. Therefore, neutrophilic airway inflammation is recognized as one feature of allergic asthma. Our observations in this study are in agreement with most of the previous studies which have determined that IL-17A participates in pathophysiology of allergic asthma. On the other hand, our data are in accordance with those of another study (Doe et al. 2010) which reported that IL-17A expression in the asthmatic airways increases in mild to moderate rather severe case. Also consistent with our result, in mice, deficiency of IL-4R is found to signal a marked increase in IL-17 concentration with inhibited eosinophil recruitment. It can be concluded that endogenous IL-17 is controlled by IL-4

[15]. This can be the answer to the question of why IL-17A increased simultaneously with IgE reduction in our study.

Th17 cells participate in allergic inflammations such as asthma [16]. In addition to predominant Th2 immunity, abnormal Th17 immunity may also be involved in the pathogenesis of allergic asthma [3]. Even though IL-17A and Th17 cells stimulate structural cells of the airways to produce cytokines essential to airways inflammation and in-vitro remodeling, deficiency of IL-17R signaling does not significantly reduce the development of airway inflammation in a murine model of mild chronic asthma [17]. As mentioned before, asthma can be divided into at least two distinct molecular phenotypes defined by degrees of Th2 inflammation. These data reveal that a significant percentage of patients with asthma have a Th2-low phenotype that manifests clinical features of asthma, airway obstruction, airway hyper-responsiveness and bronchodilator reversibility despite a paucity of Th2-driven inflammation. The causes of Th2-low asthma remain obscure, but possibilities include neutrophilic inflammation, IL-17-driven inflammation, intrinsic defects in barrier function, chronic subclinical infection by viruses, and atypical intracellular bacteria [18]. A recent analysis of bronchoalveolar lavage (BAL) T cells indicated 3 distinct subgroups of asthma: Th2 predominant, Th2/Th17 predominant and Th2/Th17 low; which still confirms the Th2 hypothesis (Th2 high and Th2 low subgroups of asthma). Moreover, Irvin et al. believe in Th2/Th17 predominance

in which there is a higher level of IL-4 production by the Th2/Th17 cells. There is a subgroup of Th2 predominant patients in which BAL Th2 cells express IL-17, too. Thus, a typical patient with a Th2 predominant endotype at the time of recurrent respiratory tract infections trends toward a Th2/Th17 predominant endotype. The severity of asthma in this subgroup can be due to increased IL-4 production. On the other hand, IL-17 production by Th2/Th17 cells is likely to change the quality of airway inflammation. Finally, Irvin et al. concluded that there is an increased frequency of dual-positive Th2/Th17 cells in BAL fluid from asthmatic patients which is associated with features of severe asthma (heightened airway hyper-reactivity and airway obstruction) [19].

Most of the previous studies indicate that Th17 cells contribute to neutrophilic inflammation in severe cases of asthma. However our findings are one of the first studies, to our knowledge, to show that Th17 is more important in mild and moderate than severe asthma. Because we performed this study to evaluate only the serum level of IL-17A and IgE, we could not determine cellular sources of IL-17A. Also, because we evaluated the serum level of IL-17A and IgE without any estimation of the amount of Th17 and Th2 in peripheral blood, sputum or BAL, we could not determine any correlation with these cells. Perhaps we can suggest that Th2 cells are normally in balance with Th17 cells in the healthy people. In inflammatory disorder such as asthma (regardless of disease subtype) when the balance tends to Th2 cells, then Th2 type

cytokines are higher than Th17 cytokines. Subsequently, more production of IL-4, IL-5 and IL-13 is plausible which is followed by IgE class switching, eosinophilic inflammation and other features of allergic asthma. Therefore allergic properties are dominant. In contrast, when Th17 cells are dominant, the levels of IL-17A, IL-17F and IL-22 increase more than Th2 type cytokines; neutrophilic inflammation is therefore indicated in addition to eosinophilic inflammation.

Class switching to IgE requires IL-4 and IL-5 as the main cytokines of Th2 cells. The importance of Th2 in asthma leads to classifying asthma into two main subclasses: Th2-high and Th2-low. In Th2-high asthma amplification in expression of Th2 cytokines can lead to symptoms of allergic asthma while in Th2-low asthma, the accumulation of neutrophils and mast cells without elevated serum IgE takes place. Based on the results of this study, we believe that both IL-17A and IgE can contribute to allergic asthma and the serum level of one of them may be in balance with the other. It was somewhat surprising to us to find that IL-17A level decreases in the serum with the increases of serum level of IgE. So in severe asthma, the ratio of Th2 and Th17 main cytokines (IL-4/IL-17A) must presumably be higher than that of moderate and mild asthma. This ratio decreases on the basis of reduction of asthma severity. By the same token, the ratio of IL-17A/IL-4 in mild asthma must presumably be higher than that of moderate and severe asthma, and this ratio decreases parallel to asthma severity. This was

confirmed by the predominance of Th2 immunity response in the pathogenesis of allergic asthma. At the end, the elevated serum level of IL-17A is important in immunopathogenesis of allergic asthma and the Th17 immunity can, in addition to Th2 immunity, contribute to asthma occurrence. Moreover, in order to understand more about how Th2, Th17 and other cells may participate in allergic asthma, elucidation of pathogenesis is required. Future studies should investigate a larger number of the population, different classifications of asthma and also further experiments on cellular mechanisms.

Conclusion

We found that IL-17A, like IgE, rises in sera of asthmatic patients though in a different manner. IgE increases in serum parallel to disease severity, though an increase of IL-17A in serum is in inverse relationship with IgE rise. It can be concluded that both have a crucial role in the pathogenesis of asthma regardless of subgroups of the disease.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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