

Assessment the Level of Interleukine-5 and Eosinophils Count in Allergic Rhinitis and Asthma Patients

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Abstract

Fifty blood samples have been collected from patients attending the center of the allergy and asthma in the province of Babylon (25 before immunotherapy treatment and 25 after treatment), in addition to 10 samples from non-allergic persons as a control . IL-5 concentrations have been measured by using ELISA, while the eosinophils have been measured by using complete blood count device. This results shows that there is an increase in the level of IL-5 and eosinophils count before allergen-specific immunotherapy treatment for allergic rhinitis patients which reached 53.1402 ± 3.67801 pg/ml and 0.8249 ± 0.20108 cell/mm³ respectively and asthmatic patients 49.8922 ± 7.43190 pg/ml and 0.6523 ± 0.25712 cell/mm³ respectively in compared with their level after treatment for allergic rhinitis patients which reached 22.7315 ± 4.03680 pg/ml and 0.3642 ± 0.14734 cell/mm³ and asthma patients 22.7177 ± 4.13433 pg/ml and 0.3645 ± 0.15743 cell/mm³ respectively, and for control which reached 24.5404 ± 8.64920 pg/ml and 0.2252 ± 0.09139 cell/mm³ respectively. The results showed that there were an increase in the level of IL-5 for allergic rhinitis patients before and after treatment in compared with asthma patients , eosinophils increase in allergic rhinitis patients in compared with asthma patients before immunotherapy treatment, but the opposite is true for their count which increase in asthma patients in compared with allergic rhinitis patients after treatment. The level of IL-5 and eosinophils count decrease gradually by increasing the period of immunotherapy treatment which divided into three groups two months , six months and one year , the level of IL-5 for these groups 26.2821 ± 3.81978 pg/ml , 21.3737 ± 2.47124 pg/ml and 20.5029 ± 4.67899 pg/ml respectively , while the count of eosinophils for these groups reached 0.4567 ± 0.15057 cell/mm³ , 0.4282 ± 0.15162 cell/mm³ and 0.2627 ± 0.06517 cell/mm³ respectively.

Keywords: Allergic rhinitis, Asthma, Allergen-specific immunotherapy, IL-5, Eosinophils

الخلاصة

تم جمع 50 عينة دم من المرضى المراجعين لمركز الحساسية والربو في محافظة بابل (25 قبل العلاج و 25 بعد العلاج) ، بالإضافة الى 10 عينات من اشخاص غير مصابين بالحساسية من النوع الاول اعتبرت كسيطرة، تم قياس تركيز IL-5 باستخدام تقنية ELISA ، في حين تم قياس تعداد كريات الدم الحمضة باستخدام جهاز عد كريات الدم الكامل. اظهرت النتائج وجود ارتفاع في مستوى IL-5 كذلك الخلايا الحمضة قبل العلاج المناعي لكل من مرضى التهاب الانف التحسسي 53.1402 ± 3.67801 pg/ml و 0.8249 ± 0.20108 خلية/ملم³ على التوالي و مرضى الربو ، 49.8922 ± 7.43190 pg/ml و 0.6523 ± 0.25712 خلية/ملم³ على التوالي، مقارنة مع مجموعة بعد العلاج لمرضى التهاب الانف التحسسي 22.7315 ± 4.03680 pg/ml و 0.3642 ± 0.14734 خلية/ملم³ على التوالي و مرضى الربو 22.7177 ± 4.13433 pg/ml و 0.3645 ± 0.15743 خلية/ملم³ على التوالي، ومجموعة السيطرة التي بلغت 24.5404 ± 8.64920 pg/ml و 0.2252 ± 0.09139 خلية/ملم³ على التوالي. اظهرت النتائج كذلك ارتفاع مستوى IL-5 بالنسبة لمرضى التهاب الانف التحسسي مقارنة مع مستواه لمرضى الربو قبل وبعد العلاج المناعي، اما بالنسبة للخلايا الحمضة فقد وجد ارتفاع في تعداد هذه الخلايا عند مرضى التهاب الانف التحسسي مقارنة مع مرضى الربو لمجموعة قبل العلاج، لكن يصبح العكس هو الصحيح حيث يرتفع تعداد هذه الخلايا عند مرضى الربو مقارنة مع مرضى التهاب الانف التحسسي لمجموعة بعد العلاج. ينخفض تركيز IL-5، والخلايا الحمضة تدريجيا بزيادة مدة العلاج المناعي التي قسمت الى ثلاث مجاميع شهرين و ستة اشهر و سنة واحدة ، بلغ تركيز IL-5 لهذه المجاميع 26.2821 ± 3.81978 pg/ml ، 21.3737 ± 2.47124 pg/ml و 20.5029 ± 4.67899 pg/ml على التوالي، في حين بلغ تعداد الخلايا الحمضة لنفس المجاميع كالتالي 0.4567 ± 0.15057 خلية / ملم³ ، 0.4282 ± 0.15162 خلية / ملم³ و 0.2627 ± 0.06517 خلية / ملم³.

