

Serum Levels of Total IgE, IL-12, IL-13 and IL-18 in Children Patients with Asthma.

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Abstract

T-cell activation and alteration of cytokine levels are involved in the pathogenesis of asthma. However, the profile of circulating T-lymphocyte subsets and related cytokines during asthmatic attacks is still unclear. We compared the serum concentrations of proinflammatory cytokines Interleukine-18(IL-18) and Interleukine-12(IL-12), T-helper 2 (Th2) cytokine Interleukine-13(IL-13) and Immunoglobuline-E (IgE) in 27 asthmatic children and 21 sex and age matched healthy control subjects. Serum cytokines and IgE concentrations were measured by enzyme-linked immunosorbent assay. Serum IL-13 , IL-18 and IgE concentrations were significantly higher in asthmatic patients than normal control subjects (IL-13: median 9.73 versus 4.43 pg/ml, $P<0.05$; IL-18: 76.81 versus 35.41 pg/ml, $P<0.05$; IgE: 225.44 versus 37.94 IU/ml, $P<0.05$. Asthmatic patients showed a decreased serum IL-12 concentration 93.57 versus 122.83 pg/ml, $P<0.05$. In conclusion, it was suggested that IL-13 and IL-18 might have a potential role in controlling lymphocyte responses in asthmatic patients specially in children through an IgE-mediated pathway.

Key words: Asthma, Interleukine-12, -13, -18, Immunoglobuline-E.

المستوى المصلي لكل من IgE الكلي والحركيات المناعية IL-12,IL-13,IL-18 لدى الاطفال المصابين بالربو

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الخلاصة

إن تحفيز الخلايا للمفاوية (T-cells) وتغاير مستويات الحركيات المناعية (السايتوكينات) لها دور في امراضية الربو . عموما ، إن انواع الخلايا للمفاوية (T-lymphocyte subsets) الموجودة في الدم والسايتوكينات التي تنتجها خلال هجمات الربو ما زالت غير واضحة. في هذه الدراسة تم مقارنة التراكيز المصلية للسايتوكينات التالية : الانترلوكين-18 (IL-18) والانترلوكين-12 (IL-12) وكذلك الانترلوكين-13 (IL-13) الذي ينتج من الخلايا للمفاوية (T-helper 2) بالاضافة الى قياس التركيز المصلي للكولوبولين المناعي- E (IgE) لدى 27 طفل مصاب بالربو و 21 طفل صحي كمجموعة سيطرة . تم قياس تراكيز الانترلوكينات والاميونوكلوبولين المناعي- E بواسطة اختبار الامتصاص المناعي المرتبط بالانزيم (ELISA). أظهر تركيز كل من IL-13 و IL-18 و IgE إزدادا معنويا لدى الاطفال المصابين بالربو مقارنة بمجموعة السيطرة (IL-13 : المتوسط 9.73pg/ml مقابل 4.43 pg/ml ; IL-18 : المتوسط 76.81 pg/ml مقابل 35.41 pg/ml ; IgE : المتوسط 226.44 IU/ml مقابل 37.94 IU/ml) ($P<0.05$)، في حين أظهر IL-12 إنخفاضا غير معنوي في تركيزه لدى الاطفال المصابين بالربو مقارنة بمجموعة السيطرة (IL-12 : المتوسط 93.57 pg/ml مقابل 122.83 pg/ml)، لذلك فإنه يعتقد ان IL-13 و IL-18 لهما دور فعال في السيطرة على استجابة الخلايا للمفاوية لدى مرضى الربو وخاصة الاطفال منهم وبطريقة يشترك خلالها IgE .

الكلمات المفتاحية: الربو ، الانترلوكين - 12 ، - 13 ، - 18 ، والاميونوكلوبولين المناعي- E.