الكلمات المفتاحية: التهاب الانف التحسسي، الربو، العلاج المناعي المتخصص بالارجين، الحركي الخلوي IL-5، الخلايا الحمضة

Introduction

Allergic diseases including allergic rhinitis and asthma are characterized by inflammation of the airway and infiltration of eosinophils and CD4⁺ T cells (Kay, 2001; Murphy and Reiner, 2002), they both represent a central feature of the late-phase allergic response (LAR) (Kay, 2001; Wills-Karp, 1999). Accumulating evidence indicates that classical Th2-cell-derived cytokines (e.g. IL-3, IL-4, IL-5, IL-9, IL-13 and GM-CSF) together with eotaxin play critical roles in the induction of airway hyper-reactivity and the development of chronic airway wall remodeling (Asquith, 2008; Rothenberg, and Hogan, 2006). Allergic rhinitis defined as an inflammation of the lining of the nose and is characterized by nasal symptoms including anterior or posterior rhinorrhea, sneezing, nasal blockage and/or itching of the nose (International Consensus Report on Diagnosis and Management of Rhinitis, 1994). Allergic asthma is recognized as a chronic inflammatory disease of the airway, it is characterized by reversible airway obstruction, airway hyperresponsiveness (AHR) to a wide variety of specific or non-specific stimuli, chronic airway inflammation, and airway remodeling (Maddox and Schwartz, 2002). The mechanisms by which allergen-specific immunotherapy exerts its effects include the modulation of both T cells and B cells responses to allergens (Larche *et al.*, 2006). Allergy immunotherapy is an effective treatment for allergic asthma and rhinitis, in addition to reducing symptoms it can change the course of allergic disease and induce allergen specific immune tolerance (Burks *et al.*, 2013).

IL-5 is produced by Th2 cells after stimulation with allergens and by mast cells upon stimulation with allergen-IgE complex (Takatsu *et al.*, 1997). IL-5 messenger RNA (mRNA) is also expressed in eosinophils, $\gamma\delta$ T cells, NK and NKT cells and non-hematopoietic cells (Takatsu *et al.*, 1994; Sakuishi *et al.*, 2007). The effects of IL-5 on eosinophils largely fall into four categories, including differentiation, migration, activation and survival (Takatsu and Nakajima, 2008; Lee *et al.*, 1997). Eosinophils possess distinctive granule proteins, including major basic protein, eosinophil peroxidase and eosinophil derived neurotoxin. In both mice and humans, IL-5 induces terminal maturation of eosinophils, prolongs eosinophil survival by delaying apoptotic death, possesses eosinophil chemotactic activity, increases eosinophil adhesion to endothelial cells and enhances eosinophil effector functions (Rothenberg, and Hogan, 2006).

The aim of this study is to investigate the differences in the level of IL-5 and eosinophils count between allergic rhinitis and asthma patients in addition to a control group. And the effect of immunotherapy on those immunological parameters.

Materials and methods

1- Patients and control

The population of this study consist of 50 allergic patients (25 before treatment and 25 after treatment), in addition to 10 persons as a control. Their ages ranged from 17-58 from both sexes. All the patients have been diagnosed under the supervision of consultant physician in allergy and asthma center in Babylon province, and they didn't take any drugs.

2- Immunological parameters

Blood samples were divided into two parts, the first used for complete blood count to estimate the eosinophils count by using complete blood count device, the second part was allowed to clot and was then centrifuged and sera was collected and stored at -20 C° until it used to estimate the level of IL-5 by using ELISA kit (Boster, USA).

Statistical analysis

Statistical analysis was done by using computer software Statistical Package for Social Science (SPSS) version 20. Results were expressed as mean \pm standard deviation (SD), and analyzed by the use of one-way ANOVA.

Results

The results shows that there is an increase in the concentration of IL-5 before treatment in patients of allergic rhinitis 53.1402 ± 3.67801 pg/ml and asthma 49.8922 ± 7.43190 pg/ml in compared with its concentration after treatment in patients of allergic rhinitis 22.7315 ± 4.03680 pg/ml and asthma 22.7177 ± 4.13433 pg/ml, and control 24.5404 ± 8.64920 pg/ml with the presence of significant differences between the first group before treatment and both after treatment and control at the level of probability $P < 0.05$. the same results for eosinophil count which increase before treatment in patients of allergic rhinitis 0.8249 ± 0.20108 cell/mm³ and asthma 0.6523 ± 0.25712 cell/mm³ in compared with its count after treatment in patients of allergic rhinitis 0.3642 ± 0.14734 cell/mm³ and asthma 0.3645 ± 0.15743 cell/mm³, and control 0.2252 ± 0.09139 cell/mm³. The results also shows that there is an increase in the concentration of IL-5 before and after immunotherapy treatment in patients of allergic rhinitis 53.1402 ± 3.67801 pg/ml and 22.7315 ± 4.03680 pg/ml respectively in compared with its level in asthma patients 49.8922 ± 7.43190 pg/ml and 22.7177 ± 4.13433 pg/ml respectively. For eosinophils, there is an increase in its count before treatment in allergic rhinitis patients 0.8249 ± 0.20108 cell/mm³ in compared with asthma patients 0.6523 ± 0.25712 cell/mm³. After treatment the opposite is true there is an increase in eosinophil count of asthma patients 0.3645 ± 0.15743 cell/mm³ in compared with allergic rhinitis patients 0.3642 ± 0.14734 cell/mm³, table (1).

Table (1) The level of IL-5 and eosinophils count for allergic rhinitis patients and asthma patients before and after immunotherapy treatment

Groups		IL-5 Mean \pm SD	Eosinophils Mean \pm SD
Before treatment	Allergic rhinitis patients	$53.1402 \pm 3.67801^*$ n = 11	$0.8249 \pm 0.20108^*$ n = 11
	Asthma patients	$49.8922 \pm 7.43190^*$ n = 14	$0.6523 \pm 0.25712^*$ n = 14
After treatment	Allergic rhinitis patients	22.7315 ± 4.03680 n = 11	0.3642 ± 0.14734 n = 11
	Asthma patients	22.7177 ± 4.13433 n = 14	0.3645 ± 0.15743 n = 14
Control		24.5404 ± 8.64920 n = 10	0.2252 ± 0.09139 n = 10
* The mean difference is significant at the level of 0.05 with after treatment group and control			

According to the period immunotherapy treatment, the results have been divided into three groups, two months, six months and one year. The concentration of IL-5 and eosinophils decrease gradually with increasing the period of treatment , for IL-5 its reached 26.2821 ± 3.81978 pg/ml , 21.3737 ± 2.47124 pg/ml and 20.5029 ± 4.67899 pg/ml respectively , while the count of eosinophils for these groups reached 0.4567 ± 0.15057 cell/mm³ , 0.4282 ± 0.15162 cell/mm³ and 0.2627 ± 0.06517 cell/mm³ respectively, figure (1).