Introduction

Asthma is a common respiratory disorder worldwide, it is a heterogenous disease, in which genetic plus environmental factors may contribute to its initiation and continuance⁽¹⁾. Allergic asthmatic patients generally develop the disease early in life , usually in infancy or childhood. The attack usually occur upon

exposure to allergens, the total serum Immunoglobuline-E(IgE) concentration is frequently elevated but sometimes remain normal⁽²⁾. Allergen-induced IgE synthesis can trigger eosinophils, basophils and mast cells to release cytokines for the differentiation of T-helper 2 (Th2) cells to secrete Interleukine-4 (IL-4)

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, Interleukine-5 (IL-5), Interleukine-10 (IL-10) and Interleukine-13 (IL-13) as well as the release of proinflammatory, vasoactive and fibrogenic factors (histamine, peptide leukotriens, platelet activating factor, tryptase, ect.) that are responsible for symptoms of asthma⁽³⁾.

Allergic airway diseases are associated with skewed Th2 cytokine production although the underlying cause of this aberrant immune response is not well understood⁽⁴⁾. Interleukine-12 (IL-12) is a critical determinant of T-helper1 (Th1) mediated immune responses, as it has been shown that deficiency in this cytokine can lead to Th2-polarized immune responses⁽⁵⁾. In peripheral lymphocytes of the Th1 type, IL-12 induces the synthesis of Interferon- γ (IFN- γ), Interleukine-2 (IL-2) and Tumor necrotic factor (TNF), the production of IL-12, TNF and IFN- γ is inhibited by IL-10⁽⁶⁾. IL-12 is also involved in the selection of immunoglobulin isotypes, it markedly inhibits the synthesis of IgE by peripheral blood mononuclear cells stimulated with IL-4⁽⁷⁾.

Interleukine-13 is an immunoregulatory cytokine generated predominantly by activated Th2 cells and it shows functional properties with IL-4⁽⁸⁾. IL-13 shares a receptor component signaling pathway and many biological activities with IL-4. In fact, IL-13 is also an anti-inflammatory cytokine that plays a unique role in the optimal induction and maintenance of IgE production and IgE-mediated allergic responses when IL-4 production is low or absent^(9,10). IL-13 has been recognized as a key cytokine mediating allergic airway inflammation and airway remodeling in asthma which is characterized by mucous hypersecretion, airway hyper-responsiveness and sub-epithelial fibrosis⁽¹¹⁾. Reports demonstrated that in asthmatics IL-4+ and IL-13+ cells present within the airway smooth muscle were predominantly expressed by mast cells, suggesting that IL-4 and IL-13 may play an important role in mast cell-airway smooth muscle interactions⁽¹²⁾.

Interleukine-18 formerly called interferon gamma inducing factor, is a novel proinflammatory cytokine related to the IL-1 family⁽¹³⁾, it is produced by a wide range of cells and involved in the pathogenesis of several inflammatory diseases such as asthma⁽¹⁴⁾. It was

recently demonstrated that IL-18 acts on T-cells to induce airway inflammation and airway hyper-responsiveness, as well as, stimulating Th1 cells to produce cytokines and chemokines responsible for the airway infiltration and inflammatory responsiveness, also activating mast cells and basophils⁽¹⁵⁾. However, there are few studies investigating the relationship between IL-12, IL-13, IL-18 and total IgE in asthmatic patients, especially in children. In this study, we studied the levels of these markers in children patients with asthma.

Materials and Methods

Study population

The population of this study consisted of 27 patients with asthma (15:males and 12:female), ages ranged from 3-12 years (Mean \pm SE: 7.0 \pm 0.89). Allergic asthmatic patients who have been attending the Zahraa allergic center of Al-Karkh hospital with clinical diagnosis of asthma, i.e.: history of recurrent wheeze, cough and dyspnea in the previous 12 months. 21 Healthy children matched for age (12:males and 9:females) were recruited as control individuals, with the following exclusion criteria: history of childhood asthma, family history of asthma, a febrile illness or chest infection within the previous four weeks, or episodes of cough and wheezing in the past 12 months, and subjects with a serum total IgE value of > 100 IU/ml.

Measurement of serum total IgE, IL-12, IL-13 and IL-18 levels

A forearm venous blood sample (5 ml) was drawn from all subjects for complete blood count, cytokine and IgE estimation upon recruitment. Blood was allowed to clot and was then centrifuged and sera was collected and divided into a number of Eppendorf tubes, stored at -20°C and thawed immediately before analysis. Serum concentration of total IgE was measured by ELISA method (Human, Germany). Serum levels of IL-12, IL-13 and IL-18 were also measured by ELISA method (Biosource, Belgium), (Immunotech, France), (MBL, Germany) respectively. The minimal detection levels of cytokines using this method were 2.0 pg/ml for IL-12, 1.5 pg/ml for IL-13 and 12.5 pg/ml for IL-18, cytokine levels below the detection limits were considered as zero.

Statistical analysis

Data on circulating IL-12, IL-13, IL-18, and IgE levels are presented as mean \pm SEM. A paired *t*-test was used to compare the serum level of the studied parameters between asthma patients and controls, also using a special ELISA software (BioRad Lab, Inc.) to draw the standard curves of IL-12, IL-13, IL-18 and IgE. Statistical analysis was assumed for *P* values lower than 0.05.

Results

Serum concentrations of IgE, IL-13, and IL-18 were significantly higher in asthmatic patients

compared to control subjects. IgE :226.44 \pm 52.33 IU/ml versus 37.94 \pm 12.42 IU/ml, $P < 0.05$; IL-13: 9.73 \pm 1.72 pg/ml versus 4.43 \pm 0.59 pg/ml, $P < 0.05$; IL-18: 76.81 \pm 22.44 pg/ml versus 35.41 \pm 3.17 pg/ml, $P > 0.05$ Fig 1.a,b,c . whereas, serum concentration of IL-12 were lower in asthmatic patients compared to control subjects IL-12: 93.57 \pm 12.17 pg/ml versus 122.83 \pm 19.16 pg/ml showed no significant differences in there results, as shown in Fig 1.d .

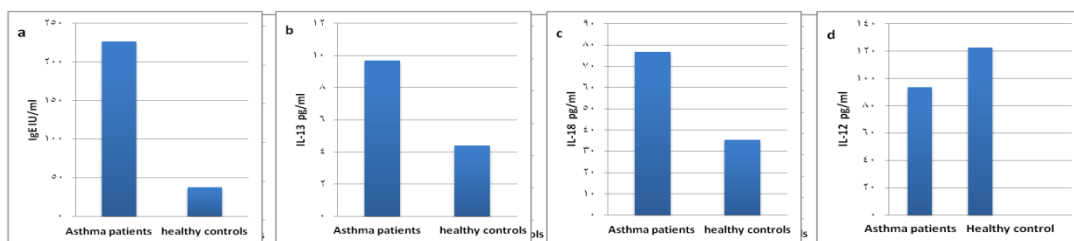


Figure 1 : Serum concentrations of a. IgE, b. IL-13, c. IL-18, and d. IL-12 in both asthma patients and healthy controls.

Discussion

Asthma is a chronic inflammatory disease of the airways characterized by mucus gland hyperplasia, basement membrane thickening and eosinophil infiltration⁽¹⁶⁾. Serum levels of IgE was shown to be higher in asthmatics compared to control subjects as a result of type 1 hypersensitivity response (Fig.1.a), the results of this study is supported by data that have been published by (Sandstrom, 2009)⁽¹⁷⁾. On the other hand, bronchial hyper responsiveness and airway inflammation can be elicited through IgE independent mechanisms as documented in an experimental model of asthma⁽¹⁸⁾. Furthermore, a genetic study in humans involving IL-13 gene polymorphism has clearly shown a relationship between susceptibility to asthma which was independent on serum IgE levels⁽¹⁹⁾. Serum IgE values decline with age in the general population, as the immune system undergoes characteristic changes with aging. Most of T-cell function are depressed in elderly individuals and the accumulation of CD45RO+memory cells in elderly individuals result in a reduced ability to respond to new antigens, and a retained ability to respond to recall antigens as long as the memory cells remained present and functional⁽²⁰⁾.