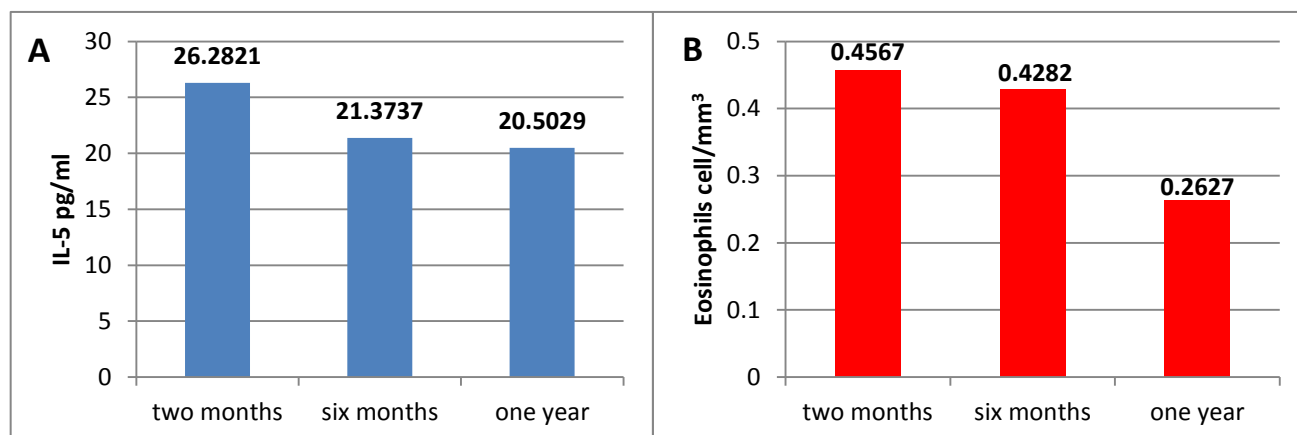


Figure (1) A- The serum level of IL-5, B- the eosinophils count of allergic patients according to the period of immunotherapy treatment

Discussion

According to these results there is an increase in the concentration of IL-5 and eosinophils in allergic patients in compared with control , the interpretation of these results , that the immune response allergic patients characterized by the dominance of Th2 cell (Akdis *et al.*,2004) , producing a number of inflammatory cytokines including IL-5, which in turn affects other cells have an effective role in the pathogenesis of allergic diseases like eosinophils (Jacobsen *et al.*,2012) , by stimulating its production , activation , and migration to the site of inflammation which is done through IL-5 association with its receptors on the surfaces of these cells (Stafford *et al.*,2002) . The result of this stimulation is a state called eosinophilia in addition to the excessive airway mucous production and stimulate allergic hyperresponsiveness(AHR) (Cohn *et al.*,1998). The concentration of IL-5 and eosinophils negatively affected by allergen-specific immunotherapy, because Immunotherapy works to change the mechanism of occurrence of the immune response from Th2 response to Th1 response and reduced the concentration of IL-5 (Passalacqua and Durham, 2007), this lead to a decrease in the eosinophils and reduced the late phase (Walker *et al.*, 2001; Wachholz *et al.*,2002), IL-10 also works on the inhibition of Granulocyte / Macrophage-Colony Stimulating Factor (GM-CSF) and surface active molecules of eosinophils and increased its apoptosis (Ohkawara *et al.*, 1996). These results also shows that the concentration of IL-5 and eosinophils are increased in allergic rhinitis patients in compared with asthma patient , this is may be due to the differences in term of exposure to allergens and noxious agents (the nose being more exposed than the lower airway) , there are also structural differences between the nasal and bronchial mucosa , the nose mucosa has a larger vascular supply whereas the latter has smooth muscle (Bousquet *et al.*,2000-2003; Barood and

Canning, 2004), this in turn may increase the migrating cells to the lining of the nose, and produce larger quantity of cytokines in compared with bronchial mucosa .

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