Interlukine-12 was shown to be involved in inhibiting IgE levels, enhancing IFN- γ production

which also inhibit IgE levels⁽⁷⁾. Serum levels of IL-12 in asthmatics showed no elevation compared to control subjects in this study, (Fig 1.d). Although high IgE levels was detected in asthmatics, and as we know asthma is a Th2 disease, in which the Th1 cytokine (IL-12) does not play a vital role in the pathogenesis of the disease. Patients with both Th1 and Th2 mediated disease like Type 1 diabetes mellitus and asthma display different pattern of IL-12 and IL-18 expression with much higher levels of both IL-12 and IL-18 compared to their levels in patients with one disease only and controls⁽²⁹⁾.

Serum levels of IL-13 was higher in asthmatics compared to control subjects as results revealed in this study, (Fig 1.b), thus pointing to an inflammatory mediator in asthma patients which was also presented in other studies^(21,22). Therefore, elevation in serum levels of both IL-13 and IL-5 in asthmatics may point towards an ongoing systemic Th2 inflammatory response⁽²³⁾. The source of elevated serum IL-13 levels in asthmatics is not clarified. However, there is evidence to suggest that the circulating PBMCs may be a major source of circulating IL-13 in asthmatics⁽²⁴⁾. In addition, the PBMCs producing IL-13 are higher in proportion after exposure to allergens⁽²⁵⁾. Increased expression of IL-13 has been documented in the bronchial

mucosa of asthmatics, therefore spillover of IL-13 from inflamed airways to peripheral blood cannot be discounted as a possibility for the raised serum IL-13 levels observed in asthmatics⁽²⁶⁾. IL-13 blocking antibody has been tested in animal models of asthma with some success⁽²⁷⁾. also, gene therapy directed against IL-4 receptors may represent another new approach in controlling airway inflammation in asthma⁽²⁸⁾.

Higher serum IL-18 levels were found in asthmatics as compared to control subjects as presented in (Fig.1.c). IL-18 serum levels may partly reflect disease activity in asthmatic patients, IL-18 is mainly secreted by activated monocytes/macrophages and kupffer cells as well as other cells⁽³⁰⁾. IL-18 and IL-12 have both been shown to be strong cofactors for Th1 cell activation and stimulate Th1 cytokines. However, it was thought that IL-18 might act as a coinducer of both Th1 and Th2 cytokines, it was suggested that elevated IL-18 levels in asthma patients did not affect IFN- γ production and might enhance T-lymphocyte-mediated inflammation⁽¹⁵⁾. It is speculated that IL-18 might partly reflect asthma exacerbation through activation of T-lymphocyte, monocyte, and granulocyte-triggered inflammation with the IFN- γ independent pathway, which may explain IL-18 contribution to Th2 cytokine dominant disease in a clinical setting.

Furthermore, histamine does dependently stimulate the production of IL-18 in human PBMCs, most of the histamine is stored in mast cells in asthmatic airway and is released quickly to bronchial tissue during IgE-dependent asthmatic response⁽³¹⁾. Increased serum IL-18 production in asthmatic patients may partly stimulated by histamine release. On the other hand, many viral and bacterial infections also may stimulate IL-18 generation⁽³²⁾. Some studies revealed detectable IFN- γ in some asthmatic patients although asthma is a Th2 cytokine dominant disease, in which Th1 cytokine production is suppressed⁽³³⁾.

In conclusion, it was suggested that IL-18 and IL-13 might have a potential role in controlling lymphocyte responses in asthmatic patients and such elevation of these cytokines might induce different immunologic responses in a Th2 cytokine dominant disease.

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